Cardiac Myosin Light Chain-2
A Novel Essential Component of Thick-Myofilament Assembly and Contractility of the Heart

Wolfgang Rottbauer, Georgia Wessels, Tillman Dahme, Steffen Just, Nicole Trano, David Hassel, Charles Geoffrey Burns, Hugo A. Katus, Mark C. Fishman

Abstract—Although it is well known that mutations in the cardiac regulatory myosin light chain-2 (mlc-2) gene cause hypertrophic cardiomyopathy, the precise in vivo structural and functional roles of MLC-2 in the heart are only poorly understood. We have isolated a mutation in zebrafish, tell tale heart (tel<sup>ml225</sup>), which selectively perturbs contractility of the embryonic heart. By positional cloning, we identified tel to encode the zebrafish mlc-2 gene. In contrast to mammals, zebrafish have only 1 cardiac-specific mlc-2 gene, which we find to be expressed in atrial and ventricular cardiomyocytes during early embryonic development, but also in the adult heart. Accordingly, loss of zMLC-2 function cannot be compensated for by upregulation of another mlc-2 gene. Surprisingly, ultrastructural analysis of tel cardiomyocytes reveals complete absence of organized thick myofilaments. Thus, our findings provide the first in vivo evidence that cardiac MLC-2 is required for thick-filament stabilization and contractility in the vertebrate heart. (Circ Res. 2006;99:323-331.)

Key Words: cardiac myosin light chain-2 • myofibrillogenesis • zebrafish heart

Inherited heart muscle diseases (cardiomyopathies) in humans, the leading cause of premature death in the young, are mainly caused by mutations in various sarcomeric proteins. Ultrastructural analysis of affected cardiomyocytes implicates disturbed myofibrillogenesis to be partially responsible for altered heart function because severe disarray of myofibrils can be observed. One of the proteins found to induce human cardiomyopathies, when mutated, is the thick-filament component cardiac regulatory myosin light chain-2 (MLC-2). However, the precise in vivo structural and functional roles of MLCs in the vertebrate heart muscle are only poorly understood.

Each thick filament in muscle includes 2 myosin heavy chains and 4 MLCs, which are wrapped like dumbbells around the α-helical neck region of myosin heavy chains. The mlc gene families differ in size among species but may generally be characterized as regulatory (ie, phosphorylatable) or essential. In vitro, regulatory MLCs appear to play an important part in the conversion of chemical energy into movement, specifically enhancing actin-filament sliding velocity. In vivo, MLCs confer different functional roles on different muscle types. In smooth muscle, phosphorylation of regulatory MLC by myosin light chain kinase is the switch for turning on the actin-activated myosin ATPase and, hence, contraction. By contrast, in vertebrate striated muscle, the actin–myosin interaction is mainly regulated through the troponin–tropomyosin complex, and regulatory MLCs are thought to have a modulatory effect on rate and magnitude of force generation. In the adult heart muscle, phosphorylation of regulatory MLCs increases tension production while decreasing stretch actuation response of cardiomyocytes. Two major regulatory MLC isoforms are coexpressed in the early stages of murine cardiogenesis. Disruption of MLC-2v causes dilated cardiomyopathy. However, there is significant compensatory upregulation of atrial-specific MLC-2 (MLC-2a) in the ventricular cardiomyocytes of MLC-2v–depleted mice. MLC-2a might partially compensate for the loss of MLC-2v, thereby masking an essential role of MLC-2v in vertebrate heart function.

We sought here the molecular cause of an ethynitrosourea (ENU)-induced recessive embryonic lethal zebrafish mutation, tell tale heart (tel<sup>ml225</sup>), which selectively perturbs early embryonic heart contractility. By positional cloning, we identify tel to encode a zebrafish cardiac regulatory MLC gene (zmlc2). zmlc2 is expressed throughout the heart, unlike the chamber-specific pattern noted for regulatory MLCs in mouse and human. In fact, zmlc2 appears to be the only regulatory mlc gene expressed in the heart of the zebrafish. Loss of this unique zmlc completely abolishes myofibrillar assembly in a cell-autonomous fashion in tel mutant hearts.
demonstrating that MLC-2 is essential for cardiac myofibrillogenesis in vivo.

Materials and Methods

Zebrafish Strains and Cell Transplantation

Care and breeding of zebrafish, Danio rerio, were as described.8 Cell transplantation was performed essentially as described9 by intercrossing telm225† fish, thus yielding a wild-type to mutant embryo ratio of 3:1.

Genetic Mapping, Positional Cloning, and Mutation Detection

DNA from 24 telm225 mutant and 24 wild-type embryos was pooled for bulked-segregation analysis.10 One hundred twenty microsatellite markers11 were tested for possible linkage. The critical genomic interval for tel was defined by genotyping 2249 mutant telm225 embryos for microsatellite markers in the area. Mapping information for expressed-sequence tag (EST) clones was derived from radiation hybrid maps (http://wwwmap.tuebingen.mpg.de and http://mgchd1.nichd.nih.gov:8000/zfrh/beta.cgi). Recombinant fine mapping analysis was performed with single-stranded conformation polymorphisms (SSCP) from the sequence of ESTs or sequenced bacterial artificial chromosome (BAC) clones.

A genomic DNA “shotgun” library of BAC 159f11, which covers the tel interval, was made essentially as described.9 Sequence tracings were read and processed by the Phred program. Six interconnected sequence contigs were assembled using Phrap and edited by Consed (http://www.phrap.org).

The cDNA sequence for zmlc-2 was determined by RT-PCR and rapid amplification of cDNA ends (Clontech) (GenBank accession no. AY057074). RNA from mutant and wild-type whole embryos was isolated using TRIzol Reagent (Life Technologies).

Histology, Transmission Electron Microscopy, RNA Antisense In Situ Hybridization, Immunostaining, and Immunoblotting

Histological analysis and electron micrographs of fish embryos were performed as described.8 Whole-mount RNA antisense in situ hybridization was performed as described9 using a full-length digoxigenin-labeled zmlc-2 riboprobe.

For whole-mount immunostaining, embryos were fixed in 4% paraformaldehyde or Dent’s fixative.12 Monoclonal antibodies against myosin heavy chain, MF20, S46,13 and α-tropomyosin (Sigma) were used.

In vitro translation of wild-type and mutant MLC-2 was performed using the TNT coupled reticulocyte lysate system (Promega). For immunoblotting, in vitro–translated proteins were separated by 15% SDS-PAGE and transferred to polyvinylidene fluoride (PVDF) membrane. Blots were probed with anti–FLAG M2 (Sigma) as primary antibody and signals detected by chemiluminescence.

Injection Procedures

Morpholino-modified antisense oligonucleotide was directed against the translational start site of zebrasfish cardiac mlc-2 (MO-mlc-2; 5′-GGTTTTTTTCTAGCCTCATTTGCACTG-3′) (GENETOOLS LLC). Embryos at the 1-cell stage were injected with 10 ng of MO-mlc-2 or a standard control oligonucleotide (MO-control) in 0.2 mol/L KCl and 0.1% phenol red.

Capped mRNA was synthesized using the mMESSAGE mMACHINE system (Ambion). Twenty-five picograms of mRNA were microinjected into 1-cell-stage embryos.

DNA from the BAC clone B159f11 was isolated using Qiagen columns, as instructed by the manufacturer, followed by additional phenol/chloroform extraction and ethanol precipitation. BAC DNA was diluted in Danieu’s medium/0.1% phenol red, and 0.5 ng was microinjected into 1-cell-stage embryos.

Results

tel Is Required for Early Embryonic Heart Contractility in a Cell-Autonomous Fashion
telm225 is an ENU-induced, fully penetrant embryonic lethal recessive mutation in zebrafish that selectively perturbs cardiac contractility during early embryonic development. Blood circulation is never established in homozygous tel mutant embryos. Aside from pericardial edema, embryos are not noticeably affected by the lack of normal blood flow during the first week of development (Figure 1A and 1B).

tel mutants form an apparently morphologically normal heart, in an anatomically correct position, with an endocardial and myocardial layer. By 36 hours post-fertilization (hpf), the 2 heart chambers are distinct (atrium and ventricle), and appropriate growth of the ventricular myocardium can be observed in tel mutant embryos by 72 hpf (Figure 1C and 1D). However, vigorous peristaltic contraction waves traversing the heart tube, as usually seen in wild-type embryos, are absent. By contrast, only weak, uncoordinated myocardial contractions of single heart cells can be observed in tel mutant embryos at 24 hpf. By 48 hpf, atrial and ventricular cardiomyocytes are silent and the fractional shortening of both chambers declines to 0 (Figure 1E and 1F). Interestingly, some cardiomyocytes of the rather extended and pronounced atrioventricular (AV) canal region continue to contract rhythmically (Movie 1 in the online data supplement, available at http://circres.ahajournals.org).

To examine the cell autonomy of the cardiac contraction defect in tel mutant embryos, we performed cell-transplantation experiments to generate mosaic hearts composed of mutant and wild-type cardiomyocytes. We found that when wild-type cells contribute to mutant hearts, they contract spontaneously and vigorously in tel ventricles and atria. In contrast, tel mutant cardiomyocytes do not contract in a wild-type host. In fact, they are thin, mechanically unstable, and bulge dyskinetically outward with each systolic contraction of the wild-type heart, forming aneurysm-like structures in the atrium and the ventricular chamber (Figure 1G and 1H), similar to titin-deficient cardiomyocytes of zebrafish pickwick mutants.15 Thus, the tel gene controls cardiomyocyte contractility in a cell-autonomous manner.

The tel Locus Encodes a Zebrafish Cardiac Regulatory Myosin Light Chain Gene

We identified the mutation causing the recessive tel mutant phenotype by a positional walk (Figure 2A). The tel mutation maps to zebrasfish linkage group 8 between microsatellite markers Z25243 and Z21115. A genetic marker derived from EST fb57f10 (GenBank accession no. AI477585), linked to the tel interval by radiation hybrid (RH) mapping,16 displays 1 recombination event in 4498 tel mutant meiotic events. A BAC clone (B159f11) containing fb57f10 was isolated and a “shot-gun” library constructed and sequenced. B159f11 contains 4 open-reading frames. Further testing of recombination events in tel mutant embryos restricts the tel interval to the zebrafish cardiac regulatory light chain gene (zmlc-2) (Figure 2A). As in other species, zmlc-2 is encoded by 7 exons.

Conceptual translation of genomic DNA and cDNA se-
quences (GenBank accession no. AY057074) reveals a high degree of homology (78%) to the human and mouse regulatory light chain 2a (MLC-2a) protein (Figure 2B).

A Splice Donor-Site Mutation Leads to Early Truncation of Zebrafish MLC-2 in tel

To identify the site of the mutation, the entire zebrafish coding sequence of zmlc-2 from wild-type and tel mutant embryos was sequenced. Three different cDNA splice products were detected in tel mutant cDNA, all transcripts leading to premature termination of translation before exon 3 or 4, respectively. As indicated in Figure 2D, tel cDNA 1 contains intron 3 sequence. tel cDNA 2 displays a new exon boundary exon 2/exon 4 attributable to skipping of exon 3, and tel cDNA 3 reveals skipping of exon 3 through exon 5 of the zebrafish mlc-2 gene (Figure 2D), all transcripts leading to a reading-frame shift and premature stop of protein translation. To further elucidate the cause of aberrant splicing in tel mutant embryos, genomic intronic sequence was determined around exon 3. A splice donor-site mutation (g→a) at the highly conserved position 1 of the splice donor site of intron 3 was detected, leading to the different splicing events in tel mutants (Figure 2C and 2D).

To confirm that loss of MLC-2 is indeed responsible for the tel phenotype, morpholino-modified antisense oligonucleotides targeted against the translational start site of zebrafish cardiac mlc-2 (MO-mlc-2) were injected into wild-type embryos (n=200) at the 1-cell stage. Ninety-seven percent of the injected embryos (194/200) displayed the tel mutant phenotype with a silent ventricle and atrium and only contractions of cardiomyocytes of the AV canal (Figure 3A through 3C and supplemental Movie 2).

To test whether wild-type DNA or RNA encoding zMLC-2 could suppress the tel mutant phenotype, tel mutant embryos (telm225/H11002/H11002) were injected at the 1-cell stage. After injection of 0.5 ng of BAC 159f11 DNA, 25% of genotypically tel mutant embryos (7/28) revealed vigorous contractions of areas of the ventricular wall by 72 hpf (Figure 3D). In 2 rescued embryos, weak circulation of blood cells could be observed. However, none of the rescued embryos survived beyond day 8 of development. Injection of capped zmlc-2 mRNA also partially rescued up to 17% of tel mutant embryos, depending on the dose injected (Figure 3D), whereas injection of tel mutant zmlc2 mRNA (tel-cDNA-1) had no effect on the tel mutant phenotype.

Truncated zMLC-2 Protein Does Not Accumulate in tel Mutant Hearts

Even though the tel mutation interferes with only RNA processing, antisense-mediated knockdown of zmlc-2 com-
pletely phenocopies \textit{tel}, implicating that even in \textit{tel} mutant hearts, zMLC-2 function is completely abolished. Therefore, to evaluate whether the \textit{tel} mutation interferes with RNA stability, we analyzed the abundance of \textit{mlc-2} mRNA by whole-mount in situ antisense hybridization. As shown in Figure 4, \textit{mlc-2} mRNA is severely reduced in the hearts of \textit{tel} embryos at 72 hpf (Figure 4A and 4B), indicating degradation of \textit{tel} mutant mis-spliced z\textit{mlc-2} mRNA most likely by nonsense-mediated RNA decay.\textsuperscript{17} However, traces of mutant RNA are still detectable in \textit{tel} hearts. To further test whether predicted truncated MLC-2 protein is stable and thereby might act as a dominant negative, we generated expression plasmids of either N- or C-terminal FLAG-tagged wild-type or mutant MLC-2 constructs. However, whereas in vitro–translated N- and C-terminally tagged wild-type MLC-2 accumulates in reticulocyte lysates, neither N- nor C-terminally tagged truncated MLC-2 proteins accumulated to detectable levels (Figure 4C). Because the majority of \textit{tel} mutant RNA is degraded, and mutant MLC-2 protein per se is not stable, lack of MLC-2 rather than a dominant-negative effect of truncated MLC-2 appears to cause the \textit{tel} mutant heart phenotype.

\textbf{\textit{tel} Is Not Expressed in a Cardiac Chamber–Restricted Pattern}

\textit{tel} appears to be most similar to the atrial isoform (MLC-2a) of human regulatory MLCs (Figure 2B). Human and mouse MLC-2a are known to be preferentially expressed in atrial cardiomyocytes.\textsuperscript{18} However, in contrast to mammals, \textit{zmlc-2} RNA is found to be expressed in both heart chambers from early embryonic development throughout adulthood (Figure 4D through 4F). Similarly, in our transgenic zebrafish

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{figure2.png}
\caption{\textit{tel} encodes cardiac MLC-2 and is highly conserved between zebrafish and human. A, Integrated genetic and physical map of the zebrafish \textit{tel} region. The \textit{tel} mutation interval is flanked by the microsatellite markers z21115 and z25210. A bacterial artificial chromosome (BAC) clone B159f11 was isolated covering the \textit{tel} interval and subjected to shotgun sequencing. Sequence analysis of the \textit{tel} mutation interval revealed 4 zebrafish genes, 1 of them highly homologous to cardiac regulatory MLC. The genomic structure of \textit{zmlc-2} is displayed at the bottom and the location of the \textit{tel} splice donor-site mutation at the exon 2–intron 3 boundary indicated by an arrow. B, Amino acid sequence alignment of zebrafish (zMLC-2) with mouse atrial MLC-2 (mMLC-2a), human atrial MLC-2a (hMLC-2a), and human ventricular MLC-2 (mMLC-2v) demonstrates high amino acid homology of zebrafish MLC-2 with both the atrial and ventricular isoform of human and mouse MLC-2. Black boxes indicate identical residues. A predicted EF-hand calcium-binding domain of zMLC2 is indicated with bars above the alignment, and the phosphorylation site at serine 19 is marked by a red-encircled P. Exon boundaries are indicated. C and D, The splice site donor-site mutation (g\textsuperscript{3}a) at the exon 2–intron 3 boundary of \textit{zmlc2} (C) results in 3 different cDNAs in \textit{tel} mutant embryos (D). Amino acid translation derived from the codons is shown below the base readouts. The mutated base is marked by an arrow. Exonic bases are displayed in uppercase; intronic bases in lowercase. D, In comparison with wild-type (wt) zMLC-2, 3 different cDNA products were identified in \textit{telm225} mutant cDNA, all predicted to lead to premature termination of translation at the indicated (asterisk) location.
\end{figure}
which drives expression of green fluorescent protein (GFP) under control of a 5.1-kb upstream region of zmlc-2, specific fluorescence is found in both the atrium and the ventricle (Figure 4 G through 4I), confirming that in contrast to mammals, there is no chamber restriction of MLC-2 in zebrafish.

In addition, after searching the zebrafish genomic and EST databases and performing consecutive RNA expression analyses, an additional cardiac regulatory zmlc gene could not be identified, indicating that similar to flies21 and frogs,22 zebrafish express only 1 regulatory mlc-2 in the heart.

**tel Is Essential for Cardiac Myofibrillogenesis**

In mammals, the structural and functional roles of cardiac regulatory MLCs are still debated. In contrast to zebrafish, 2 regulatory MLC isoforms (MLC-2a and MLC-2v) are coexpressed in heart tissue. Inhibition of zmlc-2 function by morpholino-modified antisense oligonucleotide injection phenocopies the tel mutant phenotype. A through C, Ninety-seven percent of wild-type embryos injected with MO-mlc-2, which is directed against the translational start site of zmlc-2, are indistinguishable from tel mutant embryos and display severe impairment of cardiac contractility. D, Injection of BAC159f11 DNA into tel tel mutant embryos rescues the tel mutant heart phenotype in 25%. Similarly, the tel mutant heart phenotype can be suppressed by injection of wild-type zmlc-2 RNA (capped mRNA wt), whereas injection of tel mutant cDNA-1 (capped mRNA mt) in tel tel mutant embryos had no effect on the cardiac phenotype.
pressed in the heart and seem to partially compensate for the loss of the other MLC-2 at least in the ventricle. Interestingly, knockout mice recently revealed an essential role of MLC-2a in myofibrillogenesis of atrial cardiomyocytes. Therefore, to determine whether the tel mutation renders ventricular and atrial cardiomyocytes noncontractile because of defective myofibrillogenesis, we analyzed the ultrastructure of tel embryonic hearts by transmission electron microscopy (Figure 5A through 5F). Normally, by 24 hpf, nascent myofibrillar arrays can be observed in wild-type zebrafish hearts, and by 48 hpf, clear organization of thick and thin filaments with discernible Z-discs, A-bands, and I-bands (A, C, and E), in tel mutant cardiomyocytes, no higher-order sarcomeres can be detected (B, C, and D). Only an immature assembly of thin-filament actin (Z-bodies) can be observed in tel mutant cardiomyocytes. As opposed to the hexagonal lattices of thick and thin filaments seen in cross-sections of wild-type myofibrils (E, inset), only scattered thin filaments are present in tel mutant cardiomyocytes (F, inset).

Because, as outlined above, only cardiomyocytes of the AV canal contract in tel hearts, we examined whether these cardiomyocytes, in contrast to those of the ventricle and the atrium, form regular sarcomeres. However, our detailed ultrastructural analysis of AV cardiomyocytes from tel hearts also did not reveal any organized sarcomeric structures (data not shown). This indicates that even a loose interaction of actin and myosin filaments in myocardial cells of the AV canal suffices to maintain their contractility.

To examine whether altered gene expression of key regulatory AV canal markers accounts for the changed morphology but preserved contractile function of the AV canal in tel mutant hearts, we performed whole-mount RNA expression studies for versican, an extracellular matrix protein, and bone morphogenetic protein 4 (bmp4), both of which are known to localize to the AV border during zebrafish heart development, is at normal levels and in its restricted pattern in wild-type and tel mutant hearts. Only an immature assembly of thin-filament actin (Z-bodies) can be observed in tel mutant cardiomyocytes (Figure 5F, inset).

Cardiac Thick- and Thin-Filament Components Are Expressed at Normal Levels in tel Mutant Hearts

Similar to tel mutant hearts, loss of zebrafish troponin T also inhibits cardiac myofibrillogenesis. Interestingly, troponin T deficiency also leads to significantly reduced expression of other thin-filament components, such as α-tropomyosin and troponin I, indicating interdependence of thin-filament pro-
zMLC-2 by itself is essential for cardiac myofibrillogenesis. The thick- or thin-filament sarcomeric proteins, indicating that zebrafish MLC-2 does not lead to reduced expression of other sarcomeric proteins (7H). Thus, in contrast to zebrafish tropomyosin, loss of essential components of heart muscle thick filaments such as zebrafish atrial myosin heavy chain (zamhc) (Figure 7A and 7B) and zebrafish heart muscle thick filaments such as zebrafish atrial myosin heavy chain (zvmhc) (Figure 7C through 7E) nor thin-filament components such as α-tropomyosin (α-Tm) (G and H). Lateral view of wild-type and tel mutant embryos at 48 hpf. MF20 (TRITC) stains the entire heart tube; S46 (FITC) stains only the atrium. In double exposure, the ventricle (V) fluoresces red and the atrium (A) fluoresces yellow.

Discussion

The early and rapid nature of cardiac myofibrillogenesis poses challenges for in vivo studies. Assessing early embryonic heart morphology and function is facilitated in zebrafish embryos, because they are transparent and not dependent on intact cardiovascular function during the first 7 days of development.28

Here we show, for the first time, that MLC-2 is essential for thick-filament assembly and myofibrillogenesis in the zebrafish embryonic heart. Using a forward genetic approach, we identified a recessive, embryonic lethal mutation in the zebrafish cardiac myosin light chain 2 gene, tel/tel. The mutation completely abolishes MLC-2 function, resulting in disturbance of myofibrillar assembly in a cell-autonomous fashion in tel/tel mutant hearts. Consecutively, atrial and ventricular cardiomyocytes are unable to contract.

Despite major advances in understanding the molecular basis of active contraction, surprisingly little is known about the mechanisms controlling myofibrillar formation in cardiac muscle. During early cardiac myofibrillogenesis, first I-Z-I complexes (Z-bodies) containing α-actinin, sarcomeric actin, and the amino terminus of titin are build in register. Thick filaments assemble and then incorporate into these preformed structures. Like other myofibrillar constituents, myosin first appears diffusely in the cytoplasm. Later, when M-line epitopes of titin appear, myosin thick filaments organize in a sarcomeric arrangement together with I-Z-I complexes. However, the exact mechanisms by which vertebrate sarcomeric myosins assemble into thick filaments are unknown.29 We show here, for the first time, that MLC-2 is essential for cardiac thick-filament assembly in vivo. In agreement with previous reports that thick and thin filaments assemble independently,30 initial thin-filament assembly (formation of I-Z-I bodies) proceeds normally in tel/tel mutant cardiomyocytes. However, in the absence of tel (MLC-2), thick-filament assembly is completely disrupted and organized sarcomeric units cannot be observed. This is despite the fact that cardiac myosin heavy chain is expressed at normal levels in tel/tel mutant hearts, implicating an essential role of regulatory MLCs in thick-filament assembly and sarcomereogenesis. Interestingly, loss of titin in zebrafish leads to similar alterations in cardiac ultrastructure,15 implicating potential overlapping functions of MLC-2 and titin in regulating cardiac myofibrillogenesis.

In contrast to mammals, only 1 mlc-2 gene is expressed in the zebrafish heart. Similarly, only 1 regulatory MLC gene exists in flies. Loss of mlc-2 in Drosophila melanogaster is recessive lethal and leads to a dominant flightless phenotype. Even indirect flight-muscle myofibrils from heterozygotes are aberrant, exhibiting myofilaments in disarray at the periphery. These results indicate that wild-type MLC-2 stoichiometry is required for normal flight-muscle assembly and function in flies.23 Interestingly, heterozygous tel/tel mutant embryos do not display an obvious cardiac phenotype. However, future studies of the heart morphology and function of adult heterozygous tel fish are necessary to examine the effect of heterozygous loss of zMLC on cardiac myofibrillogenesis in the adult heart.

The physiological in vivo function of regulatory MLCs in the vertebrate heart is debated. In contrast to zebrafish and flies, 2 major cardiac MLC-2 isoforms are coexpressed in the early stages of mouse cardiogenesis, a ventricular (MLC-2v) and an atrial isoform (MLC-2a).31 Both isoforms seem to be able to partially compensate for the loss of the other MLC-2. Disruption of the ventricular myosin light chain 2 gene (mlc-2v) in mice is lethal at embryonic day 12.5 as a result of
dilated cardiomyopathy. However, in contrast to tel mutant embryos, their ventricular cardiomyocytes display only slight defects in sarcomere assembly, without disturbance of thick-filament assembly, most likely because of compensatory upregulation of MLC-2a protein in the ventricles of homozygous mutant mice. By contrast, mice deficient in MLC-2a display complete absence of myofibrillar organization in their atria. Compensatory expression of MLC-2v protein in the atria is not observed. Similar to the mouse atrium, there is only 1 regulatory MLC-2 expressed in the zebrafish heart. Accordingly, loss of MLC-2 function in zebrafish tel mutant hearts cannot be compensated by another MLC-2 isoform but rather leads to complete disruption of cardiac myofibrilligenesis. However, diverging functions of zebrafish MLC-2 and the 2 mammalian MLC-2 isoforms cannot be excluded. It will therefore be of interest to generate double knockout mice for both atrial and ventricular MLC-2 to confirm the essential nature of MLC-2 for thick-filament assembly in the mammalian heart.

Autosomal-dominant inherited missense mutation in the cardiac mlc-2 genes of humans induce hypertrophic cardiomyopathy (HCM), a heart muscle disease characterized by asymmetric left-ventricular wall hypertrophy and myocytes and myofibrils in disarray. Although mutations in regulatory myosin light chains associated with HCM have been found to affect their structure, calcium binding, and phosphorylation in vitro, to our knowledge no vertebrate models exist for MLC-2–induced HCM. Studies in mouse are hindered by the ability of different MLC-2 isoforms to compensate for the function of each other. Our experiments reveal, for the first time, an essential role for MLCs in thick-filament myofibrilligenesis of the heart and make disturbed myofibrillar assembly either attributable to disturbed stoichiometry of thick-filament components (null allele hypothesis) or a dominant-negative effect of mutated MLC (poison protein hypothesis) a likely cause for the myofibrillar and myocyte disarray observed in human cardiomyopathies associated with MLC-2 mutations. Hence, further identification of essential molecules for cardiac myofibrilligenesis is key to understanding cardiomyocyte function under physiological and pathophysiological conditions.

Acknowledgments
We thank S. Marquart, H. Hosser, and A. Doherty for excellent technical assistance. We are especially grateful to M. Peters, who provided excellent technical assistance and tragically passed away during the course of this study.

Sources of Funding
This work was supported by NIH grants 5R01HL49579, 5R01DK55383, and 1R01HL63206 (to M.C.F); Deutsche Forschungsgemeinschaft Ro 2173/1-1 and Ro2173/2-1 (to W.R.); and Bundesministerium für Bildung und Forschung 01GS0108-TP5 and 01GS0420-TP-2, as well as a Klaus-Georg and Sigrid Hengstberger Stipendium (to W.R.).

Disclosures
None.


Cardiac Myosin Light Chain-2: A Novel Essential Component of Thick-Myofilament Assembly and Contractility of the Heart
Wolfgang Rottbauer, Georgia Wessels, Tillman Dahme, Steffen Just, Nicole Trano, David Hassel, Charles Geoffrey Burns, Hugo A. Katus and Mark C. Fishman

Circ Res. 2006;99:323-331; originally published online June 29, 2006;
doi: 10.1161/01.RES.0000234807.16034.fe

Circulation Research is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 2006 American Heart Association, Inc. All rights reserved.
Print ISSN: 0009-7330. Online ISSN: 1524-4571

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://circres.ahajournals.org/content/99/3/323

Data Supplement (unedited) at:
http://circres.ahajournals.org/content/suppl/2006/06/29/01.RES.0000234807.16034.fe.DC1

Permissions: Requests for permissions to reproduce figures, tables, or portions of articles originally published in Circulation Research can be obtained via RightsLink, a service of the Copyright Clearance Center, not the Editorial Office. Once the online version of the published article for which permission is being requested is located, click Request Permissions in the middle column of the Web page under Services. Further information about this process is available in the Permissions and Rights Question and Answer document.

Reprints: Information about reprints can be found online at:
http://www.lww.com/reprints

Subscriptions: Information about subscribing to Circulation Research is online at:
http://circres.ahajournals.org/subscriptions/