Identification and Genomic Cloning of CMHC1
A UNIQUE MYOSIN HEAVY CHAIN EXPRESSED EXCLUSIVELY IN THE DEVELOPING CHICKEN HEART*

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We report the identification and cloning of a unique chick myosin heavy chain (CMHC1) that is expressed exclusively in the heart during embryogenesis. Using primers specific to myosin heavy chains, we used reverse transcriptase-polymerase chain reaction to clone and isolate CMHC1 from embryonic day 10 chicken heart RNA. Sequence analysis indicated that CMHC1 was a novel member of the myosin heavy chain family. Expression of the CMHC1 transcripts was detected in Hamburger Hamilton stage 10 chick embryos in the fusing myocardium. Expression of CMHC1 was maintained at high levels throughout the tubular heart of later stage embryos. Reverse transcriptase-polymerase chain reaction and in situ hybridizations failed to detect CMHC1 transcripts in the developing somites, limb buds, or skeletal musculature at any stage of chick development. Genomic CMHC1 clones have been isolated that contain sequences approximately 5.2 kilobase upstream of the presumptive CMHC1 transcription start site. Portions of the upstream regulatory region induced a 21-fold increase in reporter gene expression in primary cardiomyocytes. Because of its unique cardiac-restricted expression, CMHC1 will provide an excellent model system to study the molecular mechanisms required for the early developmental regulation of heart-specific genes.

A unique cardiac-restricted expression is restricted to the ventricle. Expression of VMHC1 is also transiently detected in all embryonic skeletal muscle (16). AMHC1 expression differs from slow myosin heavy chain 3 expression throughout the heart until embryonic day 7 when ventricular expression is repressed. Expression of quail slow MHC 3 is also detected in embryonic slow skeletal muscles (14, 15). VMHC1 expression is detected throughout the developing chick heart until day 5 of development when its cardiac expression is restricted to the ventricle. Expression of VMHC1 is also transiently detected in all embryonic skeletal muscle (16).

Whereas great strides have been made in understanding the...
molecular regulation of cardiac differentiation, progress has been hindered by the identification of few genes that are expressed in a strictly cardiac-specific manner from the earliest stages of cardiac differentiation to the formation of a fully functional, multichambered heart. In this report, we describe a third and unique member of myosin heavy chain family that is expressed in the developing chick heart. CMHC1, isolated from embryonic day 10 chick hearts, is a member of the myosin heavy chain gene family, which is expressed exclusively in cardiac muscle. Expression of CMHC1 is first detected in stage 6 embryos with high levels of expression throughout the myocardium in all stages of embryonic development. Northern blot, in situ hybridization, and RT-PCR analysis failed to detect the presence of CMHC1 mRNA expression in any skeletal muscle tissue or skeletal muscle precursor cells. Genomic cloning of CMHC1 indicates 2.2 kb of 5′-flanking sequence from the CMHC1 transcription start site is sufficient to induce high levels of reporter gene expression in cardiomyocytes. Taken together, these results indicate that CMHC1 is one of a very limited number of genes expressed exclusively in the heart during cardiac morphogenesis.

EXPERIMENTAL PROCEDURES

Cloning and Isolation of CMHC1 cDNA—CMHC1 was isolated from cDNA produced from embryonic day 14 chicken hearts. Primers were used, which were specific to homologous regions of previously isolated chicken striated myosin heavy chain cDNAs. Two forward primers (F17, 5′-TTC AAC AGC TTT GAG CAG CCT TG-3′; F19, 5′-TTT TGG CAT GGA CCT CCA GG-3′) were used with one reverse primer (B20, 5′-TTA CCA AGA GTG CAT CCC GGC G-3′). The F17 primer was designed to lie approximately 100 bp further 5′ to the F19 primer. RT-PCR using 100 ng of mRNA was performed in a Perkin-Elmer-2400 thermocycler using a Access RT-PCR System (Promega) with the following conditions: 95 °C for 30 s; 95 °C for 30 s; 52 °C for 30 s and 72 °C for 45 s or X 25 cycles; and 68 °C for 7 min. PCR reactions were run on a 1% agarose gel. The 900-bp product from the F19-B20 primer pairs and the 100-bp product of the F17-B20 primer pairs were excised from the gel and directly cloned into the pGem T-Easy PCR cloning vector (Promega). Positive clones were sequenced using Big Dye Terminator Cycle Sequencing (Perkin-Elmer). The overlapping 900-bp of the F17 and F19 PCR products were isolated with the largest clone containing 2.5 kb of a cDNA sequence spanning into the putative untranslated region of the genomic sequence.

CMHC1 5′-RACE—The 5′-end of the CMHC1 cDNA was obtained using 1 μg of Poly(A)+ RNA from embryonic day 8 chicken hearts using a Marathon cDNA Amplification Kit (CLONTECH). Gene-specific primers were as follows: CMHC1 R1, 5′-GGG GGT TTC ATT TTT CAG GGT TTG GCC C-3′; CMHC1 R2, 5′-CAG GTG ATG TAG TAT CAG CAA TGG-3′. PCR conditions were as follows: 94 °C for 30 s; 94 °C for 5 s and 68 °C for 3 min X 30 cycles; 68 °C for 7 min. Amplified PCR products were sequenced using Big Dye Terminator Cycle Sequencing (Perkin-Elmer).

Genomic Cloning of CMHC1—To begin to understand the basic molecular regulatory mechanisms that control the cardiac-specific expression of CMHC1, chicken genomic libraries were screened to identify 5′-genomic flanking regions of CMHC1. Five independent genomic clones were isolated from a chicken genomic pWE15 library using a 800-bp probe corresponding to nucleotides 385–1185 of the CMHC1 cDNA sequence. Interestingly, all five clones began at the same site within the second intron of CMHC1. To obtain sequences further 5′ to this site, chicken genomic genewalker libraries (CLONTECH) and PCR reactions were performed using primers specific to genomic sequences contained within the second intron. A 1.5-kb fragment (S13) was isolated from the ScaI library and sequenced. The S13 fragment extended the genomic sequence of CMHC1 into the first intron. Within the S13 sequence was located a BamHI restriction site at the point in the genomic sequence where the pWE15 clones ended. A final genomic screen was performed using a 800-bp probe at the 5′-end of the S13 fragment to screen a CMHC1 genomic library (CLONTECH). Three isolated clones were positive for CMHC1 genomic sequences. Following a BamHI digestion, the S13 probe reacted to a 6.8-kb fragment from the isolated genomic clones.

Northern Blot Analysis—Total RNA was obtained from day 17 and 19 chicken embryos as described previously (17). Total RNA (15 μg) was electrophoresed on a 1% formaldehyde-agarose gel and transferred onto GeneScreen membranes. Blots were hybridized at 65 °C and probed overnight with the F19 fragment described above. Blots were washed under high stringency conditions (0.1X SSC, 0.1% SDS). In situ Hybridizations—Fertilized White Leghorn chicken eggs were obtained from Truslow Farms and incubated at 37 °C. Embryos were collected and staged according to Hamburger and Hamilton (18). In situ hybridizations were performed as described in Yutzey et al. (13) with the following exceptions. Stages 10 and 12 embryos were treated with 30 μg/ml proteinase K for 7.5 min, and stage 20 embryos were treated for 15 min. The F19 PCR fragment ligated into the pGEM T-Easy vector was used for the CMHC1 probe. The antisense CMHC1 probe was created from 1 μg of NcoI-linearized F19 vector using SP6 RNA polymerase. Following ethanol precipitation the resulting digoxigenin-labeled RNA probe was resuspended with 200 μl of hybridization solution. 50 μl of probe was then added to each in situ reaction. Following the reactions, embryos were fixed in 4% paraformaldehyde and photographed on a 1% agarose plate using a Nikon SMZ-U dissecting microscope.

RT-PCR Analysis—RT-PCR reactions for detection of CMHC1 in tissues and embryonic development were performed using the reaction conditions described previously. Primers to CMHC1, which were utilized in this experiment, were unique to CMHC1 and are not found in other myosin heavy chain sequences as confirmed by BLAST sequence searches. Primers for CMHC1 were 5′-TGA CCA GGG TGG AGA AAA G-3′ (forward) and 5′-TTG TCC TCT GGG ATT CTA CCT G-3′ (reverse), which produced a 312-bp product. Primers for chicken GAPDH were 5′-ACG CCA CTA CTA TCT TCC AG-3′ (forward) and 5′-CAG CCT TCA CTA CCC TCT TG-3′, which produced a 578-bp product. Following the RT-PCR reaction, samples were run on a 1% agarose gel, transferred to GeneScreen hybridization membrane, and probed with a random primed probe to F19 described above.

Plasmid Constructs—A 6.8-kb BamHI fragment of genomic CMHC1 DNA from the pAMEBL3 clone was cloned into the BglII site of the pGL3 Basic (Promega) luciferase reporter gene (-5205CMHC1uc). Deletions constructs were generated from KpnI-Sacl digestions of -5205CMHC1uc using an Erase-A-Base system (Promega). Resulting constructs were sequenced to determine the starting location of the plasmid.

Tissue Culture and in Situ Transfections—Primary cardiomyocytes were isolated from hearts of embryonic day 11 White Leghorn chickens (Truslow Farms) as described previously (15). NIH 3T3 cells were obtained from ATCC and grown in Dulbecco’s Modified Eagle’s medium + 20% fetal bovine serum. All cells were transfected on 35-mm plates with 2 μg of DNA (1.75 μg of reporter plasmid and 0.25 μg of CMHC1.GAL and 4 μg of pGenes3 (Roche Molecular Biochemicals) according to manufacturer’s specifications. Cell extracts were collected 48 h after transfection and assayed for luciferase and β-galactosidase activity. Luciferase results were normalized for β-galactosidase activity. Experiments were performed in triplicate using three independent isolated cell cultures.

RESULTS

Isolation of CMHC1, a Cardiac-specific Myosin Heavy Chain—To analyze the molecular mechanisms that regulate the expression of early cardiac-specific genes, attempts were made to isolate novel myosin genes that are expressed exclusively in the heart and cardiac progenitor cells. Using primers specific to known myosin heavy chains, RT-PCR was used to isolate a 1.2-kb cDNA fragment from embryonic day 10 chicken heart RNA (CMHC1). The resulting CMHC1 PCR product was sequenced and used to screen an embryonic chicken heart cDNA library. A 2.5-kb clone was isolated, which hybridized to CMHC1 sequences. This cDNA clone of CMHC1 represented a partial clone, which contained 60-bp of 5′-untranslated sequences and a deduced continuous open reading frame consisting of 884 amino acids (Fig. 1). Whereas its nucleotide sequence in the coding region and 5′-untranslated region was unique to all currently known myosin heavy chain isoforms, CMHC1

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showed a high degree of sequence identity to other myosin heavy chain transcripts that are expressed in striated muscle. CMHC1 was 72–75% identical to embryonic and pectoralis myosin heavy chain isoforms found in the chicken, as well as α- and β-cardiac myosin heavy chain isoforms found in mouse and human. Analysis of the deduced amino acid sequence of CMHC1 showed 100% identity to the amino acid sequence of the S-1 region of a cardiac myosin heavy chain protein isolated from adult chicken heart (19). The highest region of amino acid similarity between CMHC1 and other myosin heavy chain iso-
forms is in the highly conserved S-1 region of the molecule required for ATPase activity. The CMHC1 isoform contains the GESGAGKT sequence that is conserved among all myosins (20). The region between residues 118 and 191 is 84% identical to chicken embryonic myosin heavy chain and 87% identical to the α- and β-cardiac myosin heavy chain isoforms of mouse and human. These data demonstrate the high conservation of CMHC1 with other MHC family members in both avian and mammalian species.

To map the transcriptional start site of the CMHC1 message, two antisense primers specific to the CMHC1 message were designed to perform a 5′-RACE assay. The two antisense gene-specific primers, underlined in Fig. 1, were designed 782 bp and 543 bp downstream of the 5′-end of the CMHC1 cDNA clone described earlier. Using Poly(A)+ RNA isolated from ED 8 chicken hearts, these primers produced 5′-RACE products containing 782 bp and 543 bp respective to the 5′-CMHC1 sequence (Fig. 2). Similarly sized PCR products were obtained using these primers to amplify CMHC1 transcripts out of a chicken embryonic heart cDNA library (data not shown). Sequencing of both of the resulting PCR products showed no discrepancies between the two products or the initial cDNA sequence isolated from the heart cDNA library described earlier. These results suggest the CMHC1 gene contains 60 bp of an untranslated sequence upstream of the presumptive translational start site.

Expression of CMHC1 Is Detected Only In Cardiac Muscle—As CMHC1 appeared to be a novel mRNA, its expression was examined in striated muscle types; RNA was collected from striated muscle-containing tissues of day 17 and day 19 chicken embryos and analyzed by Northern blot analysis using a random-primed CMHC1 probe, which overlaps the transcriptional start site.

The intriguing cardiac-specific gene expression of CMHC1 as detected by Northern blot analysis was further tested by using the highly sensitive RT-PCR assay to detect CMHC1 transcripts in striated muscle tissue. RNA isolated from heart and skeletal muscle of day 5, 7, and 11 chicken embryos were subjected to RT-PCR, run on a 1.5% agarose gel, and probed with CMHC1. Amplified CMHC1 transcripts were detected using a random-primed CMHC1 probe, which overlaps the amplified sequences. Using this method, amplification of CMHC1 transcripts was only detected in cardiac muscle of the heart with no expression observed in skeletal muscle isolated from leg or pectoralis muscle (Fig. 4). Moreover, RT-PCR analysis failed to detect CMHC1 transcripts in smooth muscle of the gizzard or in an embryonic carcass with the heart removed. These results suggest that CMHC1 is a novel member of the myosin heavy chain family, which is expressed exclusively in the heart during the development of the chicken embryo.

Expression of CMHC1 Is Restricted to the Heart and Cardiac Progenitor Cells—Previous Northern blot and RT-PCR analyses indicated CMHC1 expression was restricted to the heart in the developing chick embryo. To determine the spatial and temporal expression pattern of CMHC1 during the earliest stages of avian cardiogenesis, whole mount in situ hybridizations were performed on early chick embryos. Hybridizations using a sense control riboprobe showed no reactivity to any embryo at any stage of avian development. By in situ hybridization, CMHC1 mRNA expression was first detected in Hamburger-Hamilton stage 8 chick embryos in the cardiac progenitor cells of the splanchnic mesoderm of the cardiac crescent (data not shown). The timing of CMHC1 expression corresponds to the induction of VMHC1 in the paired heart primordia (13). By stage 10, the fusing myocardium showed robust expression of CMHC1 in the anterior segments of the heart tube (Fig. 5A). High levels of CMHC1 expression continued in the developing heart of stage 12 embryos (Fig. 5B), and continued expression was detected throughout the looping heart of stage 20 embryos (Fig. 5, C and D).

Comparison of the embryonic localization between CMHC1 and VMHC1 transcripts indicates the unique cardiac-specific gene expression of CMHC1 that is not observed in other cardiac

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**Fig. 2.** Identification of the transcriptional start site of CMHC1 via 5′-RACE. RNA isolated from embryonic day 8 chick hearts was subjected to 5′-RACE using antisense primers corresponding to sequences underlined in Fig. 1. Lanes 1 and 3 are reactions with the linker primer (AP1) and CMHC R1 or CMHC R2, respectively. Lanes 2 and 4 are secondary PCR reactions using the internal linker primer (AP2) and CMHC R1 or CMHC R2. All fragments produced were sequenced and determined to contain identical 5′-sequences.

**Fig. 3.** Northern blot analysis of CMHC1 expression in striated muscle tissues during late stage chicken development. Total RNA was collected from heart, leg, and pectoralis muscle of day 17 and day 19 chicken embryos. Equivalent amounts of RNA was loaded into each lane (15 mg) and probed using a 900-bp fragment of CMHC1. A single band (arrow) at approximately 6 kb hybridized to the CMHC1 probe in the heart but not in the skeletal muscle of the leg or pectoralis. Positions of the 28S and 18S rRNA bands, as visualized by ethidium bromide staining, are indicated for size references.

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myosin heavy chains. Although both CMHC1 and VMHC1 are highly expressed in the myocardium of stage 15 embryos, VMHC1 transcripts are also readily detectable in the developing somites (Fig. 5, E and F). These results suggest that although VMHC1, CMHC1, and AMHC1 are highly expressed during cardiac morphogenesis, these three MHC genes are expressed in a distinct subset of cardiac and skeletal muscle cells during chick embryogenesis. Unlike most of the other known cardiac-specific structural genes such as VMHC1, CMHC1 expression was never detected in the skeletal muscle precursor cells of the somites (stage 8–12) or in the developing limb buds (stage 20).

RT-PCR analysis was utilized as a sensitive assay to examine the initial stages of detectable CMHC1 expression. RNA was isolated from chicken embryos of stages 4–11. Primers of chick GAPDH was utilized to ensure the integrity of the RNA used in this experiment. Low levels of CMHC1 were first detected in stage 5 embryos (Fig. 6). It is at this stage of avian development when expression of Nkx-2.5, SRF, and GATA4, three transcription factors implicated in the induction of cardiac-specific gene expression, are first detected (17, 21, 22). Expression of CMHC1 was readily detectable at stage 7, corresponding with the induction of other cardiac structural genes such as cardiac troponin I and VMHC1 (16, 23). By stage 11, when the heart begins to beat and a full array of cardiac structural genes are expressed, CMHC1 transcripts remain highly expressed as detected by in situ hybridization and RT-PCR analysis. These results suggest that CMHC1 expression marks one of the earliest cardiac-specific cell markers in the developing avian embryo.

**Genomic Cloning of CMHC1**—Genomic sequences containing 5’-flanking regions of the CMHC1 gene were isolated from chicken genomic libraries (see “Experimental Procedures”). Analysis of the CMHC1 genomic structure by comparison of isolated genomic and cDNA sequences indicates the first exon is composed of 149 bp containing 60 bp of the 5’-untranslated region and the translational start site. Intron/exon boundaries of the isolated genomic clones match perfectly with cDNA sequences and all contain consensus donor/acceptor splice sites. Whereas the CMHC1 gene does not contain consensus TATA box sequences, two A/T-rich motifs, typical of many promoters, were found within the −100/+1 region (Fig. 7). Sequence analysis of the CMHC1 gene revealed the presence of consensus binding sites for factors that have been shown to regulate cardiac gene expression. Within the −1900/+1 region of the CMHC1 gene, multiple consensus binding sites for GATA factors and Nkx-2.5 were observed. In addition, this region of the CMHC1 gene contained several A/T-rich motifs that may serve as putative binding sites for MEF-2 factors and SRF. Finally, a
Deletion analysis of the CMHC1 gene suggests the presence of negative-acting regulatory sequences in the -711CMHCluc and -168CMHCluc. These results suggest the cells remained at background levels in cells transfected with the other deletion constructs, luciferase activity in NIH 3T3 cells, and NIH 3T3 cells, suggesting a strong cardiac enhancer is located within the -1372 region of the CMHC1 gene. Further deletions restored high levels of luciferase expression in cardiomyocytes (-711CMHCluc and -168CMHCluc). Similar to the other deletion constructs, luciferase activity in NIH 3T3 cells remained at background levels in cells transfected with -711CMHCluc and -168CMHCluc. These results suggest the presence of negative-acting regulatory sequences in the -1372 to -711 region of the CMHC1 gene, as well as additional cardiac regulatory elements located within 168 bp of the transcriptional start site.

DISCUSSION

In order to ascertain the fundamental molecular principles underlying cardiac morphogenesis, we have now isolated three isoforms of myosin heavy chain that show distinct expression patterns within the developing chicken heart. VMHC1 expression is observed ubiquitously in early striated muscle cell types before being restricted to the ventricular chamber (16, 24). AMHC1 expression is confined to a subset of cardiomyocytes that form the atrium (13). Finally, in this report, we describe the isolation and expression of CMHC1, a novel member of the myosin heavy chain gene family that is restricted to the developing myocardium, with no expression detected in the somites or differentiated skeletal muscle tissue. The induction of CMHC1 expression in Hamburger Hamilton stage 6 chick embryos suggests it is one of the earliest structural markers of a cardiogenic cellular phenotype. Analysis of the molecular regulation of these three myosin heavy chain genes along with other cardiac-restricted genes such as cardiac troponin I will provide an excellent model system for analyzing the precise cellular and molecular mechanisms that drive cardiac-specific gene expression and chamber diversification.

The coordinated expression of cardiac structural genes in the atra and ventricles is required for proper sarcomeric formation and cardiac morphogenesis. Many cardiac structural genes in mammalian and avian species are initially expressed throughout the fusing myocardial heart tube before being confined to a specific compartment of the developing multichambered heart. The α-MHC and β-MHC isoforms in the mouse are expressed in all myocardial precursor cells before compartmentalization into the atrium and ventricles, respectively. Similarly, VMHC1 expression is localized early in all striated muscle cells and then restricted to the ventricle by day 5 of development. The functional significance on cardiac morphogenesis of these isoform switches in mouse and chicken is currently unclear. Functional differences between the three chicken MHC isoforms expressed in the heart may lie in their contractility and ATPase activities. The ATPase activity of α-MHC is 10–20-fold higher than β-MHC resulting in a greater contractile velocity in cardiac muscle strips containing α-MHC versus β-MHC (25). The ATPase activity and contractile velocities of the chicken cardiac MHCs are not currently known; however, expression of these isoforms in specific regions of the developing chicken heart may contribute to the physiological differences between the developing atrial and ventricular chambers.

Isolation of genomic CMHC1 sequences provides the first step in identifying minimal regulatory regions that confer development of cardiac-specific gene expression. The addition of the -5205 to +260 region of the CMHC1 gene was sufficient to induce reporter gene expression in primary cardiomyocytes. This promoter region also induced reporter gene expression when transfected into primary skeletal myoblast cultures. Similar results were observed with the mouse cardiac troponin I (cTNI) gene. Whereas endogenous cTNI gene expression was restricted to cardiac muscle in vivo, reporter plasmids containing the cTNI promoter conferred high levels of gene expression in both cultured skeletal myoblasts and cardiomyocytes (26).

Deletion analysis of the CMHC1 gene suggests the presence of two cardiac enhancer domains located at -2273 to -1372 and -711 to +263 and an inhibitory domain between -1372 and -711. Within the two positive-acting domains are located numerous DNA recognition sites associated with the expression of other cardiac genes. These include binding sites for GATA and MEF-2 factors, Nkx-2.5, and SRF. Cooperative combinatorial interactions between GATA-4, Nkx-2.5, and SRF have been shown to activate the transcription of the cardiac α-actin promoter in CV-1 fibroblasts (27). This concentration of putative regulatory binding sites suggests the cardiac-restricted expression of CMHC1 during chicken embryogenesis may be con-
trolled by clusters of sequences located within close proximity to the transcriptional start site.

cTNI and CMHC1 are two of the few genes isolated to date that are expressed exclusively in cardiac muscle throughout embryonic development. Positive and negative regulatory regions of these two genes are remarkably similar. Like CMHC1, deletion analysis of 4.0 kb of upstream regulatory sequences of the mouse cTNI gene on cardiomyocytes identified two positive regulatory domains separated by a negative regulatory domain (26). Transgenic mice harboring sequences of the proximal regulatory domain (2230 to 1126) of cTNI linked to LacZ conferred reporter gene expression to embryonic and adult cardiomyocytes, but not to fibroblasts. These results suggest that the positive regulatory region of cTNI contains a cardiac-specific enhancer.

**Fig. 7.** Genomic structure of CMHC1. A, a schematic representation of the genomic structure of the upstream regulatory region through exon 4 of the CMHC1 gene is shown. Isolated genomic clones contained 5.2 kb of genomic sequence upstream of the transcriptional start site (white box). CMHC1 coding sequences are represented as black boxes. Numbers correspond to the nucleotide location within CMHC1 cDNA of intron/exon boundaries. A 6.7-kb BamHI fragment of genomic CMHC1 sequences was used as the full-length reporter plasmid in Fig. 8. B, nucleotide sequence of 1882 bp of the genomic sequence immediately upstream of the transcriptional start site (+1). Potential binding sites for GATA factors, Nkx-2.5 (NKE), MEF-2 factors, SRF (SRE), and thyroid hormone receptor (TRE) are indicated.

**Fig. 8.** Transient transfections of CMHC1 upstream deletion constructs into primary cardiomyocytes and fibroblasts. Left, schematic representation of the expression constructs used in the transfection analyses. Right, relative luciferase activity of deletion constructs transiently transfected into cardiomyocytes (black bar) and NIH 3T3 fibroblasts (white bar). Results are presented in terms of luciferase activity compared to transfection efficiency as determined by the β-galactosidase activity of a cotransfected CMV-βGal control plasmid. Values represent the average of three independent transfection experiments.
hearts. Within this proximal sequence, MEF-2/Oct1, Sp1, and GATA regulatory sequences were all required for full expression in cultured cardiomyocytes (28). Whether CMHC1 and cTNI share conserved minimal sequences that are required for cardiac-specific gene expression during the initial stages of cardiac morphogenesis is not clear.

Identification and isolation of the CMHC1 gene provides a potential new model system to study the molecular regulation of the initial stages of cardiac morphogenesis. In addition, cross-species experiments introducing the minimal CMHC1 promoter in transgenic mice may prove to be an effective method for cardiac-specific gene delivery. The promoters of various avian genes have recently been shown to direct reporter gene expression to specific regions of the developing mouse heart. One region of the chick GATA-6 promoter directed gene expression exclusively to the atrioventricular canal, whereas a larger fragment induced expression in the ventricle, outflow tract, and atrioventricular canal (29). Transgene expression was detected solely in the developing atria of transgenic mice containing a reporter construct controlled by a region of the quail slow MHC3 containing a vitamin D response element (30). Therefore, because of its early and cardiac restricted expression during chick development, the CMHC1 promoter may be an ideal candidate for directing high levels of transgene expression throughout the developing murine heart tube.

Initial isolated genomic clones of CMHC1 have revealed a genomic structure that is unique to the myosin heavy chain gene family. Intron-exon boundaries of many cardiac MHC genes are conserved cross-species. However, initial genomic clones indicate the presence of unique intronic sequences in the CMHC1 gene. An exon in the CMHC1 gene, which is homologous to exon 15 of the human (31) and hamster (32) α-MHC genes and exon 16 of the chicken embryonic MHC gene, is interrupted by intronic sequences in the CMHC1 gene (data not shown). Furthermore, no other MHC genes isolated to date contain intronic insertions into this exon. The functional significance of this additional intron is unclear. However, additional variation of the CMHC1 gene organization, especially in the promoter region, may provide insight into the regulation of the highly restricted cardiac-specific gene expression of CMHC1.

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