The Myosin Gene Switching in Human Cardiac Hypertrophy

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Cardiac hypertrophy is associated with qualitative as well as quantitative changes in myocardial cells. To analyze the molecular basis of isozymic transitions of cardiac myosins in response to pressure overload, we have constructed and characterized two types of myosin heavy chain (MHC) cDNA clones, specifying $\alpha$- and $\beta$-MHCs, and two types of myosin alkali light chain cDNA clones, complementary to atrial type (ALC1) and ventricular type (VLC1) mRNAs from a human fetal heart cDNA library. Using the S1 nuclease mapping procedure, we showed that the MHC isozymic transitions from $\alpha$- to $\beta$-MHC in the pressure overloaded atria are produced by changes in the relative level of $\alpha$- and $\beta$-MHC gene expression. In addition, we observed that the expression of VLC1 gene is also induced in the atria subjected to severe pressure overload. Thus, it appears that the increased expression of VLC1 gene, together with the isogenic switch from $\alpha$- to $\beta$-MHC gene, may participate in the adaptation of myocardium to new functional requirement. Then, to get a better understanding of the genetic mechanisms involved in the regulation of isogenic expression, we have isolated and sequenced genomic clone for VLC1 isoform. Sequence analysis has identified multiple potential cis regulatory elements within a 686-bp upstream region. This region includes 28-bp alternating purine/pyrimidine sequences and two segment exhibiting homology to consensus sequence proposed for viral and cellular enhancer elements. In particular, a comparison of the VLC1 upstream gene sequence with those available for several muscle-specific genes revealed that CCG(A+T-rich)$_n$GG elements and CATTCCCT sequence are conserved. These results suggested that CARG box (−96 to −87) has an important role in the positive regulation of the VLC1 gene and this element may be involved in the co-regulation of VLC1 and cardiac $\alpha$-actin genes.

The contractile proteins of cardiac muscle, as in other muscle and non-muscle cells, are encoded by multigene families! During striated muscle development, each member of a contractile protein gene family is expressed in a highly tissue-specific, or developmental stage-specific manner. The myosin molecule, which is the principal structural component of muscle fiber and plays a major role in muscle contraction, consists of two heavy chains (MHC, of Mr about 220,000) each with a globular head region where the ATPase activity is situated, and a filamentous, rod-like tail, and four associated light chains (of Mr 15000 – 30000), two so-called regulatory light chains (MLC2) and two alkali light chains (MLC1).

Mammalian cardiac muscle is structurally and functionally heterogeneous. It contains, at least, two different MHCs, $\alpha$- and $\beta$-MHCs, and two distinct myosin alkali light

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**Key words:**
Cardiac myosin heavy chain
Myosin light chain
Cardiac hypertrophy
Gene regulation
CARG box

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### TABLE 1. SOURCES OF CARDIAC SAMPLES EXAMINED

<table>
<thead>
<tr>
<th>Patient no.</th>
<th>Age/Sex</th>
<th>Mean PCW</th>
<th>Mean RA</th>
<th>Diagnosis</th>
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<td>1</td>
<td>26/F</td>
<td>6 mmHg</td>
<td>15</td>
<td>PPH</td>
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<tr>
<td>2</td>
<td>26/F</td>
<td>13</td>
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</tr>
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<td>46/F</td>
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<td>10</td>
<td>MS, MR, TR</td>
</tr>
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</tr>
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<td>Control</td>
<td>27/F</td>
<td>ND</td>
<td>ND</td>
<td>Breast cancer</td>
</tr>
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Abbreviations used in this table: AR, aortic regurgitation; AS, aortic stenosis; ASD, atrial septal defect; IHD, ischemic heart disease; MR, mitral regurgitation; MS, mitral stenosis; PCW, pulmonary capillary wedge pressure; PPH, primary pulmonary hypertension; PS, pulmonary stenosis; RA, right atrial pressure; TOF, tetralogy of Fallot; TR, tricuspid regurgitation.

In humans, since the normal ventricle contains predominantly β-MHC, the α-to β-MHC transition may not contribute significantly to the adaptation to mechanical stress. In our previous immunofluorescence study, the transition from α- to β-MHC in pressure overloaded human atrial muscle has been described and a functional significance of myosin isozyomatic redistribution in the atria has been implied. The changes in response to increased pressure loads in human heart have been observed also in both myosin light chain 1 (MLC1) and 2 (MLC2) subunits. From the analysis of the correlation between clinical, hemodynamic, and angiographic parameters and myosin light chain compositions, it has been suggested that wall stress may be responsible for the isoform transitions. Thus, clarification of the mechanisms responsible for isoform switches of human cardiac MLC1 as well as cardiac MHCs has important implications for our understanding of the biochemical processes of cardiac hypertrophy. Nevertheless, little is known about the genes for human cardiac MHCs and MLC1, although these are very important from a clinical point of view. As an essential step toward an understanding of the complex mechanisms of isogene switching in response to pathophysiological stimuli, we have cloned and sequenced the human α-, β-MHCs, and ALC1 and VLC1 cDNAs. Using the cloned cDNAs, we have shown that in pressure overloaded atria, there is an increase in β-MHC mRNA levels and a corresponding decrease in α-MHC mRNA levels. In addition, we have found the induction of VLC1 gene expression in severely hypertrophied atria. Thus, it appears that both MHC and MLC1 transition may be involved in the adaptation to mechanical stress of myocardial cells. Furthermore, to
understand the regulatory mechanisms of MLC1 gene expression, we have isolated the human VLC1 gene from a genomic library and determined the nucleotide sequence of the gene and its flanking regions. From a comparison of the regulatory region between VLC1 and several muscle-specific genes, we have identified several potential

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Fig.1. Nucleotide and deduced amino acid sequences of pHMHC2 and pHMHC5.
Nucleotides are numbered to the right of each line. Line 1, amino acid sequence of pHMHC2; Line 2, nucleotide sequence of pHMHC2; Line 3, amino acid sequence of pHMHC5; Line 4, amino acid sequence of pHMHC5. The dashed line indicates an identical sequence to that of pHMHC2.
cis-regulatory sequence elements in 683-bp upstream of the VLC1 transcription initiation site.

**EXPERIMENTAL PROCEDURES**

**Materials** The restriction enzymes used were purchased from Takara Shuzo Co., Boehringer Mannheim, and P-L Biochemicals. Reverse transcriptase was from Boehringer Mannheim, and DNA polymerase I, RNase H, and T4 DNA ligase were obtained from Takara Shuzo Co.. The random primer labeling kits and dideoxy sequencing kits were products of Boehringer Mannheim and Takara Shuzo Co., respectively. [α-32P]dCTP and [γ-32P]ATP were obtained from Amersham Corp.

**Tissue Sources and Isolation of mRNA** Northern blot analysis reported in this paper was performed on RNA samples extracted from autopic specimens obtained from one fetus, one adult patient without clinical and pathological evidence of heart disease, and ten adult patients with hypertrophied atria. Fetal tissue was a specimen from a fetus that died as a result of an induced abortion at 17 weeks gestation. It had no evidence of cardiac pathology. For the analysis of diseased hearts, atrial tissue was obtained from ten cardiac patients, whose pertinent information is listed in Table I. As control specimen, a normal heart was obtained from a 27-year-old woman who died of breast cancer. All tissue samples obtained were frozen at −80°C no later than 6 hours postmortem.

**Construction and Screening of a Human Fetal Heart cDNA Library** Human heart poly (A) RNA extracted from a 17 weeks gestation fetus was primed with oligo (dT) and was used to direct first strand synthesis with avian myeloblastosis virus reverse transcriptase. Synthesis of the second strand was carried out using RNase H and DNA polymerase I as described by Gubler and Hoffman. Approximately 1 μg of the

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cDNA was used to construct a λ gt11 library containing 500,000 independent recombinant plaques. The library was screened using mouse a-MHC cDNA plasmid, pMHC10115 and chicken fast skeletal MLC1 cDNA plasmid, pSMA1-116 as probes. Positive plaques were purified by three successive screenings. Phage DNA was isolated by the plate lysate method. EcoRI-excised cDNA inserts were subcloned into plasmid vector pUC13 and characterized by restriction endonuclease mapping.

Isolation of Genomic Clones A human genomic library constructed by partial EcoRI digestion of high molecular weight DNA and cloned into the λ phage Charon 4A was the generous gift of Dr. T. Maniatis, Harvard University. The phage were inoculated into Escherichia coli strain LE392 and screened by in situ hybridization as described by Benton and Davis.17 The human VLC1 cDNA, pHMLC1B2, was 32P-labeled by random primer method and used as a probe.

SI Nuclease Mapping Analysis Procedure

The cDNA clone, pHMHC5 was cleaved with the restriction endonuclease SstI, 3' end-labeled with deoxyadenosine 5'-triphosphate and terminal transferase (Boehringer Mannheim), and digested with EcoRI. The resultant 420-bp SstI-EcoRI fragment contains 259-bp coding sequence, 113-bp 3' untranslated region and 48-bp poly (A) tract. The probe was hybridized in DNA excess to 35 μg of total RNA extracted from normal and hyperphophied human atria. Hybridization was in 25 μl of 80% deionized formamide, 10 mM PIPES buffered at pH 6.4, 1 mM EDTA, 0.05% sodium dodecyl sulfate for 20 h at 42°C. S1 nuclease digestion was in 300 μl for 1 h at 37°C with 200 units of enzyme (Boehringer Mannheim) in 300 mM NaCl, 30 mM Na acetate, pH 4.5, 3 mM ZnSO4. At the end of the reaction, the samples were made 10 mM in EDTA, ethanol-precipitated, resuspended in 85% formamide, and run on 6% polyacrylamide, 8.3 M urea sequencing gels.

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RESULTS

Identification of cardiac MHC cDNA clones and MLC1 cDNA clones The human fetal heart cDNA library in λ gt11 phage was constructed and screened using the PstI insert derived from the previously characterized mouse α-MHC cDNA clone, pMHC10115 and the PstI insert of chicken fast skeletal MLC1 cDNA clone, pSAM116 A total of 30 positive clones for MHC and 24 positive clones for MLC1 were obtained from about 2 × 10⁵ plaques. Then, they were divided into two types on the basis of restriction mapping results. Two MHC cDNA clones, pHMHC2 and pHMHC5, and two MLC1 cDNA clones, pHMLC1A1 and pHMLC1B2, were chosen for further analysis because of their large insert sizes and their restriction maps indicating that they might represent distinct MHC and MLC1, respectively.

DNA sequence analysis To establish unequivocally that pHMHC2 and pHMHC5 represent distinct isozymes of MHC, and pHMLC1A1 and pHMLC1B2 specify the two isoforms of MLC1, nucleotide sequences of these clones were determined by the dideoxy method. Nucleotide and deduced amino acid sequences of pHMHC2 and pHMHC5 are shown in Fig. 1. Each clone encodes the light meromyosin portion of MHC, including the entire 3'-untranslated region and a portion of the poly (A) tail. Direct comparison of the DNA sequences of clone pHMHC2 and pHMHC5 demonstrates that these cDNAs are quite homologous, exhibiting 95.1% nucleotide homology within the translated regions. However, we observed numerous nucleotide mismatches within this region. In particular, 77 nucleotides out of 1,596 compared in this study are divergent. In contrast to the generally high levels of homology observed for the translated sequences of pHMHC2 and pHMHC5, the corresponding 3'-untranslated regions show extensive sequence divergence. From a sequence comparison and tissue distribution analyzed by Northern blot using clone specific oligonucleotide probes (data not shown), we conclude that pHMHC2 and pHMHC5 represent α- and β-MHC cDNA clones, respectively.

The determined nucleotide sequence and the predicted amino acid sequence of the selected two types of MLC1 cDNA clones, pHMLC1A1 and pHMLC1B2, are shown in Fig. 2 and 3. The amino acid composition predicted from pHMLC1A1 and pHMLC1B2 shows agreement with the reported values from the analysis of purified
human ALC1 and VLC1 respectively. Moreover, the deduced primary structure of pHMLC1B2 contained a partial amino acid sequence which is the same as that reported by Klotz et al; except that 140th amino acid residue (according to their numbering) is Asn, instead of Asp, and the 190th amino acid residue is Ser, instead of Gly.

Expression of pHMHC2 and pHMHC5 in pressure overloaded atria To examine the
expression of the mRNA corresponding to \( \alpha \)- and \( \beta \)-MHC cDNAs in normal and pressure overloaded human atria by nuclease S1 mapping, we used a 420-nucleotide probe containing the 3'-terminal portion of pHMHC5. This probe comprises a 259-bp common sequence and the entire 113-nucleotide untranslated 3' end of \( \beta \)-MHC mRNA, as represented schematically in Fig. 4. The 3'-untranslated sequences of pHMHC2 and pHMHC5 are different in length and nucleotide sequence, unique in the human genome, and therefore specific for each of the two cardiac MHC genes they represent. Therefore, hybridization of the probe to the homologous mRNA should result in the protection of a labeled fragment of 372 nucleotides, while hybridization to the heterologous mRNA would result in smaller labeled fragments. It is important to emphasize that both cardiac MHC mRNAs are detected by this probe, thus allowing one to examine simultaneously the expression of the two genes with a single probe. The intensity of radioactivity in the fully and partially protected fragments will reflect the amount of each cardiac MHC mRNA present in a particular tissue.

The results of the nuclease S1 mapping with this probe are shown in Fig. 5. After hybridization with RNA from normal atria, a doublet of about 235 nucleotides is clearly observed. These fragments correspond to the distance from the labeled end to the beginning of the 3'-untranslated region, where \( \alpha \)- and \( \beta \)-MHC mRNA sequences are divergent. Thus, they presumably correspond to the \( \alpha \)-MHC mRNA represented by clone pHMHC2. Indeed, scattered nucleotide sequence divergence in that region of clone pHMHC2 and pHMHC5 (Fig. 1) produce unstable duplex regions in the heterologous MHC mRNA-DNA hybrids and explains the presence of the doublet of partially protected fragments. Together with these fragments, a band of faint intensity corresponding to the fully protected probe is also detected.

Using monoclonal antibodies specific \( \alpha \)- to \( \beta \)-MHCs, we have recently demonstrated the \( \alpha \)- to \( \beta \)-MHC transition in pressure overloaded human atria. In order to establish the level at which the regulation of these myosin transition occurs, the composition of the different MHC mRNAs in autopic specimen was determined using the S1 nuclease mapping technique. As shown in Table I, the left atria of patients 2, 3, 4, 5, 6, 7, and 9, and right atria of patients 1, 3, 4, 6, 7, 8, and 10 were pressure-overloaded chambers. Thus, we analyzed 7 normal and 14 pressure-overloaded atria in this study. Following hybridization of the probe to RNA from pressure overloaded atria, the intensity of the label in the fully protected fragment increases, while that in partially protected fragments decreases. In particular, the fully protected probe is more intense than its partially protected counterpart in the RNA from severely hypertrophied atria (left atria in case 2, 7, and 9; right
atria in case 7 and 10). As shown in Fig. 6, when the intensity of the S1 nuclease-protected bands obtained by hybridization of the normal and pressure-overloaded atrial RNA samples was analyzed by densitometry, the relative amount of α- and β-MHC mRNAs was significantly different between normal and pressure-overloaded atria (α/β; 92.5 ± 5.2%:7.5 ± 1.1% vs. 54.5 ± 7.2% /45.5 ± 3.1%, p < 0.01). These results clearly indicate that β-MHC gene is up-regulated and α-MHC gene is down-regulated by pressure overload.

Expression of VLC1 mRNA in Pressure-overloaded Human Atrium. In order to demonstrate the change in cardiac MLC1 gene expression in diseased human hearts, the 3' untranslated region of each cardiac MLC1 cDNA, pHMLC1A1 and pHMLC1B2, was hybridized to electrophoretically separated RNAs obtained from normal and pressure-overloaded atrial muscle. As shown in Fig. 7, the most significant result was the induction of VLC1 mRNA in the left atrium from a patient with severe mitral regurgitation and the right atrium from a patient with primary pulmonary hypertension. That these atria were high pressure chambers was demonstrated by cardiac catheterization. In contrast, very little, if any, hybridization to atrial muscle obtained from the normal pressure chambers was detectable. This evidence for the accumulation of VLC1 mRNA in pressure-overloaded atria unambiguously establishes that VLC1 gene expression is up-regulated by pressure overload, as is the case of β-MHC gene described above.

Structure of the Human VLC1 Gene. To analyze the molecular basis of isogene switching in pressure overloaded atria, we have cloned the VLC1 gene and determined the potential sequence elements involved in the expression of this gene. Using the VLC1 cDNA pHMLC1B2, as a hybridization probe, we screened a human genomic library and selected on VLC1 λ phage clone, designated λ MLC1 (Fig. 8). To de-
Fig.9. Nucleotide sequence of the human VLC1 gene and proximal flanking regions. The derived amino acid sequence is shown for the region encoding the protein. The AG/GT consensus signals bordering the exons are double-underlined (=). The TATA box, the CAT box, the CAG box (1–3) and polyadenylation signal are boxed. The location of DNA region capable of forming Z-DNA is underlined by a bold line. The transcription initiation site was determined by primer extension and S1 nuclease mapping experiments, and the putative initiation site (A) is marked as position +1. The enhancer consensus core sequence and enhancer sequence seen in SV40 are marked by dot(·) above the nucleotide. The CATTCCCT consensus sequence is indicated by asterisks(*) above the nucleotides. The sequence homologous to nuclear factor-1 (NF-1) consensus recognition sequence is circled. The position of (A) addition in the cDNA is indicated with an arrowhead.

termine the intron/exon structure of human VLC1 gene and localize the promoter regulatory region, an approximately 2.7 kb SacI-HindIII fragment and an approximately 6.3 kb HindIII-EcoRI fragment containing the VLC1 gene were subcloned into pUC18 for detailed restriction mapping and for production of DNA for sequence analysis. The DNA sequence analysis revealed that the human VLC1 gene span 7.0 kb and is broken into 7 exons separated by 6 introns, ranging in size from 111 bp to 3.2 kb. This procedure yielded DNA sequences for all the exons, the intron/exon junctions, and the 5'-plus 3'-flanking regions. Fig.9 presents the nucleotide sequence of the human VLC1 gene.
<table>
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<th>Regulatory element</th>
<th>Consensus sequence</th>
<th>Position</th>
<th>VLC1 sequence</th>
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<td>TATA box</td>
<td>TATA(A/T)A(A/T)</td>
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<td>ATAAA</td>
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<td>CCAAT box</td>
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<td>CArG box</td>
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<td>CAAAAT</td>
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<td></td>
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<td></td>
<td></td>
<td>−159 to −155</td>
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<td>−79 to −70</td>
<td>CCTATTGTGC</td>
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<td>SV40 enhancer core</td>
<td>(G)TGGA(A/T)(G)</td>
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<tr>
<td>SV40 TC−II</td>
<td>5’−CAGGGGTC−5’</td>
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<td>CAAAATTAGC</td>
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<td>Z−DNA</td>
<td>(TG)n</td>
<td>−158 to −152</td>
<td>CATTCCT</td>
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<td>CTF/NF−1 binding</td>
<td>TGG(A/C)NGCCAA</td>
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<td>GTGGGTATG</td>
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<td>−54 to −47</td>
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<td></td>
<td></td>
<td>−268 to −241</td>
<td>(TG)n</td>
</tr>
<tr>
<td></td>
<td></td>
<td>−89 to −76</td>
<td>TGTCCTGTCCCTA</td>
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5’ Flanking Region of the Human VLC1 Gene  The nucleotide sequence of the 5’-flanking region of the VLC1 gene was determined up to position −683. The consensus TATA box has been observed commonly in eukaryotic genes approximately 26–34 bp upstream to the RNA polymerase II transcription initiation site. In the VLC1 gene, a similar sequence, ATAAA, is found 28 bp upstream from the mRNA major cap site, which was determined by primer extension and S1 nuclease mapping procedures (data not shown). A consensus CCAAT element, which might increase the efficiency of gene transcription, has also been observed to be located approximately 70–90 bp from the cap site in many genes. No perfect match to the sequence CCAAT was found in the equivalent region of the VLC1 gene, but four closely related sequences are located further upstream (Fig. 9, and Table II). A noticeable feature in far upstream region of VLC1 gene includes the presence of 28-nucleotide-element that can form Z-DNA. Located at position −268 to −241 (28 bp, underlined in Fig. 9) is a stretch of alternating purines and pyrimidines (mainly T and G). With the aid of computer search programs we have examined the 5’-flanking sequence of the human VLC1 gene for sequences similar to known enhancers. Several potential enhancer sequence elements were identified and listed, by sequence and position, in Table II. It is remarkable that the sequence GTGGGTATG at −293 to −286 in the VLC1 promoter shows surprising sequence homology to the enhancer core consensus sequence common to most known viral and cellular enhancers, (G)TGGA(A/T)(A/T)(A/T)(G), described by Weihrauch. An additional sequence, CTGCGGTC which is very similar to the sequence motif of the SV40 enhancer, is observed at −54 to −47. Located 89 base upstream of the transcription initiation site is the sequence TGGCTGTCCCTA which matches twelve out of fourteen with CCAAT binding recognition sequence.

Comparisons of the 5’ upstream sequences of the human VLC1 gene with those of MLC1F/3F genes of chicken, mouse, and rat reveal no significant homology. However, it does contain the short sequence that shows significant similarity with upstream sequences from several other muscle genes. The 5’-flanking regions of the several muscle genes, such as sarcomeric actin genes, rat skeletal MLC2 gene, α-MHC gene and chicken cardiac troponin T gene, have been shown to contain a CC(A+T-rich)GG DNA motif, referred to as a CArG box. Three CArG elements (CArG1, 2, and 3) are found in the 683 bp of 5’-flanking sequence of the human VLC1 gene in a closely spaced region, at posi-
tions $-79$ to $-70$, $-96$ to $-87$, and $-124$ to $-115$.

Another sequence, CATTCCCT, has also been conserved in the promoter region of a number of different contractile protein genes. We have found the sequence identical to this consensus at positions $-158$ to $-152$ in the human VLC1 gene.

DISCUSSION

In the present study, we have isolated and sequenced human $\alpha$- and $\beta$-MHC cDNA clones, and ALC1 and VLC1 cDNA clones. Previously, we identified two molecular variants of cardiac MHCs, $\alpha$- and $\beta$-MHCs, in human heart and demonstrated that $\alpha$- to $\beta$-MHC transition occurs in pressure overloaded atria. Using these cDNAs as S1 mapping probes, we examined the regulation of each gene in normal and pressure-overloaded human atria. Here, we show that MHC isozyme transition is regulated at the transcriptional level of corresponding genes.

The S1 nuclease analysis did not identify additional labeled fragments in hypertrophied atrial RNA resulting from hybridization of the probe to mRNA which is different from $\alpha$- and $\beta$-MHC mRNAs. Thus, the $\alpha$- and $\beta$-MHC genes could account for the complete atrial MHC phenotype not only in normal atria but also in pressure-overloaded atria. However, since the S1 mapping analysis detects only mRNAs homologous to the probe, it is possible that mRNA, with identical 3' sequences and different 5' sequences as a result of transcription initiation from different promoters or alternate RNA splicing, may have been missed.

Recent analysis of the composition of MLCs in the rat atria showed that there is an increased expression of cardiac MLC2 gene in the spontaneously hypertensive rat atria.$^{34}$ We now demonstrate that transcripts from the VLC1 gene accumulate in the human atria subjected to severe pressure overload. Although the physiological significance of VLC1 gene induction in the pressure-overloaded atria is unknown, MLC1 as well as MHC and MLC2 may be involved in the response of myocardial tissue to new functional requirements. Since the degree of transition is different between MHC and MLC1, the up-regulation of $\beta$-MHC and VLC1 genes is not coordinated and there may be gene specific regulatory mechanisms responsible for each myosin subunit isogene switching.

Significant evidence has been accumulated to suggest that sequences in the 5'-flanking regions are important for the regulation of gene transcription. Transient expression system using CAT fusion gene as reporter gene identified the essential sequence in the VLC1 gene promoter. A short segment from $-107$ to $-94$ carried an important element for the high level expression in C2C12 cells and rat fetal cardiocytes (unpublished data). It is of particular interest to note that this region has a sequence, CCTTTTATGG, which is identified as a core sequence in the cardiac $\alpha$-actin gene expression expression. Minty and Kedes$^{35}$ reported the CC(A+T-rich)$_3$ GG motif is highly conserved in many muscle-associated genes and the results obtained by 5' deletion analyses indicate that this DNA sequence may be an important part of the regulatory elements in cardiac $\alpha$-actin gene. Recently, an in vitro transcription system and protein-DNA mobility shift assay have demonstrated that CC(A+T-rich)$_3$GG motif is a binding site for positively acting factors in human cardiac $\alpha$-actin genes.$^{36}$ It is tempting to speculate CArG box shared by striated $\alpha$-actin and VLC1 genes may be related to the induction of expression in response to pressure overloading.

Another sequence which is conserved in the 5'-flanking regions of the several muscle-specific genes, CATTCCCT identified by Nikovits et al.$^{40}$ is also found in the human VLC1 gene promoter region. Although the functional significance of this element remains unclear, evolutionary conservation and its location in the promoter region suggest that it may play a role in the transcriptional regulation of VLC1 gene.

The existence of a 28-bp segment of alternating purines and pyrimidines found at $-268$ to $-241$ raises many interesting questions regarding the possible role of lefthanded Z-DNA in the regulation of human VLC1 gene transcription. In vitro studies have shown that poly (dT-dG) poly (dC-dA)(TG-element) can form stable Z-DNA
under physiological conditions. Furthermore, when in the Z-form, TG-element is susceptible to the S1 nuclease, and Z-DNA segments in the SV 40 enhancer or other viral enhancer sequences have been demonstrated. Until recently, Z-DNA stretches have been found in several other cellular genes, such as human cardiac α-actinin gene, parathyroid hormone gene, and apo-AII genes. While no obvious common regulatory feature could be found for these genes, the effect of potential Z-DNA in the human VCL1 gene promoter region deserves to be tested.

An additional feature of the human VCL1 gene promoter region is the presence of two sequences which are very similar to regions of the SV 40 enhancer. One of these, CTGGGGTGC, located at −54, is homologous to TC-II motif in SV 40 domain B enhancer which has been shown to be a binding site for a protein or proteins present in HeLa cell nuclear extract. The other, GTGGTATG, located at −293, matches the so-called enhancer “core” sequence present in many other enhancers. Deletion and site directed mutagenesis experiments of the putative VCL1 promoter region should define the possible functional significance in the transcriptional regulation of the sequence elements described above.

In conclusion, the present study established that isozymic transition of cardiac MHC during pressure overload and subsequent hypertrophy is regulated at the transcription of their respective genes, and VCL1 gene is also up-regulated in severely hypertrophied atria. Furthermore, we showed that 5'-flanking region of the human VCL1 gene contains several putative cis-regulatory elements which are not found in MLC1 F/3 F gene, suggesting the presence of potentially novel regulatory mechanisms involved in the expression of VCL1 gene. Although the biochemical parameters involved in isoform changes during hypertrophic process are not yet known, the pathophysiological switch in cardiac MLC1 genes as well as MHC genes provides an excellent model system for the analysis of gene regulation and for dissecting the complex regulatory processes in cardiac hypertrophy.

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