

**Molecular cloning and characterization of human cardiac alpha- and beta-form myosin heavy chain complementary DNA clones. Regulation of expression during development and pressure overload in human atrium.**

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**Research Article**

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# Molecular Cloning and Characterization of Human Cardiac $\alpha$ - and $\beta$ -Form Myosin Heavy Chain Complementary DNA Clones

## Regulation of Expression during Development and Pressure Overload in Human Atrium

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### Abstract

We have constructed and characterized two types of myosin heavy chain (MHC) cDNA clones (pMHMC2, pMHMC5) from a fetal human heart cDNA library. Comparison of the nucleotide and deduced amino acid sequences between pMHMC2 and pMHMC5 shows 95.1 and 96.2% homology, respectively. The carboxyl-terminal peptide and 3'-untranslated (3'-UT) regions are highly divergent and specific for these cDNA clones. By using the synthetic oligonucleotide probes that are complementary to the unique 3'-UT regions of these cDNA clones, we demonstrate that pMHMC2 is exclusively transcribed in the atrium, whereas the mRNA for pMHMC5 is predominantly expressed in the ventricle. This result indicates that pMHMC2 and pMHMC5 code for  $\alpha$ - and  $\beta$ -form MHCs, respectively. Furthermore, we show that  $\beta$ -form MHC mRNA is expressed in adult atrium at a low level but scarcely expressed in fetal atrium. Finally, we demonstrate that MHC isozymic transition in pressure-overloaded atrium is, at least in part, regulated at a pretranslational level.

### Introduction

Mammalian cardiac muscle cells contain at least two isozymes of myosin heavy chains (MHCs),<sup>1</sup>  $\alpha$ - and  $\beta$ -MHC, specifying a subunit of the high ATPase V1 isomyosin and a component of the low ATPase V3 isomyosin, respectively (1–5). The myosin isozyme content of cardiac fiber is thought to affect contractile properties (6); the changes in myosin isozyme composition were interpreted as an adaptation of myocardial cell to new functional requirements.

The distribution of cardiac myosin isozymes in the ventricle of small animals changes not only during normal development but also in response to thyroid hormone (7–11, 12, 13), insulin (14), or increased hemodynamic load (11, 15–17). So far, the ventricular  $\alpha$ - and  $\beta$ -MHC mRNA sequences and their corresponding genes have been isolated and characterized in rat (18, 19) and rabbit (20, 21). From the observations in these animals, it has been shown that the two types of MHCs are

products of different genes and these two genes are organized in tandem (19). That is, the  $\beta$ -MHC gene is located upstream from the  $\alpha$ -MHC gene. In addition, the MHC isozyme transition in the hypertrophied ventricle is mainly regulated at the pretranslational level (22).

In man, however, in whom normal ventricular myosin contains  $\beta$ -MHC almost exclusively, myosin isozyme switches from  $\alpha$ - to  $\beta$ -MHC do not or hardly contribute to adaptation to chronic hemodynamic overload (23). In a previous immunofluorescence study, we have shown the evidence of the transition from  $\alpha$ - to  $\beta$ -MHC in pressure-overloaded human atrial muscle and suggested that myosin isozymic redistribution might have a functional implication in the atrium (24). Recently, human  $\alpha$ - and  $\beta$ -MHC genes have been isolated and partially characterized (25). They have a similar genomic organization to that seen in rat.

This study was undertaken to clarify the existence of MHC isozymes at the amino acid sequence level and to understand the molecular basis of the MHC isozyme transition in pressure-overloaded human atrium. For this purpose, we have isolated and characterized human cardiac  $\alpha$ - and  $\beta$ -MHC mRNA sequences and analyzed their expression in normal and diseased human hearts. We also showed that the expression of MHC isozyme is developmentally regulated.

### Methods

**Preparation and screening of a  $\lambda$ gt11 human fetal heart cDNA library.** Human fetal heart was kindly provided by Dr. K. Yoshida, Department of Obstetrics, San-ikukai Hospital, Tokyo, Japan. The heart was stored at  $-70^{\circ}\text{C}$  until use. RNA was extracted by the LiCl method essentially as described (26). Total polyadenylated RNA was selected by oligo(dT)-cellulose chromatography (27). Synthesis of the cDNA was carried out using the method of Gubler and Hoffmann (28). Approximately 1  $\mu\text{g}$  of the cDNA was used to construct a  $\lambda$ gt11 library containing ~ 500,000 independent recombinant plaques. The library was screened with mouse  $\alpha$ -MHC cDNA plasmid, pMHC101 (29), as a probe which was a generous gift from M. E. Buckingham, Pasteur Institute, Paris. Hybridization with  $1 \times 10^6$  cpm/ml  $^{32}\text{P}$ -labeled cDNA was performed at  $42^{\circ}\text{C}$  for 16–24 h in a solution containing 50% (vol/vol) formamide,  $2\times$  SSC ( $1\times$  SSC is 150 mM NaCl and 15 mM sodium citrate),  $5\times$  Denhart's solution ( $1\times$  Denhart's solution is 0.02% Ficoll, 0.02% polyvinylpyrrolidone, and 0.02% BSA), 0.1% SDS, 1 mM EDTA, and 100  $\mu\text{g}/\text{ml}$  salmon sperm DNA. Filters were washed to a stringency of  $0.1\times$  SSC and 0.1% SDS at  $55^{\circ}\text{C}$  and exposed for 24–48 h at  $-70^{\circ}\text{C}$  to XAR-5 film. Positive recombinant phages were purified by sequential screening at low plaque density. Phage DNA was purified according to Maniatis et al. (27). Eco RI-excised cDNA inserts were subcloned into a plasmid vector pUC13 and characterized by restriction endonuclease mapping.

**Nucleotide sequencing.** Restriction fragments were subcloned into bacteriophage M13 mp18 and mp19 and sequenced by the dideoxynucleotide chain-termination method (30), using a universal sequencing primer.

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1. Abbreviations used in this paper: MHC, myosin heavy chain; 3'-UT, 3'-untranslated region.

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**Tissue sources.** Northern blot analysis reported in this paper was performed on RNA samples extracted from autoptic specimens obtained from one fetus, one adult patient without clinical and pathological evidence of heart disease, and nine adult patients with hypertrophied atria. Fetal tissue was a specimen from a fetus that died as a result of an induced abortion at 17 wk gestation. It had no evidence of cardiac pathology. For the fetal heart, the total atrium and total left ventricle were used. For the analysis of diseased hearts, atrial tissue was obtained from nine cardiac patients, whose pertinent information is listed in Table I. As a control specimen, normal heart was obtained from a 27-yr-old woman who died of breast cancer. In the adult hearts, tissue samples were excised from the anterior wall of the right atrium, the posterior wall of the left atrium, and the free wall of the left ventricle. All tissue samples obtained were frozen at  $-80^{\circ}\text{C}$  no later than 6 h postmortem.

**Preparation of synthetic oligonucleotide probes.** Oligonucleotides were synthesized by an oligonucleotide synthesizer (model 380A; Applied Biosystems, Inc., Foster City, CA) and purified in a preparative denaturing 15% polyacrylamide gel followed by DEAE-cellulose column chromatography (31). The positions and sequences of oligonucleotides on their corresponding mRNAs are presented in Fig. 2. The probes were labeled at the 5' end using T4 polynucleotide kinase to a specific activity of  $1-8 \times 10^8$  cpm/ $\mu\text{g}$ .

**Northern blot technique with the oligonucleotide probes.** Total cellular RNA was prepared from human tissues by the LiCl method (26) and quantified by absorbance at 260 nm. Samples were denatured at  $65^{\circ}\text{C}$  for 10 min and size-separated by an 1.2% agarose formaldehyde gel (31). The fractionated RNA was then transferred to nylon membrane filters (Hybond N; Amersham Corp., Arlington Heights, IL) and hybridized for 16–24 h to  $^{32}\text{P}$ -labeled  $\beta$ -MHC specific oligonucleotide probe (specific activity  $> 1 \times 10^8$  cpm/ $\mu\text{g}$ ) at  $37^{\circ}\text{C}$  in a solution containing  $5 \times \text{SSPE}$  (0.9 M NaCl, 40 mM NaOH, 50 mM  $\text{NaH}_2\text{PO}_4$ , and 5 mM EDTA),  $5 \times$  Denhart's solution, 0.1% SDS, and 100  $\mu\text{g}$  of sonicated salmon sperm DNA per milliliter. The final hybridization wash was  $2 \times \text{SSC}/0.1\%$  SDS at  $50^{\circ}\text{C}$ . Filters were then exposed to XAR-5 film at  $-70^{\circ}\text{C}$ , and densitometric scanning was performed. The filters were subsequently stripped and rehybridized with  $\alpha$ -MHC specific oligonucleotide probe and subjected to the same autoradiographic and densitometric scanning process.

Table I. Sources of Cardiac Samples Examined

Patient no.	Age/Sex	Hemodynamic data		Diagnosis
		Mean PCW	Mean RA	
		mmHg		
1	26/F	13	4	MR
2	20/M	6	14	TOF
3	26/F	6	15	PPH
4	46/F	13	10	MS, MR, TR
5	43/M	20	12	MS, AR, TR
6	70/M	18	6	AS, AR, MS, MR
7	54/F	16	5	AR, MS, MR
8	74/M	18	5	IHD
9	25/M	10	11	PS, ASD
Control	27/F	ND	ND	Breast cancer
Normal range		<12	<6	

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.

**Indirect immunofluorescence technique.** The MAbs we used in this study were CMA19 and HMC14, specific for  $\alpha$ - and  $\beta$ -MHC, respectively. These were obtained as previously described (24). Specimens of human heart were obtained at autopsy from the patients with primary pulmonary hypertension (patient 3, Table I). Several blocks of cardiac muscle were excised from the anterior wall of the right atrium and the posterior wall of the left atrium within 6 h postmortem. Indirect immunofluorescence staining was performed according to the methods described previously (24).

## Results

**Identification of cardiac MHC cDNA clones.** A cDNA library of  $\sim 5 \times 10^5$  independent recombinants was constructed in the vector  $\lambda\text{gt}11$  by using 1  $\mu\text{g}$  of poly(A) RNA isolated from fetal human heart. About 20,000 recombinants were screened and 30 independent plaques were identified. The three cardiac MHC cDNA clones (pHMHC2, pHMHC4, and pHMHC5) that contain the long insert ( $> 1,000$  bp) were selected for further analysis. Fig. 1 shows that pHMHC4 and pHMHC5 have identical restriction maps in regions in which they overlap. They probably represent the same MHC mRNA sequence. The third clone, pHMHC2, has several altered restriction enzyme sites, although substantial homology is present between the two different cDNA sequences.

**DNA sequence analysis.** To establish unequivocally that two clones, pHMHC2 and pHMHC5, contained MHC mRNA sequences, nucleotide sequences were determined by the dideoxy method. Nucleotide and deduced amino acid sequences are shown in Fig. 2. Clone pHMHC2 consists of 1,644 MHC mRNA-derived nucleotides coding for 532 amino acids, whereas pHMHC5 contains 1,682 nucleotides coding for 524 amino acids of MHC. Each clone encodes the light meromyosin portion of MHC, including the entire 3'-untranslated (3'-UT) 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% 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. These differences are summarized in Table II, which includes percentages of silent and replacement site divergence. Compared with the generally

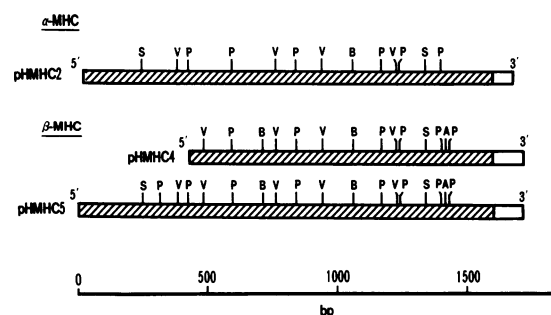


Figure 1. Restriction map of cDNA inserts in clones pHMHC2, pHMHC4, and pHMHC5 coding for  $\alpha$ - and  $\beta$ -form MHCs. The map is drawn to scale. The direction of translation is from left to right. Hatched rectangle, coding region. Open rectangle, 3'-UT regions. Restriction endonuclease sites: S, Sst I; V, Pvu II; P, Pst I; B, Bgl I; A, Acc I.



Table II. Nucleotide and Amino Acid Sequence Comparison of Human Cardiac  $\alpha$ -MHC (pHMH2) to Human  $\beta$ -(pHMH5), Rat  $\alpha$ -(pCMHC21), and Rat  $\beta$ -(pCMHC5) MHCs

MHC cDNA clone	Homology in coding region		Conservative nucleotide change	Nonconservative nucleotide change	Nucleotide homology in 3'-UT region
	Nucleotide	Amino acid			
	%		<i>n</i>		%
pHMH5	95.1	96.2	44	33	32.7
pCMHC21	90.8	93.2	74	40	66.1
pCMHC5	91.8	95.4	75	27	34.0

Percentages of overall nucleotide and amino acid sequence homology were calculated by aligning the analogous region in such a way as to maximize homology and minimize the number of deletion. Sequences of rat ventricular MHCs are from Mahdavi et al. (18).

high levels of homology observed for the translated sequences of pHMH2 and pHMH5, the corresponding 3'-UT regions show extensive sequence divergence. Alignment of DNA sequences to obtain maximum homology reveals the 3'-UT regions of pHMH2 and pHMH5 to be at least 67% divergent. However, when the pHMH2 cDNA clone was compared with the previously described  $\alpha$ -form MHC cDNA clones from rat (Table II, reference 18), rabbit (20), significant homology was seen among the 3'-UT regions. Similar results were obtained when pHMH5 was compared with the  $\beta$ -form MHC cDNA clones from other species (data not shown).

**Expression of pHMH2 and pHMH5 during development.** Since the nucleotide sequences of pHMH2 and pHMH5 are highly homologous, it is difficult to identify the specific MHC mRNA expressed in different muscle types at a particular stage of development by RNA blot analysis using cDNA coding sequence. To overcome these difficulties, synthetic oligonucleotide probes complementary to the unique 3'-UT regions of pHMH2 and pHMH5 were used in Northern blot analysis. Fig. 3 examines the expression of the

pHMH2 and pHMH5 mRNA in several muscle tissues at fetal and adult stages. The strong hybridization of fetal and adult ventricular RNAs with pHMH5 3'-UT region probe indicates that the major MHC mRNA in the ventricle in both fetal and adult life must be that which contains the same 3'-UT regions as pHMH5. Therefore, the mRNA represented by pHMH5 is not preferentially fetal specific. This conclusion is further reinforced by the fact that MHC cDNA clones isolated from an adult ventricular cDNA library (33) have identical nucleotide sequence to that of pHMH5, including 3'-UT regions, except for three nucleotide mismatches (nucleotide positions 513, 567, and 1,068 in Fig. 2). Nucleotide position 513 is a replacement change and nucleotide changes of positions 567 and 1,068 are silent. Three single-base differences in the coding region may result from sequencing or enzymatic errors during the construction of the library. Since we have sequenced this region three times, it seems more likely that a misreading of reverse transcriptase may be responsible for those differences. An alternative explanation for the sequence difference might be that the  $\beta$ -MHC gene occurs as an allelic

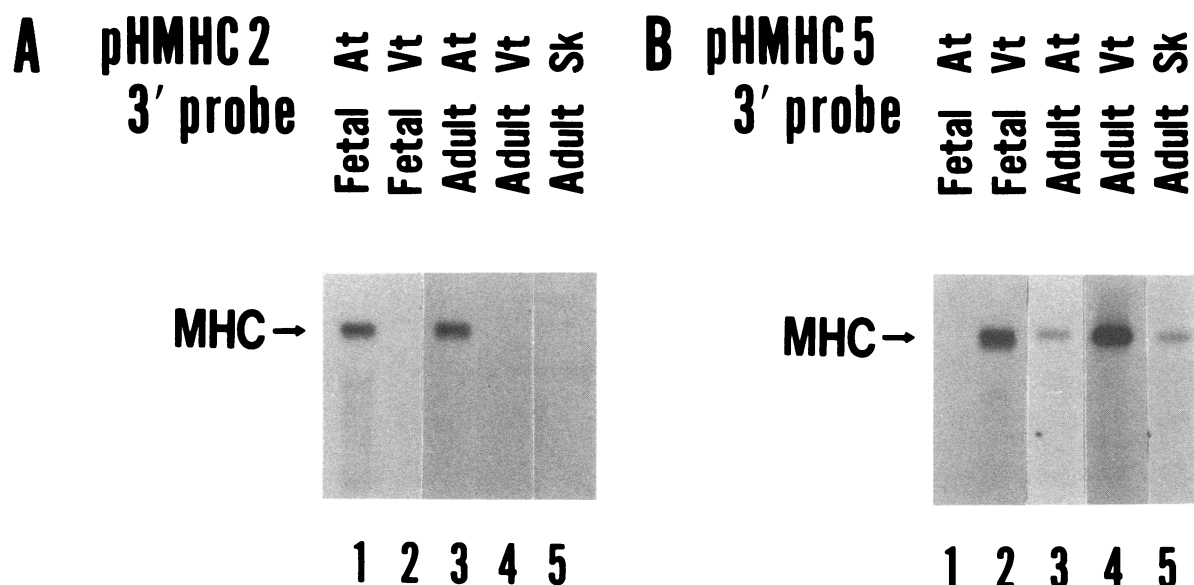


Figure 3. Autoradiographs of Northern blot showing the tissue and stage specificity of pHMH2 and pHMH5 specific probes. Hybridization of (A)  $^{32}$ P-labeled pHMH2 3'-UT or (B) pHMH5 3'-UT oligonucleotide probes to Hybond N membranes containing human muscle RNA was performed. 10  $\mu$ g of total RNA was loaded in each

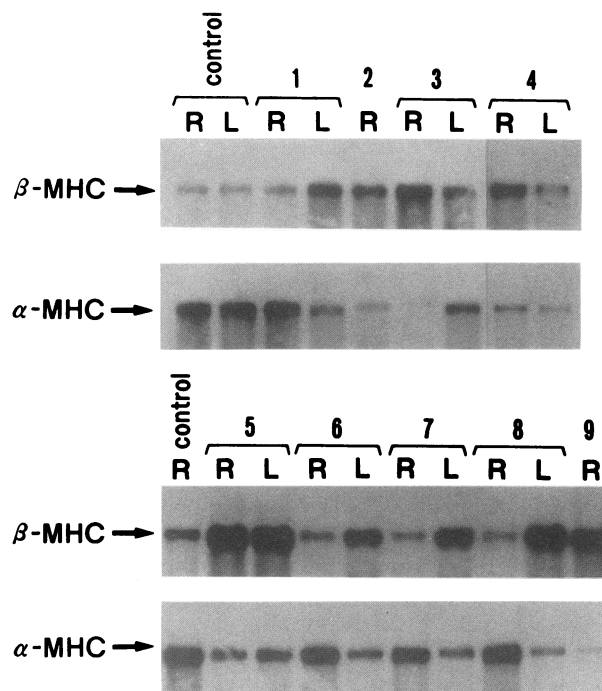
lane. The MHC mRNA band is marked on the side of the figure (MHC). Lane 1, RNA isolated from fetal atrium. Lane 2, RNA isolated from fetal ventricle. Lane 3, RNA isolated from adult left atrium. Lane 4, RNA isolated from adult left ventricle. Lane 5, RNA isolated from adult psoas muscle.

variant in some individuals. Further investigations are needed to determine the reason for these differences. Compared with the pHMHC5 probe, fetal and adult ventricles show very little, if any, hybridization with pHMHC2 probe. This indicates that pHMHC2 mRNA is expressed at a low level, if at all, in the ventricle in both fetal and adult life.

In the atrium, however, the extent of hybridization with the two probes differs between fetal and adult tissues. The RNA that is complementary to pHMHC2 remains the main form, whereas that which is complementary to pHMHC5 is apparently detected only in adult atrial muscle. Here, we observe that differential accumulation of mRNA represented by pHMHC5 occurs during atrial muscle development. When mRNA from the skeletal muscle, such as psoas muscle, is hybridized with both probes, we observe that this contains the pHMHC5 mRNA, but not the pHMHC2 mRNA. These data correlate very well with the presence of  $\alpha$ - and  $\beta$ -form MHCs. Therefore, together with the result of nucleotide sequence, we conclude that pHMHC2 and pHMHC5 represent  $\alpha$ - and  $\beta$ -form MHC sequences, respectively. Sequence analysis strongly suggests that  $\alpha$ - and  $\beta$ -MHCs are products of different genes rather than differential splicing of a transcript from a single gene, since sequence divergence and restriction map differences are distributed throughout the clones.

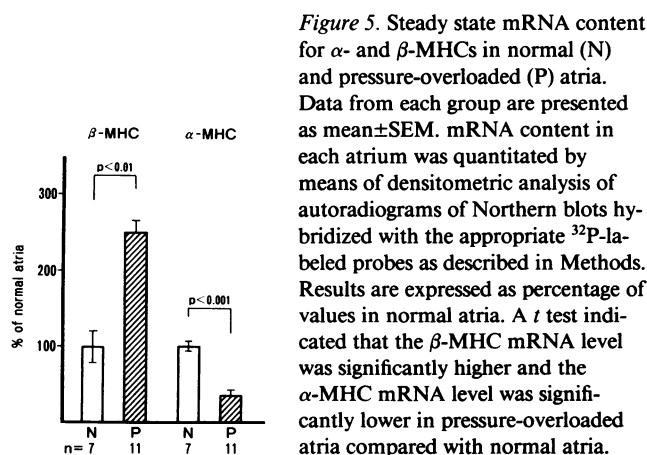
**Expression of pHMHC2 and pHMHC5 in diseased atrium.** Northern blot analyses were used to characterize expression of  $\alpha$ - and  $\beta$ -MHC genes in pressure-overloaded human atria and to assess the relative amounts of both  $\alpha$ - and  $\beta$ -MHC mRNAs in diseased atria compared with normal atria. As shown in Table I, the right atria of patients 1, 6, 7, and 8, and the left atria of patient 3 were normal pressure chambers. The right atria of patients 2, 3, 4, 5, and 9 and left atria of patients 1, 4, 5, 6, 7, and 8 were pressure-overloaded chambers. Although cardiac catheterization was not performed on the control patient, she was considered to have normal atria on the basis of clinical and pathological findings. We thus analyzed 7 normal and 11 pressure-overloaded atria in this study. RNA prepared from atria subjected to normal and elevated pressure load was hybridized with oligonucleotide probes specific for the mRNA encoding  $\alpha$ - or  $\beta$ -MHC. As demonstrated in Fig. 4, all the pressure-overloaded atria had an increased  $\beta$ -MHC mRNA content compared with the control atrium. When the blots were analyzed by densitometry, the difference of  $\beta$ -MHC mRNA content between the normal and pressure-overloaded atria was statistically significant (100 vs.  $247.9 \pm 32.3\%$  SEM;  $P < 0.01$ , Fig. 5). In contrast to the increase of  $\beta$ -MHC, the expression of  $\alpha$ -MHC mRNA in pressure-overloaded atria was significantly lower than that in normal atria (100 vs.  $34.7 \pm 6.3\%$  SEM;  $P < 0.001$ , Fig. 5). These findings indicated that  $\beta$ -MHC gene is up-regulated and  $\alpha$ -MHC gene is down-regulated by pressure overload.

To ascertain whether the changes in the pHMHC2 and pHMHC5 mRNA level under the pressure-overloaded condition are reflected in the MHC protein phenotype, the degree of transition analyzed by Northern blot was compared with that visualized by antimosin immunofluorescence in the tissue section obtained from the same patient, since pyrophosphate gel analysis failed to separate individual isozymes of human cardiac myosin. Immunofluorescence procedure was performed on the cryostat section of the right and left atria from patient No. 3. As shown in Fig. 6, all myocytes in the right



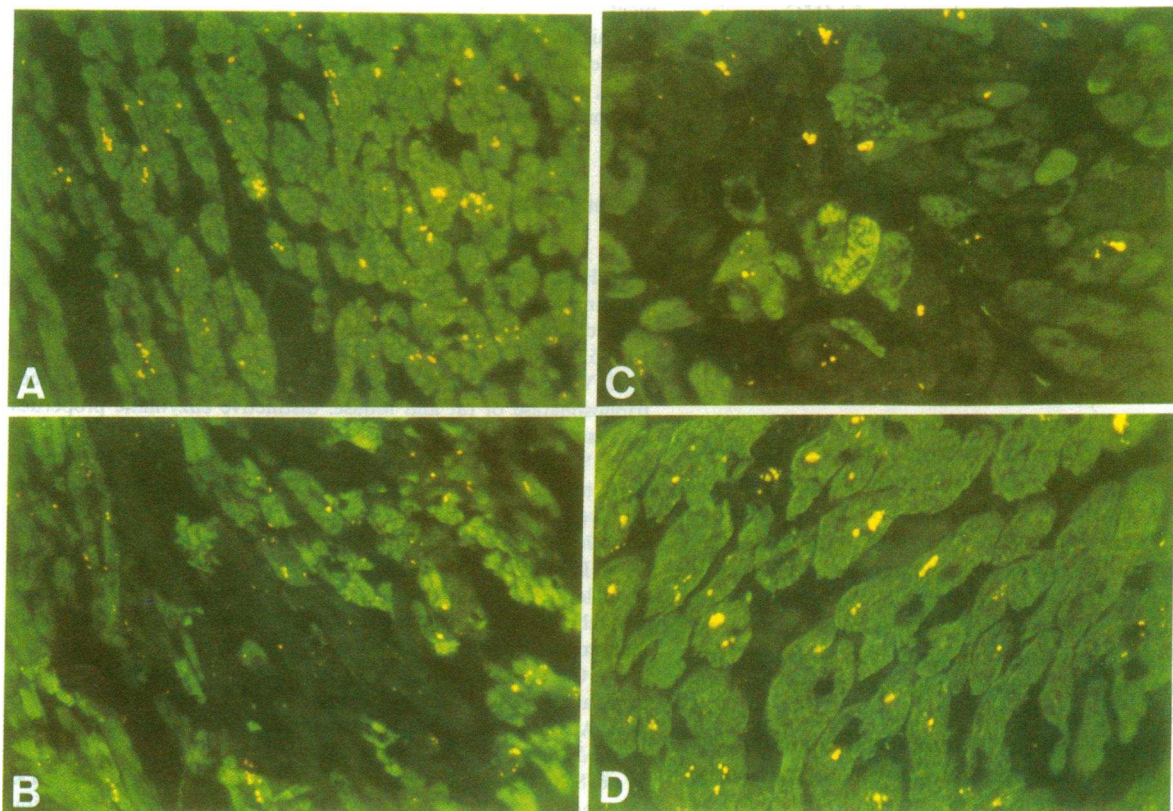
**Figure 4.** Detection of  $\alpha$ - and  $\beta$ -MHC mRNA in total cellular RNA from normal and diseased human atria. The same RNA blot was sequentially hybridized with labeled oligonucleotides specific for  $\beta$ -MHC mRNA and exposed for 24 h;  $\alpha$ -MHC mRNA was exposed for 24 h. Normal atrial RNA controls are shown in the left lanes of the figures. The lanes containing 10  $\mu$ g right (R) and left (L) atrial RNA from patients 1–9 are indicated. The MHC mRNA band is marked on the side of the figures ( $\beta$ -MHC,  $\alpha$ -MHC).

atrium were strongly reactive with anti- $\beta$ -MHC antibody and most of them were unreactive with anti- $\alpha$ -MHC antibody. The left atrium showed a moderate degree of redistribution. Although we determined only semiquantitative amounts of  $\alpha$ - and  $\beta$ -MHC proteins by immunofluorescence study, these results are consistent with the observations in Northern blot. Changes in  $\alpha$ - and  $\beta$ -MHC mRNA level thus seem to be associated with corresponding changes in the relative proportion of MHC protein.



**Figure 5.** Steady state mRNA content for  $\alpha$ - and  $\beta$ -MHCs in normal (N) and pressure-overloaded (P) atria. Data from each group are presented as mean  $\pm$  SEM. mRNA content in each atrium was quantitated by means of densitometric analysis of autoradiograms of Northern blots hybridized with the appropriate  $^{32}$ P-labeled probes as described in Methods. Results are expressed as percentage of values in normal atria. A  $t$  test indicated that the  $\beta$ -MHC mRNA level was significantly higher and the  $\alpha$ -MHC mRNA level was significantly lower in pressure-overloaded atria compared with normal atria.





**Figure 6.** Indirect immunofluorescence staining of the left and right atrium from the patient with primary pulmonary hypertension (patient 3). (A) Cryostat section of left atrium stained with CMA19. Most myofibers are labeled. (B) As in A, except that the myofibers are stained with HMC14. The heterogeneous pattern of reactivity

was seen. (C) Cryostat section of right atrium stained with CMA19. Only a small number of fibers are labeled. (D) As in C, except that the myofibers are stained with HMC14. Note that almost all myofibers are strongly labeled.

## Discussion

We have now isolated and sequenced human  $\alpha$ - and  $\beta$ -MHC cDNA clones and used them to examine the tissue distribution and developmental regulation of their gene expression. This investigation also indicates that  $\alpha$ - and  $\beta$ -MHC genes are regulated by pressure overload in human atria.

The successful cloning of  $\alpha$ - and  $\beta$ -MHC cDNAs has established the existence of the two molecular variants in human heart. Comparison of the nucleotide sequences between  $\alpha$ - and  $\beta$ -MHC cDNA clones reveals striking homology (96%). Apart from the human cardiac cDNA sequences described above, the available cardiac MHC sequence data are from rat (18), rabbit (20), and mouse (29). The sequence homology between widely separated species extends into the 3'-UT regions, when compared with the same MHC isoforms. These data provide evidence of isotype-specific preservation of MHC 3'-UT regions during evolution. It may have interesting implications for both MHC gene regulation and evolution, as postulated by Saez et al. (34).

It is well documented that the ratio of the different ventricular MHC isoforms is developmentally regulated in small animals. In rat and mouse,  $\beta$ -MHC is the most abundant myosin in late fetal life. After birth,  $\alpha$ -MHC increases and becomes the predominant form throughout perinatal and adult life (8). In comparison, in larger mammals, including humans, previous

investigations failed to detect any physical difference between fetal and adult ventricular MHC (35, 36). The results of our Northern blot strongly suggest that the same  $\beta$ -MHC gene is expressed in both fetal and adult ventricles, which supports the idea that fetal and adult  $\beta$ -MHCs are indistinguishable in human, as is the case with other mammals. The interspecies variation in the relative amount of cardiac myosin isoforms in adulthood may be due to species-dependent regulatory mechanisms in the expression of  $\alpha$ - and  $\beta$ -MHC genes. The induction of  $\beta$ -MHC gene expression in pressure-overloaded atria suggests that adaptation of the adult human atria to mechanical stress is mediated by the expression of the same  $\beta$ -MHC gene that is predominantly expressed in both fetal and adult ventricles. However, it is possible for distinct genes to have nearly identical coding and noncoding sequences. Furthermore, it is also possible that the same gene is differently transcribed, either by alternative splicing or by different promoter use.

Recently, analysis of the mRNA sequences in atrial muscle of rat (8) and rabbit (37) hearts by S1 nuclease mapping has shown that atrial  $\alpha$ -MHC mRNA is indistinguishable from ventricular  $\alpha$ -MHC mRNA in the 3'-UT regions and in a number of coding regions. It thus has been strongly suggested that atrial and ventricular  $\alpha$ -MHC mRNAs are encoded by the same gene. However, the relationship between the structure of the  $\beta$ -MHC present in the atrium and ventricle of large animals that contain a relatively large amount of  $\beta$ -MHC in the

atrium has not been established. The results of the Northern blot in this experiment indicate that the same  $\beta$ -MHC gene is expressed in both atrium and ventricle. In our previous work,  $\beta$ -MHC in the atrium is indistinguishable from that in the ventricle in peptide composition and enzymatic activities in canine heart (38). The results presented here provide consistent evidence with that of our previous work.

Although much evidence has been accumulated about the changes in the MHC isozyme composition in the ventricle, isomyosin redistribution in the atrium has not been demonstrated in sufficient detail. Previous studies indicate that each member of the MHC multigene family can be regulated by thyroid hormone in highly different modes, depending on the muscle in which it is expressed (39). For example, expression of the  $\alpha$ -MHC gene is apparently independent of thyroid hormone in the atrium but highly dependent on thyroid hormone in the ventricle. Studies described by Izumo et al. (22) demonstrated that the MHC isozyme transition during hemodynamic overload in the rat ventricle is produced by changes in the level of  $\alpha$ - and  $\beta$ -MHC gene expression. One of the important implications in our study centers on whether  $\alpha$ - and  $\beta$ -MHC genes in the human atrium also respond to hemodynamic load in the same fashion as that seen in the hypertrophied rat ventricle. The result of comparative examination by Northern blot and immunofluorescence staining, changes in the  $\alpha$ - and  $\beta$ -MHC mRNA level, are in a large part reflected by the changes in their respective proteins. Although immunofluorescence staining is not quantitative, translational and posttranslational mechanisms, if present, do not appear to play a major role in the production of the MHC isozyme switches in response to hemodynamic overload.

From the data presented here, the following important question remains to be answered. What is the biochemical signal that regulates the  $\alpha$ - and  $\beta$ -MHC gene expression in the pressure-overloaded condition? It has been shown that there is an induction of expression of the ventricular type myosin alkali light chain isoform in hypertrophied human atria (40). Significant evidence has been accumulated to suggest that sequences in the 5' flanking regions are important for the regulation of transcription. Gustafson et al. (41) have identified the 5' flanking sequences responsible for the induction by thyroid hormone in the rat  $\alpha$ -MHC gene. Furthermore, Saez et al. (25) have shown that the 5' flanking sequences of rat and human  $\alpha$ -MHC genes are highly homologous. To define the gene regulation in cardiac hypertrophy, studies of a number of muscle-specific genes whose expressions are altered by pressure overload may provide some information that will help to identify the sequences in the 5' flanking regions that are important for the regulation of transcription in the pressure overloaded condition.

In conclusion, the data presented here define the mRNA sequences coding for  $\alpha$ - and  $\beta$ -MHCs in fetal human heart. This investigation has shown that fetal and adult ventricular  $\beta$ -MHCs are products of the same gene and this is true for  $\alpha$ -MHCs present in both stages. The  $\beta$ -MHC gene is also expressed in the adult atrium and skeletal muscle. Finally, we have demonstrated that MHC isozyme transition seen in pressure overloaded atrium is produced by the increase in  $\beta$ -MHC mRNA and corresponding decrease in  $\alpha$ -MHC mRNA.

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