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Molecular cloning of mRNA sequences for cardiac α- and β-form myosin heavy chains: Expression in ventricles of normal, hypothyroid, and thyrotoxic rabbits

(DNA sequence analysis/nuclease S1 mapping/propylthiouracil/thyroxine)

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ABSTRACT We have isolated cDNA clones from thyrotoxic (pMHCα) and normal (pMHCβ) adult rabbit hearts. Restriction map analysis and DNA sequence analyses show that, although there is strong homology between overlapping regions of the two clones, they are distinctly different. The two clones exhibited 78–83% homology between the derived amino acid sequences and those determined by direct amino acid sequence analysis of rabbit fast skeletal muscle myosin heavy chains. The clones specify a segment of the myosin heavy chain corresponding to subfragment 2 and the COOH-terminal portions of subfragment 1. Nuclease S1 mapping was used to compare transcription of the two clones with expression of the α and β forms of myosin heavy chains in the ventricles of thyrotoxic, hypothyroid (propylthiouracil-treated), and normal rabbits. Thyrotoxic ventricles contained only pMHCα transcripts whereas hypothyroid ventricles contained exclusively pMHCβ transcripts. These data correlate well with the presence of α- and β-form myosin heavy chains. In the normal young adult rabbit, pMHCβ transcripts predominate, agreeing with the known β form/α form ratio of 4:1. We therefore conclude that pMHCα and pMHCβ contain sequences of the α- and β-form myosin heavy chain genes, respectively.

Myosin, a major contractile protein of skeletal and cardiac muscle, is composed of two 200,000-dalton heavy chains (HCs) and two sets of low molecular weight light chains. The active center of myosin ATPase resides in the globular head of the heavy chain. This enzymatic activity is correlated with contractile velocity in skeletal muscle (1) and thus appears to be an important determinant of contractile function. Numerous polymorphic forms of myosin HC exist, not only in different types of muscle—e.g., fast and slow skeletal and cardiac—but also within each muscle type (2–12). Expression of these forms follows a developmental pattern (12–18) that may be altered by changes in the physiological (19–21) and hormonal (10–13) milieu of the cell.

Cardiac ventricular muscle contains at least two forms—referred to as α and β (9)—of myosin HC. Electrophoresis of myosin under nondenaturing conditions reveals three bands; V1 and V3 are homodimers containing the α and β forms, respectively, whereas V2 is a heterodimer (9, 13). The ATPase activity of myosin with α-form HCs has been shown to be considerably higher than that of myosin with β-form HCs (22–24).

The expression of α- and β-form myosin HCs follows a defined developmental pattern that varies in different animal species. It has been shown that in rabbit the β form/α form ratio is ≈3:1 during the last half of the gestational period. After birth, the relative amount of the α form increases so that, during the first 2 wk postnatally, this ratio is 1:1 (12, 14). Thereafter, the α form decreases and, in the old adult, the β form is present almost exclusively (12, 14). In the young adult animals used in this study, the β form/α form ratio was ≈4:1 (12). The distribution of the α and β forms is also markedly affected by thyroid hormone administration (10–13). When thyroid hormone is given to young adults, in which more than 75% myosin HC is in the β form, there is complete conversion to the α form within 2 to 3 wk (10, 12). Conversely, after propylthiouracil (PrSur) administration, only V3 myosin containing β-form HC is detected (R. Chizzonite and R. Zak, personal communication).

To establish definitely whether α- and β-form myosin HCs are products of different genes rather than the results of post-translational modification and to elucidate the mechanisms responsible for the change in expression of these forms after thyroid hormone administration, we have cloned rabbit cardiac α and β myosin HC cDNA sequences and examined expression of the clones in the ventricles of normal, thyrotoxic, and PrSur-treated rabbits. By using nuclease S1 mapping techniques, we showed that clone pMHCβ 174 is expressed predominantly in normal hearts whereas clone pMHCα 252 is expressed in thyrotoxic hearts exclusively. We conclude that clones pMHCα 252 and pMHCβ 174 represent α- and β-form myosin HC sequences, respectively.

MATERIALS AND METHODS

Experimental Animals. Ten- to twelve-week-old male New Zealand White rabbits (1.3–2.4 kg) were used. Thyrotoxic cardiac hypertrophy was induced by daily administration of L-thyroxine (200 μg/kg, intramuscularly) for 21 days. The hypothyroid condition was produced by feeding the animals a diet containing 0.15% PrSur (Teklad Test Diet, Madison, WI) for 10 wk.

Preparation of Cardiac RNA. Total cellular RNA from the ventricles of rabbit hearts was prepared following the method of Towle *et al.* (25). The poly(A)$^+$RNA was isolated from total RNA by oligo(dT)-cellulose chromatography (26) and purified on 15–30% linear sucrose gradients as described (27).

Construction and Screening of cDNA Clones. Single- and double-stranded cDNA was synthesized by using standard procedures (28, 29). The duplex cDNA was tailed (30) with dCMP and annealed with *Pst* I-linearized deoxyguanosine-tailed plasmid pBR322 (27). The hybrid plasmid was used to transform *Escherichia coli* χ1776 (31). Clones containing inserts complementary to myosin HC mRNA were selected as described (27).

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Abbreviations: Myosin HC, myosin heavy chain; PrSur, propylthiouracil.

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All experiments with recombinant plasmids were conducted in accordance with the National Institutes of Health guidelines for recombinant DNA research.

Preparation of Plasmid DNA. Plasmid DNA was isolated from chloramphenicol-amplified cultures by Triton X-100 lysis (32) and purified on CsCl/ethidium bromide density gradients.

Restriction Endonuclease Analysis and DNA Sequence Determination. Restriction maps of the clones were constructed as described (27). DNA sequence analysis was by the method of Maxam and Gilbert (33).

Hybridization of Plasmid DNA with RNA Bound to Nitrocellulose. Cardiac poly(A)$^+$RNA was fractionated on 1.5% agarose/urea gels under denaturing conditions (34), transferred to nitrocellulose paper (35), and hybridized to nick-translated (36) ^{32}P-labeled plasmid DNA ($\approx 10^7$ cpm/μg) by the method of Wahl *et al.* (37).

Preparation of Probes for Nuclease S1 Mapping. The 350-base-pair *Bam*HI/*Bgl* I fragments (see Fig. 2) of clones pMHCβ 174 and pMHCα 252 were isolated and the strands were separated as described by Maxam and Gilbert (33). The single-stranded DNA complementary to myosin HC mRNA was 5'-end labeled with [γ-^{32}P]ATP (New England Nuclear: 3,000 Ci/mmol; 1 Ci = 3.7 $\times$ 10^{10} becquerels) and T4 polynucleotide kinase (P-L Biochemicals) (33). The specific activity of the probe was 1–5 $\times$ 10^6 cpm/μg. Hybridization and nuclease S1 digestion were carried out by the method of Orkin and Goff (38), except that the probe (0.5–1 $\times$ 10^5 cpm, 10–50 ng) was hybridized in a sealed capillary tube with either 5 μg of cardiac poly(A)$^+$RNA or 30–50 μg of cardiac total RNA. The digested products were analyzed on 5% sequencing gels (33, 38). The autoradiograms were scanned by using a soft laser scanning densitometer (model SL-504, Biomed Instruments, Chicago, IL).

RESULTS

Cloning of Cardiac Myosin HC mRNA Sequences. To clone α- and β-form myosin HC cDNA sequences, we partially purified myosin HC mRNAs from hearts of thyrotoxic and normal adult rabbits by oligo(dT)-cellulose chromatography and sucrose gradient centrifugation. These preparations were used for synthesis of single- and double-stranded cDNAs. The higher molecular weight fractions of double-stranded cDNA (>1,500 bp) (27) were inserted into the *Pst* I site of pBR322 and used for transformation of *E. coli* χ1776. Recombinant clones derived from normal heart were screened by *in situ* colony hybridization (39) with [^{32}P]cDNA transcribed from highly enriched myosin HC mRNA from embryonic chicken leg muscle (8). One clone, designated pMHCβ 174 and having a 2.1-kilobase insert, was shown to contain myosin HC mRNA sequences by blot hybridization to a 31S RNA that corresponds to the mobility of myosin HC mRNA (Fig. 1). Recombinants derived from thyrotoxic hearts were screened with ^{32}P-labeled nick-translated *Pst* I fragments from pMHCβ 174. One of these clones, designated pMHCα 252 and having a 1.4-kilobase insert, also hybridized to 31S RNA and was selected for further studies.

Restriction Endonuclease Mapping. Restriction enzyme maps of the cDNA inserts of clones pMHCβ 174 and pMHCα 252 are shown in Fig. 2. Comparison of the restriction sites of the two cDNA inserts reveals considerable homology in the regions of overlap; however, 3 out of 12 sites differ. These results indicate the presence of distinct but closely related mRNA sequences in the two clones.

DNA Sequence Analysis. To establish unequivocally that the two clones contained myosin HC mRNA sequences, the sequences of selected regions of pMHCβ 174 and pMHCα 252 were analyzed. Comparison of the derived amino acid sequence

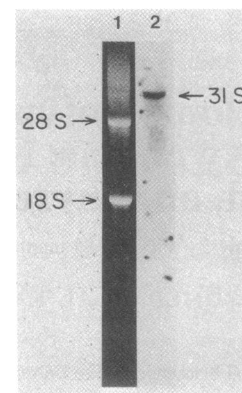

FIG. 1. Hybridization of clone pMHCβ 174 DNA to size-fractionated cardiac RNA. Two micrograms of poly(A)$^+$RNA isolated from normal rabbit ventricles was electrophoresed on 1.5% agarose/urea gels, transferred to nitrocellulose paper, and hybridized to ^{32}P-labeled nick-translated pMHCβ 174 DNA. Lanes: 1, ethidium bromide staining pattern of the RNA; 2, autoradiograph of the nitrocellulose paper after hybridization. Similar results were obtained with clone pMHCα 252.

of a portion of the *Bam*HI/*Bgl* I fragment of pMHCβ 174 with those available by direct amino acid sequence analysis of rabbit fast skeletal muscle myosin HC (M. Elzinga, personal communication) reveals 79% homology (Fig. 3*A*). The corresponding restriction fragment from pMHCα 252 shows 83% homology with fast skeletal myosin HC. The clones correspond to myosin HC sequences that include the entire length of subfragment 2 and the COOH-terminal portions of subfragment 1. The 5' ends of pMHCβ 174 and pMHCα 252 terminate 1.2 and 2.3 kilobases, respectively, from the NH_2-terminus of the myosin HC. Cloning of sequences distant from the 3' end of the mRNA may be due to either incomplete second-strand synthesis or priming by oligo(dT) at internal oligo(A) sequences during the RNA-dependent DNA nucleotidyltransferase (reverse transcriptase) reaction.

The DNA sequences of the 350-base pair *Bam*HI/*Bgl* I fragments from clones pMHCβ 174 and pMHCα 252 and the derived amino acid sequences are shown in Fig. 3*B*. When compared, pMHCβ 174 and pMHCα 252 exhibited 90% homology with respect to both the nucleotide and the derived amino acid sequences. The 10% divergence between the nucleotide sequences appeared as three tetra-, one tri-, one di-, and several single-nucleotide mismatches.

Nuclease S1 Mapping Analysis. We sought to characterize the two cardiac myosin HC clones by correlating transcription of their sequences with the form of myosin HC that is known to be present in thyrotoxic, hypothyroid, and normal adult hearts. To examine transcription, we used the nuclease S1 mapping technique of Berk and Sharp (40) as modified by Weaver and Weissmann (41) and Orkin and Goff (38). We took advantage of one of the tetranucleotide mismatches in the *Bam*HI/*Bgl* I segments (Fig. 3*B*) of the two clones. This sequence divergence is located 127 nucleotides from the *Bgl* I cleavage sites. There are no other nucleotide mismatches between the *Bgl* I site and this tetranucleotide divergence. The 350-nucleotide single-stranded probes complementary to the myosin mRNA were isolated from the *Bam*HI/*Bgl* I fragments of the two clones and 5'-end labeled with ^{32}P at the *Bgl* I site. The probes were then hybridized to poly(A)$^+$RNA or total RNA from hearts of thyrotoxic, PrSur-treated, and normal adult rabbits and digested with nuclease S1. The sequence data (Fig. 3) suggest that hybridization with homologous RNA will result in protection of the full-size probe whereas hybridization with heterologous RNA will result in a fragment of $\approx$127 nucleotides,

pMHCβ 174

pMHCα 252

0 0.5 1.0 1.5 2.0
kbp

FIG. 2. Restriction endonuclease maps of clones pMHCβ 174 and pMHCα 252. cDNA inserts are represented by solid bars. Arrows indicate the direction of transcription of the ampicillin-resistant genes of pBR322. The bracket delineates the location of sites that are not mapped exactly. ●, *Pst* I; ○, *Pvu* II; ■, *Sst* I; ▲, *Bam*HI; ▽, *Bgl* I. There are no *Eco*RI, *Hin*dIII, *Sal* I, *Xba* I, or *Xho* I sites in the inserts.

since the first sequence divergence is present at this distance from the site of 5' labeling.

The results of the nuclease S1 protection experiments with the pMHCβ 174 probe are shown in Fig. 4*A*. After hybridization with the RNA isolated from PrSur-treated ventricles, which are known to contain exclusively the β form of myosin HC, the full-size 350-nucleotide probe is protected throughout a broad range of nuclease S1 concentrations (lanes 1–4). The protected band becomes less prominent at higher concentrations of enzyme (lane 4), presumably due to trimming of the probe at its labeled end (41). With RNA from normal young rabbit ventricles, which contain myosin β and α HCs in a ratio of ≈4:1, both a full-size probe and less intense smaller fragments at ≈127 nucleotides (Fig. 4*A*, lane 6) are observed. As the enzyme concentration increases, the intensity of the bands decreases, but a consistent upper band/lower band ratio of ≈4:1 is observed (lanes 7 and 8). When RNA from thyrotoxic ventricles (α-type myosin HC) is used, the 350-nucleotide probe is trimmed by

A

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                                                            *                                                                    *   *
RABBIT FAST SKELETAL-NH2...Gly Ser Leu Glu Gln Glu Lys Lys Ile Arg Met Asp Leu Glu Arg Ala Lys Arg Lys Leu Glu Gly Asp Leu Lys Ile Leu
          pMHCβ 174-NH2...Gly Ser Leu Glu Gln Glu Lys Lys Val Arg Met Asp Leu Glu Arg Ala Lys Arg Lys Leu Glu Gly Asp Leu Lys Leu Thr
                    5'...GGA TCC CTG GAG CAG GAG AAG AAG GTG CGC ATG GAC CTG GAG CGA GCC AAG CGG AAG CTG GAG GGC GAC CTG AAG CTG ACC
                         Bam HI      10          20          30           40          50          60           70          80

         *   *           *                                       *                       *           *   *   *       *   *   *
Gln Glu Ser Thr Met Asp Ile Glu Asn Asp Lys Gln Gln Leu Asp Glu Lys Leu Lys Lys Lys Glu Phe --- Met Thr Asn Leu Gln Ser Lys Ile Glu Asp
Gln Glu Asn Ile Met Asp Leu Glu Asn Asp Lys Gln Gln Leu Asp Glu Arg Leu Lys Lys Lys Asp Phe Glu Leu Asn Ala Leu Asn Ala Arg Ile Glu Asp
CAG GAG AGC ATC ATG GAC CTG GAG AAC GAC AAG CAG CAG CTG GAT GAG CGG CTG AAA AAG AAA GAC TTT GAA CTG AAT GCC CTC AAT GCA AGG ATT GAG GAT
       90          100       110         120         130         140         150         160         170         180

                 *                                                                                   *
Glu Gln Ala Leu Met --- --- --- --- --- --- --- --- --- --- --- --- --- Ile Glu Glu Leu Glu Glu Glu Ile Glu Ala Glu Arg ... COOH
Glu Gln Ala Leu Gly Ser Gln Glu Gln Lys Lys Leu Lys Glu Leu Gln Ala Arg Ile Glu Glu Leu Glu Glu Glu Leu Glu Ala Glu Arg ... COOH
GAG CAA GCC CTG GGA AGC CAG CTG CAG AAG AAG CTC AAA GAG CTT CAG GCC CGC ATC GAG GAG CTG GAG GAG GAG CTG GAG GCC GAG CGC ... 3'
     190         200         210         220         230         240         250         260         270
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B

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                Gly Ser Leu Glu Gln Glu Lys Lys Val Arg Met Asp Leu Glu Arg Ala Lys Arg Lys Leu Glu Gly Asp Leu Lys Leu Thr Gln Glu Asn
pMHCβ 174    5'-GGA TCC CTG GAG CAG GAG AAG AAG GTG CGC ATG GAC CTG GAG CGA GCC AAG CGG AAG CTG GAG GGC GAC CTG AAG CTG ACC CAG GAG AGC
                Bam HI      10          20          30           40          50          60          70          80          90
pMHCα 252    5' GGA TCC CTG GAG CAG GAG AAG AAG GTG CGC ATG GAC CTG GAG CGA GCC AAG CGG AAG CTG GAG GGT GAC CTG AAG CTG ACC CAG GAG AGC
                Gly Ser Leu Glu Gln Glu Lys Lys Val Arg Met Asp Leu Glu Arg Ala Lys Arg Lys Leu Leu Gly Asp Leu Lys Leu Thr Gln Glu Asn

                                 *           *                       *       *   *   *   *           *   *
Ile Met Asp Leu Glu Asn Asp Lys Gln Gln Leu Asp Glu Arg Leu Lys Lys Lys Asp Phe Glu Leu Asn Ala Leu Asn Ala Arg Ile Glu Asp Glu Gln Ala
ATC ATG GAC CTG GAG AAC GAC AAG CAG CAG CTG GAT GAG CGG CTG AAA AAG AAA GAC TTT GAA CTG AAT GCC CTC AAT GCA AGG ATT GAG GAT GAG CAA GCC
            100         110         120         130         140         150         160         170         180         190
ATC ATG GAC CTG GAG AAT GAC AAG CTG CAG CTG GAG GAG AGG CTC AAG AAG AAG GAG TTT GAC ATC AGT CAG CTG AAC AGC AAG ATC GAG GAT GAG CAG GCA
Ile Met Asp Leu Glu Asn Asp Lys Leu Gln Leu Glu Glu Arg Leu Lys Lys Lys Glu Phe Asp Ile Ser Gln Glu Asn Ser Lys Ile Glu Asp Glu Gln Ala

     *   *                                   *
Leu Gly Ser Gln Leu Gln Lys Lys Leu Lys Glu Leu Gln Ala Arg Ile Glu Glu Leu Glu Glu Glu Leu Glu Ala Glu Arg Thr Ala Arg Ala Lys Val Glu
CTG GGA AGC CAG CTG CAG AAG AAG CTC AAA GAG CTT CAG GCC CGC ATC GAG GAG CTG GAG GAG GAG CTG GAG GCC GAG CGC ACT GCC AGG GCC AAG GTG GAG
        200         210         220         230         240         250         260         270         280         290
CTG GTC CTC CAG CTG CAG AAG AAG CTG AAG GAA AAC CAG GCC CGC ATC GAG GAG CTG GAG GAG GAG CTG GAG GCC GAG CGC ACT GCC AGG GCC AAG GTG GAG
Leu Val Leu Gln Leu Gln Lys Lys Leu Lys Glu Asn Gln Ala Arg Ile Glu Glu Leu Glu Glu Glu Leu Glu Ala Glu Arg Thr Ala Arg Ala Lys Val Glu

Lys Leu Arg Ser Asp Leu Ser Arg Glu Leu Glu Glu Ile Ser Glu Arg Leu Glu Glu Ala Gly Gly Ala
AAG CTG CGC TCC GAC CTG TCC CGG GAG CTG GAG GAG ATC AGC GAG CGG CTG GAG GAG GCC GGC GGG GCC-3'
    300         310         320         330         340         350 Bgl I  360
AAG CTG CGC TCC GAC CTG TCC CGG GAG CTG GAG GAG ATC AGC GAG CGG CTG GAG GAG GCC GGC GGG GCC-3'
Lys Leu Arg Ser Asp Leu Ser Arg Glu Leu Glu Glu Ile Ser Glu Arg Leu Glu Glu Ala Gly Gly Ala
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FIG. 3. (*A*) Comparison of the amino acid sequence derived from DNA sequence analysis of a portion of the *Bam*HI/*Bgl* I segment of clone pMHCβ 174 with that obtained by direct analysis of rabbit fast skeletal myosin HC protein. Coding strand sequences are shown. ★, Site of amino acid divergence; ---, sequence not determined. (*B*) Comparison of nucleotide and derived amino acid sequences obtained from clones pMHCβ 174 and pMHCα 252. Nucleotide sequences of the coding strands of the *Bam*HI/*Bgl* I restriction fragments of each clone are shown. Both strands were analyzed. Nucleotide mismatches are enclosed in boxes; ★, amino acid divergence.

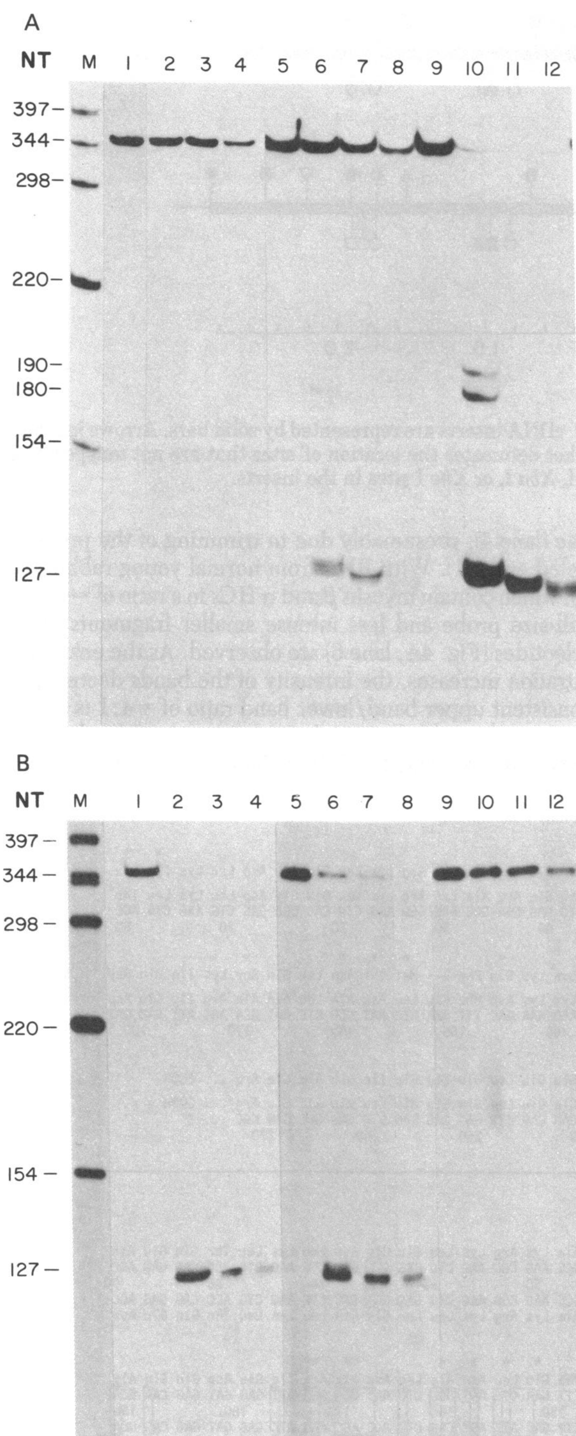

FIG. 4. Nuclease S1 mapping of cardiac RNA with cloned sequences from pMHCβ 174 (*A*) and pMHCα 252 (*B*). The 350-nucleotide single-stranded probes derived from the clones were hybridized with either 30 μg of total RNA from PrSur-treated rabbit ventricles (lanes 1–4) or 5 μg of poly(A)$^+$RNA from normal (lanes 5–8) or thyrotoxic (lanes 9–12) rabbit ventricles. The hybrids were digested with various amounts of nuclease S1 and subjected to 5% urea/acrylamide gel electrophoresis and autoradiography. Lanes: 1, 5, and 9, undigested probe; 2, 6, and 10, 1,000 units of nuclease S1; 3, 7, and 11, 2,500 units of nuclease S1; 4, 8, and 12, 5,000 units of nuclease S1; M, size markers (plasmid pBR322 digested with *Hin*fI and ^{32}P end labeled). Results shown in lanes 1–4 and 5–12 were obtained from two separate experiments. NT, nucleotides.

nuclease S1 to a fragment of ≈127 nucleotides with virtually no protection of the full-length probe (lanes 9–12). Several discrete bands are observed within the smaller protected bands, particularly after short autoradiographic exposures, presumably due to different sites of cleavage in the tetranucleotide divergent segment. Two other protected fragments of ≈180 and ≈190 nucleotides are observed at the lowest nuclease S1 concentration (lanes 6 and 10) but disappear at higher enzyme levels, probably indicating incomplete digestion of the DNA·RNA hybrid. Another protected band of low intensity is visible at ≈200 nucleotides (lanes 7 and 8), and it persists even at higher nuclease concentrations. This observation suggests the possible existence of an additional low-abundance form of myosin HC mRNA sequences in normal young rabbit ventricles.

We conclude from these results that pMHCβ 174 specifies the β form of myosin HC because transcripts corresponding to this clone correlate well with the presence of the myosin β HC isoform in cardiac tissue. This conclusion was substantiated by nuclease S1 mapping using the pMHCα 252 probe derived from thyrotoxic hearts (Fig. 4*B*). In this case, the full-size probe is protected with RNA from thyrotoxic heart, no smaller fragments being observed (lanes 9–12). On the other hand, hybridization with RNA from hearts of PrSur-treated animals results exclusively in the 127-nucleotide fragment (lanes 1–4). With RNA from normal heart, both the 350-nucleotide and the 127-nucleotide fragments are observed but, in this case, the smaller fragment predominates in a ratio of about 6:1 (lanes 5–8). Therefore, clone pMHCα 252 corresponds to gene sequences coding for the α form of myosin HC sequences.

DISCUSSION

Analysis of the expression of two clones derived from myosin HC mRNA isolated from thyrotoxic or normal adult heart indicates that the clones contain gene sequences coding for the α and β forms of myosin HC, respectively. Nuclease S1 mapping using probes derived from the two clones demonstrates an excellent correlation between the type of myosin HC transcript and the myosin isoform detected by native gel electrophoresis or immunological analysis. Thus, thyrotoxic ventricles, in which only α-form myosin HC is present, show protection of the full-sized pMHCα 252 probe but no protection of the pMHCβ 174 probe. On the other hand, RNA from ventricles of PrSur-treated animals, which contain only β-form myosin HC, detects pMHCβ 174 transcripts exclusively. The ratio of fully protected to partially digested probe in normal adult hearts also correlates well with the observed proportion of α-form to β-form myosin HC peptides. The data strongly suggest that the α and β isoforms of myosin HC are products of two different genes, the expression of which is markedly affected by the thyroid hormonal status of the animal. The possibility of differential splicing of a transcript from a single gene seems unlikely since sequence divergence and restriction map differences are distributed throughout the clones.

The nuclease S1 mapping technique is very effective in differentiating the transcription of different members of a myosin HC gene family. By using this procedure, analysis of transcription suggests that there are two major types of myosin HC transcripts. We observed protection of the full-sized probe or the appearance of a lower molecular weight band resulting from cleavage at the site of sequence divergence, or both. However, the detection of a faint intermediate-sized band that persists at high nuclease S1 concentrations suggests the presence of another low-abundance transcript. Furthermore, the possibility of additional myosin HC transcripts that differ in sequence but are sufficiently homologous to one of the probes that they are not completely cleaved by nuclease S1 cannot be eliminated. Further analysis with probes derived from other regions of myosin HC mRNAs, particularly including 3′-untranslated re-

gions, would be useful in determining whether other transcripts might be present.

The myosin HC gene family appears to be very large. In addition to the two cardiac myosin HC isoforms studied here, fast and slow skeletal muscle also express several different molecular forms of myosin HC. Thus, embryonic (2, 5, 15–18), neonatal (16, 17), and probably two adult (6, 18, 42, 43) fast myosin HCs are expressed in skeletal muscle, and there are at least two additional forms in slow skeletal muscle (5, 7, 8, 44). We have obtained evidence for a minimum of six fast myosin HC transcripts in skeletal muscle at different stages of development by using nuclease S1 techniques and probes derived from two skeletal myosin HC cDNA clones (unpublished data). Screening of gene libraries has detected clones that represent at least seven different genes (45). This number is probably an underestimate of the total myosin HC genes present since there appear to be at least 11 myosin HC peptides present in various tissues.

Thyroid hormone has a marked effect on expression of the α- and β-forms of myosin HC (10–13). It is, however, unclear whether this effect is a primary one altering the transcription of the gene directly or secondary to the multiple metabolic changes produced by the hormone. The distribution of the α and β forms of myosin HC is affected not only by thyroid hormone but also by other stimuli. Pressure-induced hypertrophy, for example, decreases α-form myosin HC expression (20, 21). Although there is a good correlation between serum thyroid hormone level and expression of α- and β-form myosin HCs during late gestational and early neonatal life (46, 47), this correlation fails during later adult life, when thyroid hormone levels remain constant despite large changes in the isoform distribution (47).

The clones that we have isolated are currently being used to screen and isolate genomic sequences specifying cardiac myosin HC. They will also be extremely useful in evaluating whether translational as well as transcriptional control contributes to alterations in expression of cardiac myosin HC genes.

We thank Dr. M. Elzinga for providing us with his unpublished amino acid sequence data for rabbit fast skeletal muscle myosin HC, Drs. R. Chizzonite and R. Zak for providing us with the PrSur-treated rabbit hearts and for their analysis of the isomyosin composition of these hearts, and Ms. J. Nigro for her expert technical assistance. This work was supported by Grants HL09172 and HL20592 from the National Institutes of Health and by a grant from the Louis Block Foundation, University of Chicago. P.K.U. is a Postdoctoral Fellow of the Chicago Heart Association. C.J.K. is a Postdoctoral Fellow on National Institutes of Health Training Grant HL07381.

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