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Human embryonic myosin heavy chain cDNA

Interspecies sequence conservation of the myosin rod, chromosomal locus and isoform specific transcription of the gene

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A 3.6 kilobase cDNA clone coding for the human embryonic myosin heavy chain has been isolated and characterized from an expression library prepared from human fetal skeletal muscle. The derived amino acid sequence for the entire rod part of myosin shows 97% sequence homology between human and rat and a striking interspecies sequence conservation among the charged amino acid residues. The single copy gene is localized to human chromosome 17 and its expression in fetal skeletal muscle is developmentally regulated. The sequence information permits the design of isoform-specific probes for studies on the structure of the gene and its role in normal and defective human myogenesis.

Myosin heavy chain cDNA; Nucleotide sequence; Amino acid sequence; Myosin rod; Chromosomal mapping; Gene transcription; (Human embryo)

1. INTRODUCTION

Isoforms of vertebrate sarcomeric myosin heavy chain (MHC) are coded by members of a multigene family, which are differentially expressed under developmental, hormonal, and neural control [1]. Comparative analyses of sarcomeric MHC genes isolated from the chicken, mouse, and rat and their expression have revealed the diversity of mRNA transcripts in developing and mature skeletal and cardiac muscle, the close physical linkage of the genes corresponding to two cardiac MHC polypeptides and the identification of *cis*-acting regulatory domains in 5′-flanking DNA sequences [2–8].

The human MHC isoforms are also encoded by a multigene family [9–12]. However, very little information is currently available about the primary structure of the different human skeletal polypeptides, and the organization and expression of their genes. Of particular interest is the human embryonic MHC (HEMHC) gene, since its expression appears to be specifically altered in Duchenne muscular dystrophy [13]. In our attempt to understand the regulation of human myogenesis in normal and pathological conditions, we have undertaken a study of characterizing the cDNAs and genes for different human myofibrillar proteins. Here we report the characterization of a cDNA clone for HEMHC isolated from a λgt11 cDNA expression library prepared from human fetal skeletal muscle. The derived primary structure of the entire rod part of HEMHC shows strik-

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ing interspecies sequence conservation. We also document that the gene is mapped to human chromosome 17 and its isoform-specific transcription in skeletal muscle is developmentally regulated.

2. MATERIALS AND METHODS

Oligo(dT)-primed cDNA libraries were constructed in λgt11 vector utilizing poly(A)$^+$ RNA from human fetal muscle as previously described [14]. The libraries were screened with the monoclonal antibody 2B6, which is specific for mammalian embryonic MHC [15]. The cDNA inserts recovered from purified recombinant phages were subcloned into the β-lactamase-encoding plasmid Bluescript (Stratagene) to facilitate restriction mapping, sequence analysis, and polynucleotide probe synthesis [16].

Northern and slot blots of RNA samples were prepared and hybridized as previously described [14,17]. The 25-mer oligonucleotide probe, CGGTCGGGAATACCTCGTCCTG-TCT was synthesized (Cyclone DNA Synthesizer, Biosearch) and was sequentially 5′-end labeled with [γ-^{32}P]ATP [18] and tailed with [α-^{32}P]dCTP [19] to achieve high specific activity. The three gel purified HEMHC cDNA restriction fragments, B, C and D (fig.1) were labeled by the 'random primer' technique with hexadeoxynucleotide primers and the Klenow fragment of DNA polymerase [20]. Southern blot analysis of genomic DNA digested with different restriction enzymes was carried out as previously described [14,21,22]. Details are given in the figure legends.

The subfragments of the cDNA (5′, 3′ and the middle region), generated by appropriate restriction enzyme digestion, were subcloned into the vector Bluescript and bidirectionally sequenced from M13 universal and reverse priming sites using the dideoxychain termination method [23]. The enzyme Sequenase (US Biochemicals) and the manufacturer's protocol were used. Synthetic primers were also used to obtain overlapping sequences of all regions of the cDNA insert at least twice in both directions.

3. RESULTS AND DISCUSSION

The monoclonal antibody 2B6 [15], which binds specifically mammalian embryonic MHC, was used to screen the λgt11 human fetal muscle cDNA library. Following immunodetection and plaque purification of recombinant phages expressing the MHC cDNA fragments, *Eco*RI restriction of DNA minipreparations identified the largest cloned fragment (designated as HEMHC-1) of about 3.6 kb size (fig.1). The size and orientation of several subfragments of HEMHC-1, generated by restriction enzyme digestion and used as hybridization probes are also shown in fig.1. D is a 5′-fragment of about 3 kb in size, whereas B and C are 3′-fragments of about 1.5 and 0.5 kb, respectively.

The nucleotide sequence of the HEMHC-1 insert (fig.2) contains the 3′-untranslated region (UT) of 119 nucleotides, the TGA stop codon at nucleotides 3502, an open reading frame that encodes 1167 amino acids corresponding to the rod sequence of HEMHC. Comparison of the nucleotide sequence in the coding region containing 3501 base pairs with the corresponding published rat genomic sequences [5] shows 366 single base substitutions indicating 89.5% homology. The derived amino acid sequences of the rod portions of human and rat embryonic MHC show 35 substitutions among a total of 1167 amino acid residues indicating a 97% homology. However, among the charged amino acid residues which constitute about 42% of the total amino acids, the interspecies sequence conservation in the rod is even more striking and amounts to 99.8%. In contrast to the coding region, the 3′-UT segments show considerable sequence diversity in rat and human. Interestingly, several homologous sequences which are either identical in position or differ only in position were observed at the 3′-UT (fig.2).

Northern blot analyses of RNA preparations from various human muscle tissues using different probes (fig.1) show interesting features. When radiolabeled 5′-subfragment D that corresponds to about 1000 amino acids of the rod portion of HEMHC (figs 1 and 2) was used as the probe, mRNA species of approx. 6 kb were detected in autoradiograms of RNA samples from human fetal skeletal, adult skeletal, ventricular and atrial muscle (fig.3B), the signal intensity being very pronounced in skeletal muscle RNA samples. To test further the specificity of the HEMHC gene transcription, a 25 base oligomer complementary to the HEMHC-1 sense strand, in a region of maximal interisoform heterology (see also section 2), was designed as a possible discriminatory probe. Under identical conditions of hybridization and washing at 52°C in 0.9 M NaCl as was used for the larger probe D, only a single mRNA species of about 6 kb in size, unique to human fetal muscle, was detected (fig.3A). These contrasting hybridization patterns obtained with the two probes indicate that the HEMHC gene is expressed in a developmentally regulated manner only in fetal skeletal muscle. The larger probe is likely to span

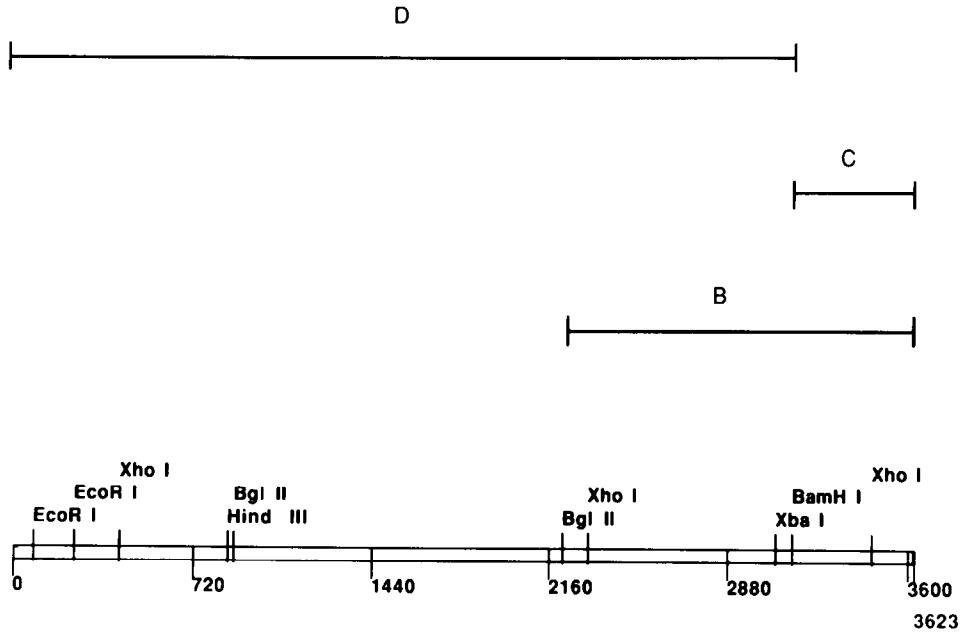
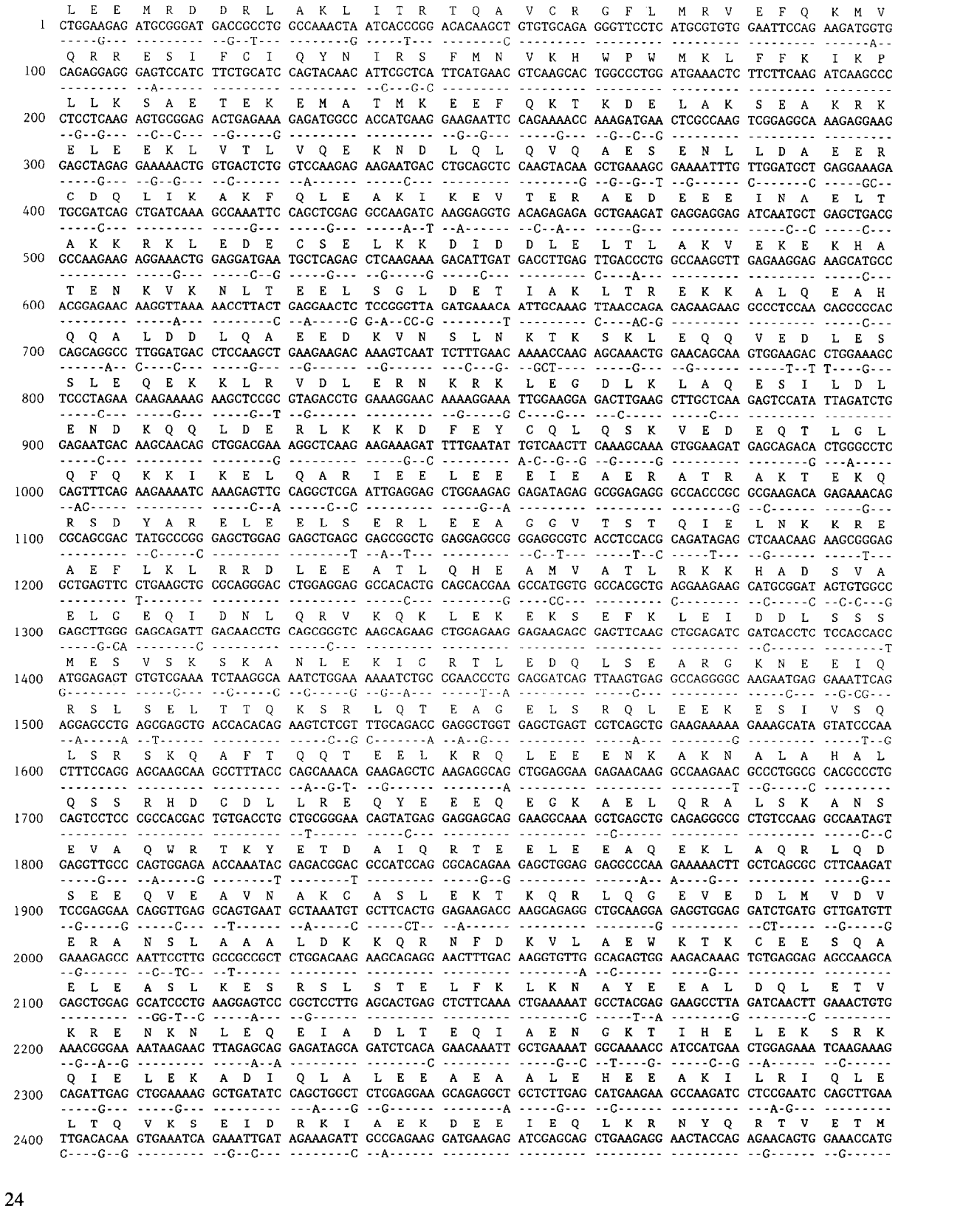


Fig.1. Restriction cleavage maps and different fragments of human embryonic MHC cDNA clone HEMHC-1. The diagram at the bottom is a restriction map of HEMHC-1 identified by immunodetection with the antibody 2B6. D is a 5′-terminal fragment, whereas B and C are 3′-terminal fragments whose size and orientation are shown. These fragments were used as hybridization probes.

regions of sufficient interisoform homology and therefore, detects other MHC mRNAs in preparations from human adult skeletal and cardiac muscle.

To identify the chromosome locus for the HEMHC gene, a Southern blot of DNA preparations from a panel of human-hamster hybrid fibroblast cell lines [21] was analyzed. The 25-mer discriminatory probe (section 2) used in mRNA hybridization assay (fig.3A) could not be used in genomic analysis, since due to its small size, we were unable to radiolabel it by the 'random priming technique' [20] to a specific activity sufficiently high enough for such an analysis. Two different 3′-subfragments of HEMHC cDNA, B and C (fig.1) were selected for gene analysis, since transcripts coding for muscle protein isoforms are known to have significant diversity at the 3′-UT segments [1,2]. The specificity of the two probes was first established by comparing their ability to hybridize with RNA from various human muscle and non-muscle tissues. Probe B which contains coding sequences corresponding to about 333 additional amino acids, gave strong hybridization signals in slot-blot analysis with RNA from human skeletal muscle, both fetal and adult (fig.4A, lanes 5 and 1), moderate signals with RNA from fetal heart (lane 4), weak signal with RNA from adult human heart (lane 2), and no detectable signal with RNA from fetal brain (lane 3) and *E. coli* (lane 6). In contrast, the 0.5 kb 3′-probe detected mRNA transcript only in fetal skeletal muscle (fig.4B, lane 5) indicating that it could serve as a discriminatory probe for the HEMHC gene. Southern blot analysis of restricted DNA preparations using probe B at moderate stringency, detected at least 6 *Bgl*II fragments, while probe C at high stringency, detected only one of these *Bgl*II fragments, about 5.5 kb (fig.5, lanes D′, D and K). The human-specific fragments detected by both probes segregate with 100% concordance only if they were assigned to chromosome 17. However, the hybrid panel illustrated does not allow discrimination between human chromosome 5 and chromosome 17 sequences. Therefore, a discriminatory panel containing restricted DNA samples from hybrids either with human chromosome 5 or 17, was hybridized with the HEMHC-specific probe C. The results using digestion with *Bam*HI (fig.6, left lanes A–E) or *Bgl*II (right lanes A–E) confirm the



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      Q  S  A  L  D  A  E  V  R  S  R  N  E  A  I  R  L  K  K  K  M  E  G  D  L  N  E  I  E  I  Q  L  S
2500  CAGAGCGCC CTGGACGCC GAGGTGCGG AGCAGGAAT GAAGCCATC CGGCTCAAG AAGAAGATG GAGGGGGAC CTGAATGAA ATCGAGATC CAGCTGAGC
      ---G----- -----T--T --------C --------- --G--G--- -----G--- --C------ -----C--- --C-----G --T------ ---------
      H  A  N  R  Q  A  A  E  T  L  K  H  L  R  S  V  Q  G  Q  L  K  D  T  Q  L  H  L  D  D  A  L  Q  T
2600  CACGCCAAC CGCCAGGCG GCGGAGACC CTCAAACAC CTCAGGAGT GTCCAGGGA CAGCTGAAG GATACGCAG CTCCACCTG GATGATGCC CTCCAAACC
      --T------ --------C --A------ A----G--- ---C------ --------- --------- --C--C--- --G------ -----C--T ----G-GG-
      Q  E  D  L  K  E  Q  L  A  I  V  E  R  R  A  N  L  L  Q  A  E  V  E  E  L  R  A  T  L  E  Q  T  E
2700  CAGGAGGAC CTGAAGGAG CAGCTGGCG ATTGTGGAG CGCAGAGCC AACCTGCTG CAGGCCGAG GTGGAGGAG CTGCGGGCT ACTCTGGAG CAGACGGAG
      --------- --------- --------- --C------ --------- --------- -----T--- --------- --------C --------- ---------
      R  A  R  K  L  A  E  Q  E  L  L  D  S  N  E  R  V  Q  L  L  H  T  Q  N  T  S  L  I  H  T  K  K  K
2800  AGGGCCCGG AAACTGGCG GAACAGGAG CTCCTGGAC TCCAACGAG AGGGTGCAG CTGCTGCAT ACCCAGAAC ACCAGCCTC ATCCACACC AAGAAGAAG
      --------- --G-----A --G------ --G------ --------- -----T--- --------C --------- --------- --------- ---------
      L  E  T  D  L  M  Q  L  Q  S  E  V  E  D  A  S  R  D  A  R  N  A  E  E  K  A  K  K  A  I  T  D  A
2900  CTGGAGACA GACCTCATG CAGCTCCAG AGTGAGGTA GAAGATGCC AGCAGGGAT GCAAGGAAC GCTGAGGAG AAGGCCAAG AAGGCCATC ACGGACGCT
      T-------T -------CA --------- --------G --G------ --------C --------- --C------ --------A --------- --T--T--C
      A  M  M  A  E  E  L  K  K  E  Q  D  T  S  A  H  L  E  R  M  K  K  N  L  E  Q  T  V  K  D  L  Q  H
3000  GCCATGATG GCGGAGGAG CTGAAGAAG GAGCAGGAC ACCAGCGCC CACCTTGAG CGGATGAAG AAGAACCTG GAACAGACG GTGAAGGAC CTGCAGCAT
      --------- --------- --A------ --------- --------- --T--G--- --------- --------- --G------ --------- --------C
      R  L  D  E  A  E  Q  L  A  L  K  G  G  K  K  Q  I  Q  K  L  E  T  R  I  R  E  L  E  F  E  L  E  G
3100  CGTCTAGAT GAGGCCGAG CAGCTGGCG CTGAAGGGC GGGAAGAAG CAGATCCAG AAACTGGAG ACCAGGATC CGAGAGCTG GAGTTTGAA CTTGAGGGA
      -----G--C -----T--- --------A --------- --C------ --------- --------- --G------ A-------A --------G --G--A--G
      E  Q  K  K  N  T  E  S  V  K  G  L  R  K  Y  E  R  R  V  K  E  L  T  Y  Q  S  E  E  D  R  K  N  V
3200  GAGCAGAAG AAGAACACA GAGTCTGTT AAGGGCCTG AGGAAGTAT GAGCGGAGG GTCAAGGAG CTGACGTAC CAGAGTGAA GAGGACAGG AAGAATGTG
      --A------ -G------- --------G --------- --------- ------C-T --------- --C--C--- --------G --------- ---------
      L  R  L  Q  D  L  V  D  K  L  Q  V  K  V  K  S  Y  K  R  Q  A  E  E  A  D  E  Q  A  N  A  H  L  T
3300  CTGAGATTG CAGGATCTG GTGGATAAA CTGCAAGTG AAAGTCAAG TCCTACAAG AGGCAGGCG GAGGAGGCT GATGAACAA GCCAATGCT CATCTCACC
      --------- --------- --------- --T------ --------- --------- --------T --------- --------- -------T- ---------
      K  F  R  K  A  Q  H  E  L  E  E  A  E  E  R  A  D  I  A  E  S  Q  V  N  K  L  R  A  K  T  R  D  F
3400  AAATTCCGG AAAGCTCAG CATGAGCTG GAGGAGGCC GAGGAACGT GCGGATATC GCAGAATCT CAAGTCAAC AAGCTCCGC GCTAAGACT CGAGACTTC
      --G------ -----C--- --------A --------- --------- --------T --------G --------T --A--G--G -----A--C --G------
      T  S  S  R  M  V  V  H  E  S  E  E
3500  ACCTCCAGC AGGATGGTG GTCCACGAG AGTGAAGAG TGAGCCAGCCCTTCTGGAGCAGGACAGAAGATATGCAAAATGTATATTTTCTTGATTCCTGACCA
      -----T--- C--------- -----T--A -----G--- ---GCATGTCCTCCTGGTGAGGGGCAGAAGATATGCAGAATGTATGTTTTCGTGGCTCCTGACCA
3602  TTGATACTTAATGTCCATGTGACTCTTTTTCACATGCAATAAACTTTGCTTTGTTTC(A)n
      TCCTGCTTAATTTCCACGTAACCCCTTTCCACATGCAATAAAATTTGCCTTGTTTCAAGT(A)n
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Fig.2. The nucleotide sequence of the cDNA clone HEMHC-1. For details see section 2. (Top lines) The nucleotide sequence and the derived amino acid sequence corresponding to the entire rod of myosin and the 3′-UT segment. (Bottom line) Corresponding published nucleotide sequence for rat embryonic MHC derived from genomic sequence [5]. Dash lines indicate identical nucleotides in the coding region. Homologous sequences at 3′-UT segment which are also identical in position are indicated by single underlines. Double underlines indicate identical sequences which differ only in position.

assignment of the HEMHC gene to chromosome 17 (compare lanes B and D for each set of DNA samples).

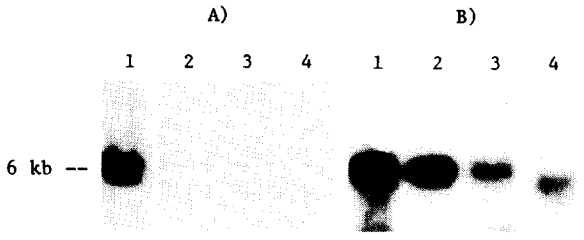


Fig.3. MHC gene transcription as revealed by Northern analysis. (A) Portion of autoradiogram of Northern blot hybridized with ^{32}P-end-labeled 25-mer discriminatory oligonucleotide probe (see section 2). RNA was isolated from: (1) 18-week human fetal skeletal muscle; (2) adult human skeletal muscle (vastus lateralis); (3) adult human left ventricle; (4) adult human left atrium. (B) Corresponding portion of autoradiogram of the same Northern blot hybridized with ^{32}P-labeled 5′-terminal 3.0 kb fragment D (fig.1).

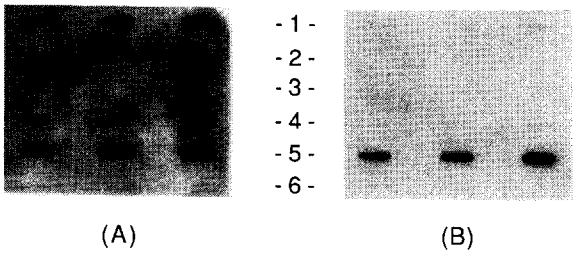
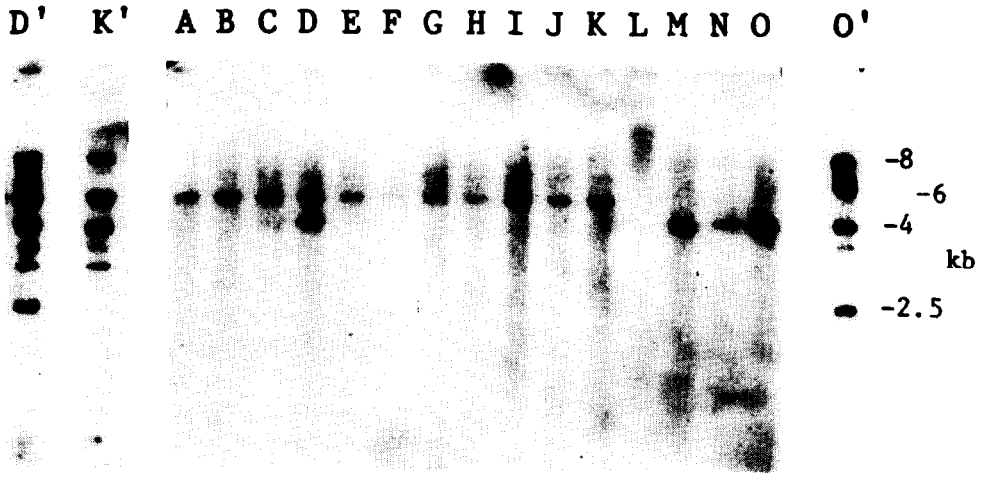


Fig.4. Specificity of 3′-terminal HEMHC cDNA fragments, B and C (fig.1) for hybridization with different human MHC mRNAs. (A) Slot-blot hybridization with ^{32}P-labeled 1.5 kb fragment B. (B) Corresponding portion of autoradiogram of the same slot-blot hybridized with ^{32}P-labeled 0.5 kb fragment C. RNA was isolated from: (1) adult human skeletal muscle (vastus lateralis); (2) adult human ventricle; (3) 17-week human fetal brain; (4) 17-week human fetal heart; (5) 17-week old human fetal skeletal muscle. Amount of RNA loaded in both panels, from left to right, are: 125, 250 and 500 ng, respectively. A single mRNA band of about 6 kb size was detected in Northern blot analysis of RNA from fetal skeletal muscle by both probes B and C (results not shown).



Hybrid		A	B	C	D	E	F	G	H	I	J	K	
Hybrids in which human specific bands appear					+								% discordance
Human chromosome content	1					+			+		+		40
	2		+			+	+						40
	3		+	+	+	+		+					40
	4		+		+								10
	5				+								0
	6			+	+			+		+			30
	7		+	+		+	+						50
	8		+							+			30
	9	+				+							30
	10		+	+		+							40
	11	+	+	+	+	+	+	+	+	+			80
	12		+		+								10
	13	+		+		+	+						50
	14		+		+		+		+				30
	15		+		+		+						20
	16	+			+	+		+					30
	17				+								0
	18				+	+			+	+			30
	19												10
	20	+	+	+									30
	21				+	+				+			20
	22	+		+	+	+							30
	X	+	+	+		+	+	+		+	+		90

Fig.5. MHC gene copy number and chromosomal localization as revealed by Southern analysis. Lanes correspond to *Bgl*II digested DNA from ten different human-hamster hybrid cell lines (A–J), one hamster (K), and three human cell lines (M–O). Lane L is a spacer gel. (A) Lanes D′, K′ and O′ are from an autoradiogram of a Southern blot hybridized with ^{32}P-labeled fragment B following a wash at 65°C in 15 mM NaCl. For details of the human chromosome content of each hybrid cell line, see [14] (nomenclature preserved) and [21]. The human specific *Bgl*II fragments detected by both B and C segregate with perfect concordance only with human chromosome 17.

Previous reports have indicated that human skeletal MHC cDNA detected sequences are localized to chromosome 17 [9,24], whereas two tightly linked cardiac MHC genes map to human chromosome 14 [11]. Our results establish that the HEMHC gene also maps to chromosome 17.

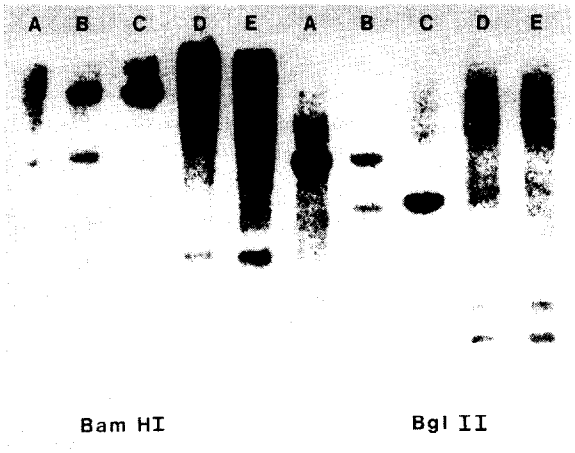


Fig.6. Southern blot analysis of discriminatory cell hybrids containing either human chromosome 5 or 17 with HEMHC-specific probe. Lanes correspond to DNA digested with *Bam*HI (A–E, left); and *Bgl*II (A–E, right). Conditions of hybridization with ^{32}P-labeled 3′-terminal 0.5 kb fragment C and wash were the same as described in legend to fig.5. Lanes represent restriction enzyme digested DNA from hamster (A); a human-hamster hybrid cell line carrying human chromosome 17 but not human chromosome 5 (B); human cell line (C); a human-murine hybrid cell line carrying human chromosome 5 but not human chromosome 17 (D); and murine fibroblasts (E). Note that lanes A–C (right; *Bgl*II-digested samples) reproduce the 4 and 5.5 kb HEMHC bands detected in fig.5 (lanes A–O).

However, the localization of all *Bgl*II fragments detected by the 1.5 kb probe B to the same chromosome, strongly suggests that other skeletal MHC genes are also detected by this probe. Since the additive molecular weight of the fragments detected with this probe is approx. 30 kb and published vertebrate sarcomeric myosin heavy chain gene sequences indicate an approximate 3:1 intron-to-exon size ratio [5], this result reflects coding sequence homology between several linked members of the human MHC gene subfamily. In contrast, the specific hybridization of the 0.5 kb 3′-end probe to a single *Bgl*II fragment in both human and hamster DNA (fig.5) indicates that this can function as an isoform-specific probe and recognizes orthologous gene sequences in other mammalian species.

In summary, a 3.6 kb HEMHC cDNA clone has been isolated and characterized from a λgt11 expression library prepared from human fetal skeletal muscle. The derived amino acid sequence of the entire rod part of HEMHC shows striking interspecies sequence conservation among the charged amino acid residues. HEMHC is coded by a single copy gene that is mapped to chromosome 17 and its expression is regulated in a developmentally controlled manner in fetal skeletal muscle. From the sequence information reported here, we also show the design of isoform specific probes which will be useful to study the structure of the gene and its expression during human myogenesis in normal and pathological conditions.

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