Proc. Natl. Acad. Sci. USA
Vol. 82, pp. 7183–7187, November 1985
Biochemistry

Genes for skeletal muscle myosin heavy chains are clustered and are not located on the same mouse chromosome as a cardiac myosin heavy chain gene

(gene localization/mouse interspecies cross)

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Communicated by François Jacob, June 26, 1985

ABSTRACT **Myosin heavy chain (MHC) genes are expressed as several distinct isoforms in a tissue- and stage-specific manner; three skeletal muscle MHC isoforms appear sequentially during development. We have isolated cDNA clones, identified by RNA blot hybridization and by nucleotide sequence determination as coding for portions of the embryonic (pMHC2.2), perinatal (pMHC16.2A), and α(V1) cardiac (pMHC141 and pMHC101) MHC isoforms. These four probes and the adult skeletal MHC probe (pMHC32) have been used on Southern blots of genomic DNA to detect restriction fragment length polymorphisms defining the alleles for these genes in mouse species *Mus musculus* and *Mus spretus*. In this way, we followed the segregation of skeletal and cardiac MHC genes in 42 offspring resulting from an interspecies backcross. We found that (*i*) the embryonic, perinatal, and adult skeletal MHC genes are clustered on chromosome 11 near the locus nude, (*ii*) the skeletal and cardiac MHC genes do not cosegregate, and (*iii*) the α(V1) cardiac MHC gene is located on chromosome 14 close to *Np-1*. This result is in contrast to that for other contractile protein genes such as the alkali myosin light chain and the actin multigene families, which are dispersed in the genome.**

The myosin heavy chain (MHC) family in mammals consists of at least 11 isoforms: 1 superfast skeletal, 2 adult fast skeletal, 1 perinatal skeletal, 1 embryonic/fetal skeletal, 2 cardiac [α(V1) and β(V3)], 1 adult slow skeletal, 1 smooth muscle, and 2 nonmuscle (1). Analysis of mRNA and genomic DNA sequences has provided evidence for the presence of different MHC genes (2, 3–7). During skeletal muscle development there is a sequential transition of different skeletal MHC isoforms from embryonic/fetal MHC to perinatal MHC and finally to adult MHC, as demonstrated in the rat (8). This phenomenon has also been shown at the mRNA level in the rat (9) and in the mouse, where a fetal MHC mRNA is replaced by an adult MHC mRNA at birth (2). A similar situation prevails for the two cardiac myosin isoforms, V1 (αα homodimer) and V3 (ββ homodimer): V3 is predominant over V1 in fetal heart ventricles and is replaced by V1 after birth in the mouse (10). In contrast to the sequential expression of MHC genes during striated muscle development, myosin alkali light chain (alkali MLC) genes and actin genes exhibit coexpression of the adult protein with the corresponding fetal isoform (refs. 11 and 12 and unpublished data). In these cases, the fetal isoform is also expressed in adult cardiac tissue. Based on a chromosomal analysis of hybrid cell lines, it has been reported that the genes coding for skeletal α-actin, cardiac α-actin, cytoplasmic β-actin, and the skeletal isoform of MLC2 are located on different mouse chromosomes (13, 14), whereas all the MHC genes are on chromosome 11 (13, 15). Using backcrosses of an interspecies *Mus* 1 (DBA/2) × *Mus* 3 (*M. spretus*) cross and following the Mendelian segregation of muscle-specific genes by restriction fragment length polymorphism (RFLP), Robert *et al.* (16) demonstrated that genes coding for the actin and myosin isoforms expressed in a given striated muscle phenotype are dispersed and that genes coding for different isoforms within the actin and alkali MLC multigene families are also not linked. The adult skeletal MHC gene was localized on chromosome 11 (16). To determine whether all the other MHC genes coding for different stage- and tissue-specific isoforms could be assigned to chromosome 11 and whether they are scattered all over chromosome 11 or clustered, we have used the same genetic approach. We report here the chromosomal localization of the skeletal and cardiac MHC genes for which specific probes have been isolated and characterized. We show that (*i*) the embryonic, perinatal, and adult skeletal MHC genes are linked and located on mouse chromosome 11 near the nude locus (*nu*), (*ii*) the skeletal and the cardiac MHC genes do not cosegregate, and (*iii*) the α(V1) cardiac MHC gene is located on chromosome 14 close to the nucleoside phosphorylase-1 locus (*Np-1*).

MATERIALS AND METHODS

Mice. DBA/2 female mice (*Mus musculus domesticus*, or *Mus* 1) were mated with SPE/Pas males (*Mus spretus*, or *Mus* 3) (17, 18). The F_1 female offspring then were backcrossed with DBA/2 males to produce a progeny of 42 individuals. These were subsequently analyzed for the segregation of 21 biochemical or coat-color markers already localized on the mouse genetic map (19) and for the segregation of MHC genes by RFLP analysis (16).

DNA and RNA Blot Hybridizations. High molecular weight DNA was extracted from spleen and lungs of DBA/2, *M. spretus*, and 42 offspring (nos. 1–20 were females, nos. 21–42 were males) as described (2). DNA (5 μg) from each mouse was digested with the appropriate restriction enzyme and analyzed by Southern blotting (20). RNA was extracted, electrophoresed, and transferred to filters as described (12, 21). After hybridization with ^{32}P-labeled probes, filters were

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Abbreviations: MHC, myosin heavy chain; MLC, myosin light chain; RFLP, restriction fragment length polymorphism; bp, base pair(s); kb, kilobase(s).

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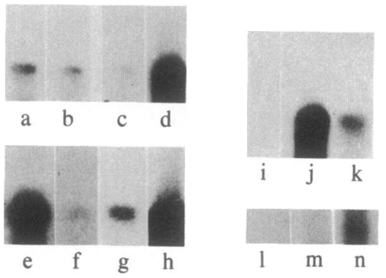


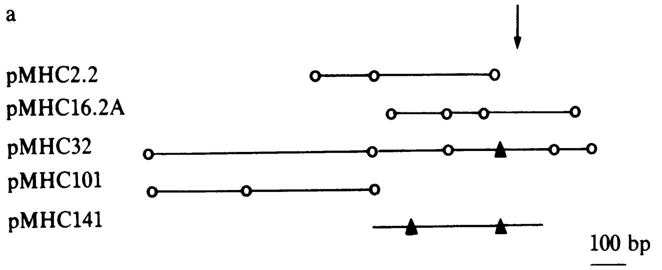
FIG. 1. Autoradiographs of RNA blots showing the tissue and stage specificity of different MHC probes. Poly(A)$^+$ RNA was isolated from T984 Cl10 myotubes [lanes a, c, i, and l (0.5 μg of RNA per lane)], from adult skeletal muscle [lanes b, d, j, and m (0.3 μg) and lanes f and h (1.5 μg)], from adult heart ventricle [lanes e and g (1.5 μg)], and from 18-day fetal skeletal muscle [lanes k and n (2 μg)], electrophoresed in agarose gels, and transferred to nitrocellulose as described (12, 21). The RNA blots were hybridized with the following nick-translated probes: the 320-bp *Pst* I–*Pst* I fragment of pMHC2.2 (embryonic skeletal MHC-specific; lanes a and b), the total insert of pMHC32 (adult skeletal; lanes c, d, g, h, i, j, and k), the 250- and 400-bp *Pst* I–*Pst* I fragments of pMHC101 [α(V1) cardiac; lanes e and f], and the 250-bp *Pst* I–*Pst* I fragment of pMHC16.2A (perinatal; lanes l, m, and n). The same lanes were successively hybridized (a,b = c,d; e,f = g,h; i,j,k = l,m,n). All the RNA blots were washed at 45°C and exposed overnight. Only the regions of the autoradiographs corresponding to the full-length mRNA (6900 nucleotides) are shown, except for lanes l–n which correspond to a cross-section through the RNA trail. Specific activity of the probes was 1–3 × 10^8 dpm/μg.

washed at different temperatures and autoradiographed as described (2).

Recombinant Plasmids. We used two different cDNA libraries. One was constructed with poly(A)$^+$ RNA extracted from whole hearts of 12-day-old mice and cloned in pBR322 (12); we constructed the second cDNA library using poly(A)$^+$ RNA isolated from myotubes of T984 Cl10 cells (22) and cloned it in pBR327. Both cDNA banks were screened with the recombinant plasmid pMHC32 (2), which encodes part of a fast adult skeletal MHC. DNA sequencing was carried out according to Maxam and Gilbert (23) and Biggin *et al.* (24). *Pst* I restriction sites were labeled as described (25). *Hin*fI restriction sites were labeled with [γ-^{32}P]ATP (5000 Ci/mmol, Amersham; 1 Ci = 37 GBq) and T4 polynucleotide kinase (Amersham).

RESULTS

Identification of the Recombinant Plasmids. From two cDNA libraries constructed with RNA from heart and from myotubes of a skeletal muscle cell line, four cDNA recombinant plasmids were obtained by cross-hybridization with the adult skeletal MHC sequence pMHC32 (2). Two recombinant plasmids have been isolated from the T984 Cl10 cDNA bank: pMHC2.2 and pMHC16.2A. The pMHC2.2 recombinant plasmid contains two *Pst* I–*Pst* I fragments of 150 base pairs (bp) and 320 bp. The 320-bp fragment was nick-translated and hybridized to poly(A)$^+$ RNA from myotubes



b

Clone pMHC2.2

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CAG GAC GCA AGG AAT GCA GAG GAG AAG GCC AAA AAG GCC ATC ACT GAC GCC GCC ATG ATG GCG GAG GAG CTG AAG AAG GAG CAG GAC ACC AGC GCC CAC CTG GAG AGG ATG
GLN ASP ALA ARG ASN ALA GLU GLU LYS ALA LYS LYS ALA ILE THR ASP ALA ALA MET MET ALA GLU GLU LEU LYS LYS GLU GLN ASP THR SER ALA HIS LEU GLU ARG MET

AAG AAG AAC CTG GAG CAG ACG GTG AAG GAC CTG CAG CAC CGT CTG GAT GAG GCT GAG CAG CTG GCG CTG AAG GGC GGC AAG AAG CAG ATT CAG AAA CTG GAG ACA CGG ATC
LYS LYS ASN LEU GLU GLN THR VAL LYS ASP LEU GLN HIS ARG LEU ASP GLU ALA GLU GLN LEU ALA LEU LYS GLY GLY LYS LYS GLN ILE GLN LYS LEU GLU THR ARG ILE

AGA GAG CTG GAG TTT GAG CTG GAA GGG GAG CAG AAG AGG AAC ACA GAG TCT GTG AAG GGC CTG AGG AAG TAT GAG CGG CGT GTT AAG GAG CTC ACA TAT CAG AGT GAG GAG
ARG GLU LEU GLU PHE GLU LEU GLU GLY GLU GLN LYS ARG ASN THR GLU SER VAL LYS GLY LEU ARG LYS TYR GLU ARG ARG VAL LYS GLU LEU THR TYR GLN SER GLU GLU

GCA --- AAG AAT GTG TTG AGA TTG CAG GAT CTG GTG GAT AAA CTC CAA GTG AAA GTC AAG TCC TAC AAG AGG CAG GCT GAG GAG GCT GAT GAA CAA GCC AAT GCT CAT CTC
ALA --- LYS ASN VAL LEU ARG LEU GLN ASP LEU VAL ASP LYS LEU GLN VAL LYS VAL LYS SER TYR LYS ARG GLN ALA GLU GLU ALA ASP GLU GLN ALA ASN ALA HIS LEU

ACC AAG TTC AGG AAA GCC CAG CAT GAG CTA GAG
THR LYS PHE ARG LYS ALA GLN HIS GLU LEU GLU
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Clone pMHC16.2A

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GAA CTT GAA GGA GAG GTC GAA AAT GAA CAG AAA CGC AAT GCT GAG GCT GTT AAA GGG TTA AGG AAG CAT GAA AGA AGA GTA AAG GAA CTT ACC TAC CAA ACT GAG GAA GAC
GLU LEU GLU GLY GLU VAL GLU ASN GLU GLN LYS ARG ASN ALA GLU ALA VAL LYS GLY LEU ARG LYS HIS GLU ARG ARG VAL LYS GLU LEU THR TYR GLN THR GLU GLU ASP

CGC AAG AAT GTG CTC CGG CTG CAG GAC CTG GTG GAC AAA TTA CAG GCG AAG GTG AAA TCC TAC AAG AGA CAG GCT GAG GAG GCT GAG GAA CAA TCC AAC GCC AAC CTG GCC
ARG LYS ASN VAL LEU ARG LEU GLN ASP LEU VAL ASP LYS LEU GLN ALA LYS VAL LYS SER TYR LYS ARG GLN ALA GLU GLU ALA GLU GLU GLN SER ASN ALA ASN LEU ALA

AAG TTC CGC AAG CTG CAG CAC GAG CTG GAG GAA GCC GAG GAG CGG GCT GAC ATC GCC GAG TCC CAG GTC AAC AAG CTG CGG GTG AAG AGC CGA GAG GTT CAC ACC AAA ATC
LYS PHE ARG LYS LEU GLN HIS GLU LEU GLU GLU ALA GLU GLU ARG ALA ASP ILE ALA GLU SER GLN VAL ASN LYS LEU ARG VAL LYS SER ARG GLU VAL HIS THR LYS ILE

AGC GCA GAG TAAACACATCTTGCAGAGGAAGGAGGCTGCCAAGGGGCTGAAGGAAAGCACAGGAGGGGCTGCTTTGGGTCGCTTGCTGGGTCACTTGCCTCTCTGGGTTTACTTTTCTCCTACTGCTGACTAAATAAAAACTAC
SER ALA GLU

AAGGCATTGGTAAATC
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Clone pMHC101

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CAG ACC TCC CTG GAT GCG GAG ACA CGC AGC CGC --- GAG GCC CTG CGG GTG AAG AAG AAG ATG GAG GGC GAC CTC AAC GAG ATG GAG ATC CAG CTC AGC CAG GCC AAT AGA
GLN THR SER LEU ASP ALA GLU THR ARG SER ARG --- GLU ALA LEU ARG VAL LYS LYS LYS MET GLU GLY ASP LEU ASN GLU MET GLU ILE GLN LEU SER GLN ALA ASN ARG

ATA GCC TCA GAG GCA CAG AAA CAC CTG AAG AAT TCT CAA GCT CAC TTG AAG GAC ACC CAG CTC CAG CTG GAT GAT GCT --- --- GCC AAT GAC GAC CTG AAG GAG AAC ATC
ILE ALA SER GLU ALA GLN LYS HIS LEU LYS ASN SER GLN ALA HIS LEU LYS ASP THR GLN LEU GLN LEU ASP ASP ALA --- --- ALA ASN ASP ASP LEU LYS GLU ASN ILE

GCC ATC GTG GAA CGG CGC AAC AAC CTG CTG CAG GCG GAC GTG GAG GAG CTG CGG GCT GTG GTG GAG CAG ACG GAG CGG TCT CGG ////CTC AAG GGC GGC AAG AAG CAG CTG
ALA ILE VAL GLU ARG ARG ASN ASN LEU LEU GLN ALA ASP VAL GLU GLU LEU ARG ALA VAL VAL GLU GLN THR GLU ARG SER ARG ////LEU LYS GLY GLY LYS LYS GLN LEU
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Clone pMHC141

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CGG GCC AAG AGC CGG GAC ATT GGT GCC AAG CAG AAG ATG CAC GAC GAG GAA TAACCTCTCCAG
ARG ALA LYS SER ARG ASP ILE GLY ALA LYS GLN LYS MET HIS ASP GLU GLU
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FIG. 2. Restriction map and nucleotide sequence of the inserts coding for different MHC isoforms. (*a*) Schematic representation of the aligned MHC-specific inserts of the recombinant plasmids. ○, *Pst* I site; ▲, *Hin*fI site. Arrow points to the stop codon; at the right of the arrow is the 3′ noncoding region. (*b*) Sequences of the different recombinant plasmid inserts. Sequencing of pMHC2.2, pMHC16.2A, and pMHC141 was carried out by the Maxam and Gilbert technique (23); sequencing of pMHC101 was done by the dideoxy technique as modified by Biggin *et al.* (24). ///, Undetermined sequence of ≈300 nucleotides; -, undetermined nucleotide. THR residue, see text.

of the mouse muscle cell line T984 Cl10 (Fig. 1, lane a) and from adult mouse skeletal muscle (lane b). The hybridization pattern was compared with that given by a nick-translated probe (pMHC32) specific for adult skeletal MHC mRNA, using the same blot (Fig. 1, lanes c and d). pMHC2.2 hybridizes relatively more strongly to RNA from cultured myotubes, showing that it does not code for the adult skeletal MHC isoform and suggesting that it might code for the embryonic MHC isoform (21). That the pMHC2.2 probe is specific for a MHC gene different from the adult skeletal MHC gene was also indicated by Southern blotting experiments, where pMHC2.2 hybridized to a distinct band (data not shown). DNA sequencing (Fig. 2*b*) confirmed that the sequence inserted in pMHC2.2 corresponds to the end of the coding region of a gene for a MHC other than the adult skeletal MHC isoform (2). Comparison with the nucleotide sequence of a rat cDNA clone (pMHC25) that encodes a portion of the embryonic MHC isoform (26, 37) shows 95% homology. From the origin of the poly(A)$^+$ RNA used to construct the bank, from the RNA blot data, and from the sequence data, we conclude that pMHC2.2 codes for a portion of the embryonic MHC isoform.

A second recombinant plasmid, pMHC16.2A (Fig. 2), with a different restriction map, was isolated from the T984 Cl10 cDNA bank. The results of RNA filter-blotting experiments show that pMHC16.2A preferentially hybridizes to RNA from fetal skeletal muscle (see Fig. 1, lanes l–n) and that the hybridization pattern is very different from that of the adult skeletal MHC probe (pMHC32) hybridized to the same filter (lanes i–k). pMHC16.2A hybridizes to a different band on Southern blots from that detected by the adult or embryonic MHC probe (data not shown). Sequencing of the pMHC16.2A recombinant plasmid (Fig. 2*b*) shows that it contains the COOH-terminal coding and 3′ noncoding sequence of another MHC mRNA. The 3′ end of the coding region shows a divergence with the adult mouse skeletal MHC mRNA at exactly the position (the codon for the Thr residue underlined in Fig. 2*b*) predicted from nuclease S1 protection experiments, using pMHC32, which identified the fetal/perinatal MHC mRNA (2). The 3′ noncoding sequence (151 nucleotides) of pMHC16.2A shows 87% homology (best fit) with the rat perinatal MHC sequence (9), whereas the coding portion of pMHC16.2A shows a homology of 95%. We conclude that pMHC16.2A codes for part of the perinatal MHC isoform, whose mRNA is a major species in fetal mouse skeletal muscle (2) but is present only as a minor species in the T984 Cl10 myotube RNA (Fig. 1) from which the insert in pMHC16.2A was cloned.

From the cardiac cDNA library two recombinant plasmids have been isolated: pMHC141 and pMHC101 (Fig. 2). Hybridization to RNA on filters showed that the insertion of pMHC101 hybridizes to a major species of cardiac mRNA (Fig. 1, lanes e and f). On Southern blots of mouse genomic DNA, two bands were detected, which were distinct from those seen with pMHC32 and pMHC2.2 (data not shown). Alignment of the pMHC101 sequence with the rat cardiac sequences (27) confirmed that pMHC101 codes for a segment of a cardiac MHC isoform, probably an α(V1) MHC isoform because the 252-bp *Pst* I–*Pst* I restriction fragment of pMHC101 is 93% homologous with this sequence, whereas the homology is 86% when pMHC101 is compared with the rat cardiac β(V3) cDNA clone.

Since pMHC101 lacks the more gene-specific 3′ end, we isolated a second recombinant plasmid, pMHC141, which contains the 3′ noncoding region (Fig. 2). Comparison of the nucleotide sequence encoding the seven COOH-terminal amino acids (Fig. 2*b*) with the rat cardiac α(V1) cDNA sequence (27) identifies pMHC141 as an α(V1)-specific cardiac MHC sequence [there is one amino acid change: Met (ATG) is in the mouse sequence in place of an Ile (ATC) in the rat sequence].

Segregation of Skeletal MHC Genes. These sequences from the MHC recombinant plasmids were used to detect RFLPs defining the corresponding alleles between two mouse species, *Mus musculus* and *Mus spretus*.

The perinatal-specific probe (250-bp *Pst* I fragment of pMHC16.2A) detects two RFLPs (one with *Bam*HI and one with *Hin*dIII) between the parental DBA/2 and *M. spretus* strains; when genomic DNA is digested with *Bam*HI, a 5.7-kilobase (kb) band and a 9.4-kb band are detected in DBA/2 and *M. spretus*, respectively (Fig. 3). When the DNA is digested with *Taq* I, the embryonic-specific probe pMHC-2.2 hybridizes to a band of 5.8 kb in DBA/2 and of 1.7 kb in *M. spretus* (Fig. 3). The adult skeletal MHC gene shows a *Bgl* I polymorphism when probed with pMHC32, as previously described (2, 16). These RFLPs were used to follow segregation of the three MHC genes in the 42 offspring. The results are shown in Table 1 and analyzed in Table 2. The perinatal MHC shows perfect cosegregation (42/42) with the adult skeletal MHC gene. The embryonic MHC gene also cosegregates with the perinatal and adult MHC genes in all cases analyzed (40/40). Therefore, these three genes must be tightly linked. They are loosely linked with two marker loci, *Hba* and *Es-3*, known to reside on chromosome 11 (19) and analyzed in the same backcross (Table 2). The analysis places the skeletal MHC gene cluster between these two loci near the locus nude. The embryonic atrial MLC1 (MLC1$_{\text{emb}}$) gene, also localized on chromosome 11 (16), shows cosegregation with the skeletal MHC gene cluster (Table 2), which would place the MLC1$_{\text{emb}}$ gene between the cluster and *Es-3*.

Segregation of the α(V1) Cardiac MHC Gene. We used the α(V1) cardiac MHC probe pMHC141 to detect two RFLPs between DBA/2 and *M. spretus* DNA, generated by *Bgl* II and *Taq* I. The RFLP generated by *Taq* I digestion (Fig. 3) shows two main bands in DBA/2, of 3.2 kb and 1 kb, and two in *M. spretus*, of 3.7 kb and 1.5 kb; other bands present varied

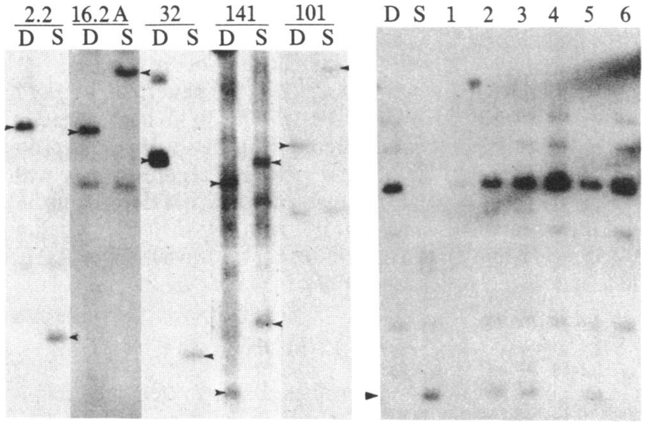

FIG. 3. Genomic Southern blots showing the different RFLPs detected by each MHC probe and an example of segregation in the backcrosses. (*Left*) Detection of RFLPs between DBA/2 and *M. spretus*. DNA samples from DBA/2 (D) and from *M. spretus* (S) were digested by *Taq* I (pMHC2.2), *Bam*HI (pMHC16.2A), *Bgl* I (pMHC32), *Taq* I (pMHC141), or *Bcl* I (pMHC101); electrophoresed in agarose gels; blotted; hybridized with the corresponding probes; washed respectively at 60°C, 65°C, 72°C, 60°C, or 72°C; and exposed 5–11 days. Sizes of the bands indicated by arrowheads are given in the text. For the pMHC141 probe, two religated *Hin*fI–*Hin*fI fragments (240 and 450 bp, overlapping in the pBR sequence) were used. (*Right*) RFLP pattern between the parental DBA/2 (lane D) and *M. spretus* (lane S) strains and their offspring nos. 1–6 (lanes 1–6, respectively). Genomic DNA was digested with *Taq* I, and the probe used was the 320-bp *Pst* I–*Pst* I fragment of pMHC2.2 (embryonic MHC-specific). The position of the *M. spretus* band is indicated by the arrowhead. Hybridization and washing were as described (2).

Table 1. Segregation of the skeletal and cardiac MHC genes in the 42 offspring of (DBA/2 × *M. spretus*)F$_1$ × DBA/2

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21
2.2	ND	+	+	−	+	−	+	−	+	+	+	+	+	ND	−	+	−	+	+	−	+
16.2A	−	+	+	−	+	−	+	−	+	+	+	+	+	−	−	+	−	+	+	−	+
32	−	+	+	−	+	−	+	−	+	+	+	+	+	−	−	+	−	+	+	−	+
Es-3 (11)	+	+	−	−	−	+	−	−	+	−	+	+	+	+	−	+	−	+	+	ND	+
Hba (11)	+	−	+	ND	+	−	+	−	+	+	+	−	+	−	ND	ND	−	+	−	−	ND
MLC1$_{emb}$ (11)	−	+	−	−	−	+	−	−	+	−	+	+	+	+	−	+	−	+	+	−	+
101	−	−	−	−	+	+	−	−	+	+	+	−	−	+	+	−	+	+	+	−	−
141	ND	−	−	−	ND	+	−	−	+	+	+	−	−	ND	+	−	+	+	+	−	−
Np-1 (14)	−	−	−	−	+	+	−	−	+	+	+	−	−	−	+	−	+	+	+	ND	−

	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40	41	42
2.2	+	+	+	+	−	−	+	+	−	+	−	−	−	+	+	+	−	+	−	+	+
16.2A	+	+	+	+	−	−	+	+	−	+	−	−	−	+	+	+	−	+	−	+	+
32	+	+	+	+	−	−	+	+	−	+	−	−	−	+	+	+	−	+	−	+	+
Es-3 (11)	−	+	+	−	+	−	+	+	−	+	−	−	−	+	+	+	−	ND	ND	ND	ND
Hba (11)	+	ND	ND	+	−	+	−	ND	ND	ND	−	−	−	+	−	−	+	+	+	ND	ND
MLC1$_{emb}$ (11)	−	+	+	+	+	−	+	−	−	+	−	−	−	+	+	+	−	−	−	+	−
101	+	+	ND	+	−	ND	+	+	+	+	+	+	+	−	−	−	−	−	−	+	ND
141	+	+	−	+	−	+	+	+	+	+	+	+	+	−	−	−	−	−	−	+	+
Np-1 (14)	+	+	−	+	−	+	+	+	+	+	+	+	+	−	−	−	−	ND	ND	ND	ND

Segregation of the skeletal MHC (2.2, embryonic; 16.2A, perinatal; 32, adult) and cardiac MHC [101 and 141, α(V1) cardiac MHC] genes and of known chromosomal markers *Es-3*, *Hba*, and the embryonic MLC1 gene (chromosome 11) and *Np-1* (chromosome 14) in the 42 offspring of (DBA/2 × *M. spretus*)F$_1$ × DBA/2 (16). The segregation of the MHC genes was followed by using the RFLPs described in the text; the segregation of the markers was followed as described in ref. 16. The mice are scored as homozygous for the DBA/2 allele (−) present in all animals or heterozygous (+). ND, not determined.

in intensity between blots. Either there is a *Taq* I site in the gene in this region or each band corresponds to a different cardiac MHC gene. This RFLP was used to follow the segregation of the α(V1) cardiac MHC gene in the 42 offspring. The cosegregation of the α(V1) cardiac MHC gene with the skeletal MHC genes (embryonic, perinatal, and adult) is not significant (cosegregation 19/39, see Table 2). The α(V1) cardiac MHC gene cosegregates with the marker allele *Np-1*, which is located on mouse chromosome 14 (cosegregation 34/34, see Table 2).

This result was confirmed by using the other cardiac MHC probe, pMHC101, which detects a RFLP generated by *Bcl* I, showing a band of 5.8 kb in DBA/2 and 10 kb in *M. spretus*. The cardiac MHC gene detected by pMHC101, most probably the α(V1) cardiac MHC gene, cosegregates totally with the α(V1) cardiac MHC gene detected by pMHC141 (36/36) and cosegregates very closely with *Np-1* (cosegregation 34/35, see Table 2). We conclude that the α(V1) cardiac MHC gene is located on chromosome 14, near *Np-1*.

DISCUSSION

The use of isoform-specific probes has permitted us to follow the segregation of MHC genes in an interspecific mouse backcross (for discussion of this genetic approach see ref. 16). We conclude that the developmental (embryonic/fetal, perinatal, and adult) skeletal muscle MHC genes are clustered on mouse chromosome 11 near *nu* and that the α(V1) cardiac muscle MHC gene is on chromosome 14 near *Np-1*. We did not investigate the location of the β(V3) cardiac MHC gene, but α(V1) and β(V3) cardiac MHC genes in the rat are organized in tandem (28).

The results of experiments based on the analysis of somatic cell hybrids (13, 15) led to the conclusion that all the MHC genes are located on mouse chromosome 11. However, no cardiac MHC probe was used and the skeletal MHC probes employed were derived from rat. We suggest that mouse cardiac MHC genes were not detected in these experiments. In humans the skeletal MHC genes have been localized on the short arm of chromosome 17 (15). Our results with the mouse raise the possibility that human cardiac genes may be located on another chromosome, and preliminary results of *in situ* hybridization suggest that some MHC genes may be located on human chromosome 7 (29).

Investigation of the chromosomal location of actin and alkali MLC genes expressed in fetal and adult skeletal and cardiac tissues has shown that the members of these multigene families are dispersed in the mouse genome (16) although expressed coordinately in a given phenotype (21, 30). The cardiac and skeletal actin genes (11, 31) and the

Table 2. Analysis of the segregation of the skeletal and cardiac MHC genes and the biochemical markers in the 42 offspring presented in Table 1

	No. of cosegregants/no. of animals compared							
	16.2A	32	*Es-3*	*Hba*	MLC1$_{emb}$	101	141	*Np-1*
2.2 (embryonic MHC)	40/40	40/40	27/35	20/29	30/40	19/37	19/39	16/35
16.2A (perinatal MHC)		42/42	27/37	21/31	31/42	20/39	19/39	18/37
32 (adult MHC)			27/37	21/31	31/42	20/39	19/39	18/37
Es-3				11/28	34/37	15/35	14/34	14/37
Hba					12/31	14/30	14/28	15/28
MLC1$_{emb}$						20/39	18/39	15/37
101 [α(V1) cardiac MHC]							36/36	34/35
141 [α(V1) cardiac MHC]								34/34

cardiac atrial and adult skeletal alkali MLC genes (12) are coexpressed during striated muscle development, whereas the skeletal MHC genes are sequentially expressed during development (2, 8). We suggest that neither coordinate expression in a distinct phenotype nor coexpression during development requires linkage of the corresponding genes (31), whereas sequential expression of genes during development may necessitate linkage, perhaps because of a requirement for a *cis*-acting regulatory mechanism (32–34). A similar situation is found for the albumin and α-fetoprotein genes (35) and for the α- and β-globin genes (32, 36).

We thank O. Jaffrézou for technical assistance; S. Alonso, A. Minty, and B. Robert for helpful discussions, A. Cohen for an RNA blot, and J. Weissenbach for constructing the T984 Cl10 cDNA library. We are also grateful to M. Periasamy and B. Nadal-Ginard for permitting us to use their unpublished sequence of pMHC25. This work was supported by grants from the Centre National de la Recherche Scientifique, the Ministère de la Recherche et de la Technologie, and the Muscular Dystrophy Association of America. P.B. was supported by a European Molecular Biology Organization fellowship, and I.G., by a Royal Society Science and Engineering Research Council fellowship.

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