
The nucleotide sequence of a rat myosin light chain 2 gene

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Received 7 May 1984; Accepted 13 August 1984

ABSTRACT

A rat myosin light chain 2 gene was characterized by nucleotide sequence and S1 mapping analyses. It contains seven exons separated by six introns. The corresponding mRNA is predicted to be 654 nucleotides long (excluding polyA sequences), with 5'-nontranslated, coding, and 3'-nontranslated lengths of 56, 510, and 88 nucleotides, respectively. The predicted amino acid sequence is identical to that from rabbit except that the rat sequence lacks one of two Gly residues located at positions 12 and 13 in the rabbit sequence. From the nucleotide sequence, nascent rat myosin light chain 2 is predicted to have Met Ala preceding Pro at the N-terminal end.

INTRODUCTION

During the development of vertebrate skeletal muscle, fusion of myoblasts to form multinucleate myotubes is accompanied by the concomitant synthesis of a variety of muscle-specific proteins, including myosin heavy and light chains, α -actin, tropomyosin, troponin, and creatine kinase [for review, see Merlie *et al.* (1)]. Because the myoblast-myotube conversion can be carried out under defined conditions *in vitro*, this system has been extensively used in studies of cell differentiation. Much of the current work in this field is focused on the characterization of genes coding for muscle-specific proteins. Here we present the complete nucleotide sequence of a rat gene coding for skeletal muscle myosin light chain 2 (MLC2). The ultimate goal of such studies is an understanding at the molecular level of events leading to the coordinate expression of a developmentally regulated set of genes.

Myosin from skeletal muscle from various vertebrates contains two heavy chains of molecular weight 200,000 and two to four light chains of molecular weight 17,000-25,000 (2,3,4,5). The composition of the light chains differs in myosin extracted from different muscle types (fast, slow, and cardiac) and from different developmental stages (6). Adult rat

skeletal muscle contains three light chains of molecular weight 23,000 (MLC1), 17,000 (MLC2), and 15,000 (MLC3) (7). The amino acid sequence of skeletal muscle MLC2 has been determined for rabbit (8), and chicken (9). Chicken contains at least three other MLC2 polypeptides, two in cardiac muscle (L2A and L2B) and one in gizzard (10). Recombinant plasmids containing cDNA to portions of MLC2 mRNA have been reported for rat skeletal muscle (11,12), quail skeletal muscle (13), and chicken cardiac muscle (14).

In the work reported here, a cDNA clone prepared from rat skeletal muscle RNA, plasmid p103 (11), was used to identify a bacteriophage λ clone containing an intact MLC2 gene. The complete nucleotide sequence of this gene and some of its flanking DNA is presented.

METHODS

Construction and Screening of Rat Genomic DNA Libraries

A library containing a partial EcoRI digest of rat DNA in bacteriophage λ (15) was a gift from T. Sargent and J. Bonner. Three other partial EcoRI rat DNA libraries were provided by A. Breiner and I. Schechter. A library containing a HindIII digest of rat DNA in λ Charon 21 was prepared by digesting rat spleen DNA to completion with HindIII and selecting DNA of size 4-6 kb for insertion into the vector. Libraries were screened for MLC2 genes as described (16,17) using nick-translated (18) plasmid p103 as probe.

Recombinant DNA Procedures

Bacteriophage λ was grown and purified as described previously (17). DNA was isolated from rat spleen (19), phage λ (17), and plasmid-containing cells (11) as described in the indicated references. Restriction endonucleases were used under conditions specified by the manufacturers (New England Biolabs, Inc.).

DNA Sequence Determination

DNA fragments containing 5'-protruding ends were labeled at their 3' ends by treatment with [α -³²P]deoxyribonucleotides and reverse transcriptase (a gift of J. Beard) (20). The sequencing procedures of Maxam and Gilbert (21) were used as modified by Smith and Calvo (20).

S1 Nuclease Mapping

RNA was isolated from cultures of L8 myoblasts and myotubes by the lithium chloride-urea extraction procedure described by Auffray and Rougeon (22). Probes were labeled at their 5' end by sequential treatment with bacterial alkaline phosphatase and polynucleotide kinase (21) or at their

3' end by treatment with reverse transcriptase (20). Single-stranded probes were obtained by fractionating denatured fragments by electrophoresis through acrylamide gels (21). Conditions for hybridization, S1 nuclease treatment, and electrophoresis of products were described by Weaver and Weissmann (23).

Computer Analysis of Sequences

The program of Fristensky *et al.* (24) was used for graphic matrix analysis of sequences (25). Sequences of 10 or 20 bases were compared with 70% or 80% homology required for printing of a dot.

RESULTS AND DISCUSSION

Plasmid p103 Carries Part of a Rat Myosin Light Chain 2 Gene

Plasmid p103 contains cDNA prepared from rat muscle polyA-containing RNA (11). It was tentatively identified as containing at least a part of a MLC2 gene on the basis of the following evidence: 1) *in vitro* translation of rat muscle RNA selected by hybridization to plasmid p103 DNA yielded a polypeptide that comigrated with MLC2 on two-dimensional gels; 2) the *in vitro* product and authentic MLC2 showed identical patterns when subjected to partial proteolysis. This assignment was confirmed by DNA sequence analysis. Plasmid p103 contains a 320 base pair insert in the *Pst*I site of plasmid pBR322. This insert codes for 76 amino acids, from amino acid 92 of MLC2 through C-terminal amino acid 167, together with 59 nucleotides of the 3'-nontranslated region of the messenger (data not shown; see Fig. 2B for numbering). Presumably, the entire 3'-nontranslated region is not present in plasmid 103 because the 3' terminus of the nontranscribed strand of the insert does not end with a run of A residues.

Genomic Clones Containing Portions of the Rat Myosin Light Chain 2 Gene

Plasmid p103, labeled to high specific activity by nick translation, was used to screen several bacteriophage lambda libraries containing inserts of rat genomic DNA (15). Several recombinant phages that hybridized strongly to the probe had similar restriction endonuclease patterns, suggesting that they were derived from the same region of the genome. Among fragments derived from phage lambda-MLC2 DNA by treatment with endonuclease *Eco*RI, only a 3 kb fragment hybridized to plasmid p103 (Fig. 1A). This 3 kb fragment was cloned into the *Eco*RI site of plasmid pBR322, generating plasmid pMLCR (Fig. 1B). Sequencing studies, described below, indicated that plasmid pMLCR lacked the 5' end of the MLC2 gene. To isolate a clone containing the 5' end of the gene, a lambda Charon 21 library

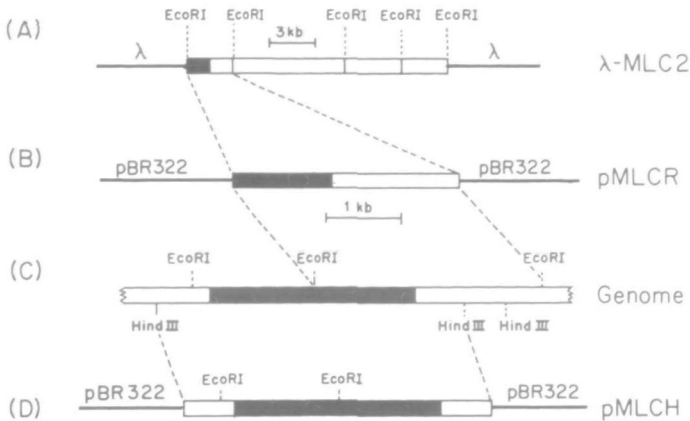


Fig. 1. Position of rat myosin light chain 2 gene within various bacteriophage λ and pBR322 derivatives. The distribution of HindIII and EcoRI sites in the genome (C) were deduced from the information in A, B and D. Bar, rat DNA; filled bar, MLC2 gene; line, vector DNA. B, C, and D are drawn to the same scale.

containing HindIII fragments of rat DNA was prepared and screened for clones hybridizing to probe p103. The HindIII insert from one such clone, which contained the entire MLC2 gene, was ligated into the HindIII site of plasmid pBR322, yielding plasmid pMLCH (Fig. 1D).

Nucleotide Sequence and Organization of the Myosin Light Chain 2 Gene

Sequencing was carried out by the procedure of Maxam and Gilbert (21) using the strategy summarized in Fig. 2A. The nucleotide sequence of the nontranscribed strand is shown in Fig. 3, with +1 assigned to the position at which transcription is presumed to initiate.

The rat MLC2 gene contains seven exons (boxes, Fig. 2B; non-italicized regions, Fig. 3) separated by six introns. Each of the boundaries of introns 3-6, the 3' boundary of intron 2, and the 5' boundary of intron 7, were readily identified by inspection, knowing the amino acid sequence of rabbit MLC2 (8). The other boundaries were established by S1 mapping experiments (23,26). RNA isolated from myotubes was hybridized separately with end-labeled probes identified in Fig. 4E. The products were treated with S1 nuclease, denatured, and fractionated by electrophoresis through acrylamide containing 50% urea. Myotube RNA protected 76 nucleotides of the transcribed strand of the 3' labeled AvaI/XbaI probe (Fig. 4A), placing the site of the polyA addition at position +2761 in the nucleotide sequence (Fig. 3). Seventeen base pairs upstream from this position is a sequence

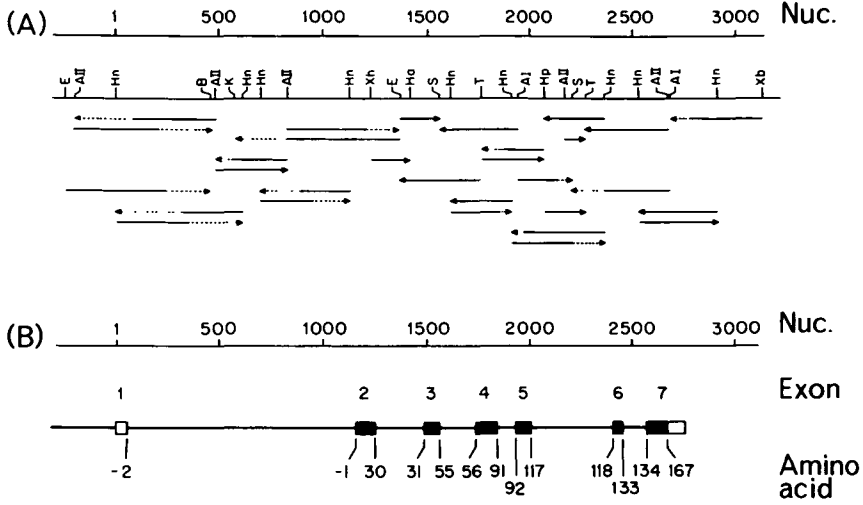


Fig. 2. A. Sequencing strategy. Arrows denote the fragments that were sequenced and the extent of sequencing (solid portion). The direction of the arrow indicates whether the relevant sequence was of the transcribed strand (arrow points leftward) or the non-transcribed strand (arrow points rightward). The tail of each arrow is aligned with the restriction site that was labeled. The restriction sites shown are those used for sequencing and do not necessarily represent all such sites. AI, *Ava*I; AII, *Ava*II; B, *Bgl*II; E, *Eco*RI; Ha, *Hae*III; Hn, *Hinf*I; Hp, *Hpa*II; K, *Kpn*I; S, *Sac*I; T, *Taq*I; Xb, *Xba*I; Xh, *Xho*I.

B. Organization of the rat myosin light chain 2 gene. Introns are lines and exons bars, with polypeptide coding regions in black and non-coding regions in white. The nucleotide numbering system (Nuc) is that in Fig. 3. The amino acid numbering system is that employed for rabbit (8) and chicken (9) in which amino acid 1 is Pro. Here, -2, -1, and 1 refer to Met, Ala, and Pro, respectively.

AATAAAA, proposed by others as a signal sequence for polyA addition (27,28).

Exon 2 codes for the known N-terminal amino acids of MLC2 from rabbit (X-NH-Pro Lys Lys) and therefore the 5' boundary of this exon could not be established by inspection. Myotube RNA protected 75 nucleotides of the transcribed strand of a *Hinf*I probe (Fig. 4B) placing the 5' boundary of exon 2 at position +1165 (Fig. 3). Since exon 2 does not contain information for a chain-initiating Met, it must be preceded by at least one other exon. The results of S1 mapping experiments shown in Fig. 4C and D identify an exon (defined here as exon 1) having boundaries at positions +1 and +59. Position +1 is an A, the nucleotide most frequently found in S1 nuclease mapping experiments at the 5' ends of transcripts (29).

-200 -150

ACACTGCAGTCCCCTTTGGGAGAAAGAACATCCCTTCTACCTAGGTCCTAACAGACTTGCATCCCAAGACCTCTCCCCTCCCCTCC

-100

TTGACTCCAAGCTCCTGCAGGCCCTCCAAGAAAGGAGAGGCCCTGAGCTGGGCATTTTAGTATCTGGGTTGGGTATCCACAGGGCTAAGGTT

-50

ACCACGGTATGTTAAGGGCAGGACTATATAACCCCAAGAAACTGCCCAAGC AGATTCTCGTTTTTCCACTCGGAGCTACTGCCTT

50 100

GCCCTCAGAGGATCTAAGAC ATG GTGAGTGAAGACCCCCCAACAGA

Met

150 200

GAGAGAGAGAGAGTGTAGCTCAGGATAGCCTCACCCCTTCCAGAAAAAAGTTGTGCTTCTCAATCCAACACTACAGGTAATAATGTGAAGT

250 300

GTCTCTTCTAGCAAACCAGAAATAAGGCCAGATAGACTGATGGGTGAAGGTTTCAAGTTAATAATGCTCAGATATCCAACCGTGATG

350 4

TATGGGGTAGGGGTAGACGTCTCAAAGTCTGTTCGGTCCCTTCTGTAATTTCTAAAGTCTCAGACCAAGTGTGGCAGTGCCTGTCA

00 450

CATTTTGTAAAGTAAAACTTGAAGTTCAGATAGGCTCAAGACTAGACTAAGCTAGCAATATGGACGCCAAAGTGGCAGGTGGAGGCTCC

500 550

TGAGAAAGTACTTGGCAGCCAGGATGAAAGCATCTGGGAGCTATAGTTGGGATTTAAGATGAAGCCAAATGAGGCCCTTAGCCAAGA

600 650

GCTACCAAGAAAAAGAGAGTTGTTTATAGAAAAGGACTCAGAGAATCTGAACTTAACTGACTAGTATGGAGGTTATGCCATAAT

700 750

TTCAACTACTTTCAAGCCTGAGGAGTGGTCTACTGGAGTCTGAGATTCAAGGCCAGCATGAGAAAATGTAAGGAGACCTGTTCGCAAAA

800 850

AAAAAAGAGGAGAAACCAAGTGTTGAAGAAATCTTTAATCTCAAGAAAGCATGGTCTATAATAATAGTCCAAAGGCTAAATCATG

900

AGACTGTCTCAGGGGTGGGGAGATTGGGTGGGGATGTAAGTCTGTTGGTGTGTTAGCCTAGCGTGCACAGCAACAAAATAAAA

950 1000

TAGGATGTGGTGCACAAGATGTAACCTGTAATGCACAATCAAGGTTAGAGATGGAGCCAAAGATGAGCAGATCAAGGTATCTCTCAG

1050 1100

TTAAGTAGCAAGTTTGGGGTAGGAGACAACCCCTCTTAAACAGAAAACAAAAGTTTGGTTTGTGCTCCCAGGAGAGATTACATGAAGT

1150 1200

GACTGTAGACTCTCTGCTCAACCTTACCCCTCAGAG CCA CCC AAG AAG GCC AAG AGA AGG GCA GCA GAA GGG

Ala Pro Lys Lys Ala Lys Arg Arg Ala Ala Ala Glu Lys

1250

AGC TCC AAT GTT TTC TCC ATG TTT GAC CAG ACT CAG ATC CAG GAG TTC AAG GAG CTGAGAGAGAGGGCTAAG

Ser Ser Asn Val Phe Ser Met Phe Asp Gln Thr Gln Ile Gln Glu Phe Lys Glu

1300 1350

GAAAGCCCCCACCCTCTCCCACCCCTCTTCCATCCCTCTCCCACCCCTCTCCCAGAAAAGTACCTGTTGAGGGAGAAGGA

1400 1450

GGGTTTAGAAATCTCCCTCCCTCGGGAAAGGTGGAGGAGACAGTGGCTCAGGGCCAGAAATCTGATCTGCTTGGGGCAATTCTCGGC

1500

TTCATCTGTCCCACCCCTACCCCTCCACAG GCT TTC ACT GTA ATT GAT CAG AAC AGG GAT GGC ATT ATT GAC AAG

Ala Phe Thr Val Ile Asp Gln Asn Arg Asp Gly Ile Ile Asp Lys

1600

GAG GAT CTT CGG GAC ACC TTT CGG GCC ATG C GTGAGCTCCTACTCCACTGTAATAATGTTGAAAGCTATGGAGTCGGAG

Glu Asp Leu Arg Asp Thr Phe Ala Ala Met G

1650 1700

GGGAACTCAGTGCTTCAGGCATTAATCTGCCAGATGAAAAATGAATGACTGATATAAGTTATGGAAAAGCAGCTCTGGTCCCCAGC

1750

ATCCAACCTGCAACCCCTGGTGTCCACCCATTGAG GC CGI CTC AAT GTG AAG AAT GAG GAA CTC GAC GCC ATG ATG

ly Arg Leu Asn Val Lys Asn Glu Glu Leu Asp Ala Met Met

1800 1850

AAG GAA GCC AGT GGG CCT ATC AAC TTC ACT GTC TTC CTG ACC ATG TTT GGG GAG AAG CTG AAG G GTGAG

Lys Glu Ala Ser Gly Pro Ile Asn Phe Thr Val Phe Leu Thr Met Phe Gly Glu Lys Leu Lys G

1900

TAAAAAGTTCTCAGTCCAAGACCGTAGTTCTCTCCAGAACTTCAGGACCTGCTAGACTCATGTGCCCTGACCCCTGCAG GT

ly

1950 2000

GCG GAC CCC GAG GAT GTG ATC ACT GGA GCC TTC AAG GTC CTG GAC CCA GAA GGG AAG GGC ACC ATC AAA

Ala Asp Pro Glu Asp Val Ile Thr Gly Ala Phe Lys Val Leu Asp Pro Glu Gly Lys Gly Thr Ile Lys

2050

AAG CAA TT GTGAGTGGCTCCTTACTGGCTTACTGTAGGCTTCTAAAATCTTCATGAAAGGTTGACCGGTTGACAGACCGCTGTGAG

Lys Gln Ph

2100 2150

CAAATAGGGGCTGGGGTGGCCAGGTTAAATTTTCTTTTCTTTTCTTTTCTTTTCTTTTCTTTTCTTTTCTTTTCTTTTCTTTTCTTTTCTTTT

2200 2250

CCAGGGCCTTGCCTTGCTAGGCAAGACTTACCACTGAGCTAAATCCCAACCCCAAGTTACATTTTCATAATACTAAAGGAAAGAAA

2300 2350

TTTCAGACAGGGCCACACAGCAATTGGGAGATGCACCAACATCTTCAGGGGAAAGGTTGATGGGAAACAAGACTGAATAAGGGGGGAC

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                2400
AGGACTCATGAAACCAATCTCACCTCCCTGAATGTCACCTTCTCAG C TTG GAG GAG CTG CTT ACC ACG CAG TGC GAC
                2450                2500
e Leu Glu Glu Leu Leu Thr Thr Gln Cys Asp
CGA TTT TCC CAG GAG GAG GTGAGCAGGGGAGAGCCAGGGAGGAAAGGACTCGGAGTCGGAAGTAAAGTGGAACTGAGGAAAGCAG
Arg Phe Ser Gln Glu Glu
                2550                2600
TGGGAGTCGGAAAGACCCGTTTGTAGACCTACTCCTCTCTCTCTACAG ATC AAG AAT ATG TGG GCA GCC TTC CCT CCA GAT
Ile Lys Asn Met Trp Ala Ala Phe Pro Asp
                2650
GTG GGC GGC AAC GTG GAC TAC AAG AAC ATC TGC TAT GTC ATC ACA CAC GGT GAC GCT AAG GAC CAG GAA
Val Gly Gly Asn Val Asp Tyr Lys Asn Ile Cys Tyr Val Ile Thr His Gly Asp Ala Lys Asp Gln Glu
                2700                2750
TAG GGGACCCGAGGCTCTCAGAAGACCCAGATAGGCTTGTAGCCGACCAACTCTCACCCCTGACCCCCAAATAAAAGTCAACTGGT CTTT
End
                2800                2850
GTTTCTTACTGGTGGCAATTTTCAGTGGGTTCACTCAGGAACTCAGGTGGGAGGCCGAAGCACCGCCAGCAAGACAGGAGTGGCTAACAGG
                2900
TGGAAAGACTAGTGGCCAGGAGGCCCTCAAAGTTCAGTTCATTCCTGCTGGGAAGAAGATTGGGGCAACTGGGGAGGCGAGGACGAGTT
950                3000
TTATTAGATGGGAACTTGGAGGAACGCTAAAACCCAACTGGAACCTGCCGTTAAGCCTTTGGAGGGCAGCCCTCTCAGAACACTATAGAA
3050                3100
GGCGGAGTCGGCTGGCCCTTAAACTCCGAAATTGGAGGCACTGATTAAAGTGAAGGCCGGCCCTACCCCTCTGGGTCTTCGTTGGG

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Fig. 3. Nucleotide sequence of the non-transcribed strand of the rat MLC2 gene. Italicized regions are either introns or 5' or 3' flanking regions. Position 1 corresponds to the presumed site of transcription initiation. Underlined regions are sequences that may be important in transcription, translation, or processing.

Transcripts of exon 1, like those of exon 2, are observed in RNA from myotubes but not myoblasts (Fig. 4B, C, and D). The nucleotides at the exon 1/intron 1 boundary, ATG/GTCAG, provide information for a Met codon and a splice junction. Twenty-nine base pairs upstream from exon 1 is a sequence, TATATAA, that is characteristic of eucaryotic promoters (29) and 62 base pairs upstream from it is a CAT sequence that may be related to the CAAT box described by Efstratiadis *et al.* (30). Taken together, these results indicate that exon 1 contributes a 56 nucleotide 5' untranslated region and a Met codon to rat MLC2 mRNA. Teem and Rosbash have also described a gene in which an intron separates information for a translational start AUG from codon 2 (31).

From the combined results of nucleotide sequence and S1 mapping, rat MLC2 mRNA is predicted to be 654 nucleotides long (excluding polyA sequences), with 5'-nontranslated, coding, and 3'-nontranslated lengths of 56, 510, and 88 nucleotides, respectively. Assuming a polyA tail of 150-200 nucleotides (32), the predicted length of MLC2 mRNA (800-850 nucleotides) is close to the estimate of 800 nucleotides obtained by gel electrophoresis (11). Thus, it is unlikely that the MLC2 gene contains exons in addition to those shown in Fig. 2. Recent studies of the human MLC2 gene, which has an exon 1-intron-exon 2 organization that is nearly identical to the rat gene, support this conclusion. The length of cDNA

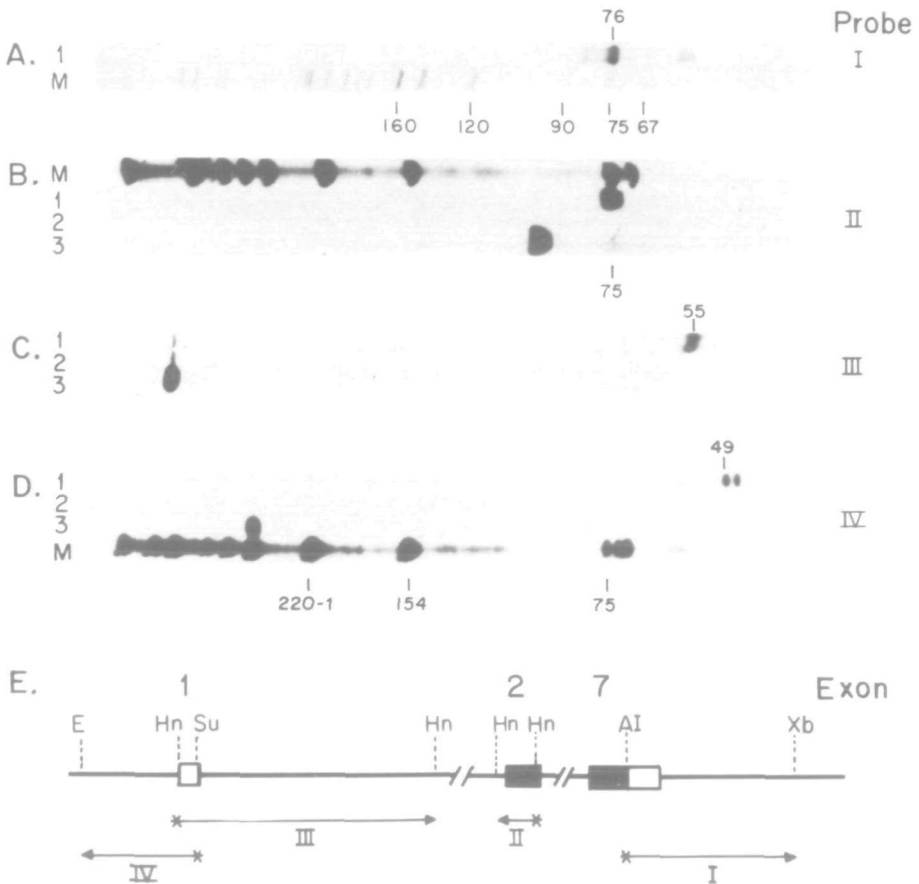


Fig. 4. Endonuclease S1 mapping of the 3' boundary of exon 7 (A), the 5' boundary of exon 2 (B), and the 3' (C) and 5' (D) boundaries of exon 1. The probes used are identified in (E), with the asterisk indicating the position of the label. Probe I, AvaI-XbaI (Fig. 3, +2684 to 3125), 3' end label, single stranded. Probe II, HinfI-HinfI (+1135 to 1233), 5' end label, double stranded. Probe III, HinfI-HinfI (+2 to 617), 3' end label, single stranded. Probe IV, EcoRI-Sau3A (-243 to +47), 5' end label, single stranded. RNA samples analyzed: A, 4.5 μ g polyA⁺ RNA from L8 myotubes M, plasmid p103 cut with HpaII. B, C, D. Lanes 1 and 2 in each case represent results using 15 μ g of total RNA from L8 myotubes and L8 myoblasts, respectively. Lane 3 in each case is the probe alone. M, plasmid pBR322 cut with HinfI. No bands were observed when the non-transcribed strands of fragments I, III, and IV were hybridized with RNA from myotubes (data not shown).

	EXON	INTRON	EXON	
1	GAG <u>ATG</u> GTGAGTGAAG---	---TTACCTCA <u>CAG</u> GCACCC	2	
2	AAG <u>GAG</u> GTGAGAGAGA---	---ACCTTCCAC <u>CAG</u> GCTTTC	3	
3	CCATGG <u>GTGAG</u> CTCCC---	---CACCCATT <u>CAG</u> GCCGTC	4	
4	TGAAGG <u>GTGAG</u> TAAAA---	---CTGACCCTG <u>CAG</u> GTGCGG	5	
5	GCAATT <u>GTGAG</u> TGGCT---	---TCACCTTCT <u>CAG</u> CTIGGA	6	
6	GAGG <u>GTGAG</u> CAGGG---	---GTCTTCCTA <u>CAG</u> ATCAAG	7	

Fig. 5. Sequences at exon-intron junctions. The sequence is of the non-transcribed strand written 5' to 3' left to right with the correct reading frame identified by underlines. Shaded regions highlight identical sequences.

synthesized in primer extension experiments employing human mRNA is just sufficient to account for exon 1 (C. Cisar, J. Calvo, and U. Nudel, unpublished data)

The predicted sequence of amino acids of rat MLC2, shown below the nucleotide sequence in Fig. 3, differs in only one respect from the known sequence of MLC2 from rabbit: the rat sequence lacks one of two Gly residues located at positions 12 and 13 in the rabbit sequence (8). The N-terminus of rabbit MLC2 is blocked, proline being the first identifiable amino acid (8). Henry *et al.* (33) suggested that the blocking group may be α -N-trimethylalanine. From the nucleotide sequence, nascent rat MLC2 is predicted to have MetAla preceding Pro at the N-terminal end. The predicted sequence of rat MLC2 differs from that of chicken skeletal muscle MLC2 by 15 amino acids, chicken cardiac muscle MLC2A and B by 45 and 44 amino acids, respectively, and chicken gizzard MLC2 by 78 amino acids (9,10).

The sequences at each of the exon-intron junctions, summarized in Fig. 5, are consistent with the general rule that an intron begins with GT and ends with AG (34). For the intron separating exons 5 and 6, correct splicing requires joining of nucleotides at two specific positions, whereas for all the other introns splicing can occur at several different positions and still generate the correct endproduct. It is interesting that all of the introns in the rat MLC2 gene have the same sequence, GTGAG, at their 5' end and CAG at their 3' end. The significance of this fact is not clear. Within a recent compilation of splice junction sequences (35), there is not a clear-cut trend of introns within a given gene containing identical

sequences at the 5' end.

Using the graphic matrix analysis of Maizel and Lenk (25), we searched the nucleotide sequence of the rat MLC2 gene for repetitive sequences and pallindromes. Centered at positions +1037 and +2143 are, respectively, C-rich (43/58 residues are C) and T-rich (40/45) regions. Beginning at position +80 is a sequence (CA)₂₂ followed immediately by (AG)₈. Sequences containing (CG)_n or (CA)_n are known to undergo a transition to the Z form of DNA under certain conditions of salt concentration and superhelical content (36,37). Further, hybridization studies indicate that (CA)_n sequences are widespread within the DNA of eucaryotes (38). Among genes that have been sequenced, (CA)_n regions are found primarily within introns, but they also exist between genes (39). The (CA)₂₂ sequence identified here is within an intron. The graphic matrix analysis did not turn up other notable cases of repetitive DNA nor any unusual pallindromic regions.

The 5' flanking region of a human MLC2 gene has recently been sequenced (C. Cisar, J. Calvo, and U. Nudel, unpublished data). The human and rat sequences show almost 80% homology within a several hundred base pair region that precedes the TATA box. This degree of relatedness, which is greater than that shown by comparisons of introns and codon silent sites between human and rodent genes (40), suggests that the conserved sequences within the 5' flanking region have a biological function. Another comparison of interest is between the rat skeletal muscle actin and MLC2 genes. Skeletal muscle actin and MLC2, and their corresponding mRNAs, are induced during fusion with similar though not identical kinetics (41). Furthermore, a 730 bp sequence 5' to the rat skeletal muscle actin gene (42; U. Nudel, unpublished data) confers cell fusion-related expression upon genes introduced into rat L8 cells (43). A detailed comparison of the rat skeletal muscle actin gene and the rat MLC2 gene, together with the nucleotide sequences of their corresponding 5' flanking regions, will be published separately. To summarize the result of this comparison (5' flanking region of actin versus the entire MLC2 gene), carried out by the graphic matrix technique (25), we found no homologies that stood out above the background level of homologies that normally occur in such comparisons. This result suggests that the elevated expression of these two genes during differentiation is in response to different signals. Of course, we cannot exclude the possibility that sequences further upstream or downstream from those analyzed share homologies or that the two genes share subtle similarities that are important for expression. Experiments to differentiate between single or multiple signals are currently underway.

ACKNOWLEDGEMENTS

We thank J. Bonner, T. Sargent, A. Breiner, and I. Schechter for rat genomic libraries, J. Beard for reverse transcriptase, and D. Yaffe, R. Zakut and D. Givol for helpful discussions. The excellent technical assistance of Ms. O. Saxel, M. Miller, and S. Neuman is gratefully acknowledged. M.S., held a fellowship from the Muscular Dystrophy Association, and J.M.C. a Fogarty International Fellowship from the National Institutes of Health. U.N. is the incumbent of the A. and E. Blum Career Development Chair in Cancer Research. This work was supported by a grant from the Muscular Dystrophy Association and by grant No. R01-GM-22767 from the National Institutes of Health.

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