Examining cDNAs for the laminin-binding \( \alpha 7 \) integrin subunit identified two different sequences (designated X1 and X2) coding for the variable region between the III and IV homology repeat domains near the putative ligand-binding site. Sequencing of a mouse \( \alpha 7 \) genomic clone established that the X1 and X2 regions are derived by mutually exclusive alternative mRNA splicing. Reverse transcriptase-polymerase chain reaction analysis of \( \alpha 7 \) mRNA indicated that the X1 and X2 isoforms were present in equal amounts in mouse skeletal myoblasts and adult heart. However, in adult skeletal muscle, the X2 variant was exclusively expressed. Amino acid sequence homologies in the III/IV segment suggest that \( \alpha 3 \) and \( \alpha 6 \) are also alternatively spliced at this site. We identified alternatively spliced exons in a human \( \alpha 6 \) genomic clone that encode X1- and X2-like segments. Analysis of the \( \alpha 7 \) cytoplasmic domain indicated that this region was also alternatively spliced and like \( \alpha 3 \) and \( \alpha 6 \) could exist as the A or B form. In mouse skeletal and cardiac muscle the B form of \( \alpha 7 \) was strongly expressed. However, we identified \( \alpha 7 A \) in neonate and adult skeletal muscle but not in cardiac tissue. High levels of \( \alpha 7 A \) were detected in differentiating myotubes, but in proliferating myoblasts only the \( \alpha 7 B \) isoform was present. These results indicate that alternative splicing of \( \alpha 7 \) mRNA is differentially regulated during development and generates variant integrin chains with structurally and presumably functionally unique ligand-binding and cytoplasmic domains.

Integrins are a family of transmembrane heterodimeric receptors that mediate cell-extracellular matrix and cell-cell interactions (reviewed in Ref. 1). Each integrin is composed of a noncovalently associated \( \alpha \) and \( \beta \) subunit. This pairing of \( \alpha \) and \( \beta \) chains is apparently required for both the transport of the receptor to the cell surface and the formation of the extracellular ligand-binding site located near the amino-terminal region of the integrin subunits. To date the amino acid sequences of 14 \( \alpha \) and 8 \( \beta \) subunits have been deduced from their corresponding nucleic acid sequences. The diversity of the integrin superfamly of receptors has been highlighted by the observations that individual \( \alpha \) subunits can pair with multiple \( \beta \) subunits (1, 2). In addition, several integrin subunits have been shown to be alternatively spliced at the cytoplasmic domain. They include \( \alpha 3 \), \( \alpha 6 \), \( \beta 1 \), \( \beta 3 \), and \( \beta 4 \) (3–8). The \( \alpha \) cytoplasmic forms of \( \alpha 3 \) and \( \alpha 6 \) have been reported to be phosphorylated on serine and tyrosine residues suggesting a possible differential regulatory and functional mechanism for the two forms (9, 10). In addition, alternative splicing in the extracellular domain of PS2c subunit has also been described (11). It was suggested that this alternative splicing in the extracellular domain may be important in determining the specificity and affinity of integrin receptors for their ligands (11).

Previously, we reported that human and mouse melanoma cells express a unique \( \alpha 7 \beta 1 \) integrin complex that bound to laminin yet contained an \( \alpha \) subunit biochemically distinct from all known \( \alpha \) subunits (2, 12, 13). The human receptor as well as the mouse homolog were purified, and the \( \alpha \) chain because of its unique NH2-terminal amino acid sequence was designated as \( \alpha 7 \) (13). This novel integrin complex, \( \alpha 7 \beta 1 \), has been shown to bind to the E8 fragment of laminin and mediate cell adhesion to this ligand (13). Recently, the cDNA for the rat homolog of \( \alpha 7 \) has been isolated, and its expression was shown to be developmentally regulated during skeletal myogenesis (14, 15).

In the present study we show that \( \alpha 7 \) is alternatively spliced both in the extracellular and cytoplasmic domains. The two extracellular isoforms, designated X1 and X2, of \( \alpha 7 \) are derived from the mutually exclusive splicing of the \( \alpha 7 \) mRNA. The X1 and X2 \( \alpha 7 \) MRNA's are differentially expressed in heart and skeletal muscle. We also show that the alternative splicing in the cytoplasmic domain of \( \alpha 7 \) gives rise to A and B variants that show similarity to those previously identified for the \( \alpha 3 \) and \( \alpha 6 \) isoforms (3–5). Furthermore, the expression of the \( \alpha 7 A \) form appears to be developmentally regulated in skeletal muscle. The existence of alternative splicing in \( \alpha 7 \) transcripts provides for a diverse set of integrin isoforms with structural and potentially functional differences.

**EXPERIMENTAL PROCEDURES**

**Cell Culture**—The mouse K1735 melanoma cell line was provided by Dr. J. Fidler (University of Texas M.D. Anderson Hospital and Tumor Institute). K1735 cells were maintained in Dulbecco's minimal essential medium (H-16) supplemented with 10% fetal bovine serum. Murine C2C12 and rat L8 skeletal myoblasts (from Dr. Douglas Cooper, UCSF) were grown in media composed of 20% M199, 80% horse serum. To induce myotube formation the C2C12 cells were plated at a density of \( 5 \times 10^6 \) cells/cm² on plastic dishes. The cultures were grown for 2
days before replacing the growth medium with Dulbecco's minimal essential medium containing 2% fetal calf serum. After 5 days >70% of the cells had fused to form myotubes as determined by phase microscopy.

RNA Isolation and Northern Blot Analysis—Total RNA from mouse myotubes was isolated by the guanidinium-isothiocynate/phenol method (16, 17). RNA samples (15 μg) were electrophoresed in a 1.2% agarose gel containing formaldehyde. After electrophoresis, the RNA was transferred to nylon membranes (Amersham Hybond N) by capillary blotting and fixed to the filter by exposure to UV light. The RNA was hybridized with a 700-bp PCR-amplified a7 cDNA fragment (see next paragraph) labeled with 32P using a Multiprime DNA labeling kit (Amersham Corp.). Hybridizations were carried out at 42 °C in 50% formamide, 5 × SSC, 5 × Denhardt's, 0.1% SDS, and 300 μg/ml salmon sperm DNA. Filters were washed twice in 1 × SSC, 0.1% SDS at room temperature and once at 65 °C in 0.1 × SSC, 0.1% SDS. Filters were exposed to x-ray film at −80 °C with intensifying screens.

Reverse Transcription and Polymerase Chain Reaction—Total RNA from cell lines, mouse neonates, and tissues was used for reverse transcription-polymerase chain reaction (RT-PCR). Briefly, the RNA sample (5 μg) was heated to 75 °C and then mixed with Moloney murine leukemia virus RNase H− reverse transcriptase (Life Technologies, Inc.), 1 mM dNTPs, 40 pmol of 5′ primer, 20 units of RNasin (Promega Corp.), 10 mM dithiothreitol, and 10 × PCR buffer (Perkin-Elmer Cetus Instruments) to a final volume of 20 μl. The reaction was incubated at 42 °C for 90 min, heated at 95 °C for 5 min, and then placed on ice. The reverse transcriptase and dNTPs were usually subjected to 30 amplification cycles (unless otherwise indicated) in 10 × PCR amplification buffer (Perkin-Elmer Cetus) containing 0.2 mM dNTPs, 40 pmol of the 5′ primer, 40 pmol of the 3′ primer, and 2 units of Taq polymerase (Boehringer Mannheim). For each set of PCR reactions a negative control, which contained the mixture above but lacked template, was always included. After denaturation for 5 min at 94 °C, the PCR reaction was amplified in a Perkin-Elmer Cetus thermocycler. Typically, cycles consisted of 1-min denaturation at 94 °C, 20-s annealing at 50–65 °C, and a 1-min extension at 72 °C. The final cycle was extended an additional 10 min at 72 °C and stored at 4 °C. Amplified fragments were analyzed in ethidium bromide-stained agarose gels.

The design and synthesis of the 5′ primer, used in the amplification of a 700-bp a7 PCR fragment probe that was subsequently used for Northern analysis and screening of the cDNA libraries, was based on the NH2-terminal amino acid sequence of the murine α7 subunit (13). A degenerate primer (A2AR), which is complementary to one of the highly conserved repeat regions (18), the sixth domain, of the α subunits, was used as the 3′ primer. Primers used in the amplification of X1 were anti-sense primer 5′-CTATCCTTGCGCATCGAGAATGAC-3′ and sense primer 5′-GTGACCAACATTGATAGAAATGAC-3′; primers used in the amplification of X2 were anti-sense primer 5′-CTATCCTTGCGCAGAATGAC-3′ and sense primer 5′-GCCAGGGTGGAGCTCTG-3′; primers used in the amplification of α-subunit were 5′-GCCAGGGTGGAGCTCTG-3′ and sense primer 5′-AGTGGGCAAGGATCCTAGCC-3′. PCR fragments from the above reactions were routinely subcloned into pCRII as per the manufacturer's instructions (TA cloning kit, Invitrogen, San Diego, CA) and sequenced to confirm the identity of each PCR product.

Amplification and Cloning of the 5′ End of α7 cDNA—Rapid amplification of cDNA ends as previously described (19) was used to isolate and clone the 5′-untranslated sequences and signal sequence of the α7 cDNA. Briefly, a reverse transcriptase reaction, as described above, was performed using 5 μg of total RNA from 50 μl of a 50-mer antisense primer (5′-CCCTCTGAGCGGTATTGC-3′) and a 50-mer sense primer (5′-GTGACCAACATTGATAGAAATGAC-3′). The membranes were washed twice in 2 × SSC/0.1% SDS at room temperature and then twice for 20 min at 55–60 °C in 0.1 × SSC and 0.1% SDS. The membranes were then exposed to x-ray film at −80 °C with intensifying screens.

Sequence Analysis—All nucleotide sequences were determined by the dideoxy chain termination methods (21) for double-stranded DNA, using dATP-5'-α-32Pthiophosphate (Amersham Corp.) and a modified T7 DNA polymerase (Sequenase 2.0; U. S. Biochemical Corp.). Primers for sequencing and PCR were synthesized and purified at the Biomedical Resource Center, University of California at San Francisco. Greater than 80% of both strands were sequenced from mouse heart cDNA clones 11a and 7a. Sequences were obtained from both strands of all other cDNA, PCR, rapid amplification of cDNA ends, and genomic clones. Any ambiguous regions were resolved using the nucleotide sequencing triphosphate. Sequence data were analyzed using DNA Strider 1.0 and MBIR programs (Baylor College of Medicine).

RESULTS

Nucleotide and Deduced Amino Acid Sequence of Mouse Heart a7 cDNA—We have previously identified and characterized a novel laminin-binding integrin designated α7β1 (2, 12, 13). Northern analysis indicated that heart tissue, and to a lesser extent skeletal muscle, expressed α7 mRNA (data not shown). To study the α subunit in more detail we isolated the cDNA for α7 using a mouse heart library and C2C12 myoblast library (see "Experimental Procedures"). The nucleotide and deduced amino acid sequence for the α7 subunit, derived from

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1 The abbreviations used are: bp, base pair(s); kb, kilobase pair(s); RT-PCR, reverse transcriptase-polymerase chain reaction; PCR, polymerase chain reaction.
overlapping mouse heart cDNA clones, is shown in Fig. 1. The approximately 3700-nucleotide sequence of the α7 cDNA, included one open reading frame, lacked the 5′-untranslated sequences and the nucleotides encoding the signal sequence. Because a full-length cDNA clone could not be identified from the two libraries screened here, rapid amplification of cDNA ends (see “Experimental Procedures”) was used to synthesize and clone the 5′ portion of the α7 cDNA. DNA sequence analysis of this fragment demonstrated that it contained 170 bp of the 5′-untranslated region of α7 as well as the nucleotides encoding the signal sequence (Fig. 1) and the NH2-terminus of the mature peptide.

Fig. 1. Mouse α7 cDNA sequence and deduced amino acid sequences. Nucleotides and amino acids are numbered from the start (position number 1) of the mature peptide; negative (−) numbers identify the signal sequence amino acids and the untranslated sequences. The three potential cleavage cation-binding domains are shaded. The likely post-translation proteolytic cleavage sites (RRRRE and RRRQ) are underlined, the putative transmembrane domain is underlined with *, and the stop codon is marked with •. The sequence data are available from GenBank under accession number L23423.
the identification of the two nonhomologous sequences for the III/IV segment in the L8 myoblast and heart α7 cDNAs suggested that the sequence of the III/IV segment is encoded by two alternatively spliced exons. This view is supported by the finding that the αPS2 fly integrin subunit is also alternatively spliced in this same variable region (11).

To prove the existence of alternative splicing in the III/IV segment of α7 it is important to determine if separate exons for the regions in question are contained within the α7 gene. Therefore, a mouse genomic library in λFix II was screened with a mouse cDNA fragment, which contained sequences that overlapped the III–IV repeat domain. One positive clone was isolated, plaque-purified, and determined to be ~17 kb in size. A subclone (designated clone G9), which by Southern blot analysis was shown to contain sequences between repeats III–IV, was examined further.

Clone G9 was directly sequenced to identify the intron/exon organization of the region spanning repeats III and IV in the α7 gene (Fig. 3, A and B). The sequence for repeat III domain is contained in a single exon of 194 bp, which is followed by a ~800-bp intron. Adjacent to this intron is an open reading frame of 132 bp that encodes an amino acid sequence (43 residues) with a high degree of homology to the published sequences of the III/IV segments of human, mouse, and chicken α6 and is identical to the corresponding region reported for the rat L8 myoblast α7 (3, 15, 22, 26). We have designated this exon X1, for extracellular (Fig. 3, A and B, and Fig. 4). Exon X1 is followed by nearly 400 bp of intronic sequences and a second exon. This exon (designated X2) encodes for a 39-amino acid segment that is completely distinct from the X1 sequence and the III/IV segment of α6 but is identical to the connecting segment between the repeat domains obtained from the mouse heart cDNA clones (Fig. 4). Thus, X1 codes for four more additional amino acids than the X2 exon; depending on which exon is spliced in, these result in mature proteins of 1196 and 1102 amino acids, respectively. Exon X2 is separated from a 205-bp exon that encodes repeat domain IV by a ~900-bp intron. Each of the intron/exon boundaries analyzed conformed to the consensus 5' and 3' splice sequences, GT/AG (27) (Fig. 3B). These results establish that the alternative forms of α7 obtained from the L8 myoblast and heart cDNAs arise through the mutually exclusive splicing of either one of two adjacent but distinct exons, X1 or X2.

Evidence for Alternative Splicing of α7 in the Extracellular Domain—Sequence analysis of the heart α7 cDNA revealed an unusual 43-amino acid stretch between repeats III/IV in α7 that was essentially nonhomologous to the corresponding region in α6 and differed in length by four amino acids (Fig. 2, amino acid residues 194–227). All four α7 cDNA clones from two different libraries contained identical sequence overlaps in this region. Furthermore, a human α7 cDNA clone from a heart library also encoded the same exact sequence in this segment between the III and IV repeats (see below). The recently published sequence for rat α7, derived from an L8 myoblast cDNA library, had a completely different sequence for the III/IV repeat segment, which in contrast to our sequence was highly homologous at the amino acid level to the corresponding region in α6 (15). Additionally, this region in the L8 myoblast cDNA was 4 amino acids longer than that in the heart α7 cDNA. A second region of approximately 19 amino acids (residues 45–64) located in repeat I of the heart α7 cDNA was also strikingly different in the L6 myoblast α7 cDNA. Our sequence has been confirmed in both a library-derived α7 cDNA clone and a genomic α7 clone (15).

The remaining amino acid sequence deduced from the heart α7 cDNA shared greater than 91% identity with that reported for the L8 myoblast α7 cDNA (15). The lack of homology in the variable regions of α6 and α7 in the III/IV segment and

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α7 subunit (13, 15). The sequences surrounding the presumptive initiator codon for α7, GATCCCATGG, are very similar to that identified in the mouse and human α6 subunit (3, 22). The context of such initiator sequences is considered rare and is thought to play a role in modulating the yield of proteins that exhibit such unfavorable initiation sites (23). We have subsequently confirmed the rapid amplification of cDNA end results by sequencing the corresponding region contained within a 17-kb mouse genomic clone containing the α-promoter region. Translation of the α7 cDNA indicated that the amino terminus of the mature protein is preceded by a signal sequence of 34 amino acids. The mature protein is comprised of 1102 amino acids, which differs from the 1060 amino acids reported by Song et al. (Ref. 15 and see below). The extracellular domain consists of 1002 amino acids followed by a single hydrophobic stretch of 24 amino acids that represents the transmembrane domain. Two putative pro tease cleavage sites, RRRRE and RRQ, are located in the extracellular domain (Fig. 1, underlined residues). In addition, there are only five potential N-linked glycosylation sites (Fig. 2, arrow marked residues). The cytoplasmic domain (77 amino acids residues) of this α7 cDNA clone shows strong homology to the α3 and α6 B cytoplasmic variants (3, 5) indicating that this clone is the B cytoplasmic isoform of the α7 subunit (see below).

The overall amino acid homology of α7 is the highest with the α6 subunit (47% identity) followed by α3 (37% identity; Fig. 2). When the deduced amino acid sequences of α3, α5, and α6 are aligned with that of α7 (Fig. 2), regions of strong and weak homology, especially in the extracellular domain, are revealed. All α subunits contain seven conserved repeat domains (I–VII) in the extracellular segment, which share 25–52% identity (24, 25). The homologies of the repeat domains in α6 and α7 are particularly striking, averaging >86% identity when the two subunits are compared. Most of the variable region segments of α6 and α7, which are located between the repeat domains, also show strong homology. These segments of α6 and α7 between repeats I/II, II/III, IV/V, V/VI, and VII/ VIII have an average identity of 50% (ranging from 34.5 to 64%).

Evidence for Alternative Splicing of α7 in the Extracellular Domain—Sequence analysis of the heart α7 cDNA revealed an unusual 43-amino acid stretch between repeats III/IV in α7 that was essentially nonhomologous to the corresponding region in α6 and differed in length by four amino acids (Fig. 2, amino acid residues 194–227). All four α7 cDNA clones from two different libraries contained identical sequence overlaps in this region. Furthermore, a human α7 cDNA clone from a heart library also encoded the same exact sequence in this segment between the III and IV repeats (see below). The recently published sequence for rat α7, derived from an L8 myoblast cDNA library, had a completely different sequence for the III/IV repeat segment, which in contrast to our sequence was highly homologous at the amino acid level to the corresponding region in α6 (15). Additionally, this region in the L8 myoblast cDNA was 4 amino acids longer than that in the heart α7 cDNA. A second region of approximately 19 amino acids (residues 45–64) located in repeat I of the heart α7 cDNA was also strikingly different in the L6 myoblast α7 cDNA. Our sequence has been confirmed in both a library-derived α7 cDNA clone and a genomic α7 clone (15).

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reported human and hamster amino acid sequences for α3 show significant homology with these conserved regions and alignments were optimized slightly by hand. The seven conserved repeat domains are (Roman numerals), and alignments were optimized and alignments were optimized.

Fig. 2. Alignment of the amino acid sequences of α3B, α5, α6, and α7B integrin subunits. The deduced amino acid sequences of the related hamster α3B (28), human α6B (3, 60), human α5 (61), and mouse α7 are compared by computer-generated alignments. To maximize homologies, gaps were introduced. The seven conserved repeat domains are indicated with boxed.
consensus regions in X1 are distinct from those in X2 suggests that these may be sites of functional divergence for the two isofoms.

Expression of the X1 and X2 Extracellular Alternative Splice Forms in a7 mRNA—To estimate the relative abundance of X1 and X2 alternatively spliced forms we used RT-PCR analysis. After isolation, total RNA from various tissues and cultured cell lines was reverse transcribed, and the reaction was divided in two and then amplified by PCR with a primer specific to exon IV and a primer specific to either of the alternative exons (X1 or X2). The primer site in X1 was near the 5' end of the exon, while the binding site of the primer to X2 was near the middle of this exon (see “Experimental Procedures”). Thus, when amplified, the X-1-IV primer pair resulted in a product of ~220 bp, while primer pair X2-IV generated a 200-bp fragment. In addition, because the binding sites of the primer pair are located in separate exons, potential amplification of contaminating DNA is identified as bands >1 kb. These fragments were readily identified when electrophoresed through a 2% agarose gel (Fig. 5). This assay assumes that the efficiency of PCR amplification for these two sequences is similar and that saturation of the products has not occurred using limited rounds of PCR amplification. The latter was confirmed by analyzing the products after 22, 25, and 30 cycles of amplification (data not shown).

Adult mouse heart and lung strongly express both mRNA forms of a7 with somewhat higher levels of X2 (Fig. 5). In contrast, for adult mouse skeletal muscle the X2 transcript was exclusively expressed (Fig. 5). Neither a7 variant could be detected in RNA isolated from mouse small intestine (Fig. 5). Overall, adult mouse skeletal muscle consistently had lower levels of PCR-generated X2 fragments than cardiac muscle; this agrees with the results for total a7 mRNA transcripts in these tissues assessed by Northern analysis (data not shown). Proliferating rat L8 and mouse C2C12 skeletal myoblasts expressed approximately equal levels of X1 and X2 (Fig. 5). PCR analysis indicated only a slight increase in the ratio of X2/X1 in C2C12 myotubes (not shown). Identical results were obtained for all tissue samples and cultured cells when the PCR reactions were repeated using poly(A) RNA.
repeats I11 and IV from the hamster and human a3 cDNAs (3x2~ and CY~X~~), respectively (28, 29, 30), chicken and mouse a6 cDNAs

Conservative changes are defined as follows: (I,

a7 chain, obtained from lysates of various cell types, after analysis by SDS-polyacrylamide gel electrophoresis and Western blotting (data not shown).

To verify the PCR results, products were routinely subcloned into pCR-11, and the ends of each fragment were sequenced. In addition, to confirm that the mRNA for a7 X2 variant exists for a7, a set of PCR primers specific for a7 was designed with the 5' primer corresponding to nucleotides 3198-3218 and the 3' primer directed to nucleotides 3546-3566 (Fig. 6B). This set of primers flank a region 5' to the transmembrane domain and to the end of the coding sequences of a7. A series of rodent tissues and cell lines was examined by RT-PCR using these primers. A major band of ~370 bp was usually present after RT-PCR, to varying degrees, in a number of tissues and cell types examined (Fig. 6A). This PCR product represented the B form of a7 as determined by sequencing analysis. A second band of ~480 bp, which was determined to be homologous to the A cytoplasmic isoform, was present in differentiated C2C12 mouse myoblasts, adult mouse muscle, and whole mouse neonates (Fig. 6A). RNA from proliferating undifferentiated L8 and C2 myoblasts also exhibited the A isoform at much lower levels, while adult heart and mouse melanoma cells (K1735) had undetectable levels of the A form after amplification. An additional band, which ran slightly above the 480-bp band in cells expressing the A isoform, was shown to be a heteroduplex resulting from re-annealing single strands of the 370- and 480-bp fragments. A similar hybrid band was seen in a3 and a6 (3, 5). The 480-bp fragment was subsequently cloned, sequenced, and found to encode a unique amino acid sequence (57 residues) that was distinct from a7B yet showed strong homology to the cytoplasmic A isoforms of a3 and a6 (Fig. 6, B and C). As with a6A and a3A, the A isoform of a7 represents an insertion immediately after the transmembrane domain (Fig. 6B). However for a3 and a6, the entire cytoplasmic A isoform appears to be generated by a putative exon that is either spliced in or out (3, 5). In contrast, the A form of a7 is encoded by a contiguous sequence, which includes not only the 113-bp alternatively spliced segment but also a 59-bp frame shifted sequence that is shared by the a7B form.

Alignment of the cytoplasmic domain sequences for the A and B forms of a3, a6, and a7 reveals major areas of sequence similarity within each variant group (Fig. 6C). While all a chains share a homologous GFFKR sequence near the transmembrane domain, there is little if any cross-isoform identity. The A forms have extensive homology particularly between a3 and a6 and, to a lesser extent, a7. Common to all three is the sequence KAXXXQPS. For the B forms the common sequence PXYHAVXIXKEER is present and of particular

3 B. L. Zoiber, J. Crawford, and R. H. Kramer, unpublished observations.
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**A**

![Image](image1)

**B**

SLEVIVRANITYKSSIKNLRLDASTVIVPVMYY

2872: tcocgtgacgcgtgcaacactacatagtgaatctcactaagaactttctttggttggcagcatccactacacttgcagttctgtgttac
LDPMAVVEGVFVVWVILGLGLVLVLALLVL

2971: tggacoccatggtccttgagaagagctccattcgtggctatctcctgggaagctctggctgggtggtttgcttagctgctggctgggtcgcctc
3070: tggagaaattggttttctgtaagctgcaagactttcatccaaacacttaacagcagcagcatctggctggctggctggctgggtcgcctc
3169: tggagaaattggttttctgtaagctgcaagactttcatccaaacacttaacagcagcagcatctggctggctggctggctgggtcgcctc
3268: tggagaaattggttttctgtaagctgcaagactttcatccaaacacttaacagcagcagcatctggctggctggctggctgggtcgcctc
3367: tggagaaattggttttctgtaagctgcaagactttcatccaaacacttaacagcagcagcatctggctggctggctggctgggtcgcctc

**C**

**Fig. 6. Identification of α7A and α7B mRNA isoforms by RT-PCR.** A, RT-PCR was performed as described under "Experimental Procedures" (amplification 35 cycles) on total RNA isolated from a mouse K1735 melanoma cell line, mouse C2C12 myoblasts, C2C12 myotubes, rat L8 myoblasts, mouse adult skeletal muscle, and whole mouse neonates. The ~370-bp fragment corresponds to the B cytoplasmic domain isoform. The ~480-bp fragment corresponds to the A cytoplasmic domain isoform. A third band that runs above the 480-bp fragment is a heteroduplex (see text). Markers are a 100-bp ladder (200–1000-bp markers are shown). The PCR products were separated on a 2.0% agarose gel and stained with ethidium bromide.

B, the nucleotide sequence of the α7 A and B cytoplasmic domains. The 113-bp insert sequence present in α7A but not in α7B is boxed. Primers used in the amplification of the PCR products seen in A are underlined.

The single amino acid sequence is shown above each codon; amino acid residues for the A and B isoforms are indicated; amino acid residues for the transmembrane domain are in italics, and stop codons are marked with (*). The sequence data are available from GenBank under accession number L23421. C, alignment of the amino acid sequences of the α3, α6, and α7 A and B cytoplasmic isoforms. The deduced amino acid sequences of the related A and B isoforms of α3, α6 (3, 5), and α7 are compared. Similar residues, either identical or conservative substitutions, in two or more α chains are shaded. Conservative changes are defined as in Fig. 4. Amino acid residues common to α3, α6, and α7 isoforms are marked (†).
interest because it contains a high density of charged and mostly acidic amino acids. Further, toward the COOH-terminal end are several additional areas of sequence overlap, which are also charged residues. The long additional segments found in the COOH terminus of aIIb and B share no homology to the isoforms of a3 and a6 or to each other. Finally, a7A has several potential tyrosine and serine phosphorylation sites that may be functionally important.

**DISCUSSION**

We have provided evidence that the a7 integrin subunit is alternatively spliced in both the extracellular and cytoplasmic domains. This allows for the generation of variant a7 subunits that are structurally and presumably functionally distinct. Furthermore, the pattern of alternative splicing at both sites appears to be developmentally regulated and tissue specific. This is the first example of alternative splicing in the ligand-binding domain in a vertebrate integrin subunit. On the other hand, the alternative spliced segments at the cytoplasmic domain of a7 are analogous to that previously described for the A and B forms of a3 and a6, confirming that these subunits belong to a closely related integrin subfamily. The differential pattern of alternative splicing of a7 during skeletal muscle development indicates that different regulatory mechanisms are operational for X1/X2 and A/B isoforms.

**Alternative Splicing of a7 in the Extracellular Domain**—The evidence supporting the existence of the alternatively spliced region between conserved repeats III–IV in the extracellular domain is substantial. Sequences corresponding to the X1 and X2 segments have been cloned from mouse (Fig. 1), human (Fig. 4), and rat cDNA libraries (15). More importantly, we have confirmed the presence and ordering of the X1 and X2 segments in a genomic clone (Fig. 3). It is clear that the two different extracellular isoforms of a7 arise through the mutually exclusive splicing of either one of two adjacent exons, X1 or X2. Finally, we have detected the expression of the X1 and X2 forms by RT-PCR in mRNA isolated from tissues and cultured cells.

The process of alternative splicing in the III/IV variable region appears to be developmentally regulated. The evidence for this was provided by estimating the relative levels of X1 and X2 in transcripts from tissues and cell lines using RT-PCR (Fig. 5). In adult mice, the relative ratio of X1 to X2 was tissue specific with some tissues lacking significant levels of a7 mRNA (e.g. intestine) while others had comparable levels of X1 and X2 (e.g. heart) or a predominance of X2 (lung). Undifferentiated myoblast cells had similar levels of X1 and X2, but only the X2 form was detected in mature skeletal muscle.

Two other a subunits have recently been shown to be alternatively spliced in the extracellular domain. A 102-bp exon was determined to be either spliced in or out of the transcripts for a1b mRNA (31). This splicing event occurred in the extracellular domain just 5' of the transmembrane region. The Drosophila aPS2 subunit that complements with the bPS subunit and binds vitronectin (32) has been shown to be alternatively spliced between domains III and IV (11). However, unlike a7, alternative splicing in aPS2 involves splicing into an internal splice acceptor site, which results in the deletion of a portion of the exon. These examples of alternative splicing in the extracellular domain of several different a subunits suggest that such processing of a transcripts may be more widespread than previously believed. In support of this conclusion, we found that a6 (Fig. 4) is also alternatively spliced in a similar manner between the III–IV domains to yield X1- and X2-like isoforms. The published a3 subunit contains an X2-like sequence (Fig. 4) at the III/IV segment, which suggests that this integrin may also be alternatively spliced at this site.

We can only speculate as to how alternative splicing at X1/X2 could influence integrin function. We hypothesize that this region is involved in defining ligand specificity and/or affinity. Several observations support this idea. The III–IV domain segment interacts with the metal-binding sites that may be functionally important. In support of this, we found that a6 and a7 integrins that bind laminin exclusively (13, 36–38). Both a6 and a7 bind specifically to the E8 fragment of A-chain laminin. Yet the two integrins differ remarkably in their affinity for laminin. Whereas a7B1 efficiently binds to immobilized laminin, a6B1 binds poorly and readily elutes with physiological salt concentrations (2, 3, 12, 13, 39). We suspect that the alternatively spliced X1/X2 segment is one region that is important in defining these activities. Preliminary evidence obtained with RT-PCR indicates that the X1 form of a6 is more commonly expressed in cultured cells, and consequently it is this form that has been identified in cDNA libraries (3, 5) also derived from cultured cells. For the a7 subunit isolated from melanoma cells, it is the X2 form that predominates, and it is this form that binds with high affinity to laminin as detected with an anti-X2 antibody.

Moreover, X1 and X2 may differ not only in their affinity for laminin but in their specificity for laminin isoforms. This possibility is particularly important because of the number of tissue-specific laminin isoforms that have recently been identified, including s-laminin (40), merosin (41), s-merosin (42), k-laminin (43), and kalinin or epiligrin (44, 45). It is now evident that integrin receptors bind differently to these macromolecules. Both a3 and a6 have been shown to bind different laminin isoforms including A-chain laminin (30, 38, 46–48) and epiligrin/kalinin (45, 49, 50). Moreover, a3 can apparently interact with fibronectin (30, 51, 52), collagens (30, 51, 52), and entactin (53) and is also involved in cell-adhesion (54). Such a diverse range of ligand binding properties could be related to the existence of X1/X2 isoforms for a6 and probably for a3.

**Alternative Splicing of a7 in the Cytoplasmic Domain**—The cytoplasmic domains of integrins interact with the cytoskeleton and potentially other cytoplasmic proteins. It is through this interface with the cytoplasmic compartment that signals are transmitted to the cell. In addition, it is now apparent that functionality of the extracellular domain of the integrin is influenced by "inside-out signaling" (55). It is also generally believed that the a cytoplasmic domain may regulate the b cytoplasmic tail interactions with the cytoskeleton (56).

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*R. H. Kramer, unpublished observations.*
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