Structure of the Bovine Elastin Gene and S1 Nuclease Analysis of Alternative Splicing of Elastin mRNA in the Bovine Nuchal Ligament[†,‡]

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ABSTRACT: Genomic clones encompassing all the translated sequences, the 3′ untranslated sequence, and 1 kb flanking the ATG translation initiation codon of bovine tropoelastin have been obtained and characterized by restriction enzyme analysis and extensive DNA sequencing. These analyses demonstrated that functionally distinct hydrophobic and cross-linking domains of the protein are segregated into separate exons throughout the gene. The putative promoter region lacks a TATA box, has an extremely high G+C content, and contains several SP1 binding sites. Comprehensive S1 analyses using probes covering the entire mRNA and RNA isolated from the nuchal ligament of bovine fetuses of different ages, neonate calves, and adult cows demonstrated that while only a single exon is alternatively spliced at high frequency, many exons are alternatively spliced at limited, variable frequencies. The results also suggest that such limited splicing is increased in the adult tissue relative to fetal and neonate tissues.

The generation of variant forms of a protein may be achieved by the existence of a family of genes coding for related but distinct proteins or by alternative splicing of the primary transcript of a single gene. Recent studies have demonstrated that both mechanisms are utilized in creating diversity within extracellular matrix proteins. In the case of collagen, a large superfamily of genes has evolved, composed of at least 20 distinct members whose gene products combine 3 at a time to form at least 12 different collagenous molecules (Martin et al., 1985). Alternative splicing has been shown to occur in more than 50 genes (Breitbart et al., 1987) including the extracellular matrix proteins fibronectin (Ruoslahti, 1988) and elastin (Yeh et al., 1987; Raju & Anwar, 1987; Indik et al., 1987a,b). In the case of fibronectin, alternatively spliced exons differentiate the cellular and plasma forms.

Sequence analyses of elastin cDNA prepared with either bovine nuchal ligament mRNA (Yeh et al., 1987; Raju & Anwar, 1987) or human fetal aorta mRNA (Indik et al., 1987a) have identified 8 exons that are subject to alternative splicing. However, cDNA sequencing does not yield quantitative information regarding the frequency of particular splicing events nor does it guarantee that all events have been detected. In order to obtain information on these important points, we have completed the isolation of the bovine elastin gene and have carried out nuclease protection experiments using mRNA isolated from the nuchal ligament of bovine fetuses of different ages, neonate calves, and adult cows. The results demonstrate that while only a single exon is alternatively spliced at high frequency, many exons are alternatively spliced at limited, variable frequencies. The results also suggest that such limited splicing is increased in the adult tissue relative to fetal and neonate tissues.

Materials and Methods

Isolation of Genomic Clones and DNA Sequencing. Overlapping genomic clones were isolated from a bovine library constructed by the insertion of genomic DNA partially digested with *Sau*3A into the *Bam*HI sites of Charon 30. Initial screening using a 0.5-kb fragment located near the 5′ end of the previously described cDNA clone, cBEL1 (Yeh et al., 1987), resulted in the isolation of clone BEL5. Further screenings were then carried out with a 0.3-kb *Bam*HI/*Hin*dIII fragment free of repetitive sequences that was located near the 5′ end of BEL5. This resulted in the isolation of clones BEL4 and BEL6. Although further screenings of libraries were carried out, the most 5′ end of the gene proved elusive and was finally isolated as follows. A 27-base oligonucleotide encoding a portion of the signal peptide at the 5′ end of the cDNA was used as the probe in Southern analysis of bovine genomic DNA restricted with *Hin*dIII in order to identify the size of the DNA restriction fragment bearing the 5′ sequence. A hybridizing 8-kb fragment was then eluted from a preparative gel and cloned into the λ Zap vector (Stratagene). Desired transformants were identified by using the same probe. The relative positions of the analyzed clones are diagrammed in Figure 1.

DNA sequencing was performed by the dideoxy method (Sanger et al., 1977) using restriction fragments cloned into appropriate M13 or pUC plasmid derivatives. Primer oligonucleotides, synthesized by using a modification of the phosphite method of Matteucci and Caruthers (1981) and employing a MilliGen 7500 automated DNA synthesizer, were purified by reverse-phase chromatography.

S1 Mapping. Bovine nuchal ligaments were obtained at the slaughterhouse and immediately frozen in liquid nitrogen. In the laboratory, the tissue was pulverized in liquid nitrogen and

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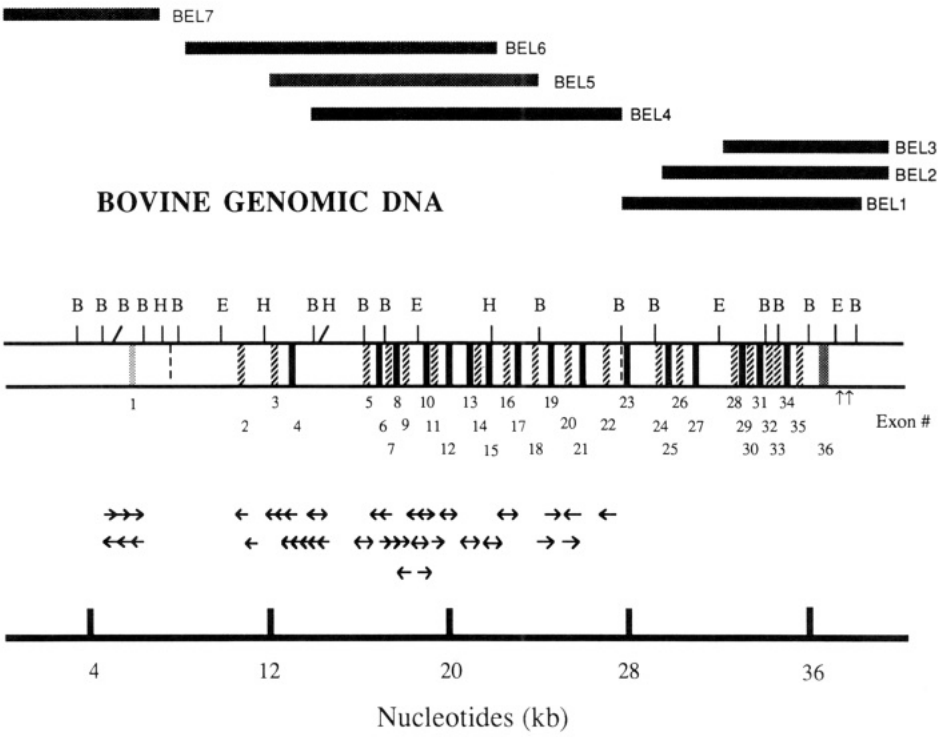

FIGURE 1: Diagram of bovine elastin gene including positions of genomic clones and sequencing strategy. Genomic clones (horizontal solid bars), isolated as described under Materials and Methods, were subjected to restriction enzyme analysis and sequenced by the deoxy method using oligonucleotides synthesized and purified in the laboratory as primers (→). Exons are not drawn to scale and have been numbered starting at the 5′ end of the gene since all coding sequences have been identified: exons encoding potential cross-linking domains (solid bars); exons encoding hydrophobic domains (crosshatched bars); exon encoding carboxy terminus (stippled bar); potential gap in intron (dashed bars). Restriction enzyme sites: B, *Bam*HI; E, *Eco*RI; H, *Hin*dIII. (↑) Polyadenylation sites.

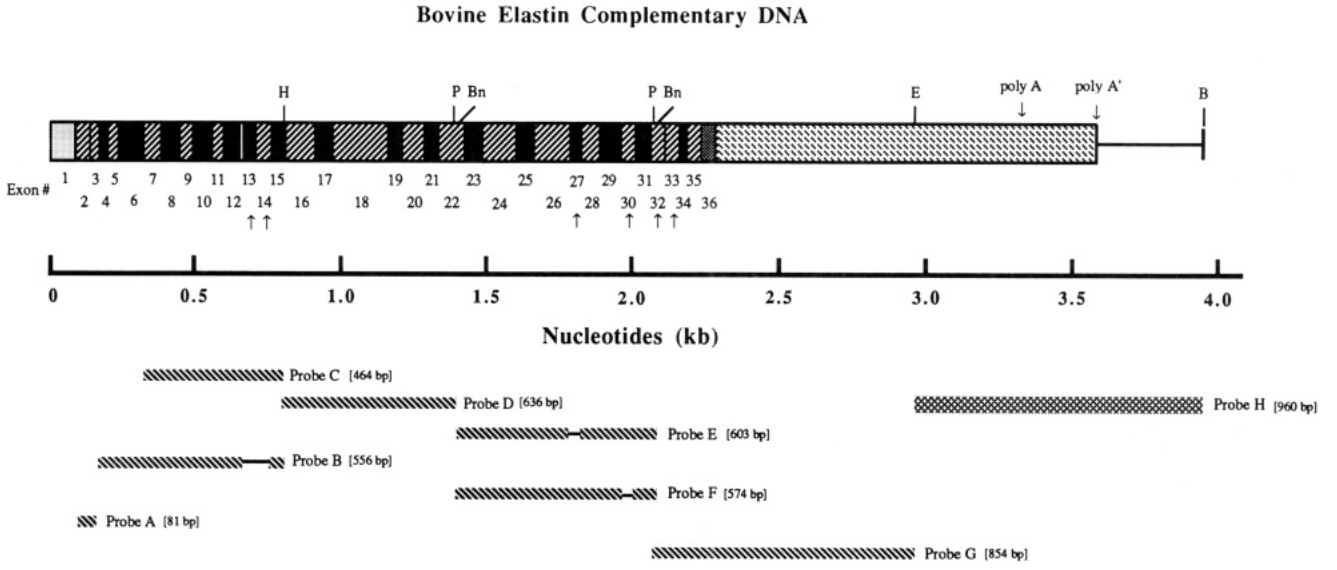

FIGURE 2: Composite diagram of bovine cDNA clones. The cDNA is divided into exons which are numbered: exons encoding hydrophobic sequences (heavy crosshatched bars); exons encoding potential cross-linking sequences (solid bars); exon encoding the signal sequence (light stippled bar); coding portion of carboxy-terminal exon (heavy stippled bar); untranslated portion of carboxy-terminal exon (light crosshatched bar). Arrows mark the exons subject to alternative splicing above a trace level (see Table I). The restriction fragments labeled B–G, isolated from cDNA clones, were used as probes for the S1 nuclease protection experiments as was a 81-mer synthetic oligonucleotide labeled A and the genomic fragment labeled H. Deletion of alternatively spliced exons in some probes is indicated (crosshatched bars at bottom of figure). Restriction enzyme sites: H, *Hin*dIII; P, *Pst*I; Bn, *Ban*I; E, *Eco*RI.

RNA isolated by the guanidine isothiocyanate/CsCl gradient method (Chirgwin et al., 1979). S1 nuclease digestion was carried out by a modification of previously described methods (Lompre et al., 1984). The concentration of elastin mRNA in individual RNA samples was estimated by dot blot hybridization using as the probe a synthetic 35-base oligonucleotide corresponding to a unique sequence in exon 36, an exon that does not appear to be alternatively spliced. For the S1 analyses, restriction fragments were isolated from previously sequenced cDNA or genomic (encoding the 3′ untranslated region) clones and used as probes. In addition, a synthetic 81-mer oligonucleotide corresponding to the amino-terminal portion of tropoelastin was also used as a probe. Collectively, these probes spanned the entire length of the cDNA, and their positions are diagrammed in Figure 2. Labeling of the 5′ end of the strands complementary to the mRNA was achieved by using T4 kinase, and the Klenow fragment of DNA polymerase was used for 3′ end labeling. Each restriction fragment probe

was labeled separately at each end for individual experiments in order to confirm the results. An estimated 5–10-fold molar excess of end-labeled DNA probe (^{32}P specific activity $5 \times 10^5 - 5 \times 10^6$ cpm/μg) was mixed with total ligament RNA, and the resultant solution was precipitated with ethanol. The pellet was resuspended in 6 μL of 2 M NaCl, 0.2 M PIPES, pH 6.5, and 5 mM EDTA, and 24 μL of formamide was added to a final concentration of 80% (final RNA concentration 0.06–3 mg/mL). Hybridization was performed at 2–4 °C above the T_m for DNA–DNA hybridization (45–60 °C range determined empirically for each probe) for 16 h and terminated by rapid addition of 9 volumes of 0.28 M NaCl, 0.05 M sodium acetate, pH 4.6, and 4.5 mM $ZnSO_4$. Eighty units of S1 nuclease was added per milliliter of digestion, and samples were incubated at 30 °C for 30 min. Ammonium acetate and EDTA were added to final concentrations of 0.4 and 0.01 M, respectively, to stop the reaction. After phenol/chloroform extraction, carrier tRNA was added, and the nuclease-resistant products were precipitated with 0.6 volume of 2-propanol. The nucleic acid pellet was rinsed with 70% alcohol and dried in vacuo and the nucleic acid subjected to polyacrylamide gel electrophoressis under denaturing conditions. Following autoradiography of the gels, the sizes of the S1-resistant bands were estimated by comparison to appropriate standards. The amounts of specific RNA fragments in the samples were estimated by densitometry and comparison to standard amounts of the probe electrophoresed on the same gel.

Results

Structure of the Bovine Elastin Gene. In order to correctly and precisely interpret the putative alternative splicing events, it is necessary to know the exact exon–intron structure of the entire bovine elastin gene. We have previously published the structure of 10.2 kb of the 3′ end of the gene which contained 14 exons (Cicila et al., 1985; Yeh et al., 1987). To complete the gene characterization, bovine genomic libraries were repetitively screened to obtain overlapping clones extending in the 5′ direction. Five clones, selected for detailed study, were analyzed by restriction enzyme mapping, followed by DNA sequencing as described in Figure 1. Correlation of the genomic sequence with the previously determined cDNA sequence permitted definition of the remainder of the genomic structure. In all, 22 exons encoding 503 amino acid residues were identified in these new clones. Thus, the bovine elastin gene consists of 36 exons contained in at least 32 kb of genomic DNA. Since all known coding sequences have been identified, the exons have been renumbered starting at the 5′ end of the gene. Two intron regions (as marked in Figure 1) have not been completely defined: (i) a gap exists in intron 1 since overlapping clones were not obtained; (ii) intron 22 may be larger than indicated since overlapping clones spanning the *Bam*HI restriction site within this intron were not obtained. However, these minor ambiguities do not compromise the analyses presented below.

Exon Description. A striking feature of the gene is the small size of the translated exons (27–186 bp) which are interspersed in large expanses of introns. The intron to coding ratio is about 15:1, which is very large even in comparison to other extracellular matrix proteins such as the fibrillar collagens, which have ratios of 8:1 (Boedtker et al., 1983). Another important characteristic of the elastin gene is that coding sequences corresponding to hydrophobic and cross-linking domains of the protein are found in separate exons, as indicated by crosshatched and solid bars in Figure 1. Except at the ends of the gene, these two types of exons alternate. Thus, the

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ctgatgcccc agggaatgac accaacaggg tctcaacccc caagccatac   -851
tgcccgcagc cacctggcct ctgcagaccc ggatcctaga ccaacccatc   -801
acccctccc cagcctcagg caggaggctg tgaccttaac ccacagagaa   -751
tggagccctg cagggcatca gggtcccctt ccctgcaccc agatccgcac   -701
gggggcagca gcaacacagt ctcaccttcc cagccattct gcccacagtc   -651
ccttgacctc tccagaccct ggcctggatg gaccccagac cccagccccc   -601
tccccagctt tggcctgtct ctggccgggt ctgggagtgg tgagggagtc   -551
tggagggctg gggtgggggg gcagaggcgc aggacagctg gcctgtgtcc   -501
ccacactggg cccgggggcc agccggagGG GCGGGGGCTG GCCACTCAGG   -451
cttggctgga gccggatttt tggccgggct gcagggccct ccctcctgct   -401
tcctctcccg agggctgtcc tggcagaggc ccccctcaca ctttctggcg   -351
ggaacagggc cagcagcgaa agaacagccg cagagggaaa gcgggagaga   -301
gatgggggga aactgtgtgt gtgtgtgtgt gcgcgtgtgt atgtgcgcgc   -251
gtgtgtttta agggAAAAAA AAAAAAAAAG agcccacagt ccagtccagt   -201
cagacccagc ctgggaggct tgcgagccgg cctttcgtaa ttgccccctc   -151
cccgtggccc cctccccgag gcctcccccc tctcccgccc tccccgcccg   -101
cctctctccc tccctccttc cctcgcagcc gacgaggcag caattacgct   - 51
ttggggataa aacgaggcgc agagagccgg ctggggcatt tctccccgag   -  1

ATG  GCG  GGT  CTG  ACG  GCT  GCG  GCT  CGG  CGG          + 30
Met  Ala  Gly  Leu  Thr  Ala  Ala  Ala  Arg  Arg

CCC  GGA  GTC  CTT  CTG  CTC  CTG  CTG  TGC  ATC          + 60
Pro  Gly  Val  Leu  Leu  Leu  Leu  Leu  Cys  Ile

CTC  CAG  CCC  TCG  CAG  CCC  GGA  G                     + 82
Leu  Gln  Pro  Ser  Gln  Pro  Gly

gtaggcctcc ccctgttccc caaggcccgc agcccttggg accgcccgct   +132
caggtggctg gaccgaccga caagaggctc cctgccctcc cagcaccctc   +182
cagggctccc aggcaggagc ccctcagcca ctggaccctc ggaaccaccc   +232
tcccgggagc cagccgtgcg gagcagaaac ctgtcatacg ttcgccccga   +282
gactcagaat ggtgatggtc ccagatcagc gccggcttct gctgccccga   +332
cagagtggat gaaccttgct ctgtcctggg gggctctccc aggatgggga   +382
tcc                                                      +385
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FIGURE 3: The 5′ flanking region of the bovine elastin gene. Note the absence of a TATA canonical sequence although a CAAT box is found at −57 to −60. The region is G+C rich and contains an A+T-rich segment (aaaa) as well as SP1 binding sites (GGGCGG) including two that are overlapping as indicated. The segment contains exon 1 encoding the signal sequence as well as a portion of intron 1.

domain structure of the protein is a reflection of the gene in which functional domains are usually segregated into separate exons. Curiously, the exon encoding the signal sequence also encodes the amino acid found at the amino terminus of the secreted tropoelastin, so that there is not a distinct separation in the gene of these two corresponding regions of the protein.

Although the exons are all multiples of three nucleotides and glycine is found usually at the exon–intron junctions, the exons do not exhibit any regularity in size as is found in the fibrillar collagen genes. It should also be noted that exon–intron borders always split codons in the same way. Thus, at the 5′ border of an exon, the second and third nucleotides of a codon are included while the first nucleotide of a codon is found at the 3′ border. This consistent structure is an important feature because it permits extensive alternative splicing of the primary transcript in a cassette-like fashion while maintaining the reading frame.

Sequence of the 5′ Flanking Region. Approximately 1 kb of the 5′ flanking region of the gene has been sequenced (Figure 3). Remarkably, this segment does not contain a TATA canonical sequence frequently found in promoters, although a CAAT sequence is found at −60 to −57. The region is also characterized by a high G+C content, the presence of an A+T-rich sequence, and several SP1 binding sites. Such features appear to be associated with promoter regions of "housekeeping genes" (Melton et al., 1986) as well as several other genes (Bird, 1986). Since the size of the elastin message, approximately 3500 nt, as estimated by Northern hybridization (Indik et al., 1987a) can be accounted for by the known, identified exons, it is likely that no other 5′ exons exist and that the 900 bp immediately upstream of the translation initiation codon do in fact contain the elastin gene promoter. This belief must remain tentative, of course, until the specific identification and functional characterization of such promoter

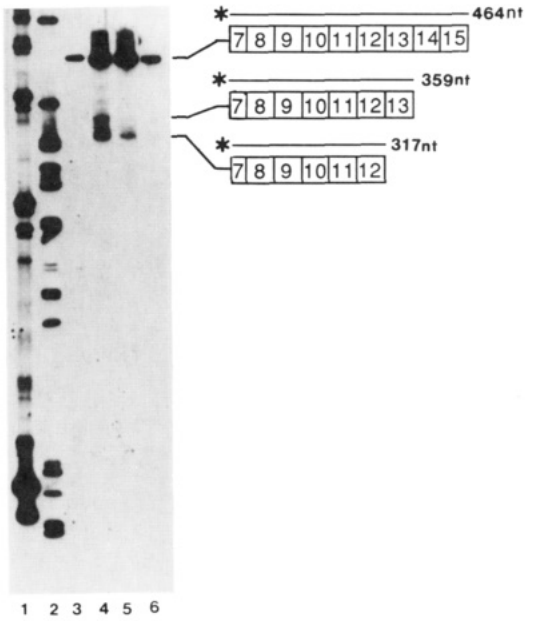

FIGURE 4: Alternative splicing of exons 7–14. Probe C (20 ng) was 3′ end labeled and hybridized to 25 μg of bovine nuchal ligament total RNA at 55 °C. After S1 nuclease digestion, the products were electrophoresed on 6% polyacrylamide gels and the gels subjected to autoradiography. (1) 123-nt ladder markers; (2) φX174 markers; (3) control, no RNA; (4) 270-day fetal RNA; (5) 14-day neonate RNA; (6) adult RNA.

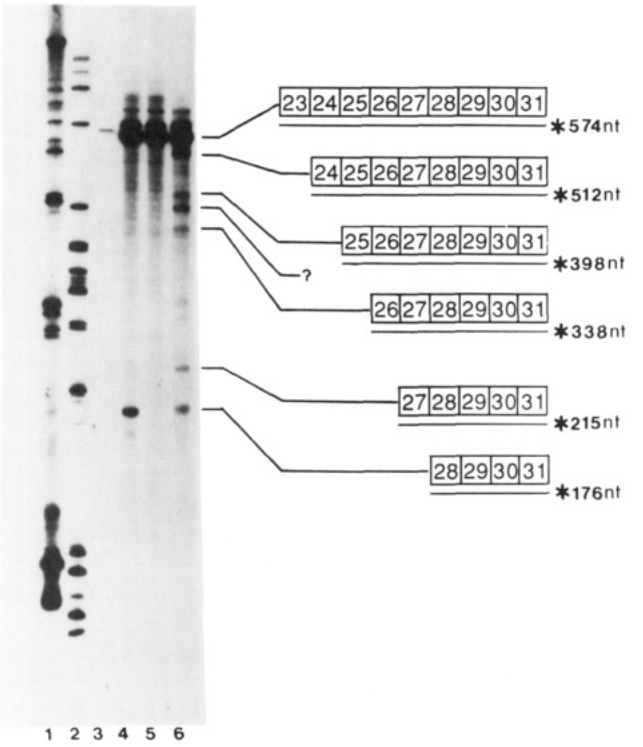

FIGURE 5: Alternative splicing of exons 23–31. Probe F (40 ng) was 5′ end labeled and hybridized to 25 μg of bovine nuchal ligament total RNA at 55 °C. After S1 nuclease, the digestion products were electrophoresed on 6% polyacrylamide gels. (1) 123-nt ladder markers; (2) φX174 markers; (3) control, no RNA; (4) 270-day fetal RNA; (5) 14-day neonate; (6) adult. The origin of the band labeled with a ? is unknown.

elements have been carried out. The features of the putative promoter in conjunction with the finding of alternative splicing suggest that the overall regulation of elastin gene expression is complex and takes place at several levels.

Quantitation of Alternative Splicing by S-1 Digestion. Although cloning of both human and bovine elastin cDNA and sequence comparisons to the corresponding genomic DNAs have conclusively proven the occurrence of alternative splicing of the primary elastin transcript, such sequence analysis does not permit quantitative estimates of the relative frequency of individual splicing events. Such estimates are essential to provide a description of the potential coding sequences found in the ensemble of mature mRNA molecules and hence in the encoded tropoelastin. S1 digestion experiments were performed in order to measure the frequency of splicing events. The appropriate cloned cDNA restriction fragments, which collectively encompassed the entire length of the elastin mRNA, were end labeled with ^{32}P at their 5′ or 3′ ends and used as probes. After hybridization of the probe to RNA isolated from the nuchal ligament of bovine fetuses of several ages, neonate calves, and adult cows, the RNA–DNA duplexes were digested with S1 nuclease and the products analyzed by quantitative autoradiography. Representative autoradiographs are illustrated in Figures 4–6. In addition to the control and experimental digest mixtures, a range of known concentrations of the particular labeled probe used in an individual experiment was also electrophoresed to provide an internal standard for quantitative densitometry.

The S1 experiments clearly demonstrated that only exon 33 is frequently subject to alternative splicing (deleted 42–65% of the time in most samples) (Figure 6). Exons 13 and 14 are spliced out to a moderate degree (10–20% of the time in all samples) (Figure 4), while exons 24 and 26–30 are infrequently spliced out in most of the RNA samples from the different aged cows (Figure 5). An interesting observation is that the RNA from adult cows displays a splicing pattern that is different from the fetal and neonate RNA. In one region

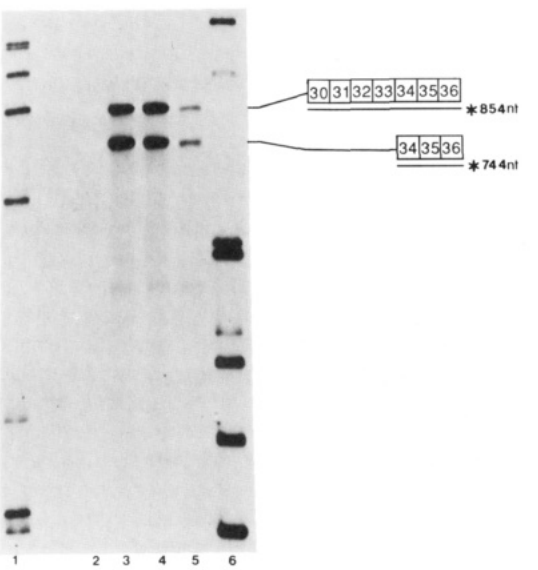

FIGURE 6: Alternative splicing of exon 33. Probe G (30 ng) was 5′ end labeled and hybridized to 25 μg of bovine nuchal ligament RNA at 50 °C and the hybridization mixture digested with S1 nuclease. The digestion products were then electrophoresed on 6% polyacrylamide gels. (1) 123-nt ladder markers; (2) digested control, no RNA; (3) 270-day fetal RNA; (4) 14-day neonate; (5) adult; (6) 1-kb ladder markers.

encompassing exons 23–27, a low but significantly higher level of splicing out of every exon was observed in the RNA from the adult while no splicing pattern was evident in the RNA from any of the other ages. An unexplained band was also seen in this region in the adult cow samples (marked with a question mark in Figure 5). The results of all these experiments are summarized in Table I.

Table I: Alternative Splicing of Individual Exons[a]

exon no.	age of cow: 175-day fetus	210-day fetus	270-day fetus	14-day neonate	adult
13	nd	9.3–10.5	4.0–7.9	0.2–3.0	1.0–2.1
14	nd	2.0–6.8	2.5–8.8	0.3–1.9	4.5–7.2
13 + 14[b]	nd	8.0–9.0	4.4–5.0	3.1–4.3	7.2–12.8
23	tr	tr	tr	tr	4.3–5.4
24	tr	tr	tr	tr	1.6–1.9
25	tr	tr	tr	tr	1.3–1.8
26	tr	tr	tr	tr	tr
27	tr	tr	7.8–12.6	1.3–1.6	1.4–4.5
28	tr	tr	tr	tr	0.8–0.9
29	tr	tr	tr	tr	tr
30	tr	tr	tr	1.5	0.5–0.6
33	68	61–74	61–65	42–50	53–55

[a] The total recovery of each probe was determined in arbitrary densitometer units by summing the recoveries in individual bands. The results in the table are the fraction of the total (expressed as a percent) corresponding to the splicing out of the individual exons. Only those exons in which there was detectable splicing out are tabulated; otherwise, no alternative splicing was detected. The results for each exon represent at least three separate determinations, and the range of values for each exon is given, except for the 175-day fetal sample in which single determinations were made. Abbreviations: nd, not determined; tr, trace (visible band, but <0.1%). [b] Represents the fraction of the molecules in which both exons 13 and 14 were deleted. The values listed beside exons 13 and 14 represent the fraction of molecules in which each exon alone has been deleted.

Use of Polyadenylation Sites. Another possible source of heterogeneity in the RNA population is the use of more than one polyadenylation site. Previous sequencing had identified two polyadenylation signals separated by 240 nt (Indik et al., 1987b). Such a small size difference would not permit clean separation of the RNA species on Northern gels. In order to estimate the relative use of each polyadenylation signal, S1 protection analyses were carried out (Figure 7). The results show clearly that the 5′ signal is consistently used about twice as frequently as the 3′ signal.

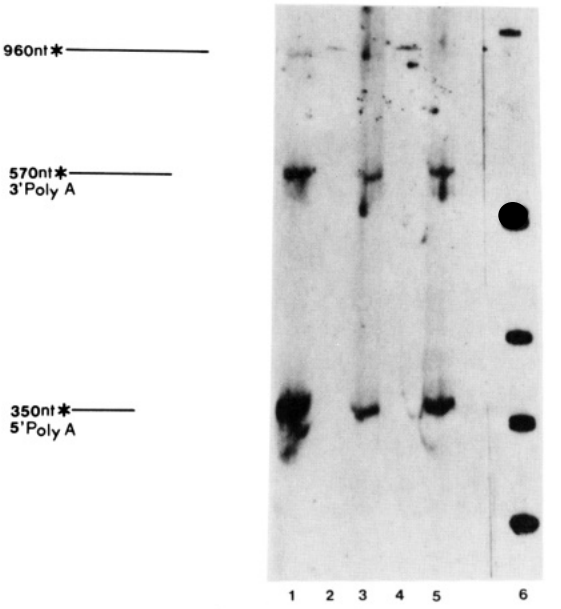

FIGURE 7: Use of polyadenylation sites. Probe H (20 ng) was 3′ end labeled and hybridized to 100 μg of total bovine nuchal ligament RNA at 60 °C and the hybridization mixture digested with S1 nuclease. The digestion products were then electrophoresed on 6% polyacrylamide gels. (1) 270-day RNA; (2) control, no RNA; (3) 14-day neonate RNA; (4) control, no RNA; (5) adult RNA; (6) 1-kb ladder markers.

Discussion

Nucleotide sequence heterogeneity clearly attributable to alternative splicing has been observed in both human and bovine elastin cDNA (Yet et al., 1987; Raju & Anwar, 1987; Indik et al., 1987a). The present results using S1 digestion have demonstrated that in the nuchal ligament of the cow, a tissue synthesizing elastin at a high level, alternative splicing is confined to a limited number of exons in the elastin gene. Furthermore, except in the case of exon 33, which is alternatively spliced roughly 50% of the time, alternative splicing of other exons is relatively infrequent, ranging from <1% to 20%.

A number of laboratories have observed that several components (usually two or three) may be observed when tropoelastin from several different species is analyzed by sodium dodecyl sulfate–polyacrylamide gel electrophoresis (Foster et al., 1980; Davidson et al., 1982; Chipman et al., 1985). The cause of this heterogeneity is presently not known with certainty, and it has been speculated that it may be caused by the existence of more than one gene for elastin, by alternative splicing of a single primary mRNA transcript, or by post-translational modification of a single primary translation product. All available evidence points to the existence of a single elastin gene per haploid genome (Emanuel et al., 1985; Indik et al., 1987b; Yeh et al., 1987; Olliver et al., 1987). Therefore, the presence of multiple genes can probably be safely ruled out as an explanation for the heterogeneity. There is no convincing evidence for any type of differential secondary modification of the initial translation product. Tropoelastin is not glycosylated and it appears to contain a unique amino terminus. In addition, the separated isoforms of tropoelastin from bovine nuchal ligament appear to contain the same carboxy terminus (Wrenn et al., 1987). This leaves alternative splicing of the mRNA as a possible explanation. Raju and Anwar (1987) have suggested that alternative splicing of a segment encompassing 102 nucleotides, shown here to encode exons 13 and 14, may account for the three nuchal ligament tropoelastin components that have been resolved so far. The present data indicate that this is an unlikely possibility since the two major protein components, accounting for greater than 90% of the tropoelastin, are found in roughly equal amounts and exons 13 and 14 are deleted only 10–20% of the time. A more likely explanation is that the presence or absence of the 15 amino acids encoded in exon 33 accounts for the two major components, which differ by approximately 2 kDa in size. One or more of the other exons subject to alternative splicing, including exons 13 and 14, may account for other isoforms.

Previously published analyses of the exon–intron borders in the bovine and human elastin genes (Yeh et al., 1987; Indik et al., 1987a,b) as well as current analyses (not shown) have shed little light on the biochemical mechanisms responsible for the alternative splicing. Relatively rare and what would appear to be minor deviations from consensus sequences were found (Breathnach & Chambon, 1981; Padgett et al., 1985). Although there was no detectable difference in which exons were alternatively spliced when late fetal and neonatal ligament RNA were compared (Table I), the alternative splicing of exons 13, 14, and 27 was significantly lower in neonatal mRNA than in 270-day fetal mRNA. This suggests that more of the tropoelastin isoforms produced in the bovine ligament after birth would be of greater molecular weight than those synthesized in utero, and this hypothesis is consistent with the relative sizes of tropoelastin isoforms produced by cell-free translation of similar RNA samples (Parks et al., 1988). It remains to be determined whether the pattern of alternative splicing is tissue specific and whether such splicing has sig-

nificant physiologic consequences. The finding of a varied pattern in the adult suggests that the precise type of monomer units synthesized in the adult could vary from that synthesized during growth. If a different type of splicing pattern were to occur in human tissues, particularly in pathological situations such as atherosclerosis, this change may contribute to the disease process.

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Lipoprotein *a* Inhibits Streptokinase-Mediated Activation of Human Plasminogen†

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ABSTRACT: Lipoprotein *a* [Lp(*a*)] inhibits human plasminogen (Pg) conversion to plasmin (Pm) by streptokinase- (SK-) mediated activation. Kinetic and binding studies indicate that Lp(*a*) inhibits Pg activation by competitive and uncompetitive inhibition. Lp(*a*) competes with Pg for SK and forms a stable complex. Lp(*a*) does not, however, inhibit Pg activation by the proteolytic SK–Pm complex. The SK–Pg and SK–Pg(act) intermediate complexes are possible targets of the Lp(*a*) uncompetitive inhibition. The competitive inhibition constant (K_{ic}) is 45 nM or 14 mg/dL, and the uncompetitive inhibition constant (K_{iu}) is 140 nM or 42 mg/dL, corresponding to physiologic and pathophysiologic Lp(*a*) concentrations, respectively.

Lipoprotein *a* [Lp(*a*)] is a low-density lipoprotein first identified by Berg (1963). Plasma levels of Lp(*a*) strongly correlate with atherosclerotic lesions [see Brown and Goldstein (1987) and Scanu (1988) for brief reviews]. Elevated levels of Lp(*a*) (greater than 30 mg/dL) are associated with a risk of atherosclerotic disease two to five times that of control subjects (Albers et al., 1977; Frick et al., 1978; Rhoads et al., 1986; Dahlen et al., 1986). The elevated Lp(*a*) levels are linked not only to coronary artery disease but also to stenosis of carotid and cerebral arteries (Murai et al., 1986; Zenker et al., 1986) and saphenous vein bypass grafts (Hoff et al., 1988).

Structural studies of Lp(*a*) demonstrate that like low-density lipoproteins it contains an apoprotein B (apo B) subunit, but also contains an apoprotein *a* [apo(*a*)] subunit linked by a disulfide bond to the apo B subunit [see, for review, Scanu (1988)]. The apo B subunit is a M_r ~510 000 polypeptide (Law et al., 1986; Knott et al., 1986), and the apo(*a*) subunit

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