Identification of a Large Region of Secondary Structure in the 3′-Untranslated Region of Chicken Elastin mRNA with Implications for the Regulation of mRNA Stability*

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Synthesis of aortic elastin peaks in the perinatal period and then is strongly down-regulated with postnatal vascular development. Our laboratory has previously shown that changes in elastin mRNA stability contribute to this developmental decrease in elastin production. Here we identify a large region of stable secondary structure in the 3′-untranslated region (3′-UTR) of chicken elastin mRNA. Reverse transcriptase polymerase chain reaction or polymerase chain reaction amplification of the 3′-UTR consistently resulted in products with an ~328-bp deletion from the central region of the 3′-UTR, suggesting the presence of secondary structure. The presence of this structure was confirmed by probing the 3′-UTR with RNases with selectivity for single- or double-stranded RNA. Gel migration shift assays using cytosolic extracts from 2-day old chicken aorta demonstrate specific binding of a cytosolic protein to riboprobes containing the 3′-UTR of elastin but not to riboprobes either corresponding to other areas of the message or containing the 3′-UTR but lacking the region of secondary structure. Binding of cytosolic protein was particularly prominent in aortic extracts from 2-day old chickens, a time when elastin message is stable, as compared with 8- and 15-week old chickens, when the elastin message is relatively unstable, suggesting that this region of secondary structure may play a role in developmental regulation of stability of elastin mRNA.

Elastin is the major matrix protein of elastic ligaments and large blood vessels, such as the aorta, and also occurs in significant amounts in lung parenchyma, skin, and elastic cartilages. The presence of elastin in these tissues gives them the physical properties of extensibility and elastic recoil which are essential for their physiological function. An unusual characteristic of elastin is that, once firmly incorporated into the extracellular matrix, this protein does not turn over at any appreciable rate (1, 2). Consistent with this lack of turnover, production of elastin in tissues such as the large arteries normally takes place only during vascular development and growth, after which synthesis and accumulation of the protein essentially ceases. For example, in the chicken thoracic aorta, elastin production is very rapid around the time of hatching but has decreased markedly by 4 weeks and can no longer be detected after 10–12 weeks of age (3).

Decreased synthesis of elastin after vascular development and growth is completed is also reflected in marked declines in steady state levels of mRNA for elastin (see Fig. 1) (3). We have previously reported that the developmental decrease in steady state levels of mRNA for elastin was due, at least in part, to decreased stability of elastin mRNA (4). That is, the half-life of aortic elastin mRNA in the 2-day old chicken, a time at which synthesis is rapid, is approximately 25 h. However, by 8 weeks after hatching, when elastin synthesis and steady state mRNA levels for elastin have fallen to low levels, the half-life of this message has decreased to approximately 7 h (4). Decreased stability of mRNA has also been reported to be important for developmental regulation of elastin synthesis in rat lung (5). Furthermore, stability of elastin mRNA has also been reported to be affected by transforming growth factor-β (TGF-β) (6, 7), phorbol esters (8), and vitamin D (9).

Although several mechanisms for regulating stability of mRNAs have been described, only a few have been well characterized (10). In general, removal of 5′-cap structures or 3′-polyadenosine tails are considered to lead to rapid degradation of messages (11, 12). Sequence elements in the 3′-untranslated region (3′-UTR) have also been implicated in regulation of the stability of many mRNAs (13–16). For example, AU-rich elements have been shown to regulate turnover of several messages, particularly those with a relatively short half-life (17, 18). Stability of other messages appears to involve C-rich sequences (19–21). In some cases, rates of turnover and rates of cleavage have been related to the formation of secondary structures such as stem-loops or hairpins, with regulatory proteins binding to these structures to alter susceptibility to RNases (22, 23).

Although stability of the elastin mRNA appears to be an important factor in the regulation of expression of this protein with development, details of the mechanism of this regulation are still not understood. The 3′-UTR of elastin is relatively large and contains regions of substantial sequence similarity among species, suggesting that these sequences may have some role to play in regulation. In addition, the 3′-UTR of elastin is unusually rich in G and C nucleotides (68% in the chicken). High GC content has been related to the ability to form stable

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secondary structures in RNA, and such GC-rich secondary structures have been postulated to be implicated in regulating stability of other messages (24). Here we report the presence of a large, stable domain of secondary structure in the 3′-UTR of chicken mRNA and provide evidence that this structure may play a role in the regulation of stability of the elastin message with development.

**EXPERIMENTAL PROCEDURES**

**RNA Isolation and Northern Analyses**—Thoracic aortas were dissected from chickens of different ages. Total RNA was isolated from these tissues by extraction with guanidine thiocyanate and centrifugation through CsCl as described previously (25).

For Northern analyses, 10 μg of total RNA was separated by 1% agarose gel electrophoresis and transferred to Hybond-N membranes (Amerham Pharmacia Biotech). RNA was cross-linked to the membranes using a UV Stratalinker (Stratagene) and baked at 80 °C for 2 h. Blots were prehybridized for 4 h at 42 °C with 50% formamide, 5x Denhardt’s buffer, 5x saline/sodium phosphate/EDTA, 0.1% SDS, and 100 μg/ml salmon sperm DNA and then hybridized for 16–18 h at 42 °C using cDNA probes radiolabeled with [32P]dCTP by random priming. This was followed by washes in 2x SSC, 0.1% SDS for 40 min at 55 °C, followed by 1x SSC, 0.1% SDS for 30–40 min at 55 °C. The blots were exposed to x-ray film (Eastman Kodak Co.) at −70 °C. Equal loading of lanes for Northern blotting was confirmed by ribosomal RNA bands visualized by ethidium bromide staining. cDNA probes for chicken elastin included pCEL, a gift of Dr. J. A. Foster, Department of Biochemistry, Boston Medical School, Boston, MA.

**Reverse Transcription of Total RNA**—Total RNA was reversed transcribed by Superscript II reverse transcriptase (RT) using the protocol recommended by the supplier (Life Technologies). 1 μg of total RNA was mixed with 0.5 μg of oligo(dT) primers in 11 μl of DEPC-treated water. This mixture was incubated for 10 min at 70 °C and quickly chilled on ice. 7 μl of master mix containing DTT, first strand buffer, and dNTPs was added. After 2 min of incubation at 42 °C, 200 units of reverse transcriptase was added, and the 25 μl mixture was incubated at 42 °C for an additional 50 min. The first strand cDNA product was incubated for 15 min at 70 °C and then diluted to 50 μl with TE buffer (pH 8.0) and stored at −20 °C until further use. A similar reaction without the addition of RT was always performed in parallel as a negative control.

**PCR Amplification of cDNA**—The PCR protocol used was adapted from that recommended for Taq Polymerase (Life Technologies). The PCR reaction mixture included 1× PCR buffer, 5% Me2SO, 0.2 mM each of dNTPs, 1.0 or 1.5 mM MgCl2, 0.5 μl of oligo(dT) primers in 11 μl of first strand cDNA was amplified using primers C and D, described previously. The upper primer (C) annealed to a sequence 586 bp downstream from the region of secondary structure. The lower primer (D) annealed to a sequence approximately 30 nucleotides upstream from the 3′-end of the region of secondary structure. PCR amplification was as described above, using 1.0 mM MgCl2 and an annealing temperature of 65 °C. PCR products were resolved on a 1.5% agarose gel, and the 280-base band was isolated using a QiaEx II gel extraction kit (Qiagen Inc.).

**Preparation of cDNA Corresponding to a Protein-coding Sequence from Chick Elastin mRNA**—Total RNA prepared from 3-day old chicken aortic tissue was reverse transcribed using an oligo(dT) primer and Superscript II reverse transcriptase, as described above. The resulting first strand cDNA was amplified using primers C and D, described previously. The upper primer (C) annealed to a sequence 586 bp downstream from the 5′-end of the region of secondary structure. The lower primer (D) annealed to a sequence approximately 30 nucleotides upstream from the 3′-end of the region of secondary structure. PCR amplification was as above, using 1.0 mM MgCl2 and an annealing temperature of 65 °C. PCR products were resolved on a 1.5% agarose gel, and the 280-base band was isolated using a QiaEx II gel extraction kit (Qiagen Inc.).

**Preparation of cDNAs Corresponding to the 3′-UTR of Elastin mRNA**—A cDNA containing the complete 3′-UTR of elastin, but lacking the region of secondary structure, was produced by PCR synthesis using pTE2 as a template and primers E and F, described above, in the presence of 1.5 mM MgCl2. The upper primer (E) annealed to the 5′-end of exon 33 of chick elastin cDNA. The lower primer (F) annealed to the 3′-end of 3′-UTR, just upstream of the poly(A) site. After digestion with HindIII and XbaI, the 780-bp cDNA product was subcloned into pGEM-4Z.

**Preparation of cDNAs Corresponding to the 3′-UTR of Elastin mRNA**—A cDNA containing the 3′-UTR of elastin, but lacking the region of secondary structure, was produced by PCR synthesis using pTE2 as a template and primers G and F, described above, in the presence of 1.0 mM MgCl2. The upper primer (G) annealed to the 5′-end of exon 32 of chick elastin cDNA. The lower primer (F) annealed to the 3′-end of 3′-UTR, just upstream of the poly(A) site. After digestion with BamHI and EcoRI, this resulting 380-bp cDNA product was subcloned into pGEM-4Z.

**A cDNA containing the complete 3′-UTR of elastin with some additional protein coding sequence was produced by PCR synthesis using pTE2 as a template and primers G and F, described above, in the presence of 1.0 mM MgCl2. The upper primer (G) annealed to the 5′-end of exon 33 of chick elastin cDNA. After digestion with BamHI and EcoRI, the 600-bp cDNA product was subcloned into pGEM-4Z.

**A cDNA containing the complete 3′-UTR of elastin without protein coding sequence was produced by PCR synthesis using pTE2 as a template and primers E and F, described above, in the presence of 1.5 mM MgCl2. The upper primer (E) annealed to the 5′-end of exon 32 of chick elastin cDNA. The lower primer (F) annealed to the 3′-end of the 3′-UTR, just upstream of the poly(A) site. After digestion with HindIII and XbaI, the 500-bp cDNA product was subcloned into pGEM-4Z.**

**A cDNA containing the complete 3′-UTR of elastin with some additional protein coding sequence was produced by PCR synthesis using pTE2 as a template and primers G and F, described above, in the presence of 1.0 mM MgCl2. The upper primer (G) annealed to the 5′-end of exon 33 of chick elastin cDNA. After digestion with BamHI and EcoRI, the 380-bp cDNA product was subcloned into pGEM-4Z.**

**A cDNA containing the complete 3′-UTR of elastin without protein coding sequence was produced by PCR synthesis using pTE2 as a template and primers E and F, described above, in the presence of 1.5 mM MgCl2. The upper primer (E) annealed to the 5′-end of the 3′-UTR.**

**A cDNA containing the complete 3′-UTR of elastin with some additional protein coding sequence was produced by PCR synthesis using pTE2 as a template and primers G and F, described above, in the presence of 1.0 mM MgCl2. The upper primer (G) annealed to the 5′-end of exon 33 of chick elastin cDNA. The lower primer (F) annealed to the 3′-end of the 3′-UTR.**

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After phenol/chloroform extraction, the transcripts were precipitated using 2.4 M ammonium acetate and ice-cold 100% ethanol for 1.5 h at 
−70 °C. The RNA pellets were resuspended in 30 μl of RNase-free TE (pH 8.0), and purified on a RNase-free G-25 RNA purification column (Boehringer-Mannheim). Determination of percent incorporation of radiolabeled nucleotides into transcripts was done by TCA precipitation. The integrity of the RNA was confirmed by 6% native polyacrylamide gel electrophoresis. Unlabeled RNA transcripts were generated in a similar manner but in the absence of [α-32P]CTP.

Preparation of Cytoplasmic Extracts—Thoracic aortas dissected from 2-day old and 15-week old chickens were rinsed with phosphate-buffered saline, cut into small pieces, and transferred to a 7-ml Dounce homogenizer containing 2 ml of ice-cold hypotonic buffer (25 mM Tris-HCl (pH 8), 0.5 mM EDTA). The tissues were then homogenized using 20 strokes with a tight-fitting pestle. Homogenates were centrifuged at 10,000 × g for 20 min at 4 °C, and the resulting supernatants were removed and quickly frozen at −20 °C. Protein concentrations were determined using the BCA protein assay reagent (Pierce) using bovine serum albumin as a standard. Total protein concentrations were determined using the BCA protein assay reagent (Pierce) using bovine serum albumin as a standard. Total protein concentrations were determined using the BCA protein assay reagent (Pierce) using bovine serum albumin as a standard. Total protein concentrations were determined using the BCA protein assay reagent (Pierce) using bovine serum albumin as a standard.

Cytoplasmic extracts containing 8–10 μg of protein were incubated at 20 °C for 30 min with 2–4 × 106 cpm of [32P]-labeled transcripts (50 μl T3 transcription buffer (pH 9.0), 300 μM MgCl2, 1 μM DTT, and 25 μg/ml yeast tRNA (Sigma-Aldrich), in a total volume of 20 μl. For competition experiments, proteins were pre-incubated with a 150-fold excess of unlabeled competitor RNA for 30 min at 20 °C, followed by incubation with the labeled RNA for another 30 min at 20 °C. Reaction mixtures were then incubated with 1:100–1:150 dilution of RNase Plus (0.83–1.25 units/ml RNase T1, 0.005–0.007 units/ml RNase A, 5 Prime—3 Prime Inc.) for a further 15 min at 37 °C. Electrophoresis of RNA-protein complexes was carried out on a 6% native polyacrylamide gel. Discrepancies between determined sequence and reported sequence for pTE2 are indicated in lowercase. Polyadenylation signals are boxed.

Fig. 2. Comparison of the reported sequence of pTE2 with the sequences of the RT-PCR product and PCR products. Only 3′-UTR regions are shown. Numbering of bases begins with the first base after the stop codon. The RT-PCR product was synthesized using primers E and F (see "Experimental Procedures" for primer sequences and sites), and total RNA was isolated from aortic tissue of 3-day old chickens. PCR products were produced using primers E and F (see "Experimental Procedures" for primer sequences and sites) with pTE2 as the cDNA template.

The secondary structure predictions were performed using Zuker's Mfold program as implemented in the GCG molecular biology software package (Wisconsin Package Version 9.0, Genetics Computer Group (GCG), Madison, WS).
RESULTS

Initially an RT-PCR approach was used to obtain a full-length 3'-UTR from chicken mRNA. For this purpose, RNA was isolated from 18-day embryo chickens, a time when elastin synthesis is rapid and elastin mRNA is abundant (Fig. 1). However, for several pairs of primers spanning the 3'-UTR region of the mRNA, RT-PCR products were always smaller than expected. Moreover, if one or both primers were situated in the central region of the 3'-UTR, no product was seen. The size discrepancy between products expected and found was consistently approximately 300 bases. However, RT-PCR products using primers located within the coding region of the mRNA were always of the expected size.

Although sequences of the protein coding region of chicken elastin had been reported previously by several investigators (26–28), only one clone reported included the sequence of the entire 3'-UTR of chicken elastin (27). This clone, designated pTE2, was obtained from Dr. Shingo Tajima, National Defense Medical College, Japan, and used in a PCR approach as an alternative method to obtain the complete 3'-UTR of chicken elastin. However, as observed using the RT-PCR procedures, while primers to the coding region of the clone yielded the expected products, primers spanning the 3'-UTR again resulted in products approximately 300 bases shorter than expected. Restriction digests of the pTE2 clone confirmed that these results were not because of a truncated 3'-UTR in the pTE2 cDNA template. Furthermore, lowering of the concentration of magnesium chloride from 1.5 to 1.0 mM in the PCR reaction resulted in the shift of the major PCR product to the expected size.

Sequencing of RT-PCR products from isolated RNA and PCR products from the pTE2 clone revealed that the size discrepancies of the products were, in fact, because of the absence of a central region of the 3'-UTR consisting of 328 bases (Fig. 2). Otherwise, the sequence corresponded to that reported for the 3'-UTR of elastin mRNA (27). The location of the missing region was consistent between RT-PCR and PCR products. Furthermore, sequencing of additional RT-PCR and PCR products using other primer pairs demonstrated a deletion at the same site (data not shown). Moreover, se-

![Fig. 3. Presence of the deleted sequence in total RNA preparations used for RT-PCR.](image)

**Fig. 3.** Presence of the deleted sequence in total RNA preparations used for RT-PCR. 10 µg of total RNA isolated from aortas of 3-day old chickens was separated by 1% agarose gel electrophoresis, transferred to Hybond-N membranes, and probed first (lane 1) with a cDNA probe corresponding to the region of the elastin 3'-UTR deleted in the RT-PCR reaction. This probe was prepared by PCR with pTE2 as the cDNA template at a MgCl2 concentration of 1.0 mM, using primers A and B (see “Experimental Procedures” for primer sequences and sites). The membrane was then stripped until only background radioactivity could be detected and was subsequently reprobed with pCEL (lane 2). Migrations positions of 28 and 18 S rRNAs, as determined using ethidium bromide staining, are indicated for reference.

![Fig. 4. Susceptibility of riboprobes (RPs) corresponding to various regions of elastin mRNA to digestion with RNases.](image)

**Fig. 4.** Susceptibility of riboprobes (RPs) corresponding to various regions of elastin mRNA to digestion with RNases. Riboprobes RP1, RP2, RP3, and RP4 (panel A) were produced using an *in vitro* transcription kit from Promega Corp. from 1 µg of linearized plasmid DNA, as recommended by the manufacturer, in the presence of [α-32P]CTP (3000 Ci/mmol). The integrity of the RNA product was confirmed by 6% native polyacrylamide gel electrophoresis. RP4 lacks the portion of the 3'-UTR deleted under RT-PCR and PCR conditions. Details of the preparation and purification of these riboprobes are given in “Experimental Procedures.” Radiolabeled riboprobes (4 × 10^4 cpm) were heated to 100 °C and cooled slowly to 20 °C. Enzymatic digests of the riboprobes (panels B and C) were carried out in 10 mM Heps buffer (pH 7.9), containing 40 mM KCl, 3 mM MgCl2, 1 mM DTT, and 0.5 µg/ml yeast tRNA, with either 0.625 units/µl RNase T1, (5 Prime-Prime, Inc.) or 9 × 10^−5 units/µl RNase V1 (Amersham Pharmacia Biotech), or a combination of both enzymes for 8 min at 37 °C. After phenol/chloroform extraction, the digested RNA products were precipitated using 2.4 M ammonium acetate and ice-cold 100% ethanol for 1–2 h at −70 °C. The RNA pellets were resuspended in sample solution (10 mM urea, 1.2 mM EDTA, 6% glycerol, 0.06% bromphenol blue, and 0.06% xylene cyanole tracking dye), heated to 70 °C for 10 min, and then cooled on ice. Electrophoresis was carried out on an 8.5 M urea, 8% polyacrylamide gel with 1× TBE running buffer. The gels were subsequently dried under vacuum at 80 °C for 3 h and exposed to a Phosphorimagier screen. RNA molecular size standards were produced by *in vitro* transcription of a mixture of RNA templates (Century Marker Plus, Ambion Inc.) in the presence of [α-32P]CTP (3000 Ci/mmole). The radiolabeled RNA markers were purified in a manner similar to that as the radiolabeled RNA transcripts used in RNA gel shift assays. Typically, 1 × 10^9–1.5 × 10^9 cpm of RNA markers were used per experiment.
Secondary Structure in 3′-UTR of Elastin mRNA

**Fig. 5.** Gel migration shift assays using riboprobes corresponding to various regions of elastin mRNA (Fig. 4A). Riboprobes RP1, RP2, RP3, RP4 were prepared as described under Fig. 4. Unlabeled RNA transcripts were generated in a similar manner but in the absence of [α-32P]CTP. Thoracic aortas from 2-day, 8-week, and 15-week chickens were cut into small pieces and homogenized in 25 mM Tris-HCl buffer (pH 8.0), containing 0.5 mM EDTA. Homogenates were centrifuged at 10,000 × g for 20 min at 4 °C, and the resulting supernatants were frozen at −20 °C. Protein concentrations were determined using the BCA protein assay (Pierce). Extracts containing 8–10 μg of protein were incubated at 20 °C for 30 min with 2–4 × 10^6 cpm of 32P-labeled transcripts in 5 mM Hepes buffer (pH 7.9), containing 0.5 mM MgCl2, 7.5 mM KCl, 0.5 mM DTT, 0.12 mM EDTA, and 200 ng/μl E. coli tRNA (Sigma-Aldrich), in a total volume of 20 μl. For competition experiments, proteins were pre-incubated with a 150-fold excess of unlabeled competitor RNA for 30 min at 20 °C, followed by incubation with the labeled RNA for another 30 min at 20 °C. Reaction mixtures were then incubated with a 1:100–1:150 dilution of RNase Plus (0.83–1.25 units/ml RNase T1, 0.005–0.007 units/ml RNase A, 5 Prime—3 Prime Inc.) for a further 15 min at 37 °C. Electrophoresis of RNA-protein complexes was carried out on a 6% native polyacrylamide gel with 0.3 × TBE running buffer, pH 8.4.

The abundance of elastin mRNA in aortic tissues of perinatal animals is consistent with the rapid synthesis and accumulation of elastin in these tissues at this stage of development (3). Similarly, the rapid decrease in steady state mRNA levels of elastin with postnatal development and growth corresponds to decreases in synthesis over this developmental period. We had previously shown that decreased stability of elastin mRNA over this developmental period contributed to the decline in steady state mRNA (4). Evidence from others also indicated that stability of elastin mRNA is likely to be an important site for regulation of synthesis of this protein (6–9).

The unusually high GC content of the 3′-UTR of chicken elastin mRNA had raised the possibility of the presence of secondary structures in this region of the elastin message. Our data, using primers spanning the 3′-UTR of elastin, demonstrated that RT-PCR or PCR products were consistently shorter than expected by approximately 300 bases. Sequencing clearly demonstrated that this shortening was because of the consistent deletion of a central region of the 3′-UTR of the elastin message. This deleted region contained 328 bases, was particularly GC-rich (79%), and was capable of forming stable secondary structures in this region of the elastin message. 

**DISCUSSION**

The presence of large regions of double-stranded RNA in the 3′-UTR of the elastin message.
Secondary Structure in 3' - UTR of Elastin mRNA

secondary structures with ~70% of the bases paired. Deletion of this region from PCR products of the elastin 3'-UTR was not because of the absence of this sequence from the pTE2 clone, as demonstrated both by restriction digests and by the fact that full-length products with the expected sequence could be obtained by PCR of this clone under conditions of lower magnesium chloride concentration. Furthermore, Northern blotting with probes specific to the missing region of the 3'-UTR showed that the inability to obtain a full-length product by RT-PCR was not because of the absence of this region from the RNA used in the reaction. Mechanisms accounting for the ability of a polymerase copying a template to skip a large region and then resume faithful transcription of the remainder of the sequence have been proposed by others to explain internal deletions occurring during DNA synthesis by DNA polymerase (29–32). In all cases, these models require that the deleted sequence contains a region of stable secondary structure. Our observations were therefore consistent with the presence of a large region of secondary structure in the 3'-UTR of elastin mRNA across which the polymerase or reverse transcriptase could skip in the PCR or RT-PCR reactions, continuing the replication of the sequence beyond the site of secondary structure. The role of secondary structure in this deletion event is supported by the fact that shortened PCR products were promoted by higher magnesium concentrations, since magnesium has been reported to promote base pairing into higher order structures (33–36).

Similar deletions in RT-PCR or PCR products using primers spanning the 3'-UTR of elastin from other species have not been described. This may be related to the fact that the 3'-UTR of chicken elastin mRNA is both the shortest and the most GC-rich of those reported. Indeed it is interesting to note that, of the three chicken elastin clones for which sequence is available (26–28), only one contained a complete 3'-UTR (27). Thus, chicken elastin message may have been a fortuitous choice to demonstrate the presence of this secondary structure. Although these structures are perhaps particularly stable and thus most easily demonstrated in chicken elastin, they may nevertheless also be present in, and important for the stability of, elastin message in other species.

Resistance of riboprobes corresponding to the 3'-UTR of elastin mRNA to digestion with RNase with specificity for single-stranded RNA offered further evidence for the presence of this region of stable secondary structure. Digestion of RP2, a riboprobe containing the entire 3'-UTR of elastin message, with RNase T1 resulted in a residual product corresponding to ~200 nucleotides. Although this is somewhat smaller than the 328 nucleotides predicted to be involved in the region of secondary structure, this can be accounted for by the fact that loops or bulges in this structure containing unpaired G nucleotides would also be susceptible to the enzyme. These data, together with the ability of a combination of RNase V1 and RNase T2 to digest RP2 completely, were consistent with the presence of a large region of secondary structure. Furthermore, the ability of RNase T1 to digest completely a riboprobe corresponding to the coding sequence of elastin mRNA (RP3) confirmed that this resistance of the 3'-UTR to digestion was not a general characteristic of all regions of the elastin message.

The presence of secondary structure in the 3'-UTR of elastin mRNA does not necessarily implicate such structures in developmental regulation of the stability of the elastin message. However, evidence from gel mobility shift assays clearly demonstrates specific binding of a cytosolic protein or complex of proteins to riboprobes containing the 3'-UTR of elastin. Such binding is not seen to riboprobes corresponding to other areas of the elastin mRNA and appears to require the presence of the region of secondary structure because a riboprobe containing the 3'-UTR sequence but lacking the deleted region showed no binding. Moreover, binding of the cytosolic protein to the 3'-UTR of elastin mRNA is particularly prominent for extracts of aortic tissue from 2-day old chickens, a period when elastin mRNA is known to be stable (4), as compared with binding of cytosolic extracts from 8- and 15-week old chickens, when the elastin message is relatively unstable. It is unlikely that this difference in binding pattern between cytosolic extracts of 2-day and 15-week old aortic tissue could be accounted for by differential protein extraction because total soluble protein recovered from these tissues was similar, patterns of proteins in the extracts were similar on Coomassie Blue staining after SDS-polyacrylamide gel electrophoresis, Western blotting demonstrated similar quantities of α-actin and α-tubulin in these cytosolic extracts, and similar amounts of total soluble protein were used in each of the gel mobility shift assays.

In summary, we have demonstrated the presence of a large region of stable secondary structure in the 3'-UTR of chicken elastin. Gel mobility shift assays indicate that binding of cytosolic proteins to this region of secondary structure is correlated with developmental periods of stability of the elastin mRNA, suggesting that such structure may play a role in developmental regulation of stability of elastin mRNA. Investigations to characterize specific binding sequences and their trans-acting proteins, and to confirm the potential role and mechanisms of these factors in determining elastin message stability, are now underway.

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