The complete sequence of the chicken $\alpha$-cardiac actin gene: a highly conserved vertebrate gene

Kun Sang Chang, Katrina N.Rothblum and Robert J.Schwartz*

Department of Cell Biology, Baylor College of Medicine, Houston, TX 77030, USA

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ABSTRACT
We sequenced the entire chicken $\alpha$-cardiac actin gene. A single intron was positioned 20 bp upstream from the initiation ATG codon in the 5' non-coding region while the coding region was interrupted by 5 introns at amino acid positions 41/42, 150, 204, 267, and 327/328. Sequencing allowed the first comparison of the $\alpha$-cardiac and $\alpha$-skeletal actin transcriptional promoters. These highly G+C rich promoters share two regions of homology which are found at position -134 (10 bp) and -296 (12 bp) in the $\alpha$-cardiac actin promoter. A smaller 9 bp motif (CCCGCCCGG) homologous to the -134 sequence was detected before, between and after the TATA and CAAT boxes of the $\alpha$-cardiac actin gene. The polyadenylation signal (AATAAA) was located 156 bp downstream from the translation termination codon. The complete length of the $\alpha$-cardiac actin mRNA excluding the poly A tail is 1370 nucleotides. The 3' noncoding transcribed portion of the chicken $\alpha$-cardiac actin gene was found to be extraordinarily conserved when compared to the human and rat $\alpha$-cardiac actin mRNA sequences.

INTRODUCTION
The actins represent a multigene family of highly conserved contractile proteins found in a broad host of eukaryotic genomes (1-5). Differences in amino acid sequence between the various actins have shown that at least six different isoforms are expressed in birds and mammals (6,7). The actins found in plants (8), protozoa (9), and invertebrates (3,4,10) are diverse, but tend to resemble the non-muscle $\beta$-and-$\gamma$ cytoplasmic actins identified in higher vertebrates (6). The first appearance of a striated actin coincides with the evolutionary emergence of the chordates (11). Urochordates and lower vertebrates such as the lamprey express the $\alpha$-cardiac actin isoform in their primitive muscles. Vandekerckhove (11) has suggested that the other striated actin, the $\alpha$-skeletal isoform, appeared during the evolution of amphibia. The smooth muscle actins which are expressed in vascular ($\alpha$-smooth muscle) and visceral ($\gamma$-smooth muscle) tissues were believed to have evolved during the later development of warm-blooded vertebrates. Hypothetically, a primitive cardiac-like actin gene might have given rise to the other striated actin
genes by a series of gene duplication steps. Since none of the striated actins seem to be closely linked to each other at the DNA (1,4,5) or even at the chromosomal (12) levels, several other events must have also transpired which dispersed these genes throughout vertebrate genomes.

We have isolated (1) and now sequenced the entire chicken α-cardiac actin gene. The coding region of this gene was interrupted by five introns which were located in the same positions as introns previously identified in other vertebrate cardiac (13) and α-skeletal actin genes (14,15). Additionally, we have identified a sixth intron in the 5' transcribed noncoding leader of the chicken α-cardiac actin gene. Sequencing of the α-cardiac actin gene has allowed the first comparison of promoter regions between two different striated actin genes. Several short regions of sequence homology were detected in the 5' promoter regions of the chicken α-skeletal and α-cardiac actin genes. Extensive regions of homology were detected in the 3' transcribed noncoding portion and the 3' flanking sequences of the chicken, rat and human α-cardiac actin genes. These highly conserved sequences may provide clues towards understanding the differential regulation of striated actin genes during embryonic development (16,17).

MATERIAL AND METHODS

Materials. Restriction endonucleases and placental RNasin were obtained from New England Biolab and Promega BioTec. AMV-reverse transcriptase was purchased from Life Sciences Inc., and E. coli DNA polymerase I large (Klenow) fragment was bought from Bethesda Research Laboratories. Radioactive nucleotides were obtained from Amersham Inc. T4 DNA ligase was a product of Collaborative Research Inc., while nucleotides and the 17bp M13 sequencing primer were from PL Biochemical.

DNA Restriction Mapping A 7.5 Kb Eco RI fragment which encompassed the entire cardiac actin gene was isolated from λAC7, a chicken genomic λ Charon 4A clone (1), and subcloned into the Eco RI site of pBR322. The restriction endonuclease enzyme map of pAC 7.5 was determined by single and double enzyme digests. Maps were also developed by the partial digestion protocol of Smith and Birnstiel (18). Reaction conditions for restriction endonuclease cleavage of DNA varied with the particular enzyme and were essentially as suggested by the supplier. Digestion products were separated by agarose slab gel electrophoresis in buffer containing 50 mM Tris-HCl (pH 7.4), 20 mM NaOAc, 18 mM NaCl, and 2 mM EDTA at 40 volts, blotted into nitrocellulose filters and hybridized with [32P]-labeled DNA probes as previously described (1).
Nucleic Acid Sequencing. The 5.6 Kb Kpn I/Eco RI fragment of pAC 7.5 which contains the entire actin coding region was mapped with a variety of restriction enzymes as shown in Figure 1. Individual DNA fragments were purified by preparative agarose gel electrophoresis. Staggered ends of the DNA fragments were filled in using the Klenow fragment of DNA polymerase I and deoxynucleoside triphosphates. The blunt ended fragments were ligated into Sma I cut M13mpl0 in both orientations. Two small overlapping M13mpl0 libraries were constructed from a 1.9 Kb Kpn I/Pst I fragment which was digested with Sin I and Dde I. The 0.7 Kb Sma I/Kpn I fragment was ligated in opposite orientations into mpl8 and mpl9. Identical clones were identified by T sequencing tracks, while clones containing DNA inserts of the opposite orientation were identified by the C-test (19). Single stranded M13 clones were sequenced by the dideoxy chain termination method of Sanger (20,21). The sequencing reaction mixtures were electrophoresed on preheated 6% polyacrylamide gels containing 8M urea (21). Gels were autoradiographed with Kodak AR film at -20°C for 4-12 hours. DNA sequences were compared to the published α-cardiac actin amino acid sequence (22) with the aid of an Apple II plus computer and the software of R. Larson and J. Messing (University of Minnesota).

Nucleotide Sequence of the 5' end of α-Cardiac Actin mRNA. The Sin I fragment cloned into M13 which overlaps the translational start site was isolated from RF M13 DNA and digested with Xma III. A 140 bp fragment was isolated, blunt ended with Klenow and subcloned into the Sma I site of M13mpl0 to produce clone XS5. The 17bp sequencing primer was end labeled with [γ³²P]-ATP and T4 polynucleotide kinase, and then used to extend a DNA chain in the presence of the single stranded clone XS5, 0.25 mM dNTP, 5 mM DTT, 10 mM MgCl₂, and 50 mM Tris-HCl (pH 7.6). The labeled extension product was digested with Hha I to produce an 84 nucleotide fragment which was complementary to a portion of the 5' end of the α-cardiac mRNA. This end labeled product was denatured in 30% DMSO and isolated on a 5% polyacrylamide gel. The single stranded labeled primer was hybridized with 100 ug of chicken cardiac poly A⁺ RNA, isolated as described in Chang et al. (1), in 40 ul of 25 mM Pipes (pH 7.5), 0.4 M NaCl and 50% formamide at 50°C for 24 hours. Hybridization was terminated by ethanol precipitation and the pellet was washed three times with 70% ethanol, once with 100% ethanol and then lyophilized. The dry pellet was resuspended in a primer extension reaction mixture which contained 10 mM DTT, 50 mM Tris-HCl pH 8.3, 0.12 M KCl, 7 mM MgCl₂, 0.25 mM dNTP, 20 units RNasin, and 25 units of AMV-reverse transcriptase in a 40 ul volume and was incubated for 1
hour at 42°C. The primer extended product was gel purified and sequenced by the Maxam and Gilbert protocol (23).

**Location of the 3' end of the α-Cardiac Actin mRNA.** In order to make an S1 nuclease probe to the 3' end of the α-cardiac actin mRNA, the 1.2 Kb Eco RI/Sin I fragment located 30 bp from the translation termination codon was blunt ended and cloned into the Sma I site of M13mp10. A clone was selected in which the destroyed Eco RI site of the insert DNA was proximal to the M13 primer. A [32P] labeled DNA was synthesized by primer extension, cleaved at the Eco RI site in the M13mplO cloning cassette, denatured, and recovered as a single stranded probe following preparative electrophoresis. This probe was hybridized with 10 μg of cardiac poly A+ RNA as described above. The S1 nuclease reaction was performed in a final volume of 250 ul which contained 0.2 M NaCl, 30 mM Na acetate (pH 4.5), 5 mM ZnSO4, and 20 μg of yeast t-RNA at 37°C for 1 hour in the presence of several concentrations of S1 nuclease. The samples were extracted with equal volumes of phenol-chloroform, and coprecipitated with carrier t-RNA (5 μg) in 70% ethanol. The size of the protected fragment was determined by comparison to thymidine sequencing ladders of an M13 clone which was co-electrophoresed on the same 6% polyacrylamide slab gel.

**RESULTS AND DISCUSSION**

Our laboratory has previously reported the isolation of the single copy α-cardiac actin gene from a λ Charon 4A chicken genomic library (1). The 7.5 Kb DNA fragment containing the coding and flanking sequences was isolated from λAC7 and subcloned into the Eco RI site of pBR322 (pAC7.5; Figure 1). The detailed map of pAC 7.5 shown in Figure 1 guided our preparation of DNA fragments for cloning into M13 vectors. From these clones we determined the linear sequence of 5463 nucleotides of the chicken α-cardiac actin gene as shown in Figure 2.

**The Amino Acid Coding Region of the α-Cardiac Actin Gene.**

Sequencing of the α-cardiac actin gene revealed the presence of 377 encoded amino acid residues, which began with a Met-Cys dipeptide (Fig. 2). This dipeptide is also found in the human α-cardiac actin gene (13) as well as the other vertebrate striated actin genes (14,15). Interestingly, this dipeptide is found in all of the Drosophila non-muscle actin genes (10) and some sea urchin actin genes (3) while the Cys codon is absent in vertebrate non-muscle actin genes (24,25). Since the NH2-terminal Met or Cys has not been detected in an actin protein yet sequenced, it is assumed that post-translational re-
Figure 1. Structural map of the chicken α-cardiac actin gene. Genomic DNA (7.5 Kb) which circumscribes the actin coding region was subcloned into pBR322 (pAC 7.5) and then mapped with the following restriction enzymes: Dde I (D); Hind III (H3); Kpn I (K); Pst I (P); Pvu II (P2); Sau 96I (S9); Sca I (Sc); Sin I (Sn); Sma I (Sm); Eco RI (R1); Sac I (S1); Xba I (Xb); Xho I (X2). These maps led to a sequencing strategy in which fragments were subcloned into M13 vectors and sequenced by dideoxy chain termination as indicated by arrows. One squiggly arrow represents a fragment sequenced by the Maxam and Gilbert (27) protocol. In the diagram above, the solid boxes represent coding exons, open boxes indicate transcribed untranslated regions and the solid lines coincide with introns and flanking DNA. The TATA box, CAATATG, initiation codon (ATG), termination codon (TAA), and the poly A addition signal (AATAAAA) are indicated.

moval of the Met-Cys dipeptide might be required for normal actin function (15).

The amino acid sequences at the NH$_2$- and COOH terminal regions provide definitive identification of each vertebrate actin isoform (7). The Asp and Glu at positions 2 and 3 as well as the Leu and Ser at positions 298 and 357 provided the 4 amino acids which differentiate the α-cardiac actin isoform from that of the α-skeletal type. We determined that the nucleotide sequence encoded a chicken protein which is absolutely identical to the bovine and human (13,22) α-cardiac actins. Thus, the sequence of the α-cardiac actin isoform has been strongly conserved during the evolution of warm-blooded vertebrates.

The coding region of the α-cardiac actin gene was split by five introns at the codons specifying amino acids 41/42 (IV 2), 150 (IV 3), 204 (IV 4), 267 (IV 5) and 327/328 (IV 6). The length and location of the introns are summarized in Table I. The sequences of the intron-exon junctions agree quite well with the consensus sequences compiled from the study of many genes (26). The positions of splice junction boundaries in the coding region are identical to those in the human α-cardiac actin gene (13) and the α-skeletal actin genes (14,15) isolated from chicken and rat. Unfortunately, only partial sequences of the human α-cardiac actin gene are available for
Figure 2. Nucleotide sequence of the chicken α-cardiac actin gene. Negative numbers indicate nucleotide positions upstream from the transcription start site. Amino acids of the actin protein are indicated above their respective codons, and numbers according to Lu and Elzinga (35). A slash represents a splice junction border, while numbers in parentheses indicate total number of nucleotides sequenced. The CAAT box, TATAAA box, and the final 3’ nucleotide in the mRNA are underscored. The cap site is indicated by a 1.
Table I
Organization of the Chicken α-Cardiac Actin Gene

| Exon/Intron | Positions (number of nucleotides) | Size (nucleotides) | Encoded region of mRNA |
|-------------|----------------------------------|--------------------|------------------------|
| Exon I      | 1-40                             | 40                 | 40bp 5' untranslated region |
| Intron 1    | 40-522                           | 482                |                        |
| Exon II     | 522-671                          | 149                | 20bp 5' untranslated region to AA 41 |
| Intron 2    | 671-1503                         | 832                |                        |
| Exon III    | 1503-1828                        | 325                | AA 42 - AA 150         |
| Intron 3    | 1828-2453                        | 625                |                        |
| Exon IV     | 2453-2615                        | 162                | AA 150 - AA 204        |
| Intron 4    | 2615-2727                        | 112                |                        |
| Exon V      | 2727-2919                        | 192                | AA 204 - AA 267        |
| Intron 5    | 2919-3917                        | 998                |                        |
| Exon VI     | 3917-4099                        | 182                | AA 267 - AA 327        |
| Intron 6    | 4099-4458                        | 359                |                        |
| Exon VII    | 4458-4778                        | 320                | AA 328 to 3' untranslated region |

comparison (13), and no extensive sequence homology was detected between human and chicken α-cardiac actin introns.

The 5' Untranslated Region. The sequence of the 5' untranslated region of the α-cardiac actin mRNA was determined by a primer extension experiment summarized by the schematic diagram in Figure 3. The [³²P] end labeled
Figure 3. Sequence determination of the 5' end of the chicken α-cardiac mRNA. The autoradiograph of the Maxam and Gilbert sequencing gel in panel A shows the 5' leader sequence of the cardiac actin mRNA. Nucleotides within the coding portion of the mRNA are divided into codons. The presumptive splice junction (sp), end labeled primer (P), and the starting point of the primer extension reaction (PE) are shown. The schematic diagram in panel B summarizes the procedure for synthesizing the primer extension probe. The 140bp Xma III/Sin I fragment was isolated, blunt ended with Klenow and ligated into M13mp10 vector at Sma I site. Single stranded DNA from this clone was annealed with end labeled M13 17bp primer and extended with Klenow. The double stranded DNA was restriction digested with Hha I, the Hha I/Xma III/17bp M13 primer fragment was separated in a strand separating 5% polyacrylamide gel; the probe was then hybridized with α-cardiac actin mRNA. See Material and Methods for a detailed description of this procedure.
fragment (84 nucleotides) which spans the 9th (Leu) to the 36th (Ser) amino acid of the actin coding region was hybridized to chicken heart poly A+ RNA, extended with reverse transcriptase, and then sequenced. The first lane of the sequencing gel shown in Figure 3 contains the non-extended primer while the second lane contains major and minor extension products of 87 and 85 additional nucleotides which may represent respectively, primary and secondary transcription start sites. The remaining lanes contain the extended primer cleaved with sequencing reagents. The sequence derived from this gel is shown beside it. The primer extension sequence exactly overlaps the coding sequence of α-cardiac actin by 27 nucleotides. Comparison of the extended sequence to that of the gene showed that an intron interrupted the 5' untranslated region 20 nucleotides upstream from the initiation methionine codon. This 5' intron must continue past the Kpn I border, since the remaining 39 nucleotides of the extended 5' untranslated region were not detected within the subcloned 5.6 Kb Kpn I/Eco RI fragment.

The location of the 5' non-coding exon of the α-cardiac actin gene was determined by the hybridization of labeled extension products (synthesized as described in the legend to Fig. 3 and Methods) to a blot of restriction enzyme digested pAC 7.5 as shown in Figure 4 panel A. In panel B the same fragments were hybridized with the non-extended DNA probe. The 0.8 Kb Smal I fragment, and the 0.7 Kb Smal I/Kpn I fragment were found to be differentially labeled by the extension products (lanes 1-3). These results directed us to sequence the Kpn I/Smal I fragment. We assigned the 5' and 3' borders of the first intron by matching the sequence of the 5' untranslated region shown in Figure 3 with that of this fragment. The signals associated with accurate initiation of transcription were identified 547 nucleotides upstream from the initiation ATG. A canonical promoter sequence (TATAAA) was found an appropriate 27 nucleotides upstream from the major extended sequence. A CAAAT box was located 79 nucleotides 5' to the transcription start site. The positions of these regulatory sequences correspond well with the positions of similar sequences found upstream from the 5' cap site of other eukaryotic genes (27).

Since the α-cardiac and α-skeletal actin genes appear to be closely related and co-expressed during embryonic cardiac and muscle development (16,17) we investigated the possibility that they might also share some common nucleic acid sequences in their flanking regions. Upon comparison, several interesting features of these regions became apparent. First, the chicken α-cardiac and the chicken and rat α-skeletal actin genes contain extraordinarily G+C-rich domains which encompass the transcriptional promoter regions. Within
the chicken α-cardiac gene a region extending a total of 298 nucleotides upstream of the cap site and another region of 522 nucleotides downstream of the cap site had base compositions of about 70% G+C, while the 100 nucleotides immediately preceding the transcriptional start site was 74% G+C. This G+C richness contribute a striking feature to the striated actin genes: an abundance of potentially methylatable sites (27).

The comparison of the nucleic acid sequences in the promoter regions of the α-cardiac and α-skeletal genes revealed several homologous sequences. The two longest homologous sequences were found at positions -296 (CCGGCCGCCGACA) and -134 (GCCGGCCGCCG) in the chicken α-cardiac actin gene. In the chicken α-skeletal actin gene these two sequences comprise a single sequence (GCCGGCCGCCGCCGACACA) found between positions -92 and -109. In the chicken α-
cardiac gene the homologous sequence located at position -134 was reiterated at positions -17, -35, and -110 as a series of nine nucleotide direct and indirect repeats (consensus sequence of CCGCGCGCGG). It is conceivable that these short nine base pair repeat sequences could potentiate transcription in the α-cardiac actin gene by either forming a stem/loop structure (positions -4 to -52) or by acting as separate regulatory elements. This hypothesis is currently being tested in our laboratory by in vitro transcription assays utilizing the Xenopus oocyte system.

The 3' Untranslated Region. A potential polyadenylation signal (AATAAA) was identified at nucleotide 5050 of the sequenced gene (Fig. 2). S1 nuclease mapping (Fig. 5) indicated that the polyadenylation site was located 20
TGAGGCGG---------CTACCTTACA-CCTCAGGACACATATTGTTTCT---GGAATCTCTGAGG
R
TAAGATGCCCTCTCTCCTACCTTCCA-TTCAGGACGACAGTATTGTGCTT-----GGAATCTCTGAGG
H
TAAGCA---------CTTTCCTACCTTACCCTACT--CTACCTTACA-TTCAGGACGACAGTATTGTGCTT
Ck
---C-CTTCTCC-CATCTCCCATACGTTTCTTCATCTACCTTCCA-GTCAGGATGACGGTATTATGCTTCTTGGAGTCTTCCAAAC
R
---C-CTTCTCC-CATCTCCCATACGTTTCTTCATCTACCTTCCA-GTCAGGATGACGGTATTATGCTTCTTGGAGTCTTCCAAAC
H
---C-CTTCTCC-CATCTCCCATACGTTTCTTCATCTACCTTCCA-GTCAGGATGACGGTATTATGCTTCTTGGAGTCTTCCAAAC
Ck
ATTATATGGCCTTTTATAAAATTAAACCCAGGCCAGG-CTTGCACCTACTCTCTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTAC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human insulin genes (31,32), or the prolactin genes (33,34) which also share common coding regions usually contain greatly divergent 3' untranslated regions. Presently, the role of highly conserved sequences in the 3' untranslated region of the cardiac actin gene is unknown, but the preservation of these sequences for over 200 million years of evolution suggests an important biological role for the expression of the α-cardiac actin genes. Currently, gene transfer experiments are being performed in our laboratory to investigate the biological function of these conserved sequences in the 5' promoter and the 3' noncoding regions of the chicken α-cardiac actin gene.

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*To whom correspondence should be addressed

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