ASBMB
JOURNAL OF LIPID RESEARCH

Evolutionary distinct mechanisms regulate apolipoprotein A-I gene expression: differences between avian and mammalian apoA-I gene transcription control regions

Stefania Lamon-Fava,*,† Rajeswari Sastry,* Stefano Ferrari,† Tripathi B. Rajavashisth,§ Aldons J. Lusis, and Sotirios K. Karathanasis[1],***

Laboratory of Molecular and Cellular Cardiology,* Department of Cardiology, Children's Hospital and Department of Pediatrics, Harvard Medical School, Boston, MA; Istituto di Chimica Biologica,† Universita' di Modena, Modena, Italy; Division of Medical Genetics,§ Harbor UCLA Medical Center, Torrance, CA; and Departments of Medicine and Microbiology,** University of California, Los Angeles, CA

Downloaded from www.jlr.org by guest, on June 18, 2012

Abstract In mammals, the apolipoprotein (apo) A-I gene is expressed predominantly in liver and intestine, while in avian species it is expressed in all tissues. Although liver and intestine are the major sites of chicken apoA-I mRNA synthesis, there are appreciable amounts of apoA-I mRNA in kidney, ovary/testes, brain, lung, skeletal, and heart muscle. In this study, the nucleotide sequences of the chicken apoA-I gene and its 5' flanking region, as well as the sequences involved in the expression of this gene, have been determined. The gene spans 1.5 kilobases and contains 4 exons and 3 introns, closely resembling the mammalian apoA-I gene. To determine the sequences involved in the expression of the chicken apoA-I gene, plasmid constructs containing serial deletions of the 5' flanking region of the chicken apoA-I gene fused to the bacterial chloramphenicol acetyltransferase (CAT) gene were transfected in human hepatoma (HepG2), colon carcinoma (Caco2), epithelial (Hela), mouse embryonal fibroblast (NIH3T3) cells, and quail myoblasts (QMLA29). The shortest deletion construct, containing 60 bp of the 5' upstream region, was sufficient for maximal transcriptional activity in all cell lines tested. This region contains a short sequence (nucleotides −60 to −54) that is highly conserved in birds and mammals, and an Sp1 binding site. Although the sequence between nucleotides −232 and −101 of the 5' region of the chicken apoA-I gene is partially homologous to the hepatic cell-specific enhancer of the mammalian apoA-I gene (located between nucleotides −222 and −110 upstream of the human apoA-I gene transcription start site), this chicken sequence is transcriptionally inactive in HepG2 cells. These results suggest that differences in the *cis*-acting regulatory elements of the apoA-I gene play a fundamental role in determining the differences in the tissue-specific expression of this gene in avian and mammalian species. —**Lamon-Fava, S., R. Sastry, S. Ferrari, T. B. Rajavashisth, A. J. Lusis, and S. K. Karathanasis.** Evolutionary distinct mechanisms regulate apolipoprotein A-I gene expression: differences between avian and mammalian apoA-I gene transcription control regions. *J. Lipid Res.* 1992. **33:** 831–842.

Supplementary key words chicken • Sp1

Apolipoprotein A-I (apoA-I) is the major protein constituent of high density lipoproteins (HDL). The plasma levels of apoA-I and HDL are negatively correlated with the risk of developing coronary artery disease (1, 2). The mechanism responsible for the protection from coronary artery disease by HDL and apoA-I is not completely understood, but it has been suggested that HDL and apoA-I exert an anti-atherogenic effect through their involvement in the reverse cholesterol transport. It has been hypothesized that, through this process, free cholesterol is collected from peripheral cells by HDL, esterified by lecithin:cholesterol acyltransferase (an enzyme activated by apoA-I), and delivered to the liver from where it is excreted either directly or in the form of bile acids (3). In avian species HDL comprise more than 80% of plasma lipoproteins and their lipid and protein composition very closely resemble that of human HDL (4, 5). In spite of this similarity, the role of HDL and apoA-I in avian lipid metabolism is not well understood.

The pattern of tissue expression of the apoA-I gene in birds is very different from that in mammals. While apoA-I synthesis occurs in most chicken tissues (6–10), in mammals the production of apoA-I is restricted to liver and intestine (11, 12). The tissue specificity of chicken apoA-I gene expression is very similar to that of the mammalian apolipoprotein E (apoE) (13, 14). Since there is no clear

Abbreviations: HDL, high density lipoprotein; CAT, chloramphenicol acetyltransferase; PCR, polymerase chain reaction; DDT, dithiothreitol.

[1]To whom correspondence should be addressed at: Laboratory of Cardiovascular Molecular Biology, Lederle Laboratories, Bldg. 200/4703, 401 N. Middletown Road, Pearl River, NY 10965.

evidence of the presence of apoE in plasma lipoproteins of birds, it has been suggested that chicken apoA-I may play a role in lipid transport similar to that of the mammalian apoE (8). These observations raise a number of questions with regard to the mechanisms involved in the regulation of the expression of apoA-I in different animal species and with regard to the role of this apolipoprotein in the lipid metabolism of birds. Expression of the mammalian apoA-I gene in liver cells depends on a powerful hepatic cell-specific enhancer, located between nucleotides −222 and −110 upstream of its transcription start site (+1) (15). This enhancer contains three distinct protein binding sites, A (−214 to −192), B (−169 to −146), and C (−134 to −119), all of which must be occupied for maximal transcriptional activity (15). Several transcription factors can bind to these sites, including several members of the steroid/thyroid receptor superfamily of ligand-inducible transcription factors (15, 16).

The current report shows that the intron-exon organization and transcription start site of the chicken apoA-I gene are very similar to those of the mammalian apoA-I gene. However, the *cis*-acting elements involved in the expression of the chicken apoA-I gene are structurally and functionally different from the elements required for the expression of the mammalian apoA-I gene in liver cells. These findings suggest that the evolutionary divergence of the patterns of tissue specific expression of the apoA-I gene between birds and mammals is due to differences in the number and organization of *cis*-acting elements within the apoA-I gene transcription control region.

MATERIALS AND METHODS

Library screening and DNA sequencing

Two chicken genomic DNA libraries, one in the λ vector Charon 4A (kindly provided by Dr. D. Engel) and another in the λ EMBL-3 vector (Clontech, CA), were screened using the chicken apoA-I cDNA as probe (8, 17), as previously described (18). The chicken apoA-I gene sequence was determined by subcloning DNA restriction fragments of positive clones in the M13 vector, followed by sequencing with the dideoxy-chain termination method (19). In some instances the nucleotide sequence was determined following the Maxam-Gilbert procedure (20).

Oligonucleotide synthesis

Oligonucleotides were synthesized with a Biosearch Model 8600 DNA synthesizer and deblocked at 55°C overnight. Oligonucleotide +1chAI is complementary to the coding strand of the chicken apoA-I gene, spanning nucleotides +1 to +21, and contains a HindIII restriction site at its 5' end. Oligonucleotides −60chAI and −311chAI span the −60 to −39, and the −311 to −290 regions, respectively, of the coding strand of the apoA-I gene and both contain an additional HindIII site at their 5' end. Oligonucleotide −232chAI corresponds to the −232 to −209 region of the coding strand, and oligonucleotide −120chAI corresponds to the −120 to −101 region of the non-coding strand of the chicken apoA-I gene. Both −232chAI and −120chAI oligonucleotides contain an additional BamHI hexanucleotide sequence at their 5' ends.

Plasmid constructions

pUC9CAT (21), a plasmid that contains the bacterial chloramphenicol acetyltransferase (CAT) gene (22) was used for most of the chicken apoA-I gene/CAT gene fusion constructs. To obtain the −60chAICAT construct, the λ #9 DNA was PCR-amplified in a Perkin-Elmer Cetus Thermal Cycler (25 cycles; each cycle: 94°C, 1 min; 37°C, 2 min; 72°C, 3 min) using −60chAI and +1chAI oligonucleotides as primers. The amplification product was cleaved with HindIII and cloned in the unique HindIII site of pUC9CAT. Similarly, for the construction of the −311chAICAT plasmid, the λ #9 PCR amplification product obtained with primers −311chAI and +1chAI was cleaved with HindIII and cloned into the pUC9CAT HindIII site. The sequence and orientation of −60chAICAT and −311chAICAT constructs were verified by nucleotide sequencing. To obtain the −139chAICAT construct, plasmid −311chAICAT was digested at the AvaI sites located at nucleotide position −139 of the apoA-I gene and in the polylinker region at the 3' end of the CAT gene. The 1.7 kb fragment was then ligated into the AvaI site of pUC9. The −3300chAICAT construct was made by cloning the 3 kb KpnI-KpnI fragment obtained from λ #9 in the KpnI site located at position −307 of the −311chAICAT plasmid. Correct orientation of the insert was verified by restriction mapping. For the construction of −520chAICAT, a 2.2 kb PstI-BamHI fragment of −3300chAICAT (containing 520 bp of the 5' upstream region of the chicken apoA-I gene in front of the CAT gene) was cloned in the PstI-BamHI sites of pUC9.

The −232 to −101 region of the chicken apoA-I gene was PCR-amplified with the −232chAI and −120chAI primers, using clone λ #9 DNA as template. The resulting DNA fragment was cleaved with BamHI and cloned, in both orientations, in the compatible BglII site of the pA10CATGem4 vector, in front of the CAT gene. The pA10CATGem4 is a SV40 early promoter/CAT gene fusion plasmid constructed by cloning the SalI-BamHI fragment of pA10CAT2 (23), lacking the SV40 enhancer, into pGem4 (Promega Biotech). pSV2CATGem4 was constructed by replacing the SV40 enhancer in front of pA10CATGem4 (15). The construction of the plasmids containing the wild-type human apoA-I liver-specific enhancer (−222/−110) and the site A mutated human apoA-I enhancer in front of pA10CATGem4 has been previously described (15).

Downloaded from www.jlr.org by guest, on June 18, 2012

Cell culture

Human colon carcinoma (Caco2), human hepatoma (HepG2), Hela, and NIH3T3 cells were grown in Dulbecco Modified Eagle Medium (DMEM, Gibco) supplemented with 10% fetal calf serum (Sigma), penicillin, and streptomycin. Cells were maintained in a 37°C incubator in 5% CO_2 atmosphere. Caco2 and HepG2 cells were seeded at 2.5×10^6 cell/100-mm dish, while Hela and NIH3T3 were seeded at 1×10^6 cell/100-mm dish 1 day before transfection. On the day of transfection a calcium-phosphate precipitate of 20 μg of test plasmid together with 5 μg of the plasmid pRSV-βGal (24) was added. Sixteen hours after transfection cell monolayers were glycerol-shocked and 48 h later cells were harvested. Cell protein extracts were made by three freeze-thaw cycles. β-galactosidase activity was determined according to the method described by Edlund et al. (24) and CAT enzyme activity was assessed as described by Gorman, Moffat, and Howard (25). For each transfection experiment, the CAT enzymatic activity was normalized to β-galactosidase activity.

Quail myogenic cells, QMLA29, transformed by the temperature-sensitive mutant ts-LA-29-PR-A of the Rous sarcoma virus (RSV) were grown as previously described (26). Cells were maintained at the permissive temperature (35°C) and were seeded at 1×10^6 cell/100-mm dish the day before the transfection. Cells were transfected with 15 μg of test plasmid and 10 μg of pRSV-βGal as described above.

Nuclear extracts and gel-retardation assay

Hela nuclear extracts were prepared from 20 confluent 150-mm dishes as previously described (27), except that all buffers were supplemented with 1 mM dithiothreitol (DTT) and 1 mM phenylmethylsulphonyl fluoride (PMSF) and buffer D was replaced by buffer G (20 mM N-2-hydroxyethylpiperazine-N'-2-ethane sulfonic acid [HEPES, pH 7.8], 0.1 M KCl, 0.2 mM EDTA, 1 mM DTT, 1 mM PMSF, 20% glycerol). Protein nuclear extracts were stored in liquid nitrogen.

Protein-DNA complexes were analyzed by electrophoresis, under nondenaturing conditions, in 6% polyacrylamide gels as previously described (28). Ten μg of Hela protein nuclear extracts was mixed with 3.5 μg of poly(dI-dC), in the presence or absence of 100-fold molar excess of different double-stranded oligonucleotide competitors, in a final volume of 27 μl and incubated in ice for 10 min. Then 20 fmoles of the DNA fragment spanning the −60 to +21 region of the chicken apoA-I gene, labeled at its 5' ends with [γ-^{32}P]ATP (New England Nuclear) and T4 polynucleotide kinase, were added and the incubation was continued for additional 30 min. The mixture was then loaded on the gel and electrophoresed for 2 h at 200 V. At the end of the electrophoresis the gel was fixed in 10% methanol–10% acetic acid, dried, and exposed to X-ray film for 3 h.

Tissue RNA isolation and chicken apoA-I mRNA quantitation

Tissue apoA-I mRNA distribution was determined in adult female (laying hens) and male chickens (age: 30–40 weeks old). For the assessment of the developmental regulation of apoA-I mRNA, liver, intestine, and breast muscle from six embryos or chicks were pooled for each time point. Chicken tissues were quickly frozen in liquid nitrogen, homogenized, and RNA was isolated by the guanidinium/cesium chloride method (18). The quality of RNA isolated from different tissues was assessed by agarose gel electrophoresis. The transcription start site of the chicken apoA-I gene was determined by primer extension assay as described by Sudhof et al. (29) using the apoA-I primer, a 30-base oligonucleotide complementary to bases 433 to 462 of the chicken gene. To determine the number of chicken apoA-I mRNA molecules per picogram of total tissue RNA, a primer extension experiment (29), using 25 μg of RNA from different tissues, was performed in the presence of an excess of ^{32}P-labeled apoA-I primer, whose specific activity had been measured. The reaction mixture was electrophoresed in an 8% polyacrylamide gel under denaturing conditions. The band corresponding to the product of primer extension was excised from the gel and its radioactivity was measured. The number of apoA-I mRNA molecules was then calculated from the specific activity of the primer.

The transcription start site of the chicken apoA-I/CAT gene transcripts was determined by primer extension using the CAT primer, a 29-base oligonucleotide complementary to bases 226 to 254 of the CAT gene (22).

RESULTS

Cloning and structure of chicken apolipoprotein A-I gene

Screening of a Charon 4A chicken genomic library with a chicken apoA-I cDNA probe (8) resulted in isolation of 17 positive clones. Two of these clones, λ #4 and λ #9 (**Fig. 1B**), were chosen for further characterization. Digestion of chicken testis chromosomal DNA with EcoRI, BamHI, HindIII, and KpnI, followed by electrophoresis, blotting, and hybridization with several DNA fragments spanning the entire length of the λ #4 and λ #9 inserts (Fig. 1A), allowed the determination of the restriction map of the chicken apoA-I gene DNA region (Fig. 1B). Screening of a EMBL-3 library resulted in the isolation of eight positive clones. One of these clones, EMBLAIA11, was found to include the entire apoA-I gene and was then chosen, as the λ #4 and λ #9 clones, for subsequent subcloning. The hybridization of probes specific for the 5' and 3' ends of the chicken apoA-I cDNA to these clones allowed the definition of the apoA-I gene direction of transcription (Fig.

Downloaded from www.jlr.org by guest, on June 18, 2012

ASBMB JOURNAL OF LIPID RESEARCH JLR

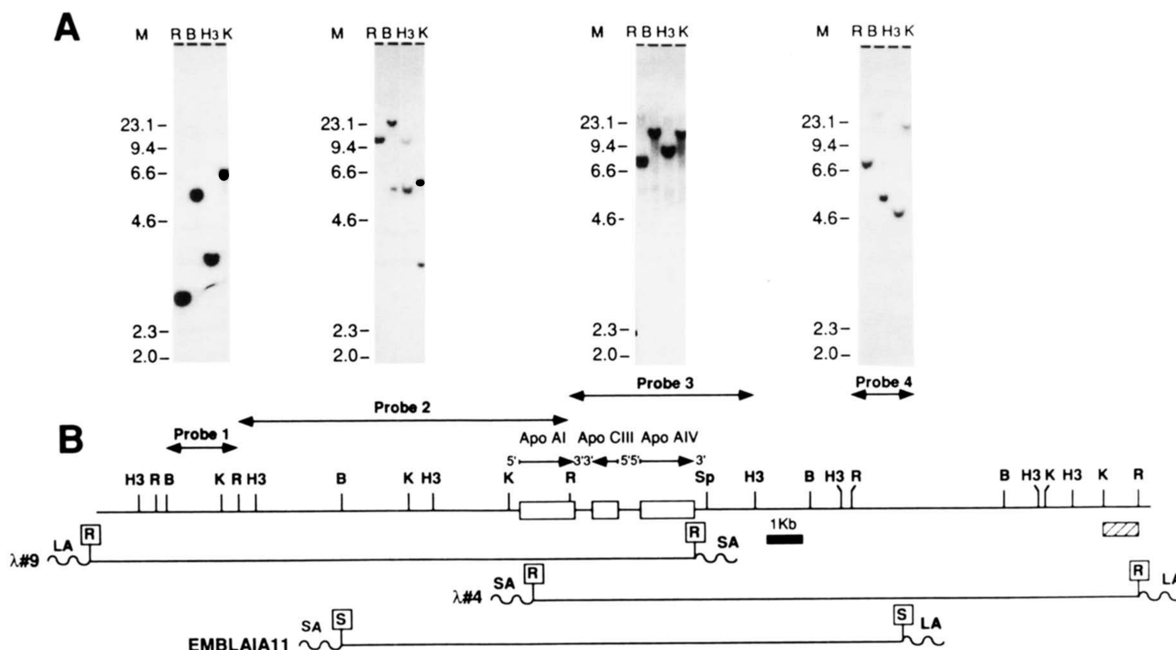

Fig. 1. Structural organization of the chicken apoA-I, C-III, A-IV gene cluster. A: Genomic DNA prepared from chicken testes was digested with EcoRI (R), BamHI (B), HindIII (H3), and KpnI (K). DNA fragments were separated by electrophoresis in 1% agarose gel, blotted into nylon filters, and hybridized with probes 1 to 4 obtained from the chicken genomic clones λ #4 and λ #9 shown below. The resulting autoradiograms are shown. Numbers on the side indicate the mobility and the molecular weight of DNA markers (M). B: Restriction maps of chicken apoA-I gene phage clones (λ #4, λ #9, and EMBLAIA11) (LA, long arm; SA, short arm; R, EcoRI; B, BamHI; H3, HindIII; K, KpnI; Sp, SphI; S, SalI). The open boxes represent the apoA-I, C-III, and A-IV genes. The direction of transcription (5' to 3') of the apoA-I, C-III, and A-IV genes is indicated by arrows. The hatched box represents a repetitive DNA CR1-like element. Its sequence is highly homologous to that of other CR1 elements reported in the chicken genome (49).

1B). In preliminary work, we have determined that the chicken gene is closely linked to the apoC-III and apoA-IV genes, which are located 3' of the apoA-I gene with a structural organization similar to that observed in mammals (Fig. 1B) (see also ref. 30). The apoA-I,C-III,A-IV gene cluster in mammals is spread over approximately 15 kb (31), while in the chicken it is contained within approximately 6 kb. The nucleotide sequence of the apoA-I gene has been determined and is similar, but not identical, to the sequence recently published by Bhattacharyya et al. (32). In fact, our nucleotide sequence, reported in **Fig. 2**, differs from the one previously reported by approximately 5% of nucleotide positions. The gene is composed by 4 exons and 3 introns, as the mammalian gene.

Structural organization and putative *cis*-acting elements in the 5' flanking region of the chicken apoA-I gene

Alignment of the chicken, rabbit (33), rat (12), and human (21) apoA-I 5' flanking sequences shows that certain regions are highly conserved (**Fig. 3**). One of these regions corresponds to the typical eukaryotic gene TATA box. Another region includes the sequence GCTTATC, which is located between nucleotides −60 and −54 of the chicken gene. High sequence homology is also observed for a sequence resembling the typical eukariotic CCAAT box, located 104 bp upstream of the chicken apoA-I start site. It has been shown that the human apoA-I contains a liver cell-specific transcriptional enhancer between nucleotides −222 and −110 (15). This enhancer contains three distinct functional sites, A (−214 to −192), B (−169 to −146), and C (−134 to −119), all of which are required for maximal expression of this gene in liver cells (15). Although certain sequences in the 5' upstream region of the chicken apoA-I gene are strikingly homologous to the mammalian site C and half of the site B, the chicken gene lacks sequences corresponding to the mammalian site A and the remaining half of site B (Fig. 3). Another difference between the upstream regions of the mammalian and the avian apoA-I genes is an insertion of 17 nucleotides between sites B and C in the chicken gene. This insertion results in separation of the chicken sites B and C by an additional 1.6 helical turns compared to the mammalian gene.

Tissue distribution and developmental regulation of chicken apoA-I mRNA

RNA blotting analysis of 25 μg of total RNA isolated from liver, intestine, kidney, ovary/testis, brain, lung, and muscle from breast, leg, and heart showed detectable

Downloaded from www.jlr.org by guest, on June 18, 2012

ASBMB

JOURNAL OF LIPID RESEARCH

JLR

```
-311  GGTACCAACCCGGCGCCCGGGCTCTGCGCCAATCCCCGGGGGCTGCGAGGGGCCCCGGCC

-251  GGGCCGGAATTTGGGTCACCCGCAGCGTAAACATCTCCTCGGCCAACGCCGCATCCCCGG

-191  GGAGCTCCTGTTTGCTGAGGCTCTGCAAAGCGGCGCTCCTTGGCCGGCTCTCCCGGGGCC

-131  GTGCGCACGATCCTTGAACTCTACGCGCCACATCGCCCGCGCCGGGAGTGATTTCTTGGG

 -71  CTGCGGCGCTGGCTTATCTCCTGCGGGAACTGCCCCTCCTGCATAAATAGCGGCGGCGGG
                                                ‾‾‾‾‾‾‾‾
                ▼                                          ^
 -11  GAACCGCGGGCTCACACGGACGAGCAGTGCGGAGCAGCGGCAGGAGAGCGCCGGTAAGA

  49  GCCCGCGCTGTGCGCCGGGACCCCCCGCAGCCCCGGGCTCTGAGCGCTCGGGGCTCCGGT
                                                ^
 109  CCTGCGCAGGGCTTCCGTGCGCCCAACTTCTCGCTCTGCCCTCAGGTTCAGCGCGAAGAT
                                                                Me  -24
                                                 ^
 169  GAGAGGCGTGCTGGTGACCCTCGCTGTGCTCTTCCTGACGGGTACGTCCGAGCAGGGCAG
      tArgGlyValLeuValThrLeuAlaValLeuPheLeuThrG                     -10

 229  CGGGGATGGGGGTGAGCCCGTGCGGGCGCTGCAGGGAGCGCTCCCGAAGCGCTCCGTGCA

 289  TTAAACCGCAGCCGAGGGCTCCCCGCAGCCCTGGAGCCCGCAGCTCCCCAGCGGCGGGGC

 349  TCTGAGCCACGGCTCCTGGGCACGGCGGTGGGGTGGGGTCCTCCCGTCTGCCCGTCCCCA
                         ^
 409  GCGTGCCGTCCGTCCCCGCAGGCATCCAGGCCCGCTCCTTCTGGCAGCACGATGAGCCCC
                          lyIleGlnAlaArgSerPheTrpGlnHisAspGluProG    4

 469  AGACGCCCCTGGACCGCATTCGGGATATGGTGGACGTCTACCTGGAGACGGTGAAGGCCA
      lnThrProLeuAspArgIleArgAspMetValAspValTyrLeuGluThrValLysAlaS  24
                                                             ^
 529  GCGGCAAGGATGCCATCGCCCAGTTCGAGTCCTCTGCTGTGGGCAAACAGCTTGAGTAAG
      erGlyLysAspAlaIleAlaGlnPheGluSerSerAlaValGlyLysGlnLeuAs       42

 589  TGGGGGGATGTGTGGTGGTGTCCCCGTGTCTCCATGTCCCCATATCCCTCTGTCCCCATA

 649  TTCCTATGTCCGGTGTCCCCACGTCCCCATTTCCCTGTCACCTCACGTCCCCACCTCTCC

 709  ACGTTTCCACATCCCCATGGCCCTACGTTCCCACATCCCCACACCACGTCTGACCCCGTG
                 ^
 769  CCGCCCTCCCCAGCCTGAAGCTGGCTGACAACCTGGACACGCTGAGTGCCGCGGCTGCCA
                  pLeuLysLeuAlaAspAsnLeuAspThrLeuSerAlaAlaAlaAlaL   58

 829  AGCTGCGTGAGGACATGGCTCCCTACTACAAGGAGGTGCGCGAGATGTGGCTGAAGGACA
      ysLeuArgGluAspMetAlaProTyrTyrLysGluValArgGluMetTrpLeuLysAspT  78

 889  CCGAGGCTCTGCGTGCTGAGCTGACCAAGGACCTGGAGGAGGTGAAGGAGAAGATCCGGC
      hrGluAlaLeuArgAlaGluLeuThrLysAspLeuGluGluValLysGluLysIleArgP  98

 949  CCTTCCTGGACCAGTTCTCTGCCAAGTGGACGGAGGAGCTGGAGCAGTACCGCCAGCGCC
      roPheLeuAspGlnPheSerAlaLysTrpThrGluGluLeuGluGlnTyrArgGlnArgL  118

1009  TGACGCCCGTGGCTCAGGAGCTGAAGGAGCTCACCAAGCAGAAGGTGGAGCTGATGCAGG
      euThrProValAlaGlnGluLeuLysGluLeuThrLysGlnLysValGluLeuMetGlnA  138

1069  CCAAGCTGACCCCGGTGGCTGAGGAGGCGCGGGATCGTCTGCGTGGGCACGTGGAGGAGC
      laLysLeuThrProValAlaGluGluAlaArgAspArgLeuArgGlyHisValGluGluL  158

1129  TGCGTAAGAACCTGGCGCCATACAGCGATGAGCTGCGGCAGAAGCTGAGCCAGAAGCTGG
      euArgLysAsnLeuAlaProTyrSerAspGluLeuArgGlnLysLeuSerGlnLysLeuG  178

1189  AGGAGATCCGCGAGAAGGGCATCCCCCAGGCTTCCGAGTACCAGGCCAAGGTGATGGAGC
      luGluIleArgGluLysGlyIleProGlnAlaSerGluTyrGlnAlaLysValMetGluG  198

1249  AGCTCAGCAACCTGCGTGAGAAGATGACGCCTCTGGTGCAGGAATTCAGGGAGCGCCTCA
      lnLeuSerAsnLeuArgGluLysMetThrProLeuValGlnGluPheArgGluArgLeuT  218

1309  CCCCCTATGCTGAGAACCTCAAGAACCGCTTGATCTCCTTCCTGGACGAACTCCAGAAGT
      hrProTyrAlaGluAsnLeuLysAsnArgLeuIleSerPheLeuAspGluLeuGlnLysS  238

1369  CCGTGGCCTGAGCTGCCAGCCCAGGGACTGACCCCAGGCCATGCTGGCTCCGGGAGTCCT
      erValAla                                                      240

1429  CGGGGACCCCTCCTTAATCTCCTCTCCCCCCCCGACCCGGAGTCCGTCTCAGCTTTGCCA
                                   ▽
1489  TTCTTTTGTCAAATAAACATGACTTAAGTTATTGGAGCTCACTCAGTCTTTGCAGTGGAT
                ‾‾‾‾‾‾‾
1549  GCTGGGGAAGGGGACCTAAGCCATGAGTGGGGACGGGTCCTTGGGGTGGGGGGCAGTAGG

1609  GGGGGGGGGGCTGCAGGGTGGGGAGCTGGGTTAGGGTTGGGTTAGGGTTAGGGTTGGGTT
```

Fig. 2. Nucleotide sequence of the chicken apoA-I gene. The TATA box and the polyadenylation signal are underlined. The arrow indicates the chicken apoA-I transcription start site. *The GT and AG sequences at the beginning and the end, respectively, of the intervening sequences are indicated* (>). The initiation and termination codons are boxed. The open triangle indicates the polyadenylation site. The amino acid sequence is shown under the corresponding coding nucleotide sequence. The positive and negative numbers along the left and right sides of the sequence indicate nucleotide and amino acid residue numbers, respectively. The nucleotide at the transcription start site and the first amino acid of the mature plasma protein are defined as +1 residues.

Downloaded from www.jlr.org by guest, on June 18, 2012

ASBMB
JOURNAL OF LIPID RESEARCH

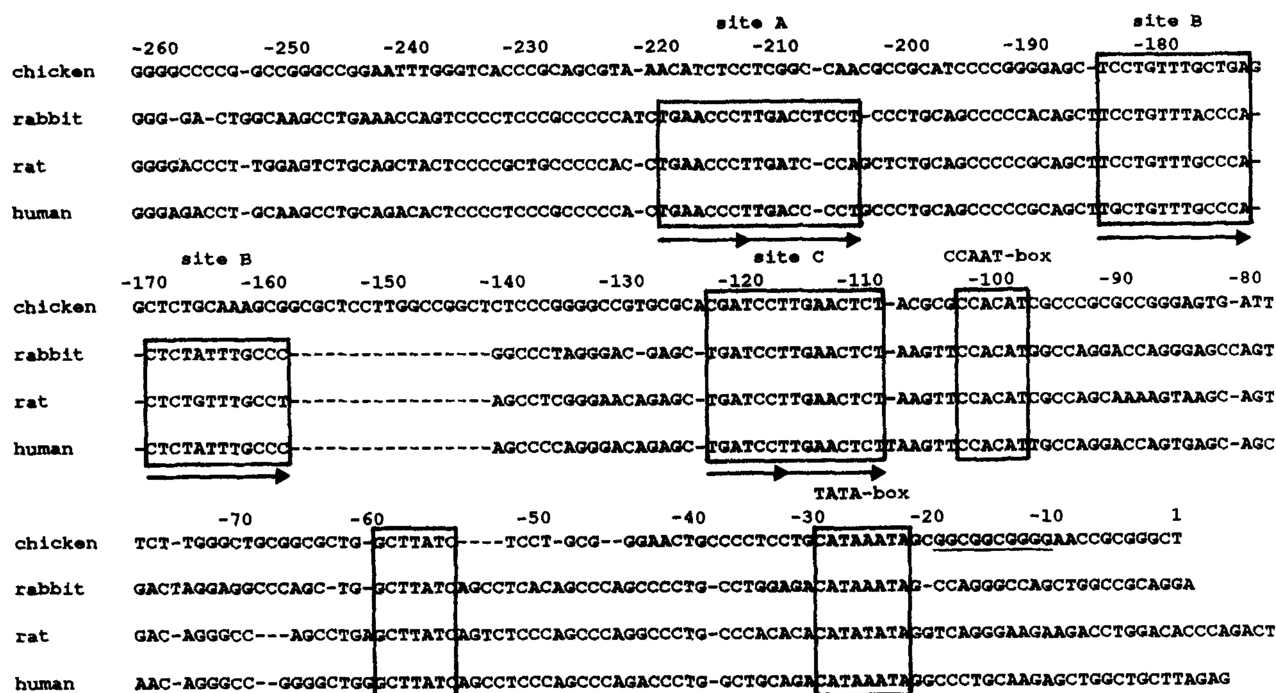

Fig. 3. Alignment of the chicken, rabbit, rat, and human apoA-I gene 5' flanking sequences. The rabbit, rat, and human sequences have been previously reported (12, 21, 33). Numbers indicate nucleotide positions relative to the chicken apoA-I gene transcription start site. Dashes indicate nucleotide deletions introduced to achieve maximal homology. The mammalian A, B, and C sites have been characterized and represent binding sites for transcription factors (15). The arrows below the A, B, and C sites indicate direct nucleotide repeats within each of these sites. The conserved sequences are boxed. The Sp1 binding site in the chicken sequence is underlined.

levels of apoA-I mRNA in every tissue tested, in both female and male adult animals (data not shown). The number of apoA-I mRNA molecules per pg of total RNA was determined in these tissues by primer extension in the presence of an excess of ^{32}P-labeled apoA-I primer (**Table 1**). The results indicate that, in adult male animals, liver and intestine have the highest levels of apoA-I mRNA. In adult females, the levels of liver apoA-I mRNA are markedly lower than the levels measured in male animals. Also the intestinal levels of apoA-I mRNA are decreased in females, as compared to males, but to a lower extent. In all other tissues, similar levels of apoA-I mRNA were measured in male and female animals. These results are consistent with the previously reported distribution and quantitation of the chicken apoA-I mRNA in different tissues of male animals, using a solution hybridization assay (8). ApoA-I mRNA concentration per pg of total RNA was also measured in liver, intestine, and breast muscle at different stages of embryonic and post-embryonic development. As shown in **Fig. 4**, the levels of apoA-I mRNA in liver from day −5 to day 13 of development is not subject to major variations, but a remarkable difference is observed in adult animals, with levels in females equivalent to one-fifth of the levels in males. In the intestine, the synthesis of apoA-I mRNA starts approximately 2 days before hatching and increases dramatically up to 3 days post-hatching. In the adult animal, the intestine is the most important source of apoA-I mRNA and the levels of specific mRNA in females are about one-half of the levels measured in males. In breast muscle, the primer extension measures detectable levels of apoA-I mRNA only around the time of hatching.

Functional analysis of the chicken apoA-I gene 5' flanking region

In order to determine the sequences of the chicken apoA-I gene involved in its expression, we made con-

TABLE 1. Number of apoA-I mRNA molecules/pg of total tissue RNA

Tissue	F	M
Liver	47 ± 4	265 ± 20
Intestine	191 ± 3	344 ± 87
Kidney	84 ± 10	90 ± 24
Ovary/testes	97 ± 24	76 ± 13
Brain	58 ± 2	52 ± 11
Lung	NQ	47 ± 7
Breast muscle	NQ	NQ
Leg muscle	NQ	NQ
Heart muscle	50 ± 13	37 ± 1

The number of apoA-I mRNA molecules per pg of tissue RNA was determined by primer extension, using the apoA-I primer labeled at its 5' end, after measuring the specific activity of the primer (F, female; M, male). NQ, not quantitated by our method. The detection limit is 35 molecules/pg of RNA.

Downloaded from www.jlr.org by guest, on June 18, 2012

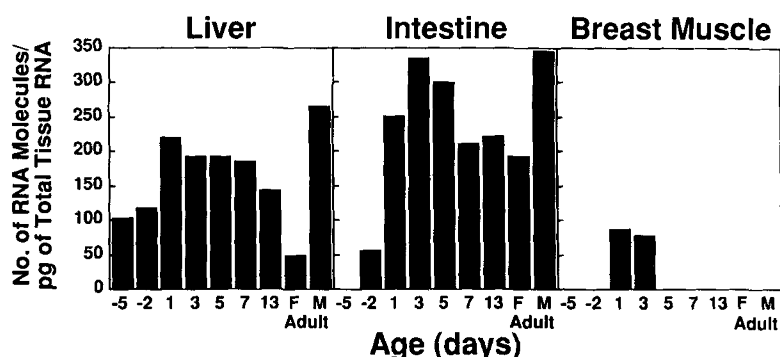

Fig. 4. Developmental variation of chicken liver, intestine, and breast muscle apoA-I mRNA steady state levels. The number of chicken apoA-I mRNA molecules per picogram of tissue RNA in liver, intestine, and breast muscle during different stages of embryonic and post-embryonic development (from day −5 to 13 of development, and during adult life) was determined by primer extension. Twenty-five μg of tissue RNA was hybridized to an excess of ^{32}P-labeled apoA-I primer, after accurate measure of the specific activity of the primer. The reaction mixture was electrophoresed under denaturing condition, the band corresponding to the product of primer extension was excised from the gel, and the incorporated radioactivity was then measured. The number of apoA-I mRNA molecules was then calculated from the specific activity of the primer. (F, female; M, male).

structs containing serial deletions of the 5' upstream region of the chicken apoA-I gene in front of the CAT gene of the CAT expression vector pUC9CAT. The expression of these constructs was determined after transient transfection in cell lines of mammalian origin such as HepG2, Caco2, Hela, and NIH3T3, as well as the quail myogenic cell line QMLA29. Expression of chicken constructs was observed in every cell line tested. The CAT activity of the chicken constructs increases steadily by decreasing the length of 5' upstream sequence, so that the −60chAICAT construct exhibits maximal level of expression, and the −3300chAICAT construct exhibits the lowest level of expression in both mammalian and avian cells (**Fig. 5**). These results suggest that a proximal element between nucleotides −60 and +21 of the chicken apoA-I gene is necessary and sufficient for expression in all cells tested.

Analysis of the DNA sequence in this region shows a GC-rich sequence, located between the TATA box and the transcription start site, between nucleotides −20 and −11. This sequence is remarkably similar to the consensus sequence for the binding of the transcription factor Sp1 (34). It has been previously reported that efficient Sp1 binding requires that at least eight nucleotides of the GGGGCGGGGC consensus sequence be conserved (34). The GC-rich sequence in the chicken apoA-I gene (GGGGCGGCGG) is, in fact, identical to this consensus sequence with the exception of two nucleotides at the 3' end. It is interesting that a sequence with homology to the Sp1 consensus sequence is absent from the corresponding region of the mammalian apoA-I genes (Fig. 3). Gel-retardation experiments, using the ^{32}P-labeled DNA fragment spanning the −60 to +21 region of the chicken apoA-I gene as probe, show that, in fact, Hela protein

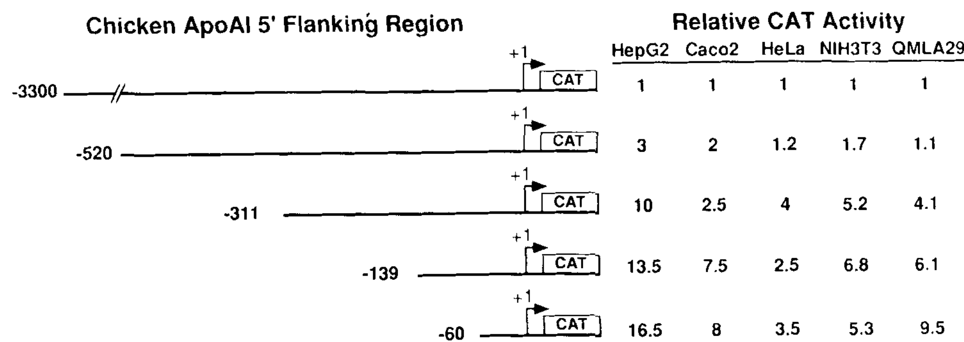

Fig. 5. Expression of the chicken apoA-I/CAT gene fusion constructs in different cell lines. Twenty μg of each plasmid construct containing serial deletions of the 5' flanking region of the chicken apoA-I gene in front of the CAT gene was transfected in HepG2, Caco2, Hela, NIH3T3, and QMLA29 cells together with 5 μg of the RSV-βGal plasmid. Cells were harvested 48 h after transfection and CAT and β-galactosidase activity were determined. CAT activity (CAT/β-galactosidase ratio) is reported for each construct, relative to that of the construct −3300chAICAT. Each value represents the mean of at least three separate experiments.

ASBMB
JOURNAL OF LIPID RESEARCH
Downloaded from www.jlr.org by guest, on June 18, 2012

nuclear extracts specifically retard the migration of this DNA fragment (**Fig. 6**). All the specific protein-DNA complexes are abolished in the presence of a 100-fold molar excess of the −60/+21 unlabeled fragment, and most of the specific protein-DNA complexes are competed by the presence of a 100-fold molar excess of the DNA fragment spanning the three 21 base pair repeats of the SV40 promoter, that has been shown to bind with high affinity the Sp1 factor. The protein-DNA complexes are not abolished in the presence of 100-fold excess of oligonucleotides spanning the transcription factor binding regions of the human apoA-I liver-specific enhancer (sites A and B), or the recognition sequence of the transcription factor C/EBP (35).

The sequence alignment in Fig. 3 indicates that there is partial homology between the human and the chicken sequences in the region corresponding to the human hepatic cell-specific enhancer. This provided the opportunity to compare the transcriptional activity of the mammalian apoA-I gene enhancer (nucleotides −222 to −110) to a structurally altered enhancer as it occurs in the corresponding region of the chicken apoA-I gene (nucleotides −232 to −101). For this comparison, a DNA fragment spanning the −232 to −101 chicken apoA-I gene region was cloned, in either orientation, 5' to the SV40 early promoter in the previously described CAT expression vector pA10CATGem4 (15). The resulting constructs, a construct containing the SV40 enhancer instead of the chicken sequence (construct $pSV_2CATGem4$, ref. 15), and two previously reported constructs containing the wild-type and the site A mutated human apoA-I gene −222 to −110 DNA region instead of the chicken sequence (15), were transiently transfected into HepG2, Hela, and NIH3T3 cells. The results shown in **Fig. 7** indicate that $pSV_2CATGem4$ expresses very high CAT activity levels compared to the pA10CATGem4 vector in all three cell lines and that the construct containing the human apoA-I gene region expresses high CAT activity only in HepG2 cells, consistent with our previous observations (15). In contrast, the constructs containing the chicken apoA-I gene fragment, in either orientation, express CAT activity levels very similar to those of pA10CATGem4 in all three cell lines. These results suggest that differences in the organization of *cis*-acting elements in the apoA-I gene transcriptional control region between birds and mammals play a fundamental role in determining the apoA-I gene tissue expression specificity in these species.

DISCUSSION

The study of transient expression of the chicken apoA-I 5' upstream region deletion constructs in both mammalian and avian cell lines indicates that activation of transcription is mediated by elements contained within a

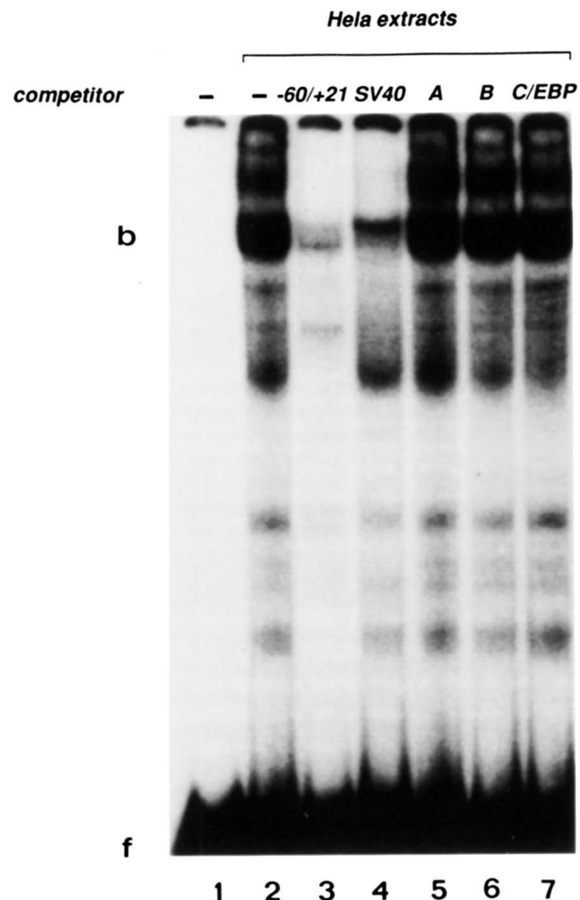

Fig. 6. Binding of Hela nuclear proteins to the −60 to +21 region of the chicken apoA-I gene. Ten μg of Hela nuclear extracts was incubated with the ^{32}P-labeled −60 to +21 DNA fragment of the chicken apoA-I gene (lanes 2 to 7), in the absence (lane 2) or presence of 100-fold molar excess of the following unlabeled oligonucleotides: −60 to +21 chicken apoA-I gene (lane 3), SV40 three 21 bp repeats (lane 4), site A (lane 5) and site B (lane 6) of the human apoA-I liver-specific enhancer (spanning the −214 to −192 and the −178 to −148 regions, respectively, of the human apoA-I gene upstream sequence; also described in ref. 15), and an oligonucleotide containing the recognition sequence 5'-CTGATTGCCCA-3' for the transcription factor C/EBP (ref. 34) (lane 7); f indicates the migration of the free probe, while b indicates the migration of the bound probe (specific protein-DNA complexes).

60 bp region upstream from its transcription start site. This DNA region contains two sequences of possible importance. First, the GCTTATC sequence motif which is located between nucleotides −60 and −54 of the chicken gene and is highly conserved in the rabbit, rat, and human genes, and second, the GGGGCGGCGG sequence motif which is located between nucleotides −20 and −11 of the chicken gene and is absent from the corresponding position of the mammalian genes (Fig. 3).

The importance of the GCTTATC motif in the expression of the avian and mammalian apoA-I genes is not understood. Since it is located in a region not required for the activity of the mammalian liver enhancer (15) it is likely that, if it is involved in transcription regulation of

Downloaded from www.jlr.org by guest, on June 18, 2012

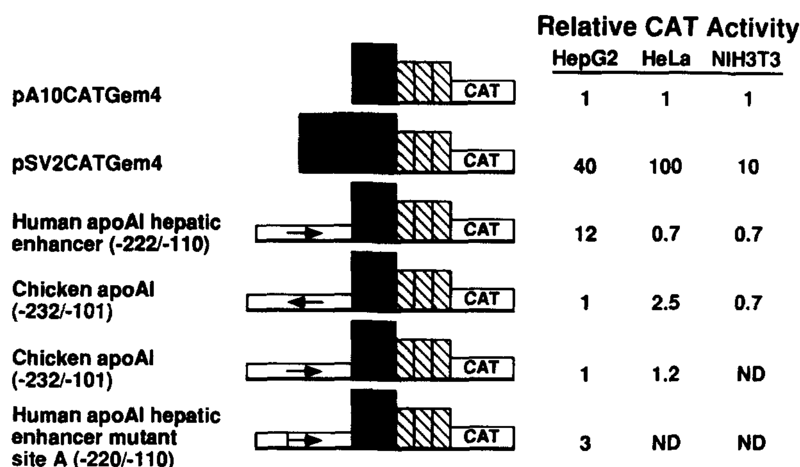

Fig. 7. Expression of the −232 to −101 region of the chicken apoA-I gene, fused to heterologous promoter and CAT gene. The human hepatic specific enhancer, spanning the −222/−110 region of the human apoA-I gene, and the corresponding region of the chicken apoA-I gene (−232/−101) were cloned in front of the CAT gene (open boxes) in the pA10CATGem4 vector. Hatched boxes represent the 21 bp repeats of the SV40 early promoter and filled boxes represent part of the 72 bp repeats of the SV40 promoter. The pSV2CATGem4 plasmid contains the entire SV40 enhancer in front of the 21 bp repeats. Arrows indicate the 5' to 3' orientation of the cloned insert. After transfection of 20 μg of plasmid constructs, together with 5 μg of the RSV-βGal plasmid, CAT and β-galactosidase activity were determined. CAT activity (CAT/β-galactosidase ratios) in HepG2, Hela, NIH3T3 cells is reported as the average of three different experiments, and relative to that of the pA10CATGem4; ND, not determined.

the apoA-I gene, it may be important for modulation rather than establishment of transcription. Whether it is essential for the transcriptional activity of the chicken gene remains to be determined. Preliminary comparison of this motif with motifs involved in binding of previously characterized transcription factors did not reveal any significant homology.

The GGGGCGGCGG motif shows remarkable homology to the binding site for the transcription factor Spl (34). It has been shown that efficient DNA binding by Spl requires that at least eight nucleotides within the binding site be identical to an Spl binding site consensus sequence (34). The GGGGCGGCGG motif matches eight of the ten nucleotides of this consensus sequence. The gel-retardation competition experiments strongly suggest that Spl, or a factor with binding affinity very similar to that of Spl, interacts with this motif. Similar gel-retardation competition experiments with the promoter region of the chicken vitellogenin II gene and the chicken liver cell line LMH nuclear extracts have suggested the presence of an homolog of Spl in the chicken (36). Since Spl is expressed in most tissues, it is possible that it may play an important role in the basal expression of the apoA-I gene in the chicken (37). It is finally noteworthy that the mammalian apoE gene, which, similar to the chicken apoA-I gene, is expressed in all tissues (7, 8), contains two Spl binding sites in its 5' flanking region (38). One of these binding sites is located 160 bp upstream of the transcription start site and is required for enhancer activity, and the other is located approximately 50 bp upstream of the start site and is required for maximal transcriptional activity of the apoE promoter.

Comparison of the chicken, rabbit, rat, and human apoA-I gene 5' upstream regions shows that certain sequences are highly conserved. The sites A, B, and C in the human gene (see Fig. 3) have been shown to play an important role in the transcription activation in hepatic cells (15). Each of these sites has no significant activity alone or when it is multimerized. However, all three together are required for maximal expression of the mammalian apoA-I gene in liver cells (15). It has been previously suggested that, since deletion or mutagenesis of site A reduces but does not eliminate expression of the human apoA-I gene in liver cells, the combination of sites B and C is sufficient for transcription activation in liver cells (15) (Fig. 7). In chicken, site A is missing but half of site B and the entire site C are conserved. In addition, the spacing between sites C and B in the chicken is increased by an insertion of a 17 nucleotide segment, which adds 1.6 DNA helical turns between these sites. Transient transfection experiments in HepG2 cells with the chicken −232 to −101 sequence placed in front of a heterologous promoter does not result in transcriptional activity above basal levels. In contrast, the human hepatic enhancer in which site A is either deleted or mutagenized exhibits activity approximately one fourth of that of the intact enhancer (15). Thus, it is conceivable that the integrity of site B and/or the spacing between sites B and C play an important role in the stereochemical alignment of the transcription factors that bind to these sites and activate transcrip-

Downloaded from www.jlr.org by guest, on June 18, 2012
JOURNAL OF LIPID RESEARCH

tion of the mammalian apoA-I gene in liver cells. These observations raise the possibility that the spacing between sites A, B, and C in the context of the mammalian apoA-I gene enhancer region influences its expression in liver cells.

Our results do not indicate the presence of a tissue-specific enhancer element in the −3300/−60 region of the chicken apoA-I gene, but they suggest the presence of a negative regulatory element located between nucleotides −3300 and −311. It is possible that regulatory elements, involved in the enhancement or modulation of apoA-I expression in different tissues, are located in other regions of the apoA-I gene, such as the introns or the 3' flanking region, as it has been shown for the human apoE gene (39, 40). In fact, it has been shown that *cis*-acting elements required for hepatic specific transcription activation of the human apoE gene are located as far as 18 kb downstream of the apoE gene and that the expression of this gene in different tissues is governed by a complex interaction of several independent tissue-specific transcriptional enhancers and silencers (40). Alternatively, other mechanisms, such as mRNA stabilization, may be involved in the regulation of the chicken apoA-I mRNA steady state levels. For example, it has been shown by run-on experiments that activation of transcription correlates with increased apoA-I mRNA steady state levels in cultured chicken myoblasts, during their differentiation into myotubes (41). However, the magnitude of the transcriptional activation is well below the magnitude of the increase of the steady state apoA-I mRNA levels under the same conditions (41). In addition, Panduro et al. (42, 43) have shown that rat apoA-I and apoE mRNA steady state levels are determined not only by activation of transcription, but also by mRNA stabilization mechanisms. Thus, it is likely that the rapid increase of steady state apoA-I mRNA levels during chicken muscle cell differentiation is due to a combination of transcription activation and mRNA stabilization.

We have observed that the variations of the steady state levels of chicken apoA-I mRNA in liver and intestine during development very closely resemble that of the rat apoA-I in liver and intestine during fetal and neonatal life (12). The only difference is that in chicken apoA-I mRNA is detectable in intestine starting only 2 days before hatching, while significant levels of apoA-I mRNA are already present in rat intestine 5 days before birth. Liver and intestine apoA-I mRNA levels are significantly lower in female than in male adult chickens. This is consistent with the modification of plasma lipoprotein and apolipoprotein profile associated with the hyperestrogenic state in the laying hen (44). In fact, either endogenous estrogens in laying hens or exogenous estrogens administered to immature female chickens have been observed to induce a marked decrease in plasma HDL and apoA-I in chickens (44, 45). Therefore, it is likely that the male–female difference in liver and intestine apoA-I mRNA levels that we have observed in adult animals is related to hormonal maturation.

From a phylogenetic point of view, most apolipoproteins have originated from duplication of an ancestor apolipoprotein gene and subsequent modification of the duplicated copy (46, 47). ApoA-I, C-III, and A-IV genes are closely arranged in a gene cluster in both birds and mammals. The analysis of the 5' upstream region of the human apoA-I gene reveals that site A closely resembles site C, and it has been shown that similar proteins may bind to these two sites (15). It is then possible that the A, B, and C organization of the human enhancer may have risen during the evolution as the duplication of the half site B and site C, as it is in the chicken gene. Alternatively, it is possible the 5' upstream region of the apoA-I gene underwent deletion of the site A and half site B, thus losing the hepatic and intestine specific expression of this gene, to accommodate a broader tissue expression. Clearly, a more complete study of apoA-I 5' flanking sequences in animals preceding the bird–mammal split 280 million years ago (48) will be helpful in resolving this question.

In conclusion, our results strongly suggest that differences in the patterns of tissue-specific regulation of the apoA-I gene in different species may have been accomplished by reorganization and/or deletion of *cis*-acting elements within the apoA-I gene transcription control region.

We thank Dr. Douglas Engel for providing the λ Charon 4A chicken genomic library and Drs. Franco Tató and Giulio Cossu for providing the QMLA29 cell line. We also thank Ms. Emily Flynn-McIntosh for the art work. This research was supported in part by CNR target projects "Biotecnologie" and "Ingegneria Genetica 2" and grants HL32032, HL42488, and HL28481 from the National Institutes of Health. Dr. Sotirios K. Karathanasis is an Established Investigator of the American Heart Association.

Manuscript received 29 August 1991, in revised form 27 December 1991, and in re-revised form 27 February 1992.

REFERENCES

1. Kannel, W. B., J. D. Neaton, D. Wentworth, et. al. 1986. Overall and CHD mortality rates in relation to major risk factors in 325,348 men screened for MRFIT. *Am. Heart J.* **112:** 825–836.
2. Miller, N. E. 1987. Association of high-density lipoprotein subclasses and apolipoproteins with ischemic heart disease and coronary atherosclerosis. *Am. Heart J.* **113:** 589–597.
3. Miller, N. E., A. La Ville, and D. Crook. 1985. Direct evidence that reverse cholesterol transport is mediated by high-density lipoprotein in the rabbit. *Nature.* **314:** 109–111.
4. Hermier, D., P. Forgez, and M. J. Chapman. 1985. A density gradient study of the lipoprotein and apolipoprotein distribution in the chicken, *Gallus domesticus. Biochim. Biophys. Acta.* **836:** 105–118.
5. Kruski, A. W., and A. M. Scanu. 1975. Properties of

Downloaded from www.jlr.org by guest, on June 18, 2012

rooster serum high density lipoproteins. *Biochim. Biophys. Acta.* **409:** 26–38.
6. Blue, M-L., P. Ostapchuk, J. S. Gordon, and D. L. Williams. 1982. Synthesis of apolipoprotein A-I by peripheral tissues of the rooster. A possible mechanism of cellular cholesterol efflux. *J. Biol. Chem:* **257,** 11151–11159.
7. Ferrari, S., E. Drusiani, S. Calandra, and P. Tarugi. 1986. Isolation of a cDNA clone for chick intestinal apolipoprotein A-I (apoA-I) and its use for detecting apoA-I mRNA expression in several chick tissues. *Gene.* **42:** 209–214.
8. Rajavashisth, T. B., P. A. Dawson, D. L. Williams, J. E. Shackelford, H. Lebherz, and A. J. Lusis. 1987. Structure, evolution, and regulation of chicken apolipoprotein A-I. *J. Biol. Chem.* **262:** 7058–7065.
9. Byrnes, L., C-C. Luo, W-H. Li, C-Y. Yang, and L. Chan. 1987. Chicken apolipoprotein A-I: cDNA sequence, tissue expression and evolution. *Biochem. Biophys. Res. Commun.* **148:** 485–492.
10. Tarugi, P., L. Albertazzi, S. Nicolini, E. Ottaviani, and S. Calandra. 1991. Synthesis and secretion of apolipoprotein A-I by chick skin. *J. Biol. Chem.* **266:** 7714–7720.
11. Smith, L. C., H. J. Pownall, and A. M. Gotto, Jr. 1978. The plasma lipoproteins: structure and metabolism. *Annu. Rev. Biochem.* **47:** 751–777.
12. Haddad, I. A., J. M. Ordovas, T. Fitzpatrick, and S. K. Karathanasis. 1987. Linkage, evolution, and expression of the rat apolipoprotein A-I, C-III, and A-IV genes. *J. Biol. Chem.* **261:** 13268–13277.
13. Blue, M-L., D. L. Williams, S. Zucker, S. A. Khan, and C. B. Blum. 1983. Apolipoprotein E synthesis in human kidney, adrenal gland and liver. *Proc. Natl. Acad. Sci. USA.* **80:** 283–287.
14. Williams, D. L., P. A. Dawson, T. C. Newman, and L. L. Rudel. 1985. Apolipoprotein E synthesis in peripheral tissues of non-human primates. *J. Biol. Chem.* **260:** 2444–2451.
15. Widom, R. L., J. A. A. Ladias, S. Kouidou, and S. K. Karathanasis. 1991. Synergistic interactions between transcription factors control expression of the apolipoprotein A-I gene in liver cells. *Mol. Cell. Biol.* **11:** 677–687.
16. Ladias, J. A. A., and S. K. Karathanasis. 1991. Regulation of the apolipoprotein A-I gene by ARP-1, a novel member of the steroid receptor superfamily. *Science.* **251:** 561–565.
17. Ferrari, S., P. Tarugi, E. Drusiani, S. Calandra, and M. Fregni. 1987. The complete sequence of chick apolipoprotein A-I mRNA and its expression in the developing chick. *Gene.* **60:** 39–46.
18. Sambrook, J., E. F. Fritsch, and T. Maniatis. 1989. Molecular Cloning. A Laboratory Manual. Cold Spring Harbor Laboratory. Cold Spring Harbor, NY.
19. Sanger, G., S. Nicklen, and A. R. Coulson. 1977. DNA sequencing with chain-terminating inhibitors. *Proc. Natl. Acad. Sci. USA.* **74:** 5463–5467.
20. Maxam, A. M., and W. Gilbert. 1977. A new method for sequencing DNA. *Proc. Natl. Acad. Sci. USA.* **74:** 560–564.
21. Sastry, K. N., U. Seedorf, and S. K. Karathanasis. 1988. Different *cis*-acting DNA elements control expression of the human apolipoprotein A-I gene in different cell types. *Mol. Cell. Biol.* **8:** 605–614.
22. Alton, N. K., and D. Vapnek. 1979. Nucleotide sequence analysis of the chloramphenicol resistance transposon Tn9. *Nature.* **282:** 864–869.
23. Laimins, L. A., G. Khoury, C. Gorman, B. Howard, and P. Gruss. 1982. Host-specific activation of transcription by tandem repeats from simian virus 40 and Moloney murine sarcoma virus. *Proc. Natl. Acad. Sci. USA.* **79:** 6453–6457.
24. Edlund, T., M. D. Walker, P. J. Barr, and W. J. Rutter. 1985. Cell-specific expression of the rat insulin gene: evidence for the role of two distinct 5' flanking elements. *Science.* **230:** 912–916.
25. Gorman, C. M., L. F. Moffat, and B. H. Howard. 1982. Recombinant genomes which express chloramphenicol acetyltransferase in mammalian cells. *Mol. Cell. Biol.* **2:** 1044–1051.
26. Tató, F., S. Alemá, A. Dlugosz, D. Boettiger, H. Holtzer, G. Cossu, and M. Pacifici. 1983. Development of "revertant" myotubes in cultures of Rous sarcoma virus-transformed avian myogenic cells. *Differentiation.* **24:** 131–139.
27. Dignam, J. D., R. M. Lebovitz, and R. G. Roeder. 1983. Accurate transcription initiation by RNA polymerase II in a soluble extract from isolated mammalian nuclei. *Nucleic Acids Res.* **11:** 1475–1489.
28. Fried, M., and D. M. Crothers. 1981. Equilibria and kinetics of Lac repressor operator interactions by polyacrylamide gel electrophoresis. *Nucleic Acids Res.* **9:** 6505–6525.
29. Sudhof, T. C., D. R. Van Der Westhuyzen, J. L. Goldstein, M. S. Brown, and D. W. Russell. 1987. Three direct repeats and a TATA-like sequence are required for regulated expression of the human low density lipoprotein receptor gene. *J. Biol. Chem.* **262:** 10773–10779.
30. Karathanasis, S. K. 1989. Apolipoprotein genes: organization, linkage and evolution. *In* Human apolipoprotein mutants 2. C. R. Sirtori, G. Franceschini, H. B. Brewer, and G. Assmann, editors. Plenum Publishing Corporation, New York, 1–7.
31. Karathanasis, S. K. 1985. Apolipoprotein multigene family: tandem organization of the human apolipoprotein A-I, C-III, A-IV genes. *Proc. Natl. Acad. Sci. USA.* **82:** 6374–6378.
32. Bhattacharyya, N., R. Chattapadhyay, A. Hirsh, and D. Banerjee. 1991. Isolation, characterization and sequencing of the chicken apolipoprotein A-I-encoding gene. *Gene.* **104:** 163–168.
33. Pan, S-c., I-Q. Hao, T-T. Yamin, P-H. Dai, B-s. Chen, S-I. Chen, A. Kroon, and Y-s. Chao. 1987. Rabbit apolipoprotein A-I mRNA and gene. Evidence that rabbit apolipoprotein A-I is synthesized in the intestine but not in the liver. *Eur. J. Biochem.* **170:** 99–104.
34. Kadonaga, J. T., A. J. Courey, J. Ladika, and R. Tijan. 1988. Promoter-selective activation of transcription by Sp1. *In* The Control of the Human Retrovirus Gene Expression. B. R. Franza, Jr., B. R. Cullen, and F. Wong-Staal, editors. Cold Spring Harbor Laboratory, Cold Spring Harbor, NY, 239–250.
35. Birkenmeier, E. H., B. Gwynn, S. Howard, J. Jerry, J. I. Gordon, W. H. Landschultz, and S. L. McNight. 1989. Tissue-specific expression, developmental regulation, and genetic mapping of the gene encoding CCAAT/enhancer binding protein. *Genes & Dev.* **3:** 1146–1156.
36. Seal, S. N., D. L. Davis, and J. B. Burch. 1991. Mutational studies reveal a complex set of positive and negative control elements within the chicken vitellogenin II promoter. *Mol. Cell. Biol.* **11:** 2704–2717.
37. Lee, W., A. Hoslinger, M. Karin, and R. Tijan. 1987. Activation of transcription by two factors that bind promoter and enhancer sequences of the human metallothionein gene and SV40. *Nature.* **325:** 368–372.
38. Chang, D. J., Y-K. Paik, T. P. Leren, D. W. Walker, J. Howlett, and J. M. Taylor. 1990. Characterization of a human apolipoprotein E gene enhancer element and its associated protein factors. *J. Biol. Chem.* **265:** 9496–9504.
39. Simonet, W. S., N. Bucay, S. J. Lauer, D. O. Wirak, M. E.

ASBMB JOURNAL OF LIPID RESEARCH JLR
Downloaded from www.jlr.org by guest, on June 18, 2012

Stevens, K. H. Weisgraber, R. E. Pitas, and J. M. Taylor. 1990. In the absence of a downstream element, the apolipoprotein E gene is expressed at high levels in kidneys of transgenic mice. *J. Biol. Chem.* **265:** 10809-10812.
40. Simonet, W. S., N. Bucay, R. E. Pitas, S. J. Lauer, and J. M. Taylor. 1991. Multiple tissue-specific elements control the apolipoprotein E/C-I gene locus in transgenic mice. *J. Biol. Chem.* **266:** 8651-8654.
41. Ferrari, S., R. Battini, and G. Cossu. 1990. Differentiation-dependent expression of apolipoprotein A-I in chicken myogenic cells in culture. *Dev. Biol.* **140:** 430-436.
42. Panduro, A., F. Shalaby, and D. A. Shafritz. 1987. Changing patterns of transcriptional and post-transcriptional control of liver-specific gene expression during rat development. *Genes & Dev.* **1:** 1172-1182.
43. Panduro, A., Y.-C. Lin-Lee, L. Chan, and D. A. Shafritz. 1990. Transcriptional and post-transcriptional regulation of apolipoprotein E, A-I and A-II gene expression in normal rat liver and during several pathophysiologic states. *Biochemistry.* **29:** 8430-8435.
44. Hermier, D., P. Forgez, J. Williams, and M. J. Chapman. 1989. Alterations in plasma lipoproteins and apolipoproteins associated with estrogen-induced hyperlipidemia in the laying hen. *Eur. J. Biochem.* **184:** 109-118.
45. Kudzma, D. J., J. B. Swaney, and E. N. Ellis. 1979. Effects of estrogen administration on the lipoproteins and apoproteins of the chicken. *Biochim. Biophys. Acta.* **572:** 257-268.
46. McLachlan, A. D. 1977. Repeated helical pattern in apolipoprotein A-I. *Nature.* **267:** 465-466.
47. Li, W-H., M. Tanimura, C-C. Luo, S. Datta, and L. Chan. 1988. The apolipoprotein multigene family: biosynthesis, structure, structure-function relationships, and evolution. *J. Lipid Res.* **29:** 245-271.
48. Romer, A. S. 1966. The vertebrate body. University of Chicago Press, Chicago.
49. Stumph, W. E., C. P. Hodgson, M-J. Tsai, and B. W. O'Malley. 1984. Genomic structure and possible retroviral origin of the chicken CR1 repetitive DNA sequence family. *Proc. Natl. Acad. Sci. USA.* **81:** 6667-6671.

Downloaded from www.jlr.org by guest, on June 18, 2012