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Structure and Expression of the Chicken Ferritin H-Subunit Gene

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Although the genomes of many species contain multiple copies of ferritin heavy (H)- and light (L)-chain sequences, the chicken genome contains only a single copy of the H-subunit gene. The primary transcription unit of this gene is 4.6 kilobase pairs and contains four exons which are posttranscriptionally spliced to generate a mature transcript of 869 nucleotides. Chicken and human ferritin H-subunit genomic loci are organized with similar exon-intron boundaries. They exhibit approximately 85% nucleotide identity in coding regions, which yield proteins 93% identical in amino acid sequence. We have identified a sequence of 22 highly conserved nucleotides in the 5′ untranslated sequences of chicken, human, and tadpole ferritin H-subunit genes and propose that this conserved sequence may regulate iron-modulated translation of ferritin H-subunit mRNAs.

Ferritin is an ubiquitous iron storage protein which maintains intracellular iron in a form which is biologically accessible and readily mobilized for cellular metabolic processes. Each ferritin molecule is an essentially spherical arrangement of 24 protein subunits encompassing a core of up to 4,500 iron atoms (see references 28 and 38 for reviews). Ferritin subunits are of two types, heavy (H) and light (L), of molecular weight 21,000 and 19,000, respectively (2). The subunits are similar in three-dimensional structure; each is a bundle of four long helices (A through D) with a fifth short helix (E) forming an acute angle to the bundle (31). The H and L subunits appear to be interchangeable within the protein shell (29), generating a large number of isoferritins, certain populations of which are characteristic for particular cell types (2).

Tissue-specific differences in the ratios of H and L subunits appear to be determined by mRNA levels (11), but synthesis of protein subunits is regulated posttranscriptionally by the intracellular iron concentration. When iron is limiting, ferritin mRNAs are stored within the cell but are not translated. In the presence of iron, these preexisting RNAs are recruited by polysomes and actively translated (4, 36, 44).

H and L subunits are derived from different genes, but the number of genes encoding each subunit is not known (22). Multiple copies of H- and L-subunit sequences exist in human, rat, and tadpole genomes (8, 11, 13, 14), but it is not clear how many loci are transcribed and processed into mature mRNAs. Many of the human genomic loci contain intronless pseudogenes flanked by direct repeats. It is therefore possible that only a single human ferritin H-subunit locus is transcriptionally active, whereas 10 to 15 other loci harbor nontranscribed pseudogenes (12). We have isolated genomic recombinants and cDNA clones for the chicken ferritin H subunit and find no evidence for H-subunit pseudogenes in the chicken genome.

MATERIALS AND METHODS

Isolation of chicken ferritin H-subunit genomic and cDNA clones. In a difference screen (34) to obtain genes expressed at high levels in erythroid cells but not in other cell types, a group of 22 nonoverlapping, non-globin-encoding λ recombinants were selected from a λ Charon 4A library of chicken chromosomal DNA (15) when the library was probed with cDNA synthesized from anemic hen reticulocyte (RBC) poly(A)$^+$ RNA. These 22 nonglobin, RBC-positive clones were further screened by DNA dot blots and RNA blot hybridization to identify recombinants whose sequences are more prevalent in RBC mRNA than in mRNA from other cell types. The genomic clone λCh4A-56D selected in this screen was subsequently shown by sequence analysis to contain the 3′ portion of the gene for the chicken ferritin H subunit.

Ferritin H-subunit cDNA clones were isolated from a λgt10 cDNA library prepared from anemic hen RBC mRNA (43) by using the exon-containing, 2.8-kilobase-pair (kbp) *Eco*RI-*Hin*dIII fragment of λCh4A-56D as probe. Of five cDNA clones plaque purified and sequenced (clones 5C, 20A, 21D, 22, and 29), two contain the entire coding sequence and 3′ untranslated region (clones 20A and 22), but none contains the entire 5′ untranslated region.

λEMBL3-56D, a genomic recombinant containing the entire ferritin H-subunit gene, was then isolated by using 5′ and 3′ subfragments of cDNA clone 21D to probe a λEMBL3 library containing 18-kbp *Mbo*I partial digestion fragments of HD6 cell chromosomal DNA (M. Yamamoto, unpublished data) constructed as previously described (10). A 16.3-kbp *Bam*HI fragment in λEMBL3-56D includes approximately 8.2 kbp 5′ and 3.5 kbp 3′ to the 4.6-kbp primary transcription unit of the ferritin H-subunit locus.

RNase and S1 nuclease protection analyses. [α-^{32}P]GTP-labeled antisense RNAs of different lengths were generated by Sp6 polymerase from a 3.9-kbp genomic *Eco*RI fragment of λCh4A-56D subcloned into pSp65 (26). For the shortest probe (0.7 kb), 10^6 cpm was used for hybridization; for the other probes, the number of counts hybridized was directly proportional to probe length. RNA probes were hybridized to 0.25 μg of anemic hen RBC poly(A)$^+$ RNA for 3 h at 37°C and then treated with RNase A and RNase T_1 at 37°C for 30 min as described previously (9, 45). Double-stranded RNA protection fragments were separated on a nondenaturing 3.5% polyacrylamide gel and visualized by autoradiography.

For the S1 nuclease protection, a 510-nucleotide *Nco*I-*Stu*I fragment from λEMBL3-56D was kinase labeled with [γ-^{32}P]ATP at the *Nco*I site and hybridized to 5 μg of anemic hen RBC total RNA at 42°C for 12 h. Hybridization reactions were quenched on ice and then digested with 1,000 U of S1

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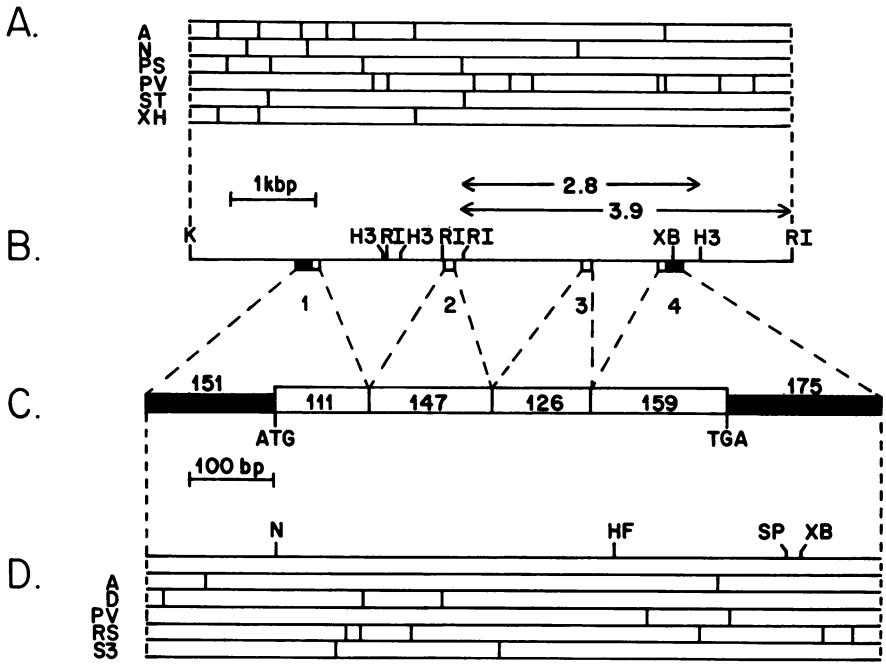


FIG. 1. Structural analysis of genomic recombinants and the transcribed region of the chicken ferritin H-subunit gene. (A) Selected restriction endonuclease maps of the chicken ferritin H-subunit genomic locus shown in B. Vertical lines indicate cleavage positions of the enzyme abbreviated at the left. (B) A map of the chicken ferritin H-subunit gene locus from λEMBL3-56D is shown, beginning at the *Kpn*I site 5′ to the first exon and terminating at the *Eco*RI site 3′ to the final exon. Boxes depict the relative positions of the four exons encoding the ferritin H-subunit within a 7.1-kbp segment of the genome. Symbols: □, coding sequence; ■, 5′ (left) and 3′ (right) nontranslated regions. The 3.9-kbp fragment common to λCh4a-56D and λEMBL3-56D is indicated above the map, together with the 2.8-kbp subfragment mentioned in the text. (C) Structure of the transcribed region of the ferritin H-subunit gene as deduced from data shown below (Fig. 2 and 3). Coding and noncoding sequences are represented as in B. (D) Selected restriction endonuclease maps of the transcribed region of the chicken ferritin H-subunit cDNA. Symbols on the first line mark the cleavage sites of enzymes which cut only once in this region. For the other enzymes listed at the left, vertical lines indicate cleavage positions. Abbreviations: A, *Ava*I; D, *Dde*I; H, *Hin*dIII; HF, *Hin*fI; K, *Kpn*I; N, *Nco*I; PS, *Pst*I; PV, *Pvu*II; RI, *Eco*RI; RS, *Rsa*I; S3, *Sau*3AI; SP, *Sph*I; ST, *Stu*I; XB, *Xba*I; XH, *Xho*I.

nuclease per ml at 30°C for 2 h. Hybridization conditions and S1 nuclease buffers were as previously described (41).

DNA sequence determination. DNA sequencing was accomplished by the dideoxy chain termination method (33) by using M13 subcloning (27) and also by using synthetic primers complementary to Sp6 and T7 promoters to prime dideoxynucleotide-truncated Klenow synthesis from alkali-denatured pGEM plasmid subclones (Promega Biotec, Madison, Wis.). Some regions of the genomic clones were sequenced by a modified chemical cleavage method (25, 37). For all sequence determinations, both DNA strands were independently sequenced.

Cells and cell lines. Chicken embryo fibroblasts (CEF) were prepared from 11-day-old embryos by the procedure of Vogt (40). MSB-1 is a lymphocytic cell line derived from a splenic lymphoma of a chick with Marek's disease (1). HD6 is a continuous definitive erythroid cell line transformed with the *ts*167-AEV allele of avian erythroblastosis virus (5, 30). Transformation of erythroid precursor cells with avian erythroblastosis virus appears to block erythroid differentiation and arrest infected precursor cells at the CFU-E stage (32).

Isolation of RNA samples. Nonerythroid cells and tissues were homogenized in guanidine thiocyanate solution, and RNA was banded in CsCl as described previously (19). For isolation of total cellular RNA from erythroid cells, a modified procedure was developed. About 2 ml of wet-packed RBC isolated from chronically anemic hens (16) were lysed in 16 ml of guanidine thiocyanate-imidazole solution (50% guanidine thiocyanate, 0.5% Sarkosyl, 25 mM sodium citrate, 0.1 M β-mercaptoethanol, 0.1% antifoam A [Sigma Chemical Co.], 0.16 M imidazole; final solution adjusted to pH 5.5 with glacial acetic acid) at maximum speed for 2 min in an Omnitron tissumizer. Cell debris was pelleted and discarded. To the supernatant was added EDTA (pH 8.0) to 25 mM, sodium acetate (pH 5) to 50 mM, and solid CsCl to 1 g/ml. After centrifugation at 12,000 × *g* for 20 min at 20°C (SS34 rotor), the protein rising to the top of the supernatant solution was discarded, as was the residual pellet. The cell lysate-CsCl solution was transferred into 38.5-ml Beckman tubes, underlaid with 3 to 10 ml of ρ = 1.8 CsCl solution, sealed, and then centrifuged at 178,000 × *g* at 20°C for 40 to 50 h (60 Ti rotor). The opalescent RNA band was removed from the side of the tube with a syringe and processed as previously described (19). Poly(A)$^+$ RNA was selected by oligo(dT)-cellulose chromatography (3).

RNA and DNA blot analysis. RNA samples were denatured in formaldehyde solution, electrophoresed on an agarose-formaldehyde gel, transferred to nitrocellulose (20, 23), and probed with radiolabeled antisense RNA generated by Sp6 polymerase transcription of the 5C ferritin H-subunit cDNA fragment cloned into pSp65 (26). The filter was washed and then treated with 8 μg of RNase A per ml in fresh RNase buffer as described previously (43). The chromosomal DNA blot was prepared by standard procedures (18) and probed with a nick-translated insert from cDNA clone 20A. The filter was washed at 42°C four times for 30 min each in 50

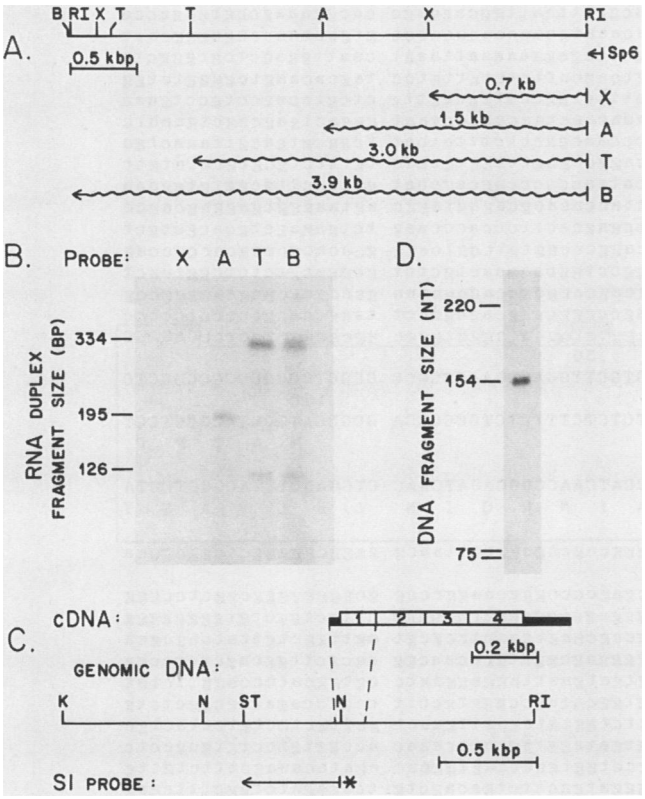


FIG. 2. RNase and S1 nuclease mapping experiments. (A) Four radiolabeled antisense RNA probes of different lengths were transcribed from a pSp65 subclone of the 3.9-kbp *Eco*RI fragment of λCh4A-56D (Fig. 1B) truncated at restriction enzyme sites within the insert fragment (X, A, T) or at the *Bam*HI site of the vector polylinker sequence (B). (B) Each probe was independently hybridized to 0.25 μg of anemic hen RBC poly(A)$^+$ RNA and then treated with RNase A and RNase T_1 to digest all nonhybridized RNA; protected fragments were then separated on a neutral 3.5% polyacrylamide gel (9). The 0.7-kb probe (X) does not hybridize to RBC mRNA. The 1.5 kb probe (A) protects a fragment of 195 nucleotides, which is not seen with the next larger probes. Thus the *Ava*I site interrupts an exon of 334 nucleotides. The 2.7-kb probe (T) protects two exons 334 and 126 nucleotides in length. No additional exons are protected by the full-length 3.9-kb probe (B). (C) Probe for the S1 nuclease mapping experiment was a 510-nucleotide *Nco*I-*Stu*I genomic fragment (nucleotides 918 to 1428 of Fig. 3) radiolabeled at the *Nco*I site, which contains the translational start codon. The cDNA map indicates coding sequences (▭) and noncoding regions (▬) of the largest isolated cDNA recombinant, clone 22, which terminates 25 nucleotides 5′ to the AUG start codon (nucleotide 127 of the transcribed sequence shown in Fig. 3). (D) Anemic hen RBC total RNA (5 μg) was hybridized to the probe shown in C, digested with S1 nuclease, and electrophoresed on a 5% polyacrylamide–50% urea gel in parallel with radiolabeled DNA restriction enzyme fragment markers (41). The protected fragment and DNA markers were visualized by autoradiography. Abbreviations: A, *Ava*I; B, *Bam*HI; K, *Kpn*I; N, *Nco*I; RI, *Eco*RI; ST, *Stu*I; T, *Taq*I; X, *Xmn*I; BP, base pairs; NT, nucleotides.

mM Tris (pH 7.5), 1 mM EDTA, 0.1% sodium dodecyl sulfate, 1 mM Na_2PO_4, and 1 mM sodium pyrophosphate.

RESULTS

Isolation and structural analysis of genomic and cDNA clones. Genomic clone λCh4A-56D was isolated from a chicken RBC chromosomal DNA library (15) by using difference methods (34; see Materials and Methods) to screen for genes transcribed at high levels in erythroid cells but at much lower levels in nonerythroid tissues and cells. A 2.8-kbp *Eco*RI-*Hin*dIII subclone of λCh4A-56D (Fig. 1B) shown to contain two exons of the chicken ferritin H-subunit gene (Fig. 2A and B) was used to probe a λgt10 cDNA library (43). Five independent cDNA clones were plaque purified and sequenced; all five clones are of identical sequence in the overlapping regions. Fragments from the 5′ and 3′ ends of cDNA clone 21D were then used to screen a λEMBL3 chicken chromosomal library to obtain a genomic clone spanning the chicken ferritin H-subunit gene (see Materials and Methods). The clone selected, λEMBL3-56D, contains four exons which encode the entire chicken ferritin H subunit (Fig. 1B).

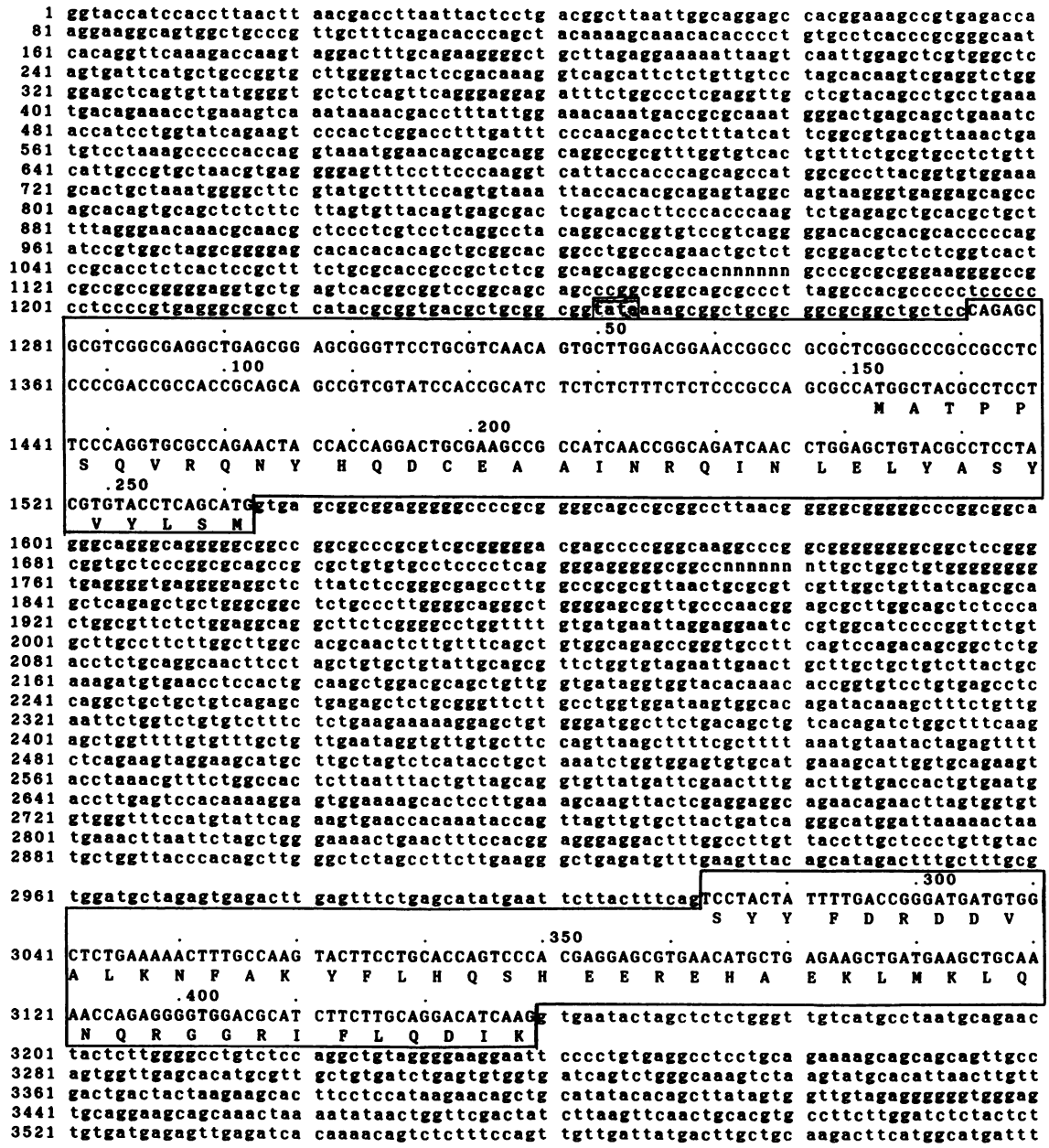
Nucleotide sequence analysis of the chicken ferritin H-subunit gene. We have sequenced a 7.1-kbp segment of the chicken genome which contains the entire ferritin H-subunit locus (Fig. 3), including 1.2-kbp 5′ and 1.3-kbp 3′ flanking sequences (Fig. 1B). The ferritin H-subunit transcript is generated from four exons, which together with three introns span 4.6 kbp in the chicken genome and are processed to an mRNA of 869 nucleotides (Fig. 1C).

A single transcriptional initiation site 154 nucleotides 5′ to the translational start codon was identified by S1 nuclease mapping (Fig. 2C and D). A standard TATA box is located 31 nucleotides 5′ to the transcriptional initiation site, but we do not find an adjacent CCAAT box (7). Immediately abutting the first exon are sequences extremely rich in guanine (G) and cytidine (C) nucleotides. Whereas the average G+C content of the chicken ferritin H-subunit genomic locus is 40 to 45%, the 100 bases 5′ to exon 1 are greater than 75% G+C, and the 150 bases 3′ to exon 1 are nearly 90% G+C.

Locations of introns within the chicken ferritin H-subunit sequence correspond precisely to their positions within the intron-containing human ferritin H-subunit gene, whose genomic sequence has also been determined (12, 21). The first intron is roughly 1.5 kbp in both chicken and human sequences, but the latter two introns are each approximately 7 times longer in the chicken gene: 1,491 versus 256 nucleotides for intron 2 and 725 versus 95 nucleotides for intron 3 (12, 21). Nucleotide sequences of the chicken and human coding regions are quite homologous, with 85, 84, 79, and 82% identity of coding regions for exons 1 through 4, respectively. Untranslated regions of the chicken and human H-subunit genes are more divergent, with only about 60% identity of the 5′ ends and about 40% identity of the 3′ ends with the algorithm of Wilbur and Lipman (42).

Although overall homology in untranslated regions is poor, a segment of 22 nucleotides toward the 5′ end is identical within the chicken and human ferritin H-subunit genes (nucleotides 41 to 62 of the transcribed region shown in Fig. 3). These 22 nucleotides occur within the context of 39 nucleotides, of which only 4 differ between the chicken and human sequences. The 22-nucleotide homology is maintained with only two substitutions (lowercase letters) within the 5′ untranslated region of the tadpole RBC ferritin sequence (14):

chicken ^{30}GGGTTCCTGCGTCAACAGTGCTTGGAC
GGAACCGGCCGC
human ^{28}GGtTTCCTGCtTCAACAGTGCTTGGACG
GAACCcGgCGC
tadpole ^{24}GaGTTCtTGCtTCAACAGTGtTTGaAC
GGAACCctCtct



Although highly conserved in ferritin H-subunit sequences, this sequence does not occur within the 5′ untranslated regions of published human and rat ferritin L-subunit cDNA sequences (6, 17, 24). We therefore propose that this conserved 22-nucleotide sequence is the region of ferritin H-subunit RNA which mediates iron induction of H-subunit protein synthesis (see Discussion).

Analysis of amino acid sequence of the chicken ferritin H subunit. The chicken ferritin H-subunit gene encodes a protein of 179 amino acids which, without posttranslational modification, has a calculated molecular weight of 20,941 and a pI of 6.1. Although the pI of 6.1 is substantially higher than the pI range of 4.2 to 5.6 determined for ferritins of other species (38), it is consistent with the pI of 6.14 determined experimentally for chicken spleen ferritin (35). The predicted chicken ferritin H-subunit sequence has 93% identity with the amino acid sequence of the human ferritin H subunit (6, 11, 12). Seventy-three consecutive amino acids (Glu-16 through Asp-88) are identical in the amino-terminal halves of the chicken and human H-subunit sequences. There are amino acid differences at 10 internal positions; Cys-15, Arg-89, Asn-94, Thr-97, Glu-122, Asp-138, Gln-146, Lys-161, Tyr-162, and Met-164 in the chicken sequence are replaced by Ser, Cys, Ser, Asn, Asp, Asn, Glu, Glu, Ser, and Leu at the corresponding positions of the human protein. H-subunit sequences vary most at amino-terminal (Ala-Thr-Pro-Pro in chicken, Thr-Thr-Ala-Ser-Thr in human) and carboxyl-terminal (Glu-Ser-Asp-Ser in chicken, Asp-Ser-Asp-Asn-Glu-Ser in human) ends.

When the chicken and human H-subunit sequences are aligned with the horse spleen L-subunit sequence and helical boundaries determined by X-ray crystallography of the L-subunit (31) are imposed upon H-subunit sequences, it is clear that the amino acid sequences of the four long helices

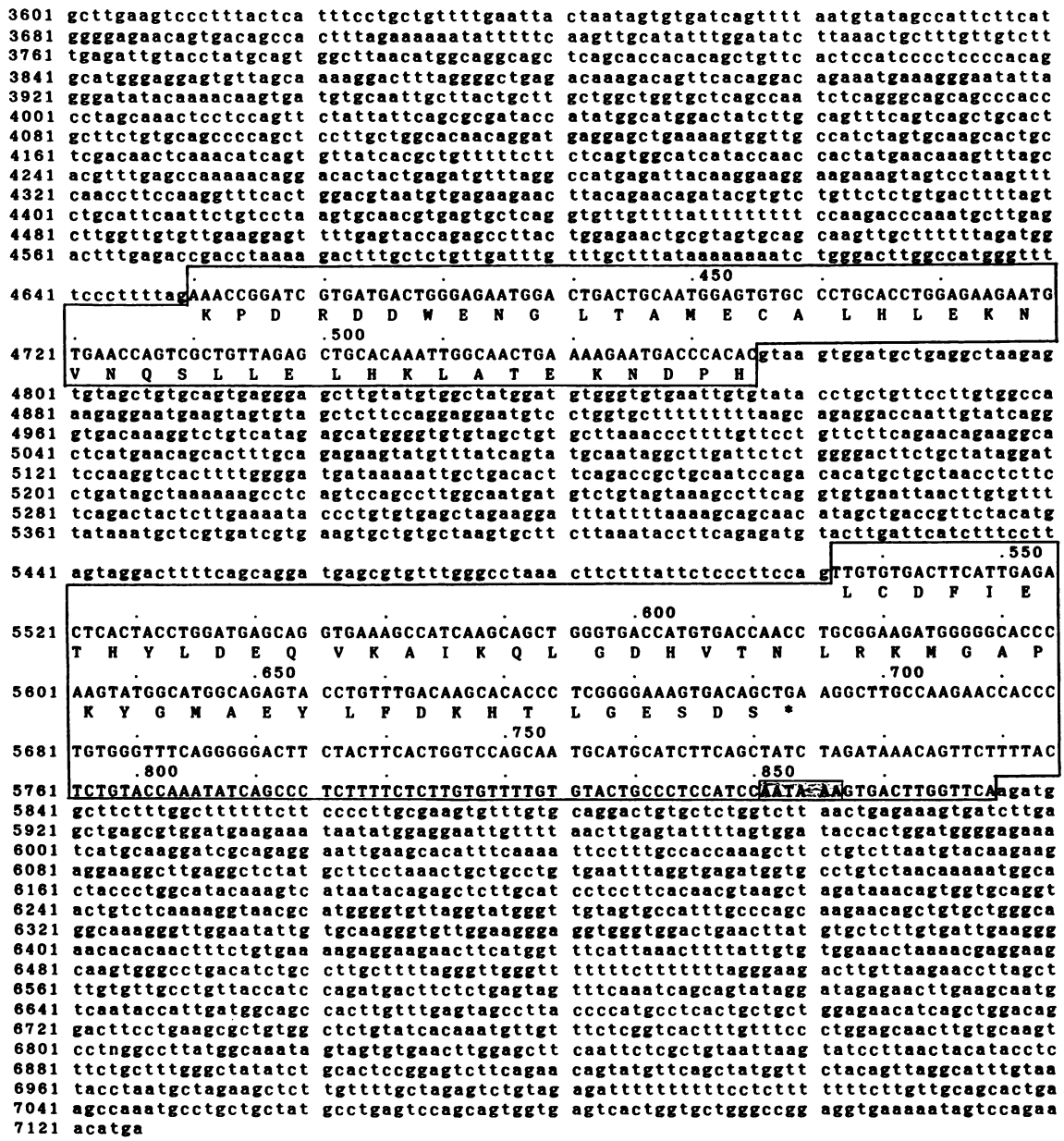


FIG. 3. Sequence of the chicken ferritin H-subunit locus. Sequence is of the 7.1-kbp ferritin H-subunit locus mapped in Fig. 1B. The four exons are individually outlined in boxes within the locus. Lowercase letters are used for nontranscribed regions; the transcribed sequence is shown in uppercase letters. The genomic sequence is numbered to the left of each line; arbitrarily, nucleotide 1 is assigned as the first base of the *KpnI* site. Above the DNA sequence, dots mark every 10th base of the mRNA sequence, with a number at every 50th nucleotide. Listed below the transcribed sequence is the amino acid sequence predicted from the single open reading frame. Amino acids are indicated by the standard one-letter code; * marks the translational stop codon. The TATA box 5′ to the transcriptional start site and the AATAAA polyadenylation signal in the fourth exon are highlighted by shading.

which form the core of ferritin subunit structure are strictly conserved between the two H-subunit sequences. Amino acids of helix B are identical at every position. Helices A, C, and E each have one or two amino acid substitutions, all within the first or last helical turns, whereas helix D sustains conservative substitutions for two internal helical residues: Asn for Asp-138 and Glu for Gln-146. It is noteworthy that the chicken ferritin H subunit maintains the sequence Asp-Pro-His-Leu-Cys-Asp-Phe at residues 125 through 131 as well as the tetrapeptide Glu-Tyr-Leu-Phe at positions 166 through 169. No amino acid variability has been observed in either of these regions in any published ferritin H- and L-subunit sequences (14).

Expression of ferritin H-subunit RNA. RNA blot analysis (Fig. 4) shows that in total cellular RNA, the chicken ferritin H-subunit transcript is expressed at essentially the same level in all erythroid cells examined: normal cells of the primitive (4.5-day embryonic RBC) and definitive (11-day embryonic and anemic hen RBC) erythroid lineages as well as transformed erythroid precursor cells (HD6). Liver RNA samples from three developmental stages (15-day embryo, 1-month-posthatching chicken, and adult hen) have approx-

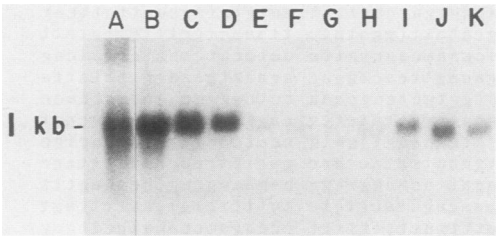


FIG. 4. Expression of ferritin H-subunit mRNA. Total cellular RNA (10 μg/lane) was electrophoresed on a 1.4% agarose–2.2 M formaldehyde gel, transferred to nitrocellulose (20, 23), and probed with an antisense RNA transcript of ferritin H-subunit cDNA clone 5C (nucleotides 202 to 869 of the transcribed region shown in Fig. 3). Lanes A through D contain erythroid RNA samples: A, 4.5-day embryonic RBC; B, 11-day embryonic RBC; C, anemic hen RBC; and D, HD6 RNA. Lanes E through H contain RNA samples from nonerythroid, nonliver cells and tissues: E, MSB-1 cells; F, 4.5-day total chicken embryo (from which embryonic RBC were perfused); G, 11-day CEF; and H, 11-day embryonic brain RNA. Lanes I through K contain liver RNA samples: I, 15-day embryonic liver; J, 1-month-posthatching chicken liver; and K, adult hen liver RNA.

imately equal levels of ferritin H-subunit RNA, although the amount of the transcript per microgram of total RNA is lower (approximately threefold) for liver RNA than for erythroid RNA. Transcripts from the ferritin H-subunit gene are also evident in each of the nonerythroid, nonliver RNA samples (MSB-1 cells, 4.5-day total embryo, 11-day CEF, 11-day embryonic brain), although at much lower levels.

Analysis of ferritin H-subunit sequences in chicken genome. When chicken genomic DNA was cleaved with various restriction enzymes and probed with an H-subunit cDNA clone at low stringency (Fig. 5), the hybridization pattern was precisely that predicted from the map of λEMBL3-56D (Fig. 1B). A single *Bam*HI fragment encompasses the entire ferritin H-subunit gene, and the 16-kbp *Xba*I fragment includes all but 95 nucleotides of the fourth exon. The 3.9-kbp *Eco*RI fragment and the 3.6-kbp *Hin*dIII fragment contain 3′ exons, whereas minor bands in these lanes at 5.8 and 5.6 kbp contain the first exon, of which only 125 nucleotides are included in the cDNA clone used as probe. The 0.24-kbp *Eco*RI fragment which contains the second exon is too small to be seen in this blot. In the chicken genome, therefore, ferritin H-subunit sequences exist in a single locus from which mRNA encoding the protein is transcribed. We find no evidence for multiple copies of the ferritin H-subunit gene in chicken, although chicken ferritin H-subunit cDNA probes detect multiple sequences in *Eco*RI-digested human genomic DNA (data not shown).

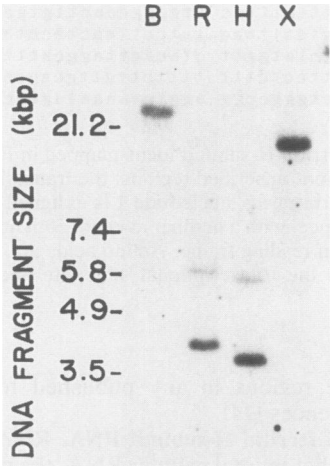


FIG. 5. Southern blot analysis of chicken genomic DNA. Chicken RBC DNA (5 μg per lane) was digested with *Bam*HI (B), *Eco*RI (R), *Hin*dIII (H), or *Xba*I (X); fragments were separated on a 0.8% agarose gel and blotted to nitrocellulose (18). The probe was nick-translated cDNA clone 20A (nucleotides 137 to 869 of the transcribed region shown in Fig. 3). DNA size markers (λ*c*I857*S*7 digested with *Eco*RI) were electrophoresed in parallel with the genomic DNA.

DISCUSSION

Our interest in the regulation of genes specifically induced during erythroid differentiation led us to isolate and characterize the chicken ferritin H-subunit gene described in this report. This gene is of special interest to us not only because its mRNA is abundant in erythroid cells but also because its expression is controlled at multiple levels.

First, RNA blot analysis of ferritin H-subunit transcripts in various chicken tissues and cell types indicates that the level of ferritin H-subunit mRNA is controlled in a tissue-specific manner. We therefore predict that the varied amounts of RNA result from differential transcription in the various tissues and cell types and that tissue-specific regulatory sequences modulate transcription from the single ferritin H-subunit gene in individual chicken tissues.

Second, other investigators have demonstrated that the ratio of ferritin H and L subunits in particular cell types appears to be determined by RNA levels for the two subunits and that the ratio of H mRNA to L mRNA may vary greatly during the course of cellular differentiation in any given tissue type (11). Although we find essentially equivalent amounts of ferritin H-subunit transcript in the total cellular RNA of erythroid cells from three different ages of organism development and from early and late stages of erythroid maturation, we have not assessed the ratio of H to L subunits in these samples. It will be of interest to determine whether transcription from the ferritin H- and L-subunit loci is regulated independently or coordinately during erythroid development and differentiation.

Third, the synthesis and accumulation of ferritin protein subunits is controlled posttranscriptionally by the level of intracellular iron (4, 36, 44). We presume that just as ferritin subunit amino acid sequences are maintained with few substitutions in species as divergent as chickens and humans, likewise the cellular mechanism for iron-modulated translational control of ferritin subunits must be highly conserved among various species. Thus it is conceivable that the 22-nucleotide identity located in the otherwise marginally homologous 5′ untranslated regions of the chicken and human ferritin H-subunit genes may be functionally conserved to effect iron-modulated sequestration and translation of ferritin H-subunit messages.

Fourth, there may exist yet another level in the control of ferritin synthesis during chicken embryonic development, specifically during the switch from the primitive to the definitive erythroid series. Ferritin protein levels have been measured to be 10-fold higher in the primitive RBC of 3-day chicken embryos than in the circulating definitive RBC of 10-day chicken embryos or adult roosters. This decline in ferritin content of the circulating RBC correlates with the replacement of the primitive embryonic erythroid lineage by the definitive adult erythroid lineage (39). Interestingly,

however, at the RNA level we do not observe the difference in ferritin content of the two different erythroid lineages. The amount of ferritin H-subunit mRNA is essentially equivalent in 4.5-day (primitive) and 11-day (definitive) chicken embryo RBC as well as in circulating definitive RBC from anemic hens (Fig. 4). To adequately dissect the various levels of control of ferritin gene expression, it will be necessary to determine whether the differing amounts of ferritin protein in cells of the primitive and definitive erythroid series are a consequence of variation in iron concentration within cells of the two lineages or whether there is an additional cellular regulatory mechanism which differentially modulates the ferritin content of primitive and definitive erythroid cells.

Other investigations of the regulation of ferritin subunit gene expression have been complicated by the fact that the rat, tadpole, and human genomes contain multiple loci which harbor sequences complementary to ferritin H- and L-subunit cDNAs. The chicken genome, however, contains only a single copy of the gene encoding the ferritin H subunit. Since our studies provide the DNA sequence of the entire chicken ferritin H-subunit locus (including flanking sequences and introns) as well as a complete transcriptional map, it should now be possible to probe the multiple levels of regulation which govern expression from this ferritin H-subunit gene.

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