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Cloning of the gene coding for human L apoferritin

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ABSTRACT

A recently reported cDNA clone coding for human promyelocytic L apoferritin, shows some differences with a liver L apoferritin cDNA. We have investigated if these differences are due to the expression of different genes or to an alternative transcription of an unique gene. In this paper we report data suggesting that a single gene is mainly expressed in several tissues examined. This gene has been cloned and characterized. Its sequence shows three introns: the exon sequence is identical to that of cDNA clone isolated from human liver. A minimum of five related pseudogenes have been also analysed. One of them is a processed pseudogene interrupted by an intron-like fragment.

INTRODUCTION

Iron storage in both prokaryotic and eukaryotic cells is assured by a specialized protein, ferritin, which maintains the iron in a non toxic and bioavailable form (1). Ferritin consists of 24 apoferritin subunits of H and L type, which are assembled in presence of iron to constitute a functional polymer (2). It has been suggested that the H/L chain ratio is responsible for the heterogeneity of ferritins isolated from different tissues (3). These isoferritins actually differ in their immunological, electrophoretical and metabolic properties, being, for instance, more acidic and rich in H subunits in heart and HeLa cells, and less acidic and rich in L chains in liver and spleen (2, 4). Even though iron storage is considered the main role of ferritin, involvement in other biological functions has been described, such as an influence on leukocyte maturation and an inhibition of lymphocyte proliferation (5, 6). Moreover, it has been suggested that a glycosilated ferritin form may be secreted and play a role in some leukocyte functions (7).

To explain the ferritin heterogeneity, two molecular mechanisms have been proposed: a) the heterogeneity is due to the tissue-specific expression of a number of genes coding for a family of related apoferritin isoforms; b) the heterogeneity is due to the tissue-specific regulation of expression of two

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unique genes coding for H and L subunit respectively, resulting in the assembly of polymers containing different H/L ratios.

We report here data supporting the hypothesis that an unique gene is expressed for human L apoferritin. The cloning and characterization of this gene will be described. A minimun of five related pseudogenes were also identified in the human genome. This is in agreement with our previous results obtained on the human H apoferritin gene family, which comprises a single coding gene and several pseudogenes (8).

MATERIALS AND METHODS

Bacterial strains, plasmids and phage vectors

Escherichia coli K12 (strains 71/18 and TG1) was used for transformation (9). The M13 phage derivatives mp18 and mp19 (10), and the plasmids puc18 and puc19 (11) were used as vectors for subcloning and sequencing. Phage EMBL3 (12) and the cosmid vector pcos2EMBL (13) were used in the construction of the human genomic libraries. Transformation and preparation of single- and double-stranded DNA were as described (14, 15). Large-scale preparation of EMBL3 and pcos2EMBL recombinants was done according to (12) and (13).

Enzymes and chemicals

Restriction endonucleases, T4 polynucleotide kinase, T4 DNA ligase, DNA polymerase I holoenzyme and Klenow fragment were purchased from B.R.L. and Biolabs. ^{32}P-labelled compounds were purchased from Amersham. AMV reverse transcriptase was purchased from Boehringer.

Cell coltures

U937, Daudi and HeLa cell lines were grown in RPMI or DMEM medium supplemented with 20% foetal calf serum. U937 cells were induced to differentiate as previously reported (16).

Oligodeoxynucleotide synthesis

Oligodeoxynucleotides were synthesized following the phosphite-amidite method (17).

DNA sequencing

Sequence analysis was done using the dideoxy chain termination method (18).

Primer elongation

Oligodeoxynucleotide primers were labelled at the 5′ end with γ-^{32}P ATP and T4 polynucleotide kinase. 1 or 3 µg of polyadenylated RNA were mixed with 9 volumes of DMSO, heated at 45° C for 20 min, ethanol precipitated and resuspended in 50 mM Tris-HCl pH 8.3, 5 mM $MgCl_2$, 50 mM KCl, 1 mM DTT, 0.4 mM

dNTPs with 0.2 pmol of kinased primer and 20 Units of AMV reverse transcriptase. Incubation was carried out at 42° C for 2 h. After phenol extraction the samples were ethanol precipitated, washed with 80% ethanol and loaded on 6% denaturing polyacrylamide gel.

S1 mapping

The PstI-PstI fragment FrL200 (Fig.1A) from the L chain cDNA, was subcloned in mp19. The L200 subclone, whose single-stranded DNA carries the coding strand of FrL200, was used as a template to synthesize the anticoding probe complementary to the L apoferritin RNA according to (19). The experimental procedure for DNA-RNA hybridization and digestion with S1 nuclease was according to Berk and Sharp (20) as modified by Ciliberto *et al.* (21).

Screening of the human libraries

The genomic library provided by Dr. G. Bensi (22) and the cosmid library provided by Dr. A.M. Frischauf were screened using as a probe the nick-translated PstI-PstI fragment FrL600 (Fig.1A) from the human liver L chain cDNA. The screening was performed according to (12) and A.M. Frischauf (personal communication).

Electron microscopy analysis

For the heteroduplex and hybrid analysis separated strands of the recombinant lambda DNAs and of the EMBL3 vector were isolated (23). Supercoiled DNA of recombinant cosmids was cut with ClaI and single strands were isolated from agarose gels. For the heteroduplex preparation equal amounts of the separated strands of the different recombinants were incubated in 50% formamide, 10 mM Tris-HCl pH 7.4, 1 mM EDTA, 0.2 M CsCl for 30 min at 37° C. Samples were spread with cytochrome C (type V, Sigma) from 30% formamide, 0.1 M Tris-HCl pH 8.5, 1 mM EDTA onto a hypophase of 0.005% octyl-glucopyranoside (24). The preparations were stained with uranyl acetate and rotary shadowed with platinum.

Hybrids were prepared by passing a mixture of complementary strands of the recombinants and of the vector through a Sephadex G-50 column (2x45 mm) equilibrated with 80% formamide, 0.1 M HEPES pH 8.3, 0.4 M NaCl, 0.01 M EDTA (25). 3 µl of human liver poly A+ RNA (0.17 mg/ml) were added and the samples were heated at 56° C in sealed Teflon tubings; temperature was decreased to 43° C in 4 h and 20 min (1° C per 20 min) and the incubation was then carried out at 43° C for 15 h. Samples were then passed through two successive Sepharose Cl-2B columns (2x45 mm): the first one washed with 0.1 M $MgCl_2$ and the second one with 0.1 M Tris-HCl pH 8, 1 mM EDTA. 20 µl aliquots were

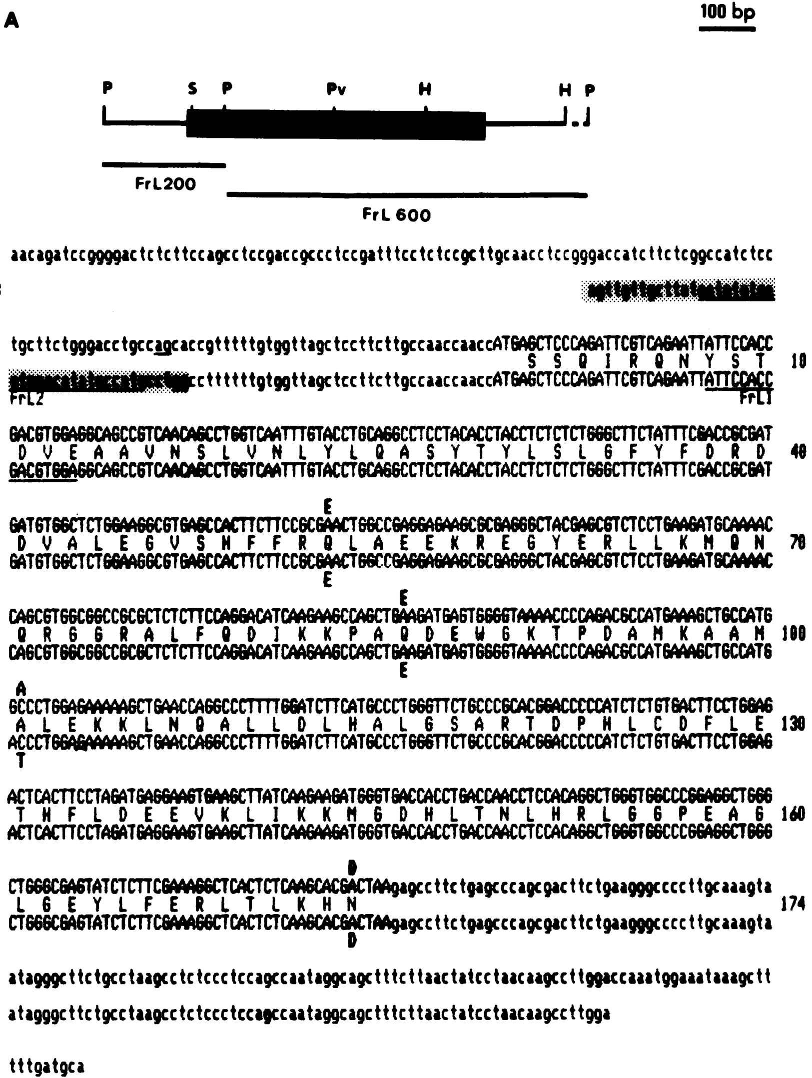

Fig. 1. Comparison between human L apoferritin cDNA sequences. A) Scheme of the human liver cDNA clone pFr36 (ref.26). The thick and thin lines represent the coding and untraslated sequences respectively. The dashed line represents the poly A tail. The two PstI-PstI fragments FrL200 and FrL600, used as probe, are shown. P: PstI site; S: SacI; Pv: PvuII; H: HindIII. B) Nucleotide and aminoacidic sequences of human L apoferritin cDNAs from liver (pFr36) and U937 cell line (p680.8; ref. 27). The deduced aminoacidic sequences are compared to the primary structure of liver L apoferritin (LFr; ref. 28). Only residue differences among the three sequences are shown. Dotted sequence corresponds to the divergent region between the two cDNAs. The two sequences complementary to FrL1 and FrL2 oligonucleotides are underlined. Capital letters represent coding sequence.

collected and 12 μl of the fractions containing the hybrids were spread in 50% formamide as described above.

RESULTS

Comparison between L apoferritin cDNA clones from different tissues and the liver L chain aminoacidic sequence.

The strategy to identify the human liver cDNA clone pFr36 has been previously reported (26). In Fig.1B its nucleotide sequence is compared to that of the cDNA clone p680.8 derived from a cDNA library of the human promyelocytic cell line U937 and recently described by Dorner et al. (27). The aminoacidic sequences deduced from the two clones are compared to the primary structure of human liver L apoferritin (28). There are two types of differences among the three aminoacidic sequences or between the two nucleotide sequences: i) aminoacidic differences between the conceptual translation products of the two cDNA clones and the actual L apoferritin

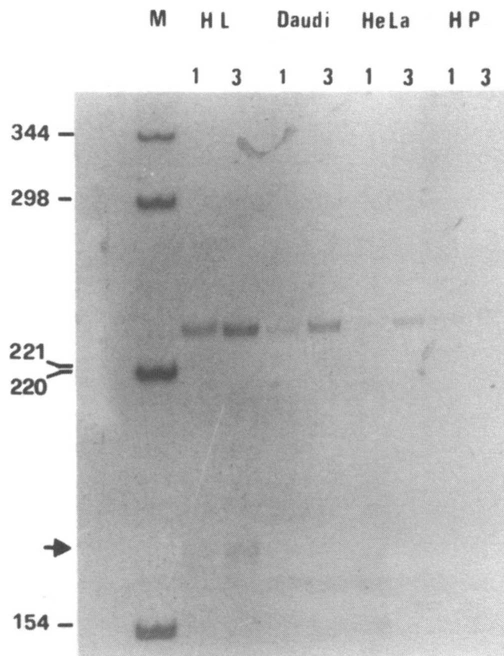

Fig. 2. Primer elongation analysis. 1 or 3 μg of poly A+ RNA from human liver (HL), Daudi cells, HeLa cells and human placenta (HP) were annealed to ^{32}P labelled FrL1 primer. cDNA synthesis was carried out as described in Materials and Methods. Size markers (M) are shown. The arrow indicates an early terminated cDNA.

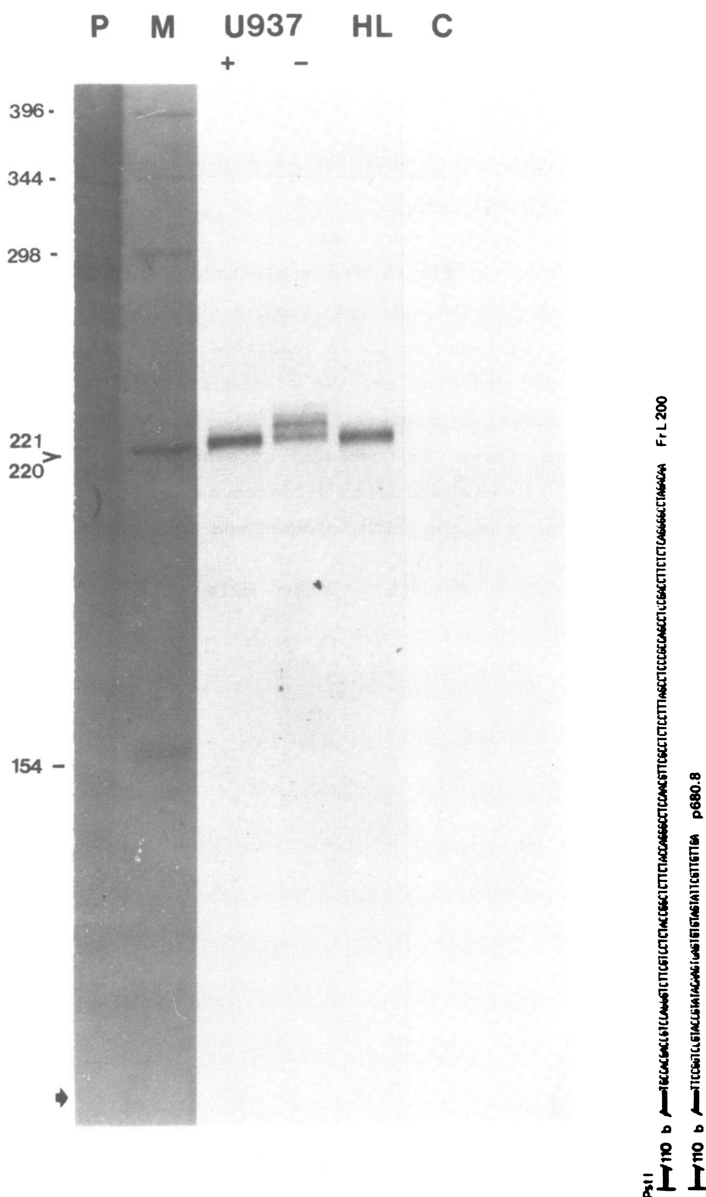

Fig. 3. S1 analysis. 50 µg of total RNA from induced (+) or uninduced (-) U937 cells, human liver (HL) and tRNA (C) were annealed to 10^6 cpm of FrL200 single stranded probe (P) and subsequently digested with S1 nuclease. The arrow indicates the size of the protected fragment expected if a mRNA corresponding to p680.8 cDNA were present. Size markers (M) and cDNA non homologous sequences are shown.

sequence; ii) nucleotide differences between the two cDNA clones. The former type is due to the ambiguity between glutamic acid/glutamine and aspartic acid/asparagine at the codons 53, 86 and 174. Since these codons are the same in the two cDNA clones, the aminoacidic differences are likely to arise from difficulties in resolving such residues during the protein sequence analysis (Crichton personal communication). As far as the nucleotide differences observed between the two cDNAs, a base pair substitution at codon 101 gives rise to threonine in clone p680.8 versus alanine in clone pFr36. Alanine is found in this position in liver L apoferritin (28). This minor difference between liver and promyelocytic L apoferritin could be due to a polymorphism within a single gene. On the other hand, the first 43 bases of the 5′ untranslated sequence of p680.8 do not show any homology with the corresponding region of the liver-type cDNA. The point of divergency is -36 bases from the AUG initiation codon of both cDNAs. pFr36 shows at this level the canonical sequence of an acceptor site for RNA splicing (Fig.1B).

Analysis of L apoferritin mRNAs from several tissues and cell lines.

Given the hypothesis of multiple genes coding for different apoferritin isoforms and the differences observed among the 5′ untranslated regions of clone p680.8 and clone pFr36, one might consider the possibility that the two cDNAs arose from tissue-specific alternative spliced mRNAs or from the transcription of different genes. However, we cannot exclude either a gene rearrangement in the cell line U937 or a cloning artifact. We have analyzed L apoferritin mRNA from several human tissues and cell lines to ascertain the presence of multiple transcription start sites. Primer elongation of L apoferritin transcripts was carried out on total RNA from human liver, placenta, HeLa and Daudi cell lines using as primer the oligonucleotide FrL1, a 17mer complementary to the 5′ proximal coding region of both p680.8 and pFr36 (Fig.1B). The results obtained by such an experiment are shown in Fig.2. A single elongation product was consistently obtained indicating that in each tissue transcription starts ≃200 bases upstream the AUG initiation codon.

The major L apoferritin transcript in U937 cell line is highly homologous to pFr36 clone.

The detection of a single 5′ extension product in different tissues suggests a common transcription start site for L apoferritin mRNAs but does not allow to exclude that p680.8 represents a transcript arisen from a rearranged gene or from an alternatively spliced mRNA. To investigate this point we carried out S1 nuclease protection analysis on RNA extracted from U937 cells using a liver cDNA probe. These cells can be induced to

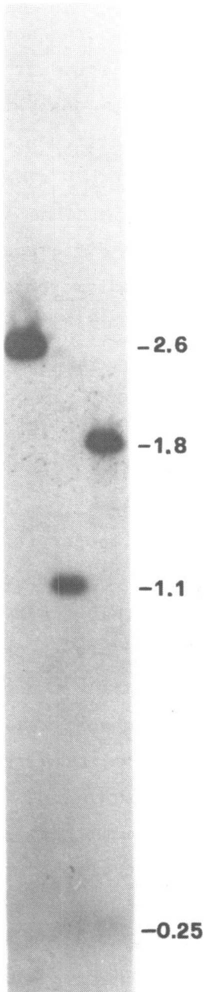

Fig. 4. cos26 DNA restricted with SacI (S), HindIII (H) and SacI plus HindIII (S-H) was blotted onto nitrocellulose filter and hybridized to FrL600 probe. The size length of hybridizing fragments is shown.

differentiate to macrophages by the action of phorbol ester derivatives (16). Because during differentiation alternative molecular mechanisms of gene expression may occur, we have analyzed total RNA from uninduced and induced cells. The cDNA probe to be protected consisted of a PstI-PstI fragment subcloned in M13 phage vector (L200) as described in Materials and Methods. This fragment spans all the 5′ untranslated region and the first 78 bases of the coding sequence of pFr36 (Fig.1A). The ^{32}P labelled single strand probe used in these experiments was synthesized by primer elongation (see Materials

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ctggcccgcctccttgccaccgcagattggccgctagccctccccgagcgccctgcctccgagggccggcgcaccataaaagaagccgcc
ctagccacgtcccctcgcagttcggcggtcccgcgggtctgtctcttgcttcaacagtgtttgacgaacagatccggggactctcttcca
gcctccgaccgccctccgatttcctctccgcttgcaacctccgggaccatcttctcggccatctcctgcttctgggacctgccagcaccg
                                     S S Q I R Q N Y S T D V E A A V N S
tttttgtggttagctccttcttgccaaccaaccATGAGCTCCCAGATTCGTCAGAATTATTCCACCGACGTGGAGGCAGCCGTCAACAGC
 L V N L Y L Q A S Y T Y L S L
CTGGTCAATTTGTACCTGCAGGCCTCCTACACCTACCTCTCTCTGgtgagtccccaggacgcccctggccctaatttcctccagctgcgc
acctccggccctcactgcacgcgccagccttctttgtgcggtcgggtaaacaagagggcggagtcccttggcctcgcctcccgctaacca
                             G F Y F D R D D V A L E G V S H F F R E L A
ttgttgcctccatctcttaccgtagGGCTTCTATTTCGACCGCGATGATGTGGCTCTGGAAGGCGTGAGCCACTTCTTCCGCGAACTGGC
 E E K R E G Y E R L L K M Q N Q R G G R A L F Q D I K
CGAGGAGAAGCGCGAGGGCTACGAGCGTCTCCTGAAGATGCAAAACCAGCGTGGCGGCCGCGCTCTCTTCCAGGACATCAAGgtaactag
tgtgtgggtaatggactacatctcccagcaggcc..../~100bp/......gcacctctgcatgcccacaacacgcggcagtccacacc
gctgcgtggtcttagggacgtatagctgtaagagctaggacagggtgcggagagtgataaatacaagctgtcacatgtctttgtggcctg
ggcctctgacccccaacgactcttgggaaatgtaggtttagttctatgtgccgagtgtgtgtattctgagccatttctcccttctatata
 K P A E D E W G K T P D A M K A A M A L E K K L N Q A L L D
gAAGCCAGCTGAAGATGAGTGGGGTAAAACCCCAGACGCCATGAAAGCTGCCATGGCCCTGGAGAAAAAGCTGAACCAGGCCCTTTTGGA
 L H A L G S A R T D P H
TCTTCATGCCCTGGGTTCTGCCCGCACGGACCCCCATgtacgtacccgctgcatccatggctacccaaccatacccctcaagcctctgct
ccctttggcaaatttccttcagagcctcattcacacctgtcacattttaatctgccaactggctgctctctccccctcttttccagggat
                                    L C D F L E T H F L D E E V K L I K K
tgggtttctaatttctccctcttctctctcagCTCTGTGACTTCCTGGAGACTCACTTCCTAGATGAGGAAGTGAAGCTTATCAAGAAGA
M G D H L T N L H R L G G P E A G L G E Y L F E R L T L K H
TGGGTGACCACCTGACCAACCTCCACAGGCTGGGTGGCCCGGAGGCTGGGCTGGGCGAGTATCTCTTCGAAAGGCTCACTCTCAAGCACG
D
ACTAAgagccttctgagcccagcgacttctgaagggccccttgcaaagtaatagggcttctgcctaagcctctccctccagccaataggc
agctttcttaactatcctaacaagccttggaccaaatggaaataaagctt
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Fig. 5. Nucleotide sequence of the human L apoferritin gene. The four exons are indicated by capital letters. The conceptual translation product is shown by single letter code. The TATAA-like sequence and the suggested start of transcription are boxed. The predicted five α-helices of ferritin (32) are underlined.

and Methods). Liver RNA protects the entire FrL200 fragment from the S1 nuclease activity (Fig.3). Instead, we would expect a protected fragment 115 bases long if a mRNA corresponding to p680.8 were present. As shown in Fig.3, RNA from U937 cells protects the same fragment as liver RNA. Thus, the 5′ untranslated sequences of L apoferritin mRNAs from human liver and U937 cells appear to be entirely homologous up to -118 bases from the initiation codon. To confirm these data, we performed primer elongation and oligonucleotide hybridization experiments on U937 cells RNA and genomic Southern blots, respectively. Oligonucleotide FrL2 (Fig.1B) used as primer or as probe, consisted of a 24mer complementary to the sequence -47 to -70 from the AUG

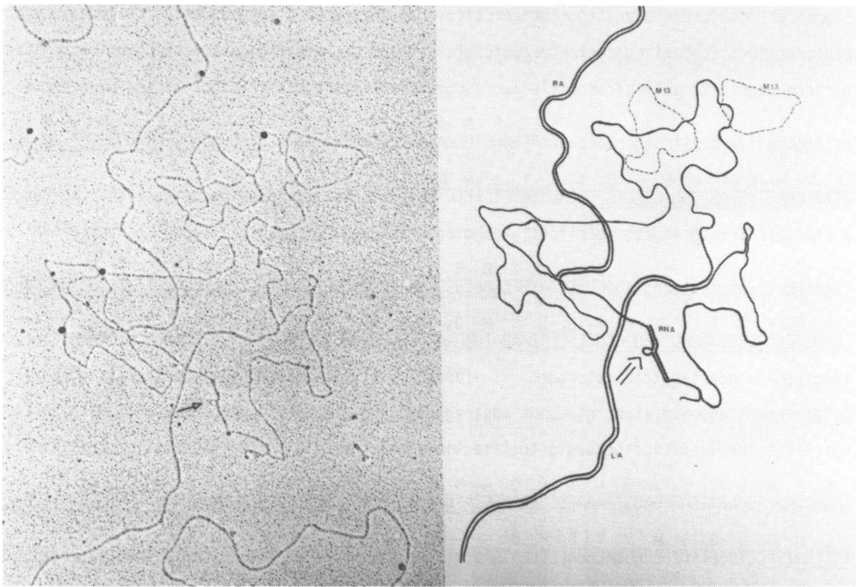

Fig. 6. R-loop analysis of clone λ16. Electron mycrograph (left) and interpretive drawing (right) of heteroduplex between λ16 DNA and poly A+ RNA from human liver are shown. The arrow indicates the single stranded DNA loop. LA: left arm of lambda vector; RA: right arm; M13: M13 single stranded DNA.

codon, unique to the p680.8 cDNA insert. We could not obtain either cDNA synthesis or specific genomic hybridization using this probe (data not shown). In light of these data we conclude that the major transcripts for L apoferritin in liver and U937 cells are highly homologous and possibly identical. Nevertheless, we cannot exclude the presence of a minor RNA form in U937 cells from which clone p680.8 may have originated. Finally, a cloning artifact should not in principle be excluded even though the sequence of the p680.8 cDNA insert does not show any peculiarities to suggest it.

Cloning of human L apoferritin genomic sequences: identification of one expressed gene.

We have previously described the complexity of the human ferritin H and L gene families and estimated that there are 5-8 independent sequences homologous to L cDNA (26). In order to collect as many as possible independent genomic clones for L apoferritin, we screened two human genomic libraries constructed in the lambda phage vector EMBL3 and in the cosmid pcos2EMBL (kindly provided by Dr. Bensi and Dr. Frishauf respectively). The PstI-PstI fragment FrL600 derived from the cDNA clone pFr36 was used as ^{32}P labelled

```
λ16    gtcttgcttcaacagtgtttggacggaagagatcctgggacttcctcttccagcctct****cactatcaatctcctctccacttgca
pFr36  c-----------------*-------c------g-----****-------------cgaccgc-ctc-g--t--------g------

       acctcagggaccatctttttagccaccttctgcttctgggtccagccaataccattgtttgtggttagctccttcttacc**agtcccAT
       -----c-----------c-cg----t--c-----------a--t----gc---g--t------------------g--aaccaa----

       GAGCTCCCAGATTCGTCAGAATTATTCCACTGAGGTGGAGGCAGCCGTGAACCACCTGCTCAATTTGCACCTGCAGGCCTCCT*CATCTG
       ----------------------------C--C-------------C---AG----G--------T--------------A--C--A

       CCTCTCTCTGGGCTTCTACTTACACTGTGACGATGTGGCTTGGGAGGGCGTGGGTCACTTCTTCCACGAATTGGCCGAGGAGAAGCGCAA
       ----------------T--CG--C-C--T---------CT---A------A-C----------G----------------------G-

       GGGTG*****CCTTAA****AGATGCAAAACCAGTATGGCGGCTGCATTCTCTTCCAGGACATCCAGAAACTGGCCCGAAATTGAGTTAG
       ---C-TACGAGCGTCTCCTG--------------CG-------C--GC----------------A----G-CA--T*************

       ATAAGACCCTGGACACGGCTGGA..../~200bp/....AGATGCTCTGCAGTGAGCTGAGATCACATCCACTGC-TCTCCAGCCTGGGC
       ***********************GAAGATGAGTGGGGT****************************************************

       TGACAAAAAAAAAATAATAATAAATAAATAAATTTTAAAAACCCTGAATGCCACGGAAGCCGCCATGGCCCTGGAGAAAAAATCTGAACC
       ************************************-------CAG-C----T-A----T--------------------G*-------

       AGGCCGCTTTTGGATCTTCAAGCCCTGAGTCCTGCCCGCACAGACCTCCATCTCTGTGACTTCCTAGAGAGTCACTTCTTAGATGAGGAA
       -----*--------------T------G--T----------G----C-----------------G----C-------C----------

       GTGAAACTCATCAAG***ATGGGCGACCACTTGACCAACCTTCCCAGGGTGGCTGGCCCCCAGGCTGGCCTGGGTGGGGTTCTCTTGGAA
       -----G--T------AAG-----T------C----------C-A----C---G------GG-------G-----C-A-TA------C---

       AGGCTCACCCACCCTCAAGCACGACtgggagcctt******************aagggcccctgccaaagtatcagggcttctgcctaa
       --------T****-------------aa-------ctgagcccagcgacttctg----------tg-------at--------------

       gcctctccctccagccactaggcagcttt*ttaattaccctg***gagccttcttcaagcttgatccaaatggaaataaagctct-tgga
       -----------------a-----------c----c--t----aaca------**********-ga------------------t-t--at

       agcaaattaaaaaaaaaacaaaacatgactcagaaagctagcgaca
       ------aa-----------a----a-
```

Fig. 7. Sequence of λ16 clone. Nucleotide sequence of λ16 is compared to those of pFr36 cDNA. Only non homologous residues of pFr36 are shown. The first nonsense codon of λ16 coding sequence and the poly A tail of pFr36 are underlined. Asterisks indicate inserted or deleted residues.

probe for screening. This fragment carries the entire 3′ untranslated region and the most part of the liver cDNA coding region (Fig.1A). We have previously shown (26) that it does not cross-hybridize under moderate strigency conditions to the H apoferritin sequences. Out of $4x10^5$ lambda recombinant plaques and $2x10^5$ colonies screened we have isolated 4 phages and 2 cosmids giving positive signals. Electron microscopy analysis carried out on D-loop hybrids of these genomic clones has shown that the six L apoferritin specific sequences lie on independent and nonoverlapping chromosomal segments (data not shown). The genomic DNA clones were restricted with the enzyme HindIII, blotted onto nitrocellulose and probed with FrL600. The rationale of such an

approach is based on the following considerations: among the several clones a polymorphism may exist at the HindIII sites and, based on the structure of the H apoferritin gene (8), there should be no intron splitting the coding and 3′ untranslated regions. As a consequence, one would expect that only clones comprising the expressed gene will give rise to a fragment identical to that obtained restricting with HindIII the cDNA clone pFr36 (see Fig.1A). As shown in Fig.4, the DNA from the cosmid clone cos26, gives in fact the predicted 250 bp fragment plus an additional 1850 bp band. These fragments were subcloned and sequenced (Fig. 5). The sequence shows three introns splitting the coding region in four exons at codons 33/34, 82/83 and 124/125. The coding sequence as well as the 5′ and 3′ untranslated sequences are 100% homologous to the liver cDNA clone pFr36. The start of transcription is located 198 base pairs upstream the AUG codon, as previously deduced from primer extension analysis (see Fig.2). In the 5′ flanking sequence of the gene a TATAA-like box is found at -30 base pairs from the start of transcription.

Clone λ16: a genomic clone carrying a processed pseudogene split by an inserted sequence.

To investigate the possibility that other genes were coding for L apoferritin, we analyzed the remaining genomic clones. D-loop and R-loop analyses of these clones show that 4 out of 5 carry intronless sequences, ≃1 kbp in length, complementary to the L apoferritin cDNA (data not shown), suggesting that these clones represent processed pseudogenes. Clone λ16, instead, when assayed by R-loop analysis with human liver polyA+ RNA, shows a double strand hybrid of ≃1 kbp in length including a 300 bases loop (Fig.6). The nucleotide sequence within the hybrid is highly homologous to that of the L apoferritin cDNA pFr36 (Fig.7). However, the coding message of λ16 is upset by several differences as insertions, deletions and base pair changes. A nonsense codon is found 190 base pairs from the initiation codon. The sequence downstream the 3′ untranslated region resembles a polyA-like stretch. The intron-like loop falls at residue 248 of the coding sequence. In this position there is no intron in the identified coding gene. Furthermore, splice consensus sequences are not present at the ends of such an intron. All these features led us to consider λ16 a processed pseudogene which has undertaken an insertional event.

CONCLUSIONS

In this report we have shown evidence suggesting that a single gene codes for human L apoferritin. Analysis of the 5′ untranslated region of L

apoferritin mRNAs from several human tissues and cell lines shows that the transcripts have a common transcription start site -198 bases from the initiation AUG codon. Since a recently described cDNA clone isolated from a cDNA library obtained from the U937 promyelocytic cell line (27) shows a striking difference between its first 43 bases and the corresponding nucleotides in the liver clone pFr36, we have further investigated the 5′ untranslated region of the L apoferritin RNA from U937 cells, performing S1 nuclease protection experiments using a fragment of the liver cDNA clone pFr36. Our data lead us to exclude that the major L apoferritin mRNA in U937 cells contains nucleotide differences in its 5′ untranslated region with respect to the liver RNA, and cannot explain the origin of the U937 cDNA.

A human gene coding for a L apoferritin was cloned and characterized. The coding sequence of such a gene is completely homologous to that of the liver cDNA clone pFr36. It is interesting to notice that the intron/exon strutcture of the L gene corresponds to the secondary structure of the protein obtained by x-ray diffraction analysis on horse spleen apoferritin (29-31) or by predictive methods on apoferritin sequences deduced from human liver cDNAs (32). The L apoferritin consists of five α-helices, A (residues 10-27), B (39-72), C (96-120), D (125-155) and E (160-174) (32). As shown in Fig. 5, the first three exons correspond to A,B and C α-helices respectively, whereas the fourth to D and E.

Five additional independent genomic clones were analyzed. They all represent processed pseudogenes. On the basis of the above evidence we conclude that L apoferritin is coded by a single gene, giving rise to a common major transcript in all tissues.

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