Structure of the 5' untranslated regulatory region of ferritin mRNA studied in solution

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ABSTRACT

Ferritin mRNAs are the first eukaryotic mRNAs for which a conserved, translational regulatory sequence has been identified. The sequence of twenty-eight nucleotides, called the IRE (iron regulatory element), is found in the 5'-noncoding region and is required for enhanced translation of ferritin mRNA by excess cellular iron; regulation occurs at initiation. The prediction of secondary structure in the IRE is a hairpin loop. We now report an analysis of the IRE structure in solution studied in natural ferritin mRNAs [H and H'(M) subunits] by primer extension, after modification or cleavage by dimethyl sulfate, RNAases T₁ and V₁, and the chemical nuclease 1,10-phenanthroline-copper (OPCu) which cleaves single-stranded and bulged regions of RNA. Overall, the structure in solution of the ferritin mRNA regulatory region is a hairpin loop, with magnesium-sensitive features, in which half the stem is provided by the IRE and half by flanking regions; only secondary structure is conserved in the flanking regions. Predicted bulges or internal loops along the stem were clearly detected by OPCu but were missed by the more bulky probe RNAase T₁, indicating the efficacy of OPCu in probing subtle features of RNA structure. Magnesium-dependent deviations from the predicted structure were observed in the stem between the hairpin loop and the bulge at C⁶. The location of the IRE in relation to the initiator AUG or the cap is variable in different ferritin mRNAs. However, the number of nucleotides in the base-paired flanking regions of known ferritin mRNAs is proportional to the distance of the IRE from the cap and places the secondary/tertiary structure 8–10 nucleotides from the cap where interference with initiation is likely.

INTRODUCTION

Ferritin synthesis has become a paradigm for translational regulation (1,2). Translational regulation is demonstrated by the observation that 30–50x increases in ferritin synthesis occur with no change in ferritin mRNA concentrations when cellular iron levels increase (3,4,5,6). [Since the biological role of ferritin is

to store large amounts of iron, in an inorganic core, control of ferritin synthesis by iron is a form of autogenous regulation.] The site of translational control of ferritin synthesis is mainly at initiation (7) with untranslated ferritin mRNA stored in the cytoplasm (6,8). When cellular iron is in excess ferritin mRNA is able to form polyribosomes (8). Ferritin mRNA translation can also be specifically regulated *in vitro* in reticulocyte lysates (9) which led to the identification and purification of a specific ferritin mRNA regulator protein (10,11). The prediction of specific mRNA-protein complexes to regulate protein synthesis in eukaryotes made twenty years ago (12) has now been fulfilled with ferritin mRNA.

What are the specific features of ferritin mRNA required for translational control? Comparison of the eight different ferritin mRNA sequences currently available, representing mammals, birds and amphibia, shows a highly conserved sequence of 28 nucleotides, 16–38 nucleotides from the cap (2) which was originally noted by Leibold and Munro in 1986. Transfection studies in whole cells have amply demonstrated the requirement of the conserved sequence for the regulation of ferritin synthesis by iron (as added hemin) (13–20). Moreover, a ferritin mRNA translational regulator protein binds to the conserved ferritin mRNA sequence (10,21). A protein of a size similar to the regulator protein has been identified in cell extracts by retardation of oligoribonucleotides during gel electrophoresis (17,22) and purified using the conserved sequence as a specific ligand (20). Sequences in the 3'-untranslated region of ferritin mRNA also contribute to the translational regulation of ferritin synthesis (9,14), but sequence conservation in the 3'-untranslated region has not been detected; however, predicted secondary structures appear to be conserved (9).

Neither the IRE structure nor the flanking regions of ferritin mRNAs have been examined except by sequence comparison and secondary structure predictions. The IRE regulatory region is predicted to form a stem consisting of ten highly conserved nucleotide pairs from flanking regions. The hairpin loop consists of six nucleotides, CAGUGU/C (Figure 1).

We now report studies of the structure in solution of both the IRE and flanking regions in ferritin mRNA using chemical modification, and both enzymatic and chemical cleavage to probe accessible single and double stranded regions. Ferritin mRNA

was analyzed in polyA⁺ RNA by primer extension. The results show that many of the bases in the IRE and flanking regions that are predicted to be paired are indeed inaccessible to probes of unpaired bases. In addition, sites of cleavage of ferritin mRNA by 1,10-phenanthroline-Cu (OPCu), a probe for single strands or loops in RNA (26), were strikingly sensitive to changes in magnesium concentration. At 0.5 mM, even some nucleotides in the stem at the base of the hairpin loop were cleaved, showing the existence of different conformers of the IRE. The combination of the IRE and the base-paired flanking sequence places the secondary/tertiary structure of the regulatory region within ten nucleotides of the cap providing a possible explanation for interference with initiation of translation.

EXPERIMENTAL PROCEDURES

RNA

PolyA⁺ RNA was isolated from reticulocytes of bullfrog tadpoles (*Rana catesbeiana*) as previously described (3). *In vitro* transcripts (IVT) of the H'(M) ferritin subunit *Rana catesbeiana* pseudogene were prepared as previously described (9); the three lengths of 3'-untranslated region tested were 24 and 94 nucleotides and full length.

Cleavage or Modification Reactions

The results of cleavage or modification were analyzed by primer extension. Specific primers which bound 15 or 23 nucleotides downstream from the ferritin mRNA regulatory region [H or H'(M) subunit mRNA] were used to examine mRNA modification or cleavage in polyA⁺ or IVT. Primer binding, incubation with reverse transcriptase and separation of DNA

fragments by electrophoresis in acrylamide gels followed procedures previously used (6).

All cleavage or methylation reactions were carried out at 30°C in 25 mM Hepes·Na (Boehringer-Mannheim), pH=7.2, 40 mM K acetate and varying concentrations of MgCl₂, and were adjusted to assure less than one modification or cleavage event per RNA molecule. The use of 80 mM K acetate did not change the results. Modified or cleaved RNA was precipitated with alcohol before analysis; enzymes were removed by phenol extraction. *V_i* RNAase, purchased from Pharmacia, was used at 7 U units/ml and RNAase T₁, the product of BRL, was used at 7 U/ml; polyA⁺ concentrations were 60–80 microg/ml. Dimethyl sulfate (DMS) was purchased from Aldrich Chemical; 50 microL reaction mixtures 88 mM in DMS and containing 2 or 0.6 microg of polyA⁺ RNA or IVT, respectively, were incubated with DMS for 30 min at 30°C. Cleavage by 1,10-phenanthroline-Cu (OPCu) followed the procedure of Yoon et al. (24) with 9 microM Cu, using mercaptopropionic acid to start the reaction (no cleavage occurred with Cu alone). Five minute reactions were terminated with a 45-fold excess of 2,9-dimethyl-1,10-phenanthroline (24). Concentrations of OPCu up to 45 microM Cu were tested with no change in the cleavage pattern.

Computer Predictions

The University of Wisconsin Genetics Computer Group Fold Program, Version 5.0, was used as developed by Zucker and Steigler (23) and Frier et al. (40).

RESULTS

To study the secondary/tertiary structure of the regulatory sequences in the IRE and flanking sequences of the 5'-untranslated region of ferritin mRNAs, chemical modification and cleavage were carried out under conditions where less than one cleavage or modification occurred/RNA molecule. Native ferritin mRNA was examined in a natural mRNA mixture using polyA⁺ mRNA from reticulocytes of the embryonic cell line of bullfrogs where the proportion of ferritin mRNA is ca 10% (3,6). Both H and H'(M) ferritin subunit mRNAs (25) were studied as well as *in vitro* transcripts (IVTs) of a genomic ferritin processed pseudogene; the pseudogene encoded the full length H'(M) amphibian ferritin subunit mRNA.

Single Stranded Regions of the 5'Untranslated Regulatory Region of Ferritin MRNA

When the results of modification or cleavage were examined under conditions similar to those used for translation or binding of specific ferritin regulatory proteins (40 mM K⁺, pH=7.2) (Table 1, Figures 2 & 3), most of the nucleotides predicted to be in single stranded regions (loops or bulges) were cleaved by RNAase T₁ or OPCu. [OpCu cleaves single-stranded regions of tRNA (26).] Single-stranded cutting reagents cleaved at positions 5, 7, 9, 14, 15, 16, 35, and 45. In addition, dimethyl sulfate alkylated A at position 13 and C at positions 6, 41, 47, and 48 (data not shown), which also are in loops or bulges in the predicted structure. Notable exceptions to the predicted structure are the alkylation of A²¹ and A²² (not shown) and the cleavage by OPCu of U¹⁸ and U¹⁹ at 0.5 mM magnesium. At 5 mM magnesium, no cleavage occurred with OPCu. The results were similar for both H and H'(M) ferritin subunit mRNAs in natural mixtures. Interestingly, although several of the OPCu cleavage

Table I. Major Modification or Cleavage Sites in the Regulatory Region of the 5'-Noncoding Segment of Ferritin mRNA (H subunit) from Bullfrog Red Cells^a

Reagent	A ^b	C ^b	G ^b	U ^b
DMS ^c	13,16 ^c ,21,22	6,41,46,48	—	—
RNAase T ₁	—	—	14,45	—
OPCu ^{c,d}	—	32	5,14,16, 20,24,25,36	8,15,18,19,35
RNAase V _i	37	—	36	1,7,35

^a The base composition of the conserved sequence and flanking sequences is pyrimidine rich: A₁₅, C₂₀, G₁₃, U₂₅ (Pyr/Pu=45/28). Similar results were obtained probing native H'(M) ferritin subunit mRNA (Figure 2) and pseudogene IVT and a 55 mer containing the IRE and blanking regions (data not shown).

^b The conserved regulatory sequence is numbered 1 to 28 starting at the 5' end; in this scheme initiation of translation begins at 149 and the cap is at -23. ^c DMS=dimethyl sulfate (data not shown)—only modified A or C can be detected by primer extension (33); OPCu=1,10-phenanthroline Cu (30). ^d Accessibility to OPCu was affected by varying the concentration of Mg. For example, G¹⁴, U¹⁵, G¹⁶, U¹⁸, and U¹⁹ were not cleaved at 0 or 5 mM Mg but were cleaved at 0.5 mM Mg. The affect of Mg is less marked in the *in vitro* transcript of the H'(M) subunit pseudogene. ^e In the transcript of the pseudogene only which has a G→A substitution at position 16.

Native ferritin mRNA, in polyA⁺ RNA naturally rich in ferritin mRNA (10%) was treated with cleavage or modifying reagents in 25 mM Hepes·Na, pH=7.2, with 40 mM KCl at 30° (Mg: 0 and 0.1 mM for RNAases and 0–5 mM for OPCu and DMS) and analyzed by primer extension (see Materials and Methods). The results were obtained from 3–5 experiments. DMS and OPCu were used with both H and H'(M) ferritin subunits and IVTs of a H'(M) subunit pseudogene (25) with similar results except that positions 21 and 22 were moderate DMS sites in the H'(M) subunit mRNA and the A substitution at position 15 in the IVTs was a strong DMS site. The results indicate a hairpin loop, with several magnesium-sensitive regions near bulges, loops, and the base of the hairpin loop, that is composed both of the conserved IRE and the flanking sequences. Possible secondary/tertiary interactions involve bases in the hairpin loop based on the differential accessibility of G residues to OPCu and RNAase.

sites near bulges or internal loops are G residues, the sites were not cleaved by RNAase T₁.

Several residues in the predicted CAGUGU/C hairpin loop had reactivities which indicate specific and magnesium-sensitive conformations. For example, G¹⁶ was cleaved poorly, if at all, by RNAase T₁ whereas G¹⁴ was cleaved readily (Figure 3). Moreover, both residues were less accessible to cleavage by OPCu in the absence of magnesium, although cleavage occurred readily in 0.5 mM magnesium (Figure 2, lanes 1 and 3). In contrast to native mRNAs, the structure of the *in vitro* transcript; (IVT), which has a G→A substitution at position 16, was much less dependent upon magnesium than the natural mRNAs (data not shown) even when a full length 3'-untranslated region (UTR) was present; the pseudogene used as a template for the IVTs was 97% identical to the ferritin M-subunit mRNA (25). OPCu cleavage patterns for the natural ferritin mRNAs and the IVTs were essentially identical in 0.5 mM magnesium. Differences in the magnesium sensitivity of the IRE regulatory region of native ferritin mRNA and the IVT probably relate to the replacement of G¹⁶ by A in the hairpin loop of pseudogene transcript; A¹⁶ was readily modified by DMS. Under the conditions used, structural effects of changing the 3'-UTR were not detected.

Double-stranded Regions

Large segments of the ferritin 5'-untranslated regulatory region are predicted to be double-stranded. Reagents accessible to open- or single-stranded regions confirmed the prediction because of the small number of reactive sites. For example, only 2 of a total of 13 G residues were readily cleaved by RNAase T₁, only 3 of 15 A and 4 of 20 C residues were highly methylated and only 13 of 73 the nucleotides examined were strong sites for OPCu cleavage at 0 or 0.5 mM magnesium (Figure 2, lanes 1 & 3). At 5 mM magnesium apparently no sites are accessible to OPCu suggesting that a stable secondary structure exists including all predicted bulges and loops.

V₁ nuclease cleavage sites were also few in number (Figure 2). However, V₁-sensitive sites were generally not reactive with single-strand specific reagents. Two exceptions are residues U³⁵ and G³⁶ which were cleaved both by OPCu and by V₁ nuclease. Such discrepancies in the specificity of V₁ have been observed before, particularly at sharp bends in a helix (27). V₁ nuclease cleavage patterns were unaffected by magnesium (0.5 mM) (Figure 2) or by increasing the ratio of V₁ nuclease/RNA.

A region of apparent conformational variation occurs near residue C⁶ and at the base of the hairpin loop, sequences which are required for iron regulation of ferritin synthesis (17). For example, the computer-predicted structure shows the G⁵-C²³ pair plus the U⁷-A²² and U⁸-A²¹ base pairs flanking the C⁶ bulge. However, A²¹ and A²² are methylated and U⁸ can be cleaved by OPCu, although U⁷ is V₁ sensitive. Additional magnesium-sensitive variations occur in bases in the predicted pairs C⁹-G²⁰, A¹⁰ and U¹⁹, and A¹¹ and U¹⁸. G²⁰ is accessible to OPCu in 0 or 0.5 mM magnesium, while U¹⁸ and U¹⁹ are cleaved by OPCu only at 0.5 mM magnesium. The stability of the stem-loop predicted for the 28 nucleotide IRE is -3.7 kcal. When the flanking region is included (-15 to 1, 28-40; Figure 1), the predicted $\Delta G^\circ = -15.1$ kcal/mole; eliminating pairing between G⁵-C²³ does not change the energy. Eliminating base pairing between U⁷-A²² changes ΔG° to -13.4 kcal/mole and to -11.3 kcal/mole for the U⁸-A²¹ pair, respectively. Similar effects were predicted when base pairing was eliminated

for A¹⁰-U¹⁹ (-12.4 kcal) and A¹¹-U¹⁸ (-14.4 kcal). The largest effect on predicted stability occurred when pairing between C⁹-G²⁰, in that middle of the stem, was eliminated. Two hairpin loop of similar stability (-9.9, -8.2 kcal) were predicted but with different loop sequences.

DISCUSSION

Ferritin mRNAs are the first eukaryotic mRNAs for which a conserved, message specific, translational regulatory element has been identified (2); the sequence is in the 5' noncoding segment. Variable copies of the translational regulatory sequence are repeated in the 3'-noncoding segment of the transferrin receptor mRNA. Both mRNAs, which encode proteins related by their function in iron metabolism, are regulated by cellular iron (e.g. 3,4,8,28,29) and the regulatory sequence is called the IRE. Specific translational regulatory proteins and IRE-binding proteins have recently been purified (10,11,20). Little is known about the structure of ferritin mRNA IRE other than the secondary-structure prediction of a stem/hairpin loop. Understanding the structure of the IRE in ferritin mRNA will provide a model for understanding eukaryotic mRNA structure and function as well as understanding the regulation of iron metabolism.

Characterizing the ferritin IRE in native mRNA was possible because of the abundance of ferritin mRNA in polyA⁺ RNA

Figure 1. Structure of the Regulatory Region in the 5'-Noncoding Segment of Ferritin mRNA (Bullfrog H Subunit). The predicted secondary structure of the conserved regulatory sequence (IRE) of ferritin mRNAs is shown with paired flanking sequences. The IRE is numbered 1-28 beginning at the 5' end. Both H and H'(M) subunits were examined. Taken together the results indicate a 20 base-pair hairpin loop that involves both the IRE and flanking sequences; stem residues bordering bulges or internal loops show magnesium sensitivity. Residues G¹⁴, U¹⁵, G¹⁶, U¹⁸, and U¹⁹ are cleaved at 0.5 mM magnesium, but not at 0 or 5 mM magnesium (Figure 2). Major cleavage sites (see Figures 2 and 3) are indicated as follows: RNAase V₁ - ■; RNAase T₁ - □; 1,10-phenanthroline-Cu (OPCu) - ●; sites of dimethyl sulfate alkylation (data not shown) - ▼.

Figure 2. 1,10-Phenanthroline-Cu and RNAase V₁ Cleavage of the 5'-Noncoding Regulatory Region of Natural Ferritin mRNA [H Subunit] at various Magnesium Concentrations Analyzed by Primer Extension. Ferritin mRNA in natural polyA + RNA mixtures from frog red cells was cleaved with chemical or enzymatic nucleases and the cleavage sites determined by primer extension using primers that bound 15–23 nucleotides downstream (depending on the subunit mRNA probed) from the conserved regulatory sequence (IRE) (see Materials and Methods). The IRE is numbered 1–28 and the base paired flanking regions extend to –15 and to 40 (see Figure 1). ACGT: Reaction mixtures with dideoxynucleotides were used to calibrate the gel. Data for the H subunit mRNA is presented but similar results were obtained with the H'(M) subunit mRNA. B-primer extension of uncleaved mRNA; lanes 1–3 OPCu in 0, 5 mM and 0.5 mM Mg; lanes 4,5 are V₁ digestion products in 0 and 0.5 mM Mg. In lane B, the control lane, transcription pause sites can be observed at positions C⁴, A¹⁰, G¹⁴, U¹⁵, G²⁴, C³⁰, G³⁸, and U³⁹ indicating secondary/tertiary structure in the stem even at 50° where the reactions were carried out. Cleavage or modification were only scored when the intensity of the autoradiogram was at least three times greater than the control. Note the sensitivity of the OPCu cleavage pattern to Mg; the sensitive sites are in sequences predicted to be at bulges, in internal loops, and around or in the hairpin loops. The results are representative of 3–5 experiments.

from embryonic red cells (3); similar results were obtained with a ribo-oligonucleotide of 55 bases containing the IRE and flanking regions (Harrell and Theil, preliminary observations). The results of cleaving or modifying ferritin mRNA with RNAases T₁ and V₁, dimethyl sulfate and the chemical nuclease 1,10-phenanthroline-Cu (OPCu) revealed several notable features: (1) The conserved IRE and flanking region combined form a hairpin loop but several magnesium-dependent conformers exist which vary at predicted bulges and the stem of the hairpin loop; (2) Base specific features appear to occur in the hairpin loop; (3) OPCu detected predicted regions of bulges of internal loops which were not sensitive to protein nucleases; (4) The combined secondary structure of the IRE and flanking region is 6 or 10

Figure 3. RNAase T₁ Cleavage of the 5'-Noncoding Regulatory Region of Natural Ferritin mRNA [H Subunits] Analyzed by Primer Extension. Ferritin mRNA in polyA + RNA from frog embryonic red cells was cleaved with RNAase T₁ and the products examined by primer extension (see Materials and Methods). ACGT—Reaction mixtures with dideoxy nucleotides to calibrate the gel. The conserved regulatory sequence (IRE) is numbered 1–28 and the base-paired flanking region extends to –15 and 40 (see Figure 1). Most of the G residues are predicted to be in the stem. Only G residues predicted to be in single-stranded regions or in the hairpin loop were cleaved. G residues near bulged or internal loop regions which were accessible to 1,10-phenanthroline-Cu (Figure 2), were inaccessible to the enzyme. Mg had no effect on the RNAase T₁ cleavage pattern. Note the absence of cleavage of G¹⁶ in the hairpin loop even though G¹⁴ is readily cleaved. The results are representative of three experiments.

nucleotides from the cap for the H'(M) and H ferritin subunit mRNAs, respectively.

The Hairpin

Conservation of the 28 nucleotides of the ferritin mRNA IRE is so striking that attention has been focused almost entirely on the IRE to the exclusion of the flanking nucleotides (e.g., 13,17,19,22). However, both the IRE and flanking regions have extensive secondary structure. For example, only a small fraction of the nucleotides in either the IRE or the 13–14 nucleotides on either side of the IRE were accessible to probes of single-stranded or open RNA, i.e., dimethyl sulfate, RNAase T₁, and 1,10-phenanthroline-Cu. Such results support the prediction of secondary structure in the IRE and flanking sequences (Figure

1). However, structural variations occur among residues at the edges of predicted bulges and internal loops. For example, G²⁴, G²⁵, U³⁵, G³⁶ are very sensitive to magnesium at physiological concentrations (Figure 2); similar data were obtained for both natural ferritin subunit mRNAs examined [H and H'(M)]. Deviations in reactivity of the IRE region from the predicted secondary structure occur at 0.5 mM magnesium in the region between C⁶ [required for function (17)] and the hairpin loop, e.g. methylation of A²¹ and A²² and OPCu cleavage of U⁷, U¹⁸, U¹⁹, and G²⁰. Moreover, computer analysis of the effect of eliminating base pairing showed only small effects on predicted stability if the disruption were near the loop or ≥ 3 base pairs away. Such results suggest that conformational heterogeneity or possibly tertiary interactions may contribute to the regulatory function of the ferritin mRNA IRE region although regulator-protein binding, measured as altered mobility during electrophoresis, was unchanged by magnesium (19,22; Harrell, Walden, and Theil, unpublished observations).

Secondary/Tertiary Structure in the Hairpin Loop

Several observations show that the hairpin loop has specific structural features. For example, two neighboring G residues in the IRE, positions 14 and 16, have different reactivities to RNAase T₁. In addition, cleavage of either G by OPCu is dependent on magnesium. When A is substituted for G at position 16 in the transcript of the H'(M) ferritin subunit pseudogene, the site is readily accessible to alkylation by dimethyl sulfate (Table 1). The importance of base sequence and structure in the loop for function is indicated by the observation that substitution of A for G at position 16 of an oligodeoxyribonucleotide containing the IRE reduces protein binding in gel-shift assays (29).

Novel Features of Cleavage by 1,10-phenanthroline-Cu(OPCu)

OPCu is an attractive probe of structure because of the lack of base specificity (30). An additional feature is the potential of OPCu to detect structural details too small to be probed by a bulky protein nuclease, as illustrated in Figures 2 and 3. Only two G residues in the ferritin mRNA region examined were cleaved by RNAase T₁ (Figure 3) suggesting that the other eleven in the sequence were in base-paired regions. However, interruptions (bulges or internal loops) are predicted in the stem near seven additional G residues. Although the predicted interruptions in stem structure around the seven G residues were transparent to RNAase T₁, five of the seven G residues were accessible to OPCu in a magnesium-dependent fashion; OPCu clearly displayed the interruptions in the base-paired stem. Finally, the independence of reactivity of OPCu on magnesium facilitated the detection of magnesium dependent changes in the hairpin loop and stem structure (Figure 2, lanes 1–3).

Location of the IRE and Paired Flanking Region in Relation to the Cap

Translational regulation of ferritin mRNA involves initiation (3,7,9,13). Both the IRE and specific cell extracts are required for regulation of ferritin mRNA translation (5,13,15,16). The location of the IRE is 16–38 nucleotides from the cap (2) which could be too far for effects on initiation (31,32). Flanking the conserved sequence are nucleotides which have extensive base pairing (Figure 1). Similar base pairing can be predicted in the eight ferritin mRNAs for which sequence is available (25,34–38)¹. The length of the predicted stem is proportional

to the distance of the IRE from the cap (39). Thus, when the IRE and the flanking regions are combined, the secondary structure is only 8–12 nucleotides from the cap in ferritin mRNA from human, rat, chicken, and frog (see Figure 1), which provides a reasonable explanation for the influence of the IRE regulatory region on translation initiation. Alternatively, the 90 kDa regulatory protein (10,11,20,21) could simply mask the entire region, but the binding site of the protein is not yet known. How the regulatory protein(s) and excess cellular iron change ferritin mRNA structure to enhance translation and why only secondary structure is conserved in the flanking regions, in contrast to constant primary structure in the IRE, are questions for the future.

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¹After submission of the manuscript, a note appeared (39) presenting the sequence of a liver ferritin cDNA from adult *Xenopus* in which the distance from the IRE to the cloning site was 157 nucleotides and the length of the sequence between the apparent initiator AUG and the cloning site was 200 nucleotides longer than any other known ferritin mRNA 5' untranslated region (5'UTR). In addition, in contrast to other known ferritin mRNAs, the *Xenopus* sequence encoded an open reading frame within the extra 200 nucleotides. Whether the cDNA clone represents a minor species of ferritin mRNA heretofore undetected or is characteristic of all *Xenopus* ferritin mRNA awaits the future analysis of the RNA.

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