There are two constructive outcomes of the above findings. First, for the parameter range used, chain motion can be characterized by several stages, all of which appear amenable to theoretical treatment (*9, 10*). Second, unlike tube models, the case of reverse field electrophoresis is not expected to give the same dependence of mobility versus chain length as the constant field case.

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Iron-Responsive Elements: Regulatory RNA Sequences That Control mRNA Levels and Translation

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The biosynthetic rates for both the transferrin receptor (TfR) and ferritin are regulated by iron. An iron-responsive element (IRE) in the 5′ untranslated portion of the ferritin messenger RNA (mRNA) mediates iron-dependent control of its translation. In this report the 3′ untranslated region of the mRNA for the human TfR was shown to be necessary and sufficient for iron-dependent control of mRNA levels. Deletion studies identified a 678-nucleotide fragment of the TfR complementary DNA that is critical for this iron regulation. Five potential stem-loops that resemble the ferritin IRE are contained within the region critical for TfR regulation. Each of two of the five TfR elements was independently inserted into the 5′ untranslated region of an indicator gene transcript. In this location they conferred iron regulation of translation. Thus, an mRNA element has been implicated in the mediation of distinct regulatory phenomena dependent on the context of the element within the transcript.

IN PROLIFERATING CELLS, THE AVAILability of iron modulates the biosynthetic rates for at least two proteins critical to cellular iron metabolism. The transferrin receptor (TfR) serves as the chief means of iron uptake, and its biosynthesis is decreased when iron is abundant and increased when it is scarce (*1*). Conversely, ferritin, which sequesters iron in the cytoplasm, is synthesized at a higher rate when iron is abundant than when it is scarce (*2*). The mechanisms involved in the control of the biosynthetic rates for these two proteins are distinct. For the TfR the regulation of biosynthetic rates can be accounted for by changes in mRNA levels (*3*). In contrast, ferritin biosynthesis is altered without a corresponding change in the level of total ferritin mRNA (*4*) by redistribution of mRNA between polysome and nonpolysome pools (*5*).

The iron-dependent modulation of the level of TfR mRNA is mediated by two regions of the gene. Together these two regions can produce >20-fold differences in transcript levels between cells treated with an iron chelator and those treated with an iron source. The transcription rate directed by 5′ flanking sequences of the TfR gene is two- to threefold higher in cells treated with the iron chelator desferrioxamine than in cells treated with hemin, an iron source (*6*). However, deletion of sequences corresponding to the 3′ untranslated region (3′UTR) of the TfR mRNA eliminates most of the iron regulation of transcript levels regardless of whether the TfR promoter region is present (*6, 7*).

The 5′ untranslated region (5′UTR) of human ferritin H chain mRNA is both necessary and sufficient to mediate iron-responsive translational regulation of ferritin biosynthesis (*8*). Moreover, the specific sequence within the ferritin 5′UTR responsible for the modulation of ferritin translation has been identified (*9, 10*). This RNA sequence, which we have termed an iron-responsive element (IRE), has the potential to form a characteristic stem-loop structure. The 5′UTR of each ferritin gene for which complete mRNA sequence data are available [human (*11*), rat (*12*), chicken (*13*), and frog (*14*)] contains a sequence capable of forming a similar stem-loop. Here we present a deletion analysis of the TfR 3′UTR that implicates strikingly similar elements in the iron-responsive regulation of TfR mRNA levels. We further show that synthetic oligonucleotides corresponding to elements of the TfR 3′UTR confer ferritin-like translational regulation when inserted in the 5′UTR of an indicator gene transcript.

We (*6*) and others (*7*) have shown that the sequences corresponding to the 3′UTR of the TfR mRNA are necessary for the full degree of iron regulation of the level of this transcript. To demonstrate the sufficiency of this region to confer TfR-like iron regulation on another gene, we have inserted a fragment containing most (2.2 kb of 2.5 kb) of the TfR 3′UTR into the 3′UTR of the structural gene for human growth hormone (hGH) and have transformed murine cells with this construct (pSVGH-TR). The hybrid construct gave rise to three major hGH/

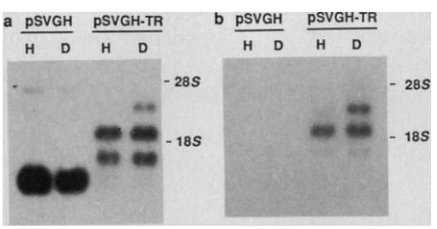

Fig. 1. The 3′UTR of the human transferrin receptor is sufficient to confer iron regulation on the accumulation of a chimeric transcript. Pools of stable transformants were prepared as described (*6*) after transfection with pSVGH or pSVGH-TR as indicated (*17*). Cells were treated for 16 hours with 6 m*M* sodium butyrate plus either 100 μ*M* hemin (H) or 100 μ*M* desferrioxamine (D) prior to isolation of cytoplasmic RNA (*6*). RNA was separated by electrophoresis in a 1% agarose-formaldehyde gel and analyzed by blot hybridization with an hGH probe consisting of the 650-bp Sty I fragment of the hGH gene (**a**) or the full-length human TfR cDNA (*18*) (**b**). The relative positions of the 28*S* and 18*S* ribosomal RNAs are indicated.

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TfR hybrid transcripts (Fig. 1). The largest of the chimeric transcripts was regulated by iron in a fashion resembling the native TfR gene, with much higher levels present after desferrioxamine treatment than after treatment with hemin. The levels of the two smaller hGH/TfR transcripts as well as the level of the hGH transcript encoded by the plasmid pSVGH, which lacks TfR sequences, were unaffected by iron availability. The largest of the hGH/TfR transcripts is of the size that would be predicted from the structure of pSVGH-TR. The relative intensities of the hybridization signals when the hGH and TfR probes were compared indicated that the two smaller chimeric transcripts lack portions of the TfR sequences that are part of the plasmid pSVGH-TR.

When hGH protein secretion into the growth medium of the pSVGH-TR transformants was assessed by radioimmunoassay, we detected no appreciable iron regulation of hGH production. This apparent absence of regulation is likely due to the ability of the two smaller hGH/TfR transcripts (which are not iron-regulated because they lack critical TfR sequences) to be used to produce hGH. In our pSVGH-TR transformants, these truncated unregulated transcripts were considerably more abundant than the full-length regulated mRNA. We have also observed multiple human TfR transcripts in murine cells transformed with the full-length human TfR cDNA. The smaller human TfR transcripts in those cells lack some or all of the 3′UTR, and only the full-length transcript was fully iron-regulated (*6*). The full-length TfR transcript in those cells was the predominant mRNA species, and iron regulation of TfR protein biosynthesis was seen. The appearance of the multiple hGH transcripts in Fig. 1 is clearly a function of the TfR 3′UTR, since the parent plasmid pSVGH that lacks the TfR 3′UTR overwhelmingly gives rise to one hGH transcript. Owen and Kühn (*7*) transferred the TfR 3′UTR to the 3′ end of the histocompatibility antigen HLA-A2 gene but, on the basis of cell surface expression of the gene's protein product, concluded that there was no iron regulation of this chimeric gene. This observation may also be a reflection of protein production from multiple hybrid transcripts that are not all iron-regulated. Our results (Fig. 1) demonstrate that no portion of the TfR protein–encoding sequences is required for iron regulation of transcript levels. Thus the TfR 3′UTR is both necessary and sufficient to confer the iron regulation of mRNA levels that is characteristic of TfR biosynthetic regulation.

Previously, we prepared stable transformants of murine cells by using a TfR "minigene" containing the full-length TfR cDNA driven by the TfR promoter (Fig. 2a, construct I) and found that expression of the human TfR in those cells was highly regulated by iron. In contrast, cells transformed with a plasmid lacking the bulk of the TfR 3′UTR (construct II) continued to express the normal human TfR protein but had lost the ability to iron-regulate this expression (*6*). In this report, the sequences within the TfR 3′UTR responsible for iron-dependent regulation are localized further by a restriction fragment deletion analysis. This analysis was first performed with a transient expression assay for the iron regulation of human TfR protein biosynthesis in transfected murine cells (Fig. 2a). Cells transfected with construct I synthesized approximately seven times as much human TfR protein after desferrioxamine treatment as after treatment with hemin. Transfection with constructs IV and V resulted in a similar degree of iron regulation of human TfR biosynthesis. We have tested constructs having smaller deletions 3′ of base 3861, and these too were iron-regulated. There was some residual iron regulation in cells transfected with constructs II and III (a less than twofold difference between desferrioxamine treatment and hemin treatment). Since all of the constructs in Fig. 2 contain 5′ flanking sequences of the human TfR gene that have been shown to mediate a two- to threefold transcriptional effect of iron (*6*), the residual regulation likely represents a manifestation of this transcriptional element. Nonetheless, the deletion analysis demonstrates that the restriction fragment bounded by TfR cDNA nucleotides 3178 and 3856 contains the region responsible for the majority of TfR iron regulation. When only this region is deleted (construct III), iron regulation is lost. When fragments flanking this region are removed (as in constructs IV and V), iron regulation is retained.

Iron regulation of TfR biosynthesis is due to alteration in TfR mRNA levels (*3*). We

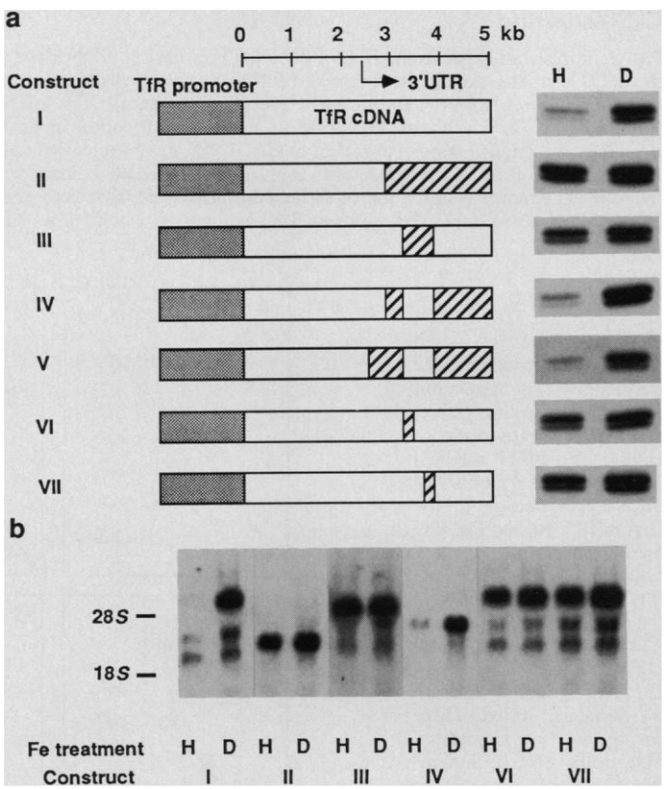

Fig. 2. The sequences responsible for iron regulation of TfR biosynthesis are contained in a 678-nucleotide fragment of the 3′UTR. (**a**) Mouse B6 cells were transfected with calcium phosphate precipitates of plasmids containing the indicated deletion constructs (*19*) as described previously (*6*). The shaded region at the 5′ end of the schematic representation of the construct denotes a fragment of genomic DNA containing the TfR promoter, and the hatched regions of each construct represent sequences of the TfR cDNA that have been deleted. After removal of the DNA precipitates, cells were placed in medium containing 6 m*M* sodium butyrate, and 8 hours later either hemin (H) or desferrioxamine (D) was added to final concentrations of 100 μ*M*. After an additional 16 hours, human TfR biosynthesis was assessed by radiolabeling with [^{35}S]methionine, immunoprecipitation with monoclonal antibody B3/25, SDS–polyacrylamide gel electrophoresis, and autoradiography as previously described (*6*). The relevant portions of these autoradiographs are shown to the right of each of the constructs tested. (**b**) Mouse B6 cells, which lack thymidine kinase, were stably transformed as described previously (*6*) by cotransfection with the herpes simplex thymidine kinase gene followed by selection of thymidine kinase–containing colonies. Six of the seven constructs depicted in (a) were used to produce stably transformed populations of murine cells that expressed human TfR. Each transformed cell population represents a pooling of multiple individual colonies of transformants. Cytoplasmic RNA was isolated and analyzed after treatment with hemin (H) or desferrioxamine (D) as in Fig. 1. The full-length human TfR cDNA was used as probe (*20*). The relative positions of the 28*S* and 18*S* ribosomal RNAs are indicated.

prepared stable transformants of murine cells with six of the seven deletion constructs of Fig. 2a and assessed the effect of iron availability on the level of the transcripts encoded by these constructs (Fig. 2b). In several instances multiple transcripts were observed. In each case, the largest human TfR transcript in a given transformant corresponded in size to the full-length transcript expected from the transfected plasmid. The largest transcript in cells transformed with construct I corresponds to the 4.9-kb full-length TfR mRNA that is normally produced in human cells (*3*). The level of this 4.9-kb transcript in the construct I transformants was highly regulated by iron availability. Deletion of the most 3′ 2.2 kb of the TfR 3′UTR (construct II) resulted in loss of the majority of the iron regulation of TfR transcript level. Deletion of the 3178 to 3856 fragment (construct III) also led to loss of full regulation of transcript level, whereas deletions involving regions flanking this fragment (constructs IV and V) had no effect on regulation of mRNA level. Thus the conclusion regarding the location of the region responsible for iron regulation that was reached on the basis of the transient expression assay for protein synthesis of Fig. 2a was confirmed by the data of Fig. 2b. In addition, the changes in mRNA levels that we observed in the regulated constructs indicated that the regulation of the biosynthesis of TfR encoded by our constructs was occurring in a fashion analogous to iron regulation of a native TfR gene (that is, by alteration of mRNA level).

Computer-aided analysis of the sequence of the human TfR mRNA corresponding to bases 3178 to 3856 of the TfR cDNA revealed that this sequence has potential to form a number of stem-loop structures. Of particular note are the sequences shown in Fig. 3. These TfR 3′UTR sequence elements are capable of folding to form structures that are strikingly similar to that predicted for the IRE located in the 5′UTR of ferritin (*9, 10*). The stem-loop structures of TfR elements B to E (Fig. 3) have in common with the human ferritin H chain IRE: (i) a loop of CAGUGX, (ii) an "upper stem" of five paired bases, (iii) an unpaired 5′ C residue separated by five bases from the loop, and (iv) a "lower stem" of variable length. This particular consensus of sequence and predicted structure is also contained as a single copy within the 5′UTR of each ferritin whose complete mRNA sequence is known (*11–14*). The only deviation from this consensus within the sequences of the human TfR 3′UTR is found in TfR element A wherein the putative loop reads CAGAGX. The 3178 to 3856 region of the TfR mRNA represents less than 30% of the TfR 3′UTR and contains five of these IRE-like elements. No such sequence elements are found in the remainder of the TfR 3′UTR.

The finding of IRE-like sequences within the TfR 3′UTR raised the question of whether these elements were capable of functioning as an IRE in translational regulation. To address this question, oligodeoxyribonucleotides corresponding to either element B or element C of the TfR 3′UTR were synthesized. Each synthetic TfR element was cloned between the ferritin promoter and the structural gene for hGH such that an mRNA would be produced that contained a single TfR element in the 5′UTR of a chimeric hGH transcript (Fig. 4). A similar approach had been used in the identification and characterization of the human ferritin H chain IRE (*9*). The regula-

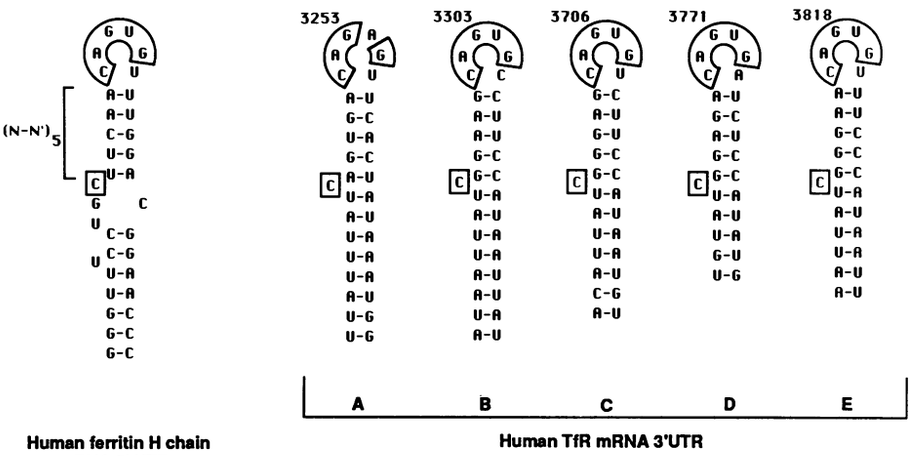

Fig. 3. Similarities between the IRE of the 5′UTR of ferritin mRNA and sequence elements present in the 3′UTR of the transferrin receptor mRNA. Sequence elements from the 5′UTR of the human ferritin H chain mRNA (*9*) and from the 3′UTR of the human TfR mRNA are depicted in stem-loop configurations (*21*). With the exception of a one-base deviation in the loop of TfR element A, all structures shown have loops consisting of CAGUGX and an unpaired C in the stem (outlined bases). The relative position of the unpaired C is invariant, in all cases being five paired bases $[(N\text{-}N')_5]$ 5′ of the loop. The numbers at the top of each of the TfR elements indicate the position in the human TfR mRNA sequence occupied by the most 5′ G in the loop according to our numbering system (*19*).

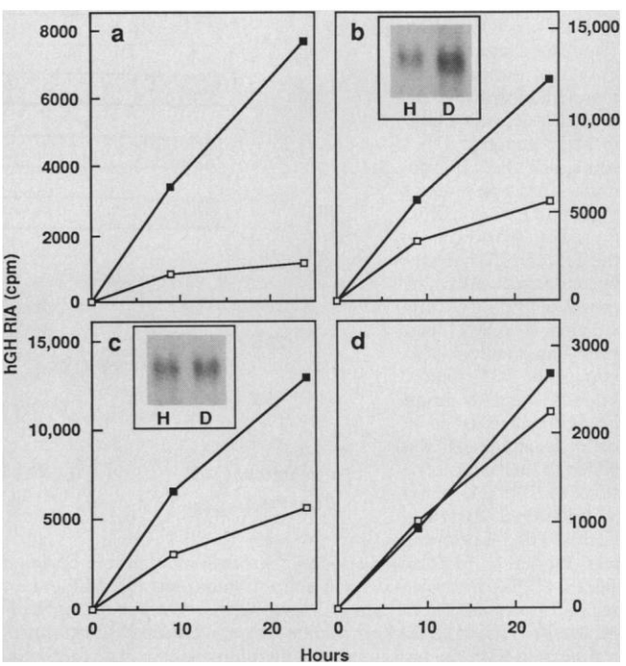

Fig. 4. Elements from the 3′UTR of the TfR mRNA are able to function as an IRE conferring ferritin-like translational regulation when moved to the 5′UTR of a chimeric transcript containing the hGH mRNA. Synthetic deoxyribonucleotides corresponding to the ferritin IRE (**a**), or TfR element B (**b**), or TfR element C (**c**) were cloned between the ferritin promoter and the structural gene encoding hGH (*22*). (**d**) An analogous plasmid lacking an IRE-like sequence element was similarly tested. These constructs were transfected into mouse B6 cells and, after treatment with either 100 μM hemin (H, ■) or with 100 μM desferrioxamine (D, □), the transient expression of hGH in the medium was assessed as previously described (*9*). Data shown are representative of at least three independent transfections with these constructs. Cytoplasmic RNA was isolated from the transfectants involving the TfR elements (*23*), separated by electrophoresis, and analyzed as described in Fig. 1. The panels (b) and (c) show the blot hybridization analysis of these RNA samples with an hGH probe. RIA, radioimmunoassay.

tion of hGH production seen under control of a synthetic ferritin IRE is shown in Fig. 4a. When in the context of a 5′UTR, each of the TfR elements tested mediated ferritin-like iron regulation of hGH production (Fig. 4, b and c). This regulation is opposite in direction from that normally seen in iron regulation of TfR biosynthesis. The presence of the ferritin promoter alone does not result in iron-dependent control of hGH biosynthesis (Fig. 4d). We have also tested two constructs that contained oligodeoxyribonucleotides with potential to form stem-loop structures not conforming to the above IRE consensus. No iron regulation was observed with these constructs. Thus, TfR elements B and C have the ability to function as translational IREs, and this ability appears to require an IRE consensus of sequence and structure.

A characteristic of the ferritin translational regulatory system is iron-dependent modulation of protein biosynthesis without corresponding alteration in the level of the mRNA (*4, 5*). To confirm that each of the TfR elements was acting as a translational IRE in the regulation of hGH production seen in Fig. 4, we assessed the level of hGH mRNA in these transfectants. In neither case (Fig. 4, b and c, insets) did we find increased levels of the hGH transcript in the hemin-treated cells that would account for their higher rate of hGH protein production. In Fig. 4c the levels of mRNA appeared to be identical in the two samples. In Fig. 4b there appeared to be somewhat more mRNA in the desferrioxamine-treated cells. If the level of mRNA is higher in these desferrioxamine-treated cells, the translational effect of iron availability would have to be more pronounced to yield the higher hGH production rate seen after hemin treatment. Thus, each of the TfR elements tested in the context of a 5′UTR was capable of mediating alterations in protein production without corresponding changes in mRNA levels.

The magnitude of the translational iron regulation mediated by each of the TfR elements (Fig. 4, b and c) appeared to be somewhat lower than that seen in the same experiment when a plasmid containing the ferritin IRE was used (Fig. 4a), but their ability to function as IREs was clear in comparison to the unregulated plasmid lacking an IRE-like sequence element (Fig. 4d). The ability of TfR element B or C to substitute for the ferritin IRE conveys information regarding the requirements for IRE function in translational regulation. Between the two synthetic oligomers corresponding to the TfR elements tested, 22 of 32 bases that are contained in the synthetic ferritin IRE oligomer have been altered and yet IRE function has been retained. It has been shown that a single base deletion in the consensus loop or a deletion that would disrupt base pairing in the upper stem each abolished iron regulation (*9*). In the proposed TfR structures depicted in Fig. 3, the nucleotides of the stems are significantly different from the ferritin IRE structure and different to a lesser extent from each other. However, in all instances where a difference in one half of the stem would prevent the base pairing needed to preserve these structures, a complementary difference exists in the other half of the stem. If the multiple elements of the TfR 3′UTR arose by duplication of a single ancestral element, it would appear that their potential to form an IRE-like stem-loop has been a more dominant feature to be conserved than the exact nature of the bases that make up the stems of the putative structures.

Although similarities exist between the ferritin and TfR elements depicted in Fig. 3, the elements function in distinct ways in their respective native contexts. One aspect of these differences relates to the number of IRE-like sequences present in the two transcripts. The IRE occurs as a single copy within the ferritin 5′UTR, whereas there are five similar elements within the human TfR 3′UTR. We have shown that a single TfR element is sufficient when moved to a 5′UTR to confer the translational regulation that is normally mediated by the ferritin IRE. However, two of our constructs having restriction fragment deletions within the critical 3178 to 3856 region indicate that a single element is not sufficient to accomplish regulation of TfR mRNA levels (Fig. 2). In construct VI, sequence elements A and B have been deleted and elements C to E are present. Conversely, construct VII contains elements A and B but not C to E. Thus each of these constructs contains an element that has demonstrated IRE function when in a 5′UTR (Fig. 4). However, after treatment with hemin or desferrioxamine, both construct VI and VII give rise to less than twofold differences between human TfR biosynthetic rates in the transient protein synthesis assay (Fig. 2a) and in the RNA levels of stable transformants (Fig. 2b). This low degree of iron regulation was not significantly greater than that seen after deletion of the entire TfR 3′UTR and is likely a reflection of the transcriptional effect described above. The insufficiency of a single element within the 3′UTR of TfR suggests that iron regulation of the TfR mRNA may involve the cooperative influence of more than one element within the TfR 3′UTR.

A portion of the chicken TfR cDNA corresponding to the 3′UTR has recently been cloned and sequenced (*15*). Our examination of the unpublished chicken sequence (*16*) revealed that the chicken TfR mRNA also contains five IRE-like sequences that correspond to elements A to E of the human TfR mRNA. These sequences are strikingly similar in the human and chicken genes; indeed, 49 consecutive bases encompassing element B are identical in the two genes. In portions of the chicken 3′UTR outside of the region corresponding to that implicated by our deletion analysis, sequence similarity to the human gene is markedly reduced. Four of the five potential stem-loops of the chicken TfR sequence also have loops of CAGUGX; the fifth (corresponding to human TfR element A) has a loop of CAGCGX. All of the potential chicken structures have five paired bases in an "upper stem" followed by a 5′ unpaired C and a lower stem of variable length. We believe that the similarity between these human and chicken TfR sequences supports our contention that these are critical elements involved in iron regulation of TfR expression.

Thus, the IRE is an RNA element capable of mediating two distinct regulatory events depending on its context within the transcript. It is conceivable that a common (or similar) regulatory molecule binds to these RNA structures such that, when in the context of a 5′UTR, translation of the transcript is attenuated and, when in the context of a 3′UTR, the transcript is protected from degradation. The putative regulatory molecule would be associated with the IRE elements when iron is scarce and dissociated when iron is abundant. Such a model would account for the opposite responses of the biosyntheses of ferritin and the TfR to a common primary stimulus (iron). Given the relatively short half-life of the TfR mRNA (*3*), this model would allow rapid changes in the biosynthetic rates for both ferritin and the TfR in response to changes in iron availability. The difference in the mechanisms of these regulatory phenomena that is apparent from assessing the effect of iron on the level of the respective transcripts would also be consistent with this model.

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19. Construct I (formerly referred to as TRmg2) and construct II (formerly referred to as TRmg3) have been described (*6*). The other constructs were prepared by restriction fragment deletion and standard cloning procedures [T. Maniatis, E. F. Fritsch, J. Sambrook, *Molecular Cloning: A Laboratory Manual* (Cold Spring Harbor Laboratory, Cold Spring Harbor, NY, 1982)]. We have numbered the bases of the TfR cDNA using the sequence data of Schneider *et al.* [C. Schneider, M. Kurkinen, M. Greaves, *EMBO J.* **2**, 2259 (1983); C. Schneider, M. J. Owen, D. Banville, J. G. Williams, *Nature* **311**, 675 (1984)] with base 1 being the transcription start site that they determined by primer extension. However, we have subtracted the 192 bases that are present near the 5′ end of their sequence but which are not in pcDTR1 (*18*) from which our constructs were derived. Using this numbering system, our deletion constructs correspond to deletions of bases: none (construct I); 2802 to the end of the cDNA (construct II); 3178 to 3856 (construct III); 2802 to 3177 plus 3861 to the end of cDNA (construct IV); 2405 to 3177 plus 3861 to the end of cDNA (construct V); 3178 to 3405 (construct VI); and 3636 to 3856 (construct VII).
20. Absolute levels of human TfR transcripts in the pools of stable transformants differed somewhat (less than fivefold) with no apparent pattern relating to the nature of the deletion. It is assumed that this represents a combination of transfection efficiency, gene dosage, and relative growth rate among members of our pools of stable transformants. For purposes of presentation, an autoradiographic exposure was selected for each transformant such that the intensities of the largest transcript in desferrioxamine-treated lanes were approximately equal.
21. We analyzed sequences of the 3′UTR of the human TfR, using an algorithm that accommodates the potential of G-U pairing in RNA [M. Zuker and P. Stiegler, *Nucleic Acids Res.* **9**, 133 (1981)].
22. Plasmid L5(+26mer)-GH (used in Fig. 4a) and its parent plasmid L5-GH (used in Fig. 4d) have been described (*9*). L5-GH contains the most 5′ six nucleotides of the human ferritin H chain 5′UTR. Two pairs of complementary deoxyribonucleotides corresponding to each of TfR elements B and C were synthesized with an Applied Biosystems DNA synthesizer. Plasmids containing these elements were prepared by cloning the double-stranded DNA between the Bam HI and Xba I sites of a plasmid derived from pUC18 with the hGH gene in its Hinc II site and the ferritin pFP_1 promoter (*8*) between its Eco RI and Bam HI sites. The correct nucleotide sequences of the insertions in the resultant plasmids were confirmed by DNA sequencing [F. Sanger, S. Nicklen, A. R. Coulson, *Proc. Natl. Acad. Sci. U.S.A.* **74**, 5463 (1977)] to be: 5′-GGATCCATTATCGGAAG<u>CAGTGC</u>CTTCCATAATTCTAGA-3′ (for the plasmid used in Fig. 4b) and 5′-GGATCCATTATCGGGAG<u>CAGTGT</u>CTTCCATAATTCTAGA-3′ (for the plasmid used in Fig. 4c). The nucleotides corresponding to the six-membered loops in the stem-loop structures proposed for mRNA arising from these constructs are underlined for purposes of orientation.
23. To increase transient expression of hGH mRNA, transfected cells were treated with 6 m*M* sodium butyrate [T. A. Gottlieb *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **83**, 2100 (1986)] in addition to either 100 μ*M* hemin or 100 μ*M* desferrioxamine beginning 24 hours after removal of precipitated plasmid DNA. After an additional 24 hours, cytoplasmic RNA was isolated (*6*). The RNA samples were treated with ribonuclease-free deoxyribonuclease for 15 minutes at room temperature to reduce their content of plasmid DNA before separation by electrophoresis and analysis by blot hybridization. Radioimmunoassay of hGH production in the cells used for RNA preparation indicated that butyrate-treated cells displayed iron regulation comparable to that seen in Fig. 4, a and b.
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