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Translation and the stability of mRNAs encoding the transferrin receptor and c-*fos*

(iron/ferritin/protein synthesis/cycloheximide/puromycin)

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ABSTRACT **Turnover of the full-length human transferrin receptor (TfR) mRNA is regulated by iron, and this regulation is mediated by the transcript's 3′ untranslated region. Alterations in the sequence of the TfR mRNA regulatory region have been identified that render the mRNA unregulated by iron and intrinsically unstable. When cells expressing this unstable mRNA are treated with inhibitors of protein synthesis (cycloheximide or puromycin), the steady-state level of the encoded human TfR mRNA is increased due to a stabilization of the transcript. A similar set of observations has been made using a chimeric mRNA in which the rapid turnover determinant of the TfR mRNA is replaced by the (A+U)-rich region from the 3′ untranslated region of c-*fos* mRNA. To distinguish between a labile protein participant in the degradation of these mRNAs and a requirement for their translation *per se*, we introduced a ferritin iron-responsive element into the 5′ untranslated region of each of these mRNAs. The presence of the 5′ iron-responsive element allowed us to use iron availability to alter the translation of the mRNAs in question without global effects on cellular protein synthesis. Although specific translation of these mRNAs could be inhibited by iron chelation to a degree comparable to that seen with cycloheximide (≈95% inhibition), no effects on mRNA turnover were observed. These data support a model in which a trans-acting labile protein is necessary for the turnover of these mRNAs rather than there being a requirement for the translation of the mRNAs themselves.**

The complex pathway involved in eukaryotic gene expression affords many points at which regulation may be imposed. Much has been learned about ways in which cells regulate the production of mRNA (1). However, it has become increasingly clear that gene regulation distal to mRNA production is also utilized extensively. In particular, the regulation of the cytoplasmic mRNA stability appears to be a widely employed mechanism for gene regulation. It has been known for some time that eukaryotic mRNAs display diverse half-lives ranging from a few minutes to many hours or even days, but the determinants responsible for this diversity in turnover rates remain largely undefined (2). It is also clear that the degradation rate of a given mRNA can be altered in response to external stimuli, and the list of examples of genes whose regulation involves mRNA stability has continued to expand. For several years, we have been investigating the regulation of transferrin receptor (TfR) mRNA levels in response to alterations in iron availability (3, 4). This regulation is achieved by cis-acting RNA sequence/structure motifs termed iron-responsive elements (IREs) and a trans-acting IRE-binding protein (IRE-BP). An interaction between the IRE and the IRE-BP is responsible for iron regulation of TfR mRNA levels and for iron regulation of ferritin mRNA translation (3–6).

There exist a number of examples of rapidly degraded mRNAs whose levels are markedly increased in cells treated with inhibitors of protein synthesis. This phenomenon has been seen with several protooncogenes, including c-*myc* (7, 8), c-*myb* (9), c-*fos* (10), and c-*jun* (11). These increases in mRNA levels appear to be the result of substantial stabilization of the mRNAs by the protein synthesis inhibitors and are seen with various types of inhibitors (i.e., inhibitors of translation initiation and of elongation that act by distinct mechanisms). It has also been reported that the human TfR mRNA expressed in mouse L cells is stabilized by cycloheximide (Chx) (12).

At present, the mechanism by which protein synthesis inhibitors stabilize certain mRNAs remains obscure. It is thought that either a highly unstable protein is involved in the degradation of these mRNAs (a so-called trans effect) or that translation *per se* is required for the decay of these mRNAs (a cis effect). These two possibilities are not mutually exclusive and each may be applicable in certain instances. The example wherein evidence for a cis effect appears most compelling is that of autoregulation of β-tubulin mRNA degradation (2). Experimental evidence obtained in this system is consistent with ribosome-bound β-tubulin mRNA being degraded after cotranslational recognition of the nascent amino-terminal peptide (Met-Arg-Glu-Ile) by unassembled dimers of α- and β-tubulin. This recognition is presumed to activate an RNase that may be ribosome-associated. A ribosome-associated nuclease has also been invoked in models for regulated turnover of histone mRNAs (2, 13, 14). In the case of c-*fos* mRNA stabilization by Chx, it has been suggested that the rapidity of the effect on mRNA stability is more consistent with a cis effect (15).

The major difficulty in distinguishing between a cis effect and a trans effect of protein synthesis inhibitors is that inhibition of global protein synthesis will, by its nature, inhibit the translation of the mRNA in question and the synthesis of the putative short-lived, trans-acting protein. We reasoned that the IRE/IRE-BP system may provide a means to distinguish between cis and trans effects of inhibition of protein synthesis. When an IRE exists in the 5′ untranslated region (UTR) of an mRNA (as it does in ferritin mRNAs) the translation of the mRNA is regulated by iron such that more translation occurs when iron is abundant and less occurs when iron is scarce (3, 5, 6). When cells are treated with an iron source, the ferritin mRNA is found associated with

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Abbreviations: TfR, transferrin receptor; IRE, iron-responsive element; IRE-BP, IRE-binding protein; Chx, cycloheximide; UTR, untranslated region; nt, nucleotide(s).

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polysomes, whereas ferritin mRNA exists as a lower density ribonucleoprotein in chelator-treated cells (16). This effect is specific for the mRNA containing an IRE in its 5′ UTR, and global protein synthesis is not affected by the experimental manipulations of iron availability. Thus, for an mRNA with an IRE in its 5′ UTR, iron can be used to attenuate translation in a way that is completely specific for IRE-containing mRNAs. If a short-lived mRNA had an IRE and were stabilized by global inhibition of protein synthesis, iron chelation should have a similar stabilizing effect only if translation of the mRNA itself were a requirement for mRNA degradation. If, instead, a short-lived protein were a necessary component of the degradation of the mRNA and this protein's disappearance accounted for the effect of global protein synthesis inhibition, then iron manipulation would be predicted to be without effect since the synthesis of this putative short-lived protein would be unaffected by iron.

MATERIALS AND METHODS

Preparation and Assessment of Plasmids. Preparation of constructs TRS-1, TRS-3, and TRS-4 and stable transfection of mouse B6 fibroblasts have been described along with the methodology for analyzing human TfR mRNA levels in these cells by blot hybridization (Northern) following experimental manipulation of iron using either bovine hemin (Sigma) or desferrioxamine (CIBA Pharmaceutical) (17–19). Human TfR was detected with a probe derived from the TfR cDNA clone termed pcD-TR1 (20). The equivalence of loading of gels utilized for blot hybridization (Northern) analysis was confirmed visually by staining of the ribosomal RNAs with ethidium bromide or by probing the blots with a cDNA probe specific for the unrelated mRNA encoding actin (American Type Culture Collection). The construct TR-Δ3′UTR was prepared by cloning the *Eco*RI–*Xba* I fragment containing the TfR promoter and protein-coding regions from the construct termed TRmg-2 (17–19) into the polylinker of the plasmid pGEM4Z (Promega) that had been cut with *Eco*RI and *Xba* I. To produce the construct TR-*fos*, an *Nsi* I–*Hin*dIII fragment containing DNA encoding the (A+U)-rich region of the c-*fos* mRNA 3′ UTR was isolated from a genomic human c-*fos* clone [pc-fos(human)-1; American Type Culture Collection] and cloned into the *Pst* I and *Hin*dIII sites of pGEM4Z. The *Eco*RI–*Xba* I fragment of TR-mg2 was then inserted as described above. To produce construct 5′IRE-TR-*fos*, *Xho* I linkers were cloned into the *Eco*RV site of the TfR cDNA (at +13 from TfR transcription start site) within the construct TR-*fos*. Synthetic double-stranded DNA was synthesized with a 5′ *Bam*HI site and a 3′ *Xho* I site flanking DNA encoding a human ferritin H-chain IRE (18). This synthetic duplex was directionally cloned into the *Xho* I linkers at the TfR *Eco*RV site using a 3′ *Xho* I site, and a 5′ *Bam*HI site produced the linker insertion. The construct 5′IRE-TRS-4 was produced from 5′IRE-TR-*fos* by replacing the latter's *Nco* I–*Eco*RI fragment with that of TRS-4.

Assessment of Protein Synthesis. The effect of protein synthesis inhibitors on global protein synthesis was assessed by measuring [^{35}S]methionine incorporation into trichloroacetic acid-insoluble material. The translation of TfR and ferritin proteins was assessed by specific immunoprecipitation following 90 min of labeling with [^{35}S]methionine as described (17–19). Immunoprecipitation of transfected human TfR employed monoclonal antibody B3/25 specific for human TfR (Boehringer Mannheim). Endogenous mouse ferritin was immunoprecipitated using rabbit polyclonal anti-human ferritin (Boehringer Mannheim) that cross-reacts with mouse ferritin.

RESULTS

In the native TfR mRNA, the regulatory region is within its 3′ UTR (17, 21). A 680-nucleotide (nt) segment of the 3′ UTR contains a rapid turnover determinant and five IREs that regulate the activity of this turnover determinant (17–19). Each of the TfR IREs competes with a ferritin IRE for binding of the IRE-BP (22). We have previously shown that a smaller 250-nt synthetic element containing only three IREs produces the full range of regulation of the TfR mRNA levels in response to manipulation of iron availability (19). This synthetic element was termed TRS-1 (Table 1), and here we directly demonstrate that the stability of an mRNA containing this element is iron-regulated (Fig. 1*A*). In cells treated with the iron source hemin, an mRNA half-life of 45–60 min is observed, whereas in cells treated with the iron chelator desferrioxamine, the TRS-1 mRNA displays a half-life of considerably greater than 3 hr.

Based on levels of mRNA expression, we previously concluded that the TRS-1 regulatory element could be altered by deletion of nucleotides and that two types of unregulated constructs could be distinguished (19). Removal of the 100 nt corresponding to the three IREs of TRS-1 produced an unregulated construct termed TRS-3. The level of expression in cells transiently transfected with TRS-3 was found to be relatively high (using a cotransfected plasmid to normalize) and completely unregulated. These results were interpreted as indicating that the nucleotides of the IREs overlap with those of the rapid-turnover determinant. In contrast, removal of three selected cytosine residues (the first cytosine in each of the three IRE loops to yield a construct termed TRS-4) also eliminated regulation but gave rise to relatively low levels of

Table 1. Summary of constructs

Construct	5′ UTR	3′ UTR	mRNA stability regulated by iron	mRNA translation regulated by iron
TRS-1	TfR	TRS-1	Yes	No
TRS-3	TfR	TRS-3	No (stable)	No
TRS-4	TfR	TRS-4	No (unstable)	No
TR-Δ3′UTR	TfR	None	No (stable)	No
TR-*fos*	TfR	c-*fos*	No (unstable)	No
5′IRE-TRS-4	TfR + IRE	TRS-4	No (unstable)	Yes
5′IRE-TR-*fos*	TfR + IRE	c-*fos*	No (unstable)	Yes

All constructs contained the promoter and coding region of the human TfR but differed from one another in their 5′ UTR and/or their 3′ UTR. All constructs were transfected into mouse fibroblasts and stable transformants were produced. The sequences of the 3′ UTR were the determinant of mRNA stability, with "unstable" being operationally defined as displaying an mRNA half-life of <1 hr and "stable" being defined as displaying a half-life of >3 hr. Only the construct TRS-1 produced an mRNA whose stability was iron-regulated. The sequences of the 5′ UTR were the determinant of mRNA translational regulation, with only those constructs that produced an mRNA containing an IRE in the 5′ UTR being regulated by iron at the level of translation.

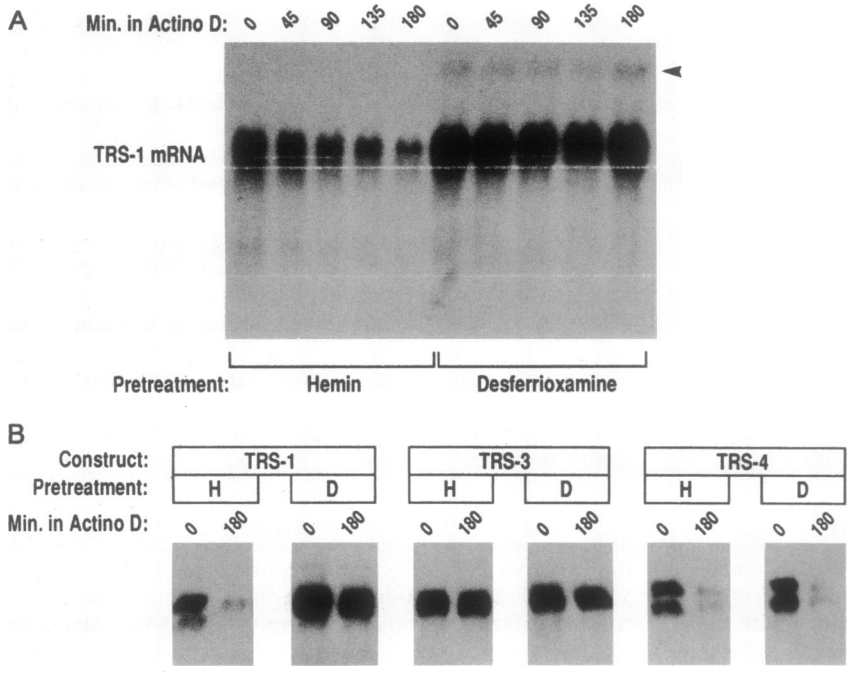

FIG. 1. The degradation of TfR mRNA is iron-regulated and alterations in sequence can result in two types of unregulated mRNAs. (*A*) Cytoplasmic mRNAs were isolated from mouse B6 fibroblasts stably expressing the human TfR by virtue of transfection with the plasmid TRS-1. These mRNAs were analyzed by blot hybridization (Northern) using a probe specific for the human TfR mRNA. Cells were treated for the indicated times with 4 μM actinomycin D (Actino D) following pretreatment for 16 hr with either 100 μM hemin (H) or 100 μM desferrioxamine (D). Densitometry indicated that the half-life of the TRS-1 mRNA in hemin-treated cells was 45–60 min, whereas in desferrioxamine-treated cells the half-life appeared to be considerably greater than 3 hr. The arrowhead represents the migration position of the endogenous 4.9-kilobase mouse TfR mRNA. (*B*) Experimental design was as in *A*. Cells expressing TfR mRNAs encoded by plasmids TRS-1, TRS-3, and TRS-4 were compared. Only the level of TRS-1 mRNA was iron-regulated and displayed a half-life that was dependent on iron status of the cell. Irrespective of iron status, TRS-3 mRNA was relatively stable, and TRS-4 mRNA was relatively unstable.

expression in the transient transfections. The removal of the first cytosine from the IRE loop abrogates high-affinity interaction with the IRE-BP (3). The role of the IRE-BP in TfR mRNA regulation is envisioned as a protection of the transcript from degradation when iron is scarce. The finding of a low level of TRS-4 mRNA in transfections was interpreted as indicating that this mRNA contained an intact rapid-turnover determinant that could not be stabilized by interaction with the IRE-BP since removal of the three cytosines eliminates IRE-BP interaction. Here we demonstrate by direct measurement of mRNA stability in stable transformants that the TRS-3 mRNA is intrinsically stable and that the TRS-4 mRNA is intrinsically unstable (Fig. 1*B*). In contrast to TRS-1 mRNA, the stabilities of TRS-3 and TRS-4 mRNAs were not affected by manipulation of cellular iron availability. A lower RNA band was seen in samples from TRS-4 cells and hemin-treated TRS-1 cells. This RNA species may represent a breakdown product since its presence correlates with rapid turnover of the TfR mRNA. We have noted a similar phenomenon involving the endogenous 4.9-kilobase TfR mRNA of a human cell line (23).

When the TRS-4 transfectants were treated with Chx, which inhibits translation elongation but leaves ribosomes on the mRNA, a marked increase in the level of TRS-4 mRNA was seen (Fig. 2*B*). A similar effect on TRS-4 mRNA level was observed when cells were treated with puromycin, which causes premature release of the peptide chain and ribosomes. Thus, the TRS-4 mRNA level is increased by inhibition of protein synthesis irrespective of whether ribosomes are present. As would be anticipated for a long-lived mRNA, no comparable effect on the intrinsically stable TRS-3 mRNA was seen. In cells expressing the iron-regulated TRS-1 mRNA, an effect of Chx on mRNA level was observed in cells that had been pretreated with the iron source hemin but not in cells treated with the iron chelator desferrioxamine (Fig. 2*B*). No comparable effect on an unrelated mRNA (actin) was seen under any regimen of iron treatment. All data are consistent with the TRS-4 mRNA being affected by the inhibitors of protein synthesis in a fashion comparable to TRS-1 mRNA in hemin-treated cells and TRS-3 mRNA resembling TRS-1 mRNA in desferrioxamine-treated cells.

We produced two constructs designed to test whether iron could be used to regulate selectively the translation of a short-lived mRNA. Each construct encoded an mRNA that contained a ferritin IRE within its 5′ UTR and each utilized the TfR protein-coding region (Table 1). In one construct (termed 5′IRE-TRS-4), the 3′ UTR was the TRS-4 element that had been shown to render the mRNA intrinsically unstable. In the second construct (termed 5′IRE-TR-*fos*), we

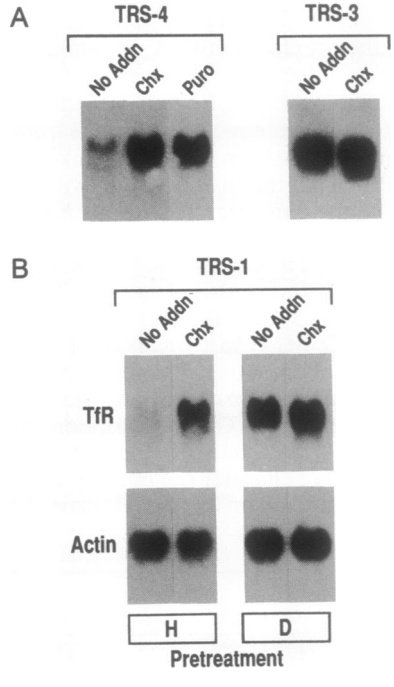

FIG. 2. The levels of the unstable TfR mRNAs are increased by treatment of cells with inhibitors of protein synthesis. (*A*) Cells expressing TRS-4 mRNA were treated for 4 hr with either 1 μg of Chx per ml or 20 μg of puromycin (Puro) per ml prior to analysis of mRNA levels as described in the legend to Fig. 1. The abundance of TRS-4 mRNA was markedly increased by both inhibitors, but no increase was seen in the level of the relatively stable TRS-3 mRNA as a result of identical Chx treatment. No Addn, no addition. (*B*) The level of TRS-1 mRNA was increased by the Chx treatment described in *A* if the mRNA was destabilized by pretreatment of cells with hemin (H). When the TRS-1 mRNA was relatively stable after treatment of cells with desferrioxamine (D), no effect of Chx treatment was observed. Neither Chx treatment or iron manipulations altered the abundance of actin mRNA in the cells expressing TRS-1 mRNA.

replaced this TRS-4 element in the 3′ UTR with a 250-nt segment of the human c-*fos* gene. This segment encodes the (A+U)-rich region that functions as one of the instability determinants in c-*fos* mRNA. When transferred to β-globin mRNA, the (A+U)-rich region of c-*fos* mRNA confers a rapid degradation rate (24, 25). We first demonstrated that the c-*fos* element destabilized the chimeric TfR mRNA (Fig. 3*A*). When the entire 3′ UTR native human TfR mRNA is deleted (in construct TR-Δ3′UTR), this truncated mRNA has a half-life considerably greater than 3 hr. A similar observation led us (16) and others (18) to conclude that the 3′ UTR contained the regulatory element responsible for regulated mRNA stability. Replacement of the native 3′ UTR with the c-*fos* mRNA element (in construct TR-*fos*) greatly enhanced mRNA decay resulting in a half-life of between 45 and 60 min. Not surprisingly, a similar half-life was seen in a construct (5′IRE-TR-*fos*) that contained an IRE in its 5′ UTR. A single IRE in the context of a 5′ UTR does not appear to influence mRNA stability.

When cells transfected with the 5′IRE-TR-*fos* construct were treated with Chx, a marked increase in the level of the encoded mRNA was seen (Fig. 3*B*). This effect was similar to that seen with the TRS-4 construct or the TRS-1 construct in iron-treated cells (Fig. 2). A comparable Chx-induced increase in mRNA level was also seen with the construct 5′IRE-TRS-4 (data not shown). The increase in 5′IRE-TR-*fos* mRNA level after Chx treatment was the result of mRNA stabilization. When cells were pretreated with Chx and then mRNA stability was assessed by decay after addition of actinomycin D, the 5′IRE-TR-*fos* mRNA was seen to be more stable in the Chx-treated cells (Fig. 3*C*). In this experiment, quantification by densitometry revealed that the mRNA level and the half-life (as judged by mRNA disappearance during 3 hr in actinomycin D) were each increased by ≈4-fold.

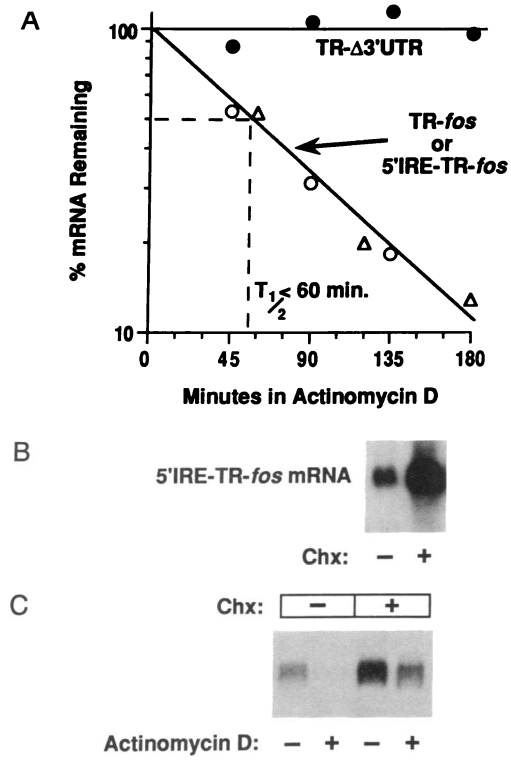

FIG. 3. A segment of the c-*fos* 3′ UTR confers instability to a TfR mRNA and the level of this chimeric mRNA is increased by Chx through mRNA stabilization. (*A*) The decays of mRNAs were analyzed by quantitative densitometry using the experimental design described in the legend to Fig. 1. The TR-Δ3′UTR mRNA represents a deletion of the native 3′ UTR of the TfR mRNA. In constructs termed TR-*fos* and 5′IRE-TR-*fos*, 250-nt segment of the *fos* 3′ UTR has been inserted. In 5′IRE-TR-*fos*, an IRE corresponding to that of human ferritin H chain has been inserted within the 5′ UTR of the mRNA. The *fos*-derived 3′ UTR confers a half-life of 45–60 min upon the TfR mRNA and this decay rate is independent of the presence of the IRE in the 5′ UTR. (*B*) Treatment of cells with 10 μg of Chx per ml for 5 hr resulted in a marked increase of the level of 5′IRE-TR-*fos* mRNA. (*C*) Cells expressing the 5′IRE-TR-*fos* mRNA were treated for 8 hr with 10 μg of Chx per ml prior to a 3-hr treatment with 4 μg of actinomycin D per ml and mRNA analysis by blot hybridization. Chx treatment was estimated by densitometry to result in an approximate 4-fold increase in mRNA level and a nearly identical increase in half-life (from ≈45 min to ≈3 hr).

Treatments of the cells expressing the 5′IRE-TR-*fos* mRNA with an iron source or an iron chelator were without effect on the level of this short-lived mRNA (Fig. 4*A*) despite a dramatic effect on the translation of this mRNA (Fig. 4*B*). Densitometry indicated that the range of translational regulation by iron of the 5′IRE-TR-*fos* mRNA was ≈20-fold. This range is somewhat lower than the range of translational regulation of the endogenous murine ferritins produced by these cells (estimated to be 30- to 50-fold regulated). More important, the 95% attenuation of translation by iron was very similar to the ≈95% inhibition of global translation produced by Chx as used in our experiments. We also found that the level of the mRNA encoded by the construct 5′IRE-TRS-4 that contains the TfR rapid-turnover determinant rather than that of c-*fos* was not affected in its abundance by iron despite an estimated 20-fold regulation of its translation by iron (data not shown). Direct measurement of the 5′IRE-TR-*fos* mRNA decay rate using actinomycin D revealed that its half-life was 45–60 min in cells treated with desferrioxamine, where it is poorly translated, and in cells treated with hemin, where it is well translated (Fig. 4*B*).

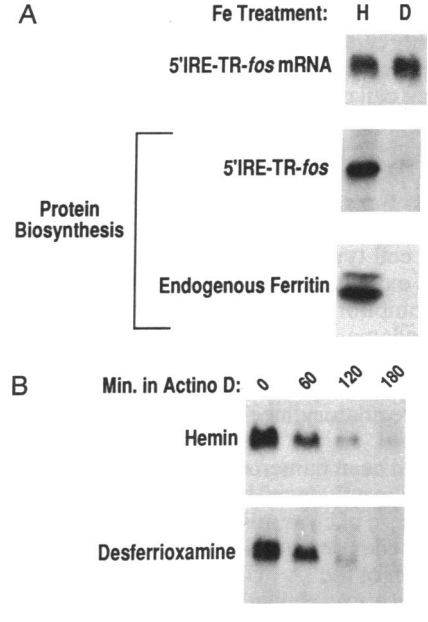

FIG. 4. The 5′IRE-TR-*fos* mRNA remains unstable despite a 95% reduction in translation caused by iron chelation. (*A*) Cells expressing 5′IRE-TR-*fos* mRNA were treated with hemin (H) or desferrioxamine (D) as described in the legend to Fig. 1. These treatments resulted in no difference in mRNA levels. In contrast, a marked difference in 5′IRE-TR-*fos* mRNA translation resulted, as judged by [^{35}S]methionine incorporation into the TfR encoded by this mRNA. For comparison, the incorporation of [^{35}S]methionine into endogenous mouse ferritin chains is presented. (*B*) Utilizing the experimental protocol described in the legend to Fig. 1, the half-life of the 5′IRE-TR-*fos* mRNA was determined to be 45–60 min irrespective of iron manipulations.

DISCUSSION

We have shown that the levels of short-lived mRNAs encoding the human TfR in mouse fibroblasts are increased in response to global inhibitors of protein synthesis. This phenomenon is seen under conditions wherein the mRNA is unstable due to cellular iron status (as with hemin-treated TRS-1 cells) or when the mRNA stability is unregulated by iron (as with any mRNA containing TRS-4 or c-*fos* rapid-turnover determinant). The full-length TfR mRNA has also been reported to be stabilized by Chx in mouse L cells (12).

In the present study, we have utilized a ferritin IRE in the 5′ UTR to modulate selectively the translation of mRNAs that have a short half-life by virtue of having within their 3′ UTR a rapid-turnover determinant derived from the mRNA of either the human TfR or c-*fos*. The levels of these mRNAs in stable transformants are markedly increased by treatment with Chx, and these increases are a result of increased stability of the transcripts when protein synthesis is inhibited. In this regard, the mRNAs resemble several other rapidly degraded transcripts that are stabilized by inhibitors of protein synthesis. The IRE within the 5′ UTR enables us to alter translation in response to iron over an estimated 20-fold range. We found no effect of the modulation of translation by iron with the transcripts having a half-life of 45–60 min irrespective of whether they were being actively translated or not. Thus, iron chelation is capable of attenuating the translation of these mRNAs to a degree resembling the global effect of Chx (≈95% inhibition) and yet has no effect on either steady-state mRNA levels or mRNA decay rate. Our results support the conclusion that the effect of global protein synthesis inhibitors on the stability of the TfR and c-*fos* mRNAs is due to a short-lived protein participant in the decay of these transcripts rather than a requirement for the translation of these mRNAs themselves. Also supporting this conclusion is the fact that we have observed that the stabilization of endogenous TfR mRNAs appears to be cell-type specific, with certain cells displaying no observable stabilization over several hours of Chx treatment (data not shown). However, all cell types examined modulate their TfR mRNA level in response to iron availability. Since the level and/or half-life of a given protein may vary from cell type to cell type (26) we interpret our observation as being indicative of these variations in the short-lived protein involved in turnover of the TfR mRNA. It would appear to be more difficult to explain the cell type variation of Chx treatment if one were to favor an explanation involving a cis effect of the protein synthesis inhibitor. One would be forced then to conclude that the fundamental mechanism by which cells modulate their TfR mRNA levels in response to iron is, in some cells, intimately tied to translation of the mRNA whereas, in others, the regulatory mechanism is independent of translation.

There have been numerous suggestions that the stability of mRNAs can be influenced by the poly(A) tract at their 3′ ends (27). In the case of c-*fos* mRNA, shortening or removal of the poly(A) precedes degradation of the remainder of the mRNA (15). Deletion of the (A+U)-rich sequences from the 3′ UTR of c-*fos* mRNA was shown to dramatically slow this process. Moreover, the removal of the poly(A) from the c-*fos* mRNA was also slowed by inhibitors of translation. Given these findings, we would infer that it is poly(A) tail shortening that requires the participation of the short-lived protein implicated here. It has also been proposed that c-*myc* mRNA turnover proceeds by translation-dependent shortening of the poly(A) tail followed by a rapid decay of the remainder of the mRNA after the poly(A) tail is shortened beyond some critical length (28). These authors also concluded that poly(A) tail shortening was the translation-dependent step in c-*myc* mRNA degradation. We have observed a decreased content of poly(A) in TfR mRNA when cells are treated with iron (unpublished observation). It remains to be determined if the length of the poly(A) tails of the mRNAs encoded by the constructs described here are affected by global or selective inhibition of translation of these mRNAs.

The utilization of a 5′ IRE for selective inhibition of an mRNA's translation may prove useful in understanding the turnover of other mRNAs (e.g., c-*myc*, c-*myb*, c-*jun*, etc.) that are affected by global protein synthesis inhibitors. Our results should also serve as an impetus to identify the short-lived participant(s) in mRNA turnover, and knowing that they are short-lived based on these findings may prove useful in the identification/characterization process.

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