Iron Differentially Stimulates Translation of Mitochondrial Aconitase and Ferritin mRNAs in Mammalian Cells IMPLICATIONS FOR IRON REGULATORY PROTEINS AS REGULATORS OF MITOCHONDRIAL CITRATE UTILIZATION

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IMPLICATIONS FOR IRON REGULATORY PROTEINS AS REGULATORS OF MITOCHONDRIAL CITRATE UTILIZATION

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Utilization of mRNAs containing iron-responsive elements (IREs) is modulated by iron-regulated RNA-binding proteins (iron regulatory proteins). We examine herein whether iron differentially affects translation of ferritin and mitochondrial aconitase (m-Acon) mRNAs because they contain a similar but not identical IRE in their 5'-untranslated regions. First, we demonstrate that m-Acon synthesis is iron-regulated in mammalian cells. In HL-60 cells, hemin (an iron source) stimulated m-Acon synthesis 3-fold after 4 h compared with cells treated with an iron chelator (Desferal). Furthermore, hemin stimulated m-Acon synthesis 2-4-fold in several cell lines. Second, we show that iron modulates the polysomal association of m-Acon mRNA. We observed m-Acon mRNA in both ribonucleoprotein and polysomal fractions of HL-60 cells. Hemin significantly increased the polysomal association and decreased the ribonucleoprotein abundance of m-Acon mRNA in HL-60 cells. Third, our results indicate that iron differentially regulates translation of m-Acon and ferritin mRNAs. A dose response to hemin in HL-60 cells elicited a 2-2.4-fold increase in m-Acon synthesis within 5 h compared with untreated cells, whereas ferritin synthesis was stimulated 20-100-fold. We conclude that iron modulates m-Acon synthesis at the translational level and that iron regulatory proteins appear to differentially affect translation of IRE-containing mRNAs.

Iron is an essential nutrient for nearly all organisms because iron-containing proteins function in a number of important cellular processes (1–4). However, organisms must simultaneously cope with two detrimental properties of iron under physiological conditions, its low solubility as the uncomplexed metal ion and its propensity to enhance production of reactive oxygen species. It is this essential but potentially toxic nature of iron that has likely served as an evolutionary driving force for the development of systems that promote efficient transport, uptake, and storage of iron in mammals, the interorgan transport, uptake, and storage of iron is performed by transferrin, the transferrin receptor, and ferritin, respectively.

Iron regulation of the synthesis of iron uptake (transferrin receptor (TfR)) and storage (ferritin) proteins represents an important avenue through which cellular iron homeostasis is modulated and maintained (1–4). Ferritin and TfR synthesis are linked to iron status through the action of two iron-regulated RNA-binding proteins, iron regulatory protein (IRP) 1 and IRP2. IRPs bind to specific stem-loop motifs (iron-responsive elements (IREs)) present in the 5'- or 3'-untranslated region (UTR) of ferritin and TfR mRNAs, respectively (1–4). Iron regulates the RNA-binding function of IRP1 and IRP2 through fundamentally different mechanisms (5–7). For IRP1, which is a bifunctional protein, iron inhibits RNA-binding activity by promoting assembly of a [4Fe-4S] iron-sulfur cluster in the binding protein, thereby converting it to cytosolic aconitase (8, 9).

In the presence of low intracellular iron levels, IRPs have high RNA-binding activity. When bound to target mRNAs containing a 5'-IRE, such as H- or L-ferritin mRNAs, IRP1 or IRP2 blocks translation of the messenger. In the case of TfR mRNA, which contains multiple IREs in its 3'UTR, IRP binding increases stability of the mRNA. Conversely, when intracellular iron levels increase, IRP RNA-binding activity is reduced, and this results in enhanced translation of ferritin mRNAs and declining TfR mRNA levels. In addition to ferritin and TfR mRNAs, the mRNA encoding the erythroid isoform of 5-aminolevulinate synthase, the rate-limiting enzyme in erythropoietic heme formation, also possesses a 5'-IRE. The evidence indicates that, like ferritin, iron stimulates translation of erythroid aminolevulinate synthase mRNA (10, 11). Taken together, IRPs are components of a sensory and regulatory network that is a critical factor in maintaining cellular and organismal iron homeostasis.

Recently, it has become apparent that IRPs may regulate other metabolic functions in mammals through their potential ability to modulate the abundance of the tricarboxylic acid cycle enzyme mitochondrial aconitase (m-Acon) (12–14). m-Acon is a [4Fe-4S] iron-sulfur protein that converts citrate to isocitrate. The identification of an IRE in m-Acon mRNA provided the first suggestion that IRPs might regulate m-Acon synthesis (15, 16). When present in a heterologous mRNA, the m-Acon IRE confers responsiveness to IRPs in an in vitro translation system, suggesting that m-Acon synthesis may be

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The abbreviations used are: TfR, transferrin receptor; IRP, iron regulatory protein; IRE, iron-responsive element; UTR, untranslated region; m-Acon, mitochondrial aconitase; Tricine, N-[2-hydroxy-1,1-bis(hydroxymethyl)ethyl]glycine; RNP, ribonucleoprotein; S.E., standard error of the mean.
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MATERIALS AND METHODS

Cell Culture—The human promyelocytic leukemia cell line HL-60, RFP2 rat embryo fibroblasts, and FTO2B rat hepatoma cells were grown as described (20, 21). The human monocytic cell line U937 was grown in RPMI 1640 medium (Life Technologies, Inc.) with 10% fetal bovine serum (Hyclone Laboratories). Penicillin and streptomycin were included in all cell culture media. Cells were lysed in buffer containing Nonidet P-40 and protease inhibitors, and a 13,000 × g supernatant was obtained as described (22). Protein concentrations were determined using the Bradford assay (23).

Metabolic Labeling and Immunoprecipitation—Cells were pulse-labeled with 50–200 μCi/ml [35S]Met/Cys (ICN) for 30 min in RPMI 1640 medium minus Met. After centrifugation, the cells were lysed in lysis buffer containing protease inhibitors (20). Between 5 and 20 × 10^6 cpm of trichloroacetic acid-precipitable material was immunoprecipitated with IgG against rat liver ferritin, bovine heart m-Acon, and rat liver IRP1 as described (22). Immunoprecipitated proteins were denatured in reducing sample buffer (24). Aconitases were resolved on 7.5–15% linear gradient SDS-polyacrylamide gels (22). Ferritin subunits were analyzed using 10% Tricine/SDS-polyacrylamide gels (25). Fluorography was as described (20).

Determination of RNA Abundance—The steady-state level of m-Acon was determined by immunoblotting as described (12).

IRE RNA Binding—IRE RNA-binding activity, in the presence or absence of 2-mercaptoethanol, was determined by electrophoretic mobility shift assay (20). Briefly, 5 μg of cell lysate protein was incubated for 10 min at room temperature with 1 nM 32P-labeled IRE-containing RNA. The RNA used for electrophoretic mobility shift assays was the first 73 nucleotides of the rat L-ferritin IRE. 5′-UTR synthesized using T7 RNA polymerase and α-32P-UTP (20). Bound and free RNAs were separated and quantitated as described (20).

Determination of RNA Abundance—The TIR mRNA level was determined by ribonuclease protection assay (Ambion Inc.) (22). m-Acon mRNA abundance in sucrose gradient fractions was also determined by ribonuclease protection assay. A cDNA encoding human muscle m-Acon was generously provided by Dr. H.-H. Jiang (Chang Gung College of Medicine and Technology, Kwei-Shan, Taiwan). The cDNA (in pBSI-I/SK) was digested with SmaI and SphI, treated with T4 DNA polymerase in the presence of all four deoxynucleotidetriphosphates, and religated. This removed the encoded poly(A) tail and 3′-UTR of the cDNA. This plasmid was digested with DdeI and transcribed with T3 RNA polymerase, allowing production of a coding region antisense RNA from nucleotides 2096 to 1930 of human m-Acon mRNA. All RNA probes were purified in 8 m urea, 5% polyacrylamide gels.

Polysome Profile Analysis—HL-60 cells (2 × 10^6 cells, 10^6 cells/ml) were treated with hemin (50 μM) or Desferal (100 μM) for 5 h before harvest. The cell pellet was lysed in 600 μl of buffer B (26), and the lysate was centrifuged at 12,000 × g for 8 min. The resulting supernatant (500 μl) was applied to a 12ml 10–50% linear sucrose gradient in 20 mM HEPES, pH 7.2, 250 mM KCl, 10 mM MgCl_2, 150 μM/μl cycloheximide, and 0.5 μM/μl heparin (26). The gradients were centrifuged at 180,000 × g in a Sorvall TH641 rotor for 135 min at 4°C and then fractionated using an Isco Model UA-6 gradient fractionator. The absorbance at 254 nm was continuously monitored. Twelve 1-ml fractions were collected, and RNA was isolated as follows. Buffer-saturated phenol (1 ml) was mixed with the sample by vortexing. Chloroform/isooamyl alcohol (24:1, 1 ml) was added, and the sample was}

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FIG. 1. Secondary structures of the ferritin and m-aconitase IREs. Shown are the secondary structures of the bullfrog H-ferritin and bovine m-Acon IRE regions. The predicted secondary structures of the first 55 and 50 nucleotides of the bullfrog L-ferritin and bovine m-Acon mRNA, respectively, were determined using the program M-fold (Genetics Computer Group, Inc., Madison, WI) (47). A, the most stable predicted secondary structure of the bullfrog H-ferritin IRE (ΔG = −10.7 kcal/mol), including the 6-nucleotide loop (CAGUGU) and the bulged nucleotide region 5 base pairs 5′ of the loop. The region of 3 base pairs at the base of this RNA helix (denoted with the phosphodiester backbone in boldface) refers to the phylogenetically conserved flanking region nucleotides shown to be important in ferritin translational regulation (1,17). The predicted structure shown here differs slightly with respect to some aspects of the structure in the flanking region from another structure predicted for the ferritin IRE (1). However, the conserved 3-base pair region is present in both structures. The sequence and structure of the loop and the bulged nucleotide region 5 base pairs 5′ of the loop influence high affinity RNA binding by IRP (19, 31, 37, 48, 49, 52). It has been suggested that the ferritin IRE loop is actually a 3-nucleotide loop due to base pairing of the first and fifth nucleotides of the CAGUGX sequence (50). B, the most stable predicted secondary structure (ΔG = −6.0 kcal/mol) of the IRE region of bovine m-Acon mRNA. The 6-nucleotide loop (CAGUGC) and 5-base pair stem are apparent. The positions of the initiation codon (AUG), which for m-Acon mRNA is found within the IRE, is also shown. C, the alternative predicted structure (ΔG = −5.4 kcal/mol) of the first 50 nucleotides of the m-Acon IRE region. The positions of the 6-nucleotide loop (CAGUGC) and initiation codon (AUG) are shown. Both of the structures for the m-Acon IRE region were predicted when the first 1400 nucleotides of the RNA were folded (data not shown). For all three structures, the beginning and the end of the highly conserved 28-nucleotide IRE sequence are denoted by arrows.

mediated by IRPs in vivo (13,14). The potential for IRP-mediated changes in m-Acon abundance suggests that a link exists between cellular iron status and mitochondrial utilization and/or export of citrate.

Compared with ferritin mRNAs, IRPs are less effective in repressing m-Acon mRNA translation in a cell-free translation system (14). Recent demonstrations of a differential dose- and time-dependent effect of dietary iron intake on ferritin and m-Acon abundance in liver support the concept of a selected (500 μM) or Desferal (100 μM) for 5 h before harvest. The cell pellet was lysed in 600 μl of buffer B (26), and the lysate was centrifuged at 12,000 × g for 8 min. The resulting supernatant (500 μl) was applied to a 12ml 10–50% linear sucrose gradient in 20 mM HEPES, pH 7.2, 250 mM KCl, 10 mM MgCl_2, 150 μM/μl cycloheximide, and 0.5 μM/μl heparin (26). The gradients were centrifuged at 180,000 × g in a Sorvall TH641 rotor for 135 min at 4°C and then fractionated using an Isco Model UA-6 gradient fractionator. The absorbance at 254 nm was continuously monitored. Twelve 1-ml fractions were collected, and RNA was isolated as follows. Buffer-saturated phenol (1 ml) was mixed with the sample by vortexing. Chloroform/isooamyl alcohol (24:1, 1 ml) was added, and the sample was
vortexed and then centrifuged for 10 min at 13,000 × g. The aqueous phase was collected, and RNA was precipitated with ethanol and NaCl.

RESULTS

Alterations in Iron Status Modulate m-Acon Synthesis—To begin to determine if alterations if m-Acon abundance could be programmed through an IRP-mediated pathway, we examined the extent to which perturbations in cellular iron status influenced the synthesis of m-Acon. HL-60 cells were treated with an iron chelator (Desferal) or an iron source (hemin) for 4 or 24 h and then pulse-labeled with [35S]Met/Cys. A rapid (4 h) and sustained (24 h) effect of cellular iron status was observed on the synthesis rate of m-Acon. After 4 h, the rate of m-Acon synthesis in hemin-treated HL-60 cells exceeded that observed in Desferal-treated cells by 3-fold (average stimulation, 3.3 ± 0.6-fold (mean ± S.E., n = 7)) (Fig. 2, lanes 1 and 2). Synthesis of cytosolic aconitase (IRP1) was not affected by hemin under these conditions (data not shown) (22). This iron-dependent difference in the synthesis rate of m-Acon was further enhanced after 24 h such that m-Acon synthesis in hemin-treated cells was 30-fold greater than in Desferal-treated cells (Fig. 2, lanes 3 and 4). The greater difference in the synthesis rate of m-Acon between hemin- and Desferal-treated cells at 24 h, compared with the 4-h time point, was mainly due to a further decline in m-Acon synthesis in Desferal-treated cells (Fig. 2, compare lanes 2 and 4).

Induction of m-Acon and Ferritin Synthesis by Hemin Does Not Require Ongoing RNA Synthesis—To determine if the action of iron required de novo synthesis of RNA, we asked if the RNA synthesis inhibitor actinomycin D could block the ability of hemin to stimulate m-Acon synthesis. HL-60 cells were pretreated with Desferal (100 μM) for 3 h. Then, actinomycin D (5 μg/ml) was added, and incubation was continued for 1 h. After centrifugation and washing of the cell pellet, the cells were resuspended in medium containing hemin (20 μM) and actinomycin D (5 μg/ml) or in medium containing Desferal (100 μM) and actinomycin D (5 μg/ml). After 2.5 h, the cells were pulse-labeled with [35S]Met/Cys for 30 min. Under these conditions, hemin treatment resulted in a 2.3-fold increase in m-Acon synthesis (Fig. 3, A, lane 2; and C, bar 2) compared with cells treated with Desferal (A, lane 1; and C, bar 1). Preimmune serum failed to immunoprecipitate any protein (Fig. 3A, lane C). In the presence of actinomycin D, the 3-h treatment with hemin resulted in ferritin synthesis being stimulated by 3-fold (Fig. 3, B, lanes 3 and 4; and C, bars 3 and 4).

Hemin Stimulates Polyribosomal Association of m-Acon mRNA—We examined the distribution of m-Acon mRNA between the translationally inactive ribonucleoprotein (RNP) pool and the translationally active polysomal pool to determine if iron regulated m-Acon synthesis translationally. HL-60 cells were treated with hemin (50 μM) or Desferal (100 μM) for 5 h. The RNP and polysome fractions of cellular mRNAs were then separated by sucrose density gradient centrifugation (Fig. 4A), and the distribution of m-Acon mRNA was determined by RNase protection assay. In Desferal-treated cells, 40% of the m-Acon mRNA was in the same region of the gradient as the 40 S ribosomal subunit, and 15% was found in the 80 S region (Fig. 4B, lanes 3 and 5). Compared with cells treated with Desferal, hemin-treated cells exhibited an ~70% decline in the amount of m-Acon mRNA in the 40 S region (Fig. 4, compare lanes 3 in B and C). In Desferal-treated cells, m-Acon mRNA was present at a low level throughout the polysome region of the gradient (Fig. 4B, lanes 7–12). However, compared with Desferal-treated cells, cells exposed to hemin had more m-Acon mRNA in the disome through tetrasome region of polysomes (Fig. 4C, lanes 7–9) and particularly in the heavy (greater than...
a tetrasome) polyosomal region (lanes 10–12). Thus, m-Acon mRNA translation is linked to cellular iron levels.

Iron Stimulates m-Acon and Ferritin Synthesis in Multiple Cell Types—We examined the effect of hemin and Desferal on the synthesis of m-Acon, cytosolic aconitase, and ferritin in three other cell lines. U937, RF2, and FTO2B cells were pretreated with Desferal (100 μM) for 3 h. They were then washed and resuspended in medium containing Desferal (100 μM) (Fig. 5, lanes a, c, and e) or hemin (50 μM) (lanes b, d, and f) for an additional 2.5 h, after which they were pulse-labeled with [35S]Met/Cys. Synthesis of m-Acon was stimulated 2.3 ± 0.6-fold (mean ± S.E., n = 7), 3.6 ± 1.2-fold (n = 3), and 2.9 ± 0.4-fold (n = 8) in U937, RF2, and FTO2B cells, respectively (Fig. 5A). In contrast to what was observed for m-Acon, biosynthesis of cytosolic aconitase was essentially unchanged in U937 and FTO2B cells (Fig. 5B, compare lanes a and e with lanes b and f, respectively), or it decreased somewhat with hemin treatment in RF2 cells (lanes c and d). In hemin-treated U937, RF2, and FTO2B cells, the incorporation of [35S]Met/Cys into cytosolic aconitase was 85 ± 5% (mean ± S.E., n = 2), 42 ± 11% (n = 2), and 110 ± 12% (n = 3), respectively, of that observed in Desferal-treated cells. Similar to what was observed for m-Acon, we also found a higher rate of ferritin synthesis in hemin-treated cells compared with Desferal-treated cells (Fig. 5C). The rate of ferritin synthesis in hemin-treated cells exceeded that measured in Desferal-treated cells by 3–7-fold.3

3 We used the Tricine/SDS system (25) to separate ferritin subunits and found differences in the migration of the subunits in human compared with rat cell lines. In the more commonly used Tris/SDS system, the L- and H-ferritin subunits from some species have been shown to migrate aberrantly (46).
Iron Translationally Regulates m-Acon Synthesis

FIG. 6. Dose-dependent effect of hemin on m-Acon and ferritin synthesis as well as on TIR mRNA level in HL-60 cells. In A–D, HL-60 cells were treated without or with hemin for 4.5 h before pulse-labeling with [35S]Met/Cys for 30 min (see “Materials and Methods”). For immunoprecipitation of m-Acon or ferritin subunits, 20 \( \times \) 10^6 or 5 \( \times \) 10^6 cpm of trichloroacetic acid-precipitable material was used, respectively. Immunoprecipitations of m-Acon (A) and ferritin subunits (C) from control cells (lane a) or cells treated with 1 \( \mu \)M (lane b), 5 \( \mu \)M (lane c), 10 \( \mu \)M (lane d), 20 \( \mu \)M (lane e), or 50 \( \mu \)M (lane f) hemin are shown. The bands on the autoradiogram were quantitated by densitometry of the entire band. The results for m-Acon (B) and L-ferritin (D) are shown. m-Acon synthesis was largely unaffected.

Iron Status Alters the Steady-state Level of m-Acon in FTO2B Cells—We determined the steady-state concentration of m-Acon in FTO2B cells treated with hemin (50 \( \mu \)M) and Desferal (100 \( \mu \)M) for 4 or 16 h. Compared with hemin-treated cells, the amount of m-Acon protein in Desferal-treated cells was 85 \( \pm \) 3.1% (mean \( \pm \) S.E., \( n = 3 \)) and 77 \( \pm \) 1.7% (mean \( \pm \) S.E., \( n = 3 \)) after 4 and 16 h, respectively. These changes in m-Acon abundance are similar to the 50% decrease in m-Acon level in liver mitochondria of rats fed an iron-deficient diet for 3 weeks (12).

Differential Effects of Iron Status on Ferritin and m-Acon Synthesis and TIR mRNA Level—To further evaluate the effect of iron status on m-Acon synthesis in relation to other targets of IRP action, we examined the dose-dependent effect of hemin on m-Acon and ferritin synthesis as well as on the abundance of TIR mRNA. HL-60 cells were cultured for 5 h in the absence of hemin or in the presence of increasing levels of the iron source ranging from 1 to 50 \( \mu \)M. Compared with control cells (Fig. 6, A, lane a; and B, first bar), m-Acon synthesis was stimulated between 2- and 2.4-fold by addition of 10, 20, or 50 \( \mu \)M hemin (A, lanes d–f; and B, fourth through sixth bars), but not when the iron source was added at 1 or 5 \( \mu \)M (A, lanes b and c; and B, second and third bars). Ferritin synthesis was also increased by hemin, but the pattern and extent of stimulation differed from those observed for m-Acon. Synthesis of the more rapidly migrating ferritin subunit, presumably the L-subunit, was slightly stimulated by 1 and 5 \( \mu \)M hemin (Fig. 6, C, lanes b and c; and D, second and third bars) and was strongly stimulated by addition of 10, 20, and 50 \( \mu \)M hemin (Fig. 6, C, lanes d–f; and D, fourth through sixth bars). Compared with untreated cells, the relative rate of L-ferritin synthesis was stimulated 20- and 100-fold in cells treated with 20 and 50 \( \mu \)M hemin for 5 h, respectively. Thus, in HL-60 cells, hemin stimulated the synthesis of both ferritin and m-Acon, but the range of regulation of ferritin synthesis greatly exceeded that of m-Acon.

To further evaluate the potential for differential effects of iron status on the targets of IRP action, we determined the effect of a 5-h hemin treatment on TIR mRNA abundance in HL-60 cells. Compared with control cells, hemin treatment reduced the level of TIR mRNA, but the effect of hemin was not apparent until its concentration reached 20 or 50 \( \mu \)M (Fig. 6, E

Thus, hemin stimulated m-Acon and ferritin synthesis in HL-60 cells and in the other cell types tested, whereas cytosolic aconitase synthesis was largely unaffected.
and 5hemin. The extent of the decrease was 19% in cells incubated with 20 μM hemin and 56% in cells incubated with 50 μM hemin.

**DISCUSSION**

Our observations provide the first direct evidence of a link between cellular iron status and translational regulation of m-Acon gene expression. We observed that perturbations in cellular iron status affected m-Acon biosynthesis in a number of cell lines and that, in HL-60 cells, this was associated with changes in the distribution of m-Acon mRNA between RNP and polyribosomal pools. Furthermore, we demonstrated that the RNA synthesis inhibitor actinomycin D failed to block induction of m-Acon synthesis by hemin in HL-60 cells. These results provide a molecular basis from which to understand previous observations in our laboratory and others that physiological decreases in iron intake lead to a reduction in m-Acon synthesis (12). Considered from a broader perspective, these observations provide a strong indication that the physiological actions of IRPs in mammalian systems extend beyond their classical roles as regulators of iron uptake and storage.

When considered together, the recent demonstrations that the IRE in the 5′-UTR of m-Acon mRNA is functional in an *in vitro* translation system (13, 14) and our observation of iron-dependent changes in translation of m-Acon mRNA in a cellular system provide strong support for the proposal that IRPs regulate m-Acon synthesis *in vivo*. In Desferal-treated cells, we observed that a significant fraction of m-Acon mRNA sedimented in the 40 S region. We believe that most of the 40 S species of m-Acon mRNA represents a translationally inactive or RNP species for the following reasons. First, in extracts of mammalian cells, the RNP form of ferritin mRNAs sediments more slowly than the 40 S ribosomal subunit (29, 30). However, m-Acon mRNA is nearly three times larger than ferritin mRNAs, so it is not surprising that the RNP form of m-Acon mRNA sediments more rapidly than the ferritin RNP. Second, we observed that the abundance of m-Acon mRNA in the 40 S region decreased by ~70% in hemin-treated HL-60 cells. This is consistent with the 40 S species of m-Acon mRNA being a translationally inactive species that is recruited to polyribosomes in response to an increase in intracellular iron concentration.

In this report, we provide clear evidence for differential effects of iron on the synthesis rates of ferritin and m-Acon. The physiological reasons for a more limited regulation of m-Acon synthesis, compared with ferritin, likely reflect the differing biological roles of these proteins. Under normal physiological conditions, inappropriately low expression of m-Acon could unduly impair cellular ATP production and would not be beneficial to cells. In contrast, excessive production of ferritin could create an undesirable deficiency of intracellular iron under normal physiological conditions. Thus, the differential regulation of the expression of m-Acon and ferritin at the translational level appears to provide cells with the means to selectively regulate expression of these targets of IRP action.

Evidence presented here and elsewhere provides a molecular basis through which IRPs can differentially affect the synthesis of proteins encoded by mRNAs containing an IRE in their 5′-untranslated regions. IRPs appear to bind more weakly to the m-Acon IRE compared with the ferritin IRE (13, 15). Furthermore, compared with m-Acon mRNA, IRPs more effectively repress ferritin mRNA translation *in vitro* (14). In agreement with this, we (12) and others (14) demonstrated a differential effect of dietary iron intake on the abundance of ferritin and m-Acon in rat liver, with ferritin expression being more extensively affected. Our current work extends these results by showing that iron can modulate ferritin synthesis over a broader range compared with m-Acon synthesis in three of the four cell lines we examined. Given our demonstration that, like ferritin mRNAs, m-Acon mRNA is translationally regulated, then it appears that IRPs can act selectively to regulate the utilization of IRE-containing mRNAs in intact cells. These differences in translational regulation of IRE-containing mRNAs may reflect differences in structure of the ferritin IRE compared with IREs in m-Acon and other mRNAs (11, 17, 19). In this context, it is of interest that the m-Acon IRE and flanking regions appear to form a stem region that contains a shorter stem and/or altered bulged nucleotide region 5 base pairs 5′ of the CAGUGX loop compared with the ferritin IRE (Fig. 1). Both stem length as well as the size and base composition of the bulged nucleotide region affect the interaction of IRPs with RNA (1, 19, 31–33). Thus, the apparent differences in the ferritin and m-Acon IREs appear to provide a structural basis for enhanced versatility of the IRE/IRP regulatory system.

What additional factors contribute to the difference in translational response of ferritin and m-Acon mRNAs to variations in iron level? First, translation of ferritin mRNAs is efficiently repressed until iron is in excess (26, 34–36). Second, in the absence of IRP action, ferritin mRNAs are efficient competitors for the translation apparatus. In fact, the ferritin IRE as well as other sequences within ferritin mRNA appear to act as positive translational control element(s) (34, 37). Thus, when considered from both perspectives, repressibility and translatability, ferritin mRNAs are efficiently regulated. These aspects of ferritin mRNA function probably reflect the wider range of ferritin expression seen in a number of cell (26) and whole animal (12, 34) systems. In contrast, iron regulation of m-Acon expression occurs over a narrower range. On the basis of this and previous reports (12–14), the more restricted regulation of m-Acon by iron appears to be due to less effective repression of m-Acon synthesis in iron deficiency and not to reduced translatability of the mRNA in the presence of iron excess.

What is the physiological basis of an IRP-mediated link between cellular iron status and m-Acon abundance? A number of proposals have been advanced concerning why m-Acon is an apparent target of IRP action. These include regulation of energy metabolism, mitochondrial oxygen radical production, and modulation of the synthesis of iron-sulfur proteins (12–14). However, it seems likely that changes in these physiological processes would occur in response to chronic changes in iron availability. We have recently shown that dietary iron deficiency can rapidly influence m-Acon abundance in rat liver (51), and as shown here, iron affects m-Acon mRNA translatability. Thus, it seems unlikely that acute changes in cellular iron status would influence the physiological processes noted above. Instead, we hypothesize that IRP-mediated changes in m-Acon abundance may represent a means to modulate the use of citrate in cellular iron metabolism.

There is abundant, albeit circumstantial, evidence for a role of citrate in mammalian iron metabolism. Citrate can bind iron (38, 39) as well as promote iron uptake and release from mammalian cells (40–42). Iron citrate is a major component of the non-transferrin-bound iron pool present in plasma in some forms of iron overload, suggesting the possibility that it may be released by some tissues such as liver (43). Furthermore, citrate influences the binding and/or release of iron to and from ferritin and transferrin (44, 45). Given the known function of IRPs in modulating iron uptake and storage, we feel it reasonable to propose that IRP mediation of m-Acon expression represents a directed effort to modulate the role of citrate in cellular iron trafficking. Future directions will include examination of the extent to which the observed changes in m-Acon...
abundance result in perturbations in mitochondrial utilization and/or export of citrate.

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