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Review

The family of iron responsive RNA structures regulated by changes in cellular iron and oxygen

R. Leipuviene[a, b, +] **and E. C. Theil**[a, b, *]

[a] Center for BioIron at CHORI (Children's Hospital Oakland Research Institute, Children's Hospital & Research Center at Oakland), 5700 Martin Luther King, Jr. Way, Oakland, California 94609 (USA), Fax: +1 510 597 71 31, e-mail: etheil@chori.org
[b] Department of Nutritional Sciences and Toxicology, University of California, Berkeley, California (USA)

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Abstract. The life of aerobes is dependent on iron and oxygen for efficient bioenergetics. Due to potential risks associated with iron/oxygen chemistry, iron acquisition, concentration, storage, utilization, and efflux are tightly regulated in the cell. A central role in regulating iron/oxygen chemistry in animals is played by mRNA translation or turnover via the iron responsive element (IRE)/iron regulatory protein (IRP) system. The IRE family is composed of three-dimensional RNA structures located in 3′ or 5′ untranslated regions of mRNA. To date, there are 11 different IRE mRNAs in the family, regulated through translation initiation or mRNA stability. Iron or oxidant stimuli induce a set of graded responses related to mRNA-specific IRE substructures, indicated by differential responses to iron *in vivo* and binding IRPs *in vitro*. Molecular effects of phosphorylation, iron and oxygen remain to be added to the structural information of the IRE-RNA and IRP repressor in the regulatory complex.

Keywords. Iron, oxygen, mRNA regulation, IRE/IRP system, iron regulatory protein, iron-responsive element.

Introduction

Gene expression in eukaryotic cells depends on mRNA-protein complexes that couple nuclear transcription and cytoplasmic translation. Recent studies of endogenous mammalian ribonucleoproteins revealed that RNA-binding proteins are associated with discrete classes and unique subpopulations of mRNAs in a dynamic manner. A corollary is the sharing of *cis*-active sequences among functionally related genes that serve as targets of specific RNA-binding proteins [1]. These elements mediate coordinated posttranscriptional regulation of otherwise independent mRNA species. To date, at least 14 different mRNA *cis* element groups are known, located in 5′ and 3′ untranslated regions (UTRs) or the coding regions of the transcripts [1]. One of the better characterized representatives of such *cis* regulatory elements in mRNA is the iron-responsive element (IRE).

The IRE mRNA encodes proteins for iron traffic and iron/oxygen mineralization. Iron and oxygen are essential to aerobic life. Proteins with iron-containing cofactors participate in DNA synthesis, oxygen transport, electron transfer, nitrogen fixation, photosynthesis, scavenging of reactive oxygen species and the metabolism of organic toxins. Paradoxically, in the

+ Present address: Fermentas UAB, V. Graiciuno g. 8, LT-02241 Vilnius, Lithuania.
* Corresponding author.

presence of O_2, free iron also promotes the generation of free radicals that damage DNA, lipids, and proteins. Due to its dual effects, the acquisition, storage, utilization, and efflux of cellular iron are tightly regulated. A central regulatory role is played by the IRE/iron regulatory protein (IRP) system during mRNA translation/protein synthesis [2–4]. Engineered mutations in IRP1, IRP2, or spontaneous mutations in the IRE mRNAs disrupt iron metabolism and can lead to iron-related abnormalities, extensively studied in humans and mice [2].

Over the past several years, a number of comprehensive reviews have been published that focus on the combinatorial nature of the IRE/IRP family [5], or on the physiological role of IRPs and iron/oxidant signals [3, 4, 6, 7]. Ligands for the RNA repressors, such as heme or, in the case of one of the IRE repressors, IRP1, the [4Fe-4S] cluster, create a link between IRE regulation, iron, oxygen, or oxidant status. The transcriptional regulation of the genes, like the control of gene expression in response to oxygen by non-heme iron dioxygenases, such as HIF-1α, is outside the scope of this work and is reviewed elsewhere [8–11]. Similarly, iron regulation of IRP activity and turnover has also been extensively reviewed [4, 6, 7, 12]. The ferritin IRE structure has been the most extensively studied, e.g., by crystallography, nuclear magnetic resonance (NMR) spectroscopy and nuclease probing. Many other IRE RNAs have been studied by some, if not all, the same approaches. In this review, we will focus on what is known about the structure and regulatory functions of the members of the IRE mRNA family.

IRE-dependent translational regulation

The posttranscriptional IRE/IRP control system for iron and oxygen metabolism has been observed in vertebrates, invertebrates, lower eukaryotes, and prokaryotes. In plants, however, iron metabolism is regulated at the transcriptional level only and neither IRE RNA nor IRPs have been detected [13–16]. When present, IRE structures regulate protein expression in two completely different ways. One mechanism is by controlling the abundance of mRNAs with rapid-turnover elements through IRE-dependent intervention in degradation; the other mechanism is by regulating rates of translation initiation of a discrete group of messages with IRE structures. The factor common to both mechanisms is IRE binding of a repressor protein, either IRP1 or IRP2. Key sequences and structures are conserved in all IREs. However, the phylogenetic conservation of the IRE in each mRNA is much higher (> 90%) compared to the conservation among IRE family members in different mRNAs of the same organism where sequence conservation is as low as 60%, suggesting additional selectivity for each RNA related to graded responses to iron or other signals [5].

A number of IREs are in the 3′ noncoding region of mRNA, a region that often controls mRNA stability and, thus, rates of protein synthesis. To date, such IRE elements have been identified in four different genes (Table 1), the transferrin receptor 1 (TfR1), divalent metal transporter 1 (DMT1), human cell division cycle protein 14A (CDC14A), and myotonic dystrophy kinase-related Cdc42-binding kinase α (MRCKα). In the TfR1 mRNA, five IRE stem-loops form the IRE stability element [17–20], which is approximately 700 nucleotides (nt); TfR1 mRNA is the only known IRE mRNA with multiple IREs. TfR1 mRNA also contains an AU-rich element in the IRE region of the 3′ untranslated region (UTR) that is common to a large family of short-lived mRNAs involved in growth regulation and cell proliferation [21] and promotes the deadenylation of the mRNA or, as in the case of TfR1, endonucleolytic cleavage [22]. IRP binding, which occurs in iron-deficient cells, increases the half-life of TfR1 mRNA to mediate increased protein production [23] and cellular iron uptake via Fe-transferrin. The effect of the IRE on the stability of DMT1, CDC14A, and MRCKα mRNA is little studied. In DMT1, the IRE occurs in only two of the four possible splice variants [24, 25]. While translational regulation of DMT1 by iron, as with TfR1, is dependent on the IRE in the 3′UTR of the mRNA, sequences inserted by alternate splicing elsewhere are also iron responsive [26, 27]. CDC14A and MRCKα were identified by sequence searches using bioinformatics, followed by cell biology studies. Both genes are involved in protein phosphorylation. CDC14A is a phosphatase that links iron metabolism and the cell cycle [28], while MRCKα is a Cdc42-binding kinase α related to cytoskeletal reorganization and appears to be involved in iron uptake via transferrin [29]. Since phosphorylation can regulate IRP activity [5, 30–32], it is tempting to speculate that in the future, a physiological relationship between the IRP kinase and phosphatase, and the activity of CDC14A and MRCKα proteins, coordinated through IRE-dependent mRNA decay, will be uncovered.

The IREs involved in translation initiation are found in the 5′UTR of the mRNAs. To date, seven different genes are known to contain IRE sequences in the 5′UTR (Table 1), ferritin H (FTH), ferritin L (FTL), mitochondrial aconitase (m-acon), ferroportin1 (FPN), erythroid amino levulinate synthase (eALAS), succinate dehydrogenase (SDH), and hypoxia-inducible factor α (HIF-1α), recently detected

Table 1. IRE containing genes in eukaryotes.

Location in mRNA	Gene name	Protein	Function in iron metabolism	References
Degradation: IRP represses mRNA nucleolytic turnover[1]				
3′UTR	TFRC	transferrin receptor protein 1	iron acquisition	17, 18, 23, 37
	DMT1/DCT1/ SLC11A2/ NRAMP2	divalent metal transporter 1	iron acquisition	24, 26
	CDC14A	dual-specificity protein phosphatase CDC14A	?	28
	CDC42BPA/ MRCKα	myotonic dystrophy kinase-related Cdc42-binding kinase α	?	29
Initiation: IRP represses mRNA translation initiation				
5′UTR	FTH1/FTH/ FTHL6/PIG15	ferritin heavy polypeptide 1	iron concentration and storage	35, 54, 131, 132.
	FTL	ferritin light polypeptide	iron concentration and storage	35, 54, 131, 132.
	ALAS2/ ALASE/ASB	drythroid 5′-aminolevulinic acid synthase	iron utilization	41, 42
	SLC40A1/ FPN1/IREG1/ SLC11A3	ferroportin	iron efflux	43–45
	SdhB/SDH	succinate dehydrogenase subunit B from *Drosophila melanogaster*	iron utilization	46,48
	ACO2/acon	mitochondrial aconitase	iron utilization	42, 46, 133
	EPAS1/HIF-1α	endothelial PAS domain, hypoxia-inducible factor 1-α	transcription changes in response to hypoxia	33

[1] Shown experimentally only for the TfR1 mRNA.

using bioinformatics before translational regulation was revealed [33]. In contrast, the ferritin IRE, the oldest known, was observed by translational regulation [34] before the IRE sequence was identified by comparison of cloned sequences [18, 35–37]; the ferritin IRE remains the most studied. In both ferritin subunit mRNAs, FTH mRNA and FTL mRNA, the IRE is located close to the cap; FT IRE function is lost if the distance is more than 60 nt from the cap [38, 39]. When IRE RNA is bound by IRP under low-iron conditions, the proximity of the IRE/IRP complex to the cap and cap-binding complex eIF4F prevents translation initiation by blocking contact between eIF4F and the 43S ribosomal subunit [40]. The physiological result is inhibition of ferritin protein synthesis, which minimizes sequestering iron in the mineral concentrate during phases of high iron demand. eALAS mRNA translation is also regulated by inhibition of ribosome binding under low-iron conditions (high IRP repression), which reduces rates of heme biosynthesis [41, 42] and incorporation of iron into heme. Another IRE is located in the 5'UTR of the FPN mRNA, which controls the synthesis of the iron efflux protein on the basolateral side of the gut. The FPN IRE binds IRP *in vitro* [41, 42], but further studies are required to determine directly the role of the IRE in FPN synthesis and to understand the apparent tissue-specific contributions to iron regulation of FPN [43–45]. Two genes encoding mitochondrial proteins in the citric acid cycle have 5'UTR-IRE mRNAs: the iron-sulfur protein subunit of SDH in *Drosophila melanogaster,* and aconitase in mammals [42, 46–48], linking iron, oxygen, and energy metabolism.

Similarities of RNA structure among the IRE mRNA family members that confer recognition by IRP are complemented by gene-specific, conserved IRE sequence differences that coincide with a range of iron responses *in vivo* [49] and a range of IRE/IRP stabilities *in vitro* [26, 50]. The IRE context also varies among IRE family members. For example, in FTH and FTL mRNAs, which have large responses to iron signals, the IRE is flanked by sequences that base pair. The base pairs in the ferritin IRE-flanking region influence the magnitude of iron regulatory effects [51, 52]. Another example is m-acon, where the IRE contains the AUG translation start codon [47]. Recent data suggest that the Alzheimer's amyloid precursor protein (APP) mRNA is regulated by a divergent IRE sequence (IRE type II) in the 5'UTR of the transcript [53].

Many of the genes regulated by the IRE/IRP system function in the steps of iron metabolism that are closely interwoven with oxygen metabolism. For example, in ferritin, Fe^{2+} is coupled to O_2 to be stored as a hydrated ferric oxide mineral inside the protein shell [54]. m-acon and SDH participate in the tricarboxylic acid cycle, an important part of the sugar oxidation pathway in aerobic organisms. Moreover, SDH is also part of the respiratory chain where electron flow to molecular oxygen drives ATP synthesis. Another example is HIF-1α, recently shown to

have an IRE mRNA [33]. HIF-1α encodes a transcription factor that controls expression of genes induced by hypoxia and emphasizes the regulatory connections between iron and oxygen homeostasis. Still another IRE is found in the mRNA of one of the heme biosynthetic enzymes catalyzing the rate-limiting step, eALAS. Finally, IRE mRNAs, such as TfR1, FPN, and DMT1, encode proteins that participate in iron traffic crucial for the synthesis of iron and heme proteins.

In specialized cell types and tissues or under abnormal conditions, the 'normal' IRE/IRP mechanisms that coregulate iron uptake (TfR1, DMT1) and iron efflux/storage in opposite directions are bypassed [55–60]. For example, maturing erythroid cells accumulate iron by upregulating both transferrin receptor and ferritin, before heme and globin synthesis are expanded, and use the ferritin iron for heme synthesis [61, 62]. In later phases of erythroid maturation, expression of TfR1 is sustained, expression of ferritin repressed, and synthesis of eALAS increases for efficient hemoglobin production [63]. In addition, in mouse liver, when the putative mitochondrial efflux protein Abcb7 ATPase was functionally deleted, iron accumulated and both TfR1 and ferritin were upregulated [64] in order for TfR1 to increase iron uptake and to create an iron reserve for the entire organism.

The IRE/IRP system is usually described as a feature of animal cells, although IRE-like sequences have been identified in bacteria as well. *Bacillus subtilis* was shown to contain aconitase that could bind to rabbit ferritin IRE and to IRE-like sequences that were located in *B. subtilis* operons encoding the major cytochrome oxidase and an iron uptake system [65]. Moreover, an analogous mRNA/aconitase interaction was linked to efficient sporulation of *B. subtilis* [66]. Finally, *Escherichia coli* aconitases A and B bind to stem-loop structures in the cognate mRNAs, stabilizing them during oxidative stress [67, 68]. In the case of aconitase B, switching between the catalytic and mRNA-binding modes of protein activity was mediated by iron availability [69]. Such RNA-protein interactions in bacteria and animals could indicate an ancient origin for regulation of proteins important in iron and oxygen metabolism that was lost in plants after plastid infection [15], or may reflect the independent evolution of mRNA regulation for analogous proteins in animals and contemporary bacteria.

IRE structure

Stabilities of IRE/IRP complexes are in the picomolar range, much more stable than typical enzyme-substrate complexes. Sequence comparisons of different animal IREs and 'footprinting' reveal that the IRP-binding site is about 30 nt long [26, 50, 51]. In spite of the fact that all IRE sequences bind IRPs, and have high phylogenetic conservation for each mRNA (> 95%), differences among the IREs in a single organism range from 36 to 85% [5, 70]. Secondary and tertiary structures of IRE RNAs have been determined by mutagenesis, nuclease and chemical probing *in vitro* and *in vivo*, as well as by solution NMR [71–81].

The conserved structural features of all members of the IRE family are a double-stranded RNA helix of 9–10 base pairs with a terminal hexaloop, -C_{14}AGUGU/C_{19}-, and an unpaired C_8 residue in the helix creating a five-base-pair upper stem and a lower stem of variable length (Fig. 1). In the terminal loop, C_{14} and G_{18} are base paired and create a pseudotriloop of -$A_{15}G_{16}U_{17}$- [71, 74, 76, 77, 82–84]. Sequence and base pairing around the unpaired C_8 residue varies among different IREs, separating IRE mRNAs into two groups, one with an isolated, unpaired C, and the other with an internal loop constructed from the unpaired C_8, an unpaired base at position 6, 5′UTR to the unpaired C, and an additional paired base between them (Fig. 1) [19, 77, 79]. Helix distortion around the conserved C_8 residue plays an important role in selective repressor binding, especially for IRP2 [19, 26, 78]. For example, the ferritin IRE and the TfR1 IRE, which in the native context of four other IREs has a large helix loop like the ferritin IRE, form complexes with IRP1 and IRP2 that have comparable stability [19]. In contrast, the IREs from eALAS, DMT1, FPN, m-acon mRNAs, or an isolated, single IRE from TfR1 mRNA, all contain a single C bulge and form complexes with IRP2 that are much less stable than complexes with IRP1 [26, 50]. Several of the newly discovered IRE mRNAs, e.g., HIF-1α [50, 78], have a predicted structure within bulge/loops analogous to the ferritin IRE, but structural information is incomplete. The importance of helix structure around the unpaired C_8 is illustrated by the effect of deleting a single unpaired U_6, characteristic of ferritin IRE RNA, which decreases the stability of the IRP1 and IRP2 complexes significantly, with the consequence of decreasing translational repression [50, 78].

Variation among members of the IRE-RNA family also includes IRE context. The influence of IRE context on IRP repressor binding is particularly clear for TfR1 mRNA [19, 20]. Even though the predicted secondary structure of the TfR1 IRE is a single, unpaired C bulge in the helix, in the native context of multiple IREs and connecting linkers, an internal loop was detected by chemical nuclease probes. In addition, IRP1 and IRP2 binding were equal, con-

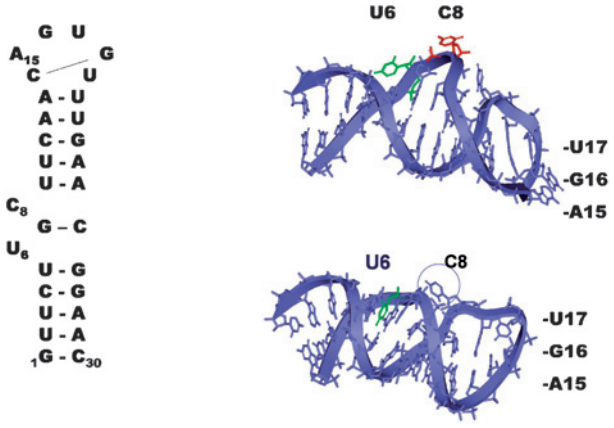

Figure 1. Comparison of a ferritin IRE RNA in free solution and complexed to a protein repressor IRP1. Secondary structure of the IRE (left) shown in the NMR, and crystal structures (right). The 30 nt long IRE RNA used is the ferritin H (frog) with the ferritin-specific unpaired U6 that increases IRP2/RNA stability [76]. The crystal structure of an IRE RNA is shown (top right) complexed in the IRP1 complex using data from Walden et al. [84] pdb file2IPY. The NMR solution structure of the free IRE RNA is shown (bottom right) to facilitate comparison of changes in RNA conformation induced by protein binding. A15, which is stacked over the helix in the free RNA (NMR), is flipped out in the IRP1 complex with U17 capping the base stack of the helix; the unpaired C8 is disordered (blue circle) in the free RNA and could be modeled in any position, flipping in and out of the stack. By contrast, C8 (red) is in a pocket of IRP1 in the protein/RNA complex (crystal). U6 (green), which is ferritin IRE specific and important in IRP binding [65], is ordered both in free RNA and in the crystal of the IRP repressor complex, but changes conformation.

trasting with a single Tfr1 IRE-RNA [19]. IRE flanking sequences serve an important role in ferritin mRNA as well, where, unlike other IRE-family members, the flanking sequences are complementary and base pair to elongate the IRE regulatory region from the 30 nt to a 55 nt structure. The length of the base-paired flanking region differs among different ferritin mRNAs and is roughly proportional to the distance between the cap and the IRE. Together, the IRE plus flanking base pairs place the IRE regulatory structure 10–20 nt from the m^7G cap, a distance associated with effective translation regulation [51, 52, 71]. Upon IRP binding to ferritin IRE, the flanking regions increase their helicity (RNAse V1 accessibility), indicating structural integration between IRE and the flanking region helix [51]. In addition, a set of three base pairs is embedded within the flanking region helix and is phylogenetically conserved in higher vertebrates; when the flanking region base pairs are mutated, IRP repression is decreased [52]. The sequence of the triplet of base pairs differs between FTL and FTH mRNAs, indicating a potential for differential regulation of the two subunits of ferritin, which are expressed in tissue-specific concentrations [85], at ratios which differ markedly from the FTH-FTL protein ratio [86].

Structural and functional variations among IRE members defined by RNA solution studies, complemented with *in vitro* protein binding, translation *in vitro*, translation regulation in tissue culture, and in whole animals [49–51, 71–83, 87, 88], lacked structural information on the RNA:protein complex. Recently, FTH IRE-RNA was successfully crystallized in a complex with IRP1 [89] and the X-ray crystal structure solved [84]. The results show that the basis for the high selectivity of IRE RNA/IRP protein interaction resides in two spatially separated binding sites, at the terminal loop and unpaired C in the helix, indicated as important by the earlier studies. Each site involves a very large number of contacts between the RNA and protein. The presence of two RNA-binding sites in a single protein, as for IRP1, is relatively rare, except among tRNA synthetases [90].

A number of changes occurred in the RNA structure complexed to the IRP1 repressor protein. First, terminal loop residues A_{15} and G_{16} were extruded from the IRE molecule and extended into a large protein cavity, with U_{17} completing the helix stack (Fig. 1). In solution, by contrast, A_{15} was stacked over the C_{13}-G_{18} base pair, and G_{16} with U_{17} were disordered. At the second RNA-binding site, the unpaired C_8 in the helix, which was disordered in free RNA in solution [76, 77], was extended completely away from the stem and inserted into a protein pocket, while unpaired U_6, which was stacked in an RNA cavity of the free RNA, was flipped out toward the protein surface in the IRP1 complex (Fig. 1).

Differences between terminal loop and C bulge solution conformations and the conformation adopted in the IRE/IRP complex indicate a mutually induced fit mechanism of binding [84]. In the protein/RNA crystal, IRP1 is bound to a convex surface of the IRE-RNA, assuming an L shape, and leaving the concave surface of the RNA exposed, possibly for long-range interactions with RNA or protein.

The importance of correct IRE stem-loop sequence and structure is exemplified in the human genetic disorder hereditary hyperferritinemia-cataract syndrome, which arises from point mutations or deletions within the IRE or flanking regions in the FTL 5'UTR. The result of the IRE mutations is increased rates of FTL translation and the accumulation of excess protein [91, 92]. The mutations clustered in the terminal loop and within the helix loop of FTL IRE affect IRP repressor binding. Stronger clinical symptoms, i.e., higher serum ferritin levels and early occurrence of FTL crystals in the lens [92, 93], were correlated with the quantitative variations in IRE/IRP complex stabilities. If similarly altered IRE regulation

could occur in patients with iron overload from transfusion therapies of hemoglobinapathies, such as sickle cell disease and thalassemia, an increase in ferritin synthesis could be beneficial. The development of small molecules that selectively disrupt ferritin-IRE/IRP binding, like those recently described [94], have such therapeutic potential.

IRP1 and IRP2 proteins

The IRE RNA proteins, IRP1 and IRP2, that repress either mRNA translation (IRE 5′UTR) or mRNA degradation (IRE 3′UTR), are homologous to aconitases, which are found in a wide range of organisms, including bacteria [65, 95, 96], plants [97, 98], vertebrates, and invertebrates [99–101]. In animal models with ablated IRP1 or IRP2, *Irp2*$^{-/-}$ mice have neurological and hematopoietic defects [102–104] that become more pronounced in *Irp1*$^{+/-}$ *Irp2*$^{-/-}$ double mutants [105]. IRP1 deletion causes no detectable changes, indicating that IRP2 can functionally substitute for IRP1, but the detectable, albeit milder phenotype with *Irp2*$^{-/-}$indicates that IRP1 can only partially substitute for IRP2. Complete loss of both proteins is lethal [106]. Oxygen affects IRP function, exemplified by changes in IRP2 activity when oxygen levels change in cultured cells [107, 108], and destabilizes the FeS form of IRP1 (c-acon) as discussed below. IRP protein regulation has been extensively reviewed [3, 6, 7] and is discussed only briefly here, except as related to RNA interactions.

IRP1 is a bifunctional protein with cytosolic aconitase (c-acon) and RNA-binding activities that are mutually exclusive [109–113]. Aconitase activity in c-acon/IRP1 requires the presence of an [4Fe-4S] cluster, while the apo-protein binds to RNA. IRP2, unlike IRP1, which shares 65% sequence identity (75% similarity), lacks the ligands to form an [Fe-S] cluster, and thus lacks the potential for aconitase activity [114, 115]. The predicted structures of IRP1 and IRP2 are similar, based on ‘generic’ aconitase structures, with three domains, and a linked fourth domain; IRP2 has an additional 73 amino-acid insertion near the N terminus. Confirmation of the structure prediction for an [Fe-S] complex of IRP1 has been recently obtained from a crystal structure of [Fe-S]-IRP1 (c-aconitase) [116]. IRPs are ubiquitously expressed, though under physiological conditions IRP2 expression predominates over IRP1 expression in most tissues, and when IRP1 is abundant, it is mainly in the aconitase form [19, 102, 117].

A number of mechanisms for IRP turnover or regulation are shared by IRP1 and IRP2. For example, recent evidence shows that an [Fe-S] cluster-free IRP1 undergoes iron-dependent degradation as for IRP2 [6 31, 32, 115, 118–121]. The degradation of both IRP repressors is also stimulated by heme [122,123] that, in addition to serving as an iron source, can bind IRPs directly to target degradation [124]. Furthermore, phosphorylation regulates IRP1 and IRP2. IRP1 is phosphorylated preferentially in the apo-protein form [125], which blocks formation of c-acon and enhances accumulation of the RNA-binding form; phosphorylation of S138 also destabilizes [Fe-S] clusters [126], which can increase the IRP1 pool size. In the case of IRP2, phosphorylation increases IRP2 binding to RNA two-fold [30]. The fact that protein kinases can modulate IRP1 and IRP2 activities links IRE/IRP regulation to signals that control many fundamental aspects of cell biology. A recent addition to the IRE family, MRCKα, is a serine/threonine kinase [29], and another new IRE family member is CDC14A, a cell cycle phosphatase [28]. Such IRE mRNAs emphasize the connection of the IRE/IRP regulatory family to other phophorylation/dephosphorylation systems. It may be that the ‘iron signal’ causes modification of surface and trafficking of proteins in analogy to other signal transduction pathways.

Reactive oxygen species $O_2^{\bullet -}$ and H_2O_2, or reactive nitrogen species such as NO^- or $ONOO^-$, disrupt the [Fe-S] cluster in c-acon, providing another set of signals that change the distribution of the IRP1 peptide between catalytically active and RNA-binding conformations. The distribution change has often been observed at the high dioxygen levels commonly used with cultured mammalian cells [reviewed in 6]. At physiological oxygen tensions (~3%), where c-acon is stable, and IRP2 RNA-binding activity increases relative to the higher dioxygen concentrations used in tissue culture [102, 108], IRP2 will be the dominant regulator [19, 102]. IRP1 expression appears to dominate in conditions where [Fe-S] cluster synthesis/stability is inhibited, such as extreme iron deficiency, e.g., treatment with iron chelators, defective [Fe-S] cluster assembly/repair machinery, or in the human neurodegenerative disease, Friederich’s ataxia [127, 128].

Many models of the coincident changes in c-acon and IRP1 RNA-binding activities include a direct conversion of the [Fe-S]/protein complex (c-acon) and the RNA/protein complex. Recent crystal structures of both [Fe-S]/c-acon and IRE/IRP complexes suggest that the direct conversion from one protein conformation to another may be only one of several appropriate models, since comparisons of IRP1 protein in two complexes, FeS and RNA bound, reveal major structural differences in the protein (Fig. 2). The wide distribution of RNA contacts along the linear IRP1 polypeptide explains the absence of RNA

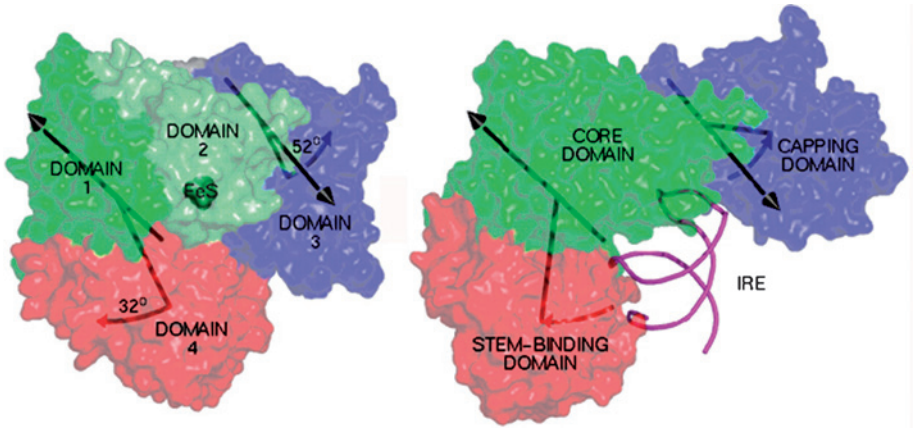

Figure 2. Differences in domain positions between c-acon and the IRP1/IRE RNA complex. The figure is from Walden et al. [84]. Domains 1 (dark green) and 2 of c-aconitase (light green) (left) correspond to the core in the IRP1/IRE complex (fused yellow and green sections) (right). The two molecules are displayed in the same orientation to show the displacements of domains 3 (blue/purple) and 4 (red). If the c-acon and IRP1 are in 'equilibrium' as proposed in some models, domains 3 and 4 might pivot in the direction shown by the arrows; arcs show the possible motion of the centers of mass of domains 3 and 4 in the 'switch' model. In the IRP1 complexed with the ferritin IRE, there is no long interface between the red and blue protein domains of c-acon, and the contact between the green and blue domain of c-acon is absent. Such differences suggest an alternative model to the "switch" model from c-aconitase to IRP1, supported by the disordered structure of the apo-IRP1 in solution [129], where the [Fe-4S] and RNA structures of IRP1 may reflect alternate folding pathways of the polypeptide.

binding by IRP1 subdomains or short peptides. In the RNA/protein complex, two of the domains in c-acon (1 and 2) are folded into a single domain in IRP1. Two other contiguous, c-acon domains (3 and 4) are separated by more than 30 Å in the RNA complex, with the elimination of any contacting surfaces (Fig. 2) [84, 116]. Moreover, many of the ligands of the active site in c-acon are in different conformations in the RNA complex, becoming part of the cavity which contributes numerous bonds to the extruded A_{15} and G_{16} bases of the IRE-RNA terminal loop.

Crystals of the apo-IRP1 protein, without either the IRE-RNA or [Fe-S] cluster, have been elusive, making the resolution of the structural relationships among polypeptide, IRP1 and c-acon conformations difficult. However, circular dichroism and small angle X-ray scattering demonstrate that IRP1 without either [FeS] cluster or IRE RNA is a less organized protein structure than in either c-acon or the RNA/protein complex [129, 130]. Such data emphasize the importance of considering, as an alternative to the model of a direct equilibrium between the [Fe-S]/c-acon and IRP1/IRE complexes, alternate folding pathways for the less organized apo-IRP1 that are induced by either the [Fe-S] cluster or IRE RNA.

Summary and perspective

Synthesis of key enzymes and transporters of iron and oxygen is regulated by the interplay between non-coding mRNA structures and repressor proteins that either inhibits translation or mRNA degradation. Diversity of IRE substructures among IRE mRNA family members, coupled with tissue-specific, or even cell-type-specific expression of IRP repressor proteins, coupled with levels of phosphorylation, and a variety of cellular signals such as iron, oxygen, and cytokines, create a combinatorial array of interactions [5]. The different combinations of RNAs and proteins with or without phosphorylation generate the observed quantitative range of mRNA-specific signal responses within each cell [49]. The IRE RNA structural basis for the graded responses appears to relate, at least in part, to RNA structural variations in the IRE helix bulge, to IRE base pair sequences, and to IRE context. Numerous bonds between the protein and ferritin IRE RNA occur at both the RNA terminal loop and the mid-helix bulge contacting IRP1 protein sites that are separated by 30 Å. Potentially, alternative IRP1 configurations exist that bind the single unpaired C site compared to the bulge loop in the recent crystal structure. The structural differences between IRP1 and IRP2/IRE RNA complexes are questions for the future. Another unsolved problem is how previously repressed mRNAs reenter the translation cycle, and how or whether IRP is released from mRNA or moves out of the way for incoming ribosomes. Since in a ferritin IRE/IRP1 crystal structure, a surface of the RNA is exposed, other protein- or RNA-binding partners may contribute to the observed translational regulation. Such novel macromolecular interactions could explain why under some specific conditions, like differentiating

erythroblasts, or heavily iron-loaded hepatocytes, the 'standard' IRE/IRP regulation is bypassed.

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1 Keene, J. D. and Tenenbaum, S. A. (2002) Eukaryotic mRNPs may represent posttranscriptional operons. Mol. Cell 9, 1161 – 1167.
2 Andrews, N. C. and Schmidt, P. J. (2006) Iron homeostasis. Annu. Rev. Physiol. 69, 69 – 85.
3 Hentze, M. W., Muckenthaler, M. U. and Andrews, N. C. (2004) Balancing acts: molecular control of mammalian iron metabolism. Cell 117, 285 – 297.
4 Pantopoulos, K. (2004) Iron metabolism and the IRE/IRP regulatory system: an update. Ann. N. Y. Acad. Sci. 1012, 1 – 13.
5 Theil, E. C. and Eisenstein, R. S. (2000) Combinatorial mRNA regulation: iron regulatory proteins and iso-iron-responsive elements (Iso-IREs). J. Biol. Chem. 275, 40659 – 40662.
6 Wallander, M. L., Leibold, E. A. and Eisenstein, R. S. (2006) Molecular control of vertebrate iron homeostasis by iron regulatory proteins. Biochim. Biophys. Acta 1763, 668 – 689.
7 Rouault, T. A. (2006) The role of iron regulatory proteins in mammalian iron homeostasis and disease. Nat. Chem. Biol. 2, 406 – 414.
8 Hintze, K. J. and Theil, E. C. (2006) Cellular regulation and molecular interactions of the ferritins. Cell. Mol. Life Sci. 63, 591 – 600.
9 Theil, E. C. (2007) Coordinating responses to iron and oxygen stress with DNA and mRNA promoters: The ferritin story. Biometals 20, 513 – 521.
10 Ozer, A. and Bruick, R. K. (2007) Non-heme dioxygenases: cellular sensors and regulators jelly rolled into one? Nat. Chem. Biol. 3, 144 – 153.
11 Schofield, C. J. and Ratcliffe, P. J. (2004) Oxygen sensing by HIF hydroxylases. Nat. Rev. Mol. Cell Biol. 5, 343 – 354.
12 Dunn, L. L., Rahmanto, Y. S. and Richardson, D. R. (2007) Iron uptake and metabolism in the new millennium. Trends Cell Biol. 17, 93 – 100.
13 Rothenberger, S., Mullner, E. W. and Kuhn, L. C. (1990) The mRNA-binding protein which controls ferritin and transferrin receptor expression is conserved during evolution. Nucleic Acids Res. 18, 1175 – 1179.
14 Wei, J. and Theil, E. C. (2000) Identification and characterization of the iron regulatory element in the ferritin gene of a plant (soybean). J. Biol. Chem. 275, 17488 – 17493.
15 Proudhon, D., Wei, J., Briat, J. and Theil, E. C. (1996) Ferritin gene organization: differences between plants and animals suggest possible kingdom-specific selective constraints. J. Mol. Evol. 42, 325 – 336.
16 Briat, J. F. and Lobreaux, S. (1998) Iron storage and ferritin in plants. Met. Ions Biol. Syst. 35, 563 – 584.
17 Koeller, D. M., Casey, J. L., Hentze, M. W., Gerhardt, E. M., Chan, L. N., Klausner, R. D. and Harford, J. B. (1989) A cytosolic protein binds to structural elements within the iron regulatory region of the transferrin receptor mRNA. Proc. Natl. Acad. Sci. U. S.A. 86, 3574 – 3578.
18 Casey, J. L., Hentze, M. W., Koeller, D. M., Caughman, S. W., Rouault, T. A., Klausner, R. D. and Harford, J. B. (1988) Iron-responsive elements: regulatory RNA sequences that control mRNA levels and translation. Science 240, 924 – 928.
19 Erlitzki, R., Long, J. C. and Theil, E. C. (2002) Multiple, conserved iron-responsive elements in the 3'-untranslated region of transferrin receptor mRNA enhance binding of iron regulatory protein 2. J. Biol. Chem. 277, 42579 – 42587.
20 Schlegl, J., Gegout, V., Schlager, B., Hentze, M. W., Westhof, E., Ehresmann, C., Ehresmann, B. and Romby, P. (1997) Probing the structure of the regulatory region of human transferrin receptor messenger RNA and its interaction with iron regulatory protein-1. RNA 3, 1159 – 1172.
21 Brennan, C. M. and Steitz, J. A. (2001) HuR and mRNA stability. Cell. Mol. Life Sci. 58, 266 – 277.
22 Binder, R., Horowitz, J. A., Basilion, J. P., Koeller, D. M., Klausner, R. D. and Harford, J. B. (1994) Evidence that the pathway of transferrin receptor mRNA degradation involves an endonucleolytic cleavage within the 3' UTR and does not involve poly(A) tail shortening. EMBO J. 13, 1969 – 1980.
23 Mullner, E. W. and Kuhn, L. C. (1988) A region in the 3' untranslated region mediates iron dependent regulation of transferrin receptor mRNA stability in the cytoplasm. Cell 53, 815 – 825.
24 Gunshin, H., Mackenzie, B., Berger, U. V., Gunshin, Y., Romero, M. F., Boron, W. F., Nussberger, S., Gollan, J. L. and Hediger, M. A. (1997) Cloning and characterization of a mammalian proton-coupled metal-ion transporter. Nature 388, 482 – 488.
25 Lee, P. L., Gelbart, T., West, C., Halloran, C. and Beutler, E. (1998) The human Nramp2 gene: characterization of the gene structure, alternative splicing, promoter region and polymorphisms. Blood Cells Mol. Dis. 24, 199 – 215.
26 Gunshin, H., Allerson, C. R., Polycarpou-Schwarz, M., Rofts, A., Rogers, J. T., Kishi, F., Hentze, M. W., Rouault, T. A., Andrews, N. C. and Hediger, M. A. (2001) Iron-dependent regulation of the divalent metal ion transporter. FEBS Lett., 309 – 316.
27 Hubert, N. and Hentze, M. W. (2002) Previously uncharacterized isoforms of divalent metal transporter (DMT)-1: implications for regulation and cellular function. Proc. Natl. Acad. Sci. U. S.A. 99, 12345 – 12350.
28 Sanchez, M., Galy, B., Dandekar, T., Bengert, P., Vainshtein, Y., Stolte, J., Muckenthaler, M. U. and Hentze, M. W. (2006) Iron regulation and the cell cycle: identification of an iron-responsive element in the 3'-untranslated region of human cell division cycle 14A mRNA by a refined microarray-based screening strategy. J. Biol. Chem. 281, 22865 – 22874.
29 Cmejla, R., Petrak, J. and Cmejlova, J. (2006) A novel iron responsive element in the 3'UTR of human MRCKalpha. Biochem. Biophys. Res. Commun. 341, 158 – 166.
30 Schalinske, K. L. and Eisenstein, R. S. (1996) Phosphorylation and activation of both iron regulatory proteins 1 and 2 in HL-60 cells. J. Biol. Chem. 271, 7168 – 7176.
31 Fillebeen, C., Chahine, D., Caltagirone, A., Segal, P. and Pantopoulos, K. (2003) A phosphomimetic mutation at Ser-138 renders iron regulatory protein 1 sensitive to iron-dependent degradation. Mol. Cell Biol. 23, 6973 – 6981.
32 Clarke, S. L., Vasanthakumar, A., Anderson, S. A., Pondarre, C., Koh, C. M., Deck, K. M., Pitula, J. S., Epstein, C. J., Fleming, M. D. and Eisenstein, R. S. (2006) Iron-responsive degradation of iron-regulatory protein 1 does not require the Fe-S cluster. EMBO J. 25, 544 – 553.
33 Sanchez, M., Galy, B., Muckenthaler, M. U. and Hentze, M. W. (2007) Iron-regulatory proteins limit hypoxia-inducible factor-2alpha expression in iron deficiency. Nat. Struct. Mol. Biol. 14, 420 – 426.
34 Shull, G. E. and Theil, E. C. (1982) Translational control of ferritin synthesis by iron in embryonic reticulocytes of the bullfrog. J. Biol. Chem. 257, 14187 – 14191.
35 Leibold, E. A. and Munro, H. N. (1987) Characterization and evolution of the expressed rat ferritin light subunit gene and its pseudogene family. Conservation of sequences within noncoding regions of ferritin genes. J. Biol. Chem. 262, 7335 – 7341.
36 Leibold, E. A. and Munro, H. N. (1988) Cytoplasmic protein binds *in vitro* to a highly conserved sequence in the 5'

untranslated region of ferritin heavy- and light-subunit mRNAs. Proc. Natl. Acad. Sci. U. S.A. 85, 2171 – 2175.
37 Casey, J. L., Di Jeso, B., Rao, K., Klausner, R. D. and Harford, J. B. (1988) Two genetic loci participate in the regulation by iron of the gene for the human transferrin receptor. Proc. Natl. Acad. Sci. U. S.A. 85, 1787 – 1791.
38 Dix, D. J., Lin, P. N., Kimata, Y. and Theil, E. C. (1992) The iron regulatory region of ferritin mRNA is also a positive control element for iron-independent translation. Biochemistry 31, 2818 – 2822.
39 Goossen, B. and Hentze, M. W. (1992) Position is the critical determinant for function of iron-responsive elements as translational regulators. Mol. Cell Biol. 12, 1959 – 1966.
40 Muckenthaler, M., Gray, N. K. and Hentze, M. W. (1998) IRP-1 Binding to ferritin mRNA prevents the recruitment of the small ribosomal subunit by the cap-binding complex eIF4F. Mol. Cell 2, 383 – 388.
41 Cox, T. C., Bawden, M. J., Martin, A. and May, B. K. (1991) Human erythroid 5-aminolevulinate synthase: promoter analysis and identification of an iron-responsive element in the mRNA. EMBO J. 10, 1891 – 1902.
42 Dandekar, T., Stripecke, R., Gray, N. K., Goossen, B., Constable, A., Johansson, H. E. and Hentze, M. W. (1991) Identification of a novel iron-responsive element in murine and human erythroid δ-aminolevulinic acid synthase mRNA. EMBO J. 10, 1903 – 1909.
43 Donovan, A., Brownlie, A., Zhou, Y., Shepard, J., Pratt, S. J., Moynihan, J., Paw, B. H., Drejer, A., Barut, B., Zapata, A., Law, T. C., Brugnara, C., Lux, S. E., Pinkus, G. S., Pinkus, J. L., Kingsley, P. D., Palis, J., Fleming, M. D., Andrews, N. C. and Zon, L. I. (2000) Positional cloning of zebrafish ferroportin1 identifies a conserved vertebrate iron exporter. Nature 403, 776 – 781.
44 McKie, A. T., Marciani, P., Rolfs, A., Brennan, K., Wehr, K., Barrow, D., Miret, S., Bomford, A., Peters, T. J., Farzaneh, F., Hediger, M. A., Hentze, M. W. and Simpson, R. J. (2000) A novel duodenal iron-regulated transporter, IREG1, implicated in the basolateral transfer of iron to the circulation. Mol. Cell 5, 299 – 309.
45 Lymboussaki, A., Pignatti, E., Montosi, G., Garuti, C., Haile, D. J. and Pietrangelo, A. (2003) The role of the iron responsive element in the control of ferroportin1/IREG1/MTP1 gene expression. J. Hepatol. 39, 710 – 715.
46 Gray, N. K., Pantopoulos, K., Dandekar, T., Ackrell, B. A. and Hentze, M. W. (1996) Translational regulation of mammalian and Drosophila citric acid cycle enzymes via iron-responsive elements. Proc. Natl. Acad. Sci. U. S.A. 93, 4925 – 4930.
47 Kim, H. Y., LaVaute, T., Iwai, K., Klausner, R. D. and Rouault, T. A. (1996) Identification of a conserved and functional iron-responsive element in the 5'-untranslated region of mammalian mitochondrial aconitase. J. Biol. Chem. 271, 24226 – 24230.
48 Kohler, S. A., Henderson, B. R. and Kuhn, L. C. (1995) Succinate dehydrogenase b mRNA of *Drosophila melanogaster* has a functional iron-responsive element in its 5'-untranslated region. J. Biol. Chem. 270, 30781 – 30786.
49 Chen, O. S., Schalinske, K. L. and Eisenstein, R. S. (1997) Dietary iron intake modulates the activity of iron regulatory proteins and the abundance of ferritin and mitochondrial aconitase in rat liver. J. Nutr. 127, 238 – 248.
50 Ke, Y., Wu, J., Leibold, E. A., Walden, W. E. and Theil, E. C. (1998) Loops and bulge/loops in iron-responsive element isoforms influence iron regulatory protein binding: fine-tuning of mRNA regulation? J. Biol. Chem. 273, 23637 – 23640.
51 Harrell, C. M., McKenzie, A. R., Patino, M. M., Walden, W. E. and Theil, E. C. (1991) Ferritin mRNA: interactions of iron regulatory element with translational regulator protein P-90 and the effect on base-paired flanking regions. Proc. Natl. Acad. Sci. U. S.A. 88, 4166 – 4170.
52 Dix, D. J., Lin, P.-N., McKenzie, A. R., Walden, W. E. and Theil, E. C. (1993) The influence of the base-paired flanking region (FL) on structure and function of the ferritin mRNA iron regulatory element (IRE). J. Mol. Biol. 231, 230 – 240.
53 Rogers, J. T., Randall, J. D., Cahill, C. M., Eder, P. S., Huang, X., Gunshin, H., Leiter, L., McPhee, J., Sarang, S. S., Utsuki, T., Greig, N. H., Lahiri, D. K., Tanzi, R. E., Bush, A. I., Giordano, T. and Gullans, S. R. (2002) An iron-responsive element type II in the 5'-untranslated region of the Alzheimer's amyloid precursor protein transcript. J. Biol. Chem. 277, 45518 – 45528.
54 Liu, X. and Theil, E. C. (2005) Ferritins: dynamic management of biological iron and oxygen chemistry. Acc. Chem. Res. 38, 167 – 175.
55 Cairo, G. and Pietrangelo, A. (1994) Transferrin receptor gene expression during rat liver regeneration. Evidence for post-transcriptional regulation by iron regulatory factorB, a second iron-responsive element-binding protein. J. Biol. Chem. 269, 6405 – 6409.
56 Cairo, G., Tacchini, L. and Pietrangelo, A. (1998) Lack of coordinate control of ferritin and transferrin receptor expression during rat liver regeneration. Hepatology 28, 173 – 178.
57 Testa, U., Kuhn, L., Petrini, M., Quaranta, M. T., Pelosi, E. and Peschle, C. (1991) Differential regulation of iron regulatory element-binding protein(s) in cell extracts of activated lymphocytes versus monocytes-macrophages. J. Biol. Chem. 266, 13925 – 13930.
58 Testa, U., Petrini, M., Quaranta, M. T., Pelosi-Testa, E., Mastroberardino, G., Camagna, A., Boccoli, G., Sargiacomo, M., Isacchi, G., Cozzi, A., Arosio, P. and Peschlet, C. (1989) Iron up-modulates the expression of transferrin receptors during monocyte-macrophage maturation. J. Biol. Chem. 264, 13181 – 13187.
59 Chan, R. Y., Seiser, C., Schulman, H. M., Kuhn, L. C. and Ponka, P. (1994) Regulation of transferrin receptor mRNA expression. Distinct regulatory features in erythroid cells. Eur. J. Biochem. 220, 683 – 692.
60 Busfield, S. J., Tilbrook, P. A., Callus, B. A., Spadaccini, A., Kuhn, L. and Klinken, S. P. (1997) Complex regulation of transferrin receptors during erythropoietin-induced differentiation of J2E erythroid cells – elevated transcription and mRNA stabilisation produce only a modest rise in protein content. Eur. J. Biochem. 249, 77 – 84.
61 Peto, T. E., Rutherford, T. R., Thompson, J. L. and Weatherall, D. J. (1983) Iron metabolism in murine erythroleukaemic cells. Br. J. Haematol. 54, 623 – 631.
62 Vaisman, B., Fibach, E. and Konijn, A. M. (1997) Utilization of intracellular ferritin iron for hemoglobin synthesis in developing human erythroid precursors. Blood 90, 831 – 838.
63 Schranzhofer, M., Schifrer, M., Cabrera, J. A., Kopp, S., Chiba, P., Beug, H. and Mullner, E. W. (2006) Remodeling the regulation of iron metabolism during erythroid differentiation to ensure efficient heme biosynthesis. Blood 107, 4159 – 4167.
64 Pondarre, C., Antiochos, B. B., Campagna, D. R., Clarke, S. L., Greer, E. L., Deck, K. M., McDonald, A., Han, A. P., Medlock, A., Kutok, J. L., Anderson, S. A., Eisenstein, R. S. and Fleming, M. D. (2006) The mitochondrial ATP-binding cassette transporter Abcb7 is essential in mice and participates in cytosolic iron-sulfur cluster biogenesis. Hum. Mol. Genet. 15, 953 – 964.
65 Alen, C. and Sonenshein, A. L. (1999) *Bacillus subtilis* aconitase is an RNA-binding protein. Proc. Natl. Acad. Sci. U. S.A. 96, 10412 – 10417.
66 Serio, A. W., Pechter, K. B. and Sonenshein, A. L. (2006) *Bacillus subtilis* aconitase is required for efficient late-sporulation gene expression. J. Bacteriol. 188, 6396 – 6405.
67 Tang, Y. and Guest, J. R. (1999) Direct evidence for mRNA binding and post-transcriptional regulation by *Escherichia coli* aconitases. Microbiology 145 (Pt 11), 3069 – 3079.

68 Tang, Y., Quail, M. A., Artymiuk, P. J., Guest, J. R. and Green, J. (2002) *Escherichia coli* aconitases and oxidative stress: post-transcriptional regulation of sodA expression. Microbiology 148, 1027 – 1037.
69 Tang, Y., Guest, J. R., Artymiuk, P. J. and Green, J. (2005) Switching aconitase B between catalytic and regulatory modes involves iron-dependent dimer formation. Mol. Microbiol. 56, 1149 – 1158.
70 Johansson, H. E. and Theil, E. C. (2002) Iron-response element (IRE) structure and combinatorial RNA regulation. In Molecular and Cellular Iron Transport (Templeton, D., ed. eds), pp. 237 – 253. Marcel Dekker, Inc., New York.
71 Wang, Y.-H., Sczekan, S. R. and Theil, E. C. (1990) Structure of the 5' untranslated regulatory region of ferritin mRNA studied in solution. Nucleic Acids Res. 18, 4463 – 4468.
72 Bettany, A. J.E., Eisenstein, R. S. and Munro, H. N. (1992) Mutagenesis of the iron regulatory element binding protein further defines a role for RNA secondary structure in the regulation of ferritin and transferrin receptor expression. J. Biol. Chem. 267, 16531 – 16537.
73 Henderson, B. R., Menotti, E., Bonnard, C. and Kühn, L. C. (1994) Optimal sequence and structure of iron-responsive elements: selection of RNA stem-loops with high affinity for iron regulatory factor. J. Biol. Chem. 269, 17481 – 17489.
74 Sierzputowska-Gracz, H., McKenzie, R. A. and Theil, E. C. (1995) The importance of a single G in the hairpin loop of the iron responsive element (IRE) in ferritin mRNA for structure: an NMR spectroscopy study. Nucleic Acids Res. 23, 146 – 153.
75 Butt, J., Kim, H.-Y., Basilion, J. P., Cohen, S., Iwai, K., Philpott, C. C., Altschul, S., Klausner, R. D. and Rouault, T. A. (1996) Differences in the RNA binding sites of iron regulatory proteins and potential target diversity. Proc. Natl. Acad. Sci. U. S.A. 93, 4345 – 4349.
76 Addess, K. J., Basilion, J. P., Klausner, R. D., Rouault, T. A. and Pardi, A. (1997) Structure and dynamics of the iron responsive element RNA: implications for binding of the RNA by iron regulatory binding proteins. J. Mol. Biol. 274, 72 – 83.
77 Gdaniec, Z., Sierzputowska-Gracz, H. and Theil, E. C. (1998) Iron regulatory element and internal loop/bulge structure for ferritin mRNA studied by cobalt(III) hexammine binding, molecular modeling, and NMR spectroscopy. Biochemistry 37, 1505 – 1512.
78 Ke, Y., H., S.-G., Gdaniec, Z. and Theil, E. C. (2000) Internal loop/bulge and hairpin loop of the iron-responsive element of ferritin mRNA contribute to maximal iron regulatory protein 2 binding and translational regulation in the iso-iron-responsive element/iso-iron regulatory protein family. Biochemistry 39, 6235 – 6242.
79 Ke, Y. and Theil, E. C. (2002) An mRNA loop/bulge in the ferritin iron responsive element (IRE) formed *in vivo*, and detected by radical probing with Cu-phen and protein (IRP) footprinting. J. Biol. Chem. 277, 2373 – 2376.
80 Horowitz, J. A. and Harford, J. B. (1992) The secondary structure of the regulatory region of the transferrin receptor mRNA deduced by enzymatic cleavage. New Biol. 4, 330 – 338.
81 Leibold, E. A., Laudano, A. and Yu, Y. (1990) Structural requirements of iron-responsive elements for binding of the protein involved in both transferrin receptor and ferritin mRNA post-transcriptional regulation. Nucleic Acids Res. 18, 1819 – 1824.
82 Hall, K. B. and Tang, C. (1998) ^{13}C relaxation and dynamics of the purine bases in the iron responsive element RNA hairpin. Biochemistry 37, 9323 – 9332.
83 McCallum, S. A. and Pardi, A. (2003) Refined solution structure of the iron-responsive element RNA using residual dipolar couplings. J. Mol. Biol. 326, 1037 – 1050.
84 Walden, W. E., Selezneva, A. I., Dupuy, J., Volbeda, A., Fontecilla-Camps, J. C., Theil, E. C. and Volz, K. (2006) Structure of dual function iron regulatory protein 1 complexed with ferritin IRE-RNA. Science 314, 1903 – 1908.
85 Arosio, P. and Levi, S. (2002) Ferritin, iron homeostasis, and oxidative damage. Free Radic. Biol. Med. 33, 457 – 463.
86 Jenkins, Z. A., Hagar, W., Bowlus, C. L., Johansson, H. E., Harmatz, P., Vichinsky, E. P. and Theil, E. C. (2007) Iron homeostasis during transfusional iron overload in β-thalassemia and sickle cell disease: changes in iron regulatory protein and ferritin expression. Pediatr. Hematol. Oncol. 24, 237 – 243.
87 Jaffrey, S. R., Haile, D. J., Klausner, R. D. and Harford, J. B. (1993) The interaction between the iron-responsive element binding protein and its cognate RNA is highly dependent upon both RNA sequence and structure. Nucleic Acids Res. 21, 4627 – 4631.
88 Kikinis, Z., Eisenstein, R. S., Bettany, A. J. and Munro, H. N. (1995) Role of RNA secondary structure of the iron-responsive element in translational regulation of ferritin synthesis. Nucleic Acids Res. 23, 4190 – 4195.
89 Selezneva, A. I., Cavigiolio, G., Theil, E. C., Walden, W. E. and Volz, K. (2006) Crystallization and preliminary X-ray diffraction analysis of iron regulatory protein 1 in complex with ferritin IRE RNA. Acta Crystallograph. Sect F Struct. Biol. Cryst. Commun. 62, 249 – 252.
90 Beuning, P. J. and Musier-Forsyth, K. (1999) Transfer RNA recognition by aminoacyl-tRNA synthetases. Biopolymers 52, 1 – 28.
91 Cazzola, M. (2005) Role of ferritin and ferroportin genes in unexplained hyperferritinaemia. Best Pract. Res. Clin. Haematol. 18, 251 – 263.
92 Cremonesi, L., Foglieni, B., Fermo, I., Cozzi, A., Paroni, R., Ruggeri, G., Belloli, S., Levi, S., Fargion, S., Ferrari, M. and Arosio, P. (2003) Identification of two novel mutations in the 5'-untranslated region of H-ferritin using denaturing high performance liquid chromatography scanning. Haematologica 88, 1110 – 1116.
93 Allerson, C. R., Cazzola, M. and Rouault, T. A. (1999) Clinical severity and thermodynamic effects of iron-responsive element mutations in hereditary hyperferritinemia-cataract syndrome. J. Biol. Chem. 274, 26439 – 26447.
94 Tibodeau, J. D., Fox, P. M., Ropp, P. A., Theil, E. C. and Thorp, H. H. (2006) The up-regulation of ferritin expression using a small-molecule ligand to the native mRNA. Proc. Natl. Acad. Sci. U. S.A. 103, 253 – 257.
95 Mengaud, J. M. and Horwitz, M. A. (1993) The major iron-containing protein of Legionella pneumophila is an aconitase homologous with the human iron-responsive element-binding protein. J. Bacteriol. 175, 5666 – 5676.
96 Prodromou, C., Artymiuk, P. J. and Guest, J. R. (1992) The aconitase of *Escherichia coli.* Nucleotide sequence of the aconitase gene and amino acid sequence similarity with mitochondrial aconitases, the iron-responsive-element-binding protein and isopropylmalate isomerases. Eur. J. Biochem. 204, 599 – 609.
97 Peyret, P., Perez, P. and Alric, M. (1995) Structure, genomic organization, and expression of the Arabidopsis thaliana aconitase gene. J. Biol. Chem. 270, 8131 – 8137.
98 Navarre, D. A., Wendehenne, D., Durner, J., Noad, R. and Klessig, D. F. (2000) Nitric oxide modulates the activity of tobacco aconitase. Plant Physiol. 122, 573 – 582.
99 Saas, J., Ziegelbauer, K., von Haeseler, A., Fast, B. and Boshart, M. (2000) A developmentally regulated aconitase related to iron-regulatory protein-1 is localized in the cytoplasm and in the mitochondrion of *Trypanosoma brucei*. J. Biol. Chem. 275, 2745 – 2755.
100 Gourley, B. L., Parker, S. B., Jones, B. J., Zumbrennen, K. B. and Leibold, E. A. (2003) Cytosolic aconitase and ferritin are regulated by iron in *Caenorhabditis elegans*. J. Biol. Chem. 278, 3227 – 3234.
101 Nichol, H., Law, J. H. and Winzerling, J. J. (2002) Iron metabolism in insects. Annu. Rev. Entomol. 47, 535 – 559.

102 Meyron-Holtz, E. G., Ghosh, M. C., Iwai, K., LaVaute, T., Brazzolotto, X., Berger, U. V., Land, W., Ollivierre-Wilson, H., Grinberg, A., Love, P. and Rouault, T. A. (2004) Genetic ablations of iron regulatory proteins 1 and 2 reveal why iron regulatory protein 2 dominates iron homeostasis. EMBO J. 23, 386 – 395.

103 Cooperman, S. S., Meyron-Holtz, E. G., Olivierre-Wilson, H., Ghosh, M. C., McConnell, J. P. and Rouault, T. A. (2005) Microcytic anemia, erythropoietic protoporphyria, and neurodegeneration in mice with targeted deletion of iron-regulatory protein 2. Blood 106, 1084 – 1091.

104 Galy, B., Ferring, D., Minana, B., Bell, O., Janser, H. G., Muckenthaler, M., Schumann, K. and Hentze, M. W. (2005) Altered body iron distribution and microcytosis in mice deficient in iron regulatory protein 2 (IRP2). Blood 106, 2580 – 2589.

105 Smith, S. R., Cooperman, S., Lavaute, T., Tresser, N., Ghosh, M., Meyron-Holtz, E., Land, W., Ollivierre, H., Jortner, B., Switzer, R., 3rd, Messing, A. and Rouault, T. A. (2004) Severity of neurodegeneration correlates with compromise of iron metabolism in mice with iron regulatory protein deficiencies. Ann. N. Y. Acad. Sci. 1012, 65 – 83.

106 Smith, S. R., Ghosh, M. C., Ollivierre-Wilson, H., Hang Tong, W. and Rouault, T. A. (2006) Complete loss of iron regulatory proteins 1 and 2 prevents viability of murine zygotes beyond the blastocyst stage of embryonic development. Blood Cells Mol. Dis. 36, 283 – 287.

107 Hanson, E. S., Rawlins, M. L. and Leibold, E. A. (2003) Oxygen and iron regulation of iron regulatory protein 2. J. Biol. Chem. 278, 40337 – 40342.

108 Meyron-Holtz, E. G., Ghosh, M. C. and Rouault, T. A. (2004) Mammalian tissue oxygen levels modulate iron-regulatory protein activities *in vivo*. Science 306, 2087 – 2090.

109 Kennedy, M. C., Mende-Mueller, L., Blondin, G. A. and Beinert, H. (1992) Purification and characterization of cytosolic aconitase from beef liver and its relationship to the iron-responsive element binding protein. Proc. Natl. Acad. Sci. U. S.A. 89, 11730 – 11734.

110 Haile, D. J., Rouault, T. A., Harford, J. B., Kennedy, M. C., Blondin, G. A., Beinert, H. and Klausner, R. D. (1992) Cellular regulation of the iron-responsive element binding protein: disassembly of the cubane iron-sulfur cluster results in high-affinity RNA binding. Proc. Natl. Acad. Sci. U. S.A. 89, 11735 – 11739.

111 Hirling, H., Henderson, B. R. and Kuhn, L. C. (1994) Mutational analysis of the [4Fe-4S]-cluster converting iron regulatory factor from its RNA-binding form to cytoplasmic aconitase. EMBO J. 13, 453 – 461.

112 Philpott, C. C., Haile, D., Rouault, T. A. and Klausner, R. D. (1993) Modification of a free Fe-S cluster cysteine residue in the active iron-responsive element-binding protein prevents RNA binding. J. Biol. Chem. 268, 17655 – 17658.

113 Brazzolotto, X., Gaillard, J., Pantopoulos, K., Hentze, M. W. and Moulis, J. M. (1999) Human cytoplasmic aconitase (Iron regulatory protein 1) is converted into its [3Fe-4S] form by hydrogen peroxide in vitro but is not activated for iron-responsive element binding. J. Biol. Chem. 274, 21625 – 21630.

114 Guo, B., Brown, F. M., Phillips, J. D., Yu, Y. and Leibold, E. A. (1995) Characterization and expression of iron regulatory protein 2 (IRP2). Presence of multiple IRP2 transcripts regulated by intracellular iron levels. J. Biol. Chem. 270, 16529 – 16535.

115 Guo, B., Yu, Y. and Leibold, E. A. (1994) Iron regulates cytoplasmic levels of a novel iron-responsive element-binding protein without aconitase activity. J. Biol. Chem. 269, 24252 – 24260.

116 Dupuy, J., Volbeda, A., Carpentier, P., Darnault, C., Moulis, J. M. and Fontecilla-Camps, J. C. (2006) Crystal structure of human iron regulatory protein 1 as cytosolic aconitase. Structure 14, 129 – 139.

117 Brown, P. H., Daniels-McQueen, S., Walden, W. E., Patino, M. M., Gaffield, L., Bielser, D. and Thach, R. E. (1989) Requirements for the translational repression of ferritin transcripts in wheat germ extracts by a 90-kDa protein from rabbit liver. J. Biol. Chem. 264, 13383 – 13386.

118 Wang, J., Fillebeen, C., Chen, G., Biederbick, A., Lill, R. and Pantopoulos, K. (2007) Iron-dependent degradation of apo-IRP1 by the ubiquitin-proteasome pathway. Mol. Cell Biol. 27, 2423 – 2430.

119 Guo, B., Phillips, J. D., Yu, Y. and Leibold, E. A. (1995) Iron regulates the intracellular degradation of iron regulatory protein 2 by the proteasome. J. Biol. Chem. 270, 21645 – 21651.

120 Iwai, K., Klausner, R. D. and Rouault, T. A. (1995) Requirements for iron-regulated degradation of the RNA binding protein, iron regulatory protein 2. EMBO J. 14, 5350 – 5357.

121 Samaniego, F., Chin, J., Iwai, K., Rouault, T. A. and Klausner, R. D. (1994) Molecular characterization of a second iron-responsive element binding protein, iron regulatory protein 2. Structure, function, and post-translational regulation. J. Biol. Chem. 269, 30904 – 30910.

122 Goessling, L. S., Mascotti, D. P. and Thach, R. E. (1998) Involvement of heme in the degradation of iron-regulatory protein 2. J. Biol. Chem. 273, 12555 – 12557.

123 Goessling, L. S., Mascotti, D. P., Bhattacharyya-Pakrasi, M., Gang, H. and Thach, R. E. (1994) Irreversible steps in the ferritin synthesis induction pathway. J. Biol. Chem. 269, 4343 – 4348.

124 Jeong, J., Rouault, T. A. and Levine, R. L. (2004) Identification of a heme-sensing domain in iron regulatory protein 2. J. Biol. Chem. 279, 45450 – 45454.

125 Schalinske, K. L., Anderson, S. A., Tuazon, P. T., Chen, O. S., Kennedy, M. C. and Eisenstein, R. S. (1997) The iron-sulfur cluster of iron regulatory protein 1 modulates the accessibility of RNA binding and phosphorylation sites. Biochemistry 36, 3950 – 3958.

126 Brown, N. M., Anderson, S. A., Steffen, D. W., Carpenter, T. B., Kennedy, M. C., Walden, W. E. and Eisenstein, R. S. (1998) Novel role of phosphorylation in Fe-S cluster stability revealed by phosphomimetic mutations at Ser-138 of iron regulatory protein 1. Proc. Natl. Acad. Sci. U. S.A. 95, 15235 – 15240.

127 Seznec, H., Simon, D., Bouton, C., Reutenauer, L., Hertzog, A., Golik, P., Procaccio, V., Patel, M., Drapier, J. C., Koenig, M. and Puccio, H. (2005) Friedreich ataxia: the oxidative stress paradox. Hum. Mol. Genet. 14, 463 – 474.

128 Stehling, O., Elsasser, H. P., Bruckel, B., Muhlenhoff, U. and Lill, R. (2004) Iron-sulfur protein maturation in human cells: evidence for a function of frataxin. Hum. Mol. Genet. 13, 3007 – 3015.

129 Brazzolotto, X., Timmins, P., Dupont, Y. and Moulis, J. M. (2002) Structural changes associated with switching activities of human iron regulatory protein 1. J. Biol. Chem. 277, 11995 – 2000.

130 Yikilmaz, E., Rouault, T. A. and Schuck, P. (2005) Self-association and ligand-induced conformational changes of iron regulatory proteins 1 and 2. Biochemistry 44, 8470 – 8478.

131 Aziz, N. and Munro, H. N. (1987) Iron regulates ferritin mRNA translation through a segment of its 5' untranslated region. Proc. Natl. Acad. Sci. U. S.A. 84, 8478 – 8482.

132 Hentze, M. W., Caughman, S. W., Rouault, T. A., Barriocanal, J. G., Dancis, A., Harford, J. B. and Klausner, R. D. (1987) Identification of the iron-responsive element for the translational regulation of human ferritin mRNA. Science 238, 1570 – 1573.

133 Zheng, L., Kennedy, M. C., Blondin, G. A., Beinert, H. and Zalkin, H. (1992) Binding of cytosolic aconitase to the iron responsive element of porcine mitochondrial aconitase mRNA. Arch. Biochem. Biophys. 299, 356 – 360.