

Iron Responsive mRNAs: A Family of Fe²⁺ Sensitive Riboregulators

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CONSPECTUS

Messenger RNAs (mRNAs) are emerging as prime targets for small-molecule drugs. They afford an opportunity to assert control over an enormous range of biological processes: mRNAs regulate protein synthesis rates, have specific 3-D regulatory structures, and, in nucleated cells, are separated from DNA in space and time. All of the many steps between DNA copying (transcription) and ribosome binding (translation) represent potential control points. Messenger RNAs can fold into complex, 3-D shapes, such as tRNAs and rRNAs, providing an added dimension to the 2-D RNA structure (base pairing) targeted in many mRNA interference approaches. In this Account, we describe the structural and functional properties of the IRE (iron-responsive element) family, one of the few 3-D mRNA regulatory elements with known 3-D structure. This family of related base sequences regulates the mRNAs that encode proteins for iron metabolism.



We begin by considering the IRE-RNA structure, which consists of a short (~30-nucleotide) RNA helix. Nature tuned the structure by combining a conserved AGU pseudotriloop, a closing C-G base pair, and a bulge C with various RNA helix base pairs. The result is a set of IRE-mRNAs with individual iron responses. The physiological iron signal is hexahydrated ferrous ion; in vivo iron responses vary over 10-fold depending on the individual IRE-RNA structure.

We then discuss the interaction between the IRE-RNA structure and the proteins associated with it. IRE-RNA structures, which are usually noncoding, tightly bind specific proteins called IRPs. These repressor proteins are bound to IRE-RNA through C-bulge and AGU contacts that flip out a loop AG and a bulge C, bending the RNA helix. After binding, the exposed RNA surface then invites further interactions, such as with iron and other proteins. Binding of the IRE-RNA and the IRP also changes the IRP conformation. IRP binding stabilities vary 10-fold within the IRE family, reflecting individual IRE-RNA paired and unpaired bases. This variation contributes to the graded (hierarchical) iron responses in vivo.

We also consider the mechanisms of IRE-mRNA control. The binding of Fe²⁺ to IRE-RNA facilitates IRP release and the binding of eukaryotic initiation factors (eIFs), which are proteins that assemble mRNA, ribosomes, and tRNA for translation. IRE-RNAs are riboregulators for the inorganic metabolic signal, Fe²⁺; they control protein synthesis rates by changing the distribution of the iron metabolic mRNAs between complexes with enhancing eIFs and inhibitory IRPs.

The regulation of mRNA in the cytoplasm of eukaryotic cells is a burgeoning frontier in biomedicine. The evolutionarily refined IRE-RNAs, although absent in plants and bacteria, constitute a model system for 3-D mRNAs in all organisms. IRE-mRNAs have yielded "proof of principle" data for small-molecule targeting of mRNA structures, demonstrating tremendous potential for chemical manipulation of mRNA and protein synthesis in living systems.

1. Overview of mRNA Regulation

Messenger RNA regulation complements DNA regulation to control the amount of specific proteins synthesized by living cells. The multiple sizes, shapes, domain functions, and metabolic histories of mRNAs contrast with the relatively

constant sizes and overall shapes of tRNAs and rRNAs. In prokaryotic cells (without nuclei), DNA transcription to form mRNA and mRNA translation (protein biosynthesis) are coupled. By contrast, in nucleated cells, transcription and translation are separated in space and time, by the nuclear

membrane and by RNA “processing” (e.g., editing, splicing, 5’ “capping”, and 3’ “polyA tailing”) and transport to ribosomes in the cytoplasm, providing many opportunities to regulate mRNA translation. Subcellular compartments such as mitochondria and plant plastids, which contain their own DNA and transcribe their own mRNAs for localized protein synthesis, have novel features not considered here.

The variety of mRNAs in each cell type, for example, skin cells versus muscle cells, is specific. Thus, RNA provides a very rich set of targets that are more cell-specific than DNA. The rapid growth in RNA interference methods underscores the potential of RNA targeting, although selectivity with RNA_i is based on primary sequence and secondary structure.

RNA regulators, primarily described in bacteria,¹ are a heterogeneous group of molecules that modulate a wide range of physiological responses. Of most relevance here are riboswitches, noncoding, structured mRNA regions that undergo conformational changes upon binding small metabolites, creating an RNA molecular sensor for cellular and/or environmental changes in metabolite concentrations. The RNA structural changes lead to changes in gene expression (protein accumulation). In addition to bacteria, a thiamine-pyrophosphate-binding riboswitch occurs in plants and fungi.² Riboregulator RNA contrasts with riboswitches by the absence of changed helix base pairs. An intensely studied set of mRNA regulatory structures, the internal ribosome entry site, is found in viruses and normal cells.³ Here we describe a family of riboregulators in animal mRNAs that bind an inorganic metabolite, iron, to regulate gene expression; there are no base pair changes but RNA helix bending is important.

Generally, mRNA regulatory structures are noncoding, and in the untranslated region (UTR) of mRNAs, either before (5’UTR) or after (3’UTR) the coding region. The most extensively characterized animal (including humans) mRNA regulatory structures are a family of related sequences named IRE (iron responsive element) because of the response to changes in environmental iron.⁴ IRE-containing mRNAs code for a variety of proteins involved in iron metabolism and transport. The vast majority of studies are about the extensively reviewed iron repressor proteins, IRP1/2, for example.^{4–8} Only recently, the iron metabolite signal is identified as a ferrous ion/RNA interaction.⁹

IRE-RNA sequences fold into 3-D structures selectively recognized by the specific repressor proteins (IRPs) and possibly by translation initiation proteins.¹⁰ They occur in both the 5’UTR to control ribosome binding/translation initiation, responding to elevated iron, and in the 3’UTR to

control mRNA stability during iron deprivation. Much more is known about 5’UTR IRE structures, the focus of this discussion. The evolutionarily oldest IRE in the 5’ UTR is in ferritin mRNAs,¹¹ and it has been studied by chemical and nuclease probing, secondary structure prediction, mutagenesis, and NMR spectroscopy as well as X-ray diffraction of IRE-RNA/protein repressor crystals.^{4,6,12–15} Chemical nucleases, models for selective, small RNA-targeting molecules, confirmed constant IRE structure between native ferritin mRNA in solution and cells,¹⁶ full length in vitro transcripts and short oligoribonucleotides.^{17,18}

2. IRE-RNA: The Conserved Core Structure

Repressor protein (IRP) recognition of IRE-RNAs depends on the conserved IRE-RNA core structure (30 nt) described below (Figures 1 and 2). The protein repressors (IRP1 and 2) bind IRE-mRNA subdomains (Figure 3) to inhibit protein synthesis (5’UTR) or mRNA degradation (3’ UTR).^{4–6} IRP1 and 2 are degraded by iron-dependent processes.^{7,8} IRP1 is converted to c-aconitase by iron–sulfur cluster insertion at the RNA site.^{6,14,15} IRE sequence is more than 90% conserved for IRE-mRNAs coding for the same protein in different animals, but sequence conservation of the element in IRE–mRNAs coding for different proteins of the same animal varies as much as 40%.^{13,15,19}

NMR studies of an IRE-RNA 30 base oligoribonucleotide, representing the IRP1 “footprint”,^{20,21} showed the following: (1) both well-defined and conformationally disordered regions; (2) well-defined terminal loop residues C14, A15, and G18 and the bases in helix pairs below and above the C8 bulge; (3) base pairs were folded into the RNA A helix conformation; (4) the orientation of helix sections flanking the C8 bulge was not fixed with respect to each other, allowing conformational changes during IRP binding that are indicated by differences between the IRE-RNA solution NMR structure and in X-ray diffraction of IRP/IRE crystals.¹²

All IRE-RNAs have five base pairs in the upper stem.²² While the constant number of base pairs may seem like a “molecular ruler” for spacing of the two protein contact sites at the bulge C8 residue and the upper pseudotriloop, the identity of the base pairs in the upper stem vary in an IRE-mRNA specific way. Moreover, IRE-RNAs of at least 30 nucleotides, the RNA length protected by IRP binding,²³ show that the natural variations in base pairs of the upper stem influence IRP binding.^{24–26} In the IRE-RNA/IRP complex, the RNA helix is bent as much as 30° from the helix axis,¹² contrasting with IRE-RNA in solution.^{20,27} Likely, the individual sets of five base pairs in the upper RNA stem

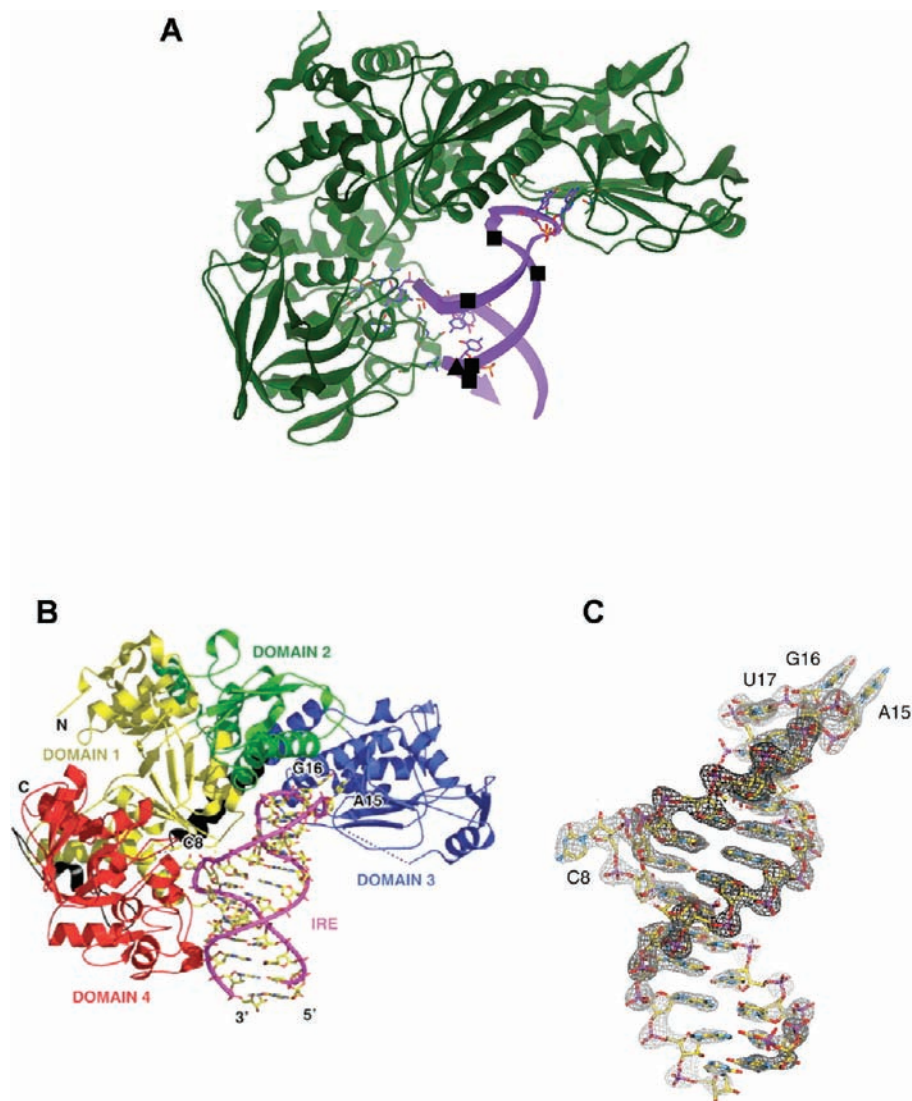


FIGURE 1. IRP1 binding to FRT IRE-RNA. The RNA helix bends, and contact bases C8 and triloop bases A15 and G16 are flipped out. Panel A: (■) hydrated Mg^{2+} , determined by solution NMR; (▲) Cu^{1+} -1.10-phenanthroline, determined by RNA cleavage in O_2 . Figure prepared from Protein Data Bank file 2IPY. (Modification of figure originally published in ref 12.) Panel B: IRE:IRP complex showing IRP domains (1, yellow; 2, green; 3, blue; 4, red) and contacts with IRE-RNA. Panel C: Electron density of IRE in IRE:IRP complex. (Modification of figure originally published in ref 12.)

control the kinetics of IRE-RNA helix bending during IRP binding.

The apical loop of all IRE-RNAs, one of the two IRP contact sites (Figures 1, 2, and 3B), has a C14-G18 base pair that creates an AGU pseudotriloop.^{12,20,21,27,28} G16 and U17 are disordered. In the IRP/RNA protein complex A15 and G16 are flipped out of the helix, penetrating deep into the IRP structure.¹² The C14-G18 base pair is crucial for loop structure/stability, since G18A IRE-RNA has a lower T_m ; an IRE loop with U14-A18 retains high affinity for IRP1.^{21,29} Apparently, variable base 19 cannot be G, since an alternate base pair to C14-G19 would occur.

Binding specificity in the IRP1/H ferritin IRE-RNA complex is achieved with only two, widely separated, contact sites.¹² In the complex, the RNA is inserted between protein domains I–II and IV (Figure 3), using the numbering of aconitases. Aconitases are globular structures with close interactions among all four domains, whereas in the IRE-RNA/IRP complex IRP protein is L-shaped and the RNA is bent. Twenty-two bonds hold the RNA and protein together: 10 contacts are formed between A15 and G16 in the pseudotriloop at the RNA terminus (Figure 3B) and amino acids, such as residues 371 and 379 in a pocket generated in domain III at a site that is blocked by domains I and II in the globular form (Figure 3B). Eight bonds are

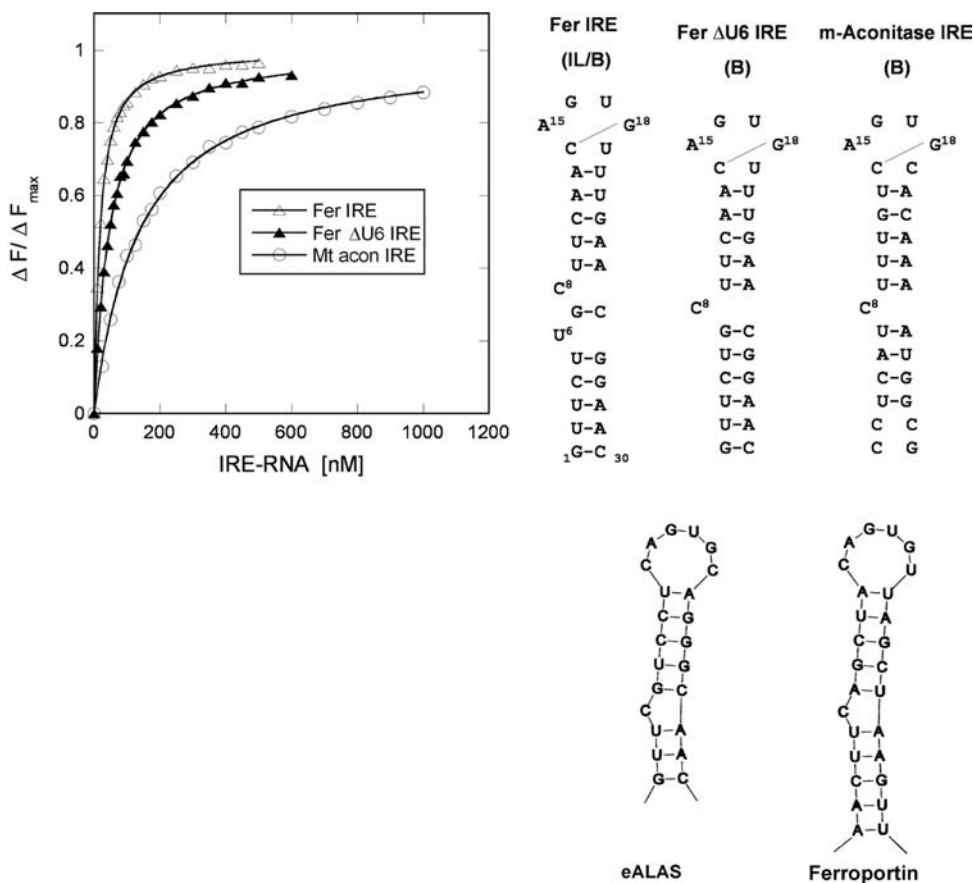


FIGURE 2. IRP1/IRE-RNA solution binding compared for three IRE-RNAs. IRP1 preferentially binds to the FRT IRE-RNA compared with ACO2 IRE-RNA with nM affinity: binding curve (protein fluorescence quenching) on the left and conserved IRE secondary structures on the right (Reprinted with permission from ref 9. Copyright 2009 American Society for Biochemistry and Molecular Biology).

formed between IRE-RNA C8 and amino acids in Domain IV of IRP1; four additional bonds occur between amino acids in domain IV of IRP1 and the IRE stem below C8 (Figure 3C). In addition to flipping out of terminal loop bases A15 and G16 and helix bulge C8,²⁰ in the protein/RNA complex, the RNA backbone is distorted by a sharp midhelix turn, unpaired U19 in the hexaloop is flipped out of the RNA helix, and unpaired U6 is tucked into the minor groove (Figure 1,3). The differences between the solution structure of free IRE-RNA and protein bound IRE-RNA require conformational changes in the RNA, and likely of the unliganded protein; while the structure of apo-IRP is not yet known, it is more disordered than in either the RNA or [4Fe-4S] complexes.³⁰ In the protein/RNA crystal structure,¹² a large surface of the IRE-RNA is exposed free for interactions with other proteins, metal ions, or RNA.

3. IRE-RNA Structure: Natural Variations and Protein Repressor (IRP) Binding

The conserved structure of the IRP contact sites in IRE-RNAs, the terminal loop and bulge C, means that differences in

RNA/IRP complex stability must depend on structural differences in helices. IRP binding affinity is altered by increasing the length of the upper stem or by disrupting the helices above and below the C bulge.^{22,23,31,32} Natural variations, which occur in helix base pairs of IRE-containing mRNAs coding for different proteins, coincide with quantitative differences in IRP binding affinity and the magnitude of the iron response in vivo. It is as if Nature has created a set of graded "dimmer" switches in the IRE family, using helix sequences that vary as much as 36%, rather than an on/off switch, so that IRE-mRNAs can have a range of sensitivities to iron.

Ferritin H and MT-aconitase (ACO2) IRE-mRNAs^{9,26} differ by at least an order of magnitude in the in vivo response to iron levels and show the largest differences in IRP1 binding in solution^{24,33} (Figure 2). The relative affinity of ferritin IRE-RNA and ACO2 IRE-RNA with IRP1 differ by 9-fold whether by mobility shift in gel electrophoresis or fluorescence quenching in solution; the picomolar binding constants from gel shifts contrast with nanomolar binding constants for solution fluorescence; this difference may be

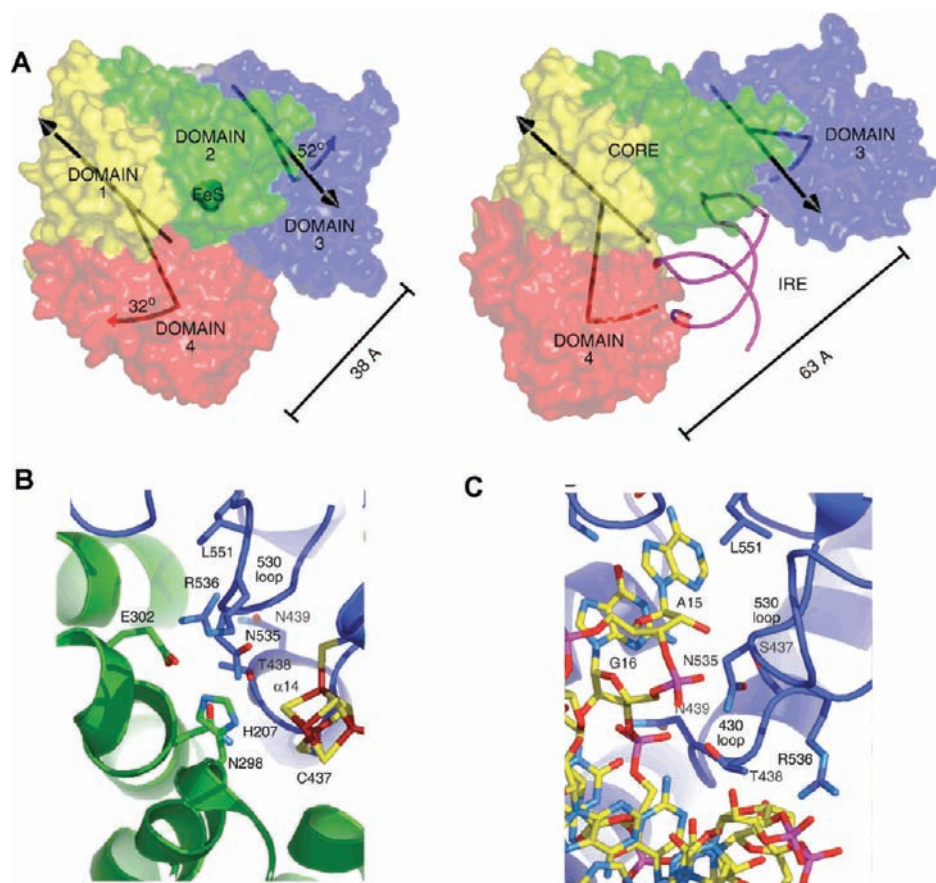


FIGURE 3. Crystal structure of IRP1 as c-aconitase and complexes with IRE-RNA. (A) Differences in protein domain positions between c-aconitase and IRP1:IRE-RNA complex. Left, FeS-apo-IRP; right, IRE-RNA/IRP. RNA, magenta; protein, blue, green, red, yellow. (B) Close up of the protein–RNA contacts at the RNA triloop. (C) Close-up of the protein–RNA contacts at the RNA bulge C8. (Modification of figure originally published in ref 12.)

caused by an adsorptive component in the gels. Ferritin IRE-RNA with the additional U6 bulge (Figure 2) binds IRP the most tightly, and may reflect faster kinetics of helix bending during protein binding. When large numbers of IRE-RNAs were compared, a binding hierarchy was defined by Goforth et al.,²⁶ also described earlier as combinatorial regulation,³⁴ since different combinations of variable elements (base pairs, helix bulges) and constant elements (terminal loop, C bulge) are combined in each phylogenetically conserved IRE-RNA. IRE-RNA base pairs can change protein/RNA stability as much as 14-fold,²⁶ even though there is little or no contact between IRP protein and most of the helix base pairs (Figure 2), likely reflecting the contributions of helix pitch, flexibility and binding dynamics.

The array of similar but distinct structures among IRE-RNA family members, selectively recognized by IRP binding, suggests an RNA scaffold, similar to tRNAs, presenting key residues for interaction with recognition proteins. Recognition of tRNA by tRNA synthetase depends on core elements, as does IRE recognition by IRP1; tRNAs also contain minor

elements that fine-tune the interactions.^{35–37} Like the tRNAs, IRE-RNAs have both nonconserved sequences, particularly in the stem region that contributes to IRP binding,¹¹ and core elements. However, the binding affinities do not have the range of discrimination or energetics observed in tRNAs^{35,37,38} which have to be recognized by individual synthetases. Different IREs are recognized by the same IRP proteins.

IRE-RNAs were originally associated with iron and oxygen metabolism. Recently, however, IRE-mRNAs have been identified that encode a number of other proteins such as hypoxia inducible transcription factor,³⁹ cell cycle phosphatase,⁴⁰ α -hemoglobin stabilizing protein,⁴¹ MT- p75 protein,⁴² and Alzheimer amyloid precursor (AAP) protein.^{43,44} The AAP IRE-mRNA secondary structure is predicted to contain the pseudotrilloop and also contains the conserved C8 residue. While predicted structure for APP IRE-RNA does not predict the C8 residue to be a bulge base, G7 is predicted to be a bulge base.⁴³ The binding stability of the complex appears to be similar to the ferritin IRE-RNA/IRP1 complex. However, AAP IRE

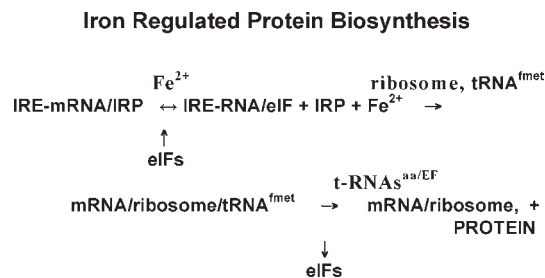
does not bind IRP2.⁴³ The greater selectivity of IRP2/IRE-RNA interactions has also been observed for the IRE-mRNAs involved in iron metabolism.^{24,45} It is likely that additional IRE structures differentially binding IRP1 and IRP2 will be identified.

4. IRE-mRNA Translation

Effects of changes in environmental iron (solutions of inorganic salts) on ferritin mRNA function and protein synthesis in animals and cells were first observed in the 1970s and 1980s,^{5,46} long before cloning, recombinant mRNAs, and discovery of the conserved IRE sequences.^{47,48} The effect was later extended to many other IRE-mRNAs in iron metabolic pathways; the environmental signals were also extended to include heme, oxygen (anoxia), and oxidants (hydrogen peroxide) as reviewed in refs 4, 6, and 15. Because IRE-RNAs are located in two different locations in mRNA, iron can have opposite effects on different IRE-mRNAs. In Type 1 IRE regulation, the IRE structure is in the 5'UTR and regulates ribosome binding. Iron signals release IRP to allow ribosome binding and translation of the mRNA increases. In Type 2 IRE regulation, the IRE is in the 3'UTR, to regulate nuclease binding and mRNA degradation. Most IRE-RNAs that have been characterized to date regulate ribosome binding rather than nuclease binding.

How cellular iron signals change the affinity of IRP for IRE-RNA and remove translational repression was not understood until very recently.⁹ Moreover, ribosome binding is a very complex process requiring the binding of many protein "factors" to form an initiation complex of mRNA, initiator tRNA, and both ribosomal subunits. The exact sequence of events in releasing IRP and assembling an initiation complex with an IRE-RNA remains unclear. Some features suggest IRE structures function synergistically with other elements of the mRNA structure based on a short distance between the beginning of the mRNA and the IRE,^{10,49} in some IRE-RNAs such as ACO2, the initiator AUG is embedded in the IRE-RNA; the functional significance of initiation at the IRE is not known. The initiation of protein synthesis begins with the binding of a large protein, eukaryotic initiation factor (eIF) 4F, to the 5' cap of mRNA; eIF4F is actually a supramolecular complex of a helicase (4A), RNA binding protein (4B), and scaffolding protein (4G) that may also contact IRP.⁵⁰ eIF4F binding can facilitate rapid responses to cellular iron levels, since it binds competitively with IRP to IRE-RNA, indicating the two proteins occupy the same or overlapping binding sites (Ma, Khan, Merrick, Haldar, Theil, and Goss, to be published). Many of the details of the assembly of an active protein synthesis initiation complex remain to be elucidated.

The IRE-RNA binds metal ions, which include physiologically relevant Fe^{2+} , to decrease IRE-RNA/IRP stability, indicating a direct role for metal ions in dissociation of the IRP1/IRE complex.



The impact of Fe^{2+} , the physiological iron signal on IRE-mRNA translation, i.e., protein biosynthesis, is shown in eq 1. The reaction, a series of sequential, supramolecular interactions between IRE-mRNA with IRP (iron regulatory protein), Fe^{2+} , eIFs (initiation factor proteins), rRNA/protein complexes (ribosome), and tRNA/protein (EF, elongation factor) complexes is required for Iron-induced protein biosynthesis as shown in eq 1. Not shown is the consumption of GTP at every $\text{tRNA}^{\text{aminoacid}}$ binding step, which makes ribosome-dependent protein biosynthesis the most bioenergetically expensive reaction in growing cells.

5. Metal Ions and IRE-RNA/IRP Binding Interactions

The addition of iron chelators, exemplified by a recent study of the Alzheimer amyloid precursor protein (AAP) IRE, illustrates the effects of changing iron concentrations: cells exposed to the Fe(III) chelator desferrioxamine increased binding of IRP1 to the IRE-RNA,⁴³ as observed for many IRE-RNAs in a variety of cultured cell types.^{32,51–53} The molecular mechanism was not clear until the recent observations that low concentrations of ferrous ions (anaerobic) weakened IRE-RNA/IRP1 complexes; other metal ions have selective effects.⁹ The magnitude of the metal ion effects varies with individual IRE-RNA sequences/structures. For example, ferritin IRE/IRP1 binding affinity decreased 5–10 times while ACO2 IRE/IRP1 binding decreased only 2–5 times over the concentration ranges used,⁹ indicating the impact of Nature's modulation of riboregulation among IRE-RNAs.

Metal ions bind directly to the IRE-RNA based on ethidium bromide displacement, effects on NMR spectra, binding of metal complexes,^{9,17,27,54} and the absence of predicted metal ion binding sites on IRP beyond the [4Fe-4S] cluster insertion site. Moreover, eIF4F binding to IRE-RNA is metal

ion sensitive (Ma, Khan, Merrick, Haldar, Theil, and Goss, to be published). Finally, a number of RNA protein bonds involve sites that are hypersensitive to cleavage by Fe^{2+} -EDTA,²³ suggesting specific interactions with Fe^{2+} -EDTA, solvent, or both. The fact that metal ion effects were greatly reduced when the conserved ferritin IRE-RNA bulge U6 was deleted⁹ and the earlier studies of Cu-1,10-phenanthroline and modeling of Co(III)hexamine binding^{27,54} support the idea of the IRE-RNA midhelix bulge as the binding site of regulatory metal ions. Bulge bases are metal ion binding sites in other RNAs.^{55,56} The metal selectivity of Fe^{2+} on IRP binding to IRE-RNAs, which is 200× more effective than Mg^{2+} ,⁹ indicates that Fe^{2+} is the physiological signal that targets the IRE-RNA riboregulator to decrease IRP binding and increase translation of the IRE-mRNA.

6. IRE-mRNA as Fe^{2+} Riboregulator

Riboswitches and riboregulators regulate gene expression with target molecules, usually small metabolites that cause RNA conformational changes upon binding. Often a polymerase or ribosome binding site is exposed as a result. In the IRE-RNAs, based on solution structures of free RNA and a crystal structure of RNA bound to repressor proteins,^{12,20,27} structural changes induced by Fe^{2+} binding involve changes in the orientation of unpaired bases and helix bending with retention of base pairing. Riboregulator activity can depend on kinetics, thermodynamics (steady state binding stability) or a combination of both, depending on ligand concentrations. In IRE-RNAs, the conformational changes caused by Fe^{2+} binding results in the release of the IRP repressor protein. In addition, Fe^{2+} -induced RNA conformational changes enhance binding of the eukaryotic initiation factor 4F to increase translation. If only equilibrium effects are considered, a change in ligand concentration of ~80-fold is required for a change in the fraction of an RNA/protein complex from 10 to 90%. However, many riboswitches fine-tune ligand sensitivity by changing binding kinetics.⁵⁷ Riboregulators that are kinetically controlled rely on the occupancy of the RNA site; gene expression will depend on the dissociation rate of the ligand, or for the Fe^{2+} -IRE-RNA/IRP, dissociation rate of the IRP, association rate of eIF-4F and ribosomes, and dissociation rate of Fe^{2+} . If initiation factor binding is rapid, expression (protein synthesis of IRE-encoded proteins) will be sensitive to small changes in Fe^{2+} concentrations and regulation will be dominated by kinetics of IRP release and not by equilibrium binding because eIF binding will occur before equilibrium is established.

7. Kinetics and Mechanisms of IRP Binding to Ferritin and ACO2 IRE-RNAs

The role of IRP/IRE-mRNA binding kinetics and direct Fe^{2+} /RNA interactions in controlling downstream gene expression, that is, rates of mRNA translation, have been little considered. However, a characteristic of a kinetically operated riboregulator, and indeed of life itself, is genetic decisions made under nonequilibrium conditions for RNA and ligand. Rate constants for association and dissociation are, thus, more important than the equilibrium constants, albeit experimentally more difficult to measure in complex biological systems. When we investigated the rates of association and dissociation of IRP1 to ferritin and ACO2 IRE (Ma, Khan, Haldar, Merrick, Theil, and Goss, to be published), we found that the smaller K_d for ferritin IRE-RNA/IRP1 binding, compared to ACO2, depended on a much faster association rate; the dissociation rate constants were comparable. In the presence of Fe^{2+} (anaerobic) or the oxygen-resistant Fe^{2+} -surrogate, Mn^{2+} , in air, both the k_{on} and k_{off} rates were changed, more so for the ferritin IRE-RNA than for ACO2 IRE-RNA; whether Mn^{2+} /IRE-RNA interactions are physiologically relevant is not known. The lifetime of the IRP-IRE complex ($1/k_{\text{off}}$), in the presence of Mn^{2+} , decreased for both the ferritin IRE-RNA/IRP and ACO2 IRE-RNA/IRP complex. Since the concentrations of IRE-mRNAs and IRP repressor proteins vary in different types of cells, kinetic contributions to the concentration of IRE-RNA/IRP complexes are predicted to be large and cell specific.

The interaction of IRE-mRNAs with translation initiation factors (eIFs) and/or ribosomes is a second potential set of targets for riboregulator- Fe^{2+} control. The association of eIF4F increases approximately 5-fold in the presence of 50 μM Mn^{2+} or Fe^{2+} , an effect opposite that for the IRP (Ma, Khan, Haldar, Merrick, Theil, and Goss, to be published). Thus, when cellular concentrations of free or loosely bound Fe^{2+} increase, IRE-RNA- Fe^{2+} complexes are in a conformation unfavorable to IRP repressor binding and favorable to eIF4F binding. The IRE riboregulator in the presence of metals increases protein synthesis by two mechanisms: facilitating IRP repressor release and eIF4F binding, explaining earlier observations that the presence of an IRE-RNA had positive effect on protein synthesis rates.¹⁰ Nature has tuned the IRE-RNA riboregulator in different IRE-mRNAs by changing the base pairs, which results in changes in IRP binding stability.^{9,24,26,33}

8. Small Molecule Drug Targeting of IRE-RNA and Other mRNA Regulatory Structures

Current development of RNA drugs, for example, based on RNA_i, depends heavily on RNA secondary structure, whereas

for proteins 3-D structure is the more common drug target. RNA therapies are advantageous because of the smaller target size (number of molecules/cell) compared to proteins. Small molecules, for example, Cu-1,10-phenanthroline and yohimbine, bind to selective sites in the IRE-RNA in solution altering mRNA function in solution and in cultured, human cells.^{16,17,58} Such results showed that the small RNA binding molecules can enter living cells and bind to folded target RNA structures with the same selectivity as in solution. In one case, such as IRP-inactivated ferritin mRNA, a small molecule could activate the fraction of mRNA repressed in iron overload, and decrease the accumulation of damaged ferritin (toxic hemosiderin). In another case, small molecules could target riboregulators in oncogene mRNAs or mRNAs important in metabolic diseases. All that is needed is identification of more riboregulatory mRNA structures and the expansion of small molecule targeting of 3-D structures of mRNAs.

9. Summary and Perspective

MRNA regulation in the cytoplasm of eukaryotic (nucleated cells) is a burgeoning frontier in the understanding of Chemistry and Biology. Here we describe an example of a group of three-dimensional RNA structures, iron responsive elements (IRE-RNAs), in normal cellular mRNAs. These structures selectively bind iron to regulate the stability of a protein repressor complex that impedes ribosome binding and protein synthesis (mRNA translation). Positive regulation occurs through binding of protein synthesis initiation factor eIF4F. The IRE-RNA is described in terms of the IRE-RNA loop and bulge common to each IRE-mRNA that forms the separated protein binding sites. Variations in the sequence of individual IRE-RNA helix base pairs modulate both repressor protein (IRP) binding and the metabolite Fe²⁺ binding, tuning protein synthesis to in vivo environmental iron. Future studies to characterize the Fe²⁺ binding site on the IRE will identify target regions of the IRE-RNA for future drug design. IRE-RNAs, evolutionarily old in animals, are absent in plants and bacteria, but form a model system for other 3-D mRNAs in any organism. IRE-mRNAs have yielded “proof of principle” data for small molecule targeting of mRNA structures that illustrate the untapped potential for chemical manipulation of mRNA and protein synthesis in living systems.

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BIOGRAPHICAL INFORMATION

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Elizabeth C. Theil is a biochemist/bioinorganic chemist, educated in the public schools of New York City, and Cornell and Columbia Universities. Her work, spanning DNA, mRNA, and protein structure/function, has relevance to iron nutrition and medicine and is focused on the ferritin family of protein nanocages. Currently, she is Senior Scientist at CHORI: Children's Hospital Oakland Research Institute and Prof. Nutr. Sci. & Tox. (adj) UC-Berkeley.

FOOTNOTES

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