

Living with Iron (and Oxygen): Questions and Answers about Iron Homeostasis

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1. Introduction

Mechanisms to regulate iron homeostasis are very likely billions of years older than those for oxygen homeostasis since contemporary microbes regulate iron in the absence of oxygen and may model ancient organisms that lived before atmospheric oxygen appeared.¹ Moreover, proteins that manage iron and oxidants such as the mini-ferritins in contemporary bacteria, also called Dps (DNA protection during stress) proteins, are expressed in anaerobic archaea.² Mini-ferritins are 12 subunit protein cages from archaea and bacteria that contrast with maxi-ferritins, the 24 subunit cages from bacteria, animals and plants, which use iron and dioxygen as substrates, by consuming iron and hydrogen peroxide as substrates to make the mineral inside protein nanocages. Such peroxide-consuming ferritins may be progenitors of modern ferritins and may have contributed to the

transition from anaerobic to aerobic life with iron and oxygen.

Iron and oxygen homeostasis, in animals, integrate DNA/mRNA controls; regulation of these two processes intersect along many pathways. Iron homeostasis occurs within cells and between cells to confer balance throughout tissues and the organism. Each cell or tissue type will have a different iron set point for homeostasis that reflects the specific role of each cell type. For example, animal red blood cells need much more iron than epithelial cells because of the synthesis of hemoglobin.³ Similarly, in plants, leaves contain much more iron than flowers because of the synthesis of ferredoxins important in photosynthesis.⁴ Growing animals or plants will also have different requirements for iron than aging animals or senescent plants. Thus, the conditions that cause changes in iron homeostasis may vary depending on age or specialized function, explaining, in part, quantitatively different results that can be obtained with cultured cell lines derived from different tissues.

Metal ion homeostasis has common features shared by all the metal ions that include cell uptake, cell efflux, and intracellular transport. However, the chemical properties of iron require additional iron-specific homeostatic features compared to other metals, because of the hydrolytic properties of ferric ion under physiological condition. Hydrated ferric ions are relatively strong acids; protons in water coordinated to ferric ions have a $pK_a \approx 3$. The conjugate bases of hydrated ferric ions form multinuclear species rapidly, accounting for the low solubility of aqueous ferric ions (10^{-18} M) and for rust formation. In addition, living cells and organisms use much more iron than other metals. For example, the human body contains ~ 3.5 g of iron compared to 100 mg of copper.

Iron concentrations in cells are much higher than the solubility of free ferric ions in aqueous solution in air. Average iron concentrations in cells are $\sim 10^{-4}$ M requiring, since the solubility of free ferric ions in water/plasma is 10^{-18} M, a concentration gradient of 100 trillion between aqueous, external environments and intracellular environments. The concentration difference is achieved using transporters (membrane), intracellular carriers and concentrators, cellular exporters, and, in multicellular organisms, extracellular carriers. Except for iron concentrators such as ferritins, the uses of receptors, transporters, and intracellular carriers is shared with other metal ions, such as copper, manganese, etc. The ferritins, which concentrate and store intracellular iron at concentrations far above the solubility of the free ion, are unique for iron homeostasis, compared to other metal ions.

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Educated in the public schools of New York City, Elizabeth C. Theil received her baccalaureate degree at Cornell University and her doctoral degree at Columbia University, supported by public funds. After part-time postdoctoral studies in Chemistry at Florida State University and Biochemistry at North Carolina State University reflecting child-raising responsibilities, she was appointed Assistant Professor of Biochemistry, Associate and Full Professor in 1979, and The University Professor of Biochemistry and Physics in 1988. Recognition she has received includes the O. Max Gardner Award (Univ. of North Carolina), the Olin-Garvan Medal (ACS), and election as a Fellow of the AAAS. Currently she is a Senior Scientist at CHORI (Children's Hospital Oakland Research Institute) and Professor (Adj.) at North Carolina State University and the University of California-Berkeley. Her research interests included the ferritin model for nanomineralization, iron transport through proteins, diiron/oxygen protein catalyst structure/function, DNA and mRNA iron/oxidant-regulated promoters for understanding iron in health and disease.



After early education in a one-room schoolhouse in rural Nebraska, Dixie J. Goss obtained her B.S. degree from Nebraska Wesleyan University and her Ph.D. degree from the University of Nebraska. Following a year of postdoctoral work at the University of Georgia, she returned to the University of Nebraska. She joined the Chemistry faculty of Hunter College, City University of New York, in 1984, was promoted to full professor in 1991, and was appointed the Gertrude Elion Endowed Scholar in 2002. Her research interests include macromolecular assembly, regulation of translation, and kinetics of protein–RNA interactions.

Many different chemical species of iron exist in the environment that are available for biological absorption. Examples include iron–protoporphyrin IX, iron chelates, inorganic iron salts, polynuclear iron, and, at least in animals, iron minerals stored in ferritin.^{5,6} Iron acquisition related to homeostasis is discussed elsewhere in this issue. Studies on iron homeostasis are rarely related to the chemical species of iron used. The working model of iron in transit is Fe(II) bound to chaperone or carrier sites of varying kinetic and equilibrium stabilities. Thus, this review will focus on iron homeostasis independently of iron speciation.

2. Iron Distribution in Cells and Organs

The largest amount of iron in cells is found in protein cofactors such as heme, iron–sulfur clusters, and di-iron or monoiron cofactors. Specialized cells in multicellular organisms and subcellular components rich in proteins with iron cofactors, or proteins that store iron for cofactor synthesis and growth, will contain the highest concentrations of iron. Thus, the distribution of iron in a particular cell type or tissue will depend both on the function and the stage of development.

2.1. Cells, including Bacteria and Archea

In single-celled organisms without recognizable organelles, i.e., most bacteria and archea, iron will be distributed relatively uniformly throughout the cytoplasm either in protein cofactors or in ferritins. However, in bacteria with both cell walls and cell membranes, the cell wall and membrane surfaces and the compartment in between, called the periplasmic space, have multiple sets of iron acquisition and transport proteins because of the scarcity of iron in most environments and the need to use many different chemical species of iron.⁷ The iron distribution in single-celled organisms with internal compartments, such as photosynthetic plastids in *Chlamydomonas* or mitochondria in *Saccharomyces* and *Chlamydomonas*, is uneven with much higher iron concentrations in the organelles. In plastids, for example, high concentrations of iron are needed for the electron transfer by iron sulfur ferredoxins for photosynthesis and, in mitochondria, for the electron transfer by heme proteins of mitochondrial oxidative metabolism. In specialized cells of multicellular organisms, the iron concentration in the cytoplasm is usually lower than in the mitochondria, although in some specialized cells, e.g., mature red blood cells, cytoplasmic hemoglobin has the major amount of cell iron. Nitrogen fixing-nodules in plants are another exception, with large amounts of leghemoglobin in the cytoplasm and iron concentrated in nitrogenase itself in the bacteroids. Ferritins are in plant and animal mitochondria, animal cytoplasm, and plant plastids.

Iron acquisition by mitochondria appears to depend on redundant carriers and transporters as does iron acquisition in single-cell organisms, plants, and likely the intestinal surfaces of animals.^{4,7–9} Among the mitochondrial iron carriers currently known are mitoferrins¹⁰ and frataxin, a small mitochondrial protein which donates iron to the biosynthetic pathways for Fe–S clusters (the ISC proteins)¹¹ and possibly to ferrochelatase, the catalyst for iron insertion into protoporphyrin IX to synthesize heme.¹²

2.2. Organisms

The distribution of iron among tissues or cells in multicellular organisms is not uniform. When the specialized role of a tissue requires large amounts of iron, the cellular iron concentration can be an order of magnitude higher than in a more average tissue or cell. Extracellular iron transport molecules can recognize cell or tissue iron need, which is often communicated by the total number of surface receptors that bind extracellular iron complexed to protein or chelator transporters

2.2.1. Animals

In animals, tissues with specialized functions in iron or oxygen homeostasis have the highest iron concentrations.

The most iron-rich part of animals is the blood, followed by spleen, liver, and kidney. Changes in gene expression maintain iron homeostasis during iron deficiency or iron excess. Some changes in expression of iron homeostatic genes have been known for fifty years and are currently being monitored by customized iron (microarray) "chips".¹³ New genes are being discovered by microarray analyses, positional cloning, and genome mining, reviewed, e.g., in refs 3 and 14. While in blood, the majority of the cellular iron is in the cytoplasm of mature red cells as hemoglobin. In other iron-rich tissues, the majority of the iron is in ferritin. Liver iron is a major body iron storage site for emergencies, e.g., iron loss from hemorrhages. Spleen iron is mainly iron in the process of recycling from old, phagocytosed erythrocytes, since excretion of old iron through the kidneys is precluded by the low solubility of iron. Trillions of liters of water, or gallons or orange juice with citrate, would be required each day if the iron from one day's worth of old red cells were to be excreted in solution.¹⁵ Instead iron from the hemoglobin of old red cells is recycled with ~90% efficiency; some iron is also lost due to sloughing of epithelial cells. The iron recycling process usually takes ~24 h. Ferritin is an intermediate site in macrophages, except during inflammation or iron overload, when hepcidin depresses iron release from macrophages and iron-rich ferritin accumulates. The regulation of iron efflux from spleen and liver by hepcidin, the peptide hormone, is modulated differently than in intestinal enterocytes. The chemical nature of the signaling molecules that trigger hepcidin-mediated changes in iron flux are not yet known. Kidneys, which have high concentrations of iron, have the major function of separating dissolved blood components from excreted metabolites, but the role of the iron in kidneys remains largely unexplored.¹⁶

Iron distribution in vertebrate and some invertebrate animals is mediated by transferrin, a serum protein synthesized mainly in liver. Transferrin is a member of a family of two-domain (two-"lobed") proteins that bind iron extremely tightly, $K_d \approx 10^{-20}$ M; the family includes lactoferrin in human tears and ovotransferrin in egg albumin.¹⁷ A common feature of all the family members is antibacterial activity that relates to the ability to outcompete many bacterial mechanisms for iron acquisition. Serum transferrin is subsaturated (~30%) with ferric iron under normal conditions and provides a buffer for changes in serum iron concentrations. The ferric iron transport activities of transferrin depend on cell receptors where surface numbers relate to iron need in cells. An unusual feature of transferrin receptor-mediated endocytosis, one of the early endocytotic pathways identified, is the high stability of apotransferrin/receptor after iron release that results in delivery of apotransferrin to the cell surface for repeated cycles of iron uptake. Many other carrier proteins, by contrast, are targeted for intracellular degradation, usually in the lysosome. Transferrin binds iron so tightly that delivery without degrading the protein was a puzzle until the structure of the receptor-transferrin complex was obtained.¹⁸ Apparently the acidic pH of endosomes that weakens the iron-protein bond and conformational changes in transferrin induced by receptor binding overcome the stability of the iron-transferrin complex in serum. DMT1 and a ferrireductase, Steap3, participate in the transfer of iron from the iron/transferrin/receptor complex in the endosomes through the endosomal membrane to the cell cytoplasm.^{3,8}

The hepcidin propeptide,^{3,8,19,20} secreted by the liver, is a hormone that coordinates whole body responses to changes in iron and oxygen (inflammatory oxidants). Two pathological conditions with altered iron homeostasis, the anemia of chronic disease and hereditary hemochromatosis, can be traced to abnormal hepcidin metabolism.

The anemia of chronic disease is caused by increased hepcidin and reflects an innate immune response thought to protect the host by diminishing both transferrin bound iron and pathogen iron acquisition. This response has the side effect of causing a mild anemia in the host, an anemia due to a change in the iron distribution within the body. The result is that the red cells are iron-deficient but macrophages have excess iron.

Hereditary hemochromatosis (HH) is associated with a decrease in serum hepcidin that leads to continued efflux of absorbed iron by enterocytes (gut epithelial cells) mediated by the efflux protein ferroportin on the basolateral (serum) surface of the intestine and a defect in sensing body iron accurately. When hepcidin binds to ferroportin, localized iron efflux is blocked and iron-deficiency signals, for which the molecular properties remain unknown, are released. The deficit in iron sensing results in continued uptake of iron from food at the apical surface of the cells even when the amount of iron in the body is normal or excess. As a result, more nutritional iron is absorbed than necessary for iron balance and toxic levels of iron accumulation in tissues, damaging liver, pancreas, and skin. Symptoms of hereditary hemochromatosis appear later in life (young to mid-adult) as iron homeostatic mechanisms are breached, e.g., synthesis and mineralization of ferritin reach a maximum. The cyclical loss of iron in blood usually delays the onset of HH symptoms in women (after menopause). Another name for HH is "bronze diabetes" because of iron damage to the pancreas and iron related pigmentation in the skin. In the case of the red cells, the iron deficiency is real, since macrophage iron recycling is inhibited, but in the spleen and other organs such as skin and pancreas, iron is in excess of normal requirements. The changes in the hepcidin concentrations in disease such as HH, which has a relatively high frequency in populations of Northern European ethnicity, have focused recent attention on the regulation of hepcidin transcription, translation, and secretion.^{3,8,20}

2.2.2. Plants, Including Yeast and Fungi

The most iron-rich parts of plants are the leaves, seeds, and, in the case of nitrogen-fixing legumes, the nodules. Iron homeostasis can be monitored by changes in gene expression.²¹ In the leaves, at different stages of chloroplast maturation, the iron in the plastids is concentrated in ferritin (proplastid and senescent plastids) and in ferredoxins (mature chloroplasts). Seeds accumulate iron for the next generation, the growing embryo, in either ferric chelates (phytates, oxalates) or in legume seeds mineralized in ferritin. In legumes, nitrogen-fixing nodules that form on roots are an unusually iron-rich part of the plant because nitrogenase has 32 iron atoms/molecule and because nodules synthesize (leg)hemoglobin for intranodule oxygen transport. During early nodule development, the need for iron is so great in legume plants that root tissues inoculated with nitrogen-fixing bacteria display iron-deficient behavior.^{22,23} The extra iron absorbed by the root nodules is stored in nodule ferritin before nitrogenase and heme are synthesized.²⁴ During nodule

senescence, when the plant flowers, the accumulated iron is recycled to the leaves and the developing seeds.²⁵

Iron is transported in the xylem and phloem of plants often as small-molecule iron-chelates such as the siderophore, mugineic acid.²⁶ Many new genes required for iron transport have recently been identified in a variety of plants.⁴

3. Iron/Dioxygen Regulated Transcription

The intimate metabolic relationship between iron and oxygen is emphasized by the multiple effects of both iron and oxygen metabolism on the transcription of genes involved in oxygen or iron homeostasis. An example is genetic anemias in humans where the metabolic oxygen deficiency, caused by diminished amounts of hemoglobin and respiratory heme proteins, increases iron absorption even though the body has sufficient, and sometimes excessive, amounts of iron.^{27,28} Another example of the intimacy of iron and oxygen metabolism and signaling is the regulation of genes that respond to low oxygen (anoxia) by degradation of a repressor protein (hypoxia-inducible factor) regulated by iron and oxygen dependent enzyme activity.^{29–31}

3.1. Iron Deficiency and Homeostasis

Decreased synthesis of hemoglobin and the consequent cellular oxygen deficiency, are the major effects of iron deficiency in animals where hemoglobin contains ~85% of the body iron. Iron deficiency affects 30% (2 billion) of the world's human population, even in developed countries where access to suitable foods is unlimited and ~180 million people are iron-deficient. In humans and other animals, the main target to reestablish homeostasis is increased iron absorption through the gut. Increases occur in iron uptake proteins, such as DMT1, iron transport across the gut, and iron efflux to serum transferrin, mediated by increased ferroportin activity. In addition, DMT1 contributes to iron distribution within some cells, such as red blood cells where mutated DMT1 causes decreased hemoglobin synthesis and anemia.³ Humans have a particular weakness in iron homeostasis because changes in kidney excretion/retention are more limited than in other mammals, increasing dependence on regulation of gut iron absorption to maintain iron homeostasis. Heparin regulates expression of gut DMT1, as well as ferroportin efflux activity.^{32,33} Thus, when hepcidin concentrations decrease in iron deficiency,^{3,8,19,20} both intestinal iron uptake (DMT1) and efflux (ferroportin) increase.

The molecular structure of the signals that change hepcidin synthesis in animals are not known, and the pathway is complex. Multiple protein/protein interactions occur in the hepcidin signaling pathway that include bone morphogenetic factor (BMF) protein, which recognizes a DNA promoter sequence in the hepcidin (HAMP) gene,³⁴ and hemojuvelin (HFE 2), an iron regulatory protein that binds to BMF protein and to the neogenin receptor.³⁵ The environmental signals that decrease hepcidin expression are related to oxygen signals since genetic anemias, which create an oxygen deficit with normal or excess body iron, increase gut iron absorption.^{27,28} Moreover, hypoxia or inflammation, which induces many antioxidant responses, changes hepcidin expression: excess iron without anemia/hypoxia increases hepcidin expression.³⁶

Plant iron deficiency induces two types of responses in the roots, the main site for iron absorption in plants.³⁷ The first type of response has three effects: (i) increased iron

solubility with proton pumps to acidify (dissolve) ferric iron in soil; (ii) increased expression of ferric reductases to produce soluble ferrous, ferrous transporters; (iii) increased root surface area (root-hair proliferation). Plants of this type are exemplified by tomatoes, soybeans, and the model for dicots, *Arabidopsis*. The second type of iron-deficiency response in plants is accompanied by increased synthesis of siderophores to chelate soil iron and is exemplified by the grasses. However, there is mechanistic overlap, under some conditions, between the two types of responses. Iron deficiency in plants is called chlorosis because iron limitation decreases ferredoxin synthesis and the coordinately regulated genes for the green magnesium porphyrin, chlorophyll. As a result, leaves of iron-deficient plants are pale green or yellow. During the Renaissance, physicians also used the term chlorosis to describe their patients with iron-deficiency anemia because of their pallor.

3.2. Antioxidant Response Proteins and Inflammation

Oxidant stress and inflammation in animals has two phases. In the most rapid, the acute or phase I response, genes are transcribed at increased rates that encode inflammatory cytokines such as TNF- α or IL-1, IL-6, INF- γ , glucocorticoids, vasopressin, and several serum proteins, e.g., CRP that aids phagocytosis of invading pathogens. Only in phase II of inflammation are genes for antioxidant repair transcribed at increased rates. The genes encoding phase II proteins contain a common DNA promoter element, ARE (antioxidant response element) that is recognized by a protein repressor, Bach 1.³⁸ Transcription is blocked when Bach1 binds to maf-DNA. The result is that Nrf-2 cannot bind to maf-DNA to allow transcription. ARE genes regulated by Bach 1 include: (i) NADPH quinone oxidoreductase I and thioredoxin reductase I, which repair oxidation and increase the concentrations of reductant in the cell; (ii) ferritins H and L, the subunits of the cytoplasmic ferritins, where antioxidant activity is conferred by consuming thousands of iron and oxygen atoms to make the internal iron mineral inside the protein cage; (iii) heme oxygenase1, which degrades heme and releases iron, carbon monoxide and bilirubin; and (iv) the β -subunit of hemoglobin in red cells, which transports dioxygen. All the ARE sequences are regulated by Bach 1, and all the encoded proteins contribute to reestablishing normal iron and oxygen homeostasis.

The homeostatic mechanisms for ARE gene regulation are best understood when heme is the signal. Bach 1/ARE-DNA interactions are regulated through heme in two ways. First, heme binds directly to Bach 1 protein and prevents/reverses Bach 1 binding to DNA.³⁹ Second, heme decreases the amount of Bach 1 in the nucleus,³⁸ possibly entering the nucleus on the types of intracellular heme transporters that have been recently identified.⁶ The mechanism of action of other known ARE gene activators such as *t*-butyl hydroquinone (TBHQ) or sulforaphane, a phenethylisothiocyanate naturally occurring in cruciferous vegetables and studied for potential antitumor activity,⁴⁰ are not known; whether there are possible intersections with the heme pathway are problems for future research.

3.3. Hypoxia Inducible Proteins

Plants respond to decreased oxygen (hypoxia/anerobiosis) with a variety of changes in gene expression, some of which

can relate to iron metabolism.^{41,42} Much of the information on hypoxic stress in plants is derived from studies related to crop production where stress is induced by drought and increases in salinity. The stress response has two phases, a rapid (osmotic) phase followed by a slower (ionic phase). The three types of plant adaptations to salinity are (i) osmotic stress tolerance; (ii) exclusion of Na^+ or Cl^- dependent on the HKT gene family for Na^+ ; and (iii) increased tissue tolerance to Na^+ and Cl^- .⁴³ Molecular knowledge of plant responses to decreased oxygen and to salinity and drought are only partially identified^{43,44} but will play a critical role in the future as growing conditions are changed by the impact of contemporary civilization on the environment.

Animals have a variety of responses to hypoxia that have been studied extensively because of the induced hypoxia associated with surgery and because the hypoxia experienced by rapidly growing tumors is a target for developing new cancer therapies.⁴⁵ Hematological research also has a significant focus on hypoxia because of the induced hypoxia from hemoglobin deficiency.

Responses to hypoxia in animals are coordinated by transcription factor proteins, HIFs (hypoxia inducible factors). One of the HIFs, HIF-1 α , is stabilized during hypoxia.³¹ HIF-1 α interacts with HIF-1 β to allow transcription of genes for a variety of oxygen sensitive reactions. When oxygen concentrations are too high for the oxygen-sensitive reactions, HIF-1 α is degraded and the transcription of genes encoding the oxygen-sensitive proteins is prevented. Many of the genes required for efficient function in hypoxic conditions are controlled by HIF. A range of oxygen affinities among oxygen-sensitive proteins creates a hierarchy of responses to anoxia with HIF-1 α function being the most sensitive.

At normal oxygen levels, HIF- α is degraded rapidly in a cascade of reactions that begin with hydroxylation of a proline residue embedded in conserved sequences called the oxygen-dependent degradation domain (ODD). The prolyl hydroxylase uses iron directly bound to the protein as a cofactor, with dioxygen as one of the substrates and the protein ODD proline as the other.⁴⁶ Once the specific proline residues in HIF are hydroxylated, another protein, VHL (von Hippel–Lindau tumor suppressor gene protein) binds. Since VHL is a subunit of a ubiquitin ligase, VHL/HIF interactions result in ubiquitinated HIF, which target HIF to proteasomes for digestion.^{31,47} When oxygen decreases, the hydroxylases, which bind oxygen more weakly than a number of other oxygen-sensitive proteins, become inactive. The advantage, if any, of synthesizing HIF under normal oxygen conditions, and then degrading it, is not known but may relate to quick responses to changes in oxygen concentration. Since HIF affects transcription of genes in so many processes, including development and inflammation, HIF-responsive genes are targets for drug discovery.

3.4. Iron Excess and Homeostasis

Iron excess in animals increases both serum hepcidin^{3,20} and cell accumulations of ferritin protein; however, the iron content/ferritin protein cage increases as well, indicating that ferritin synthesis is not linearly proportional to cellular iron content. Hepcidin downregulates iron uptake and transport mediated by ferroportin and DMT1, which were identified by using cloning methods available in the 1990s. Regulation of ferritin by iron was understood long before gene profiling because the ferritin minerals inside the protein cage were large enough to be observed by conventional electron

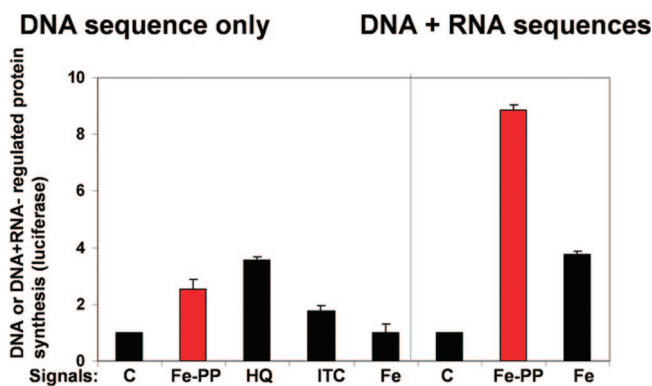


Figure 1. Dual regulation of mRNA and DNA by heme–repressor (bach 1 or IRP) interactions. Luciferase activity, encoded in plasmids under the control of the human ferritin L DNA–ARE promoter or the human ferritin L DNA–ARE promoter plus the mRNA–IRE 5′UTR regulator (promoter), was measured in HeLa cells as described in ref 59. Antioxidant response inducers: Fe-PP, Fe–protoporphyrin IX (heme); HQ, *t*-butylhydroquinone; ITC, 4-methylsulfanylbutyl isothiocyanate (sulforaphane; iron inducer is ferric citrate (1:10)),⁵⁹ and the data are from ref 59 with the error as the standard deviation.

microscopy, reviewed in ref 48, and with biochemical probing,^{49,50} reviewed in refs 15 and 51. Iron responses related to derepression of stored mRNA⁵² were discovered decades ago, using tissues such as liver or embryonic red cells, before cloning techniques emerged where the iron response was very large. In addition, ferritin mRNA was so abundant in embryonic erythrocytes and reticulocytes, that the poly A RNA itself could be isolated and the ferritin mRNA studied without any amplification.⁵² Later, similar mRNA regulatory structures were identified in other tissues and mRNAs and are discussed in section 4. As a result of the unusual mRNA regulation by iron signals, effects of iron on transcription of iron homeostatic genes have been less studied and, in the case of ferritin genes, occur under such extremes of iron excess that oxidative damage or inflammation may be the more significant signals^{54–56} (see Figure 1).

The effects of iron on ferritin gene transcription were clarified when an antioxidant element (ARE) was found in the ferritin H^{57,58} and ferritin L genes⁵⁹ that linked their transcriptional regulation to the antioxidant response genes NAHPH-quinone oxidoreductase, thioredoxin reductase, and heme oxygenase. (see section on antioxidant responses and inflammation). Transcription of ferritin genes was much more sensitive to *t*-butylhydroquinone and sulforaphane than to iron, except iron in protoporphyrin X (heme).^{59,60} Ferritin protein synthesis is unusually sensitive to heme because heme binds both to bach 1, the DNA protein repressor to increase ferritin mRNA synthesis,³⁹ and to IRP1 and IRP2, the mRNA protein repressors, to increase ferritin mRNA translation (Figure 1). The dual genetic targets cause heme to have an unusually large effect on synthesis.⁵⁹

In plants, sensitivity to excess environmental iron is variable for similar concentrations of iron, but whether the differences relate to iron transport in the roots or managing iron in other plant tissues is not clear.⁴ However, iron clearly increases ferritin gene transcription exemplified by soybean, *Arabidopsis*, and *Chlamydomonas*.^{61–63} Since plants can store iron in vacuoles as well as in ferritin, the role of ferritin was not clear until the ferritin contributions to antioxidant responses were identified in *Arabidopsis*.⁶² The antioxidant role of ferritins in animals, bacteria and Archea, and plants

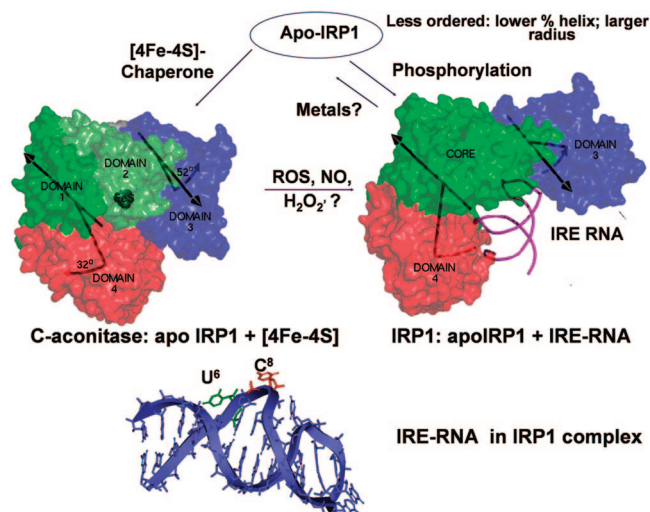


Figure 2. Structure of an IRE RNA and complexed with IRP1. Data are modified from ref 97; apo-IRP has not been crystallized to date and appears to have disordered regions.¹⁵⁴ The ribbon diagram of the IRE-RNA used PDB file 2IPY.

is now clear.^{57–59,62,64} Iron regulation of ferritin genes in *Chlamydomonas* and in maize, which each have two iron-responsive ferritin genes, indicates that one ferritin gene selectively participates in iron homeostasis and the other participates in oxidant protection,^{63,65} emphasizing the role of ferritins in both iron and oxygen homeostasis.

4. Iron/Dioxygen Regulation of Translation

Post-transcriptional changes in the synthesis of proteins encoded in iron homeostasis genes are induced in living cells or animals by iron (solutions of inorganic iron salts or heme) and oxygen (anoxia) and oxidants (hydrogen peroxide). The effect is mediated by noncoding mRNA structures (Figure 2) that bind the IRP repressor proteins, reviewed in refs 14 and 66–69. IRP repressors inhibit ribosome binding and translation when the IRE is in the 5'UTR (Type 1 IRE regulation) or inhibit nucleolytic degradation of mRNA when the IRE is in the 3'UTR (Type 2 IRE regulation). In plants, no evidence for iron regulation targeted to mRNA has been detected.^{41,70} Iron homeostasis in plants, as currently understood, is maintained entirely by changes in transcription⁶¹ and post-translational protein degradation.^{63,65}

4.1. IRE–mRNA Family

Recognition of ferritin mRNA by *trans* factors that regulate translation was demonstrated over 25 years ago^{52,71} and suggested by indirect experiments over 30 years ago.⁵³ Later, cloning and sequencing identified conserved sequences in the 5' untranslated region (UTR) of both ferritin H and ferritin L mRNA that controlled the quiescent pool of ferritin mRNA in the cell and the rapid translational response to abundant iron levels.^{72,73} The UTR sequences, named IRE (iron responsive element), form stable hairpins with a characteristic secondary structure, predicted from thermodynamic studies.⁷⁴ Secondary and higher-order structure was demonstrated in the natural (polyA⁺) ferritin mRNA, by metal nuclease and protein nuclease probing.⁷⁵ Cytoplasmic IRE–mRNA binding proteins, called IRP (iron regulatory proteins) were discovered,^{73,76–78} isolated,⁷⁹ and shown to “footprint” (protect IRE-RNA from degradation) along the entire IRE.⁸⁰ Binding of IRP *trans* translation regulatory

proteins to *cis* regulatory IRE–mRNA structures is analogous to binding of *trans* transcription factors to *cis* promoter elements in DNA.

The IRE/IRP RNA protein complex (Figure 2) coordinately controls iron metabolism by regulating the expression of mRNAs encoding proteins for concentrating and storing iron (ferritin H and ferritin L), for iron uptake (transferrin receptor 1, TfR1 and DMT1), and for iron export (ferroportin). IRE/IRP complexes also control translation of mRNAs encoding proteins of oxidative metabolism [(FeS)-cluster protein, mitochondrial aconitase, heme synthesis protein, erythroid 5'-aminolevulinic acid synthase (eALAS), succinate dehydrogenase (*Drosophila melanogaster*), hypoxia-inducible factor 2 (HIF2)], and phosphate signaling (kinase MRCK α and protein phosphatase, CDC14A and refs 81–83). Two of several recent reviews describing the IRE family are refs 14 and 69.

Mutations in IRP1 or IRP2 or in the mRNA IRE element, extensively studied in mice and humans, reviewed in ref 67, lead to abnormal iron metabolism that is detrimental to cell and organism. The flow of iron into animals is coordinately regulated by four IRE containing genes: (i) DMT1, on the intestinal cell apical membranes for nutritional iron uptake and, in some cells, both iron acquisition and endosomal iron release; (ii) cytoplasmic ferritin (FRT) to concentrate iron; (iii) FPN1, on the basolateral side of gut and other cells for iron export to serum; and (iv) TfR1a, on the basolateral surface of epithelial cells and the surface of other cells that import circulating iron needed for cell metabolism. Differences in IRE structure/function generally result in excess iron increasing FRT, and FPN synthesis (using RNA–ribosome complexes) while decreasing DMT1 and TfR1 synthesis (decreasing mRNA concentration). However, differentiation programs that change DMT1 expression,^{84,85} for example, and environmental signals that selectively target DNA promoters or both DNA and mRNA repressors^{59,86} (Figure 1) combine to differentially influence the expression of each protein (see section 2).

The two mechanisms of regulation in the IRE family of mRNAs reflect the context of the IRE: type 1, when the IRE is in the 5'UTR, controls translation of IRE–mRNA, i.e., ribosome/mRNA binding, and type 2, when the IRE is in the 3'UTR, controls mRNA turnover and controls mRNA abundance. IRP binding, thus, has opposite effects on the synthesis of proteins encoded in 5'UTR or 3'UTR IRE–mRNAs. Whether the IRE–RNA is in the 5'UTR or 3'UTR, IRP 1 and 2 binding activity is high under iron-deficient conditions in the cell (Figure 3). Inhibition of translation by 5'UTR IRE/IRP complexes reflects decreased binding of the 43S ribosome to the mRNA.⁸⁷ Inhibition of mRNA degradation by 3' UTR IRE/IRP complexes permits continued ribosome binding and mRNA translation.⁸⁸ Since 5' IRE–mRNAs encode proteins of iron efflux or storage (FPN and FRT) and 3' IRE–mRNAs encode proteins of iron uptake (TfR1 and DMT1), under iron-deficient conditions coordination of iron flow can be achieved. When IRP activity is low, for example, the combined effects of IRE/IRP interactions decrease iron efflux (FPN) and storage (FRT) and increase iron uptake (DMT1, TfR1) and intracellular iron distribution (DMT1). On the other hand, when iron is plentiful and IRP binding decreases, translation of mRNA encoding iron storage and efflux proteins increases and translation of mRNA encoding proteins for iron uptake decreases because the mRNA is degraded. Intestinal iron uptake by the

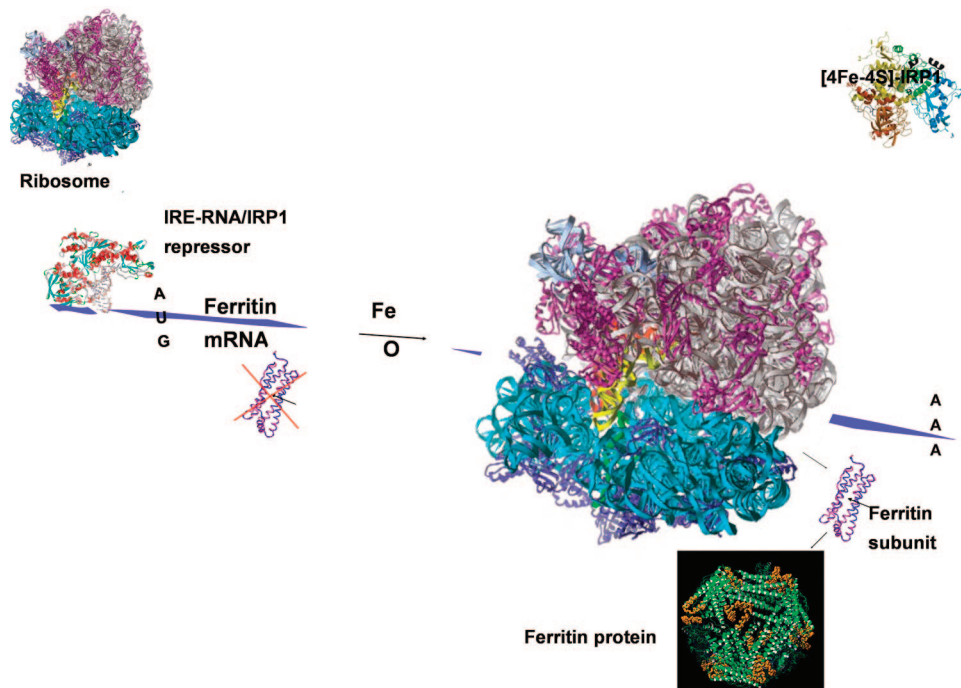


Figure 3. Model for IRE/IRP-regulated translational control. Structures incorporated into the illustration are taken from IRE-RNA/IRP complex,⁹⁷ ribosome,¹⁵⁵ ferritin subunit,¹⁵⁶ and ferritin nanocage.¹⁵⁷

specialized, epithelial enterocyte, key to maintaining human iron homeostasis because of the low ability to excrete excess iron, is regulated by five IRE-containing genes, DMT1, ferritin (FTN)-H, FTN-L, ferroportin (FPN), and transferrin receptor 1 (TfR1).

IRP binds to 30 nucleotides of the IRE RNA regulatory element. The RNA sequence is highly conserved, >90%, for each specific mRNA. However, in a specific organism, the IRE sequences among the IRE-RNA family members are much less conserved with differences up to 40%.^{89,90} The canonical IRE structure (Figure 2) is composed of a 6 nucleotide terminal loop, CAGUGU/C separated by a five base pair helix from an unpaired C residue on the 5' strand of the stem that creates an asymmetrical bulge. The IRE helix below the C bulge has a variable length.^{91–97} Sequence and base pairing around the C⁸ bulge varies among IRE-mRNA family members. In addition to the group of IRE-mRNAs with a single C⁸ bulge, a second group of IRE structures contain an internal loop composed of the unpaired C⁸, and an unpaired base at position 6, separated by paired bases at position 7.^{94,98,99} Helix structure around the unpaired C⁸ is important for selectivity in repressor binding, especially for IRP2.^{93,98,100} Those IRE-mRNAs such as mRNA coding for ferritin and the set of five IRE structures and linkers in TfR1, which have a large distortion around C⁸, form complexes with IRP1 and IRP2 of comparable stability.⁹⁸ In contrast, IRE-RNA structures with a single C bulge, such as eALAS, mitochondrial (mt-) aconitase, or DMT1 IRE-RNAs form more stable complexes with IRP1 compared to IRP2,^{101,102} indicating greater sensitivity of IRP2 binding to distortions in the midhelix region, compared to IRP1. The contribution of the unpaired U in the stem of ferritin IRE RNA was shown by deletion; ΔU^6 IRE-RNA had decreased IRP1 and IRP2 binding, with a much larger effect on IRP2 binding, and also had less IRP-dependent translation repression.^{100,101}

The context of the IRE element varies considerably among members of the IRE-mRNA family. For example, the ferritin IRE-RNA flanking sequences are complementary

and base pair to elongate the base-paired flanking sequence and extend the lower helix of the IRE-RNA to create a regulatory structure near the 5' cap,^{75,80,103} a distance associated with effective translation regulation; disruption of several base pairs in the flanking sequence decreased IRP repression.¹⁰³ A mutation in the IRE structure of L-ferritin mRNA results in unregulated ferritin synthesis, although the consequences are relatively mild: high serum ferritin levels and early onset cataracts.^{104,105} (L-ferritin, a ferritin subunit encoded in an animal-specific gene, has lost residues required for catalysis¹⁰⁶ and contrasts with all other ferritins, which are designated H-ferritin; L ferritin subunits coassemble with H subunits in tissue-specific ratios.) Variations in the stem-loop between the IRE in ferritin and mitochondrial aconitase mRNAs correlate with different IRP binding stabilities and graded responses to iron signals *in vivo*.¹⁰⁷

4.1.1. 5' IRE (Type 1 IRE Regulation)

The 5' UTR IRE-mRNA translation regulator studied most extensively is in ferritin mRNAs. The ubiquitous protein cages of ferritin, containing iron oxide mineral with thousands of iron and oxygen atoms, initiate mineralization by coupling two Fe(II) with dioxygen at protein catalytic sites in the cage. The IRE-RNA has only been found in animal ferritin mRNAs close to the 5' terminal cap and a variable distance from the initiator AUG. IRE-RNA; IRE function is lost if the distance between the IRE-RNA and the mRNA cap structure is more than 60 nucleotides.^{108,109} IRP repressor binding to the IRE under low iron conditions blocks ribosome binding and mRNA translation by preventing contact between the cap binding complex and the 43S ribosomal subunit.⁸⁷ The physiological consequence is a reduction in ferritin synthesis to minimize diversion of iron to storage when iron is limiting.

The mRNA for erythroid δ -amino levulinate synthase (eALAS), the first and rate-limiting enzyme of erythroid heme biosynthesis, is also regulated by IRE-dependent IRP

repression of ribosome binding.^{110,111} In iron deficiency, eALAS synthesis is repressed to maintain constitutive iron metabolism and divert iron away from heme biosynthesis for hemoglobin; the molecular consequence of eALAS repression is iron-deficiency anemia.

Ferroportin (FPN1) mRNA contains a functional IRE in the 5' UTR.¹¹² In FPN mRNA, encoding the protein for iron efflux in the intestine, an IRE binds IRP in vitro.¹¹³ IRP regulation of ferroportin expression,^{114,115} which is complemented by hepcidin regulation of ferroportin activity, demonstrates the linking of the hepcidin and IRE/IRP regulatory systems. The relative contributions of IRE and hepcidin to cell and tissue-specific FPN function are currently subjects of intense study. The two other iron-trafficking proteins encoded in IRE-mRNAs, transferrin receptor 1, which regulates delivery of serum iron to cells, and DMT1, a transporter of divalent cations that include ferrous, have 3'UTR IRE regulatory elements and are discussed with the other 3'UTR mRNAs.

Two proteins of the citric acid cycle,^{116–118} mitochondrial aconitase in mammals and succinate dehydrogenase from *Drosophila melanogaster*, are encoded in IRE mRNAs in the 5' UTR. Mt-aconitase is encoded in an mRNA with a short 5'UTR and an IRE that includes the initiator AUG. When ferritin and mt-aconitase protein synthesis are compared in the same tissue in whole animals, the response to iron is much smaller for mt-aconitase than for ferritin.¹⁰⁷ Such results indicate the selective influence of each IRE-RNA structure on regulation.

Control of the synthesis of proteins encoded in mRNAs with 5'UTR IRE-RNA structures (Type 1 IRE regulation) is closely coupled to oxygen metabolism through the citric acid cycle, an important part of sugar oxidation pathway. In addition, iron linked to oxygen delivery through eALAS synthesis of the oxygen transport cofactor heme in hemoglobin, and ferritin, through consumption of thousands of oxygen molecules in the oxidation of ferrous substrates to form the hydrated iron oxide mineral inside the protein cage.

4.1.2. 3' IRE (Type 2 IRE Regulation)

There are four IRE-RNA structures known in the 3'UTR of mRNAs. The IRE structure in the transferrin receptor 1 was the first identified in ref 88 followed by DMT1,^{102,119} CDC14A,⁸³ and MRCK α .⁸² IRE sequences in the 3'UTR are in mRNAs encoding cellular iron traffic or phosphate signaling. Tfr1 and the splice variant of DMT1 with the IRE, which participate in iron traffic and intracellular iron distribution, have well-known sensitivity to changes in cellular iron. In contrast, the effect of iron on the stability of IRE-mRNAs CDC14A, encoding a phosphatase involved in human cell division and MRCK α , a myotonic dystrophy kinase-related Cdc42-binding kinase α , are less studied.^{82,83} Tfr1 on cell surfaces captures iron on transferrin, and DMT1, and transports low molecular weight Fe(II) and other divalent cations into cells.^{88,102,119,120} Effects of environmental iron of Tfr1 and DMT-1 mRNAs are IRP dependent.

When the IRE RNA is in the 3'UTR, the increased mRNA stability conferred by IRP/IRE complexes increases synthesis of the encoded protein. Tfr1 mRNA contains five IRE stem loops to form the stability element^{74,98,120,121} and, currently, is unique in having more than one IRE-RNA structure. The Tfr1 mRNA IRE structures are embedded in an AU-rich sequence,^{122,123} with an AU-rich element (AURE).¹²⁴ The AURE is commonly found in short-lived mRNAs involved

in growth and cell proliferation and is believed to promote deadenylation and 3' endonuclease degradation of the mRNA.^{124,125} Transferrin was considered a growth factor in the early development of tissue culture media, which reflects the role of iron and transferrin receptors in cell growth and division. IRP binding to Tfr1 mRNA increases the half-life of the mRNA, resulting in increased protein production and iron uptake.⁸⁸ Mouse models support the role of the IRP/IRE regulation in controlling iron uptake in erythroid cells, since mice lacking IRP2 have diminished levels of Tfr1 mRNA, which hampers iron uptake and leads to microcytosis.^{126,127} The lower levels of Tfr1 mRNA in the absence of IRP2 are consistent with IRP2 protection of the mRNA from degradation and also suggest the IRP1 cannot substitute for IRP2 in this system.

Higher-order RNA structure and IRP binding are different for a Tfr-IRE in the context of the five IRE sequences and linkers, compared to a single Tfr-IRE.⁹⁸ Structure of the ~700 nucleotide Tfr1 3'UTR IRE regulatory region is more complex than for any other known IRE-mRNA,^{98,128} and much of the information contained in the multiple loops and linkers remains to be determined.

4.2. IRP1 and IRP2, the IRE-RNA Binding Proteins

IRP1 and IRP2 share 65% sequence identity and are homologous to aconitases, which are widely distributed in nature. The biological advantage of two IRP proteins remains a subject for speculation and study. Clearly the cell specificity of expression is a factor, since the ratio of mRNAs encoding the two proteins varies widely among cell and tissue types.⁹⁸ Gene alterations have given only limited information. For example, targeted disruption of IRP1 gene in mice produces no apparent phenotypic abnormalities,^{129,130} suggesting IRP2 can partially substitute for IRP1. However, disruption of IRP2 leads to changes in iron homeostasis and is characterized by hypochromic anemia, and possibly late onset neurodegenerative disorder,^{126,127,131,132} strain-specific features contribute to the phenotypes. Deficiencies of IRP2 induced by genetic manipulation in mice cause abnormalities in iron metabolism and anemia resulting from defective red cell maturation.^{126,127,133} Disruption of both IRP1 and IRP2 genes is embryonic lethal, thereby establishing the importance of the proteins;^{114,134} retention of both genes and proteins may provide redundancy needed for a critical function. IRP2 lacks residues that confer dual functions on IRP1, leading to the conjecture of a more specialized evolutionary descendent of IRP1.

IRP1 is a bifunctional protein that serves as either a cytosolic aconitase or an IRE-RNA binding protein. Aconitase activity requires insertion of a [4Fe-4S] cluster, which prevents IRE-RNA binding.^{135–139} The apo (no metal cofactor) form of the protein is designated IRP1 and binds IRE-mRNA to regulate translation. IRP2 lacks ligands to form an [4Fe-4S] cluster and thus cannot acquire aconitase activity.^{140,141} Nevertheless, under physiological conditions, IRP2 expression predominates over IRP1 expression in most tissues. When IRP1 is the more abundant IRP protein in a tissue, as in the liver,^{98,130} it is largely in the aconitase form.¹⁴² The predicted structure of IRP2 is similar to IRP1 but with a 73 amino acid insertion near the N terminus.¹⁴³ Crystal structures of IRP1 as an aconitase and in a complex with ferritin IRE-RNA show major structural differences between

the protein folding complexed to the FeS cluster or to the IRE–RNA (Figure 2) and references.^{97,143,144}

The IRE–RNA/protein complex has clusters of 10–12 contacts between the RNA and proteins at two spatially distinct sites⁹⁷ (Figure 2). At each site, bases have been flipped out either from disordered conformation (C⁸) or from stacking over the main helix (A¹⁵ and G¹⁶ in the free RNA) in the protein complex; the long axis of the IRE–RNA helix deviates significantly from that of a typical RNA A-helix.

IRP1 complexed to the [4 Fe-4S] cluster,¹⁴³ i.e., c-aconitase, has the same four contiguous domains as the other aconitases. In the IRP1/IRE–RNA complex,⁹⁷ however, only domains 1 and 2 of the aconitase structure form the central core of the protein (Figure 2). Domains 3 and 4 are extended into an L-shaped complex that embraces two sites on the RNA. IRP1 domains 3 and 4 are separated by more than 30 Å in the RNA complex, while they are contiguous in c-aconitase. Many of the ligands involved in formation of the [4Fe-4S] cluster in aconitase are part of the cavity that contributes bonds to A¹⁵ and G¹⁶ bases of the IRE–RNA terminal loop.⁹⁷ Examination of these structures suggests that direct conversion of the aconitase form of the protein to the RNA binding conformation may not occur. The pathway could involve alternate folding of the less-ordered apoIRP1 and proceed through an intermediate involving the less structurally organized apoprotein (no [Fe–S] cluster, no RNA).

Changes in both iron and oxygen homeostasis alter IRP1 and 2 activity and concentration, which influences repression of 5'UTR IRE–mRNAs and stabilization (turnover repression) of 3'UTR IRE–mRNAs. There are a number of signals that disrupt the [Fe–S] cluster in cytosolic aconitase and change the distribution between the enzymatic and RNA binding forms. Reactive oxygen species and reactive nitrogen species both disrupt the [Fe–S] cluster.^{68,145,146} When IRP1 and c-aconitase protein expression are compared in the same tissue, IRP1 appears to dominate under conditions where the [Fe–S] cluster is inhibited, such as iron deficiency or in the human neurodegenerative disease, Friederich's ataxia.^{147,148}

Much less is known about the structure of IRP2 than IRP1 since IRP2 is less stable when isolated. IRP2 binding to IRE–RNA predominates under physiological oxygen conditions where the [FeS] cluster in aconitase is stable.^{98,130} Both proteins, apo IRP1 and IRP2, undergo iron-dependent degradation and the degradation of both proteins is stimulated by heme binding.^{141,145,149,150}

Phosphorylation of IRP1 and IRP2 links iron homeostasis to phosphorus-regulated pathways. IRP1 is preferentially phosphorylated compared to apo-IRP1. Moreover, phosphorylation prevents insertion of the FeS cluster and formation of c-aconitase, which increases the fraction of IRP1 available for RNA binding.¹⁵¹ IRP2 is also a target for phosphorylation, and phosphorylation directly increases the RNA binding affinity.¹⁵² Thus phosphorylation increases RNA binding by both proteins, either indirectly, by increasing the amount of IRP1 protein in the RNA-binding form, or directly, by increasing the IRP2 binding affinity for IRE–RNA.

4.3. Mechanisms of Translational Regulation

Cellular iron levels are balanced by the coordinated expression of proteins involved in iron uptake, export, storage, and distribution. Genetic control is exerted at multiple steps, but balance is predominantly achieved by the

IRE/IRP regulatory network. While repression of mRNA translation is known to occur by binding of IRP1 or IRP2 to 5' UTR, and IRP binding inhibits ribosome binding, the step(s) in assembling the initiation complex that are blocked remain obscure. While the requirement for proximity of the IRE and the mRNA 5' cap moiety is known and IRP inhibition of eIF4F protein/mRNA binding is demonstrated,^{108,109} the molecular basis for communication among mRNA cap, IRE–RNA, and/or the binding proteins remains elusive. Since binding of eIF4F to the cap residue is thought to be the rate-limiting step for initiation of protein synthesis, it is interesting to speculate that binding of the eIF4F in the presence of IRP is necessary for a rapid response to changes in cellular iron, allowing immediate activation of the mRNA for translation once the IRP is released.

The mechanism of release of the IRP from the mRNA has been little studied. In the case of IRP1, the conformation of the protein bound to [Fe–S] cluster precludes IRE–RNA binding because of both the protein conformation and the fact that some FeS cluster ligands are also RNA ligands.^{97,143,144} The crystal structure of the ferritin IRE–RNA bound form of the protein raises questions about the mechanism of dissociation of the protein from the RNA that is different from simply [FeS] cluster displacement of the RNA. In the crystal structure, a large IRE–RNA surface is available for binding of additional proteins, small molecules, or metal ions that can change the IRE and/or protein conformation for dissociation and subsequent insertion of the [Fe–S] cluster into the protein. Phosphorylation/dephosphorylation is a possible mechanism for regulating the stability of the IRE–RNA/IRP complex.^{145,150,152} In addition, preliminary data indicate that metal ions known to bind in the region of the RNA exposed in the IRP1/IRE–RNA crystals weaken the IRE–RNA/IRP1 interaction in solution (Goss, Khan, Walden, and Theil, to be published), suggesting a direct role for metal ions in the dissociation of the mRNA/repressor protein complex.

Selective IRP binding to different IRE–RNAs may modulate IRE/IRP activity, as suggested by differences in the % IRE–RNA bound by IRP1 and IRP2 in Electrophoretic Mobility Shift Analysis (EMSA).^{101,102} The IRE context, which varies among IRE family members, may contribute to the fact that the conserved IRE sequence and context differences coincide with a range of IRE/IRP stabilities in vitro.^{101,102,153} For example, the iron response of ferritin synthesis, which is much larger than mt-aconitase synthesis, coincides with greater ferritin IRE–RNA/IRP stability than the mt-aconitase IRE–RNA/IRP complex, and both context and the structural differences in the two IRE–RNA structures.^{101,102} The ferritin IRE–RNA, for example, is flanked by sequences that base pair and together bring the structure closer to the cap. Moreover, changing the ferritin IRE–RNA structure by deleting the single unpaired U⁶ in ferritin IRE RNA decreases the stability of the IRP1 or IRP2 complex and also decreases the translational repression.^{100,101} Finally, IRP2 binding to a single TfR1–IRE is weaker than to the same TfR–IRE in the context of the native RNA linkers and the other four other TfR1–IRE structures in the full 3'UTR regulatory element.⁹⁸ In spite of such clear relationships between IRE–RNA structure and function for a few IRE–mRNAs, the full extent to which the IRE mRNA context and IRE structure contribute to IRP binding selectivity remains only partly explored.

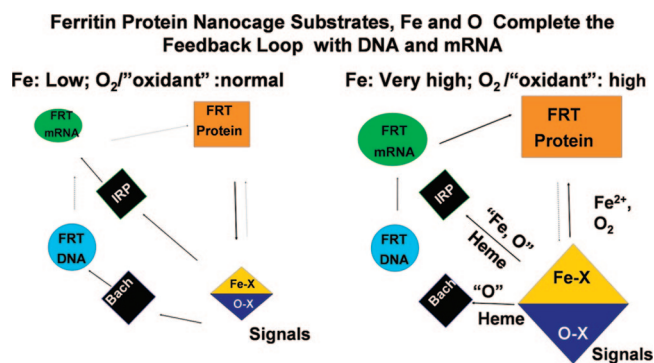


Figure 4. Links between oxygen and iron homeostasis through ferritin: The Fe/O feedback loop. Frt = ferritin; IRP = iron regulatory protein, binds ferritin IRE–RNA; IRE = iron responsive element; bach-1 = a transcription factor that binds ARE–DNA; ARE = antioxidant response element.

5. Summary and Perspective: Links between Iron and Oxygen Homeostasis

The overlap between iron signals and oxygen signals in regulating genes of iron storage and trafficking is an underlying theme in iron homeostasis. Iron and oxygen signals directly coordinate the synthesis of ferritin for iron storage, and antioxidant responses use common genetic targets. Ferritin shares the ARE DNA sequence with other antioxidant response proteins such as NADPH quinone reductase, thioredoxin reductase, and heme oxygenase; ARE DNA is regulated by the heme binding transcription factor, bach1. Ferritin also shares the IRE–RNA sequence with iron-trafficking proteins ferroportin, transferrin receptor 1, and DMH, using heme-binding IRP proteins as well as the heme biosynthetic protein eALAS. IRE–RNA is regulated by heme sensitive IRP1 and IRP2; a form of IRP1 with an FeS cluster is also oxygen sensitive. Moreover, some IRE–mRNAs also have indirect responses to changes in iron and oxygen/inflammation, mediated by the serum peptide hormone, hepcidin. Since ferritin protein consumes iron and oxygen as substrates, the iron and oxygen signals that induce ferritin mRNA and ferritin protein synthesis are depleted when more ferritin protein is synthesized, creating a feedback loop (Figure 4). Determining the “gain” for each step in the feedback loop, the molecular identity of the iron and oxygen signals and the full pathway from environmental iron to protein repressor dissociation for the combined DNA–ARE and mRNA–IRE sequences that activate ferritin transcription and translation are future directions of investigation.

Regulation of mRNA translation and degradation by the IRP/IRE network is the first example of coordinated mRNA regulation. The IRE–mRNA system controls the synthesis of key enzymes and transporters for iron homeostasis. While much progress has been made in understanding the structural basis for IRP1 recognition of IRE–RNA, the significance of the differences between IRP1 and IRP2 remain relatively unexplored. The full functional effects of different IRE–RNA secondary and three-dimensional structural properties, e.g., the helix bulge, the base pair sequences, and the IRE context, need to be learned. How IRP is released from the repressed IRE-containing mRNA is not understood, although the development of small molecules selectively targeted to specific IRE–mRNAs requires such knowledge. The results of such understanding can lead to therapeutic interventions that will recruit the ferritin mRNA that is unused (~50%) during iron overload to minimize toxic hemosiderin ac-

cumulations. (Hemosiderin is ferritin that is degraded when the amount of iron in the nanocages is abnormally high; normally ferritin cages are only 1/3 full. Since the average iron content/ferritin protein cage increases during iron overload, there is a mismatch between increased ferritin synthesis and increased amounts of iron that could be alleviated if unused ferritin mRNA were translated. Damaged ferritin, i.e., hemosiderin, is a site for redox chemistry that produces reactive oxygen and oxidative damage.) How phosphorylation of the eIF proteins and/or the IRP proteins alter IRE/IRP interactions is only partly understood. Finally, the interplay of the IRE/IRP network with hepcidin and other effectors remains to be fully elucidated.

Many of the questions about the targets for iron and oxygen signals that regulate iron homeostasis have been answered. It is clear, for example, that oxidants and possibly oxygen itself maintain iron status by coordinating transcription of antioxidant genes, including ferritin; oxygen also regulates hepcidin, a peptide hormone that controls iron distribution. Iron and oxygen responses also depend on noncoding mRNA structures that coordinate mRNA function, translation, or degradation, among a group of iron-trafficking proteins in addition to ferritin. The identity of some of the DNA and RNA genetic elements is known, ARE promoter sequence in ferritin DNA, e.g., and the IRE regulatory structure in mRNAs. Several RNA and DNA repressors important in iron homeostasis have also been identified such as ferritin DNA–ARE binding protein bach 1 and the IRE–RNA binding proteins IRP1 and 2. Structural information for IRP repressor complexed to an IRE–mRNA regulatory structure is available, but others are needed to fully understand mechanisms of type 1 (5'UTR IRE) and type 2 (3'UTR) regulation. Moreover, questions about mechanisms remain abundant. For example, how fast do the DNA–ARE/bach1 and IRE–RNA/IRP complexes dissociate? How do the repressor DNA or RNA complexes prevent transcription factor/polymerase activity or translation factor/ribosome binding? Does hepcidin only regulate cells expressing the iron efflux protein ferroportin, or are there other protein targets or other hormones that control iron efflux in other cell types? Finally, what are the molecular identities of the iron and oxygen/oxidant signals? Do the signals include hydrated inorganic species such as ferrous iron, hydrogen peroxide, or dioxygen? What is the role of small iron complexes or protein chaperones? Do the oxidant signals diffuse through the cells, or are they transported on chaperones/sensors? The answers to the questions, which can extend beyond bioinorganic chemistry of iron proteins to other metalloproteins and organic cofactor proteins, will increase understanding of iron and oxygen homeostasis and life in air.

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