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Review

Cellular regulation and molecular interactions of the ferritins

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Abstract. Controlling iron/oxygen chemistry in biology depends on multiple genes, regulatory messenger RNA (mRNA) structures, signaling pathways and protein catalysts. Ferritin, a protein nanocage around an iron/oxy mineral, centralizes the control. Complementary DNA (antioxidant responsive element/Maf recognition element) and mRNA (iron responsive element) responses regulate ferritin synthesis rates. Multiple iron-protein interactions control iron and oxygen substrate movement through the protein cage, from dynamic gated pores to catalytic sites related to di-iron oxygenase cofactor sites. Maxi-ferritins concentrate iron for the bio-synthesis of iron/heme proteins, trapping oxygen; bacterial mini-ferritins, DNA protection during starvation proteins, reverse the substrate roles, destroying oxidants, trapping iron and protecting DNA. Ferritin is nature's unique and conserved approach to controlled, safe use of iron and oxygen, with protein synthesis in animals adjusted by dual, genetic DNA and mRNA sequences that selectively respond to iron or oxidant signals and link ferritin to proteins of iron, oxygen and antioxidant metabolism.

Key words. Iron; oxygen; mRNA regulation; DNA regulation; ferritin.

Introduction

The dependence of aerobes on iron and oxygen for life is an apparent paradox in biology. On the one hand, both elements are absolutely necessary for central reactions in respiration. On the other hand, iron and oxygen chemistry produce free radicals that damage DNA, lipids and proteins, and have been implicated in the etiology of cancer and aging [1]. As a result, cellular iron is tightly regulated and compartmentalized by nature to maximize controlled reactions with oxygen in cells, just as gasoline is compartmentalized to maximize reactions with oxygen in combustion engines. The ferritin family of nanocage proteins is central to the natural regulation of iron in cells. Thousands of iron atoms combined with oxygen atoms are concentrated inside the proteins as solids that overcome the 10^{14}-fold gradient between iron concentrations in ferritin and the aqueous environment to provide physiological iron concentrations for biochemical reactions. In addition, excess iron is trapped with oxygen in the ferritin mineral to minimize radical chemistry and reactive oxygen species (ROS) [2]. Deletion of a ferritin gene is lethal in mice [3], and several human diseases are associated with mutations in ferritin [4, 5]. In animals, ferritin occurs as a mixture of subunits comprised of the more generic H type, and the animal-specific L type. When assembled, ferritin with each type of subunit mixture differs in the rates and mechanisms for mineralizing iron [6–10]. *In vivo,* each cell type synthesizes a specific ratio of H-ferritin and L-ferritin protein subunits during differentia-

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tion; the H:L protein subunit ratio is usually stable except during chronic iron overload [11–13]. H-ferritin subunit proteins have multiple catalytic sites, one/H-subunit, that oxidize and couple two ferrous atoms within milliseconds, >80,000× faster than an L subunit protein, reviewed in reference [8]. The products of the active site in animal H-ferritins are a diferric-oxo mineral precursor and hydrogen peroxide [10, 14]; the peroxide product is both a genetic signal [15–17], and a dangerous metabolite causing radical chemistry and cell damage. The role that hydrogen peroxide, generated during ferritin mineralization, plays in cell homeostasis is not clear, but could relate to the evolution of the animal gene for catalytically inactive L-ferritin to avoid overwhelming antioxidant response systems. The cell specificity of the H:L protein subunit ratio could reflect, for example, part of the cell-specific control of hydrogen peroxide concentration and signaling.

A multi-layered system of genetic controls has evolved to regulate ferritin synthesis under the control of both iron and oxygen, which emphasizes the key role played by ferritin in controlling iron and oxygen interactions. Both H-ferritin and L-ferritin synthesis are known to be controlled at the level of transcription and translation in response to iron and oxygen, creating an intricate system of regulation and revealing the importance of sensitive signal responses for ferritin expression.

Cellular regulation of ferritin transcription

Most studies examining transcriptional regulation of ferritin have focused on H-ferritin genes. In the early studies of Torti and colleagues, cytokine regulation was the focus (reviewed in [18]). Where they observed that induction of H-ferritin by tumor necrosis factor α [19] and interleukin 1α [20], required an nuclear factor κB (NF-κB) sequence located 4.8 kb upstream of the transcription start site [21]. Others showed differentiation-dependent increases in transcription rate required a *cis*-acting NF-Y sequence 0.077 kb upstream of the transcription start site [22] or thyroid hormones [23]. In contrast, c-myc decreased H-ferritin expression [24].

Both oxidants and antioxidant response inducers regulate H-ferritin gene transcription, in addition to cytokines and hormones. For example, hydrogen peroxide activates the murine gene [25]. Antioxidant response inducers (phase II enzyme-inducing compounds) such as *tert*-butylhydroquinone, oltipraz and 1,2-dithiole-3-thione have similar effects [26]. The DNA sequence responsible for the hydrogen peroxide response is an antioxidant responsive element (ARE), 4.1 kb upstream of the transcription start site. Deletion of the H-ferritin ARE promoter sequence ablated the transcriptional response to the oxidant stressors [26]. The ARE is found in the promoter of several antioxidant response and detoxification genes such as thioredoxin reductase and quinone reductase and allows for a concerted defense against oxidative insults.

Recent studies from our lab [27] on the L-ferritin gene identified a functional ARE 1.4 kb upstream from the transcription start site which contrasts with the H-ferritin gene where the ARE is located at 4.1 kb [26]. The ARE in the human L-ferritin gene has high homology to other functional AREs [27], and also contains a Maf recognition element (MARE) subdomain, which is known to regulate heme-responsive genes through the heme binding repressor Bach 1 [28]. ARE inducers which activate transcription of L-ferritin include those for the murine H-ferritin gene and other antioxidant response genes such as *tert*-butylhydroquinone or sulforaphane [27]. Quantitative effects of the antioxidant response inducers of the human L-ferritin gene were comparable to thioredoxin reductase and quinone reductase [27, 29]. Iron inducers (ferric ammonium citrate) had little effect on the DNA-ARE, in contrast to antioxidant response inducers for other DNA-AREs, or iron for the iron responsive element (IRE) messenger RNA (mRNA) regulator. The antioxidant inducer *tert*-butylhydroquinone had little effect on the IRE, indicating that the responses of the DNA and mRNA regulatory elements to iron and oxidant signals were selective and complementary [27].

Iron induction of L-ferritin gene transcription only occurs after chronic or acute exposure to very high iron doses, and has been observed using nuclear run-on assay in HeLa cells and rats [12, 30], and Northern blots in rats and frogs [11, 13]. The effects of high concentrations of iron on ferritin mRNA concentrations, shown by using microarray analyses, were a 1.5-fold increase in L-ferritin with no change in H-ferritin [31]; treatment with the iron chelator deferoxamine decreased L-ferritin mRNA 1.5-fold. The iron effect was specific to the L-ferritin subunit; the mechanism for the differential regulation by iron of L-ferritin and H-ferritin is not known.

Hemin (ferric-protoporphyrin IX chloride), which induces MARE/ARE genes such as heme oxygenase and β-globin [32, 33], affected ferritin expression in cultured cells [22, 34–36]. In our recent studies we showed that the hemin effect on transcription required the native MARE/ARE sequence and was iron independent, since the results for hemin and protoporphyrin IX were comparable [27]. Moreover, when both the MARE/ARE and the mRNA regulator, the IRE, were present, the hemin response was synergistic, possibly because of direct effects of hemin on DNA and mRNA protein repressors [27, 37, 38]. Ferritin linked to other iron genes such as ferroportin and the transferrin receptor by the mRNA-IRE, and to oxygen and antioxidant responses genes by the DNA-ARE, is at the center of iron and oxygen regulation.

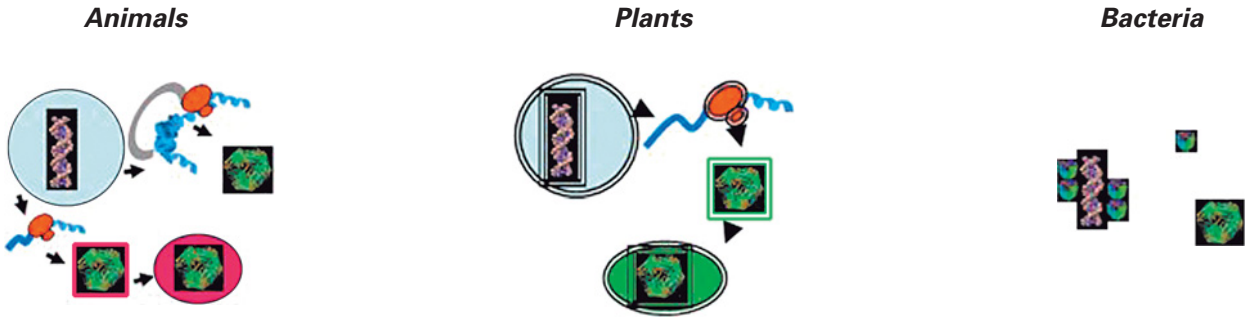
Cellular regulation of ferritin mRNA translation

Ferritin synthesis rates respond to changes in cellular iron and oxygen, or oxidants in plants, animals and bacteria [18, 39–43] (fig. 1). However, the dual control points for synthesis of ferritin protein, transcription (concentration of mRNA) and translation (utilization of mRNA for protein synthesis), appear at this time to be specific to cytoplasmic ferritin in animals. Thus, the genetic mechanisms and the gene structure vary, as well as the cellular location of the ferritin protein itself, even though the signals and the nuclear gene product (ferritin protein) are conserved (fig. 1). Whether organelle/nuclear cross-talk eliminates the need for mRNA control, or whether incorporation of information for organelle targeting overrides the mRNA control, is not known.

In animal ferritin mRNA, a specific 3D loop-helix-loop-helix structure called the IRE is defined by sequence analysis, nuclear magnetic resonance (NMR) spectroscopy and chemical probing [44–47]. The IRE structure, through binding a protein repressor, IRP-1 or IRP-2, controls rates of ribosome binding to ferritin mRNA. Synthesis of a group of proteins for iron or oxygen metabolism is controlled by variant, mRNA-specific IRE structures, which create a hierarchal/graded set of responses to iron or oxygen signals [48]. Ribosome binding is regulated when the IRE is in the 5′ untranslated (UTR) region, by interfering with eIF4F/ribosome interactions [49], but when an IRE is in the 3′ UTR, embedded in AURE sequences as in the transferrin receptor mRNA, protein synthesis is controlled through degradation/turnover of the mRNA. The family of mRNA-specific IRE structures, and the iron-responsive element binding proteins 1 and 2 (IRP-1, IRP-2), repressors of translation or degradation, form a natural, combinatorial array of RNA/protein complexes [48] where responses to the same signals vary quantitatively.

Interactions between the IRE and IRP-1 or -2 reflect cell concentrations of iron, hydrogen peroxide, nitric oxide and dioxygen, as these inorganic compounds or elements influence the formation of mRNA/repressor complexes. Sensing mechanisms for IRP-1 and -2 are not fully understood but have a number of potential links to other metabolic systems. For example, phosphorylation, a com-

Ferritin Regulation and Subcellular Distribution



	Protein Location*	Gene regulation	Signals
Animals	Cytoplasm, Mitochondria	DNA, mRNA	Fe, O_2, H_2O_2
Plants	Plastid	DNA	$Fe^{3+}(Cit)_6$ or Fe^{3+} + ascorbate
Bacteria	Cytoplasm, DNA complex	DNA	Stress, Starvation

* All cells, varied with development/environment

Figure 1. Comparison of the genetic target and signals that regulate ferritin synthesis in plants animals and bacteria. Modified from X. Liu, K. Hintze, B. Lonnerdal and E. Theil [Biochemical Research, in press]. Symbols: blue circles, nuclei with double-stranded DNA; red squares, ferritin (animal mitochondrial precursor); red circles, animal mitochondria; green squares, plant plastid precursor; green ovals, plant plastid or in the cytoplasm; complexes of blue helices with red ovals, mRNA/ribosome complexes; grey circle and blue stem loop, IRE (iron responsive element)/IRP (iron regulatory repressor protein) complex of ferritin mRNA. In bacterial cells, ferritin is shown in the cytoplasm along with mini-ferritin (Dps protein); mini-ferritin is also shown complexed to DNA as in bacterial chromatin.

monly used regulatory signal, was shown by Eisenstein to alter formation of a FeS cluster that inhibits RNA binding and confers aconitase activity on the protein [50, 51]; IRP-1 also binds heme [37, 52]. The heme precursor, δ-aminolevulinic acid, has been shown to promote IRP-1 in RNA binding activity [53] which suppresses ferritin translation.

IRP-2/IRE interactions are regulated by protein turnover, in apparent contrast to IRP-1, and are dependent on an iron or oxygen-dependent ubiquitination and proteasomal degradation pathway. A 73-amino acid sequence in IRP-2 absent in IRP-1 was, at one time, considered as a site for modification by iron or heme binding [54, 55], but elimination of the domain had little effect on iron or heme-mediated IRP-2 degradation; mRNA binding was altered, however [56]. An alternative IRP-2 degradation mechanism, particularly important during anoxia, recently studied by Leibold and co-workers, is an iron- and oxygen-dependent 2-oxo-glutarate hydroxylase [57]. In another oxygen-related study, Rouault and co-workers, using macrophages isolated from either IRP-1 or -2 knockout mice cultured in either 3 or 21% oxygen, observed altered L-ferritin expression in 3% oxygen when cells lacked IRP-2 but not IRP-1 [58]. Such data suggest that IRP-1 and -2 can complement each other at high oxygen tension, but that IRP-2 is the only functional repressor at low oxygen tensions. Redundancy of IRP-1 and -2 functions may only be relevant in cell types exposed to the higher levels of oxygen, and indicates the importance of oxygen as a signal in ferritin expression, a conjecture supported by antioxidant response elements in both H- and L-ferritin genes.

Oxidative stress can also alter IRP/IRE interactions. For instance, IRP-1 RNA binding is diminished by xanthine oxidase-generated oxidative stress in rat liver lysates [59]. However, the opposite effect has been observed using intact cells and the hydrogen peroxide generator glucose oxidase, and was preventable by protein phosphatase inhibitor treatment, indicating cell- or membrane-dependent variation and sensitivity [60]. Both IRP-1 and -2 can be phosphorylated by protein kinase C, which increases IRE binding [61]. An example of oxidative stress that alters the IRE/IRP-1 complex occurs when cells are exposed to hydrogen peroxide. IRP-1 exists without or with a peroxide-sensitive FeS cluster that, when disrupted, converts IRP-1 to the mRNA binding and explains, at least in part, observations of increased IRP-1 RNA binding after exposure to hydrogen peroxide [60].

The IRE-mRNAs are a family that encodes proteins of iron metabolism, with common, noncoding regulatory elements that are very highly conserved phylogenetically (95%), but which have mRNA-specific variations (~70–85% sequence conservation) among the IRE sequences in a single organism [48]. The mRNA-specific differences in the IRE family affect IRP-1 and -2 binding [62–64] and relate to bulges or loops in the middle of the IRE helix; the specific folds observed *in vitro*, can also be observed by IRE 'footprinting' *in vivo* [65]. IRE/IRP interactions display selectivity analogous to interactions of combinatorial DNA promoters with repressors [66]. Higher-order structural features of some IRE-mRNA structures have been revealed by NMR spectroscopy [45–47] and nucleases sensitive to RNA folds (reviewed in reference [48]). IRP-2 binding is more selective than IRP-1, and is particularly sensitive to the structure in the middle of the base-paired stem, the bridging base pair across the terminal loop and flanking regions [62, 64, 67]. Specific base pairs in the stem contribute smaller effects than changing the loop or bulge/loop structures [68–70]. Additional effects of RNA context are known, such as base-paired flanking regions [71], but the full picture of the contribution of IRE-mRNA structure itself to regulation remains to be developed. The differences among the mRNA-specific IRE structures corresponded to differences observed in sensitivity to iron *in vivo* [72].

Structure and function of the ferritin family

Ferritins share a unique protein cage structure, 8–12 nm in outer diameter [7, 8], that most closely resembles small, spherical viruses. How nature encodes the information for assembly of protein subunits into hollow cages is not fully understood. But in the case of ferritin, the structure has been preserved throughout evolution, even though the primary sequence can vary so much as to be almost unrecognizable. Bacterial ferritin from *Escherichia coli*, for example, was thought to be completely unrelated to eukaryotic ferritins, but has a similar crystal structure and function to human ferritin [73]; eukaryotic ferritins share 65–90% sequence identity. In the case of bacterial mini-ferritins or Dps proteins [8], which were named for DNA protection during starvation, intra-genus sequence can be as high as 85%, e.g. in *Bacilli;* however, the conservation of *E. coli*/*Bacillus anthracis* sequence is only 28% [X. Liu and E. C. Theil, unpublished results]; the secondary and quaternary structures of all Dps proteins are very similar (see [8, 74, 75] for a review and e.g. [74–78]).

Polypeptide subunits of ferritin spontaneously fold into four helix bundles and assemble into hollow spheres with inner cavities 5–8 nm in diameter, depending on whether there are 12 (mini-ferritins) or 24 (maxi-ferritins) subunits. When there are only 12 subunits in a ferritin, as in the bacterial Dps mini-ferritin proteins, the catalytic site for iron oxidation and hydrogen peroxide or dioxygen reduction is created between two subunits with residues contributed by each subunit [75–78]. In the case of maxi-ferritins, whether in bacteria, plants or animals, the reaction sites are within each subunit, buried deep within the

helix bundles, and with iron residues contributed by each helix in the subunit. A gene duplication in animals encodes the catalytically inactive L-ferritin subunit, a protein with a very different charge distribution [8] created by an apparently small number of changes in amino acids [9]. Each tissue has a specific ratio of H:L subunits in ferritin proteins [6–8].

The three types of iron sites identified in ferritins are: (i) ferroxidase, (ii) nucleation and (iii) iron entry/exit pores [7, 79]. Catalytic activity is a property of mini- and maxi-ferritins and both form ferric-oxy minerals. Kinetic products of the Fe^{2+} oxidation reactions and the acceptors of the reactions between substrates appear to differ [80–83]. The ferroxidase sites in maxi-ferritin subunits, which occur in the center of the helix bundle of ferritin-H subunits in animals, plants and bacteria [6–8], have been mainly characterized in cytoplasmic ferritins of animals. There, the catalytic site couples two ferrous ions with dioxygen, via a diferric peroxo intermediate, e.g. [68], to form two products: a diferric oxo/hydroxo precursor of the ferritin mineral and hydrogen peroxide [8, 10, 14, 68, 84, 85]. Peroxide can be a signaling molecule [15], and might influence the increased expression of the animal-specific L-ferritin subunit, which increases during chronic iron overload [11–13].

The ferroxidase activity of maxi-ferritins has been studied extensively by our group and those led by Arosio, Chiancone, Chasteen and Harrison. In eukaryotic ferritins, the catalytic site binds two iron atoms, one each in sites A and B, which are about 15 Å from the entry pore. The path taken by iron ions to the ferroxidase site is unknown. But the distance is traversed rapidly (ms) [9]. Site A, in maxi-ferritins, has three invariant amino acid residues contributed from helices A, B and C (E23, E58, H61, or E27, E62 and H65 in an alternate numbering system). In a number of other di-iron sites such as those in the related oxygenase families, exemplified by ribonucleotide reductase, Δ9-fatty acid desaturases and methane monoxygenase, both iron atoms are bound by E,ExxH, ligand sets, and the iron remains at the active site as a cofactor. In ferritins, by contrast, iron is a substrate that leaves the active site; the second iron site, site B, is much weaker. Site B has three invariant amino acids, E58, E103, and Q137, plus a fourth iron ligand at residue 140 that is variable; the relationship of residue 140 to Fe has been observed in ferritin chimeras [9] and in other metal ion co-crystal structures [86–88]. Examples of the distribution of residue 140 in natural eukaryotic ferritins are D in *Arabidopsis* or frog liver, A in human liver, pig, bean, rice or maize, and S in soy, frog, rat, mouse cytoplasm and human/mouse mitochondrial ferritins. The variation in residue 140 of site B in the ferritin ferroxidase site explains at least in part the observed kinetic differences between ferritins *in vitro* [9, 85, 89–91]. While the physiological significance of the different active sites in H-ferritin subunits at residue position 140 remains unknown, the cell or organelle specificity of expression [11, 92] indicates a biological role.

In the mini-ferritins, each ferroxidase site is formed by amino acids from two subunits, at the subunit dimer interface facing the mineralization cavity [74–76, 81]. The sites have been less well characterized than in eukaryotic ferritins [82], but it is clear that the iron and oxygen chemistry appear to have different physiological roles in the two proteins based on the oxidative sensitivity of Dps mutants. In maxi-ferritins, especially in eukaryotic maxi-ferritins, oxygen is used to concentrate and mineralize iron, both of which are substrates. In bacterial Dps mini-ferritins, iron is used to detoxify oxygen (or hydrogen peroxide) [81–83]. The ferroxidase site Fe ligands are currently being studied, e.g. [82]. How the iron moves from the entry pores to the ferroxidase sites, from the ferroxidase sites to the mineralization cavity and from the mineral in the cavity to the exit pores remains a mystery. The active sites in the mini-ferritins are just beginning to be probed by mutagenesis [82], and the mechanisms for the switch between dioxygen hydrogen peroxide electron acceptors is under current investigation.

Nucleation sites in ferritins are clusters of conserved carboxylate residues that face the inner cavity of ferritins, where the mineral forms. In maxi-ferritins, substitution of the nucleation site carboxylate with alanine in L-ferritin subunits completely inactivated protein-dependent mineralization [9, 93]. However, in H-ferritin subunits with catalytically active ferroxidase sites, the mineralization rates were unchanged by mutations at the nucleation sites [94], presumably because formation of the diferric mineral precursors at the ferroxidase sites was sufficient for mineralization at normal rates. Future experiments will be required to fully define the routes taken by iron after leaving the ferroxidase site.

Gated pores through which iron, thought to be Fe^{2+}, enters and leaves the protein nanocage are formed by six sets of helix segments, a pair from each of three subunits, which create eight equivalent, gated pores in maxi-ferritins and four pores in mini-ferritins (fig. 2). Mutation analysis has shown that the pore residues D127 and E130 [95, 96] in maxi-ferritins control entry of ferrous iron to the di-iron catalytic sites, but there are no crystal structures of the mutant proteins. In contrast, R72/D122 and L110/L134, the pore gates for iron exit, control electron transfer to the ferric ferritin mineral and/or chelation of iron dissolved from the mineral, and mutated pores have been observed in protein crystals [97] (fig. 2). Mutation of any of the four pore gate residues increases rates of iron chelation [98]; in one mutant, L134P, the mineral was removed from the protein nanocages 30-fold faster than wild type. High electrostatic negativity around the pores in mini- and maxi-ferritins [8] is thought to attract the iron cations.

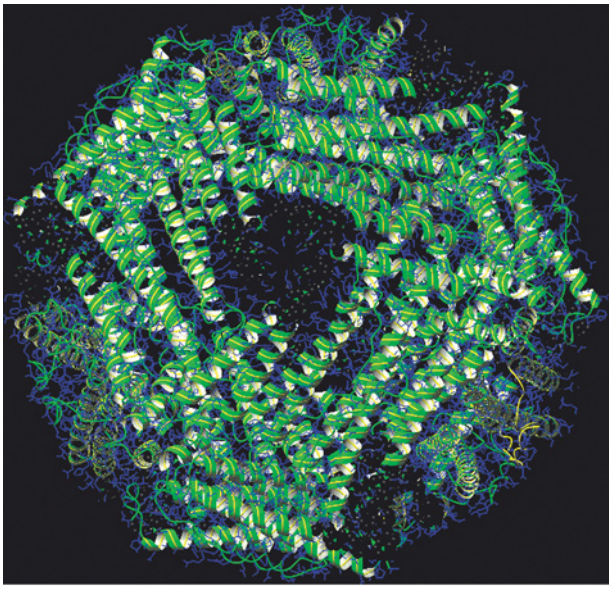


Figure 2. A ferritin pore, unfolded by mutation of a pore gate residue, L134P. The pore appears black because the peptide chain is disordered and does not diffract X-rays in the protein crystal. Pores can be unfolded by physiological concentrations of urea (1 mM) or temperatures which do not unfold the protein nanocages, shown in green and white [99]. Redrawn from data in reference [97]. The figure was created from PDB 1BG7file by X. Liu.

Ferritin is a normal part of foods, such as legumes, and the iron in ferritin is well-absorbed [100]. Recent experiments in humans confirm those in model animals [101–104] and confirm earlier preliminary observations [105]. The resistance of soy ferritin to digestion and the detection of soybean ferritin inside cultured cell models of gut enterocytes indicate that the ferritin macromolecule interacts directly with the gut cells and raise the possibility that soybean ferritin pores may play a role in iron absorption.

Ferritin and infection

Two types of ferritin play specific roles during infection and inflammation. Ferritin in host cells is the canonical maxi-ferritin with 24 subunits that has accumulated iron resulting from diminished iron recycling via hepcidin signaling [106–108]. A mini-ferritin in the cells of the invading bacteria has 12 subunits [8].

Macrophage ferritin accumulates during inflammation, when serum iron decreases and iron in specific cells increases, leading to ferritin with more iron/protein cage. The protein cages of such ferritin, when compared with protein isolated from the same tissue in control animals, has increased iron uptake; when both types of ferritins are mineralized *in vitro*, rates of iron release are decreased compared with the ferritin from control tissue [109]. The differences in the protein account, at least in part, for the increased amount of iron/protein in the molecule, but the molecular changes responsible for the altered function of ferritin protein are unknown [110, 111]. Lysosomes in cells accumulating iron during inflammation, also have increased amounts of ferritin and the ferritin degradation product hemosiderin. Whether lysosomal ferritin protects the lysosomes, represents the result of increased cytoplasmic ferritin, is damaged ferritin targeted for degradation or all of the preceding possibilities is the subject of some discussion [112–114]. Worth noting is the fact that most experimental studies of tissues with increased iron and ferritin use as a stimulus external 'elemental iron' such as ferrous sulfate or ferric ammonium citrate or iron carbonyl. Given the emerging complexity of iron signaling [106, 108, 115], changes in iron homeostasis induced by excess elemental iron, which increases total body iron, may be inadequate to model changes in iron homeostasis when iron distribution changes among specific cells, with total body iron remaining relatively constant (inflammation, chronic disease and infection). When maxi-ferritins use dioxygen to concentrate iron by conversion to the mineralized, hydrated ferric oxide, dioxygen is coincidently removed from the cytoplasm, which may itself change expression of the proteins of iron homeostasis.

Pathogen ferritins that are particularly important to host protective mechanisms are the mini-ferritins [8], or Dps proteins, that protect DNA [40] when exposed to ferrous ions and hydrogen peroxide *in vitro* [74, 81] and confer cellular resistance to oxidative damage *in vivo* [77, 78]. When the Dps protein structure was determined for the *E. coli* Dps protein, the mini-ferritin relationship to eukaryotic ferritins was immediately recognized [74] and confirmed as a mini-ferritin in subsequent studies with Dps proteins from a variety of human pathogens, e.g. *Helicobacter, Streptococcus, Listeria*, and *Bacillus* [75–78, 116]. Mini-ferritins using iron to detoxify oxygen/hydrogen peroxide by conversion to a mineral, hydrated ferric oxide, coincidently remove iron from the cytoplasm. Siderophores are synthesized in response to the deficit of environmental iron and are often associated with virulence [117]. During infection or inflammation, it is the 'iron deficiency' created by the host redistribution of iron, with a deficit in serum and an increase in macrophages, that is considered as the main stimulus to bacterial siderophore production. However, an induced iron deficit in the bacterial cytoplasm caused by Dps activity may also contribute to siderophore induction in the invading bacterium. Evolution of natural actions and reactions between hosts and pathogens on the oxygen and iron battlefield appear to depend on multiple layers of regulation including both gene (DNA) and template (mRNA) activity.

Summary and perspective

Taming the corrosive chemistry of iron and oxygen, which we take for granted in daily life, depends on an array of genes, regulatory mRNA structures, signaling pathways, protein catalysts and subcellular compartments. Ferritin, a center for controlling iron and oxygen, is a protein nanocage with highly conserved and unique properties that is coordinately regulated by both DNA (ARE/MARE) and mRNA (IRE) sequences to link ferritin with members of three gene families: those for iron and dioxygen metabolism and antioxidant responses. Multiple iron-protein interactions in the ferritins control the entry and exit of iron, which concentrates in a large, hydrated mineral form of Fe_2O_3 in the protein cavity; mineralization begins with coupling of two iron atoms with oxygen at protein binding sites related to those of di-iron oxygenases, but differing in the role of iron as a substrate rather than a cofactor. Maxi-ferritins (24 subunits) concentrate iron for protein synthesis and to protect cells from iron/oxygen radical chemistry. Mini-ferritins (12 subunits) in bacteria, long known as Dps proteins, protect bacterial DNA and play a role in minimizing host attempts to damage invading bacteria with oxidants. The unique ferritin structure and function, synthesized at rates controlled by both DNA and mRNA mechanisms, integrates iron, oxygen and antioxidant metabolism, illuminates the complexity of matching inorganic chemistry to life and models regulatory biology for all elements.

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