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# Iron at the center of ferritin, metal/oxygen homeostasis and novel dietary strategies

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**ABSTRACT**

Bioiron – central to respiration, photosynthesis and DNA synthesis and complicated by radical chemistry with oxygen – depends on ferritin, the super family of protein nanocages (maxi-ferritins in humans, animals, plants and bacteria, and mini-ferritins, also called DPS proteins, in bacteria) for iron and oxygen control. Regulation of ferritin synthesis, best studied in animals, uses DNA transcription *and* mRNA translation check points. Ferritin is a member of both the "oxidant stress response" gene family that includes thioredoxin reductase and quinine reductase, and a member of the iron responsive gene family that includes ferroportin and mt-aconitase ferritin DNA regulation responds preferentially to oxidant response inducers and ferritin mRNA to iron inducers; heme confers regulator synergy. Ferritin proteins manage iron *and* oxygen, with ferroxidase sites and iron + oxygen substrates to form mineral of both Fe and O atoms; maxi-ferritins contribute more to cellular iron metabolism and mini-ferritins to stress responses. Iron recovery from ferritin is controlled by gated protein pores, possibly contributing to iron absorption from ferritin, a significant dietary iron source. Ferritin gene regulation is a model for integrating DNA/mRNA controls, while ferritin protein function is central to molecular nutrition cellular metabolism at the crossroads of iron and oxygen in biology.

Beneficial effects of iron and oxygen for bioenergetics (respiration and photosynthesis) are dependent on effective transport of iron and oxygen and in the case of iron, concentrators to match cellular need to low iron solubility. Proteins that correct for products of iron/oxygen chemistry, which escape transporters and concentrators or which are side products of catalytic reactions such as occur with cytochrome p-450s, or cytochrome oxidase in mitochondrial metabolism, include peroxidases, superoxide dismutases, and reductases.

Ferritin is a concentrator protein at the center of iron and oxygen metabolism (2, 5, 13, 14, 18, 20). The protein is a nanocage of α-helices, assembled from 12 or 24 subunits of 4-α-helix bundles, that surrounds a solid iron containing thousands of Fe and O atoms per protein. In nature, ferritins have a range of mineral sizes or may be devoid of iron and filled with water/buffer (4, 19, 23, 25). The diameter of the cavity that accommodates the ferritin mineral inside the ferritin protein cage is 5-8 nm (Fig. 1). The functions of the ferritin protein can be grouped in the categories of iron entry into the protein (oxidation, coupling though an oxo bridge, translocation to the cavity, and mineralization) and iron exit or release (reduction, hydration, and chelation by a biological or synthetic chelator). Cells regulate the amounts of ferritin protein both during cell differentiation (DNA target) and in mature cells (mRNA or postsynthetic targets). The function of ferritin as a natural nutritional source of iron in legumes, indicated as long as thirty years ago, recently has been recognized more completely (1, 6, 17, 20).

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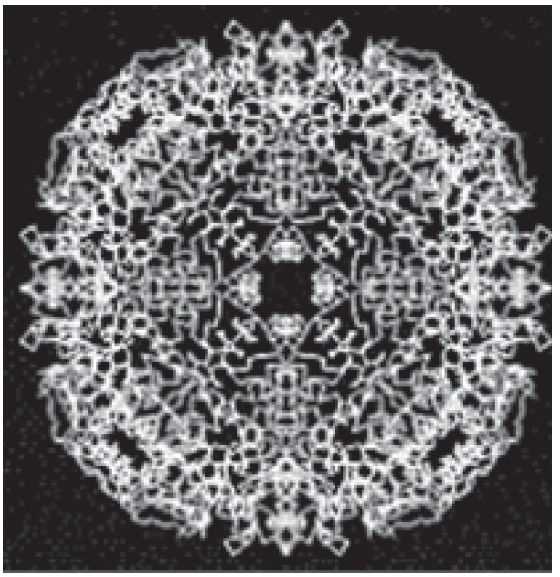

**Figure 1. Structure of ferritin from protein crystals (PDB 1FR ) described in (26)**. Points in the carbon backbone of ferritin polypeptides were etched in lead crystal using a CAD-controlled, short laser beam developed by B. Grossman (www.bathsheba.com). Frog M ferritin, Pdb: 1MFR

*Regulation of ferritin synthesis*

Cellular iron and oxygen signals regulate the expression of ferritin (Fig. 2) at multiple sites. Transcription of ferritin DNA changes during differentiation and is very sensitive to oxidants and hemin, but only very high levels of non-heme iron have effects; all are targeted to an antioxidant response element or ARE, first discovered by the Torti group in the mouse H ferritin gene (22, 24). We recently have found an MRAE/ARE in the human L ferritin gene where hemin appears to coordinate regulation synergistically for the combined DNA element and the mRNA element (12). The only *trans* factors that have been identified to date are relatively generic and target a number of genes in addition to the ferritin.

Translation of ferritin mRNA has been studied much more extensively than transcription and has revealed a combinatorial family of mRNA noncoding structures that create a set of quantitative different, or hierarchal, responses withly two specific repressor proteins, sensitivity to both inorganic iron and oxygen signals, and interactions with one *trans* factor, eIF-4F (3, 7, 10, 11, 16, 21). In plants, animals and bacteria, the gene product of the ferritin family of genes – the protein nanocages is highly conserved. In contrast, subcellular location of ferritin is variable (organelles or cytoplasm or DNA-bound) (2, 5, 13, 14), the genetic regulatory targets and gene structure have diverged (18) (DNA or DNA + mRNA) and the regulatory signals can be Fe, oxy derivatives, or both (11, 21) (Table 1) .

*Iron uptake in ferritin*

Among the many functions of ferritin the first in the series is protein-catalyzed, Fe oxidation reactions with $Fe^{2+}$ and $O_2$ to form diferric oxo mineral precursors. The catalytic coupling site is called the ferroxidase site and is present in every subunit of plant and bacterial (24 subunit) maxi-ferritins. In contrast, in animals a second gene encodes a catalytically inactive subunit, called L in a historic nomenclatures in which the active subunit is called H. Ferritin in animals cells is a mixture of ferroxidase active and inactive subunits, where ferritin in each cell type is a specific, distinct mixture. Bacteria also have a 12 subunit ferritin, or mini-ferritin, historically called a dps (DNA protection during starvation) protein, that is synthesized in response to stress, such as the production of hydrogen peroxide by a host or when nutrients are depleted. Mini-ferritins share with maxi-ferritins the reactions of iron oxidation, oxygen and peroxide consumption and iron biomineralization (26), but the effect is to protect DNA, in some cases by forming long chains of the protein nanocages that DNA wraps around to make a "bacterial chromatin" (8).

Amino acids at the ferroxidase active site of maxi-ferritins that are required for the formation of the first detectable reaction intermediate between ferrous ion and dioxygen, the blue diferric peroxo complex, were recently identified using protein chimeras (14). The catalytic sites in maxi-ferritins, molecule are related to oxygenases with di-iron cofactor sites, except that in ferritin both Fe and $O_2$ are the substrates

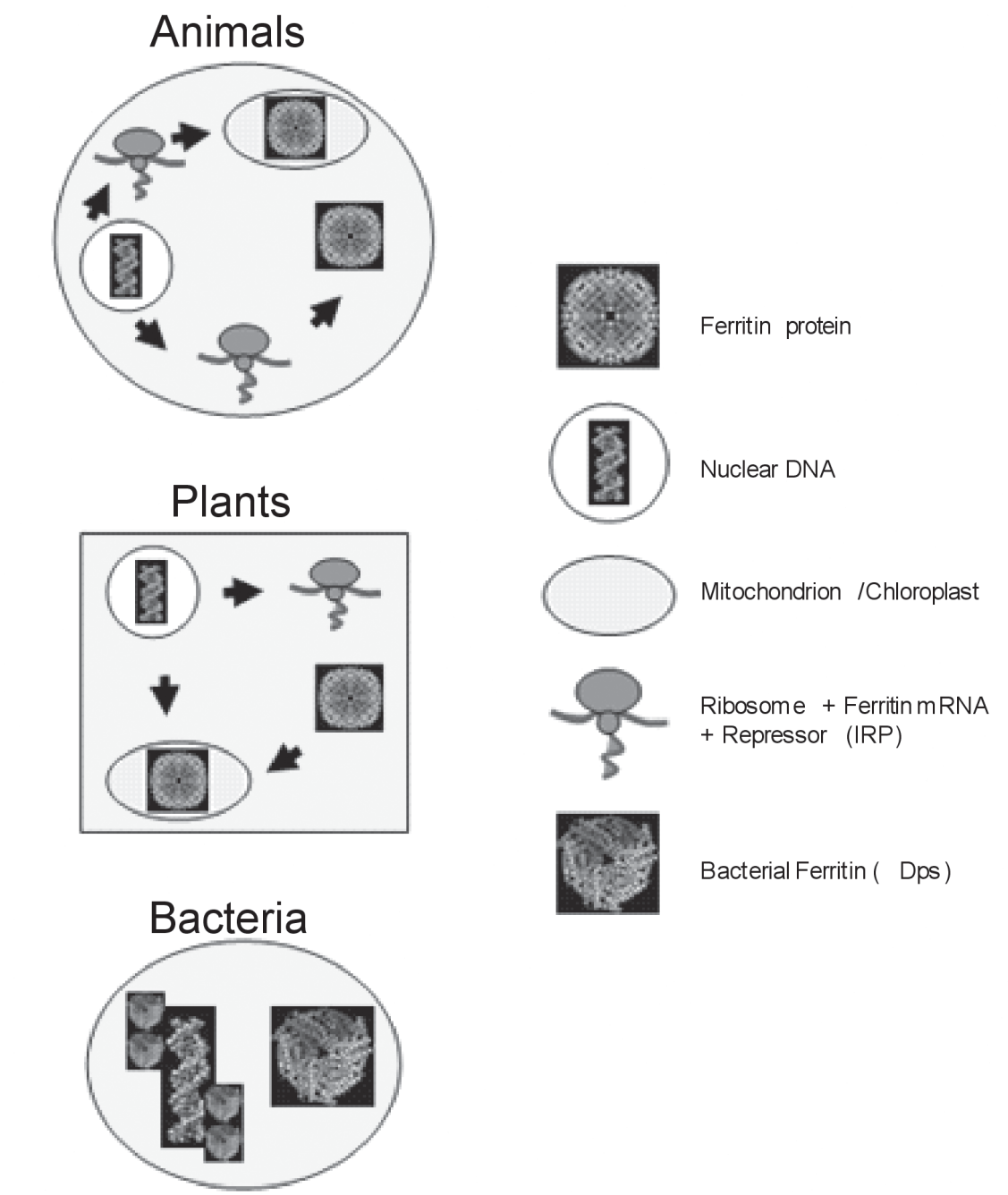


**Figure 2. The different genetic regulatory mechanisms, signals and cellular location for the conserved ferritin family of protein nanocages with FeO minerals catalyzed by di-iron ferroxidase sites.** Data are presented or reviewed in references 2, 5, 11, 18 and 21 and are tabulated in Table I.

TABLE 1

Similarities and Differences Among the Ferritin Family: Conserved Protein Nanocages with Iron Minerals and Ferroxidase Activity

| | Gene regulation | Signals | Cell Location* |
|---|---|---|---|
| Animals | DNA, mRNA | Fe, $O_2$, NO, $H_2O_2$ | Cytoplasm, Mitocdria |
| Plants | DNA | Fe, Fe+ Vit C | Plastid |
| Bacteria | DNA | Stress, Starvation | Cytoplasm, DNA bound |

* All cells, varied with development/environment. The data are reviewed in references 2, 5, 11, 18 and 21.

(21), in contrast to oxygenases where $O_2$ and organic molecules are the substrates. Little direct information is available on the ferroxidase sites of mini-ferritins.

*Iron release from ferritin*

The sites for reduction and chelation of ferritin iron mineral (8/molecule) are related to other gated ion channels that unfold more readily (1 mM urea, temperatures of 56°C (15), compared to the rest of the molecule, which resists temperatures up to 80°C and 7M urea.

Nutritional availability of Fe has been demonstrated in humans from both purified ferritin or from whole soybeans, where most of the Fe is in ferritin (2, 18). The stability of ferritin protein to conditions of digestion, and the sequestration of seed ferritin inside the plastid membrane and the accumulation of ferritin in the hulls, indicate that food ferritin iron is absorbed, at least partly, at the enterocyte membrane surface. Understanding the mechanisms of absorption of iron from food ferritin and interactions with other food components are crucial to realizing the full potential of whole legumes as natural, sustainable dietary iron sources for battling global dietary iron deficiency (5).

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