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Mitochondrial ferritin in animals and plants

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1. ABSTRACT

Ferritins play a role in preventing Fe toxicity because of their ability to sequester several thousand Fe atoms in their central cavity in a soluble, non-toxic bioavailable form. The identification of ferritin in mitochondria, an organelle with a constant generation of O_2^- as a by-product of the electron transfer, and the presence of a mitochondrial nitric oxide synthase activity opened up brand new metabolic interactions to be analyzed. In spite of cytosolic ferritins in mammals being ubiquitous, mitochondrial ferritin (mtF) expression is restricted to the testis, neuronal cells, islets of Langherans, and as recently described to mice normal retinas. None was detected in major storage organs such as liver and spleen. MtF has about 80% identity to cytosolic H-chain and 55% to L-chain in its coding region. There has been reported some differences in the Fe binding and oxidation properties between mtF and cytosolic H-ferritin suggesting that mtF functions differently as an Fe storage protein within the mitochondria and perhaps has other function(s) in Fe homeostasis as well. Recently it was also presented evidence for the presence of ferritins in plant mitochondria. The understanding of the role of mitochondrial ferritin in Fe oxidative metabolism may be useful in approaching clinical situations such as the treatment of Friedreich's ataxia, X-linked sideroblastic anemia, and in other neurodegenerative disorders.

2. INTRODUCTION

Fe is an essential element for the growth and well-being of almost all living organisms, except for some strains of lactobacillus, where the role of Fe may be assumed by another metal (1). It is involved in many biological functions since by varying the ligands to which it is coordinated, Fe has access to a wide range of redox potentials and can participate in many electron transfer reactions, spanning the standard redox potential range. It is also involved in O_2 transport, activation, and detoxification, in N_2 fixation and in several of the reactions of photosynthesis (2). However, there are problems in the physiological management of Fe, such as that in spite of its overall abundance, usable Fe is in short supply because at physiological pH under oxidizing conditions, Fe is extremely insoluble. Anytime Fe exceeds the metabolic needs of the cell it may form a low molecular weight pool, referred to as the labile iron pool (LIP), which catalyzed the conversion of normal by-products of cell respiration, like superoxide anion (O_2^-) and hydrogen peroxide (H_2O_2), into highly damaging hydroxyl radical ($HO^{\cdot}$) through the Fenton reaction (reaction 1) or by the Fe^{2+} catalyzed Haber-Weiss reaction (reaction 2), or into equally aggressive ferryl ions or oxygen-bridged Fe^{2+}/Fe^{3+} complexes. Fe^{3+} can be reduced either by O_2^- (reaction 3) or by ascorbate leading to further radical production.

$Fe^{2+} + H_2O_2 \Rightarrow Fe^{3+} + HO^- + HO^\cdot$ (1)
(Fe)
$O_2^- + H_2O_2 \Rightarrow O_2 + HO^- + HO^\cdot$ (2)
$Fe^{3+} + O_2^- \Rightarrow Fe^{2+} + O_2$ (3)

In vertebrates, defense against the toxic effect of Fe and O_2 mixtures is provided by two specialized Fe-binding proteins: the extracellular transferrins and the intracellular ferritins. Both retain Fe in the form of Fe^{3+} which unless mobilized will not be able to efficiently catalyze the production of free radicals. Fe is stored mainly intracellularly, where its potentially damaging effects are greatest. Thus, ferritins play a key role in preventing Fe toxicity because of their ability to sequester several thousand Fe atoms in their central cavity in a soluble, non-toxic bioavailable form. However, there are indications that ferritins may have other functions, not described yet, in addition to the well assessed role in storing intracellular Fe, moreover the origin and function of the extracellular ferritins found in mammals are not yet identified.

3. FERRITIN AND THE LABILE IRON POOL (LIP)

The labile Fe pool is defined as a low-molecular-weight pool of weakly chelated Fe that rapidly passes through the cell. It likely consists of both forms of ionic Fe (Fe^{2+} and Fe^{3+}) associated with a variety of ligands with low affinity for Fe ions. LIP represents only a minor fraction of the total cellular Fe (3-5%) (3). It has been proposed that Fe is complexed to diverse low-molecular weight chelators, such as citrate and other organic ions, phosphate, carbohydrates and carboxylates, nucleotides and nucleosides, polipeptides and phospholipids (4-6). However, the actual nature of the intracellular ligands participating in LIP formation remains obscure. The accessibility of cellular Fe to chelators (such as desferrioxamine) is commonly used as the criterion of 'lability'. It was suggested that the LIP actually represents relatively weakly Fe bound to prosthetic groups in functional sites of Fe-containing proteins, such as non-transferrin and non-ferritin proteins which functions are not yet known (7). Regarding intracellular distribution, substantial amounts of LIP were measured by Petrat et al. (8) in mitochondria (5 ± 2 microM), nucleus (7 ± 3 microM) and lysosomes (16 ± 4 microM).

Ferritin plays a dual role in LIP homeostasis. In Fe rich conditions it acts as Fe sequestering protein, protecting cells against Fe toxicity and at low Fe conditions it acts as a source of Fe ions necessary for Fe-containing protein synthesis. However, the physiological mechanism of Fe release from ferritin remains obscure. Ferritin-Fe is reduced effectively by reductants with redox potentials more negative than -200 mV, thus O_2^- (redox potential -300 mV) has the potential to reduce ferritin in a hydrofobic environment. Cytochrome P_{450} complex seems to be a major microsomal enzyme responsible for the release of Fe from ferritin (9). On the other hand, nitric oxide (NO) chelates labile Fe in a form which decreases its potential to yield reactive intermediates, and NO reacts with, and scavenges free radicals. In this scenario, the prevention of the ferritin-dependent stimulation of microsomal chemiluminescence by NO (10) suggested that the antioxidant capacity of NO could also involve its ability of decreasing the activity of Fe-heme compounds preventing the release of catalytic active Fe from storage forms and thus decreasing the cellular ability to generate free radicals involved in cytotoxicity.

It is important to point out that mitochondria, with a constant generation of O_2^- as a by-product of the electron transfer, in conjunction with the presence of an active mitochondrial nitric oxide synthase (mtNOS) activity and the recently identified internal ferritin, represent a critical locus to understand the complex pathways of the oxidative metabolism of Fe.

4. GENERAL FEATURES OF FERRITIN

Ferritin is a nanobox protein designed to contain and maintain in solution up to a few thousands Fe atoms, which otherwise would aggregate in toxic precipitates. Ferritins originated early in phylogenesis and are present in archeobacteria, eubateria, plants, invertebrates, and mammals (11). Although ferritins from different origin may have largely different sequences, with identities as low as 15%, their three-dimensional structures are remarkably highly conserved (11).

4.1. Cytosolic ferritin in mammals

The ferritin molecule is a hollow protein shell (outside diameter 12-13 nm, inside diameter 7-8 nm, Mr about 500000), composed of 24 polypeptide chains and capable of storing up to 4500 Fe^{3+} atoms of inorganic complex. Ferritin isolated from mammalian tissues consist of a mixture of isoferritins with a range of subunit compositions and Fe contents. It has been described that isoferritins from human placenta are constituted by 80% L: 20% H chains, human spleen by 90% L: 10% H, human liver by 50% L: 50% H, human heart by 10% L: 90% H (12), human serum by 100% L: 0% H (1), horse spleen 90-95% L: 10-5 % H and rat liver by 66% L: 34% H chains (2). In general, L-rich ferritins are characteristics of organs storing Fe (liver and spleen) and these ferritins usually have a relatively high average Fe content (1500 Fe atoms/molecule or more). H-chains rich ferritins which are characteristics of heart and brain have relatively low average Fe content (less than 1000 Fe atoms/molecule) (1). Such a high Fe:protein ratio (200 times that in hemoglobin) is made possible by sequestering Fe as a compact mineral. The protein is not just a passive reservoir for Fe but provides a microenvironment within the protein shell that influences the mineral phase that is formed.

Apoferritin catalyzes the oxidation of Fe^{2+} to Fe^{3+} state. Although oxidation at specific site on the protein is involved in the initial phase of core formation, it is generally accepted that once a critical nucleus of Fe^{3+} ions has been formed, and has begun to hydrolyze, it will then act in an autocatalytic manner to promote crystal growth on the surface of the initial biomineral core. There is now considerable evidence supporting the hypothesis that H and L subunits have complementary functions, ferroxidation,

Table 1. Biochemical properties of ferritins

	Mammal ferritin	**Plant ferritin**
Localization	Cytosol	Plastids
Number of subunits	24	24
Type of subunits	One H chain with ferroxidase activity and one L chain	One H chain with ferroxidase activity H_1 and H_2 subunits
Core	ferrihydrite ($5Fe_2O_3.9\ H_2O$)	Hidrated ferric phosphate amorphous
Crystalinity	Good	Very low
Peptides identified after SDS-PAGE	H chain ~ 21 kDa L chain ~ 20 kDa	H_1 subunit 26.5 kDa H_2 subunit 28 kDa
Synthesis control by Fe	Post-transcriptional level	Transcriptional level

References: 1, 2, 11, 26, 28, 29, 35, 66, 67, 68

and mineralization, respectively (13). The large activation energy of 63-67 kJ/mol measured for the effusion/penetration of small nitroxide spin probe radicals and the small diffusion coefficient, D ~ $5x10^{-22}$ m^2/s at 20ºC, corresponding to a time of ~ 60 min for traversing the ferritin shell, is consistent with the kinetics of diffusion being largely controlled by the restrictive porosity of the protein itself (14).

The stoichiometries of O_2 consumption and Fe^{3+} hydrolysis have been determined by Yang et al. (15), and in those experiments the consumption of two Fe^{2+} per O_2 for the protein-based reaction implied that H_2O_2 is the product of the ferroxidase activity in human ferritin-H chain. Catalase supplementation changed the measured stoichiometry, and one H^+ was released per Fe^{2+}. The overall reaction at the ferroxidase center, postulated by Yang et al. (15), is as shown in reaction 4, and the net reaction for the transfer of Fe^{3+} to the core is as shown in reaction 5.

$$\text{Protein} + 2\,Fe^{2+} + O_2 + 3\,H_2O \Rightarrow \underset{\text{ferroxidase complex}}{\text{Protein-}[Fe_2O(OH)_2]^{2+}} + H_2O_2 + 2\,H^+ \quad (4)$$

$$\text{Protein-}[Fe_2O(OH)_2]^{2+} + H_2O \Rightarrow \text{Protein} + 2\,FeOOH_{(core)} + 2\,H^+ \quad (5)$$

Hydrolysis produces one more H^+ per Fe atom and the ferroxidase center is vacated. Once sufficient core is developed (>100 Fe atoms), the alternative mineral surface Fe^{2+} oxidation and hydrolysis on the surface of the growing core may now proceed according to reaction 6.

$$4\,Fe^{2+} + O_2 + 6\,H_2O \Rightarrow 4\,FeOOH_{(core)} + 8\,H^+ \quad (6)$$

In most ferritin molecules some of the HO^- ions of the core are replaced by orthophosphate ions, the majority of these residing in the core surfaces (16). Thus, ferritin has evolved a molecular design that limits the Fe chemistry within its interior, avoiding nonspecific Fe oxidation and hydrolysis reactions from occurring within the cytosol of the cell. In this way, other proteins and nucleic acids are protected from the toxic effects of labile Fe. The general features of mammal ferritin are summarized in Table 1.

4.2. Plant ferritin

In plants, mineral nutrition is one of the factors involved in growth and development and, therefore, in crop productivity (17). Fe plays an important role because of its implication in fundamental processes such as photosynthesis, respiration, N_2 fixation and DNA synthesis. In addition it acts as a cofactor of key enzymes involved in plant hormone synthesis (*e.g.* lipoxygenases and ethylene forming enzymes) (18,19) which are involved in various pathways controlling both development events and responses to multiple environmental variations. Mechanisms controlling subcellular distribution of Fe in the various organelle of the plant cell are unknown, although it has been suggested that an active Fe-citrate transporter could participate in Fe uptake by leaf chloroplasts (20). The Fe storage in plant cell occurs in the vacuole (21), and as in animal cells, in the ferritin. However, the cellular distribution is not identical to that observed in animal cells since animal ferritin is a cytosolic protein and plant ferritin is found in the plastids (17,22).

Plant and animal ferritins have very similar three-dimensional structure and are formed by 24 subunits arranged to form a protein coat able to sequester up to 4500 Fe atoms in a non-noxious form (22,23). Plant ferritins are homopolymers of a single subunit type and combine a pattern of carboxylate residues on their cavity surfaces like the mammalian L-chains (24) with conserved ferroxidase centre residues typical of H-chains. Recombinant pea seed ferritin has been shown to exhibit ferroxidase activity (25). Recently, Masuda et al. (26) have identified a novel soybean ferritin subunit (H-2), whose maturation process is different from that of the originally described subunit (H-1).

The ferritin Fe-phosphate mineral characteristic of plants appears to be formed in plastids after protein transport to the plastids (27) (Table 1). Under physiological conditions, plant ferritin synthesis is developmentally regulated being undetectable in the plastids of vegetative organs like roots and leaves (28). Ferritins accumulate in non green plastids such as protoplastids, etioplasts, and amiloplasts and are found in specific tissues such as the shoots, root apex, seeds or nodules (29). While ferritin accumulates in developing nodules, cotyledons and embryo axes (28) of soybean and pea, it has not been detected in green leaves of bean by Lobréaux and Briat (28). However, van der Mark et al. (30,31) reported that ferritin was detectable in normal green leaves of bean. Interestingly, these authors provided preliminary evidence for the existence of multiple subunits of ferritin expression in bean (32). It was reported that exogenous treatment with ozone or ethylene, as well as impaired photosynthesis or Fe overload, also induce ferritin accumulation in chloroplasts (29,33). It was shown that NO mediates ferritin regulation by acting downstream of Fe in the induction of ferritin transcript accumulation (23). These data suggest that both

Table 2. Main differences between mammals Hf and mtF ferritin

	Cytosolic HuHF	mtF
Functionality of ferroxidase centers	48 Fe^{2+} bind and oxidized (2 Fe^{3+} per ferroxidase center)	24 Fe^{2+} bind and oxidized (1 Fe^{3+} per ferroxidase center)
Mineralization properties	hyperbolic kinetics protein catalyzed reaction	sigmoidal kinetics autocatalytic mineral surface mechanism
Detoxification reaction ($2Fe(II)+H_2O_2$)	present	absent
Regeneration of ferroxidase activity	0.31 s^{-1} and 0.20 s^{-1} for the first and second 48 Fe^{2+}/shell addition at 40 min	0.017 s^{-1} and 0.014 s^{-1} for the first and second 24 Fe^{2+}/shell addition at 24 h
Renaturation after full denaturation	reassembled quantitatively	only a minor fraction is reassembled

References: 40, 69

development and environmental signals, are involved in plant ferritin gene regulation. It was recently reported that Fe accumulation does not parallel the high expression level of ferritin in transgenic rice seeds (34), and although a high storage ability of Fe could be achieved by ferritin over-expression, the limiting factor for Fe accumulation may be Fe uptake/or transport. Moreover, the synthesis of ferritin in vertebrates is regulated during traslation, whereas the expression of plant ferritin is regulated primarily at the transcriptional level in response to Fe administration (35).

5. MITOCHONDRIAL FERRITIN

5.1. Mitochondrial ferritin in mammals

Since its discovery, the mitochondrion has been known as an essential and dynamic component of cellular biochemistry. The complexity of the mitochondrion has been gradually revealed by the study of a variety of genetic diseases associated with its function. Thus, it is clear that Fe plays a crucial role in many facets of mitochondrial metabolism and the consequences of disruption to these pathways are catastrophic. Therefore, it would seem clear that the mitochondrion, a site of dynamically active electron transfer and redox activity, would possess sufficient measures for the safe trafficking and metabolism of Fe. However, until recently, knowledge of the Fe metabolism of the mitochondrion has been largely confined to the heme synthesis pathway.

In 2001, the description of a new human ferritin type was reported. It was named mitochondrial ferritin (mtF) after the demonstration that it localizes specifically in this organelle (36). The origin of the mtF is obscure. It is suggested that evolved sometimes before the separation of rodents and primates from an H-type sequence before the L gene, since its non-coding regions have less homology to H than to L gene (37). Up to now, homologues have been found in primates, mouse and rat, but not in other species (37). The human mtF gene is encoded by an intronless sequence on chromosome 5q23.1 and has characteristics of a processed pseudogene: no introns, vestiges of a polyA tail, and suggestions of direct flanking repeats (37). It has about 80% identity to cytosolic H ferritin and 55% to L ferritin in its coding region and its C terminus is at the same position as that of HF but its N terminus extends further 60 residues. There are no other homologous sequences in the present human genomic database and this uniqueness was confirmed by Southern Blot experiments by Drysdale et al. (37). Transcripts for a mtF have also been identified in mice (36). However, in spite of cytosolic ferritins in mammals being ubiquitous mtF expression is restricted mainly to the testis, neuronal cells and islets of Langherans. None was detected in major storage organs such as liver and spleen but sensitive RT-PCR techniques detected mtF mRNA in other tissues (37). Recently, Hahn et al. (38) have reported that mice normal retinas have subtle mtF in the mitochondria-rich inner segments of photoreceptors and diffusely throughout the inner retina, and that this mtF increases with Fe accumulation.

Mitochondrial ferritin is expressed as a precursor and the mature protein has structural and functional properties analogous to the well-characterized cytosolic ferritins (39) and is a homopolymer of 24 subunits that has a high degree of sequence homology with human H-chain ferritin and similar diFe ferroxidase centers. By following the synthesis of mtF *in vivo* and *in vitro*, it was shown that mtF is first synthesized as a 30 kDa peptide that is proteolytically processed into a 22 kDa peptide in mitochondria where it forms shells similar to the 21 kDa H chain and the 19 kDa L chain and can form heteropolymers with both *in vitro*. However, this is very unlikely to occur in cytosol because its 30 kDa precursor cannot form shells without being proteolytically processed in mitochondria (37). However, in contrast to human H-chain ferritin, mtF has considerably slower ferroxidation and mineralization activities. Mitochondrial ferritin exhibits sigmoidal kinetics of mineralization more characteristic of an L-chain than an H-chain ferritin (40). Table 2 shows the main functional differences between human H-chain Ferritin (HuHF) and mtF. Unlike HuHF where 48 Fe(II) bind and form dimeric Fe(III) species (two Fe^{3+} per ferroxidase center), mtF binds and oxidizes only 24 Fe(II), indicating that only half of the 24 ferroxidase centers of this protein are functional. The two proteins also differ markedly in their mineralization properties, since HuHF displays hyperbolic kinetics of mineralization characteristics of a protein catalyzed reaction, whereas the kinetics form of mtF are sigmoidal, as expected for an autocatalytic mineral surface mechanism similar to that seen with L-chain ferritin. Also, the so called 'detoxification reaction' between 2 Fe^{2+} and H_2O_2, that is seen in HuHF, is absent in mtF (40). Moreover, it was described that minimal $HO^{\cdot}$ amounts are produced during the oxidative deposition of Fe in mtF using O_2 as the oxidant (40). These major differences in the Fe binding and oxidation properties suggest that mtF functions differently as an Fe storage protein within the mitochondria and perhaps has other function(s) in Fe homeostasis as well. As indicated in Table 2, HuHF and mtF differ in their ability to regenerate ferroxidase activity. Bou-Abdallah et al. (40) reported that HuHF regenerated up to 65% of its initial ferroxidase activity after allowing the protein to stand 40 min after the initial addition of Fe^{2+} at a Fe^{2+}/protein ratio

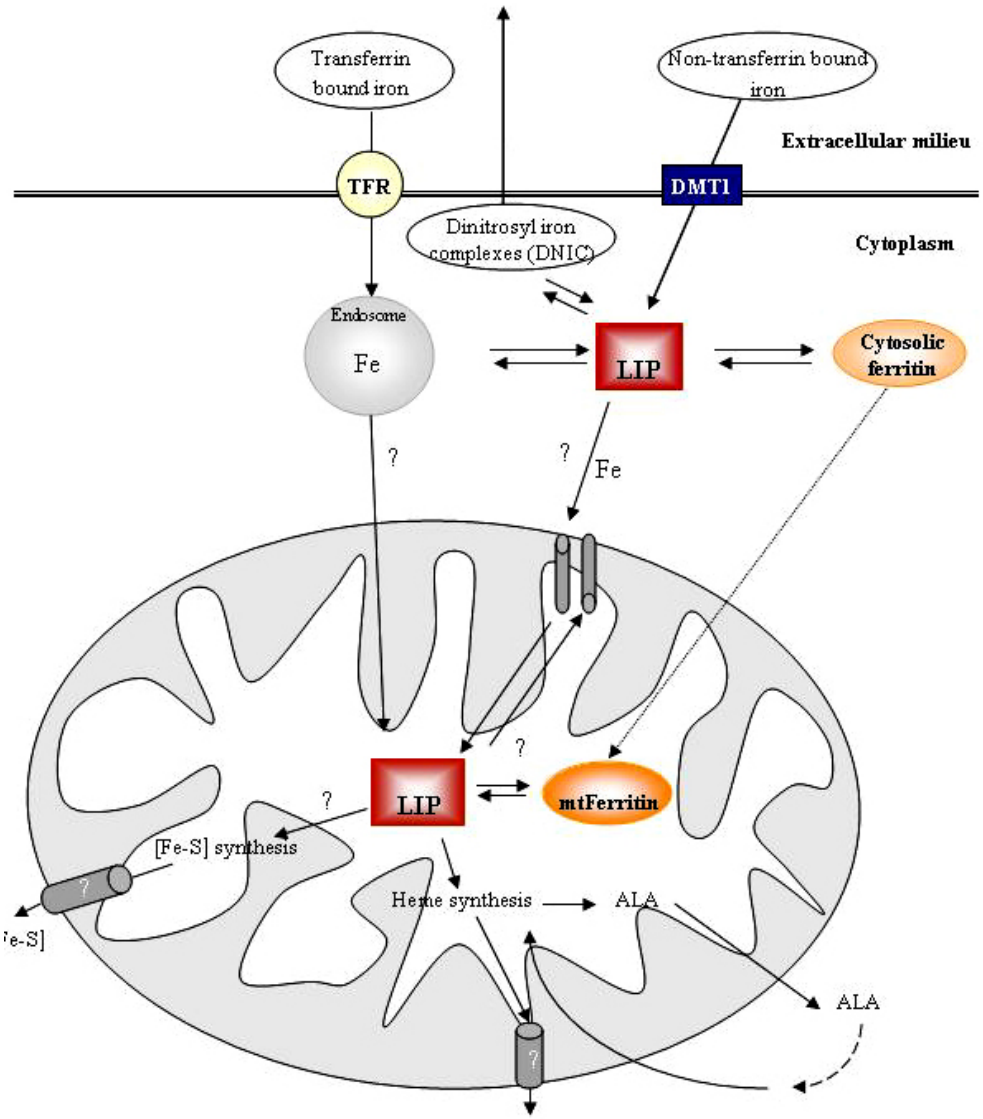

Figure 1. Scheme of mitochondrial Fe metabolism and the potential sources of LIP. TFR, transferrin receptor; DMT1, divalent metal transporter 1; mtFerritin, mitochondrial ferritin; ALA, δ-aminolevulinic acid; and [Fe-S], Fe sulphur cluster. Dashed lines indicate the Fe acquisition from cytosolic ferritin Fe by mtFerritin. Reproduced with modification from (55).

just enough to saturate the ferroxidase centers (*i.e.* 48 Fe^{2+}/shell). However, under the same experimental conditions mtF did not shown an initial ferroxidase activity before 24 h after the initial addition of Fe^{2+} at a Fe^{2+}/protein ratio just enough to saturate the ferroxidase centers (*i.e.* 24 Fe^{2+}/shell).

However, *in vitro* studies showed that the relative rate and extent of Fe uptake into cytosolic and mitochondrial ferritins from HeLa cells transfected with mtF were similar (37). The mechanism allowing external Fe to gain access to mitochondria as quickly as it does to cytosol remains to be determined. Even more surprising is the observation that mtF is able to retain more Fe than HF even when Fe stores are depleted by chelating with desferal (41). Obviously, mtF should have such a high affinity for Fe that increases in its expression result in an apparent Fe deficiency in the cytosol, shown as decreases in the cytosolic LIP and in the levels and synthesis of HF protein and increased levels of transferrin receptors (37). The avidity of mtF for Fe could be understood as the reason why mtF level is kept so low in most cells.

Figure 1 briefly summarizes the main pathways involved in the trafficking and storage of Fe in the mitochondria. How Fe is incorporated into mitochondria is still not clear. Physical contact between the mitochondrion and the endosome has been suggested (42), but up to date there has been no evidence of either LIP acting as a kinetic intermediate (43) or for direct contact between the mitochondrion and the endosome. Once Fe reaches the mitochondrial matrix it can participate in a) the synthesis of

[2Fe-2S] and [4Fe-4S] clusters for mitochondrial, cytosolic and also nuclear proteins that play a main role in many metabolic functions including the regulation the uptake of Fe itself; b) in the heme biosynthesis or c) it may be stored in mtF. Nie et al. (44) have demonstrated that mtF is capable of a very efficiently acquisition of Fe from endosomal, transferrin-bound Fe as well as cytosolic Fe inserted in ferritin. Moreover, the induction of mtF compromises the function of at least some nonheme Fe proteins and also appears to interfere with Fe delivery to heme. Furthermore, compared to cytosolic ferritin, Fe inserted in mtF is less available for chelation. Mitochondrial Fe level must be well regulated because an inadequate supply of Fe would impair the metabolic and respiratory activities of the organelle, whereas excess labile Fe in mitochondria would promote the generation of harmful reactive oxygen species, which are produced as a side reaction of mitochondrial electron transport (45).

Immature erythroid cells have an exceptionally high capacity to synthesize heme that is, at least in part, the result of the unique control of Fe metabolism in these cells. To get access to mitochondria the vast majority of Fe released from the endosomes in the erythroid cells must cross both the outer and inner mitochondrial membranes to reach ferrochelatase, which inserts Fe into protoporphyrin IX (46). The chemical nature of the non-heme Fe which accumulates in mitochondria of hemoglobin-synthesizing cells is unknown, but it is possible that only Fe in excess may be stored in mitochondrial ferritin (36). This model proposed by Ponka et al (46) is a quite attractive hypothesis since Fe^{2+} ions would by-pass an O_2-rich cytosol in hemoglobin synthesizing cells. This possibility seems interesting as a protective mechanism since Scott and Eaton (47) showed that LIP is capable of establishing a self-amplifying and self-propagating redox reaction with hemoglobin that can eventually lead to red cell destruction. Hence, the chaperone-like function of endosomes may be one of the mechanisms that keeps concentrations of Fe at extremely low levels under physiological conditions. At least in these cells with an extremely rapid incorporation of Fe into hemoglobin and an intricate and highly organized structure of the intracellular matrix, it is feasible that a tightly coordinated and efficient mechanisms could be operative to minimize the potential damage of freely diffusible and potentially toxic cytosolic Fe pool. However, Fe in mtF is abundant in the Fe-loaded mitochondria of erythroblasts of patients with sideroblastic anemia (48). The sideroblastic anemias are characterized by ring sideroblasts, that are red cell precursors with mitochondrial Fe accumulation. Cazzola et al. (48) compared the distribution of stainable Fe with cytosolic and mitochondrial ferritins in erythroblasts from patients with different forms of anemia and have shown that mtF is almost exclusively expressed in sideroblastic anemia. The nature of the excess of mitochondrial Fe of ring sideroblasts have remained an enigma for many years, however these observations on the incorporation of Fe in the protein shells of mtF could have considerable pathophysiological and clinical implications since specific immunodetection of mtF should allow the development of diagnostic tools for sideroblastic anemias (48).

Friedreich ataxia (FA) is the most common inherited spino/cerebellar ataxia resulting in confinement to a wheelchair and death during middle age due to cardiomyopathy (49), that is characterized by Fe accumulation in mitochondria, especially in tissues of high mitochondrial content, such as nerve and cardiac tissue (50). An excess of labile Fe in the redox-active environment of the mitochondrion of FA patients has been suggested to play a role in the pathogenesis of this disease (51). In this regard, free radical scavengers, such as idebenone, have been shown to be protective against the cardiomyopathy observed in this disease (52). This observation lead to the hypothesis that tissue injury could be due to the participation of Fe in Fenton chemistry resulting in damage to essential biologic molecules (53). Frataxin, a 210-amino acid protein has been investigated as a potential iron-binding protein (54) and was suggested to perform the function of a 'mitochondrial ferritin' (55). In FA, frataxin expression is low and this thought to lead directly or indirectly to mitochondrial Fe accumulation in nonerythroid cells (49,50). The tissues affected in FA are composed of nonerythroid cells (*e.g.* neurons and cardiomyocytes) that have a basal level of heme synthesis (42). Considering this, Napier et al (55) proposed that in the nonerythroid cells of FA patients where, because there is no intense demand for heme synthesis the excess Fe is not used for [Fe-S] cluster synthesis but is actively incorporated into mtF. Thus, initially the Fe accumulation in mtF may be protective and could explain the delay in pathogenesis of the disease until many years after birth (56). However, in the absence of intense Fe utilization in nonerythroid cells for heme synthesis, mtF may potentially degrade to hemosiderin-like molecule that is redox active, leading to the subsequent mitochondrial damage seen in FA (57). However, at present it is unknown how mtF would be degraded to form a hemosiderin-like material, since in the cytosol this may be accomplished by lysosomes (58). Moreover, observations by Campanella et al. (59) indicated that mtF expression rescued the respiratory deficiency caused by the loss of frataxin, protecting the activity of [Fe-S] clusters enzymes and enable a frataxin-defficient cells to grow in non-fermentable carbon sources. Furthermore, mtF expression prevented the development of mitochondrial Fe overload, preserved mtDNA integrity and increased cell resistance to H_2O_2.

5.2. Mitochondrial ferritin in plants

Zancani et al. (60) presented evidence for the presence of ferritins in plant mitochondria. Mitochondria were isolated from etiolated pea stems and *A. thaliana* cell cultures. The proteins were separated by SDS/PAGE and a protein with an apparent molecular mass of approximately 25-26 kDa (corresponding to that of ferritin), was cross-reacted with an antibody raised against pea seed ferritin. The mtF from pea stems was also purified by immunoprecipitation. The mitochondrial localization of ferritin was also confirmed by immunocytochemistry experiments in isolated mitochondria and cross-sections of pea stem cells. These data from Zancani et al. (60) indicate a mitochondrial localization for ferritins in *P. sativum* and *A. thaliana* and could be understood as the protein being targeted to both plastids and mitochondria, similarly to

what was shown for several plant proteins (61). This feature can be accomplished by alternative transcription, alternative translation starts, alternative exon splicing (or a combination of the above), or the presence in the N-terminus of an ambiguous presequence (61); thus prediction programs could just be unable to detect such a dual targeting. On the other hand, a similar situation has been previously described for ferrochelatase I in *A. thaliana* (62).

As in animals, plant mitochondria possess an electron transport chain where O_2^- may be generated by univalent reactions at the level of complexes I and III (63). Thus, mitochondria have evolved systems to appropriately scavenge deleterious radical species or to prevent their formation (64), but sequestration of potential harmful Fe^{2+} ions has not been described (60). In this context, the main role of ferritins could concern Fe sequestration. Overexpression of this protein, in either the cytoplasm or plastids of transgenic tobacco, leads to an increase of Fe sequestration that induces an activation of the Fe transport systems (65). Therefore, they are crucial in controlling Fe homeostasis and storage in plant cells. Other functions of plant ferritins are still obscure. It has been suggested that sequestering of intracellular Fe may protect from oxidative damage induced by a wide range of conditions, such as photoinhibition or ozone exposure (33). As a consequence, the sequestration of Fe by ferritins in chloroplasts and mitochondria, two of the major sites of oxygen-radical generation in plant cells (64) can constitute and additional strategy to prevent damage.

6. CONCLUSIONS AND PERSPECTIVES

Among the functions performed by the mitochondria in animals and plants, the processing of Fe to heme and [Fe-S] clusters is unique in being essential for maintenance of mitochondria and for the production of ATP. When these biosynthetic pathways are corrupted a massive oxidative damage not only to the mitochondria but also to the cell and tissue is observed. Even though the biological role of the mtF is not yet clear, its localization in an organelle with a critical function that shows a high Fe flux strongly suggest that it plays a main role in Fe trafficking. Its ferroxidase activity and high affinity for Fe are well suited for Fe storage and detoxification. It is also important to point out the possible clinical applications of mtF induction for therapeutic purposes. The increased expression of mtF in Fe-loaded erythroblasts makes it a valid marker of sideroblastic anemia, and can therefore be employed to facilitate the diagnosis of different forms of myelodysplastic syndrome. This may be useful in some other clinical situations such as in the treatment of Friedreich's ataxia, X-linked sideroblastic anemia, and in some other neurodegenerative disorders such as Parkinson's and Alzheimer's diseases. It will be also specially interesting to explore the possibility of using mtF high affinity for Fe as a tool to limit the oxidative damage, that results in a mitochondria decay in other conditions such as aging, exposure to toxins, or in genetic diseases. However, more extensive analyses are required to draw therapeutic protocols, since for example, the abundance of mtF in spermatids, endocrine cells and embryos, seems less related to Fe distribution than to mitochondrial localization and function. Thus, a better understanding of mtF function, especially in nonhematopoietic tissues, should be mandatory before exploring the possible clinical use of this new player in Fe metabolism.

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