Characterization of Two Homologous Yeast Genes That Encode Mitochondrial Iron Transporters

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Two different yeast genes were identified that when overexpressed suppressed the low iron growth defect of a mutation in the endoplasmic reticulum iron binding enzyme methyl sterol oxidase. These genes were determined to be novel and highly related. The deduced amino acid sequences indicated that both were membrane proteins having two identical histidine-rich motifs. The predicted proteins, while not ABC transporters, are homologous to a widely distributed family of transition metal transporters present in all kingdoms. Subcellular fractionation and fluorescence microscopy localized these gene products to mitochondria. Based on this result we term these genes Mitochondrial Fe Transporters (MFT). Cells with disruptions in both genes show a growth defect on low iron medium, suggesting that these genes have redundant function and can affect cytosolic iron levels. Measurement of mitochondrial iron in cells grown in iron-rich medium overexpressing MFT1 or MFT2 show a 2–5-fold increase in iron compared with mitochondria from control cells. These results suggest that the mitochondria may act as a reservoir for iron that can be mobilized and used for cytosolic purposes.

In the past few years, enormous strides have been made in understanding the mechanisms that mediate iron accumulation in the budding yeast Saccharomyces cerevisiae. Genes for both high (1, 2) and low affinity plasma membrane (3) iron transport systems and their regulators (4) have been identified. While these studies have resulted in characterization of plasma membrane iron transport at the molecular level, much less is known about intracellular iron transport. Iron is distributed among different intracellular compartments: the vacuole in which iron may be stored, the mitochondria where the terminal steps of heme biosynthesis occurs, and the cytosol where many iron-utilizing enzymes are located. To date, little is known about the molecules or transporters responsible for intracellular iron movement.

We previously identified the yeast gene ERG25 by a screen that, selected for mutants, showed poor growth on low iron medium (5). This gene encodes a methyl sterol oxidase that was deduced to be an oxo-diiron-containing enzyme based on its sequence and the observation that the mammalian methyl sterol oxidase requires iron for activity. This enzyme is responsible for an oxygen-mediated dimethylation of 4,4′-dimethylzymosterol, an intermediate in yeast and mammalian sterol biosynthesis (6). The mutant erg25 allele, termed fet6-2, has a missense mutation that appears to affect the ability of the protein to bind iron; the protein has normal activity in iron-replete medium but is nonfunctional in low iron medium. We screened for genes that when overexpressed could rescue the fet6-2 mutant phenotype of poor growth on low iron medium. We report here the identification and characteristics of two homologous genes that suppress the mutant phenotype. We determined that the products of these genes affected the mutant protein indirectly by increasing cytosolic iron concentration. Subcellular fractionation studies localized the gene products to mitochondria and hence, we refer to them as Mitochondria Iron (Fe)Transporters (MFT).

We report here the identification and characteristics of two homologous yeast genes that encode mitochondrial iron transporters. Our data suggest that the MFT genes are involved with mitochondria iron movement and that the mitochondria may act as a reservoir for iron.

MATERIALS AND METHODS

S. cerevisiae and Escherichia coli Strains—The yeast strains employed in this study were derived from DY150 and DY1457 as described previously (7). The isolation and phenotype of the fet6-2 strain is described in Li and Kaplan (5). The cells were grown in YPD (1.0% yeast extract, 0.2% peptone, 2.0% glucose), or in YPD made iron-limited by the addition of banthophenanthroline sulfonate (BPS)1 (1), or in LIM medium, a synthetic medium that has defined concentrations of iron or other transition metals (8). Spheroplasts and a membrane preparation were obtained as described previously (5). Iron transport and ferrireductase activity was assayed as described in Eide et al. (8).

DNA Sequencing—DNA was sequenced using the dye deoxy sequencing method using Sequenase from U. S. Biochemical Corp. and the Applied Biosystems automated sequencer. The primers were universal M13 primers and primers prepared using previously obtained sequences. Both strands were sequenced.

Identification and Subcloning of MFT1 and MFT2—DNA transformations of E. coli and S. cerevisiae were performed by standard procedures (9, 10). DNA fragments were isolated using Wizard Miniprep (Promega). The shuttle vector pTF63 was derived from YEplac195 and contained the Bluescript II polylinker (11). The Sau3A genomic library used in the complementation studies was described previously (5) and was the gift of Drs. D. Stillman and W. Ming. The high copy genomic library was transformed into fet6-2 and colonies were isolated that complemented the growth defect on low iron medium. Plasmids were isolated and analyzed by restriction enzyme analysis. Two different complementing plasmids were sequenced using M13 primers. One plasmid (p20) contained a 3410-base pair segment of chromosome XIII,

1 The abbreviations used are: BPS, banthophenanthroline sulfonate; YPD, yeast peptone dextrose; SNG, streptonigrin; ORF, open reading frame; PCR, polymerase chain reaction; GFP, green fluorescent protein.
while the other plasmid (p21) contained a 9094-base pair region of chromosome XVI. The only ORF that was identified on the p21 fragment was YMR177W. A number of different ORFs were present on the p21 plasmid. An ORF YPI224C was shown to be responsible for the complementation of fet6-2 was proven by isolating the ORF using a HindIII site on the 5′ end of the ORF and the polymerase chain reaction and primers on the 3′ end of the ORF. The isolated ORF was subcloned into pTF63 and was shown to complement the low iron growth phenotype of fet6-2.

**Construction of Deletion Strains**—A double fusion PCR technique (12) was used for construction of ΔMFT1::URA3 and ΔMFT2::HIS2. The primers for amplification of the 5′ end of ΔMFT1 were: 5′-GCC CGT TAT AAA GAT COT TC-3′ and 5′-GTC GTG ACT GGG AAA ACC CTG GCG TTG TAG TCT CGT ATC GTG CC-3′. The primers for amplification of the 3′ end of ΔMFT1 were: 5′-TGC TGT GTG AAA TTA TTC TCC GCT TCC AGC CTA CCT AGA GAT AG-3′ and 5′-GAG TAT TCG TAT GGT GTG GC-3′. The primers for amplification of the 5′ end of ΔMFT2 were: 5′-CTA TCA AGC AAT TCG GCT GT-3′ and 5′-GTC GTG ACT GGG AAA ACC CTG GCG TTG GCT GTA GTG CTA GTG AC-3′. The primers for amplification of the 3′ end of ΔMFT2 were: 5′-TCC TGT GTG AAA TTG TTA TCC GCT TCT TAT CCT CAC TGA AGG AG-3′ and 5′-CAT CAA CAA ACT CGA CTT GC-3′. The URA3 gene was used as the selectable marker for the ΔMFT1 disruptant while the HIS3 gene was used as the selectable marker for the ΔMFT2 disruptant (12). The PCR conditions for the 5′ end and 3′-end-flanking sequences of two gene deletions were: 0.5 μM primer mixture, 3 μM Mg, denatured at 94 °C, annealing at 50 °C, elongating at 70 °C, 20 s for 35 cycles in Idaho Technology Air Thermo-Cycler. The PCR conditions for the first fusion of the upstream fragment to the marker and the second fusion to the downstream fragment were performed in Perkin Elmer PCR machine at conditions of denaturing 94 °C, 40 s, annealing 60 °C, 40 s, elongating 72 °C, 3 min, for 35 cycles.

The purified double fusion PCR products were transformed into a wild-type diploid strain DY1640. After sporulation and dissection, spores containing the disruption were selected either by growth on Ura− or His− media. Correct integration was tested by colony PCR and Southern blotting.

**Construction of Epitope-tagged and Green Fluorescent Protein (GFP) Fusion Proteins**—Both MFT1p and MFT2p were epitope-tagged with MYC at their carboxyl terminus using the polymerase chain reaction. For MFT1p, an 180-base pair fragment that encompassed a unique Bsm36II restriction site to the 3′ end of the gene was amplified using two primers: 5′-CGT TAC TGT CCT CAG CAG ATT TAC GCG-3′ and 5′-AAG GAA AAA AGC GGC CGG TTA ATG CAA CTC TCT TGG AGA AAG CGT-3′. The downstream fragment were performed in Perkin Elmer PCR machine at conditions of denaturing 94 °C, 40 s, annealing 60 °C, 40 s, elongating 72 °C, 3 min, for 35 cycles.

The purified double fusion PCR products were transformed into a wild-type diploid strain DY1640. After sporulation and dissection, spores containing the disruption were selected either by growth on Ura− or His− media. Correct integration was tested by colony PCR and Southern blotting.

**Identification of MFT1 and MFT2**—Incubation of fet6-2, an erg25 mutant, in low iron medium results in defective sterol synthesis leading to a marked growth deficit (5). To isolate extragenic suppressors, fet6-2 was transformed with a high-copy yeast genomic library and colonies that demonstrated enhanced growth on low iron medium were isolated. After screening 20,000 colonies, a plasmid was isolated (p20) that allowed partial complementation of the fet6-2 growth defect on iron limited medium (Fig. 1). The gene responsible for the low iron growth enhancement was identified by subcloning as ORF fet6 - 02

**Wild Type**

Addition of 5.0% non-fat dry milk dissolved in 20 mM Tris-HCl, 0.9% NaCl, 0.1% Tween 20 (pH 8.0). The blots were incubated with the primary antibody for 20 min at 37 °C, washed and incubated with a horseradish peroxidase-conjugated goat anti-rabbit antibody (Jackson Immuno Research Laboratories Inc.) for 1 h at room temperature. The filters were washed and developed using the ECL procedure (Amer sham Life Science) as per the manufacturer’s instructions. Samples were treated with endoglycosidase H according to the manufacturer’s instructions (New England Biolabs).

**Immunofluorescence**—Cells in log phase were harvested and reincubated in 3.7% formaldehyde for 1 h at 30 °C and then resuspended in fixative buffer that contained 4.0% paraformaldehyde (pH 6.5) over night at 30 °C. The fixed cells were then washed and treated with 200 mM Tris-HCl, pH 8.0, 20 mM EDTA, 1.0% β-mercaptoethanol for 10 min and then incubated with 1 mg/ml olateixase. The cells were permeabilized by the addition of 2.0% SDS in 1.2 M sorbitol for 2 min, washed in sorbitol buffer, and then allowed to settle on polycry-llysine treated coverslips for 30 min. The cells were washed three times with 0.5% bovine serum albumin-phosphate-buffered saline and then incubated with a 1:500 monolonal mouse anti-Myc antibody for 10 h. The coverslips were washed and then incubated with a 1:200 dilution of a Texas Red-conjugated goat anti-mouse antibody (Molecular Probes) for 2 h at room temperature. The cells were visualized using an Nikon inverted fluorescence microscope with a Zeiss 100× oil immersion objective. Images (512 × 512) were acquired using a Photometrics cooled CCD camera and a Macintosh workstation running OncorImage 3-D cytom etry software. A multiday filter set was used in which excitation filters of 575 nm (Texas Red) were selected from a computer-controlled filter wheel in conjunction with a multi-wavelength emitter and dichroic filter set (640 nm; XF56 set from Omega Optical). Out-of-focus blur was removed by adjacent plane fast Fourier deconvolution using the inverse of the modulation transfer function of the microscope. Adjacent planes were taken at 0.5-μm intervals under control of the OncorImage software package. Prior to deconvolution, all images were corrected for background and flatfield. Images were then scaled to 256 levels of gray before output to a film recorder.

**RESULTS**

**Mitochondrial Iron Transporters**

**Fig. 1. Partial complementation of fet6-2 by p20 and p21.** Cells were streaked on iron-limited medium YPD (BPS5). Wild type cells; fet6-2 transformed with p20; fet6-2 transformed with p21; fet6-2. Note that transformation with p20 or p21 resulted in only partial complementation of the low growth phenotype of fet6-2.

**Additional**—Wild type cells; fet6-2 transformed with p20; fet6-2 transformed with p21; fet6-2. Note that transformation with p20 or p21 resulted in only partial complementation of the low growth phenotype of fet6-2.
YMR177W. This gene, when placed in a multicopy plasmid, could by itself suppress the low iron growth deficit of fet6-2. The gene, however, when placed in a centromeric plasmid was unable to rescue the low iron growth phenotype. Using the same library, a different plasmid (p21) was isolated that also complemented the low iron growth phenotype of fet6-2. Subcloning determined that the responsible ORF, YPL224C, when expressed from a multicopy but not a single copy plasmid, suppressed the fet6-2 low iron growth defect.

Examination of the deduced amino acid sequence of these two genes revealed that they are highly homologous (52% identity and 75% similarity) and novel (Fig. 2). They appear to encode membrane proteins with six putative transmembrane domains. The sequences show no identifiable leader or endoplasmic reticulum retention signal. Prosite analysis of either protein suggests that the amino terminus could be a mitochondrial targeting sequence. There is, at best, one potential N-glycosylation site. A feature of interest is two conserved histidine-rich clusters found in both proteins. In particular, the sequence HTHSHX strongly suggests a metal binding motif.

These genes show extensive homology to other genes identified as mitochondrial iron transporters.

**Fig. 2. Deduced amino acid sequence of the complementing open reading frames in P20 and P21.** Subcloning experiments localized the complementing activity of plasmid p20 to open reading frame YMR177W of chromosome 13 and of p21 to open reading frame YPL224C on chromosome 16. A, Conceptual translation of YMR177W and YPL224C. B, Kyte-Doolittle hydropathy analysis (16) of the amino acid sequence of YMR177W and YPL224C. The underlined sequences are the histidine-rich domains.
fied as transition metal transporters, particularly for zinc, cadmium, or cobalt. These genes are found in diverse species, including bacteria, yeast, and mammals. These transporters have been localized to different organelles; mitochondria, endosomes, and plasma membrane (Fig. 3). For example Cot1p, which is involved in cobalt resistance in yeast is mitochondrial (17). The mammalian zinc transporters, ZNT2 and ZNT3, are located in endosomes or synaptosomes, respectively (18, 19). For all of these transporters there are no motifs that indicate that they are ATP dependent transporters.

**Homology of YMR177W and YPL224C to other transition metal transporters.** These sequences were compiled using Blast analysis and the cluster W algorithm. For reasons described below, we refer to YMR177W as MFT1 and YPL224C as MFT2. COT1 is a cobalt resistance gene found in *S. cerevisiae* (17), ZNT2 (18), and ZNT3 (19) are mammalian zinc transporters, czcd is a cobalt, zinc, cadmium resistance gene found in *A. eutrophus*, a Gram-negative bacteria (20). The *E. coli* sequence (F313, accession no. 1786966) and the *Caenorhabditis elegans* sequence (T18d3.3, accession no. 1082146) were the result of genome sequencing projects.
Myc-epitope marked YMR177W carried on a centromeric plasmid did not reveal any specific band. A similar analysis, however, on cells transformed with a multicopy plasmid containing the Myc-tagged gene showed a specific band migrating at a molecular mass of 56 kDa on SDS-polyacrylamide gel electrophoresis. When the presence of the Myc-epitope is taken into consideration, the observed molecular mass is the same as that predicted from the deduced sequence. Treatment of samples with endoglycosidase H had no effect on size of the protein, indicating that the protein does not contain any N-linked carbohydrate.

The Myc-tagged YMR177W was quantitatively recovered in a 10,000 × g membrane pellet, as expected from the presence of hydrophobic domains in the deduced sequence. To further de-
fine the location of the protein a low speed supernatant (800 \( g \)) from homogenized cells was applied to a Percoll density gradient. Fractions were collected and applied to SDS-polyacrylamide gel electrophoresis, and Western blots were probed using specific antibodies to defined membrane proteins. Most of the epitope-marked protein was found in the same fractions that were enriched for mitochondria as defined by the presence of the outer mitochondrial membrane protein porin. These fractions were depleted of endoplasmic reticulum (Erg25p), plasma membrane (Fet3p), and vacuoles (Cpyp) (Fig. 4). To further confirm the location of YMR177W, cells transformed with a high copy plasmid encoding a GFP-tagged fusion protein was examined by fluorescence microscopy. The protein is localized to mitochondria as its distribution is identical to that of the mitochondrial protein porin (Fig. 5). Similar results were obtained using cells transformed with YMR177W containing a carboxyl-terminal Myc-tag, in which the localization was examined by indirect immunofluorescence (data not shown). A YPL224C fusion protein containing a carboxyl-terminal Myc-tag was also localized to the mitochondria (Fig. 6). Based on their localization to mitochondria and their homology to transition metal transporters, we have termed these genes Mitochondrial Iron Transporters in which YMR177W is \( \text{MFT1} \) and YPL224C is \( \text{MFT2} \).

**Evidence that MFT1 and MFT2 Affect Cytosolic Iron Concentration**—Both MFT1 and MFT2 were identified due to their ability to suppress the poor growth of fet6-2 in low iron medium. We considered that they could exert this effect by either being involved in the direct insertion of iron into this oxo-diiron protein, or by increasing the cytosolic concentration of iron and, thereby, indirectly providing more iron to the defective methyl sterol oxidase. Since these gene products are localized to mitochondria, we hypothesize that their effect on fet6-2 is indirect and results from an increase in cytosolic iron concentration.

Several experiments indicate that the latter hypothesis is...
were more resistant than cells overexpressing cells were more resistant to SNG than wild type cells, which concentration. When grown on low iron medium, ence of SNG can be used as an assay for cytosolic iron concen-
tration. Thus, cell viability in the pres-
Reduced SNG in the presence of ferrous iron and oxygen gen-
aminoquinone antibiotic that diffuses into cells and is reduced. select cells that had defects in iron transport (7). SNG is an
by accessing internal pools.

Evidence That MFT1 and MFT2 Affect Mitochondrial Iron Accumulation—Many of the members of the extended gene family to which MFT1/MFT2 belonged were identified as metal resistance genes. Expression of these genes confer resistance to the specific transition metals by either transporting them out of cells or into the lumen of intracellular vesicles. Consequently, the action of these genes permits cells to grow in supernormal levels of transition metals. To determine if MFT1 or MFT2 functioned in a similar manner, cells were incubated in supernormal levels of iron, and both whole cell and mito-
chondrial iron concentration determined by atomic absorption spectroscopy (Table I). Cells that overexpressed either gene showed a higher accumulation of iron than wild type cells. Further, this increased iron could be quantitatively accounted for by an increase in mitochondrial iron. Incubation of cells in increased concentrations of either cobalt, zinc, or manganese did not result in either increased cellular or mitochondrial levels of these transition metals.

**DISCUSSION**

We identified two different genes, MFT1 and MFT2, which when overexpressed were capable of partially overcoming the growth defect attributed to a mutation in an endoplasmic reticulum iron-binding enzyme, methyl sterol oxidase. We believe that the iron binding site of Erg25p is in the cytosol and hence is sensitive to cytosolic iron. Overexpression of these two gene products in cells grown in low iron medium did not increase plasma membrane iron transport. Rather, the data suggest that overexpression increased cytosolic iron as a result of mo-
obilization of iron from mitochondrial pools. In the absence of any exogenous iron, cells transformed with multicopy plasmids
containing either MFT1 or MFT2 grew longer than control cells, and were more susceptible to the iron mediated toxicity of SNG. Conversely, cells in which both genes were deleted were more SNG resistant than control cells and showed a lowered rate and degree of growth in iron free media. The fact that cells grew longer in iron free media supports the view that the effect of these genes is not on plasma membrane iron transport but on redistribution of iron from intracellular compartments.

Based on subcellular fractionation and immunofluorescence both MFT1 and MFT2 encode mitochondrial membrane proteins. The major caveat to these studies is that overexpression is required to detect the tagged proteins and that overexpression may lead to mislocalization. In this instance that concern is lessened for two reasons. First, the protein is exclusively localized to a single organelle. Second, to our knowledge there is no evidence from published studies that overexpressed mitochondrial proteins are missorted to other organelles.

The MFT genes show amino acid homology to a gene family that encode transition metal transporters. All family members have six potential transmembrane domains and are not members of the ABC family. While a number of these proteins are simply anonymous open reading frames, many have definable functions or are associated with phenotypes. For example COT1, a yeast gene that encodes a mitochondrial protein, was identified through a screen that selected for genes that when overexpressed conferred resistance to increased Co (17). The yeast ZRC1 gene that confers resistance to cadmium and zinc (21) is also a member of this family. Similarly, cccd was identified as a gene on a plasmid that conferred resistance to cadmium, zinc, and cobalt in the bacteria Alcaligenes eutrophus (20). This gene has homology to genes found in E. coli and other

**TABLE I**

| Strain* | Whole cell iron | Mitochondrial iron |
|---------|-----------------|-------------------|
|         | nmol Fe/mg protein |                  |
| WT/vector | 2.04 ± 0.08 | 5.38 ± 1.52 |
| WT/MFT1   | 5.41 ± 0.04 | 33.99 ± 2.53 |
| WT/MFT2   | 5.35 ± 0.09 | 25.43 ± 0.50 |

* WT, wild type.

**FIG. 7.** Growth of cells that overexpress MFT1 or have a deletion in MFT1 and MFT2 in iron-free-medium. Wild type cells or cells carrying either a multicopy plasmid expressing MFT1, or a strain with a double deletion (ΔMFT1, ΔMFT2) were grown in YPD. The cells were washed and resuspended in media made iron-deficient by the addition of BPS to YPD medium (BPS0). At the specified times, cell multiplication was assayed by measuring the optical density of the cultures using a wave length of 600 nm.

**FIG. 8.** SNG sensitivity of cells that either overexpress MFT1 or have a deletion in the MFT1/MFT2 genes. Wild type cells or cells carrying either a multicopy plasmid expressing MFT1, or a strain with a double deletion (ΔMFT1, ΔMFT2) grown in YPD were transferred to iron-limited medium (YPD BPS5) for 24 h. The cells were washed and exposed to the specified concentrations of SNG in iron-free medium for 2 h. The cells were then plated on to YPD medium and the number of colonies determined.
bacteria, and with lower degrees of homology, to genes in the Archea. Other homologous genes include endosomal or synap- 
tosomal zinc transporters present in mammalian cells (18, 19). 
Based on their location, as well as direct physiological studies 
it is suggested that these proteins may be H⁺/metal co-trans-
porters (20). Proteins located in either the bacterial membrane 
(Czcd) or mitochondrial membrane (Cot1p, MFT1p, MFT2p) 
may also utilize a pH gradient for transport activity (22). The 
proteins encoded by the mammalian zinc transporters, ZNT2 
and ZNT3, are located in subcellular organelles which also 
may be acidic (18, 19). Each one of these proteins contains a histi-
dine-rich sequence. The exact sequence is different for each of 
the family members, although the histidine rich region in 
MFT1 and MFT2 are extremely similar to each other. The role 
of this sequence is unknown. Two likely hypotheses are that 
this sequence is required to bind metals prior to transport or 
that it is a metal binding domain responsible for regulating the 
activity of these transporters.

That the MFT genes act as “metal resistance” genes is sug-
gested by the observation that when cells expressing these 
genes were placed in high iron medium iron was accumulated 
above that seen in control cells. The ability to accumulate metal 
also seen in yeast overexpressing the 
COT1 gene that medi-
ates cobalt resistance (17). In cells that overexpress MFT1 or 
MFT2, the increased cellular iron is located within the mito-
chondria. The fact that the double deletion strain can grow well 
on both glycolytic and respiratory substrates indicates that 
under normal physiological conditions these genes are not 
responsible for the delivery of iron to either ferrochelatase or 
mitochondrial iron binding proteins.

The action of the MFT genes allows cells to utilize iron when 
cells grown in high iron are placed in iron-restricted medium. 
Based on this observation, we surmise that mitochondrial iron 
can be utilized for purposes other than heme biosynthesis. This 
line of reasoning suggests that mitochondria can act as a res-
ervoir for iron. Iron, particularly in the presence of reactive 
oxygen intermediates, can be toxic. Indeed, the mitochondria 
is the major source of reactive oxygen intermediates. Cells over-
expressing MFT1 or MFT2 are perfectly capable of growth on 
respiratory substrates, suggesting that the stored iron is not 
toxic. Conditions exist, however, in which mitochondrial iron 
accumulation can lead to toxicity. This has recently been dem-
onstrated in cells bearing a deletion in the YFH1 gene, a gene 
that is homologous to the mammalian gene Frataxin (23). De-
fects in Frataxin are responsible for Friedreich’s ataxia, a le-
thal disease. Cells with a defective YFH1 gene also accumulate 
iron in mitochondria. Such cells show a respiratory defect and 
an increased sensitivity to H₂O₂. The observation that cells with 
overexpressed MFT genes in high iron conditions also 
accumulate mitochondrial iron suggest that iron accumulation per se need not be toxic. These observations suggest 
that strains overexpressing MFT1/MFT2 or carrying a deletion in 
YFH1 may accumulate iron into different mitochondrial sub-
compartments or that YFH1 may affect the conditions in which 
mitochondrial iron is stored. Additionally, the amount of iron 
found in mitochondria in Δyfh1 (10×) is much greater than in 
MFT1/MFT2 overexpressors (2–5×). This may suggest that 
toxicity is defined by the absolute magnitude of mitochondrial iron accumulation. Experiments are in progress to distinguish 
between these hypotheses.

Note Added in Proof—The family of genes described here fit the 
characteristics of the cation facilitator family described by Paulsen and 
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