Defects in the Yeast High Affinity Iron Transport System Result in Increased Metal Sensitivity because of the Increased Expression of Transporters with a Broad Transition Metal Specificity*

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Yeast with defects in vacuolar pH show increased sensitivity to high concentrations of transition metals. This sensitivity has been presumed to result from defective metal storage. We demonstrate that mutations that result in a defective high affinity iron transport system, such as a deletion in the surface ferrooxidase FET3, also result in increased metal sensitivity independent of vacuolar function. Multiple copies of transition metal transporter resistance genes, such as COT1 or ZRC1, do not reduce the metal sensitivity of fet3 mutations. Increased metal sensitivity is because of an increased cellular accumulation of transition metals resulting from the increased activity of low affinity iron transporters, such as FET4, that mediates the transport of other transition metals. In cells lacking a high affinity iron transport system, the increased transition metal uptake can be prevented by increased extracellular iron. These results suggest that vacuolar function may not be required for transition metal sequestration.

The yeast vacuole is considered to be a site for transition metal storage since yeast mutants defective in vacuolar pH show an increased sensitivity to a wide variety of transition metals (1–3). This increased sensitivity has been ascribed to an inability to store transition metals in defective vacuoles. These mutants also show a decreased ability to grow on low iron medium and an inability to grow on respiratory substrates. Similar phenotypes, including metal sensitivity, are seen in ups mutants that show defective vacuolar formation (4). Recently we (5) and Yuan et al. (6) demonstrated that many ups mutants show defective iron metabolism because of the inability to form a functional high affinity iron transport system. Iron transport relies on a cell surface multicopper oxidase, Fet3p, that converts ferrous iron to ferric iron, which is then transported across the plasma membrane by the permease Ftr1p (7). The construction and phenotype of the Δfet3 strain is described in Askwith et al. (7). The construction and phenotype of the Δups41 strain is described in Radisky et al. (5). A deletion of the FET4 gene was generated using a deletion plasmid provided by Dr. D. Eide (University of Missouri, Columbia, MO). This plasmid contains a LEU2 gene cloned into the PauI site in the FET4 gene. Deletions in the SMF1 gene were generated using a double PCR deletion technique (10). The primers were (SMF1) CTCCTTCACATTTTGTGCCC, (SMF2) GTCGTGACTGGAAAAACCTGGCCAACCCTTACCACTTATTGAC, (SMF3) TCCCTGTAATAATAATGGCTACCATCC. The isolation and phenotype of the gef1–1 mutant is described in Green et al. (11). The gef1–2 strain is a gef1 mutation in strain background DY1457. The cells were grown in YPD (1.0% yeast extract, 0.2% peptone, 2.0% glucose). In YPD made iron-limited by the addition of benthophenanthroline sulfonate, or in CM medium, a synthetic medium of yeast nitrogen base, amino acids, and glucose. DNA transformations of Escherichia coli and Saccharomyces cerevisiae were performed by standard procedures (12, 13).

Construction of Epitope-tagged Protein—Both COT1p and ZRC1p were epitope-tagged with myc at their carboxyl terminus using the polymerase chain reaction and ligated into the multicopy vector pTFS63 (7). Each 5′ primer included an upstream promoter region, and the 3′ primer included a myc epitope. Two primers for COT1-myc were: 5′-CCG GAA TTC TAT TAC CCA CCA ATG TCT GTC TTC TCA GCA ATC TGC-3′. The two primers for ZRC1-myc were: 5′-CCG GAA TTC TAT TAC CCA CCA ATG TCT GTC TTC TCA GCA ATC TGC-3′. The PCR reactions were performed in a Perkin-Elmer PCR machine at conditions of denaturation 92 °C, 30 s, annealing 60 °C, 30 s, elongation 72 °C, 3 min, for 35 cycles. The constructed plasmids were sequenced at the junction of the myc-tagged 3′ end and were also tested for their ability to decrease zinc/cobalt toxicity.

Metal Toxicity Test—Metal sensitivity of yeast strains were tested in CM liquid medium supplemented with transition metals at the specified concentrations. Cells were allowed to grow for 20 h in 5 ml of culture at which point A600 was measured. Iron in the form of ferrous sulfate (500 μM) was added to the cultures as specified.

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Atomic Absorption Assay—Cells were grown in 20.0 ml of CM medium with transition metals as specified for 20 h. Log phase cells were collected and washed by centrifugation with 50 mM Tris-HCl, pH 6.5, 10 mM EDTA. Cell pellets were digested in 200 ml of 5:2 nitric acid: perchloric acid at 80 °C for 1 h. After digestion, the samples were diluted to 1.0 ml with deionized water and then flamed in a Perkin-Elmer ICP atomic absorption spectrometer. All samples were measured in duplicate, and the experiment was performed at least twice.

Northern Blotting—Total RNA was isolated from yeast grown for

Fig. 1. Growth of wild-type cells and gef1–2 cells in different concentrations of transition metals. Cells of the specified genotype are grown in CM medium containing designated concentrations of metals. After 20 h of growth, the A600 was measured. A, cells grown in Zn supplemented medium; B, cells grown in Co supplemented medium.

Fig. 2. Immunofluorescent localization of Cot1p or Zrc1p in wild type and gef1–2 cells. Wild type cells or gef1–2 cells were transformed with multicopy plasmids containing epitope marked Cot1p or Zrc1p. The cells were harvested and the proteins localized by immunofluorescence as described under “Materials and Methods.” Shown are localization of Cot1p-myc in wild type cells (A) and in gef1–2 cells (B) and localization of Zrc1p-myc in wild type cells (C) and in gef1–2 cells (D). A photomicrograph of wild type cells that are not expressing a myc epitope protein that have been stained with both the primary and secondary antibody is shown (E).
15–20 h in CM medium containing specified supplements. RNA was separated by agarose gel electrophoresis, blotted onto nitrocellulose paper, and probed with FET4, SMF1, SMF2, and ACT1 (actin), which were randomly labeled with \(^{32}\)P. The blot was washed with 0.5 M SSC, 0.1% SDS at 65 °C and then subjected to autoradiography at ~70 °C.

**S1 Analysis of RNA**—An oligonucleotide probe for FET4 was designed: 5'-CCG AAT TCT TCG TAC TGT TTG CAG TCA ACA GTA GGT GCT CTA TGA TGA ACG TCA GGC CTA GCA CCC AAG CC-3'. The probe was labeled at the 5'-end with \([\gamma^{32}\text{P}]\text{ATP}\) by T4 Kinase. The analysis of transcript level was performed as described in Iyer and Struhl (14). Total RNA was isolated from yeast grown for 15–20 h with the specific transition metals. RNA (50 ng) was hybridized overnight at 55 °C with both the FET4 probe and a probe for calmodulin. The RNA was then incubated with 50 units of S1 nuclease (Life Technologies, Inc.) and applied to an 8% denaturing polyacrylamide gel. The gel was dried onto 3-mm Whatman paper and either exposed to x-ray film or analyzed by use of a PhosphorImager.

**Immunofluorescence**—Cells transformed with plasmids encoding myc-tagged proteins 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) for 6 h 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 o-xylyl-case. The cells were permeabized by addition of 2.0% SDS in 1.2 M sorbitol for 2 min, washed in 1.2 M sorbitol, and then allowed to settle on poly-L-lysine-treated coverslips for 30 min. The cells were washed three times with 0.5% bovine serum albumin in phosphate-buffered saline and then incubated with a 1:100 monoclonal mouse anti-myc antibody (9E-10) overnight at room temperature in a humid chamber. 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 a Nikon inverted fluorescence microscope with a Nikon ×100 oil immersion objective. Fluorescent and Nomarski images were acquired using a Princeton Instruments cooled CCD camera and a Macintosh workstation running OpenLab-Improvision cytometry software from a computer-controlled filter wheel in conjunction with a multi-wavelength emitter and diachronic filter set (Omega Optical).

**FIG. 3.** Effect of different metals on the growth of wild type and Δfet3 cells. Cells were incubated in the designated concentrations of metals for 20 h at which point the A\text{\textsubscript{600}} was determined.
RESULTS

Previous studies demonstrated that mutants defective in either vacuolar pH or in \textit{GEF1}, a gene that has homology to a human voltage-regulated chloride channel, show increased metal sensitivity (12, 15). This result is confirmed by the data shown in Fig. 1 in which a \textit{gef1}-2 mutant shows an increased sensitivity to Co (Fig. 1A) or Zn (Fig. 1B), as well as Cu and Mn (data not shown). Both vacuolar morphology and pH appear normal in \textit{Δgef1} strains. The \textit{GEF1} gene product is localized to a prevacuolar compartment, suggesting that its affects on vacuolar function must be indirect. It may be possible that the \textit{gef1} defect results in the missorting of vacuolar proteins that affect transition metal resistance. \textit{COT1} and \textit{ZRC1} are genes that when overexpressed result in increased resistance to Co and Zn, respectively (Refs. 16 and 17, and Fig. 1). Although Cot1p was thought to be localized to the mitochondria, examination of the distribution of epitope-tagged protein reveals it to be localized to the vacuole (Fig. 2A). Epitope-tagged Zrc1p is also localized to the vacuole (Fig. 2C). No increased metal resistance is seen, however, when these genes are overexpressed in a \textit{gef1–1} strain (Fig. 1). Examination of the subcellular distribution of these proteins in a \textit{gef1–1} strain shows no change from their vacuolar location (Fig. 2, C and D). Thus, it is unlikely that mistargeting of vacuolar membrane proteins can explain the defective metal resistance in \textit{gef1} strains.

One phenotype common to \textit{gef1}, \textit{vps}, and \textit{uma} mutants is a defective high affinity iron transport system as a result of improper copper loading of Fet3p (5, 6, 18). We questioned whether deletion of \textit{FET3} would result in increased metal sensitivity. As shown in Fig. 3, \textit{Δfet3} strains show a markedly increased sensitivity to transition metals. Similar results were obtained for \textit{vps 41}, which shows a Class B vacuolar morphology (data not shown). This mutation also results in a defective high affinity iron transport system resulting from a defect in copper loading of Fet3p (5).

Cells lacking the high affinity iron transport system can grow because of the expression of other plasma membrane transport systems, which act as low affinity iron transporters. These transporters have a broad specificity and transport other transition metals. We hypothesized that the increased expression of these low affinity iron transporters may account for the increased metal sensitivity. Besides Fet3p there are (at least) three cell surface transporters that may mediate iron transport: Fet4p (Co, Cd, Cu) (19), Smf1p (Mn), and Smf2p (Mn, Co) (20). Smf1p and Smf2p are homologous to the mammalian divalent cation transporter NRAMP2 that is responsible for both intestinal transport and reticulocyte endosome-mediated iron transport (21–23). NRAMP2 expressed in \textit{S. cerevisiae} can complement a \textit{Δsmf1Δsmf2} deletion strain (24). Strains with

![Fig. 4. Effect of genetic background on the ability of cells to grow in Co-enriched medium.](image)

Wild type cells, \textit{Δfet3}, \textit{Δfet4}, \textit{Δfet3/Δfet4}, and \textit{Δfet3/Δfet4/Δsmf1} were streaked on YPD plates in the absence (A) and presence (B) of 50 μM Co. The plates were incubated at 30 °C for three days and then photographed.

![Fig. 5. Effect of transition metals on the level of \textit{FET4} transcript in wild type and \textit{Δfet3} cells.](image)

Wild type and \textit{Δfet3} cells were incubated in CM containing either Co (50 μM), Cu (500 μM), Mn (5 mM), or Zn (5 mM) for 20 h. Cells were harvested, RNA was extracted, and transcripts for \textit{FET4} and calmodulin were analyzed by \textit{S1} analysis. The value under the autoradiogram is the amount of \textit{FET4} transcript relative to calmodulin transcript as quantified by phosphoimager analysis.
multiple deletions in membrane transport genes grow poorly in iron-limited medium even without added transition metals. An increase in medium iron (YPD as opposed to CM) improves the growth of strains with multiple deletions. Addition of 50 μM Co to YPD plates does not affect the growth of wild type cells but has a severe affect on the growth of Δfet3 cells (Fig. 4). Deletion of the FET4 gene allows the Δfet3 strain to grow in the presence of Co. An additional deletion in the SMF1 gene has no effect.

If deletion of the FET4 gene suppresses the transition metal toxicity in Δfet3 strains, then there may be an increased expression of FET4 transcripts in strains incubated in transition metals. Analysis of FET4 transcription by either Northern or S1 analysis reveals an increased level of transcript in Δfet3 cells relative to wild type (Fig. 5). In several different experiments, the increase in transcript level was usually no greater than 2-fold. Addition of transition metals to the Δfet3 cells resulted in a 2–5-fold increase in FET4 transcript level, depending upon the metal, whereas no increase in transcript is seen in wild type strains. We did not observe any increase in SMF1 or SMF2 transcripts in Δfet3 cells, or in Δfet3 cells incubated with

Fig. 6. Effect of iron addition on the growth of wild type and Δfet3 cells in the presence of transition metals. Wild type and Δfet3 cells were incubated in CM medium containing either Co (0.1 mM), Mn (5 mM), Cu (500 μM), or Zn (5 mM) with the specified concentrations of iron for 20 h at which point A600 was measured.
Yeast Transition Metal Transport

### Table I

| Cell type | Co  | Cu  | Fe  | Zn  | Mn  |
|-----------|-----|-----|-----|-----|-----|
| WT        | 0.000 | 0.053 | 0.343 | 1.390 | 0.035 |
| Δfet3     | 0.000 | 0.058 | 0.040 | 1.384 | 0.024 |
| WT/Cu     | 0.416 | 0.062 | 0.349 | 1.482 | 0.029 |
| Δfet3/Cu  | 1.182 | 0.382 | 0.001 | 1.798 | 0.050 |
| WT/Cu/Fe  | 0.357 | 0.072 | 2.946 | 1.648 | 0.027 |
| Δfet3/Cu/Fe | 0.367 | 0.066 | 1.405 | 1.237 | 0.021 |
| WT/Zn     | 0.000 | 1.369 | 0.412 | 1.353 | 0.013 |
| Δfet3/Zn  | 0.000 | 4.918 | 0.000 | 0.837 | 0.019 |
| WT/Zn/Fe  | 0.000 | 1.502 | 0.927 | 1.300 | 0.013 |
| Δfet3/Zn/Fe | 0.000 | 1.227 | 0.259 | 1.060 | 0.011 |
| WT/Mn     | 0.000 | 0.055 | 0.333 | 10.930 | 0.027 |
| Δfet3/Mn  | 0.000 | 0.267 | 0.000 | 17.683 | 0.035 |
| WT/Mn/Fe  | 0.000 | 0.063 | 3.265 | 11.663 | 0.028 |
| Δfet3/Mn/Fe | 0.000 | 0.073 | 1.617 | 11.137 | 0.021 |

Wild type and Δfet3 cells were grown in CM medium for 20 h in the presence or absence of transition metals Co (10 μM), Cu (200 μM), Zn (1.0 mM), Mn (2.0 mM), and additional iron. The cells were washed and prepared for elemental analysis using a Perkin-Elmer ICP (inductively coupled plasma) atomic absorption spectroscope. The data are expressed as ppm normalized to cell number. This experiment was repeated twice with similar results.

### Discussion

This result suggests that increased transition metal accumulation results from the increased activity of low affinity iron transporters. Accordingly, addition of iron to Δfet3 cells should reduce transition metal toxicity. As demonstrated in Fig. 6, addition of iron to Δfet3 cells completely prevents the toxicity of any of the transition metals tested. Similar results were obtained using either gef1 or cps4 strains (data not shown). If these metals are competing with iron for transport, then Δfet3 cells should show reduced iron levels when incubated with transition metals. Conversely, if iron competes with the other transition metals for uptake, additional iron should raise cellular iron levels and lower the content of transition metals. Incubation with transition metals resulted in an increased content in Δfet3 cells as compared with wild type cells (Table I). As expected, Δfet3 strains had a lower cellular iron level than wild type cells. Incubation of Δfet3 with transition metals results in a further decrease in cellular iron content. When Δfet3 cells were incubated with iron, cellular iron content increased, and there was a decrease in the content of the specific transition metal. Exposure of Δfet3 cells to Co, Zn, or Mn resulted in an increase in accumulation of cellular copper that was reduced by added iron. An increased concentration of Mn resulted in decreased cellular iron in the Δfet3 strain, and added iron also restored iron content. There was, however, little difference in the cellular accumulation of Mn in either wild type or Δfet3 cells. Addition of iron to cells incubated in Mn had little effect on the cellular accumulation of Mn.

### Intracellular transition metal concentration in wild type and Δfet3 cells

Intracellular transition metal concentrations in wild type and Δfet3 cells were determined by atomic absorption spectrometry. The data are presented as ppm normalized to cell number. This experiment was repeated twice with similar results.

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### DISCUSSION

Our results suggest that any mutation that results in defective Fet3p function (missorting, defective copper loading, or gene deletions) results in increased metal sensitivity. Without Fet3p, the iron requirements of cells are met by increased expression of other transporters. These transporters mediate the uptake of not only iron but other transition metals. Although there are transporters that are highly selective for specific transition metals, such as Cu (CTR1, CTR3) (25, 26), Zn (ZNT1) (27), and iron (FET3/FTR1) (7), many transporters show a lower affinity and a broader specificity. In the absence of the high affinity iron transport system, the cellular need for iron is met by these lower affinity Fe(II) transport systems. This conclusion is supported by the observation that strains with multiple gene deletions grow poorly in iron-limited medium (Δfet3Δfet4), whereas strains with single deletions grow well. Furthermore, transcription of the FET4 gene is increased under conditions of iron starvation.

There may be two reasons why increased transition metals result in increased cell toxicity in strains that are lacking high affinity iron transport. First, because of the low transporter specificity, increased concentrations of Co, Zn, and Cu compete with iron for uptake. This competition results in a further decrement in intracellular iron content (Table I). Second, in the face of high concentrations of transition metals, increased expression of these transporters will lead to increased accumulation of transition metals (Table I). Addition of iron to these cells results in an increased cellular iron level and a decreased uptake of potentially toxic transition metals. Our data also indicate that incubation of the Δfet3 strain with increased concentrations of Co, Zn, or Mn results in increased accumulation of Cu, which is decreased by the additional iron. An increase in Cu sensitivity in both vascular acidification strains and in a Δfet3 strain has been seen by Szczypka et al. (3). These authors suggested that increased FET3 may play a role in copper detoxification and that copper may affect FET3 transcription. Our results do not address the issue of whether Cu affects transcription of FET3. There are differences between our results and those of Szczypka et al. (3) as to whether there is increased copper accumulation in mutants that show a defective Fet3p. Many of these differences can be ascribed to strain, medium, and experimental differences. Further experiments are required to determine whether the increased Cu uptake observed is because of increased expression of high affinity or low affinity copper transporters.

Addition of Mn also results in increased metal sensitivity in strains lacking a high affinity iron transport system. Similar to other transition metals, this increased sensitivity is also abrogated by the addition of iron. Addition of Mn, however, does not lower intracellular iron, and Δfet3 strains exposed to high Mn do not accumulate more Mn than wild-type strains. Perhaps Mn toxicity results from a competition with iron at an intracellular level since intracellular iron transporters or enzymes might also recognize Mn. Iron may be displaced from proteins when the intracellular iron concentration is low and intracellular Mn is high. As intracellular iron is increased, the effect of Mn would be reduced.

Our data offer an explanation for increased metal sensitivity that is independent of the role of the vacuole in metal storage. Genetic and biochemical evidence suggests that there is a vacuolar transport system for Cd-glutathione conjugates (28). Studies have also reported an increased transition metal content in vascular-enriched fractions (29). The transition metal resistance genes COT1 and ZRC1, when overexpressed, are localized to the vacuole and clearly provide resistance to high levels of transition metals. Yet when there is increased expression of transition metal transporters at the cell surface, there is no increased transition metal resistance even in the face of overexpression of vascular transporters. Perhaps the role of Cot1p or Zrc1p is to retain in the vacuole metals brought in by endocytosis. In wild-type cells that are iron replete there may be little expression of low affinity transporters, certainly the expression of FET4 is reduced (30). In metal-replete wild type cells, additional transition metals may only enter through the endocytic system. It may be that the role of Cot1p or Zrc1p is...
to retain metals within the vacuole. Metals brought in through the cytosol by surface transporters may exceed the capacity of vacuolar transporters. Alternatively, due to the orientation of the vacuolar transporters, cytosolic metals may not be recognized.

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REFERENCES
1. Kitamoto, K., Yoshizawa, K., Ohsumi, Y., and Anraku, Y. (1988) J. Bacteriol. 170, 2687–2691
2. Ramsay, L. M., and Gadd, G. M. (1997) FEMS Microbiol. Lett. 152, 293–298
3. Szczypka, M. S., Zhu, Z., Silar, P., and Thiele, D. J. (1997) Yeast 13, 1423–1435
4. Klionsky, D. J., Herman, P. K., and Emr, S. D. (1990) Microbiol. Rev. 54, 266–292
5. Radisky, D. C., Snyder, W. B., Emr, S. D., and Kaplan, J. (1997) Proc. Natl. Acad. Sci. U. S. A. 94, 5662–5666
6. Yuan, D. S., Dancis, A., and Klausner, R. D. (1997) J. Biol. Chem. 272, 25787–25793
7. Askwith, C., Eide, D., Van Ho, A., Bernard, P. S., Li, L., Davis-Kaplan, S., Sipe, D. M., and Kaplan, J. (1994) Cell 76, 403–410
8. Stearman, R., Yuan, D. S., Haile, D., Askwith, C., Elde, D., Moehle, C., Kaplan, J., and Klausner, R. D. (1994) Cell 76, 393–402
9. Knight, S. A., Labbe, S., Kwon, L. F., Kosman, D. J., and Thiele, D. J. (1996) Genes Dev. 10, 1917–1929
10. Huang, L., and Gitschier, J. (1997) Nat. Genet. 17, 292–297
11. Dancis, A., Yuan, D. S., Haile, D., Askwith, C., Elde, D., Moehle, C., Kaplan, J., and Klausner, R. D. (1994) Cell 76, 393–402
12. Knight, S. A., Labbe, S., Kwon, L. F., Kosman, D. J., and Thiele, D. J. (1996) Genes Dev. 10, 1917–1929
13. Huang, L., and Gitschier, J. (1997) Nat. Genet. 17, 292–297
14. Bode, H. P., Dumshat, M., Garetti, S., and Fuhrmann, G. F. (1995) Eur. J. Biochem. 228, 337–342
15. Dix, D., Bridgman, J., Broderius, M., and Eide, D. (1997) J. Biol. Chem. 272, 11770–11777