Zinc suppresses the iron-accumulation phenotype of *Saccharomyces cerevisiae* lacking the yeast frataxin homologue (Yfh1)

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Analysis of *Saccharomyces cerevisiae* cell transcriptome revealed that iron deprivation/supplementation affects genes other than those of the iron regulon (controlled by Aft proteins). Several genes regulated by zinc (induced by zinc deprivation) were induced by iron. Cells lacking the yeast frataxin homologue Yfh1 accumulate large amounts of iron in their mitochondria. We have shown that the zinc metabolism of these cells is also impaired: zinc uptake and zinc accumulation were both much lower in $\Delta yfh1$ cells than in wild-type cells. Excess zinc in the growth medium also influenced the phenotypes of $\Delta yfh1$ cells. It prevented the

accumulation of iron in the mitochondria of $\Delta yfh1$ cells and increased the growth rate of these cells and their resistance to oxidative stress. However, zinc did not restore the deficiency of Fe–S and haem proteins of $\Delta yfh1$ cells. Zinc inhibited mitochondrial respiration and protected Yfh1p, the mitochondrial ferredoxin. These results suggest that zinc nutrition may be important in the aetiology of Friedreich's ataxia.

Key words: Fe–S protein, iron, mitochondria, YAH1, YFH1, zinc.

INTRODUCTION

The yeast *Saccharomyces cerevisiae* has become an important model for studying iron metabolism in the eukaryotic cell. This yeast has two high-affinity iron-transport systems and one low-affinity one (reviewed in [1]). One high-affinity iron-transport system is called the reductive system because the first step involves the reduction of iron at the external surface of the cell by transmembrane reductases encoded by the reductase genes *FRE1* and *FRE2* [1]. These enzymes make the ferric chelators in the extracellular medium labile so that the resulting ferrous iron can be taken up by the permease–oxidase complex of the plasma membrane encoded by the genes *FTR1* and *FET3* [1]. The reductive iron-uptake system is remarkable because it involves the metabolism of both iron and copper. Fet3p is a multi-copper ferroxidase that is required for iron translocation through the plasma membrane, so that copper is required for reductive iron uptake [2]. There is a similar iron–copper connection in humans. The multicopper oxidase ceruloplasmin is required to mobilize iron to transferrin by converting the ferrous iron into ferric iron [3]. Individuals suffering from the inherited disease aceruloplasminemia completely lack ceruloplasmin and their iron homeostasis is defective [4].

The second high-affinity iron-uptake system is non-reductive and copper-independent. Cells take up iron bound to siderophores excreted by other micro-organisms [1]. The siderophore transporters are encoded by four genes (*SIT1*, *ARN1*, *TAF1* and *ENB1*) and belong to the major facilitator superfamily (MFS). Production of the components of the reductive and non-reductive iron-uptake systems is induced under iron-deficient conditions by the homologous transcriptional activators Aft1p and Aft2p [5,6]. In addition to these high-affinity transport systems, iron can be taken up by a third, low-affinity, transporter encoded by *FET4* [7]. Fet4p transports both ferrous iron and other divalent cations like Zn^{2+} [8]. Zinc is also taken up by the high-affinity transporter encoded

by *ZRT1* [9], whose transcription is controlled by the Zap1p activator [10].

The molecular events of iron and zinc uptake by *S. cerevisiae* cells have been well characterized, but our understanding of intracellular iron and zinc metabolisms is still poor. During these last few years, several genes have been described whose deletion results in the accumulation of iron in mitochondria (reviewed by [11]); however, the underlying mechanisms have not been elucidated. One of these genes, *YFH1* is the yeast homologue of the human *FRDA* gene that encodes the protein frataxin. Mutations of *FRDA* associated with decreased frataxin production are responsible for Friedreich's ataxia, a neurodegenerative disease associated with cardiomyopathy [12]. Deletion of *YFH1* causes a redistribution of the intracellular iron pool, with depletion of the cytoplasm and accumulation in mitochondria [13]. As a consequence, the Aft1p regulator is activated and iron-transport genes are induced, leading to increased iron uptake [13,14]. The accumulation of iron in mitochondria may be responsible for reduced respiratory activity and increased sensitivity to oxidative stress [13,15]. Yfh1p has been postulated to mediate the efflux of iron from the mitochondria into the cytoplasm [13,16]. But exactly how Yfh1p participates in this movement has never been described. Iron accumulates only in the cardiac tissues of Friedreich's ataxia patients, but the lack of Fe–S enzymes is general [17,18]. Studies on mouse models indicate that the Fe–S protein deficit and onset of the disease precede iron accumulation, suggesting that iron accumulation is a secondary event [19]. Recent reports propose that Yfh1p plays a role in the synthesis of Fe–S clusters [17,19–21], possibly by making iron available for biosynthesis. Thus, Yfh1p could be an iron donor protein, as it can form multimers and store iron [22,23]. Our recent studies on the role of Yfh1 in haem synthesis showed that $\Delta yfh1$ mutants are impaired for haem synthesis because iron is not available to ferrochelatase [24]. However, zinc, a competitive substrate of iron for ferrochelatase [25], is readily incorporated into protoporphyrin

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IX to form Zn-protoporphyrin IX [24]. The observation that Zn-protoporphyrin IX is synthesized rather than haem in $\Delta yfh1$ cells led us to investigate further the relationships between iron and zinc metabolisms. The present study reports an iron–zinc connection in *S. cerevisiae* that could have important implications for our understanding of the role of Yfh1 and frataxin.

EXPERIMENTAL

Strains, media and growth conditions

The *S. cerevisiae* strains used in this study were S150-2B (*MATa*, *his3-Δ1*, *leu2-3,112*, *trp1-289*, *ura3-52*), S150-2B *yfh1Δ::TRP1* and GAL-Yfh1 (*MATa*, *his3Δ200*, *ura3-52*, *lys2-801*, *ade2-101*, *cyh2*, *yfh1Δ::TRP1*, *GAL-YFH1::URA3*) [24]. Unless otherwise stated, cells were grown at 30 °C in complete YPD medium (1 % yeast extract, 1 % peptone and 2 % dextrose) or in complete YPR medium (1 % yeast extract, 1 % peptone and 2 % raffinose). The iron-deficient medium was minimal YNB-glucose medium (yeast nitrogen base without copper or iron; Bio 101) plus the required amino acids and 1 μM Cu₂SO₄. Iron-deficient and zinc-deficient media were minimal YNB-glucose medium (yeast nitrogen base without copper, iron or zinc; Bio 101) plus the required amino acids and 1 μM Cu₂SO₄.

RNA isolation and Northern blot analysis

RNA extraction, Northern blotting and hybridization [at 42 °C in 50 % (v/v) formamide] were done essentially as described in [24]. The 661-bp DNA fragment used as a probe for the *ZRT1* genes was amplified by PCR using 5'-GGTGGAAACGAGTACAATGG-3' and 5'-GATGGAACACTAAGACAGGG-3' as primers. A 1.2-kb *Bam*HI–*Hind*III fragment was used for the *ACT1* gene [24].

Microarray hybridization and analysis

Cultures were grown overnight in minimal YNB-glucose medium for iron-deficient conditions and YNB-glucose medium plus 50 μM ferric citrate for iron-rich conditions. The cells were diluted 5-fold in the same media for iron-rich conditions, and 100 μM bathophenanthroline disulphonate was added for iron-deficient conditions. Cultures were grown until the *D*₆₀₀ was 0.8–1.0 and then RNA was extracted. The resulting RNA was hybridized with *S. cerevisiae* Yeast GeneFilters® microarrays (Research Genetics, Invitrogen Corporation) according to the Research Genetics protocols. The data from the Phosphorimager images were analysed using Pathways 4 software (Research Genetics). A ≥ 2.0-fold change in quantity was considered significant. All ratios presented here have been log transformed (base 2). The complementary tools used for data analysis were found at the web server <http://www.justbio.com/arrays>. The *Saccharomyces* Genome Database (<http://genome-www.stanford.edu/Saccharomyces>) was used for gene annotation. The complete data are available from the corresponding author on request. Microarray data from zinc-regulated genes [where wild-type cells under low-zinc (61 nM ZnSO₄) and high-zinc (3 mM ZnSO₄) conditions were compared] have been described in [26] and are available at <http://genome-www.stanford.edu/zinc>.

Iron- and zinc-uptake assays

Iron uptake was assayed using cells washed twice with water and suspended in 50 mM citrate (tri-Na⁺) buffer, pH 6.5 containing 5 % glucose. Cells were incubated for 2 min at 30 °C with

stirring. Iron [as ⁵⁵Fe(II)-ascorbate] was then added to give a final concentration of 1–5 μM. Aliquots (100 μl) were withdrawn at various times and added to 100 μl of 1 mM cold ferrous ascorbate kept on ice in the wells of a microtitre plate. The cells were collected with a cell harvester (Brandel) and washed on a filter. Iron accumulated by the cells was measured by adding various amounts of ⁵⁵Fe-citrate to the growth medium. Cells were harvested in mid-logarithmic phase, washed twice with 10 mM EDTA (pH 7) and twice with water and counted by liquid scintillation. Zinc uptake and accumulation was measured as described in [9].

Other methods

Protoplasts were lysed and fractionated to purify isolated mitochondria as described previously [27]. Low-temperature spectra (–191 °C) of whole cells were prepared as described in [28]. Antibodies were obtained, as described previously, against Yfh1p [29], Hem15p [25], Nfs1p [30], Aco1p, Isu1p and Yah1p [31]. Antibody against Cyc1p was a gift from Debukmar Pain (Department of Pharmacology and Physiology, New Jersey Medical School, Newark, NJ, U.S.A.). Aconitase activity was measured spectrophotometrically on mitochondrial extracts of cells grown in YPR [32].

RESULTS

Zinc homeostasis is impaired in $\Delta yfh1$ cells

Analysis of the transcriptome of a wild-type *S. cerevisiae* strain grown with or without iron showed that 351 genes were significantly regulated by iron (results not shown). As expected [5], genes belonging to the Aft1 regulon were induced by iron deprivation (see below). In addition, 52 genes were induced by the presence of iron (results not shown). Almost half of these genes (Table 1) had been described as zinc-regulated genes, as their transcription is repressed by excess zinc in the medium [26]. These genes encode proteins involved in such functions as metabolism, respiration, oxidative stress defence, protein folding and translocation across the secretory pathway. The transcription of the *ZAP1* gene was induced by both zinc deprivation and by iron supplementation (Table 1). These data suggest that there could be a connection between iron and zinc metabolisms. The molecular basis of such a connection and its physiological implications remain to be determined. These questions are currently under investigation in our laboratory. In the present work, we wanted to focus on a possible involvement of zinc in the deleterious phenotypes of cells lacking the yeast frataxin homologue (*YFH1*). We analysed the transcriptome of $\Delta yfh1$ cells compared with wild-type cells. In agreement with others [14], we found that the genes of the iron regulon were constitutively more active in $\Delta yfh1$ than in wild-type cells (results not shown). As shown in Table 2, several zinc-dependent genes were also deregulated in $\Delta yfh1$ cells compared with wild-type cells. Notably, a number of genes of the zinc regulon that were not regulated by iron in the wild-type were repressed in $\Delta yfh1$ cells. This is the case for *ZRT1* (Table 2 and Figure 1), encoding the high-affinity zinc transporter. These observations raise the question of whether the zinc metabolism was affected, and to what extent, in cells deleted from the *YFH1* gene. The initial rate of zinc uptake from 1 μM ZnSO₄ by mid-logarithmic phase $\Delta yfh1$ cells was 1.4 fmol/min per 10⁶ cells, whereas that of wild-type cells was 54 fmol/min per 10⁶ cells. Similarly, $\Delta yfh1$ cells grown with 3.2 μM ZnSO₄ to mid-logarithmic phase accumulated 13 pmol of Zn/10⁶ cells while wild-type cells

Table 1 Iron regulation of zinc-dependent genes

Microarray data are expressed as logarithmic (base 2) values and for the comparison of the presence and absence of iron were obtained as described in the Experimental section. The data for high and low zinc exposure were reported previously [26]; zinc concentrations in the comparison of WT (wild-type) cells were (– Zn; low) 61 nM and (+ Zn; high) 3 mM. SRP, signal-recognition particle.

Gene	Biological process	Expression ratio	
		WT + Fe/– Fe	WT – Zn/+ Zn
Zinc-responsive regulator			
<i>ZAP1</i>	Zinc-responsive transcriptional activator	1.67	3.64
Metabolism/respiration			
<i>MCD4</i>	Glycosylphosphatidylinositol anchor biosynthesis	2.40	2.49
<i>ADH4</i>	Fermentation	3.41	5.06
<i>SDH2</i>	Tricarboxylic acid cycle; oxidative phosphorylation	1.14	2.00
<i>SDH3</i>	Tricarboxylic acid cycle; oxidative phosphorylation	1.87	1.29
<i>SDH4</i>	Tricarboxylic acid cycle; oxidative phosphorylation	1.67	1.25
<i>CIT2</i>	Citrate metabolism; glutamate biosynthesis; glyoxylate cycle	1.46	1.15
<i>QCR10</i>	Oxidative phosphorylation	1.10	1.76
<i>BIO3</i>	Biotin biosynthesis	3.34	1.12
<i>THI5</i>	Thiamine biosynthesis	1.35	1.69
<i>GAP1</i>	Amino acid transport	1.13	1.84
Stress			
<i>TSA1</i>	Response to oxidative stress	2.29	1.94
<i>HYR1</i>	Response to oxidative stress	1.18	1.09
Protein maturation and traffic			
<i>KAR2</i>	SRP-dependent, co-translational membrane targeting, translocation; karyogamy	1.35	1.29
<i>SLS1</i>	SRP-dependent, co-translational membrane targeting, translocation	1.08	2.25
<i>ERO1</i>	Protein folding; protein thiol–disulphide exchange	1.04	2.40
<i>LHS1</i>	Protein transport	1.00	1.56
Unknown			
<i>YOR387C</i>	Unknown	3.72	5.06
<i>YGL258W</i>	Unknown	3.54	4.64
<i>YOL154W</i>	Unknown, weak similarity to zinc metalloproteinases	1.97	4.64
<i>YJL048C</i>	Unknown	1.18	1.60

accumulated 40 pmol of Zn/10⁶ cells (data are means from three experiments with S.D. < 10 %). Thus our results show that $\Delta yfh1$ cells suffer from deregulation of zinc homeostasis. This defective zinc metabolism of $\Delta yfh1$ cells had features that were the opposite to the defective iron metabolism of these cells. Iron accumulates to toxic concentrations in $\Delta yfh1$ cells, but the cells behaved as if they were iron-deficient and induced all the iron-uptake systems. In contrast, the zinc-deficient $\Delta yfh1$ mutant behaved as if it was zinc-replete, and the genes of the Zap1p regulon (e.g. *ZRT1*, *ZRT3*, *YOL155C*, *YOR387C*) were repressed.

Effect of zinc on growth, iron uptake/accumulation and resistance to oxidative stress

We tested the effect of excess zinc in the medium on the phenotypes of $\Delta yfh1$ and wild-type cells. Adding up to 0.5 mM zinc slightly improved the growth of $\Delta yfh1$ cells, and higher concentrations of zinc were much less toxic for $\Delta yfh1$ cells than for wild-type cells (Figure 2A). The effect of zinc was much more pronounced when there was excess iron (100 μ M ferric citrate) in the medium (Figure 2B). This completely inhibited the growth of $\Delta yfh1$ cells unless zinc was added to the medium, while zinc inhibited the growth of wild-type cells. The growth rates of wild-type and $\Delta yfh1$ cells were the same at about 0.75 mM zinc (Figure 2B).

We measured the total intracellular iron accumulation in wild-type and $\Delta yfh1$ cells as a function of the extracellular zinc concentration. The intracellular iron in $\Delta yfh1$ cells decreased rapidly as the extracellular zinc concentration increased, and was as low as in wild-type cells, or even lower (Figures 2C and 2D). In contrast, zinc had little effect on the amount of iron accumulated

by wild-type cells (Figures 2C and 2D). The great decrease in intracellular iron in $\Delta yfh1$ cells grown in excess zinc reflected a decrease in the mitochondrial iron pool, not in the cytosolic iron pool (results not shown). Adding zinc to the growth medium not only affected the total iron in stationary-phase $\Delta yfh1$ cells, but the rate of iron uptake by resting cells was also changed according to the zinc content of the medium (Figure 3). $\Delta yfh1$ cells grown in a zinc-enriched medium and then washed and suspended in citrate buffer took up iron more slowly than $\Delta yfh1$ cells grown without added zinc (Figure 3), reflecting repression of the high-affinity reductive uptake system by zinc.

It has been proposed that the accumulation of iron in mitochondria is the cause of the sensitivity of $\Delta yfh1$ cells to oxidative stress [13,15]. Therefore, we tested the effect of adding zinc on the resistance of $\Delta yfh1$ cells to oxidative stress. Excess zinc clearly improved the resistance of $\Delta yfh1$ cells to iron, copper and H₂O₂ (Figure 4). Zinc had the opposite effect on wild-type cells, whose resistance to oxidative stress decreased with increasing extracellular zinc concentration (Figure 4). A large excess of zinc (5 mM) made $\Delta yfh1$ cells even more resistant to H₂O₂ and copper than wild-type cells (Figure 4).

Effect of zinc on haem synthesis and the assembly of Fe–S clusters

Although $\Delta yfh1$ cells grown with excess zinc grew better, did not accumulate iron and were resistant to oxidative stress in the same way as wild-type cells, not all of the deleterious phenotypes of these cells were improved by zinc. The Fe–S clusters assembly and haem synthesis were not improved by adding zinc to the growth medium. $\Delta yfh1$ cells grown with or without excess zinc all had the same very low content of aconitase protein and

Table 2 Genes repressed in the $\Delta yfh1$ mutant that are induced under zinc deficiencyData as in Table 1. WT and $\Delta yfh1$ cultures were tested in the presence of 50 μ M ferric citrate.

Gene	Biological process	Expression ratio	
		$\Delta yfh1$ /WT	WT – Zn/+ Zn
Zinc transport			
<i>ZRT1</i>	High-affinity zinc ion transport	– 1.46	5.64
<i>ZRT3</i>	Vacuolar zinc ion transport	– 1.15	2.74
Metabolism/respiration			
<i>BIO3</i>	Biotin biosynthesis	– 3.73	1.12
<i>YOL155C</i>	Cell-wall organization and biogenesis	– 3.23	2.00
<i>MCD4</i>	Glycosylphosphatidylinositol anchor biosynthesis	– 2.77	2.49
<i>GCV1</i>	Glycine metabolism; one-carbon compound metabolism	– 2.13	1.00
<i>ADE17</i>	<i>De novo</i> inosine 5' -monophosphate biosynthesis	– 1.65	2.74
<i>OAR1</i>	Aerobic respiration; fatty acid metabolism	– 1.54	1.25
<i>HXT7</i>	Hexose transport	– 1.52	1.12
<i>AUS1</i>	Sterol transport	– 1.34	1.15
<i>ADE2</i>	Purine base metabolism	– 1.33	1.09
<i>GAP1</i>	Amino acid transport	– 1.17	1.84
<i>SDH3</i>	Tricarboxylic acid cycle; oxidative phosphorylation	– 1.17	1.29
<i>ALD4</i>	Ethanol metabolism	– 1.09	2.12
<i>PGM2</i>	Glucose 1-phosphate and glucose 6-phosphate utilization	– 1.08	1.22
Stress			
<i>TSA1</i>	Response to oxidative stress	– 2.77	1.94
<i>TSL1</i>	Response to stress; trehalose biosynthesis	– 1.58	1.32
<i>MSN4</i>	Response to stress; regulation of transcription, DNA-dependent	– 1.21	1.00
Protein catabolism			
<i>PRC1</i>	Vacuolar protein catabolism	– 1.25	1.12
Unknown			
<i>YOL154W</i>	Unknown, weak similarity to zinc metalloproteinases	– 4.03	4.64
<i>YOR387C</i>	Unknown	– 3.60	5.06
<i>YIL169C</i>	Unknown	– 3.39	1.51
<i>YAR068W</i>	Unknown	– 2.71	1.89
<i>YGP1</i>	Unknown	– 1.53	2.40
<i>YHR214W-A</i>	Unknown	– 1.31	1.60
<i>YGR226C</i>	Unknown	– 1.23	1.29
<i>SPI1</i>	Unknown	– 1.14	2.12
<i>YKR046C</i>	Unknown	– 1.11	1.51

Figure 1 Effect of zinc on *ZRT1* expression

Total RNA was probed with *ZRT1* and *ACT1* by Northern blotting. Strains were grown to mid-logarithmic phase in YPD media with or without zinc supplementation (0.75 mM ZnSO_4). wt, wild type.

activity, and the same low cytochrome content (results not shown). We created a yeast strain in which the sole copy of *YFH1* was under the control of a galactose-induced promoter to determine the effect of zinc on parameters affected by *YFH1* gene expression. The regulated expression of *YFH1* in this strain allowed us to correlate phenotypes with the concentrations of different proteins. When *YFH1* expression was repressed in standard growth conditions (no zinc added), the mitochondrial iron content of the cells was high (Figure 5A). The mitochondrial iron content decreased when *YFH1* gene expression was induced by galactose (Figure 5A). Induction of *YFH1* also led to the progressive recovery of the cytochromes, Fe-S proteins (aconitase, Yah1p, Nfs1p) and ferrochelatase (Hem15p), and to increased mitochondrial respiration (Figure 5A). The initial iron content of the

Figure 2 Effect of zinc on growth and iron accumulation by wild-type and $\Delta yfh1$ cells

Cells were grown in complete YPD medium with various amounts of ZnSO_4 added and either 1 μ M (A, C) or 100 μ M (B, D) $^{55}\text{Fe(III)}$ -citrate. Cell growth (A, B) was determined as the D_{600} ('OD') of the cultures after 16 h of growth at 30 °C. Cell iron accumulation (C, D) was determined by liquid scintillation after cell harvesting and washing. Strains: ●, wild-type; ○, $\Delta yfh1$. Means $\pm$ S.E.M. from three experiments are shown.

Figure 3 Effect of excess zinc on iron uptake by $\Delta yfh1$ cells

Cells of the wild-type strain (□, ■) and the $\Delta yfh1$ strain (○, ●) were grown to mid-logarithmic phase in complete YPD medium with no addition (□, ○) or supplemented with 0.5 mM ZnSO₄ (■, ●). Cells were harvested, washed and resuspended in 50 mM citrate buffer (pH 6.5) containing 5% glucose and 1 μ M ⁵⁵Fe(II)-ascorbate. Iron uptake by the cells was followed. Data are from a single representative experiment.

Figure 4 Effect of zinc on the resistance to oxidative stress of wild-type and $\Delta yfh1$ cells

Cells were sequentially 5-fold-diluted and then plated on YPD agar medium with (A) 1 mM H₂O₂, (B) 100 μ M ferrioxamine B or (C) 1 mM CuSO₄, in the presence (lanes 3 and 4) or not (lanes 1 and 2) of excess ZnSO₄ (5 mM). Strains: lanes 1 and 3, wild-type; lanes 2 and 4, $\Delta yfh1$.

mitochondria (before *YFH1* induction) was low when there was excess zinc in the growth medium, and it was further decreased after *YFH1* induction (Figure 5B). The cell aconitase, Nfs1p and cytochrome contents were all initially low and recovered normal values after *YFH1* induction (Figure 5B), as in the absence of added zinc (Figure 5A). However, two parameters changed markedly in the presence of excess zinc, in addition to the cell iron content. First, there was a significant amount of Yah1 protein in the mitochondrial fraction when *YFH1* was repressed (Figure 5B), while this protein was almost undetectable without added zinc (Figure 5A). Second, the respiration of the cells was strongly inhibited, even after induction of *YFH1* (Figure 5B). The inhibition of the respiratory electron-transport chain by zinc is well documented [33], but how it does so is still unknown. This is the first report that zinc can protect the mitochondrial ferredoxin Yah1p. This result could be significant for understanding how zinc affects mitochondrial iron accumulation, since a lack of Yah1p results in mitochondrial iron accumulation [34].

DISCUSSION

The present work shows that there is an iron–zinc connection in *S. cerevisiae*. The physiological and molecular bases of this iron–zinc connection are still completely unknown. The only transcription regulators described so far in iron and zinc metabolisms are activators that respond to iron (Aft) or zinc (Zap) deprivation. The molecular mediators that induce the transcription of genes in response to excess iron or zinc remain to be determined. In *Bacillus subtilis*, it was recently shown that zinc uptake is specifically induced by oxidative stress, together with other genes of the PerR regulon [35]. This observation provides evidence for the important role of zinc in resistance to oxidative and disulphide stresses. No direct relationship between oxidative stress and zinc uptake has been shown in yeast, but cells lacking the copper-zinc superoxide dismutase (Sod1) have an increased zinc content and an increased content of ‘free’ (EPR-detectable) iron compared with wild-type cells [36]. Thus an iron–zinc connection could have evolved to protect the cells from putative oxidative damages induced by iron. This hypothesis is under investigation in our laboratory. The existence of a close relationship between iron and zinc metabolism could have important implications, as shown here with cells lacking the frataxin homologue (Yfh1). $\Delta yfh1$ cells accumulate large amounts of iron [13] and all the iron-uptake mechanisms are induced in these cells, although they are overloaded with iron. Here we show that $\Delta yfh1$ cells also suffer from disorders of zinc metabolism, so that zinc transport and accumulation are much lower than in wild-type cells. Here too, the cells behave paradoxically, since *ZRT1* and *ZRT3* genes, that encode zinc transporters, are repressed as in zinc-replete wild-type cells. It is generally admitted that Aft-dependent genes are up-regulated in $\Delta yfh1$ cells because their cytosolic iron is low and most of the iron is in the mitochondria [14]. This work shows that Zap1-dependent genes are down-regulated in $\Delta yfh1$ cells. We are currently trying to determine if this results from some abnormal distribution of zinc in the cells.

Some of the deleterious phenotypes of mutant $\Delta yfh1$ cells are believed to result from excess mitochondrial iron. Decreasing the iron concentration in the medium improves the growth of $\Delta yfh1$ cells and increases their resistance to oxidative stress ([37] and E. Lesuisse and R. Santos, unpublished work). We have now shown that $\Delta yfh1$ cells recover normal or even sub-normal intracellular concentrations of iron when grown in medium containing excess zinc. Adding zinc to $\Delta yfh1$ cells clearly improves their growth and resistance to oxidative stress. However, these cells remain very sick, with low growth and respiratory rates and deficient in Fe–S and haem proteins. This clearly establishes that the iron accumulation phenotype can be separated from the other phenotypes associated with Yfh1p deficiency. Hence, the effect of zinc on iron accumulation by $\Delta yfh1$ mitochondria does not indicate that Yfh1p is directly or indirectly involved in the export of mitochondrial iron [13,16]. Rather, iron accumulation in $\Delta yfh1$ mitochondria seems to be a secondary phenotype. Puccio et al. [19] analysed the sequence of events in tissues of transgenic mice following frataxin depletion. They found that the Fe–S assembly defect appeared prior to the accumulation of iron in mitochondria. Our results suggest that the same is true for *S. cerevisiae*.

The mitochondrial ferredoxin encoded by *YAH1* is a vital protein that contains a Fe–S cluster [38]. A decrease in the Yah1p concentration results in iron accumulating in mitochondria [34], and the concentration of the holoprotein is very low in mutants lacking Yfh1p [39]. For some reason, excess zinc protects Yah1p in $\Delta yfh1$ cells and prevents iron accumulation in $\Delta yfh1$ mitochondria. These two events may be related. Zinc

Figure 5 Effect of excess zinc in the growth medium on various parameters during induction of *YFH1* in a $\Delta yfh1$ strain (GAL-*Yfh1*)

Cells of the GAL-*Yfh1* strain were grown for 24 h on YPR, diluted 20-fold into fresh YPR medium without (A) or with (B) added zinc (0.5 mM ZnSO_4) and grown for another 16 h. At this time ($t=0$), part of the cells were harvested and galactose (2%) was added to the culture. Cell aliquots were harvested 2 and 5 h after galactose addition. Each aliquot was assayed for (1) mitochondrial iron content, (2) mitochondrial respiration (in the presence of 1 mM succinate and 1 mM ADP), (3) cytochrome content (low-temperature spectra of the cells) and (4) abundance of Aco1p, Nfs1p, Hem15p, Yah1p, Yfh1p and Isu1p proteins.

may decrease mitochondrial iron accumulation by protecting Yah1p. Conversely, the accumulation of iron in the mitochondria of $\Delta yfh1$ cells may result directly from the lack of Yah1. Zinc could protect Yah1p via its effect on respiration. Zinc has long been known to inhibit the respiratory electron-transport chain [33]. Respiration can generate superoxide ions under some

conditions, especially in the presence of free iron [40], and these could destabilize iron-sulphur proteins like Yah1p. These are still speculations and our further work will aim to determine experimentally whether the Yah1p defect that occurs in $\Delta yfh1$ mutants is the primary cause of iron accumulation in these mutants. If Yfh1p is an iron-binding protein, as suggested by several

recent studies, there should be 'free' iron in Yfh1-deficient mitochondria. This iron could catalyse superoxide generation, resulting in the destabilization of some Fe–S protein involved in iron signalling. There could well be such an iron signal mediated by a mitochondrial Fe–S protein, since Atm1p is expected to export Fe–S proteins from the mitochondria to the cytosol [41], and $\Delta atm1$ mutants accumulate iron in their mitochondria [42]. Whatever the mechanism involved, the effect of zinc in preventing mitochondrial iron accumulation in $\Delta yfh1$ cells may aid iron sensing by Aft1p in these cells, leading to decreased iron-uptake rate by the cells.

Even though the role of frataxin has not been determined, it is clear that oxidative stress plays a major role in Friedreich's ataxia. Several reports account for the reduced protection against oxidative stress in tissues of Friedreich's ataxia patients [43]. But all attempts to date to treat Friedreich's ataxia have employed antioxidant drugs, and idebenone seems promising [44]. Zinc has antioxidant properties (reviewed in [45]) and prolonged deprivation renders the body more susceptible to oxidative stress injury [45]. On the other hand, chronic zinc ingestion leads to the synthesis of the antioxidant metallothioneins [46]. To our knowledge there are no reports of Friedreich's ataxia patients suffering from zinc disorders. Nevertheless, our findings that zinc homeostasis is disturbed in the yeast $\Delta yfh1$ mutant could stimulate research in the involvement of zinc in Friedreich's ataxia. Moreover, our observation that excess zinc suppresses some phenotypes of $\Delta yfh1$ cells suggests that zinc nutrition may be important in the aetiology of Friedreich's ataxia.

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