Saccharomyces cerevisiae glutaredoxin 5-deficient cells subjected to constitutive oxidizing conditions are affected in the expression of specific sets of genes*

Gemma Bellí‡, María Micaela Molina‡, José García-Martínez§, José E. Pérez-Ortín§ and Enrique Herrero‡¶

‡Departament de Ciències Mèdiques Bàsiques, Facultat de Medicina, Universitat de Lleida, Rovira Roure 44, 25198-Lleida, Spain, and §Departamento de Bioquímica y Biología Molecular, Facultad de Ciencias Biológicas and Servicio de Chips de DNA-S.C.S.I.E., Universitat de València, 46100-Burjassot, Valencia, Spain

Running title: Transcriptome of Grx5-deficient yeast cells

Keywords: Yeast, Glutaredoxin, Aft1, Hap4, Oxidation

¶To whom correspondence should be addressed: Departament de Ciències Mèdiques Bàsiques, Facultat de Medicina, Universitat de Lleida, Rovira Roure 44, 25198-Lleida, Spain. Tel.: 34-973-702409; Fax: 34-973-702426; E-mail: enric.herrero@cmb.udl.es

*This work was supported by grants GEN2001-4707-CO8-06 and GEN2001-4707-CO8-07 from the Spanish Ministry of Science and Technology (to E.H. and J.E.P-O. respectively) and by grant 2001SGR00305 from the Generalitat de Catalunya (to E.H.). M.M.M. received a predoctoral grant from the Generalitat de Catalunya.

[1] The abbreviations used are: ROS, reactive oxygen species; PCR, polymerase chain reaction; KAPA, 7-keto 8-aminoperlargonic; *t*-BOOH, *t*-butyl hydroperoxide; MAP kinase: mitogen-activated protein kinase

SUMMARY. The *Saccharomyces cerevisiae GRX5* gene codes for a mitochondrial glutaredoxin involved in the synthesis of iron/sulfur clusters. Its absence prevents respiratory growth, and causes the accumulation of iron inside cells and constitutive oxidation of proteins. Null *Dgrx5* mutants were used as an example of constitutively oxidized cells, as opposed to situations in which oxidative stress is instantaneously caused by addition of external oxidants. Whole transcriptome analysis was carried out in the mutant cells. The set of genes whose expression was affected by the absence of Grx5 does not significantly overlap with the set of genes affected in respiratory petite mutants. Many Aft1-dependent genes involved in iron utilization that are upregulated in a frataxin mutant were also upregulated in the absence of Grx5. *BIO5* is another Aft1-regulated gene induced both upon iron deprivation and in *Dgrx5* cells; this links iron and biotin metabolism. Other genes are specifically affected under the oxidative conditions generated by the *grx5* mutation. One of these is *MLP1*, which codes for a homologue of the Slt2 kinase. Cells lacking *MLP1* and *GRX5* are hypersensitive to oxidative stress caused by external agents and exhibit increased protein oxidation in relation to single mutants. This in turn points to a role for Mlp1 in protection against oxidative stress. The genes of the Hap4 regulon, that are involved in respiratory metabolism, are downregulated in *Dgrx5* cells. This effect is suppressed by *HAP4* overexpression. Inhibition of respiratory metabolism during constitutive moderately oxidative conditions could be a protective response by the cell.

INTRODUCTION

Cells growing aerobically are subjected to oxidative stress caused by reactive oxygen species (ROS)[1] produced at the respiratory chain in the presence of oxygen [1]. Cells have developed a number of enzymatic and non-enzymatic mechanisms to counteract the damage that ROS cause to the different cellular macromolecules. In the yeast *Saccharomyces cerevisiae*, addition of external oxidants such as hydrogen peroxide or menadione (a generator of superoxide anion) cause a rapid and generally transient transcriptional response [2,3]. This consists of the induction of genes involved in hydrogen peroxide and superoxide detoxification, as well as those related to redox homeostasis within the cell. In the case of hydrogen peroxide, observations at the transcriptome level confirm studies carried out at the proteome level [4,5]. Two transcriptional regulators that respond to oxidative stress, Yap1 and Skn7, are important in such a response to hydrogen peroxide [5,6]. The Yap1 regulon involves two types of proteins: those required for antioxidant scavenging and enzymes that participate in the metabolic pathways that regenerate cell reducing power in the form of glutathione and NADPH. The first subset of proteins is also Skn7-dependent, while the second is not [5]. Msn2 and Msn4 are two transcription factors that respond to different stresses, including oxidative stress, by regulating gene expression through binding to STRE promoter elements [7,8]. The defense function of Msn2/4 partially overlaps with that of Yap1. However, Yap1 seems to be more important for the adaptative response induced by moderate concentrations of hydrogen peroxide, while Msn2/4 would be preferentially implicated in recovery from acute exposure to the oxidant [9]. This is consistent with the specific role that Msn2/4 has in regulating expression of genes involved in protein degradation pathways [9].

Most studies into the effect of oxidative stress on yeast cells have involved adding external oxidants to cell cultures. However, this does not necessarily reproduce the physiological effect caused by aerobic respiration or other types of oxidation at moderate though constant levels. Proteins of *S. cerevisiae* cells growing under aerobic conditions are more exposed to constitutive oxidation than proteins from anaerobic cultures [10]. The latter demonstrated permanent oxidative stress under the former conditions. However, the gene expression pattern of cells that shift from fermentative to respiratory metabolism at the diauxic transition [11,12] is very different from that of cells subjected to external aggression by oxidants such as hydrogen peroxide, menadione or diamide [2,3]. Thioredoxins and glutaredoxins are two thiol oxidoreductases that play an important role in protecting sulphydryl groups in proteins against oxidation, and therefore in maintaining the protein activity under these conditions [13]. Grx5 is a monothiol glutaredoxin of *S. cerevisiae* (a single cysteine at the active site) whose absence leads to high sensitivity to oxidative agents and to constitutive carbonylation of proteins (a parameter that measures protein oxidation [10]) even in the absence of external oxidative injury [14]. Accumulation of glutathionilated proteins has also been observed in the absence of Grx5 [15,16]. This monothiol glutaredoxin is located at the mitochondria, where it is involved in the formation of iron-sulfur clusters [17]. As occurs with other proteins of the iron-sulfur assembly complex, lack of Grx5 causes iron accumulation within the cell. This could be related to the observed constitutive protein oxidation due to the formation of high levels of ROS via the Fenton reaction [17]. A *Dgrx5* mutant is therefore an appropriate model for studying the effect of permanent oxidative stress on gene expression. In this work, we carried out transcriptome analysis of the Grx5-defective mutant. The results show that under these conditions the expression program was significantly different from

the transcription pattern described in response to external oxidants, the inhibition of respiratory genes being a relevant response.

EXPERIMENTAL PROCEDURES

Strains and Growth Conditions. Wild type *S. cerevisiae* strain W303-1A (*MAT***a** *ura3-52 leu2-3,112 trp1-1 his3-11,15 ade2-1*) was employed in this study. MML100 is a *Dgrx5::kanMX4* derivative of W303-1A [17]. MML511 is an isogenic derivative of W303-1A that contains the *Dpet117::kanMX4* disruption. It was constructed using the short-flanking homology approach following PCR-amplification of the *kanMX4* cassette (resistance for geneticin) from plasmid pFA6a-kanMX4 with appropriate oligonucleotides [18]. The resulting *PET117* deletion covers exactly from the initial to the stop codon. Disruption was confirmed by PCR analysis [18]. The same strategy was employed for disruption of *YKL161c* (*MLP1*) in the W303-1A background, although in this case the *natMX4* marker cassette (resistance for nourseothricin) was used [19]. The resulting *Dmlp1::natMX4* strain (MML524) was crossed with the *MATα Dgrx5::kanMX4* mutant MML289 [17] and the double W303-1A *Dmlp1::natMX4 MATα Dgrx5::kanMX4* mutant (MML533) was segregated. The W303-1A *aft1-D5::URA3* strain (MML348) has been previously described [17]. Plasmid pMT15 is a derivative of the multicopy vector pCM189 [20] that contains *HAP4* under the control of the doxycycline-regulated $tetO_7$ promoter.

Cells were generally grown in rich YPD medium (1% yeast extract, 2% peptone, 2% glucose) at 30ºC. In some experiments 3% glycerol was used instead of glucose (YPGly medium). Synthetic complete medium (SC) contains 0.67% yeast nitrogen base (Difco), 2% glucose and the amino acids and nitrogen

base additions indicated in Ref. 21. In some experiments, SC medium was prepared by adding the individual components at the standard concentrations, with the exception of biotin, which was included at the indicated final concentrations.

Array Hybridizations and Data Analyses. Experiments representing a particular situation were indepedently carried out in triplicate. Total RNA from W303-1A wild type strain and the *Dgrx5* and *Dpet117* mutants was used to obtain labelled cDNA. About 30-40 μg RNA were retrotranscribed into cDNA by adding 200 units of RT polymerase SuperScript II (Invitrogen), 500 ng of oligo dT primer (5'-T_{15}VN-3'), 1μl RNaseOUT (Invitrogen), 6 μl 5X First Strand Buffer (Invitrogen), 1.5 μl dNTP mix (16 mM dATP, dTTP, dGTP, and 100μM dCTP) and 5 μl ^{33}P-dCTP (10 mCi/ml) in a final reaction volume of 30 μl. Labelling reaction was allowed for 1 h at 43ºC. One microlitre of EDTA 0.5 M was added to stop the reaction. The labelled sample was purified using an S300-HR MicroSpin column (Amersham BioSciences). After pre-hybridizing nylon membranes for 1 h in 5X SSC, 5X Denhart's, 0.5% SDS, hybridizations were performed using the same solution containing labelled cDNA (3.5 x 10^6 dpm/ml) for 16-18 h. After hybridization, the filters were washed once in 2X SSC, 0.1 % SDS for 30 min, and twice in 0.2 X SSC, 0.1 % SDS for 30 min. They were exposed to a imaging plate (BAS-MP, Fujifilm) for 24 h and read in a Phosphorimager (FLA-3000, Fujifilm) at 50 μm resolution.

The images were quantified by using ArrayVision 7.0 software (Imaging Research, Inc.). Each mutant replicate was normalized against the corresponding wild type hybridized in the same membrane (to eliminate the membrane variability factor) following the Lowess method. Reproducibility of the replicates was tested by the ArrayStat software (Imaging Research, Inc.). The data were

considered as independent and the program was allowed to take a minimum of at least two valid replicates in order to calculate mean and standard deviation values for every gene. A Z-test for independent data was applied in order to detect differences in individual gene expression between each mutant and the wild type. Then, a Z-score was obtained for every gene. A p-value of 0.05 and the False Discovery Rate method were used to monitor the overall false positive error rate.

Database Analyses. Data on the expression of individual genes from global genome analyses were obtained from the Yeast Microarray Global Viewer (yMGV, http://transcriptome.ens.fr/ymgv). Analysis of genes whose expression is modulated throughout the cell cycle [22] was carried out with the Saccharomyces Genome Database (http://genome-www.stanford.edu).

Northern Blot Studies. RNA electrophoresis, probe labelling with digoxigenin, hybridization, and signal detection were carried out as previously described [20]. Signals were quantified using the Lumi-Imager equipment (Roche) software. Gene probes were generated from genomic DNA by PCR, using oligonucleotides designed to amplify internal ORF regions.

Sensitivity Tests. Exponential cultures in YPD medium at 30ºC (about 2 x 10^7 cells per ml) were serially diluted (5-fold dilution factor), and two microlitre drops of each dilution were spotted onto YPD plates containing inhibitory agents at various concentrations. Growth was tested after three days of incubation at 30ºC. In parallel, growth was also recorded in YPD plates without an inhibitor after incubation in the same conditions.

Protein Carbonylation Analyses. Protein carbonyl groups in cell extracts were derivatized with 2,4-dinitrophenilhydrazine. This was followed by SDS-polyacrylamide gel electrophoresis and the immunodetection of peptides using an anti-dinitrophenylhidrazone antibody, according to Ref. 10.

RESULTS

Wild type and isogenic *Dgrx5* cells were grown exponentially in rich YPD medium at 30ºC. Transcriptome analyses were carried out in three independent experiments in these growth conditions for each of the strains. Table I lists the genes that showed expression levels in *Dgrx5* cells that were at least twice as high as in wild type cells. Table II shows the genes whose expression levels in *Dgrx5* cells were at least 50% lower than the expression levels in wild type cells. Cells lacking *GRX5* exhibit a petite phenotype, that is, they do not grow under respiratory conditions [17]. It has also been shown that *S. cerevisiae* mitochondrial DNA rhoº mutants have an altered expression profile [23]. As expression pattern for *Dgrx5* cells could have been influenced by their petite defects, we carried out a parallel transcriptome analysis of *Dpet117* cells with the same W303-1A genetic background. The total number of genes either up or downregulated in the *Dgrx5* mutant was considerably lower than in the *Dpet117* cells (Fig. 1A). Both Table I and II indicate the genes affected in *Dgrx5* cells that were respectively up or downregulated in *Dpet117* with respect to wild type cells. The proportion of these genes was very low in both cases, and we can therefore conclude that the transcriptome profile of Grx5-deficient cells was not significantly influenced by the petite character of the mutant. As revealed by FACS analysis[2],

[2] M. A. de la Torre, G. Bellí and E. Herrero, unpublished data

the *Dgrx5* mutant accumulated a large proportion of cells at the G1 stage of the cell cycle. This fact could have influenced the expression profile of those genes whose expression changed throughout the cell cycle [22]. However, the proportion of these cell cycle-dependent genes whose expression is up or downregulated in the *Dgrx5* cells was low (Tables I and II), and overall, this did not significantly affect the analyses.

Overexpressed Genes in Dgrx5 Cells. A total of 99 genes showed at least two-fold induction of expression in cells lacking Grx5 as opposed to wild type cells (Table I). For some of these genes, the results were confirmed by Northern analysis (Fig. 2A). A group of genes implicated in ion homeostasis, especially in iron and copper transport, was induced in *Dgrx5* cells. Most of these genes were controlled by the transcriptional activator Aft1, which responds to iron deprivation by inducing the expression of genes coding for plasma membrane metalloreductases (*FRE1-3*), the multicopper ferroxidase (*FET3*), the iron permease (*FTR1*), copper ion transporters (*ATX1, CCC2*), components of the siderophore-iron uptake system (*ARN1-4*), cell wall-associeted facillitators of iron uptake (*FIT1-3*), and heme oxygenase (*HMX1*) [24-29]. Most of these genes are also upregulated in a yeast *Dyfh1* mutant, which lacks the yeast homologue of human frataxin [27]. Absence of frataxin causes mitochondrial accumulation of iron in a form that is not available to the cells [30], and this probably signals the activation of Aft1 [27]. We had previously shown that disruption of iron-sulfur cluster assembly through Grx5 inactivation also caused iron accumulation [17], and the results presented in Table I support the idea that this iron pool is also in a form that is not available to the cell.

Two genes associated to biotin transport were upregulated in Grx5-deficient cells, *VTH1* and *BIO5*. *VTH1* has been characterized as coding for a

plasma membrane high-affinity biotin transporter, and its expression is repressed in media with high biotin levels [31]. It has recently been reported that this gene is an Aft1 target [29]. This suggests that its upregulation in *Dgrx5* cells could also be a consequence of iron deficiency in these cells. More intriguing is the upregulation of the *BIO5* gene (Table I and Fig. 2A). *S. cerevisiae* cells require an external source of biotin as they lack the first enzyme of the pathway that converts pimelic acid into biotin [32]. However, they are able to grow on the biotin vitamers 7-keto 8-aminoperlargonic acid (KAPA), 7,8-diamino-pelargonic acid and dethiobiotin, which are sequential intermediates in the pathway that leads to biotin [32]. The last enzyme in the pathway (biotin synthase) converts dethiobiotin into biotin and is the product of *BIO2* in *S. cerevisiae*. This enzyme contains iron/sulfur centers [33,34] and its activity therefore would be very low in *Dgrx5* cells. We have observed that *Dgrx5* cell growth was no more dependent on biotin concentration in the SD medium (from 20 to 0.1 mg/liter) than growth of wild type cells under the same conditions (Fig. 2B). This would seem to dissociate the absence of an active Bio2 protein from the biotin requirement. The *BIO5* product has been characterized as the transporter of KAPA, which is a vitamer that is probably not present in either natural or laboratory yeast cell growth media [32]. We examined whether *BIO5* expression was also dependent on biotin levels in the growth medium, as in the case of *VTH1*. However, this was not the case (Fig. 2C). On the other hand, *BIO5* expression was upregulated upon iron chelation by ferrozine, similarly to the well known Aft1-dependent gene *FET3* (Fig. 2D). This upregulation did not occur in a null *aft1* mutant (Fig. 2D), which showed that *BIO5* must be a member of the Aft1 regulon. Altogether, these results confirm the previous suggestion [29] that biotin synthesis and transport are related to iron metabolism in yeast cells.

SNZ1 transcription was increased in *Δgrx5* cells (Table I and Fig. 2A). This gene is involved in vitamine B_6 (piridoxine) biosynthesis and has orthologues in various prokaryotes and eukaryotes [35]. Its upregulation in the absence of Grx5 (from almost undetectable levels in wild type cells) could be related to the proposed effect of piridoxine as a quencher of singlet oxygen [36]. This hypothesis is supported by the fact that yeast *snz* mutants are hypersensitive to the singlet oxygen generator methylene blue [37].

YKL161C (*MLP1*) is one of the genes that showed highest relative induction-fold in *Δgrx5* cells (Table I). The Mlp1 protein is homologous to the Slt2 (Mpk1) MAP kinase, although it lacks the conserved active site of MAP kinases [38]. Overexpression of *MLP1* suppresses the caffeine-sensitivity phenotype of a *bck1* mutant in the signal transduction pathway leading to Slt2, while a null *mlp1* mutant has additive effects on caffeine sensitivity when combined with a *slt2* mutation [38]. However, a single null *mlp1* mutant does not show apparent phenotype. These observations suggest that Mlp1 acts in a pathway parallel to the Slt2 pathway in the regulation of downstream targets involved in cell integrity such as Rlm1 [39]. On the basis that the *grx5* mutant overexpresses *MLP1*, we reasoned that the absence of the latter could enhance defects in cells lacking *GRX5*. The double *Δgrx5 Δmlp1* mutant (strain MML533) was no more sensitive to caffeine, to the cell wall inhibitor calcofluor white or to heat shock (growth at 38ºC) than either the single *Δmlp1* mutant or the wild type cells (not shown). The latter are phenotypic defects characteristic of cells where the Slt2 MAP kinase pathway is affected. However, the double mutant was more sensitive than the single *Δgrx5* mutant to *t*-BOOH and menadione (Fig. 3A). The *MLP1* function could therefore play a protective role against oxidative stress in *Δgrx5* cells. Importantly, hypersensitivity to external oxidants was not observed in the single *Δmlp1* mutant (Fig. 3A). We then tried to correlate sensitivity to oxidants with

protein oxidation for the different genetic backgrounds. Carbonylation of protein residues (see Experimental Procedures for determination) was used as a measure of protein oxidation [10,14]. As expected from previous studies [14], under normal growth conditions *Dgrx5* cells contained more carbonyl groups in proteins than wild type cells. In contrast, *Dmlp1* cells exhibited even fewer carbonyl groups in total protein extracts than wild type cells (Fig. 3B). Protein carbonylation in the double *Dgrx5 Dmlp1* mutant was slightly higher than in the single *Dgrx5* mutant (Fig. 3B). When total carbonyl groups were quantified [10,14], the doble mutant consistently gave values about 30% higher than *Dgrx5* cells. These differences were much larger after treatment with *t*-BOOH: under these conditions we observed an additive effect between the two mutations on protein carbonyl content (Fig. 3B). It is also interesting that protein oxidation in the treated single *Dmlp1* mutant was greater than in wild type cells, though less than in *Dgrx5* treated cells. Altogether these results support the protective role of *MLP1* against external oxidants, particularly when the Grx5 function is absent.

Genes of the Yap1 and Msn2/4 Regulons were not Constitutively Induced in the Absence of Grx5. In yeast, oxidative stress by external agents such as hydrogen peroxide, menadione or diamide causes upregulation of genes involved in glutathione and thioredoxin-based defence and repair systems against oxidative damage, and also of other antioxidants such as superoxide dismutases and catalases (see Ref. 6 for details). This induction is regulated by one or several of the transcriptional factors Msn2/4, Yap1 and Skn7. None of the above genes appeared to be upregulated in the experiments shown in Table I for *Dgrx5* cells. We confirmed these results by Northern blot analysis for a number of genes that are known to be upregulated by external oxidants: *DDR2* [40], *GSH1* [41], *HSP12* [42], *CTT1* [40], *TRX2* [43] and *TRR1* [5]. Basal expression in *Dgrx5* cells

did not differ significantly from wild type cells in any of these cases (Fig. 4A). However, the Yap1 and Msn2/4 regulatory systems remained functional in the mutant, since *GSH1* (whose induction by oxidants is Yap1-dependent [5,41]) and *DDR2* (Msn2/4-dependent [7, our unpublished observations]) were inducible by hydrogen peroxide in wild type and *Dgrx5* cells (Fig. 4B).

Repressed Genes in Dgrx5 Cells. A total of 64 genes had an expression level in *Dgrx5* cells that was 50% or less with respect to wild type cells (Table II). Thirty-four of these genes were also downregulated by external oxidants, as shown in Ref. 2 and 3. This confirmed that the *Dgrx5* cells were subjected to metabolic oxidative stress. However, the remaining 30 genes in Table I were specifically downregulated in the mutant. These included the *YFH1* frataxin gene. Decreased expression of the latter was confirmed by Northern blot analysis (Fig. 2A). Twenty of the downregulated genes were nuclear genes involved in mitochondrial functions, mostly in respiration (Table II). We confirmed the downregulation of *CYC1* and *COX13* by Northern blot analysis (Fig. 2A). It has been reported that petite cells display decreased expression of a number of genes related to mitochondria [23]. However, none of the genes reported in that study were among the genes downregulated in the present work. Only three of the mitochondrial genes in Table II (*CYC1, COX5A* and *COX17*) displayed decreased expression in the *Dpet117* mutant. We therefore concluded that for most of these mitochondrial function genes the decreased expression is specific to the null *grx5* mutant. It can be deduced that this is a specific response of genes involved in respiration and other mitochondrial energy metabolism functions caused by the constitutive generation of ROS in Grx5-deficient cells. Hap4 is a transcriptional factor that upregulates *S. cerevisiae* respiratory functions and that is therefore important in the shift from fermentative to

respiratory metabolism which occurs at the diauxic shift [12,44]. We analyzed previous reports [11,12] to determine whether there were any changes in the expression pattern of mitochondrial function genes downregulated in *Dgrx5* cells during the diauxic shift [11] or after overexpression of Hap4 [12]. In fact, the expression of 14 of these genes is upregulated after the diauxic shift and/or by Hap4 overexpression (Table III). Two of the unknown function open reading frames (*YDR316w* and *YER182w*) showing strong downregulation in *Dgrx5* cells (Table II) are also Hap4-dependent [12].

We studied the expression of one of these genes, *CYC1*, in conditions in which *HAP4* was overexpressed in order to demonstrate that downregulation of Hap4-dependent genes in cells deficient in Grx5 was not indirect. As expected from previous studies [45], overexpressing *HAP4* in wild type cells led to an increased expression of *CYC1* (Fig. 5A). Under the same conditions, *Dgrx5* cells expressed *CYC1* at almost the same level (only 20% reduction) as the wild type cells, contrasting significantly with the situation in non-overexpression conditions (which experienced a three-fold reduction) (Fig. 5A). This confirmed that the observed downregulation of Hap4-dependent genes occurred as a direct consequence of the absence of Grx5.

Based on the hypothesis that downregulation of respiratory genes is a response to the constitutive oxidation of cell molecules which occurs in the absence of Grx5, it would be expected that a shift to respiratory metabolism by *HAP4* overexpression would have a negative effect on the physiology of *Dgrx5* cells. Although these cells have no functional respiratory chains due to defects in iron-sulfur cluster biogenesis [17], ROS could still be produced as a consequence of incomplete electron transfer. The overexpression of *HAP4* caused increased carbonylation of cell proteins even in a wild type background, although this effect was higher in *Dgrx5* cells (Fig. 5B). This confirmed that respiratory conditions are

a source of ROS able to cause oxidative damage on proteins, and also that there are additive effects between the absence of Grx5 activity and the increased expression of respiratory genes. In accordance with these observations, overexpression of *HAP4* in the *Dgrx5* mutant significantly lowered growth rate compared to mutant cells displaying normal levels of *HAP4* expression. In contrast, this effect of *HAP4* overexpression on cell growth was not observed in wild type cells (Fig. 5C). Altogether, these observations support the biological significance of the observed downregulation of respiratory genes in the absence of Grx5.

DISCUSSION

In this work we used a Grx5-deficient mutant as a model for yeast cells constitutively subjected to moderate oxidative stress, in order to study the genes whose expression was significantly modified under these conditions. As the *Dgrx5* mutant exhibits a petite phenotype [17], we had to first distinguish between the genes whose modified expression was specifically due to the petite character of the cells and those genes affected by other physiological effects of the *grx5* mutation. Very few of the genes affected in the *Dpet117* mutant were also affected in the *Dgrx5* mutant cells (Tables I and II). Furthemore, there was no overlap with the genes shown to be differentially expressed in rho^o cells in a previous study [23]. Therefore, most of the expression changes seen in *Dgrx5* cells do not seem to be due to the respiratory deficiencies in these cells. It is also interesting that a small set of genes displayed significantly altered expression in opposite ways when both mutants were compared (Fig. 1B). Most of the twelve genes that were upregulated in *Dgrx5* cells and downregulated in the petite mutant are of unknown function, although the group also included two genes

(*FIT3* and *ARN2*) related to iron uptake. Of the sixteen genes upregulated in the *Dpet117* mutant and downregulated in the *Dgrx5* cells, four (*ILV3*, *LYS4*, *BAT1* and *GAP1*) are involved in amino acid metabolism and transport. This could be related to the fact that up to thirty two genes involved in amino acid metabolism and transport were induced in the *Dpet117* mutant. This is a situation that extends to genes for purine and pirimidine biosynthesis (data not shown in detail). The upregulation of genes in these functional categories was not observed in rho^o cells [23].

A subset of the genes upregulated in the absence of Grx5 is also upregulated in the frataxin *yfh1* mutant [27]. These genes are regulated by the transcriptional activator Aft1. The latter responds to iron deprivation by regulating its nuclear localization and activating genes that participate in iron and copper utilization [24-29, 46]. In this study we added *BIO5* to the list of genes that are regulated by Aft1 in a way that is dependent on iron concentration in the medium. This confirmed the previously reported relationship between biotin metabolism and iron assimilation [29]. The upregulation of Aft1-dependent genes in the yeast frataxin mutant was taken as evidence that the iron accumulating in the mitochondria of the mutant cells is in a non-metabolizable form, and that *yfh1* cells are therefore nutritionally depleted of iron [27]. Similarly, iron accumulates intracellularly at abnormally high levels in the *Dgrx5* mutant [17], probably as a consequence of the disruption of the Grx5 function in the iron-sulfur cluster assembly. The results shown here suggest that the accumulated iron in the *grx5* cells is not available for cell metabolism. There is, however, an alternative explanation, which is based on the fact that the *YFH1* gene was downregulated in cells lacking Grx5 (Table I and Fig. 2A). This could make the *Dgrx5* cells phenotipically similar to a *Dyfh1* mutant. In this case, iron accumulation in the *Dgrx5* mutant would not be a direct consequence of disruption of iron-sulfur

cluster assembly, but secondary to depletion of Yfh1 molecules. We, however, favour the first alternative, since iron accumulation is common to many mutants in genes involved in the assembly of iron-sulfur clusters [47]. At this respect, recent studies indicate that the primary function of yeast frataxin is the synthesis of iron-sulfur clusters [48-50] and heme groups [51]. In order to carry out this function, Yfh1 would act as an iron chaperone and an iron store to provide Fe(II) [52] to the assembly complexes.

Two genes that could be related to protection against the oxidative stress generated in these conditions were upregulated in the absence of Grx5. One of these is *SNZ1*, through its role in the biosynthesis of piridoxine [35], a vitamin that acts as a protector against single oxygen [36]. The second is *MLP1*, which codes for a homologue of the Slt2 MAP kinase [38]. Slt2 plays a central role in the *S. cerevisiae* cell integrity pathway by activating at least two transcriptional factors: Rlm1 and SBF [53]. These factors respond to stimuli that alter the integrity of the cell envelope by respectively activating the expression of cell wall biosynthesis genes and cell cycle genes [54,55]. However, genes that protect against oxidants do not appear to be targets of Rlm1 or SBF. Genetic interactions between *SLT2* and *MLP1* suggest the existence of some parallel functions between the two gene products [38], but the hypothetical targets for the Mlp1 function are unknown. Our results showed additivity between the *Dgrx5* and *Dmlp1* mutants in their sensitivity to oxidants and in their levels of both constitutive and oxidant-induced protein carbonylation. This supports a functional relationship between Grx5 and Mlp1. The single *Dmlp1* mutant was no more sensitive to oxidative stress than wild type cells. Therefore, the Mlp1 hypothetical protective role against oxidative stress was only manifested under the constitutive stress conditions occurring in the absence of the Grx5 function. Transcriptome analysis of the *mlp1* mutant and of cells overexpressing *MLP1*

could help to reveal more details about the role of Mlp1 relative to oxidative stress.

On the other hand, Msn2/4, Yap1 and Skn7 regulon genes were not constitutively upregulated in *Dgrx5* cells. This indicates that either the stress signal generated in the glutaredoxin mutant was not sufficient to activate the respective transcriptional activators, or that these were in fact only acting in response to instantaneously generated external signals and not to a sustained stress signal.

A significant number of genes whose expression was downregulated in the *Dgrx5* background are Hap4-regulated. Hap4 is the activator component of a complex in which the Hap1/2/3/5 proteins also participate. These are necessary for binding of the complex to specific promoter sites. The Hap complex is required for expression of respiratory genes [56]. Overexpression of Hap4 is sufficient to cause the shift from fermentative to respirarory metabolism, through induced expression of respiratory genes [12,45]. We have shown in the present work that overexpression of *HAP4* suppresses the downregulation of the Hap4-dependent gene *CYC1* in *Dgrx5* cells. This supports the idea that expression of the respiratory genes is repressed in the mutant cells through the inhibition of Hap4 activity. Heme could initially be proposed as the mediator involved in the process. In fact, it is required for the activation of Hap4-dependent genes [29,56]. In *Dgrx5* cells, depletion of metabolically available iron and consequent upregulation of the heme oxygenase gene *HMX1* [29] leads to heme depletion and eventually to Hap4 inactivation. However, the downregulation of this set of genes is not observed in yeast cells that lack the Yfh1 frataxin, in which non-available iron also accumulates and *HMX1* is also repressed [27]. The specificity of the response in cells without Grx5 therefore points to additional factors being

responsible for regulating Hap4 activity under conditions of moderate constitutive oxidative stress.

Based on the role of Grx5 in the biogenesis of iron-sulfur clusters, the respiratory deficiency of *Dgrx5* cells [17] could be initially explained by the absence of Rip1 activity. Rip1 is the iron-sulfur Rieske protein that is part of complex III in the yeast respiratory chain [57]. *S. cerevisiae* cells have not respiratory complex I, which in mammalian cells also contains iron-sulfur proteins. In yeast, complex I is replaced by the mitochondrial inner membrane Ndi1 NADH-dehydrogenase [58,59]. In mammalian cells, both complex I and complex III are sources of ROS, whose production is enhanced by cytochrome oxidase inhibition [60,61]. It can therefore be hypothesized that incomplete respiratory chains in Grx5-deficient cells are sources of ROS with sustrates such as NADH or succinate, unless all chain components are depleted, for instance through Hap4 inhibition. The situation observed in cells lacking Grx5, in which expression of the different components of the respiratory chain (including the *NDI1* gene) was inhibited, could be extrapolated to other situations in which ROS-mediated stress exists. This could be a general response to prevent respiratory production of ROS. Other models for metabolically-generated constitutive oxidative stress need to be tested to confirm this hypothesis.

Acknowledgments- We thank Lidia Piedrafita for her excellent technical assistance.

REFERENCES

1. Storz, G., and Imlay, J.A. (1999) *Curr. Opin. Microbiol.* **2,** 188-194
2. Gasch, A.P., Spellman, P.T., Kao, C.M., Carmel-Harel, O., Eisen, M.B., Storz, G., Botstein, D., and Brown, P.O. (2000) *Mol. Biol. Cell* **11,** 4241-4257
3. Causton, H.c., Ren, B., Koh, S.S., Harbison, C.T., Kanin, E., Jennings, E.G., Lee, T.I., True, H.L., Lander, E.S., and Young, R.A. (2001) *Mol. Biol. Cell* **12,** 323-337
4. Godon, C., Lagniel, G., Lee, J., Buhler, J.-M., Kieffer, S., Perrot, M., Boucherie, H., Toledano, M.B., and Labarre, J. (1998) *J. Biol. Chem.* **273,** 22480-22489
5. Lee, J., Godon, C., Lagniel, G., Spector, D., Garin, J., Labarre, J., and Toledano, M.B. (1999) *J. Biol. Chem.* **274,** 16040-16046
6. Toledano, M.B., Delaunay, A., Biteau, B., Spector, D., and Azevedo, D. (2003) in *Topics in Current Genetics, vol. 1, Yeast Stress Responses*, eds. Hohmann, S., and Mager, P.W.H. (Springer-Verlag, Berlin), pp. 241-303
7. Martínez-Pastor, M.T., Marchler, G.C.S., Marchler-Bauer, A., Ruis, H., and Estruch, F. (1996) *EMBO J.* **15,** 2227-2235
8. Schmitt, A.P., and McEntee, K. (1996) *Proc. Natl. Acad. Sci. USA* **93,** 5777-5782
9. Hasan, R., Leroy, C., Isnard, A.D., Labarre, J., Boy-Marcotte, E., and Toledano, M.B. (2002) *Mol. Microbiol.* **45,** 233-241
10. Cabiscol, E., Piulats, E., Echave, P., Herrero, E., and Ros, J. (2000) *J. Biol. Chem.* **275,** 27393-27398
11. De Risi, J.L., Iyer, V.R., and Brown, P.O. (1997) *Science* **278,** 680-686
12. Lascaris, R., Bussemaker, H.J., Boorsma, A., Piper, M., van der Spek, H., Grivell, L., and Blom, J. (2002) *Genome Biol* **4,** R3.1-10
13. Trotter, E.W., and Grant, C.M. (2003) *EMBO Rep*. **4,** 184-188

14. Rodríguez-Manzaneque, M.T., Ros, J., Cabiscol, E., Sorribas, A., and Herrero, E. (1999) *Mol. Cell. Biol.* **19,** 8180-8190

15. Shenton, D., Perrone, G., Quinn, K.A., Dawes, I.W., and Grant, C.M. (2002) *J. Biol. Chem.* **277,** 16853-16859

16. Shenton, E.W., and Grant, C.M. (2003) *Biochem. J.* **374,** 513-519

17. Rodríguez-Manzaneque, M.T., Tamarit, J., Bellí, G., Ros, J., and Herrero, E. (2002) *Mol. Biol. Cell* **13,** 1109-1121

18. Wach, A., Brachat, A., Pöhlmann, R., and Philippsen, P. (1994) *Yeast* **13,** 1793-1808

19. Goldstein, A.L., and McCusker, J.H. (1999) *Yeast* **15,** 1541-1553

20. Bellí, G., Garí, E., Piedrafita, L., Aldea, M., and Herrero, E. (1998) *Nucleic Acids Res* **26,** 942-947

21. Sherman, F. (1991) *Meth. Enzymol.* **194,** 3-21

22. Spellman, P.T., Sherlock, G., Zhang, M.Q., Iyer, V.R., Anders, K., Eisen, M., Brown, P.O., Botstein, D., and Futcher, B. (1998) *Mol. Biol. Cell* **9,** 3273-3297

23. Traven, A., Wong, J.M.S., Xu, D., Sopta, M., and Ingles, C.J. (2001) *J. Biol. Chem.* **276,** 4020-4027

24. Yamaguchi-Iwai, Y., Dancis, A., and Klausner, R.D. (1995) *EMBO J.* **14,** 1231-1239

25. Yamaguchi-Iwai, Y., Stearman, R., Dancis, A., and Klausner, R.D. (1996) *EMBO J.* **15,** 3337-3384

26. Yun, C.W., Tiedman, J.S., Moore, R.E., and Philpott, C.C. (2000) *J. Biol. Chem.* **275,** 16354-16359

27. Foury, F., and Talibi, D. (2001) *J. Biol. Chem.* **276,** 7762-7768

28. Protchenko, O., Ferea, T., Rashford, J., Tiedman, J., Brown, P.O., Botstein, D., and Philpott, C.C. (2001) *J. Biol. Chem.* **276,** 49244-49250

29. Protchenko, O., and Philpott, C.C. (2003) *J. Biol. Chem.* **278,** 36582-36587

30. Babcock, M., De Silva, D., Oaks, R., Davis-Kaplan, S., Jiralerspong, S., Montermini, L., Pandolfo, M., and Kaplan, J. (1997) *Science* **276,** 1709-1712
31. Stolz, J., Hoja, U., Meier, S., Sauer, N., and Schweizer, E. (1999) *J. Biol. Chem.* **274,** 18741-18746
32. Phalip, V., Kuhn, I., Lemoine, Y., and Jeltsch, J-M. (1999) *Gene* **232,** 43-51
33. Ugulava, N.B., Gibney, B.R., and Jarrett, J.T. (2001) *Biochemistry* **40,** 8343-8351
34. Mühlenhoff, U., Richhardt, N., Gerber, J., and Lill, R. (2002) *J. Biol. Chem.* **277,** 29810-29816
35. Rodríguez-Navarro, S., Llorente, B., Rodríguez-Manzaneque, M.T., Ramne, A., Uber, G., Marchesan, D., Dujon, B., Herrero, E., and Pérez-Ortín, J.E. (2002) *Yeast* **19,** 1261-1276
36. Bilski, P., Li, M.Y., Ehrenshaft, M., Daub, M.E., and Chignell, C.F. (2000) *Photochem. Photobiol.* **71,** 129-134
37. Padilla, P.A., Fuge, E.K., Crawford, M.E., Errett, A., and Werner-Washburne, M. (1998) *J. Bacteriol.* **180,** 5718-5726
38. Watanabe, Y., Takaesu, G., Hagiwara, M., Irie, K., and Matsumoto, K. (1997) *Mol. Cell. Biol.* **17,** 2615-2623
39. Jung, U.S., Sobering, A.K., Romeo, M.J., and Levin, D.E. (2002) *Mol. Microbiol.* **46,** 781-789
40. Marchler, G., Schüller, C., Adam, G., and Ruis, H. (1993) *EMBO J.* **12,** 1997-2003
41. Wu, A., and Moye-Rowley, W.S. (1994) *Mol. Cell. Biol.* **14,** 5832-5839
42. Charizanis, C., Juhnke, H., Krems, B., and Entian, K.D. (1999) *Mol. Gen. Genet.* **261,** 740-752
43. Kuge, S., and Jones, N. (1994) *EMBO J.* **13,** 655-664
44. Gancedo, J.M. (1999) *Microbiol. Mol. Biol. Rev.* **62,** 334-361

45. Blom, J., De Mattos, M.J., and Grivell, L.A. (2000) *Appl. Environ. Microbiol.* **66,** 1970-1973

46. Yamaguchi-Iwai, Y., Ueta, R., Fukunaka, A., and Sasaki, B. (2002) *J. Biol. Chem.* **277,** 18914-18918

47. Lill, R., and Kispal, G. (2000) *Trends Biochem. Sci.* **25,** 352-356

48. Chen, O.S., Hemenway, S., and Kaplan, J. (2002) *Proc. Natl. Acad. Sci. U.S.A.* **99,** 12321-12326

49. Muhlenhof, U., Richter, N., Gerber, J., and Lill, R. (2002) *J. Biol. Chem.* **277,** 29810-29816

50. Duby, G., Foury, F., Ramazzotti, A., Herrmann, J., and Lutz, T. (2002) *Hum. Mol. Genet.* **11,** 2635-2643

51. Lesuisse, E., Santos, R., Matzanke, B.F., Knight, A.A.B., Camadro, J.M., and Dancis, A. (2002) *Hum. Mol. Genet.* **12,** 879-889

52. Park, S., Gakh, O., O´Neill, H.A., Mangravita, A., Nichol, H., Ferreira, G.C., and Isaya, G. (2003) *J. Biol. Chem.* **278,** 31340-31351

53. Hohmann, S. (2002) *Microbiol. Mol. Biol. Rev.* **66,** 300-372

54. Jung, U.S., and Levin, D.E. (1999) *Mol. Microbiol.* **34,** 1049-1057

55. Lagorce, A., Hauser, N.C, Labourdette, D., Rodriguez, C., Martin-Yken, H., Arroyo, J., and Francois, J (2003) *J. Biol. Chem.* **278,** 20345-20357

56. Kwast, K.E., Burke, P.V., and Poyton, R.O. (1998) *J. Exp. Biol.* **201,** 1177-1195

57. Beckmann, J.D., Ljungdahl, P.O., Lopez, J.L., and Trumpower, B.L. (1987) *J. Biol. Chem.* **262,** 8901-8909

58. Schagger, H., and Pfeiffer, K. (2000) *EMBO J.* **19,** 1777-1783

59. Joseph-Horne, T., Hollomon, D.W., and Wood, P.M. (2001) *Biochem. Biophys. Acta* **1504,** 179-195

60. Young, T.A., Cunningham, C.C., and Bailey, S.M. (2002) *Arch. Biochem. Biophys.* **405,** 65-72

61. Chen, Q., Vazquez, E.J., Moghaddas, S., Hoppel, C.L., and Lesnefsky, E.J. (2003) *J. Biol. Chem.* **278,** 36027-36031

LEGENDS TO THE FIGURES

FIG. 1. **Comparative expression of *S. cerevisiae* genes in *Dgrx5* and *Dpet117* cells.** *A,* expression of each individual gene (marked by a square) in *Dgrx5* cells (MML100) in relation to its expression in *Dpet117* cells. Values result from normalizing the expression level of the gene in the respective mutant by its expression in wild type cells (log_2 of the ratio). *B*, genes whose relative expression was significantly modified in divergent ways in *Dgrx5* and *Dpet117* mutants. Only induction and repression ratios (mutant vs. wild type) of two or more were considered significant.

FIG. 2. **Representative genes differentially expressed in wild type and *Dgrx5* cells.** *A*, Northern blot analysis of some of the genes that were induced (Table I) or repressed (Table II) in the *Dgrx5* mutant (strain MML100) relative to wild type cells (W303-1A). The snU2 mRNA served as a loading control, and *ACT1* is representative of genes whose expression did not vary in the mutant. Samples were taken from exponential cultures in YPD medium at 30ºC. *B*, Growth levels (measured by optical density units at 600 nm) of wild type cells (solid bars) and *Dgrx5* mutant cells (open bars) after two days at 30ºC in liquid SC medium with biotin at the indicated final concentrations. Initial inocula contained 10^5 cells per ml. Growth levels in cultures of both strains containing biotin at 20 μg per litre were respectively given unit value. *C,* Northern blot analysis of *BIO5* expression in exponential cultures of wild type and *Dgrx5* cells in SC medium at 30ºC, with biotin at the indicated concentrations. *D*, Northern blot analysis of *FET3* and *BIO5* expression in wild type (W303-1A) and *aft1-D5* (MML348) cells. Samples were obtained from exponential cultures in YPD medium at 30ºC; ferrozine was

added at 2 mM final concentration at time 0. Same amounts of RNA were loaded per lane, as determined by snU2 mRNA analysis (not shown).

FIG. 3. **Sensitivity of null mutants in the *GRX5* and *MLP1* genes to oxidative stress.** *A*, wild type (W303-1A), *Dgrx5* (MML100), *Dmlp1* (MML524) and *Dgrx5 Dmlp1* (MML533) cells were tested for sensitivity to *t*-BOOH or menadione, on YPD plates containing the oxidative agent at the indicated concentration. *B*, protein oxidative damage in the indicated strains, as measured by the presence of side carbonyl groups. Total protein extracts were obtained from untreated or *t*-BOOH-treated cultures, and analyzed by Western blot (20 mg of total protein per lane) using antibodies against anti-2,4-dinitrophenylhidrazones [10].

FIG. 4. **Expression of oxidative stress-inducible genes in a *Dgrx5* genetic background.** *A*, Northern blot analysis of the indicated genes in exponentially-growing wild type (W303-1A) and *Dgrx5* (MML100) cells in YPD medium. SnU1 mRNA was included as a loading control. *B*, Induction of *DDR2* and *GSH1* expression by menadione (Md) at the indicated concentrations, in wild type and *Dgrx5* cells growing exponentially in YPD medium. The expression of a non-inducible gene (*ACT1*) is also shown.

FIG. 5. **Effect of *HAP4* overexpression in wild type and *Dgrx5* cells.** Wild type (W303-1A) and *Dgrx5* (MML100) cells were transformed with plasmid pMT15 (that overexpresses *HAP4* under the *tet* promoter). Non-transformed and pMT15-transformed cells were grown in liquid SC medium at 30ºC. Samples were taken in exponential conditions. *A*, Northern blot analysis of *HAP4* and *CYC1* expression. SnU2 mRNA is shown as a loading control. *B*, carbonyl groups in total cell proteins, after Western blot analysis (20 μg of total protein per lane). *C*,

growth rates of the respectives cultures (mean of three experiments). Values over bars indicate the optical density (at 600 nm) doubling times in minutes, in exponential growth conditions.

Table I

Genes induced in Δgrx5 cells

Values are averages for three independent experiments

Gene	Induction fold	Function/characteristics	*Δyfh1*[a]	Aft1-dependent[b]	Cell cycle[c]
Ion homeostasis					
FIT3	52.2	Siderochrome transport	+	+	M
ARN2	10.4	Siderochrome transport	+	+	
FRE3	10.2	Ferric chelate reductase	+	+	
FIT1	7.9	Siderochrome transport	+	+	
ARN1	7.3	Siderochrome transport	+	+	
FIT2	6.0	Siderochrome transport	+	+	
FTR1*	5.4	Iron ion transport	+	+	M
SIT1	3.8	Siderophore transport	+	+	M
PCA1	3.2	Cooper ion homeostasis			
FTH1	3.0	Ftr1 homologue	+	+	
HMX1	2.9	Heme oxygenase, iron homeostasis	+	+	
CCC2	2.8	Copper ion transport	+	+	
CTR2	2.3	Cooper ion transport	+		
ATX2	2.2	Manganese ion homeostasis	+		
CUP1-1	2.1	Cooper ion binding			
Transport					
*MUP3**	8.1	Methionine permease	+		
AUS1	4.1	Sterol transport			
VHT1	3.4	Biotin transporter		+	M
HXT2	2.1	Hexose transporter			
Biosynthesis					
BIO5	7.3	Biotin biosynthesis		+	
SNZ1	3.0	Vitamin B6 biosynthesis			
THI21	2.9	Thiamine biosynthesis			
NCP1	2.7	NADP-cytochrome P450 reductase, ergosterol biosynthesis			
ADE16	2.2	Purine biosynthesis			
CYS3	2.2	Cysteine biosynthesis			
Carbon and lipid catabolism					
AMS1	3.5	Vacuolar alpha-manosidase			
IDH2	2.5	Isocitrate dehydrogenase subunit II			
IDH1	2.4	Isocitrate dehydrogenase subunit I			M
OLE1	2.3	Stearoyl-CoA desaturase			M/G1
PDA1	2.2	Piruvate dehydrogenase			
Mitochondrial biogenesis and functions					
MRS4	3.4	Mitochondrial carrier protein			
ISU1	3.3	Iron-sulphur cluster assembly	+	+	
MMT2	2.2	Mitochondrial iron transport			
Protein targeting					
AKR1	2.6	Palmotoyltransferase, endocitosis	+		
PMT3	2.2	Dolichyl-phosphate-mannose protein mannosyltransferase			G1
SEC61	2.2	Protein transporter			
STV1	2.2	Hydrogen-transporting ATPase, Golgi			

		apparatus			
Protein degradation					
LAP4	3.0	Aminopeptidase I	+		G1
UBC8	2.4	Ubiquitin conjugating enzyme			
PRE2	2.4	Proteasome endopeptidase			
PRB1	2.3	Serine-type endopeptidase	+		S
Stress responses					
HSP26	8.5	Heat shock protein			S
SLT2	2.1	PKC-dependent MAP quinase			
Cell wall					
TIR3	8.3	Mannoprotein, cold shock-induced			M
TIR1	6.4	Mannoprotein, cold shock-induced			
TIR2	3.2	Mannoprotein, cold shock-induced			
GSC2	2.8	Beta-1,3 glucan biosynthesis			
Others					
APG16*	14.8	Autophagy			
ENT4	2.7	Cytoskeletal adaptor			
Unknown function					
DAN1	15.0	Induced under anaerobic conditions			
YKL161C	11.0	Strong similarity to Slt2			
PAU7	8.7	Member of the seripauperin protein/ gene family			
YHL035C	7.4				
YDR476C	7.0				
YOL161C	6.9				
YPL272C	5.9				
*LSB3**	5.4				
YPR076W*	5.4				
YMR325W	5.3				
PRY1	5.2				M
YGL039W	5.2	Dihydrokaempferol 4-reductase			
PAU1	5.1	Member of the seripauperin protein/ gene family			
TIS11	5.1		+	+	
PAU3	4.9	Member of the seripauperin protein/ gene family			
YBR047W	4.9				
PAU6	4.7	Member of the seripauperin protein/ gene family			
YIR041W	4.5				
PAU2	4.4	Member of the seripauperin protein/ gene family			
YPR039W*	4.3				
YFR024C	4.2				
YGR294W	4.1				
YGL261C	4.0				
PAU5	3.8	Member of the seripauperin protein/ gene family			
YHL046C	3.7				
YKR104W	3.6				
NFT1	3.6	Putative ABC transporter			
YOR394W	3.6				
YNL190W	3.6				
YIL176C	3.6				
YGR160W	3.4				

*YKL162C**	3.3	
YDR271C	3.2	
YLL064C	3.2	
YDR319C	3.1	
YOL087C	3.0	
PRM4	3.0	Pheromone-regulated membrane protein
YMR041C	2.7	
YHR199C	2.6	
YOR389W	2.4	
YGR160W	2.4	
YOR385W	2.3	
YNL208W	2.3	
YCL027C	2.2	
YPL278C	2.1	
YPR090W	2.1	
NOG1	2.1	Nucleolar GTPase
YKL224C	2.0	
DAN3	2.0	Putative cell wall protein
SNA3	2.0	

---

[a] Genes upregulated in a *Dyfh1* mutant [27] are marked with a + sign
[b] Genes whose expression is Aft1-dependent are marked with a + sign
[c] For genes with a cell cycle-dependent expression [22], the stage with the greatest expression is indicated

Asterisks indicate genes upregulated in a *Dpet117*-defective mutant.

Genes in boldface type were not induced by hydrogen peroxide, menadione or diamide treatment in experiments reported in Ref. 2 and 3. Induction was considered to exist only when at least two consecutive time points gave significantly increased levels under the experimental conditions employed in these studies

Table II

Genes repressed in Dgrx5 cells

Values are averages for three independent experiments

Gene	Change	Function/characteristics	Cell cycle[b]
Ion homeostasis			
YFH1	3.3	Frataxin, iron homeostasis	
CCC1	3.3	Fe, Ca and Mn ion homeostasis	
Biosynthesis			
CYB5	7.7	Sterol biosynthesis	M
GLT1	7.4	Glutamate synthase	
ILV3	4.7	Isoleucine and valine biosynthesis	
DPH2	3.2	Diphtamide biosynthesis	
LYS4	2.6	Lysine biosynthesis	
ERG11	2.0	Sterol metabolism	
Carbon and lipid catabolism			
DLD1	2.8	D-lactate dehydrogenase	
PDC5	2.1	Pyruvate decarboxylase	
Mitochondrial biogenesis and functions			
MEF2	25.0	Mitochondrial translation elongation factor	
CYC1[a]*	9.6	Cytochrome c isoform 1	
CYT1	7.0	Electron transporter	G2
QCR10	6.7	Ubiquinol-cytochrome c oxidoreductase subunit	
MHR1	6.2	Mitochondrial transcription regulator	
RIP1	5.9	Ubiquinol-cytochrome c reductase	
COX7	5.7	Cytochrome c oxidase subunit	
QCR8	5.0	Ubiquinol-cytochrome c oxidoreductase subunit	
NDI1	4.2	NADH dehydrogenase	M
COX6	3.8	Cytochrome c oxidase subunit	
SDH4	3.3	Succinate dehydrogenase	
QCR7	3.7	Ubiquinol-cytochrome c oxidoreductase subunit	
QCR6	3.1	Ubiquinol-cytochrome c oxidoreductase subunit	
COX5A[a]	3.1	Cytochrome c oxidase subunit	
QCR9	2.9	Ubiquinol-cytochrome c oxidoreductase subunit	
COX13	2.7	Cytochrome c oxidase subunit	
COX12	2.6	Cytochrome c oxidase subunit	
COX9	2.5	Cytochrome c oxidase subunit	
OAC1	2.4	Oxalacetate carrier activity	
COX17*	2.3	Cytochrome c oxidase assembly	
Cell cycle and mating			
AGA2	3.5	a-agglutinin subunit	M/G1
APC5	3.4	Subunit of anaphase-promoting complex	
*MAD1**	2.9	Mitotic spindle checkpoint	
TEC1	2.8	Transcription factor involved in pseudohyphal growth	M/G1
BAR1	2.6	alpha-factor protease	
BUB1	2.6	Mitotic spindle checkpoint	G1
MOB2	2.4	Establishment of cell polarity	

Stress responses			
GRX4	4.8	Glutaredoxin, oxidative stress response	
PHO5	3.0	Acid phosphatase, response to phosphate starvation	M
Others			
*PDR15**	3.4	ABC transporter involved in multudrug resistance	
*MLP2**	3.4	Nuclear protein targeting	
CWP2	3.1	Cell wall organization	G2
PHO3	2.6	Acid phosphatase, thiamine uptake	M
BAT1	2.6	Branched chain amino acid degradation	G2
GAP1	2.4	General amino acid transport	
PHO12	2.1	Acid phosphatase	
Unknown function			
*YDR316W**	4.2		
YER182W*	3.8		
YER156C[a]*	3.7		
YJL200C	3.1		
*KRR1**	3.0		
CTH1	3.0		
YEL033W	2.9		
YJL018W	2.9		
*YMR102c**	2.9		
ABM1	2.8		
YAL046C	2.7		
YNL144C*	2.7		
*YPL146C**	2.7		
YBR028C	2.6		
YLR198C	2.5		
YOL109W	2.5		
YHR045W	2.4		
YBR025C	2.1		

[a] Genes also repressed in a *Dyfh1* mutant [27]

[b] For genes with a cell cycle-dependent expression [22], the stage with the greatest expression is indicated

Asterisks indicate genes downregulated in a *Dpet117*-defective mutant

Genes in boldface type were not repressed by hydrogen peroxide, menadione or diamide treatment in experiments reported in Ref. 2 and 3. Repression was only considered when at least two consecutive time points gave significantly reduced levels under the experimental conditions employed in these studies

Table III

Expression of Δgrx5-repressed genes involved in mitochondrial functions under respiratory conditions

Gene	Post-diauxic phase[a]	Hap4 overexpression[b]
COX5A	+	
COX6	+	+
COX9	+	+
COX13	+	
COX17		+
CYC1	+	+
CYT1	+	+
NDI1	+	+
QCR6	+	+
QCR7	+	
QCR9		+
QCR10		+
RIP1	+	+
SDH4	+	+
YDR316W		+
YER182W		+

[a] Data from Ref. 11
[b] Data from Ref. 12
+ denotes significant induction under the experimental conditions reported

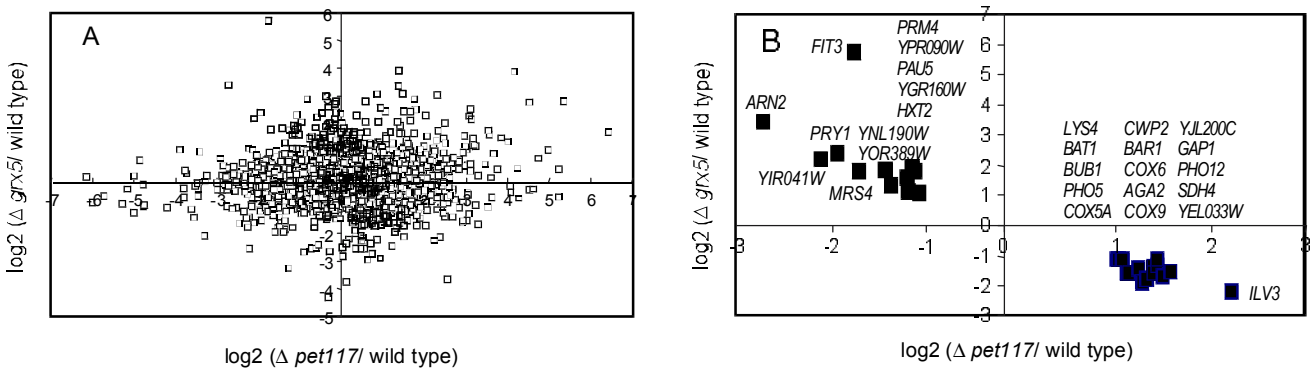
A
log2 (Δ grx5/ wild type)
log2 (Δ pet117/ wild type)
B
FIT3
PRM4
YPR090W
PAU5
YGR160W
HXT2
ARN2
PRY1
YNL190W
YOR389W
YIR041W
MRS4
LYS4
CWP2
YJL200C
BAT1
BAR1
GAP1
BUB1
COX6
PHO12
PHO5
AGA2
SDH4
COX5A
COX9
YEL033W
ILV3
log2 (Δ grx5/ wild type)
log2 (Δ pet117/ wild type)

Fig. 1

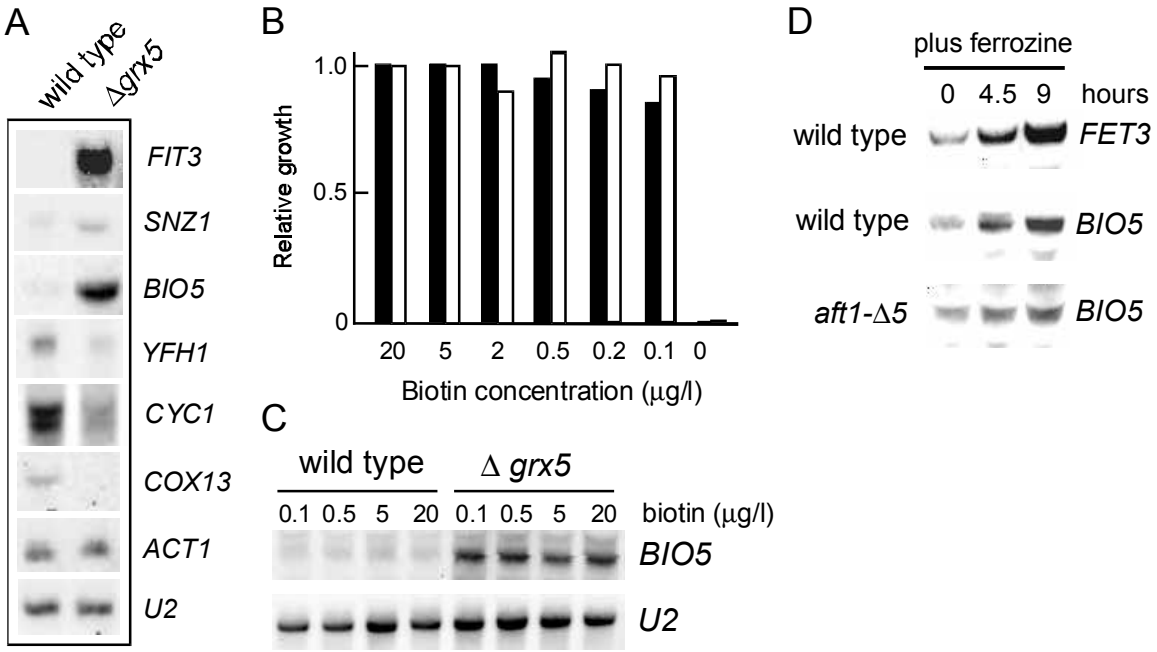
A
wild type
Δgrx5
FIT3
SNZ1
BIO5
YFH1
CYC1
COX13
ACT1
U2
B
Relative growth
1.0
0.5
0
20 5 2 0.5 0.2 0.1 0
Biotin concentration (μg/l)
C
wild type
Δ grx5
0.1 0.5 5 20 0.1 0.5 5 20 biotin (μg/l)
BIO5
U2
D
plus ferrozine
0 4.5 9 hours
wild type
FET3
wild type
BIO5
aft1-Δ5
BIO5

Fig. 2

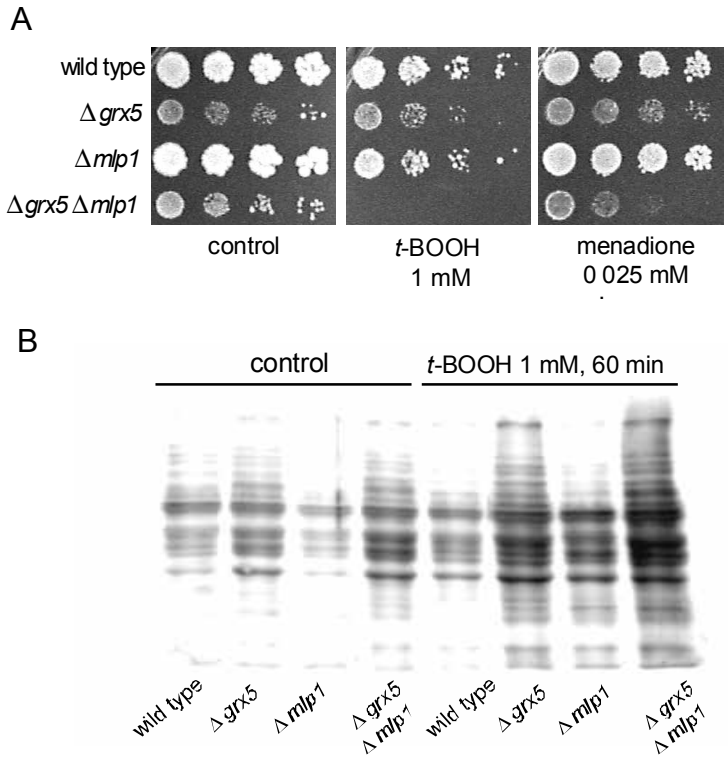
A
wild type
Δgrx5
Δmlp1
Δgrx5 Δmlp1
control
t-BOOH
1 mM
menadione
0 025 mM
B
control
t-BOOH 1 mM, 60 min
wild type
Δgrx5
Δmlp1
Δgrx5
Δmlp1
wild type
Δgrx5
Δmlp1
Δgrx5
Δmlp1

Fig. 3

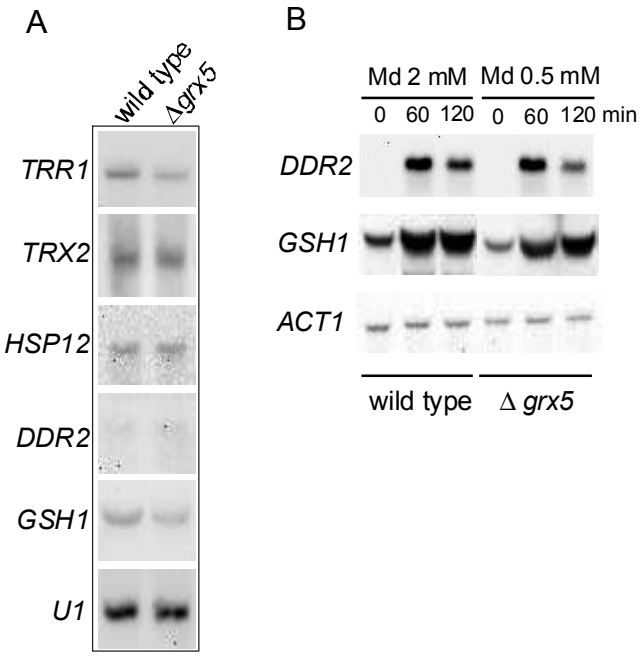
A
wild type
Δgrx5
TRR1
TRX2
HSP12
DDR2
GSH1
U1
B
Md 2 mM
Md 0.5 mM
0 60 120 0 60 120 min
DDR2
GSH1
ACT1
wild type
Δ grx5

Fig. 4

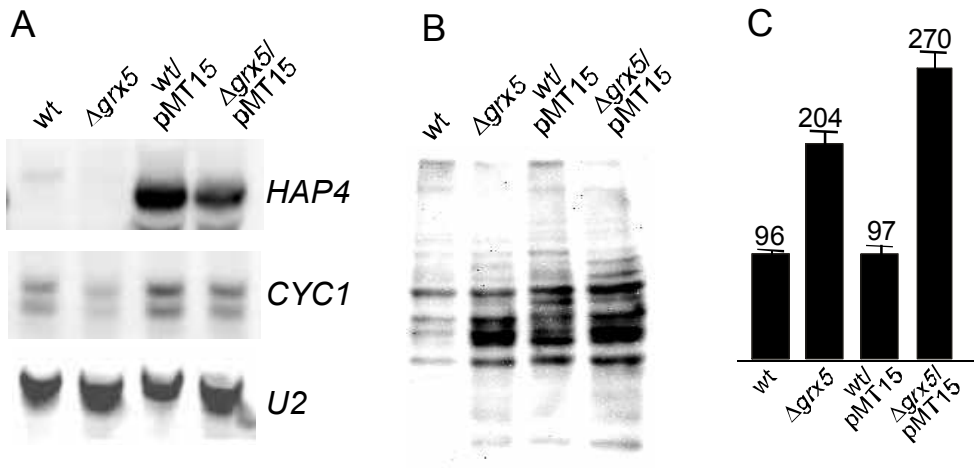
A
wt
Δgrx5
wt/ pMT15
Δgrx5/ pMT15
HAP4
CYC1
U2
B
wt
Δgrx5
wt/ pMT15
Δgrx5/ pMT15
C
96
204
97
270
wt
Δgrx5
wt/ pMT15
Δgrx5/ pMT15

Fig. 5