Quantitative transcriptome, proteome, and sulfur metabolite profiling of the Saccharomyces cerevisiae response to arsenite

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Submitted 27 October 2006; accepted in final form 22 February 2007

Thorsen M, Lagniel G, Kristiansson E, Junot C, Nerman O, Labarre J, Tamás MJ. Quantitative transcriptome, proteome, and sulfur metabolite profiling of the Saccharomyces cerevisiae response to arsenite. Physiol Genomics 30: 35–43, 2007. First published February 27, 2007; doi:10.1152/physiolgenomics.00236.2006.—Arsenic is ubiquitously present in nature, and various mechanisms have evolved enabling cells to evade toxicity and acquire tolerance. Herein, we explored how Saccharomyces cerevisiae (budding yeast) respond to trivalent arsenic (arsenite) by quantitative transcriptome, proteome, and sulfur metabolite profiling. Arsenite exposure affected transcription of genes encoding functions related to protein biosynthesis, arsenic detoxification, oxidative defense, redox maintenance, and proteolytic activity. Importantly, we observed that nearly all components of the sulfur assimilation and glutathione biosynthesis pathways were induced at both gene and protein levels. Kinetic metabolic profiling evidenced a significant increase in the pools of sulfur metabolites as well as elevated cellular glutathione levels. Moreover, the flux in the sulfur assimilation pathway as well as the glutathione synthesis rate strongly increased with a concomitant reduction of sulfur incorporation into proteins. By combining comparative genomics and molecular analyses, we pinpointed transcription factors that mediate the core of the transcriptional response to arsenite. Taken together, our data reveal that arsenite-exposed cells channel a large part of assimilated sulfur into glutathione biosynthesis, and we provide evidence that the transcriptional regulators Yap1p and Met4p control this response in concert.

DNA microarray; proteomics; glutathione

Arsenic is a highly toxic metalloid that considerably threatens the environment and human health. The most striking example is the epidemic of arsenic poisoning observed in Bangladesh and West Bengal, where arsenic contaminates the drinking water through geological sources and thereby affects millions of people (10, 26). Chronic arsenic exposure causes cardiovascular diseases, neurological disorders, and liver injury and is associated with cancers of the skin, bladder, liver, and lung. Despite its toxicity, arsenic trioxide is currently used as a treatment for acute promyelocytic leukemia, and it might also be employed against other hematological and solid cancers (7, 28).

All organisms have been exposed to toxic agents since the origin of life, and tolerance mechanisms arose early during evolution. The unicellular model eukaryote Saccharomyces cerevisiae (budding yeast) evades arsenic toxicity by increasing efflux of trivalent arsenite (As(III)) through the plasma membrane protein Acr3p (12, 38), by sequestering glutathione-conjugated As(III) in the vacuole through the ATP binding cassette (ABC) transporter Ycf1p (12) and by reducing As(III) influx through the aquaglyceroporin Fps1p (34, 39). In addition, cells may acquire tolerance by adjusting cytosolic redox and glutathione levels in response to As(III) (31). Two AP-1-like transcription factors have been shown to be important for yeast As(III) tolerance; Yap8p controls expression of the arsenic-specific detoxification genes ACR2 and ACR3, whereas Yap1p controls transcription of genes encoding proteins with antioxidant properties. In addition, Yap1p contributes to YCF1 and ACR3 control (17, 24, 40).

Sulfur assimilation is essential for all organisms. In yeast, extracellular sulfate is taken up and metabolized through the sulfate assimilation pathway where sulfide is the reduced end product (33). Sulfide can then either go through the methyl cycle or into the cysteine/glutathione biosynthesis pathway (Fig. 1). Hence, the fate of assimilated sulfur is principally biosynthesized from the sulfur-containing amino acids methionine and cysteine and the low-molecular-weight thiol molecules S-adenosylmethionine and glutathione (GSH) (33). GSH is a key factor in the cell’s defense against oxidative stress and metal toxicity. GSH may detoxify metals by 1) chelation followed by vacuolar sequestration; 2) protecting against oxidation caused by metals, since GSH serves as the main redox buffer of the cell; and 3) binding to reactive sulfhydryl groups on proteins (protein glutathionylation), thereby protecting them from irreversible metal binding and/or oxidative damage (13, 27). Whether protein glutathionylation occurs in response to metals has not been investigated. Transcription of the genes encoding enzymes in the sulfite assimilation/GSH biosynthesis pathways is principally controlled by the transcriptional activator Met4p. Met4p is recruited to target promoters by the DNA binding proteins Met28p, Met31p, Met32p, and Cbf1p, forming the complexes Met4p-Met31p/Met32p and Met4p-Cbf1p-Met28p (33). Interestingly, Met4p has been shown to play a central role in cadmium tolerance by controlling expression of sulfur assimilation and GSH biosynthesis genes (8).

The aim of this study was to gain detailed insight into the yeast response to arsenite. We demonstrate that As(III)-exposed cells channel a large part of assimilated sulfur into GSH
biosynthesis and provide evidence that the transcriptional regulators Yap1p and Met4p control this response in concert.

**MATERIALS AND METHODS**

Yeast strains, plasmids, and growth conditions. *S. cerevisiae* strains used in this study are summarized in Table 1. All deletion mutants were constructed according to Ref. 14, and metal sensitivity assays were carried out as previously described (39). The metals used were sodium arsenite (Sigma), cadmium chloride (Sigma), and potassium antimonyl tartrate (Acros). Yeast strains were grown at 30°C on minimal YNB medium (0.67% yeast nitrogen base) supplemented with auxotrophic requirements and 2% glucose as a carbon source or on SC medium (YNB containing 2% glucose).

RNA isolation, cDNA synthesis, microarray hybridization, and analysis. Total RNA was isolated as described previously (4) from exponentially growing yeast cells that were either untreated or exposed to sodium arsenite (Sigma), cadmium chloride (Sigma), and potassium antimonyl tartrate (Acros). Yeast strains were grown at 30°C on minimal YNB medium (0.67% yeast nitrogen base) supplemented with auxotrophic requirements and 2% glucose as a carbon source or on SC medium (YNB containing 2% glucose).

RNA isolation, cDNA synthesis, microarray hybridization, and analysis. Total RNA was isolated as described previously (4) from exponentially growing yeast cells that were either untreated or exposed to sodium arsenite; 20 μg of total RNA were primed with 3 μg of random hexamer (Invitrogen) and 3 μg of anchored oligo(dT)20 primer (ABgene) and labeled in a reverse transcription reaction with Cy3-dUTP or Cy5-dUTP (Amersham Pharmacia Biotech) in a volume of 30 μl, according to standard protocols [http://cgm.physiology.org/](http://cgm.physiology.org/). Labeled cDNA was cleaned (microcon YM-30 columns, Milipore), combined, vacuum-dried, and resuspended in 80 μl of DIGeasy hybridization buffer (Roche Diagnostics). The hybridization mix was placed at 100°C for 2 min and then at 37°C for 30 min. Before hybridization, the microarray chip (Yeast 6.4k array from University Health Network Microarray Centre, Toronto, Canada) was prehybridized with 1% BSA in DIGeasy hybridization buffer at 42°C for 1 h. Hybridization was performed at 42°C for 12–18 h. After hybridization, the slides were washed at room temperature in 2× SSC plus 0.1% sodium dodecyl sulfate for 5 min, in 1× SSC for 5 min, and in 0.1× SSC for 5 min and then blow dried with N2. The slides were scanned (VersArray ChipReader, Bio-Rad) at laser intensity and photomultiplier tube voltage settings giving the best dynamic range for each chip in the respective channel. Image segmentation and spot quantification were performed with ImageJ software (BioDiscovery, CA). The microarray data were analyzed using the linear models for microarray data (LIMMA) package [http://www.bioconductor.org](http://www.bioconductor.org) in the statistical language R [http://www.R-project.org](http://www.R-project.org). The data were normalized by subtracting a loess line from the M-value to remove intensity-dependent trends (41). The genes were ranked by the moderated t-statistic to avoid

**Table 1. Saccharomyces cerevisiae strains used in this study**

<table>
<thead>
<tr>
<th>Strain</th>
<th>Genotype</th>
<th>Source</th>
</tr>
</thead>
<tbody>
<tr>
<td>W303-1A</td>
<td>MATa ura3-1 leu2-3/112 trp1-1 his3-11/15 ade2-1 can1-100 GAL</td>
<td>Ref. 32</td>
</tr>
<tr>
<td>RW124</td>
<td>W303-1A yap1Δ::loxP</td>
<td>Ref. 40</td>
</tr>
<tr>
<td>CC849-1B</td>
<td>MATa his3 leu2 trp1 ura3 met4Δ::TRP1</td>
<td>Ref. 29</td>
</tr>
<tr>
<td>RW104</td>
<td>W303-1A acr3Δ::loxP-kanMX-loxP</td>
<td>Ref. 29</td>
</tr>
<tr>
<td>YPDahl166</td>
<td>W303-1A acr3Δ::KanMX met4Δ::TRP1</td>
<td>Present study</td>
</tr>
</tbody>
</table>

![Fig. 1. Outline of the sulfur assimilation and glutathione (GSH) biosynthesis pathways in *Saccharomyces cerevisiae*.](http://cgm.physiology.org/) Induction levels (fold induction) of genes/proteins in the pathways in response to arsenite are indicated within brackets as follows: gene expression at 0.2 mM trivalent arsenite [As(III)] for 1 h, gene expression at 1.0 mM As(III) for 1 h, protein level at 0.2 mM As(III) for 4 h. ND, not done.
false positives (30). Each comparison consists of at least three independent experiments. Minimum information about a microarray experiment (MIAME)-compliant microarray data have been deposited in the microarray database Gene Expression Omnibus (GEO: http://www.ncbi.nlm.nih.gov/geo/) (6) with the accession number GSE6129.

Northern blot analysis. Northern analysis was performed as previously described (40). Exponentially growing cells were exposed to 0.2 mM sodium arsenite, and total RNA was extracted at the indicated time points. Blots were hybridized with 32P-labeled PCR fragments of MET3, MET25, and MET14. 18S rRNA was used as a loading control. Primer sequences are available on request.

Proteome analysis. Exponentially growing yeast cells in YNB medium were exposed to 0.2 mM sodium arsenite for 1 h and then labeled for 30 min with [35S]methionine. Protein extraction, two-dimensional gel electrophoresis, and gel analysis were performed as previously described (36).

Metabolite measurements and metabolic flux analysis. Exponentially growing yeast cells (in YNB medium) were exposed to 0.2 mM sodium arsenite. Cells were collected before and at the indicated time points after treatment, and metabolites were extracted as previously described (20). The intracellular concentrations of sulfur metabolites were determined by liquid chromatography/mass spectrometry/mass spectrometry (LC/MS/MS) using a pool of 15N metabolites as internal standards (20). GSH and protein synthesis rates were determined as previously described (19).

Testing for overrepresented transcription factor binding sites. In the data set by Clifton et al. (3), promoters from >5,200 genes in S. cerevisiae together with corresponding promoters from orthologous genes in S. mikatae, S. kudriavzevii, S. bayanus, S. kluveri, and S. castelli are available. For each transcription factor binding site described in the Saccharomyces Genome Database (SGD) (http://www.yeastgenome.org), we searched for genes with the consensus motif present in the promoter of S. cerevisiae and in at least one-half of the available promoters of the other species. To test whether these hits were spread equally among all genes or whether they were overrepresented among the regulated genes from the microarray study [0.2 mM As(III), 1 h], a generalized additive model (GAM) with a two-dimensional gel electrophoresis, and gel analysis were performed as previously described (36).

Here, Prob is probability, $X_g$ is a dichotomous random variable indicating whether gene $g$ has the motif present (according to the search above), and $Y_g$ is a dichotomous variable indicating whether gene $g$ is regulated (fold change $>1$). $L_g$ is the length of the promoter in S. cerevisiae, and $f$ is an unknown smooth function. The coefficient $\beta$ and the function $f$ are estimated from data using the mgcv package (37) in the statistical language R (http://www.R-project.org), and $P$ value is calculated based on the test of $\beta = 0$. The reason for using a GAM instead of a less complex hypergeometric test is to avoid problems with unequal promoter length between the regulated genes and the rest of the genome. Indeed, the average promoter lengths in S. cerevisiae of the up- and downregulated genes were 576 nucleotides and 579 nucleotides, respectively, which should be compared with an average length of 493 nucleotides for the rest of the genes in the genome. Thus the use of a hypergeometric test in this situation would lead to biased P values.

RESULTS

Transcriptional profiling of As(III)-exposed cells. The transcriptional response of yeast cells exposed to sodium arsenite was analyzed using two different concentrations: 0.2 mM, which has a moderate effect on growth, and 1 mM, which severely affects growth of wildtype cells [the minimal inhibitory concentration of As(III) on the W303-1A strain used here is 1.2 mM (39)]. Total RNA was isolated at various time points, and gene expression profiles were analyzed using cDNA microarrays. Exposing cells to 0.2 mM As(III) for 1 h altered the expression of 761 genes (differentially expressed $>2$-fold); mRNA levels for 428 genes were less abundant in exposed cells, whereas mRNA levels for 333 genes were more abundant (Supplemental Table S1; supplemental data are available at the online version of this article). The bulk of downregulated genes encode functions related to protein biosynthesis, i.e., genes encoding RNA, tRNA, ribosomal proteins, and elongation factors. Among those whose expression is stimulated by As(III), we found genes related to arsenic detoxification, oxidative stress defense, redox maintenance, and proteolytic activity as well as genes encoding structural components of the sulfur assimilation and GSH biosynthesis pathways (Supplemental Table S1). The response to As(III) was largely transient: mRNA levels of most responsive genes started to change within the first 15 min of exposure, peaked at 60 min, and then stabilized at a new steady-state expression level once cells had adapted (Supplemental Table S2, A–D; see also Fig. 4A).

When comparing the transcript profiles of cells exposed to 0.2 or 1 mM As(III), we found similar responses in terms of the identity of the genes whose expression was either up- or downregulated (Supplemental Tables S1 and S2). However, the lower concentration triggered a faster transcriptional response than the higher concentration. For example, the arsenic detoxification genes ACR2 and ACR3 responded earlier at 0.2 mM than at 1 mM As(III). In contrast, the amplitude of gene expression levels was generally larger at the higher concentration (compare Supplemental Tables S1 and S2). During the course of this study, Haugen et al. (17) reported a detailed analysis of the transcriptional response to arsenite. Since our gene expression data reported here largely confirm their results, a full analysis of gene expression changes will not be provided. Instead, we focus on characterizing the response and the control of the sulfur assimilation/GSH biosynthesis pathways in more detail.

As(III) stimulates expression of sulfur assimilation and GSH biosynthesis genes. We noted that As(III) strongly stimulated expression of genes encoding components of the sulfur assimilation and GSH biosynthesis pathways. In fact, mRNA levels of genes encoding basically all the enzymatic steps required for sulfate uptake, its reduction to sulfide, and further conversion into cysteine and GSH were elevated (Fig. 1; Supplemental Tables S1 and S2). In general, expression of these genes was induced $\sim$2- to 5-fold at 0.2 mM As(III), whereas expression of some genes (MET3, MET14, and MET16) was induced up to 15- to 20-fold at 1.0 mM As(III). In addition to the sulfate permease-encoding genes SUL1 and SUL2, expression of the high-affinity S-methylmethionine permease-encoding gene MMP1 as well as MUP1 and MUP3, encoding methionine permeases, was enhanced (Supplemental Tables S1 and S2). CY54 and GSH2 were the only two genes in the pathway whose expression was not stimulated at least twofold by As(III).

Similarly, expression of genes in the methyl cycle (MET6, SAM1, SAM2) was not significantly enhanced by As(III), whereas SAHI expression was reduced (Fig. 1; Supplemental Tables S1 and S2).
Proteome analysis of As(III)-exposed cells. To analyze whether the observed transcriptional changes in response to As(III) would be translated into similar changes at the proteome level, we performed two-dimensional gel analysis and quantified the abundance of selected proteins. Proteome analysis confirmed increased levels of proteins in the sulfur assimilation and GSH biosynthesis pathways (Fig. 1; Supplemental Table S3). In particular, Cys3p levels increased very strongly. On the other hand, Met6p levels were largely unaffected. Proteome analysis also confirmed enhanced levels of several proteins with antioxidant properties including Ahp1p and Sod2p, and the amount of overoxidized Tsa1p increased during As(III) exposure (Supplemental Table S3). Taken together, there appears to be a good correlation between the response of the transcriptome and the proteome in As(III)-treated cells, at least when it comes to the genes/proteins in the sulfur assimilation/GSH biosynthesis pathways. Importantly, the data furthermore suggested that As(III)-exposed cells may channel assimilated sulfur into cysteine and probably GSH biosynthesis.

Kinetics of sulfur metabolites in response to As(III). To address how As(III) affects the metabolites of the GSH biosynthesis pathway, we monitored sulfur metabolite levels in a time course experiment. The pools of homocysteine, cystathionine, cysteine, γ-glutamylcysteine, and GSH started to increase within the first 30 min of exposure and continued to rise over time (Fig. 2A). The strongest effect was observed for γ-glutamylcysteine, which is the precursor of GSH, increasing >10-fold after 3 h. Also, the total GSH content increased considerably (~7-fold after 4 h). In contrast, methionine levels remained largely unchanged, while S-adenosyl-homocysteine increased slightly (2-fold after 4 h). Collectively, these results are consistent with the notion that As(III) stimulates the sulfur pathway and that assimilated sulfur may be redirected toward GSH biosynthesis in As(III)-treated cells. We also noted that the ratio of oxidized to reduced GSH (GSSG/GSH) remained constant throughout the course of this experiment (Fig. 2B and below).

Flux in the sulfur pathway increases in response to As(III). The strong boost of the GSH pool, the end product of the sulfur pathway, suggested that the flux in the pathway may increase in As(III)-challenged cells. To test this, we performed a direct measurement of the GSH synthesis rate before and during As(III) exposure. The method was based on [35S]sulfate label-
incorporated into proteins or GSH. Nos. at top Table 2. Transcription factors with overrepresented DNA binding site in the promoters of up- and sites in the promoters of As(III)-regulated genes. We next

exposed cells channel a large part of assimilated sulfur into proteome in response to As(III). We conclude that As(III)-
decrease of the sulfur amino acid utilization in the global
reflect a decrease of the protein synthesis rate and/or a
reduction of sulfur incorporation into proteins, which may
increase and quantification of newly synthesized GSH and proteins
by counting the radioactivity in GSH and protein fractions
(19). This analysis evidenced a strong increase in GSH syn-
thesis in As(III)-exposed cells. In particular, we observed a
sevenfold raise in GSH synthesis following exposure to 0.2 mM As(III) (Fig. 3). Concurrently, we found a significant
represented compared with the promoters of the entire genome
(Table 2, top). Of those, Yap1p was previously shown to be
regulated by and implicated in the transcriptional response to arsenite (17, 24, 40). Since Yap2p-Yap5p and Yap7p share a
DNA binding site with Yap1p (9), it is no surprise that these
proteins were also identified. In addition, our analysis pin-
pointed Cbf1p, Met31p, and Met32p, which together with
Met4p control expression of sulfur assimilation/GSH biosyn-
thesis-encoding genes. For genes with at least twofold down-
regulated transcripts, we discovered binding motifs for 13 transcription factors to be highly over-
represented compared with the promoters of the entire genome
(Table 2, bottom). Three of these regulate expression of genes encoding ribo-
somal proteins.

Comparative genomics reveals transcription factor binding sites in the promoters of As(III)-regulated genes. We next
sought to identify transcription factors that mediate the tran-
scriptional response to As(III). For this, we analyzed the
promoter sequences of As(III)-regulated genes with the aim of
revealing motifs that are common among groups of genes that
display similar expression patterns. Such analyses can reveal
regulatory elements that may function as transcription factor
binding sites involved in activation/repression of gene expres-
sion. We found 132 regulatory motifs reported in the literature
(and curated by the SGD; http://www.yeastgenome.org) re-
lated to 90 different transcription factors. Using the complete
current knowledge of DNA binding sites for transcriptional
regulators, one can do an unbiased search for proteins that may
control the transcriptional response to As(III). To this end, we
searched for conserved stretches in the promoters of five
related Saccharomyces species (S. mikate, S. kudriavzevii, S.
bayanus, S. kluyveri, and S. castelli) to assess whether a motif
in a given S. cerevisiae promoter is conserved in the corre-
sponding promoters in these related species.

In the promoter sequence of all genes with transcripts that
were found upregulated more than twofold, we discovered
binding motifs of 13 transcription factors to be highly over-
represented compared with the promoters of the entire genome
(Table 2, top). Of those, Yap1p was previously shown to be
regulated by and implicated in the transcriptional response to arsenite (17, 24, 40). Since Yap2p-Yap5p and Yap7p share a
DNA binding site with Yap1p (9), it is no surprise that these
proteins were also identified. In addition, our analysis pin-
pointed Cbf1p, Met31p, and Met32p, which together with
Met4p control expression of sulfur assimilation/GSH biosyn-
thesis-encoding genes. For genes with at least twofold down-
regulated transcripts, we discovered binding motifs for six
transcription factors to be highly overrepresented compared
with the promoters of the entire genome (Table 2, bottom). Three of these regulate expression of genes encoding ribo-
somal proteins.

Expanding this analysis to include combinatorial control, we
found 50 promoters in the entire genome that have both a

![Graph](image.png)

**Fig. 3.** Balance of sulfate utilization in As(III)-exposed cells. Exponentially growing cells in minimal medium containing 1 mM sulfate were divided into three culture aliquots: one untreated culture (control) and two cultures exposed to the indicated As(III) concentration. After 1 h of exposure, cells were labeled by counting the radioactivity in GSH and protein fractions

**Table 2.** Transcription factors with overrepresented DNA binding site in the promoters of up- and downregulated genes in arsenite-challenged cells

<table>
<thead>
<tr>
<th>Transcription Factor</th>
<th>P Value</th>
<th>Target Genes Encode Proteins with Function in . . .</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Transcription factors with overrepresented DNA binding site in the promoters of upregulated genes</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rpn4p</td>
<td>1.94 x 10^{-21}</td>
<td>Proteasome function</td>
</tr>
<tr>
<td>Yap2p (Cad1p)</td>
<td>9.12 x 10^{-21}</td>
<td>Stress response</td>
</tr>
<tr>
<td>Yap7p</td>
<td>2.91 x 10^{-09}</td>
<td>Unknown</td>
</tr>
<tr>
<td>Msn4p</td>
<td>6.36 x 10^{-09}</td>
<td>Environmental stress response</td>
</tr>
<tr>
<td>Msn2p</td>
<td>6.36 x 10^{-09}</td>
<td>Environmental stress response</td>
</tr>
<tr>
<td>Yap3p</td>
<td>6.65 x 10^{-09}</td>
<td>Unknown</td>
</tr>
<tr>
<td>Yap5p</td>
<td>6.65 x 10^{-09}</td>
<td>Unknown</td>
</tr>
<tr>
<td>Yap4p (Cin5p)</td>
<td>6.65 x 10^{-09}</td>
<td>Unknown</td>
</tr>
<tr>
<td>Yap1p</td>
<td>2.14 x 10^{-06}</td>
<td>Resistance to oxidative stress</td>
</tr>
<tr>
<td>Aft1p</td>
<td>2.17 x 10^{-05}</td>
<td>Peroxisomal function, utilization of alternative carbon sources</td>
</tr>
<tr>
<td>Met32p</td>
<td>1.34 x 10^{-04}</td>
<td>Sulfur metabolism</td>
</tr>
<tr>
<td>Met31p</td>
<td>1.34 x 10^{-04}</td>
<td>Sulfur metabolism</td>
</tr>
<tr>
<td>Cbf1p</td>
<td>3.64 x 10^{-03}</td>
<td>Sulfur metabolism</td>
</tr>
<tr>
<td><strong>Transcription factors with overrepresented DNA binding site in the promoters of downregulated genes</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sfp1p</td>
<td>5.92 x 10^{-21}</td>
<td>Ribosomal function</td>
</tr>
<tr>
<td>Rap1p</td>
<td>4.61 x 10^{-17}</td>
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<td>Fhl1p</td>
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</tr>
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<td>Spt23p</td>
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<td>Unknown</td>
</tr>
<tr>
<td>Aft2p</td>
<td>1.33 x 10^{-05}</td>
<td>Iron homeostasis, resistance to oxidative stress</td>
</tr>
<tr>
<td>Hap1p</td>
<td>6.27 x 10^{-04}</td>
<td>Response to cellular heme and oxygen levels</td>
</tr>
</tbody>
</table>
conserved Yap1p binding site and a binding site for one of the Met4p-recruiting factors. Of the 50 genes controlled by these promoters, the transcription of 11 was upregulated (at least 2-fold) in response to As(III). Interestingly, 8 of these 11 promoters regulate genes with products related to sulfur metabolism (MET1, MET2, MET3, MET14, MET16, GSH1, SUL2, and GTO1). Transcription of GSH1 has previously been reported to be controlled by both Met4p and Yap1p in response to cadmium (35). Met4p has been shown to be regulated by Yap1p in response to H2O2 (3) and in response to GSH depletion (35). Transcription of MET4 has been reported to be controlled by both Met4p and Yap1p in response to cellular stress - metabolites (21) and by Met4p in response to cadmium (8). The other three genes (HSV2, ICY2, and BNA3) encode products with no apparent role in sulfur metabolism, although ICY2 and BNA3 are upregulated under sulfur starvation (2).

Yap1p and Met4p control sulfur assimilation/GSH biosynthesis pathway genes in concert. Comparative genomics strongly suggested that Yap1p and Met4p control the sulfur assimilation/GSH biosynthesis pathways in concert. To test this, and to identify gene targets of Yap1p and Met4p under As(III) exposure, we compared global gene expression profiles of yap1Δ and met4Δ mutants to that of the wildtype using microarray analysis.

Seventy-two genes displayed twofold lower expression in yap1Δ compared with wildtype at 0.2 mM As(III), whereas the number of Yap1p-dependent genes was larger at 1 mM As(III) (Supplemental Table S4, A and B). Many of those genes are known Yap1p targets and encode antioxidant defense functions. Importantly, lack of YAP1 also reduced As(III)-stimulated expression of most genes related to sulfur uptake and assimilation at 0.2 mM As(III) (Fig. 4A; Supplemental Table S4A). Curiously, induced expression of these genes appeared to be Yap1p independent at the higher concentration.

We next analyzed the transcriptome of met4Δ and found 70 genes with at least twofold lower expression than in the wildtype at 0.2 mM As(III) (Supplemental Table S5); most of these genes encode functions in sulfur metabolism and functions related to protein biosynthesis (Fig. 4A and Supplemental Table S5). Remarkably, MET4 deletion also resulted in enhanced expression of many genes; these genes appear to be stress responsive, and many of them are actually controlled by Yap1p. Hence, lack of MET4 may result in hyperactivation of Yap1p. In fact, we previously observed the same phenomenon in cells with elevated cellular As(III) levels, e.g., in cells lacking ACR3 (40). Although MET4 deletion does not appear to affect As(III) uptake/efflux systems or to alter intracellular As(III) levels (data not shown), the amount of free As(III) is probably higher in met4Δ, since GSH synthesis is likely to be defective in this mutant. Finally, Northern blot analyses confirmed that MET3, MET14, and MET25 transcripts are elevated in response to 0.2 mM arsenite and that As(III)-stimulated expression of these genes depends on both Yap1p and Met4p (Fig. 4B). However, while YAP1 deletion only affected induction, MET4 deletion affected both basal mRNA levels and As(III)-induced transcription of these genes (Fig. 4B). Taken together, our data demonstrate that Yap1p and Met4p activate gene expression in the sulfur/GSH pathways in concert when cells are exposed to a moderate As(III) concentration, whereas Met4p may play a more prominent role during severe As(III) stress.

Mutations in the sulfur/GSH pathways render cells As(III) sensitive. To assess the physiological importance of the sulfur/GSH pathways for As(III) detoxification/tolerance, we scored growth of mutants lacking pathway components in the presence of arsenite. We found that deletion of MET3, MET14, MET16, MET25, CY3, or CYS4 sensitized cells to As(III), whereas gsh1Δ cells were found hypersensitive (data not shown), confirming the importance of a functional sulfur/GSH pathway for As(III) tolerance. Also, met4Δ cells displayed As(III) sensitivity, although this sensitivity was not as strong as in the presence of cadmium or antimonite [Sb(III)] (Fig. 5). We hypothesized that the Met4p-mediated response of the sulfur/GSH pathways might be masked by the action of Acr3p, which efficiently mediates As(III) efflux. Corresponding cadmium- or Sb(III)-specific efflux systems have not been described. To test this, we scored growth of acr3Δ met4Δ cells in the presence of metals. Indeed, growth tests evidenced a clear additive As(III) sensitivity of the acr3Δ met4Δ double mutant compared with...
systems. The most As(III)-sensitive strains tested by us were those that cells are oxidized in response to As(III). Since GSH is produced in its reduced form, this finding might be expectedly found to remain constant in As(III)-exposed cells. The ratio of oxidized to reduced GSH (GSSG/GSH) was unexpectedly found to remain constant in As(III)-exposed cells. The role of the sulfur/GSH pathways under As(III) exposure. Yeast cells responded to As(III) by stimulating the sulfur assimilation/GSH biosynthesis pathways at both gene and protein levels. Furthermore, a rapid increase of the pools of all the intermediates in the GSH biosynthesis pathway was observed. The metabolites continued to accumulate over time, and the sulfur may eventually be metabolized all the way to GSH. This finding was further underscored by a sulfur flux analysis that evidenced a strong increase in GSH synthesis concomitantly with a significant reduction of sulfur incorporation into proteins. Hence, As(III)-exposed cells channel a large part of the assimilated sulfur into GSH biosynthesis. This response is likely to provide more GSH for metal conjugation, for cellular redox buffering, and possibly also for protein glutathionylation. The physiological importance of the sulfur/GSH pathway for As(III) tolerance is highlighted by the sensitivity of mutants in the pathway.

In mammals, arsenite exposure may lead to increased production of reactive oxygen species (22). Therefore, it is likely that cells launch defense mechanisms that protect the cytosol from oxidation. Indeed, we observed increased expression of several genes encoding antioxidant functions including GSH1 and GLR1 (GSH reductase; Ref. 40 and present study). Similarly, we observed increased levels of proteins with antioxidant properties. However, despite a strong increase in GSH levels, the ratio of oxidized to reduced GSH (GSSG/GSH) was unexpectedly found to remain constant in As(III)-exposed cells. Since GSH is produced in its reduced form, this finding might indicate that a continuously increasing number of GSH molecules are oxidized in response to As(III).

By comparing As(III) sensitivities of deletion mutants, one may get insight into the relative importance of various defense systems. The most As(III)-sensitive strains tested by us were yapΔ and acr3Δ (39, 40), suggesting that the main line of defense is probably the Yap8p/Acr3p-mediated response. Yap1p deletion caused a moderate sensitivity (40), implying that the antioxidative defense and/or the Ycf1p-mediated defense is less critical for tolerance. Here, we showed that met4Δ cells were moderately As(III) sensitive, probably because the Acr3p-mediated defense system is generally sufficient to ensure almost wildtype tolerance. Indeed, when MET4 was deleted in an acr3Δ background, we observed a clear additive effect in terms of As(III) sensitivity. Hence, although the Met4p-mediated defense is important for As(III) tolerance, it is not as critically required as it is for cadmium or antimonite tolerance, possibly because of the lack of specific inducible cadmium or antimonite detoxification systems.

**DISCUSSION**

In this study, we explored the response of *S. cerevisiae* to arsenite. By combining transcriptome, proteome, and metabolite profiling with comparative genomics and physiology, we demonstrate that stimulation of the sulfur assimilation/GSH biosynthesis pathways represents an important step in cellular As(III) tolerance acquisition and that the transcription factors Yap1p and Met4p control this response in concert.

**Role of the sulfur/GSH pathways under As(III) exposure.** Yeast cells responded to As(III) by stimulating the sulfur assimilation/GSH biosynthesis pathways at both gene and protein levels. Furthermore, a rapid increase of the pools of all the intermediates in the GSH biosynthesis pathway was observed. The metabolites continued to accumulate over time, and the sulfur may eventually be metabolized all the way to GSH. This finding was further underscored by a sulfur flux analysis that evidenced a strong increase in GSH synthesis concomitantly with a significant reduction of sulfur incorporation into proteins. Hence, As(III)-exposed cells channel a large part of the assimilated sulfur into GSH biosynthesis. This response is likely to provide more GSH for metal conjugation, for cellular redox buffering, and possibly also for protein glutathionylation. The physiological importance of the sulfur/GSH pathway for As(III) tolerance is highlighted by the sensitivity of mutants in the pathway.

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**Regulation of the sulfur/GSH pathways under As(III) exposure.** By combining transcriptome analyses with comparative genomics, we confirmed previous reports implicating Yap1p in the transcriptional response to As(III) challenge (17, 24, 40). Furthermore, we established a role for Met4p in transcriptional activation of genes in the sulfur/GSH pathways and demonstrated that this protein contributes to As(III) tolerance. Previous studies indicated that Met4p and Yap1p jointly control GSH1 expression; induced GSH1 expression is regulated by Yap1p in response to oxidative stress, whereas GSH1 expression is co-regulated by Yap1p and Met4p during cadmium exposure (5) and GSH depletion (35). Here, we show that this joint control in fact extends to most genes of the sulfur/GSH pathways; deletion of either *YAP1* or *MET4* resulted in reduced gene expression, and promoter analysis confirmed the presence of Yap1p and Met4p DNA binding sites in these promoters. Hence, both transcription factors contribute to As(III)-stimulated expression of sulfur/GSH pathway genes.

During sulfur starvation, transcription of sulfur/GSH pathway genes is regulated in response to changes in the cysteine pool (15, 23). In As(III)-exposed cells, we found an increase in the cysteine pool without a downregulation of Met4p gene targets. Similarly, Met4p activation is also independent of the cysteine pool under cadmium exposure (1, 19, 42). Met4p is regulated by ubiquitination, and the SCF<sup>Met30</sup> (Skp1/Cullin/F-box protein, where Met30p is the F-box protein) ubiquitin ligase complex is responsible for ubiquitination (and hence inactivation) of Met4p in response to adequate cysteine levels (18, 29). Cadmium inhibits Met4p ubiquitination by preventing proper formation of the SCF<sup>Met30</sup> ubiquitin ligase complex (1, 42). Interestingly, the need for Yap1p to fully induce expression of sulfur/GSH genes at low concentrations of As(III), but not at high concentrations, may also be explained by the regulation of Met4p. Ubiquitination and degradation of Met4p are inhibited in response to As(III); however, high concentrations are needed to completely abolish Met4p ubiquitination (42). So when cells are exposed to low arsenite concentration, Met4p may still be partially ubiquitinilated, and full induction of sulfur/GSH genes then requires Yap1p as an additional trans-activating factor.

**Other factors required for As(III) tolerance?** Besides Yap1p and Met4p-requiring factors, comparative genome analysis pinpointed a number of transcription factors that might control the transcriptional response to As(III). These factors include Msn2p, Msn4p, and Rap1p, which have been implicated in the regulation of the so-called “environmental stress response” (11), as well as Rpn4p, which controls As(III)-stimulated...
expression of proteasome genes (17). However, the physiological roles of these transcription factors and their molecular mechanisms of action under As(III) challenge remain to be revealed. Our promoter analysis did not identify Yap8p, despite the fact that this transcription factor is critically involved in mediating arsenic tolerance by activating ACR2 and ACR3 expression (40). The exact DNA binding site of Yap8p is not known, and, hence, Yap8p was not retrieved by this analysis.

A common response to thiol-reactive metals? Yeast cells respond in a similar way to both As(III) and cadmium; expression of genes and enzymes of the sulfur/GSH pathways is strongly induced, GSH synthesis and pathway flux increase, and several pathway mutants display arsenite and cadmium sensitivity (Refs. 8, 19, 25, and 36 and present study). Since both arsenite and cadmium are thiol-reactive metals, the observed stimulation of the sulfur/GSH pathways might be of a general nature in response to this class of metals. Consistent with this notion, metΔ is antimonite sensitive, and Yap1p is involved in the cellular response to antimonite (40). Although yeast cells respond to cadmium and arsenite in a similar way, metal-specific responses also exist. For instance, ACR2 and ACR3 respond only to As(III), while a similar cadmium-induced detoxification system has not been described. In response to cadmium, yeast launch a so-called sulfur sparing program; highly abundant proteins involved in carbohydrate metabolism (e.g., pyruvate decarboxylase and enolase) are replaced by isoenzymes with a low sulfur amino acid content, possibly to permit allocation of more sulfur to GSH production (8). Here, we did not find any clear evidence for a sulfur sparing program in response to arsenite. Whether this response is absent altogether or masked by the action of Acr3p remains to be revealed.

To conclude, this study confirms and extends previous reports on the S. cerevisiae response to arsenite. First, our transcriptional analysis largely corroborates previously reported gene expression data (17). Haugen et al. (17) integrated phenotypic and transcriptional profiling and mapped the data onto metabolic and regulatory networks. By using this approach, they suggested that arsenic-exposed cells channel sulfur into GSH biosynthesis (17). We now demonstrate that this is indeed the case by sulfur metabolite and metabolic flux analyses. Second, our expression data combined with the promoter analysis clearly establish that Met4p and Yap1p act together as transcriptional activators of the sulfur assimilation/GSH biosynthesis pathways in As(III)-challenged cells. Finally, we show that quantitative transcriptome, proteome, and metabolite profiling combined with comparative genomics and physiology provides a powerful means to obtain “systems level” insight into the role and regulation of entire metabolic pathways. Arsenite has a profound impact on the environment and on human health, as both a causative and a curative agent of disease, and a full understanding of global and specific responses may prove of value for use in medical therapy.

ACKNOWLEDGMENTS

We acknowledge L. Kuras (Centre National de la Recherche Scientifique, Gif-sur-Yvette, France) for providing strains, P. Dahl (Gothenburg University, Gothenburg, Sweden) for constructing the acr3Δmet4Δ mutant, and R. Genet (CEA/Saclay) for providing [15N]ammonium sulfate.

GRANTS

This work was supported by grants from the Programme Toxicologie Nucléaire Environnementale, to J. Labarre; the Swedish National Research School in Genomics and Bioinformatics, to O. Nerman and M. J. Tamás; and the Swedish Research Council, to M. J. Tamás.

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