A Proteome Analysis of the Cadmium Response in
Saccharomyces cerevisiae*

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Cadmium is very toxic at low concentrations, but the basis for its toxicity is not clearly understood. We analyzed the proteomic response of yeast cells to acute cadmium stress and identified 54 induced and 43 repressed proteins. A striking result is the strong induction of 9 enzymes of the sulfur amino acid biosynthetic pathway. Accordingly, we observed that glutathione synthesis is strongly increased in response to cadmium treatment. Several proteins with antioxidant properties were also induced. The induction of nine proteins is dependent upon the transactivator Yap1p, consistent with the cadmium hypersensitive phenotype of the YAP1-disrupted strain. Most of these proteins are also overexpressed in a strain overexpressing Yap1p, a result that correlates with the cadmium hyper-resistant phenotype of this strain. Two of these Yap1p-dependent proteins, thioredoxin and thioredoxin reductase, play an important role in cadmium tolerance because strains lacking the cor-
den strains overexpressing Yap1p and Skn7p are yeast transcription factors that regulate the adaptive response to oxidative stress (9–11). Strains lacking either transcription factor are sensitive to H2O2 and are defective in the induction by H2O2 of several enzymes with antioxidant properties (9). Yap1p is also important in cadmium tolerance because yap1-deleted strains are very sensitive to cadmium, and strains overexpressing YAP1 are hyper-resistant to this toxic metal (12). The contribution of Skn7p to the cadmium response is more complex, because skn7-deleted strains are hyper-resistant to cadmium (9), suggesting that Skn7p is not only dispensable for this response but might also repress genes that are important for cadmium tolerance.

Using two-dimensional gel electrophoresis, we have identified several proteins induced by cadmium in Saccharomyces cerevisiae, providing a framework to the mechanisms of Cd2+ toxicity and cellular protection against this toxic metal. In particular, our results highlight the importance of both glutathione and of the thioredoxin/thioredoxin reductase system in the cellular defense against cadmium.

Heavy metals represent major environmental hazards to human health. In particular, cadmium is very toxic and probably carcinogenic at low concentrations. However, the biological effects of this metal and the mechanism of its toxicity are not yet clearly understood. It has been proposed that Cd2+ ions might displace Zn2+ and Fe3+ in proteins (1), resulting in their inactivation and in the release of free iron, which might generate highly reactive hydroxyl radicals (OH·) (2). In support of this hypothesis, a major effect of cadmium is oxidative stress (3), particularly lipid peroxidation (1). However, it is not known whether these effects are responsible for the extreme toxicity of the metal.

Living organisms use several mechanisms to counter cadmium toxicity. In bacteria, efflux pumps are able to export toxic ions outside the cell (4). In higher eukaryotes, Cd2+ is sequestered by metallothioneins through their high cysteine content (5). Cadmium can also be detoxified by chelation to GSH or to phytochelatin, a glutathione polymer of general structure ([Glu-Cys]n-Gly) synthesized from GSH in plants and in the yeast Schizosaccharomyces pombe. Cd2+-phytochelatin and Cd2+-(GSH)2 complexes are transported into the vacuole by ATP-binding cassette transporters (6–8).

Yap1p and Skn7p are yeast transcription factors that regulate the adaptive response to oxidative stress (9–11). Strains lacking either transcription factor are sensitive to H2O2 and are defective in the induction by H2O2 of several enzymes with antioxidant properties (9). Yap1p is also important in cadmium tolerance because yap1-deleted strains are very sensitive to cadmium, and strains overexpressing YAP1 are hyper-resistant to this toxic metal (12). The contribution of Skn7p to the cadmium response is more complex, because skn7-deleted strains are hyper-resistant to cadmium (9), suggesting that Skn7p is not only dispensable for this response but might also repress genes that are important for cadmium tolerance.

Using two-dimensional gel electrophoresis, we have identified several proteins induced by cadmium in Saccharomyces cerevisiae, providing a framework to the mechanisms of Cd2+ toxicity and cellular protection against this toxic metal. In particular, our results highlight the importance of both glutathione and of the thioredoxin/thioredoxin reductase system in the cellular defense against cadmium.

MATERIALS AND METHODS

Strains and Growth Conditions—Studies were performed with the wild-type strain YPH98 (13) [MATa ura3–52 lys2–801 amber ade 2–101(ade2–1) trp1Δ1 leu2–Δ1]. All disruptants and strains used in this study were isogenic derivatives of YPH98. The strain overexpressing YAP1 and the mutant skn7Δ:TRP1 were previously described (9). Strains trp1Δ:TRP1, trx1Δ:LEU2, trx1Δ:TRP1-trx2Δ:LEU2 and trx1Δ:TRP1-trx2Δ:LEU2 were previously described (14). Strains yap1Δ:TRP1, gre2Δ:TRP1, ahp1Δ:TRP1 and gpx3Δ:TRP1 were constructed in this work. The strain gsh1Δpro2–2 has been described. The strain overexpressing YAP1 (mcYAP1) was obtained by transformation of strain YPH98 by a 2-μm plasmid pRS425 carrying a 2.5-kilobase EcoRI genomic YAP1 fragment. Cells were grown in liquid medium containing 0.67% yeast nitrogen base without amino acids (YNB, Difco), 2% glucose supplemented with uracil, adenine, lysine, tryptophan, and leucine at a final concentration of 30 mg/liter. The YAP1-overexpressing strain was grown in the same medium but without leucine.

Survival Assays—Aliquots from an exponential growth culture (A600, 0.5) were serially diluted in water (10-fold at each step). Ten μl were spotted onto rich broth (YPD) plates containing different cadmium sulfate concentrations: 0, 25, 50, 75, 100, 150 and 200 μM. Plates were incubated at 30 °C for 2 to 5 days, and the colony-forming units were counted.

35S Labeling—Ten-μl cultures were inoculated with a colony and cultivated overnight aerobically at 30 °C with shaking. Two-μl aliquots of mid-log phase culture (A600, 0.4) were withdrawn and treated with 0, 0.5, 1, or 1.5 mM cadmium sulfate. 15 or 45 min after cadmium addition, cells were labeled with [35S]methionine (200 μCi) for 15 min for analysis of protein expression or for 40 min for analysis of glutathione synthesis rate.

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35S Labeling—Ten-μl cultures were inoculated with a colony and cultivated overnight aerobically at 30 °C with shaking. Two-μl aliquots of mid-log phase culture (A600, 0.4) were withdrawe...
transcriptase-PCR products were used as internal standards. ACT1 was performed essentially as previously described (19).

The Yeast Genomic Response to Cadmium—We analyzed the proteomic response to cadmium to identify activities, which could be potentially important for the resistance to this toxic compound. Exponentially growing cells were untreated or treated with 1 mM cadmium for 60 min, pulse-labeled with [35S]methionine, and analyzed by comparative two-dimensional gel electrophoresis (Fig. 1). The changes in spot intensity between untreated and treated cells were quantified by phosphorimager and software analysis (see “Materials and Methods”). More than 50 proteins were induced by a factor greater than 2 after cadmium treatment (Table I). Concomitantly, about 40 other proteins were significantly repressed. A lower dose of cadmium (0.5 mM) or shorter period of treatment (15 min) gave a similar pattern of protein expression, but with lower induction levels. Proteins induced by cadmium included enzymes with antioxidant properties, heat shock proteins, proteases, enzymes of the sulfur amino acid biosynthesis pathway, carbohydrate metabolism enzymes, and other unclassified proteins or with unknown function. Conversely, proteins repressed by cadmium were mainly components of the translational apparatus and metabolic enzymes.

Induction of Glutathione Synthesis—The particularly high induction level of enzymes of the sulfur amino acid pathway (Fig. 2) suggested an increased synthesis of cysteine and perhaps of GSH, which is essential in the cellular detoxification of cadmium. To test this hypothesis, we directly measured GSH synthesis rate by TLC after [35S]methionine pulse labeling. After cadmium treatment, the synthesis rate of GSH was increased more than 4-fold (Fig. 3A, lanes 1 and 2). As expected, in extracts from cells lacking GSH1 (encoding the rate-limiting enzyme of GSH biosynthesis), no GSH was produced (Fig. 3A, lanes 3 and 4). The production of another [35S]-labeled compound was also augmented in response to cadmium treatment, but we could not identify this molecule.

The stimulation of GSH biosynthesis correlated with a more than 10-fold increase of GSH1 mRNA level after 1 h of cadmium treatment (not shown and Ref. 24). As metallothioneins are cysteine-rich and potentially important for cadmium detoxification, we also analyzed CUP1 mRNA levels and found no increased expression of this gene in response to cadmium, but rather a slight repression. These results strongly suggest that the cysteine biosynthesis pathway was induced to allow enhanced glutathione synthesis.

Cadmium Response Is Altered in Regulatory Mutants—Strains lacking the transcriptional activator Yap1p are very sensitive to cadmium and conversely, strains overexpressing this regulator are hyper-resistant (12). However, strains lacking the transcriptional activator Skn7p are hyper-resistant to...
this toxic metal (9). We therefore analyzed the cadmium protoco-

tomic response in strains lacking either YAP1 (yapΔ) or SKN7 (skn7Δ) or overexpressing TAP1 (mcYAP1) (Fig. 4). The

comparative measure of the proteomic response between these

mutants and the wild-type cells is given in Table I. The yapΔ strain had a weaker induction of 9 of the 57 responsive pro-
noteins, by a factor of 2 or more. These included four antioxidant
defense proteins (the superoxide dismutase Sod2p, the alkyl
dydroperoxidase Ahp1p, the thioredoxin Trx2p, and the thioc-

teroxidase Tsa1p) and five other proteins (Sam1p, Oye3p,

Gre2p, YNL134C, and YNL274C). Except for Sam1p, all these

proteins were significantly overexpressed in response to cad-
mium in mcYAP1. Additionally, most of the heat shock proteins

and proteasome subunits were also overexpressed in mcYAP1.

In contrast, only two proteins (Cdc48p and Shm2p)

were not properly induced by cadmium. However, surprisingly,

\[\text{Table I} \]

| Protein class | Gene name | Stimulation index$^a$ | Protein function |
|---------------|-----------|-----------------------|-----------------|
| Enzymes with antioxidant properties | AHP1 | 3.0 | alkyl hydroperoxide reductase |
| | CCP1 | 2.6 | cytochrome c peroxidase |
| | CTT1 | 1.0 | catalase T |
| | TRR1$^b$ | 0.9 | thioredoxin reductase |
| | TRX2 | 3.3 | thioredoxin |
| | TSA1 | 3.1 | thioperoxidase |
| | PRX1 | ≥5 | thioperoxidase, homolog to TSA1 |
| | SOD1$^c$ | 0.7 | Cu/Zn superoxide dismutase |
| | SOD2 | 3.8 | Mn superoxide dismutase |
| Heat shock proteins and chaperones | HSP12 | 2.0 | heat shock protein |
| | HSP26$^d$ | >3.0 | heat shock protein |
| | HSP42 | >10 | heat shock protein |
| | HSP82 | 5.1 | heat shock protein |
| | HSP104 | >10 | heat shock protein |
| | KAR2 | >20 | heat shock protein |
| | SSA1 | 4.8 | heat shock protein |
| | SSA2 | 6.9 | heat shock protein |
| | SSA4 | >10 | heat shock protein |
| | STI1 | 5.1 | heat shock protein |
| Proteases | HSP78 | 6.8 | mitochondrial protease |
| | PRE1 | 3.1 | proteasome subunit |
| | PUP2 | 5.4 | proteasome subunit |
| | MPRI | 3.8 | proteasome subunit |
| | UBA1 | 5.4 | ubiquitin activating enzyme |
| | YTA1$^e$ | 3.0 | proteasome subunit |
| Sulfur amino acid and glutathione biosynthesis enzymes | CYT3 | 14.3 | cystathionine-gamma-lyase |
| | CYT4 | 2.2 | cystathionine-beta-synthase |
| | GSH1$^f$ | >10 | gamma glutamyl cysteine synthase |
| | MET16 | 5.2 | PAPS reductase |
| | MET25 | 3.5 | homocysteine synthase |
| | MET3 | 6.3 | ATP sulfurylase |
| | ECM17 | 2.5 | sulfite reductase (beta subunit) |
| | SAM1 | 4.4 | S-adenosylmethionine synthetase |
| | SAM2 | 2.1 | S-adenosylmethionine synthetase |
| | SS4 | 2.6 | cytochrome c peroxidase |
| | YNL134C | 3.1 | unknown function |
| | YNL274C | 3.5 | similar to alpha-ketoisocaproate reductase |
| | YOR007C | 3.0 | similar to Tfs1p |
| | YLR179C | 3.0 | homolog to 14–3-3 protein |
| | YM1 | 2.0 | transcriptional regulator |
| | RNR4 | 5.6 | ribonucleotide reductase |
| | OYE3 | 6.1 | NADPH-dehydrogenase |
| | GRE2 | 1.6 | similar to dihydroflavonol-4-reductase |
| | CDC48 | 2.3 | ATPases family |
| | IPP1 | 1.8 | inorganic pyrophosphatase |
| Unclassified proteins | BHY2 | 1.8 | homolog to 14–3-3 protein |
| | CDC48 | 2.3 | ATPases family |
| | DDR48 | 7.0 | DNA damage response protein |
| | GRE2 | 1.6 | similar to dihydroflavonol-4-reductase |
| | OYE3 | 6.1 | NADPH-dehydrogenase |
| | RNR4 | 5.6 | ribonucleotide reductase |
| | WTM1 | 2.0 | transcriptional regulator |
| | YST2 | 4.1 | ribosomal protein |
| | YNL134C | 3.1 | unknown function |
| | YOR007C | 2.3 | unknown function |

$^a$ The stimulation index is the ratio of protein expression in cadmium versus standard conditions.

$^b$ Results for Ctt1p, Sod1p, and Trr1p were reported though these enzymes were not induced by cadmium.

$^c$ Proteins identified in this work by mass spectrometry.

$^d$ Hsp26p does not contain methionine or cysteine residues, and the induction was measured at the mRNA level by RT-PCR analysis.

$^e$ Gsh1p has not been identified on two-dimensional gels, and the induction was measured at the mRNA level by RT-PCR analysis.

$^f$ Sam1p, Oye3p, Gre2p, YNL134C, and YNL274C. Except for Sam1p, all these proteins were significantly overexpressed in response to cadmium in mcYAP1. Additionally, most of the heat shock proteins and proteasome subunits were also overexpressed in mcYAP1 (Table I). In skn7Δ, only two proteins (Cdc48p and Shm2p) were not properly induced by cadmium. However, surprisingly,
Cadmium Response in Yeast

**DISCUSSION**

To gain insight into the biological effects of Cd\(^{2+}\) and to identify activities relevant to its detoxification, we have analyzed the proteomic response to this toxic metal. Fifty-seven proteins were found to be induced by Cd\(^{2+}\), including enzymes of the cysteine and glutathione biosynthesis pathway and proteins with antioxidant properties. In addition, the induction of several of these proteins is controlled by the transcription factor Yap1p.

Cadmium Stimulates the Biosynthesis of Cysteine and Glutathione—The proteomic response to cadmium has revealed a strong induction of eight enzymes of the sulfur amino acid and GSH biosynthesis pathways, particularly the last enzyme of the cysteine biosynthesis pathway, Cys3p, and the rate-limiting enzyme of glutathione biosynthesis, GSH1. This increased gene expression was correlated with a strong stimulation of GSH biosynthesis.

These results are consistent with the notion that GSH acts as a first line of defense against cadmium toxicity by chelation and sequestration of the toxic metal. In yeast, cadmium is sequestered in the vacuole upon transport of Cd\(^{2+}\)(GSH)\(_2\) complexes by the membrane ATP-binding cassette Ycf1 (6). The importance of this detoxification system is demonstrated by the hypersensitive phenotype resulting from the deletion of either YCF1 (26) or GSH1 (27). The cysteine biosynthetic pathway is also important as demonstrated by the cadmium hypersensitive phenotype of strains deleted for CYS4 (data not shown). This pathway is probably essential for the increased production of GSH under these stress conditions.

The coordinated regulation of GSH1 expression with enzymes of the sulfur amino acids pathway may suggest that a common transcriptional mechanism is involved in these inductions. Consistent with this idea, a recent work (28) has shown that the GSH1 induction by cadmium is dependent upon the transcription factors Met4p, Met31p, Met32p, and Chb1p, which belong to the transcriptional complex of MET genes. It was also found that the GSH1 promoter contains functional elements typical of MET genes (28).

Interestingly, the sulfur amino acid pathway is not induced but rather repressed under most of the other stress conditions examined, including the oxidative stress (19), the osmotic stress (29), and the heat shock response (30). Furthermore, it is remarkable that among the enzymes involved in amino acid metabolism, only those of the sulfur amino acid biosynthetic pathway are induced, which is consistent with a very specific control mechanism.

**Does Cadmium Cause Oxidative Stress?**—Cd\(^{2+}\) is not a redox active metal ion. However, it could cause oxidative stress and
lipid peroxidation, perhaps by displacing protein-bound Fe$^{2+}$, allowing this ion to become available for the Fenton reaction. This hypothesis is based on previous studies showing an increase in lipid peroxidation products after exposure to Cd$^{2+}$ (1, 31–34). In addition, yeast strains deleted for cytosolic copper and zinc and mitochondrial manganese superoxide dismutases (SOD1 and SOD2) are hypersensitive to cadmium (3). The observation that several antioxidants such as Ahp1p, Ccp1p, Tsa1p, and Sod2p are induced by cadmium is consistent with this hypothesis. We also found that strains deleted for AHP1 or GPX3, which encode two main organic peroxide-scavenging activities, are not sensitive to cadmium, which does not support the idea that cadmium toxicity is related to the cellular generation of lipid peroxidation products. However, we cannot rigorously rule out the possibility that yeast tolerance to cadmium-generated lipid peroxidation products involves specific activities other than Ahp1p or Gpx3p, which have not yet been elucidated.

It is also possible that cadmium indirectly contributes to oxidative stress by affecting the cellular thiol redox balance. We indeed found that Trx2p is significantly induced by cadmium and that strains deleted for both TRX1 and TRX2 or for TRR1 are hypersensitive to this metal. In support of such a mechanism, a recent work has shown that cadmium inactivates thioredoxins, thioredoxin reductases, and glutathione reductases (35). Therefore, given the essential nature of these systems (36), thiol transferase inactivation could be the primary deleterious effect of cadmium. In addition, the inactivation of the thiol transferase pathway could also result in an increase in cellular lipid peroxidation products as a consequence of their unavailability of the main electron donors for the thiol and GSH peroxidases.

Yap1p Target Genes—The observation that YAP1-deleted strains were hypersensitive to cadmium (9, 12) and strains overexpressing YAP1 were hyper-resistant (27), suggested that this transcriptional activator might control genes important for
cadmium tolerance. YCF1 and GSH1 are two of them (27, 8). Given the importance of the Ycf1p-GSH detoxification system, these two genes are probably the primary targets by which Yap1p exerts a control of cadmium tolerance. In accord with this notion, we found that cadmium-induced GSH synthesis is controlled by Yap1p. We also discovered that nine other genes required the presence of Yap1p for their proper induction by cadmium. These mostly include antioxidant defense genes also induced by H$_2$O$_2$ in a Yap1p-dependent manner (9). Several other proteins of the Yap1p H$_2$O$_2$-inducible regulon (Ccp1p, Ssa1p, Hsp78p, Hsp82p, Mpr1p, Uba1p, Cys3p, Tal1p, Zwf1p, Dak1p, and Tps1p) were still induced by Cd$^{2+}$ in yap1Δ (see Table I), suggesting the existence of other control pathways for the cadmium response. This pathway does not involve the Yap1p homologous factor Yap2p, because a strain deleted for yap1 and yap2 had the same proteomic response to cadmium than the yap1Δ strain (not shown).

Interestingly, most of the cadmium-inducible Yap1p-dependent proteins were also overexpressed in a YAP1 multicopy strain after cadmium treatment. This YAP1 gene dosage effect, which was not observed in the H$_2$O$_2$ response, strongly correlate well with the hyper-resistance of the YAP1-overexpressing strain toward Cd$^{2+}$ but not toward H$_2$O$_2$.

**Skn7p Acts As a Repressor in Cadmium Response**—Skn7p is an important regulator of the H$_2$O$_2$ response that cooperates with Yap1p to activate the expression of several genes in response to H$_2$O$_2$ (9). However, although Yap1p is also important for the cadmium response, the function of Skn7p in this response is more complex because skn7Δ is hyper-resistant to this metal (9). This resistance phenotype could not be explained by a defective Cd$^{2+}$ intracellular transport or alternatively, by the overproduction of metallothioneins (data not shown) or glutathione (this work). However, this could be explained by the observation that several proteins of the cadmium stimulus were superinduced in the skn7Δ mutant, supporting the idea that Skn7p may act to repress these genes upon cadmium treatment. This was particularly striking for heat shock proteins and for some Yap1p-dependent proteins of unknown function (YNL134C, Gre2p, or Oye3p). Interestingly, the YAP1 multicopy strain overexpresses nearly the same set of proteins, suggesting a correlation between the cadmium hyper-resistance phenotype and the overexpression of these proteins. Some of these proteins, yet to be studied, might be important for cadmium resistance.

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3. Godon, J. Lee, J. Labarre, and M. Toledano, unpublished results.

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