The \( \text{H}_2\text{O}_2 \) Stimulon in \textit{Saccharomyces cerevisiae}

(Received for publication, January 30, 1998, and in revised form, June 12, 1998)

Christia\-n Godon†, Gilles Lagniel†, Jaekwon Lee§, Jean-Marie Buhler‡, Sylvie Kieffer³, Michel Perrot², Hélia\-n Boucherie, Michel B. Toledano², and Jean Labarde*²

From the *Service de Biochimie et Génétique Moléculaire, CEA-Saclay, F-91191 Gif-sur-Yvette Cedex, France, ‡Department of Pharmacology and Toxicology, College of Pharmacy, Rutgers University, Piscataway, New Jersey 08855, ¶Institut de Biochimie et de Génétique Cellulaires, UPR CNRS 9026, 33077 Bordeaux Cedex, France, and §Département de Biologie Moléculaire et Structurelle, CEA-Grenoble, F-38054 Cedex 9, France

The changes in gene expression underlying the yeast adaptive stress response to \( \text{H}_2\text{O}_2 \) were analyzed by comparative two-dimensional gel electrophoresis of total cell proteins. The synthesis of at least 115 proteins is stimulated by \( \text{H}_2\text{O}_2 \), whereas 52 other proteins are repressed by this treatment. We have identified 71 of the stimulated and 44 of the repressed targets. The kinetics and dose-response parameters of the \( \text{H}_2\text{O}_2 \) genomic response were also analyzed. Identification of these proteins and their mapping into specific cellular processes give a distinct picture of the way in which yeast cells adapt to oxidative stress. As expected, \( \text{H}_2\text{O}_2 \)-responsive targets include an important number of heat shock proteins and proteins with reactive oxygen intermediate scavenging activities. Exposure to \( \text{H}_2\text{O}_2 \) also results in a slowdown of protein biosynthetic processes and a stimulation of proteolytic pathways. Finally, the most remarkable result inferred from this study is the resetting of carbohydrate metabolism minutes after the exposure to \( \text{H}_2\text{O}_2 \). Carbohydrate fluxes are redirected to the regeneration of NADPH at the expense of glycolysis. This study represents the first genome-wide characterization of a \( \text{H}_2\text{O}_2 \)-inducible stimulon in a eukaryote.

Aerobic organisms have to maintain a reduced cellular redox environment in the face of the prooxidative conditions characteristic of aerobic life. The incomplete reduction of oxygen to water during respiration leads to the formation of redox-active oxygen intermediates (ROI), such as the superoxide anion radical \( \text{O}_2^- \), hydrogen peroxide \( \text{H}_2\text{O}_2 \), and the hydroxyl radical \( \text{OH}^- \) (for review, see Refs. 1–3). ROI are also produced during fatty acid oxidation, and upon exposure to radiation, light, metals, and redox-active drugs. Oxidative stress results from abnormally high levels of ROI which perturb the cell redox status and leads to damage to lipids, proteins, DNA, and eventually cell death. Living organisms constantly sense and adapt to such redox perturbations by the induction of batteries of genes or stimulons whose products act to maintain the cellular redox environment (4). In \textit{Escherichia coli}, two distinct stimulons exist, one for \( \text{H}_2\text{O}_2 \) and the other for \( \text{O}_2^- \), consisting of a set of 30–40 proteins (for review, see Refs. 2, 5, and 6). The genes encoding nine of the \( \text{H}_2\text{O}_2 \)-inducible proteins are controlled by the transcriptional regulator \text{OxyR} and include \text{katG} (catalase), \text{ahpCF} (an alkyl hydroperoxide reductase), \text{gorA} (glutathione reductase), and \text{aps} (a nonspecific DNA binding protein). The genes encoding nine of the \( \text{O}_2^- \)-inducible proteins are controlled by the SoxRS transcriptional regulators and include \text{sodA} (manganese superoxide dismutase), \text{zuf1} (glucose-6-phosphate dehydrogenase), \text{nfo} (DNA repair exonuclease IV), \text{fumC} (fumarase C), and \text{micR} (an antisense RNA regulator). Most of the remaining proteins of these stimulons are unknown, but their identification would increase our understanding of the mechanisms of cellular redox control and ROI metabolism. The yeast \textit{Saccharomyces cerevisiae} can also adapt to both \( \text{H}_2\text{O}_2 \)- and \( \text{O}_2^- \)-generating drugs (7, 8) by the induction of two distinct but overlapping stimulons for \( \text{H}_2\text{O}_2 \) and \( \text{O}_2^- \) (9, 10). Yeast has the same defense mechanisms as higher eukaryotes (for review, see Refs. 11 and 12) and offers the power of genome-wide experimental approaches owing to the availability of the complete sequence of its genome. It therefore represents an ideal eukaryotic model in which to study the cellular redox control and ROI metabolism.

We recently established a general method to identify yeast proteins based on two-dimensional gel electrophoresis (13). We used this genome-wide experimental approach to characterize proteins whose expression is altered upon exposure to low doses of \( \text{H}_2\text{O}_2 \). Such an oxidative stress challenge results in a dramatic genomic response involving at least 167 proteins. Identification of these proteins and their mapping into cellular processes give a global view of the ubiquitous cellular changes elicited by \( \text{H}_2\text{O}_2 \) and provides the framework for understanding the mechanisms of cellular redox homeostasis and \( \text{H}_2\text{O}_2 \) metabolism.

MATERIALS AND METHODS

Strains and Growth Conditions—The yeast strain YPH98 (14) (MATa ura3-32 lys2-801 amber ade2-101 ochre trp1 leu2-3,112) was used for the analysis of the \( \text{H}_2\text{O}_2 \) response. The strain S288C (15) was used for protein spot identification. Strains were grown at 30 °C in a medium containing 0.67% yeast nitrogen base without amino acids (Difco), 2% glucose, buffered to pH 5.8 with 1% succinate and 0.6% NaOH. For YPH98, uracil, adenine, lysine, tryptophan and leucine (30 mg/liter) were added to the culture medium.

Identification of Protein Spots on Two-dimensional Gels—All the 32 new protein identifications were performed in the S288C strain background. The data, following the publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked “advertisement” in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

‡ To whom correspondence should be addressed: Service de Biochimie et Génétique Moléculaire, Bât 142, CEA-Saclay, F-91191 Gif-sur-Yvette Cedex, France. Tel.: 33-1-69-08-22-31; Fax: 33-1-69-08-47-12; E-mail: labarre@jonas.saclay.cea.fr.

* This work was supported in part by the Groupement de Recherche et d’Études sur les Génomes (GREG) (to J. L. and H. B.) and by the New Jersey Commission for Cancer Research (to M. B. T.). The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked “advertisement” in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

† The abbreviations used are: ROI, redox-active oxygen intermediate; RT, reverse transcription; PCR, polymerase chain reaction.
containing 2700 protein sequences of codon bias index $>0.1$ with the help of a specific algorithm program. Eight different double labelings were performed here: $[^{35}S]$Met/$[^{3}H]$Leu, $[^{35}S]$Met/$[^{3}H]$Lys, $[^{35}S]$Met/$[^{3}H]$Phe, $[^{35}S]$Met/$[^{3}H]$Tyr, $[^{35}S]$Met/$[^{3}H]$Trp, $[^{35}S]$Met/$[^{3}H]$His, $[^{35}S]$Cys/$[^{3}H]$Leu and $[^{35}S]$Cys/$[^{3}H]$His. We analyzed 280 spots out of which 124 already identified spots were used as internal standard for the establishment of calibration curves. Six of these 124 proteins devoid of Cys (Sac1p, Pgi1p, Hsp12p, Hsp82p, Hsc82p, and Cys3p) were used to estimate the metabolic interconversion of Cys to Met which is about 15%. Reciprocally, two proteins devoid of Met (Tpi1p and Tsa1p) were used to estimate the interconversion from Met to Cys which is about 33%.

We identified spots were used as internal standards for the establishment of calibration standard for each spot by adding an equal aliquot of $[^{3}H]$-labeled cells to the $[^{35}S]$-labeled cultures. This allows one to express any change in the rate of protein synthesis as the ratio between the $[^{35}S]/[^{3}H]$ ratios of corresponding spots from two different gels and hence to correct for any variation not related to the H$_2$O$_2$ treatment (see “Materials and Methods”). Preliminary experiments comparing two-dimensional gels from two identical cultures showed that the variations observed in the protein quantitation were below a factor of 1.3 for 96% of the 400 control spots analyzed and never exceeded 1.5 (Fig. 2). Therefore, differences by a factor greater than 1.5 between treated and control cultures were considered to be significant.

Accordingly, 115 proteins were specifically induced by H$_2$O$_2$ with a stimulation index ranging from 1.5 to 20 (see Table 1). Conversely, 52 other proteins were repressed with a repression index ranging from 0.65 to 0.15 (Table 1). Almost identical results were observed with two related yeast strains S288C and YPH89.

Proteins Induced by H$_2$O$_2$—The identity of 71 of the 115 proteins induced by H$_2$O$_2$ is given in Table 1 along with their stimulation index. 39 of them were previously identified on two-dimensional maps (for review, see Ref. 18). The 32 other spots were identified in this work by amino acid analysis as described by Maillet et al. (13) or by mass spectrometry (16).

H$_2$O$_2$-responsive proteins were sorted into seven different functional classes (see Table 1). (i) Proteins directly related to the cellular antioxidant defense: This class shows a high stimulation index which ranges from 3 to 20 depending on the protein. It comprises the major oxidant scavenging enzymes cytochrome c peroxidase (Ccp1p), cytosolic catalase (Ct1p), Cu/Zn and Mn superoxide dismutases (Sod1p and Sod2p), thioperoxidase (Tsa1p), thioredoxin (Trx1p or Trx2p), NADPH-dependent thioredoxin reductase (Trr1p), and glutathione reductase (Glr1p). Four newly identified proteins were included in this functional class in the basis of their homology to known oxidant scavenging enzymes and/or predicted antioxidant defence properties. YDR453Cp is one of the two other Ahp/Ct/Tsa family members identified in the yeast genome. YCI035Cp is 86% similar and 68% identical to TTR1-encoded glutaredoxin (thioltransferase). YLR109Wp and YOL151Wp were classified here on the basis of their role in the tolerance to tert-butyldihydroperoxide and diamide, respectively (see “Discussion”). (ii) Heat shock proteins:
This class also shows a strong stimulation index for several of its members. (iii) Proteases and proteasome subunits: The stimulation index is significant but not as high as in the two previous classes. (iv) Translation apparatus components: This class contains only two H$_2$O$_2$-inducible proteins but several H$_2$O$_2$-repressed targets (see below). (v) Carbohydrate metabolism enzymes. (vi) Enzymes involved in amino acid metabolism. (vii) Unclassified proteins and open reading frames of unknown
function: These include two putative regulators, Cdc37p and Mpr1p. MPR1 is the S. cerevisiae homologue of Schizosaccharomyces pombe Pad1+, a global positive regulator of transcription implicated in chromatin structure and identified as a positive regulator of Pap1p, the S. pombe homologue of Yap1p (18). Cdc37p has been shown to be a chaperone acting with Hsp90p and other chaperones to promote the folding/activity of a series of kinases (19).

Proteins Repressed by H2O2—44 out of these 52 proteins had been previously identified on two-dimensional maps (17). Each was assigned a repression factor and sorted into functional classes (Table I). Most of these proteins are translational apparatus components and metabolic enzymes. They include the translation initiation factors eIF4A (Tif1p) and eIF5A (Tif51Ap) and the translation elongation factors EF1-β (Efb1p), EF-2 (Eft1p), and EF-3 (Yef3p), which are dramatically repressed. Rpa0p, Rpa2p, Rpa4p, and Rps5p are acidic ribosomal proteins that act both at the initiation and the elongation steps (20). Ssb1p and Ssb2p are heat shock proteins of the 70-kDa superfamily that are ribosomal-associated and have a role in the folding of nascent polypeptides emanating from the ribosome (21, 22). Metabolic enzymes repressed by H2O2 include enzymes involved in glycolysis, the Krebs cycle, purine and amino acid biosynthesis, sulfur metabolism, S-adenosylmethionine, and polyamine biosynthesis. Ilv2p(a) (acetolactate synthase) appears repressed by a factor of 0.35. Interestingly, we could identify a H2O2-responsive spot of pi 6.7 and Mr 67,000 with a very good match with Ilv2p with regard to its amino acid composition, and therefore it is indicated as Ilv2p(b) in Fig. 1 (13). It may represent an Ilv2p precursor form. The apparent Ilv2p repression seen here may thus be related to the decreased maturation/mitochondrial translocation of its precursor. The remaining eight H2O2-repressed targets have not yet been identified.

Kinetics and Dose-Response Profiles of the H2O2 Response—The kinetics of the genomic response to H2O2 was analyzed for 36 H2O2-responsive targets by pulse-labeling cells for 2 min at various times after the H2O2 treatment. Two-dimensional gel electrophoresis was then performed to determine the relative rate of synthesis of several proteins. Except for a few targets, the H2O2 response was very rapid and transient. We could define three kinetic classes (Fig. 3A). Proteins of class A responded as early as 2 min after induction with a peak at approximately 15 min and a complete return to the baseline after 1 h. Proteins of class B had a very similar kinetic profile but initiated their response with a lag period of at least 4 min. Class c proteins had a somewhat different kinetic profile with a relatively delayed response and a peak at 45 min or even at 1 h for Uba1p. Repression by H2O2 was similarly transient with a nadir at approximately 15 min after stress imposition.

We also tested the H2O2-dose-genomic response profile (Fig. 4). Synthesis rates were determined 15 min after exposure to 0.2, 0.4, or 0.8 mM H2O2 for 61 H2O2-responsive targets. Trr1p and several other proteins were equally induced by each of the three H2O2 doses tested (Fig. 4). Interestingly, Ccp1p and several other proteins were maximally induced by 0.2 mM H2O2. In contrast, most of the heat shock proteins exhibited their maximal response at 0.8 mM. These kinetics and dose-response differences may be related to distinct regulatory mechanisms.

Alterations in mRNA Levels and Protein Synthesis Rates Are Parallel after H2O2 Treatment—The alterations observed in the expression of target proteins in response to H2O2 are likely to include a transcriptional component. We therefore evaluated the mRNA levels of eight selected H2O2-responsive targets. Total poly(A) transcripts were purified from control untreated cells and from cells treated with 0.4 mM H2O2 for 15 or 60 min and quantified by PCR. The results for TRR1, CCP1, YDR453C, YNL134C, and YOL151W are shown in Fig. 5. Levels of all the transcripts analyzed were increased by 5–37-fold at 15 min after H2O2 exposure and had returned close to their basal levels at 60 min. We also analyzed by Northern blot the kinetic profile of the TRR1 message levels at several points after H2O2 treatment (Fig. 3B). The kinetics of the message levels and protein synthesis rates are strikingly parallel after H2O2 treatment. These data corroborate those obtained from the two-dimensional gel analysis and strongly suggest that the dramatic genomic response to H2O2 involves, at least in part, a transcriptional control.

DISCUSSION

ROI are obligate by-products of aerobic life which can inflict structural damage to a wide variety of cell components, thus leading to oxidative stress and cell death. Stress-inducible defense or adaptive response mechanisms act to protect cells from these oxidative threats (2, 4, 5). For instance, the exposure of bacteria or yeast to low levels of H2O2 or O2·−-generating drugs switches on within minutes a resistance to toxic doses of these oxidants. These adaptive stress responses are produced by the induction of distinct batteries of genes or stimulons. However, the genes which constitute these stimuli are, for the most part, not yet identified. We have attempted here a systematic identification of the gene products of the S. cerevisiae H2O2 stimulon. An intense gene activity occurs within minutes of exposure to H2O2, resulting in a transient alteration in the synthesis of at least 150 proteins. The identification of 71 stimulated and 44 repressed proteins and their assignment to specific cellular processes has given a distinct picture of the way in which yeast cells adapt to oxidative stress. The cellular functions that are primarily affected by these changes are antioxidant defenses, heat shock and chaperone proteins, translational apparatus, proteases, and carbohydrate metabolism.

Antioxidant Defense Activities—As expected, several primary antioxidants were induced by H2O2. They include cyto-
| Gene name | Repression$^a$ | Induction$^b$ | Protein function |
|-----------|---------------|---------------|------------------|
| I. Proteins with antioxidant scavenging/defense properties |
| CCP1      | 6             |               | Cytochrome c peroxidase |
| CTT1      | 14.7          |               | Catalase $^T$ |
| GLR1      | 2.1           |               | Glutathione reductase$^{d}$ |
| SOD1      | 4.3           |               | Cu/Zn superoxide dismutase |
| SOD2      | 5.9           |               | Mn superoxide dismutase |
| TRX1      | 12.2          |               | Thioredoxin reductase/NADPH dependent$^c$ |
| TRX (1/2) | 11.5          |               | Thioredoxin 1 or 2$^c$ |
| TSA1      | 5.9           |               | Thiol-specific antioxidant protein/thioperoxiode |
| YCL035C   |               | 5             | Similarity to thioltransferase (glutaredoxin)$^c$ |
| YDR453C   | $>15$         |               | Similarity to Tsa1p$^c$ |
| YLR101W   | 3.1           |               | Similarity to C. boidinii peroxisomal protein A and B |
| YOL151W   | 5.9           |               | Similarity to plant dihydroflavonol-4-reductases$^c$ |
| II. Heat shock and chaperone proteins |
| CPR3      | 2.3           |               | Cyclophilin$^c$ |
| DDR48     | 7.4           |               | Heat, salt, and DNA damage inducible |
| HSC82     | 0.62          | 82-kDa heat shock protein |
| HSP104    | 14.9          | 104-kDa heat shock protein |
| HSP12     | 10            | 12-kDa heat shock protein$^c$ |
| HSP26     | $>5$          | 26-kDa heat shock protein |
| HSP42     | 4             | 42-kDa heat shock protein$^d$ |
| HSP90     | 2.3           | 82-kDa heat shock protein |
| PD1       | 2.8           | Protein-disulfide isomerase |
| SSA1      | 2.7           | Heat shock protein |
| SSA2      | 0.33          | Heat shock protein |
| SSA3      | 4.2           | Heat shock protein |
| III. Proteases |
| CIM5      | 1.7           | Proteasome subunit |
| PRE1      | 2.3           | Proteasome subunit$^c$ |
| PRE3      | 1.9           | Proteasome subunit |
| PRE5      | 3.4           | Proteasome subunit$^c$ |
| PRE8      | 1.5           | Proteasome subunit |
| PRE9      | 2             | Proteasome subunit$^c$ |
| PUP2      | 2.9           | Proteasome subunit$^c$ |
| SCL1      | 2.5           | Proteasome subunit$^c$ |
| UBA1      | 1.6           | Ubiquitin-activating enzyme |
| UBI4      | 1.75          | Ubiquitin$^c$ |
| HSP78     |               | 4.9           | Mitochondrial protease$^c$ |
| PEP4      |               | 2.9           | Vacuolar protease A |
| IV. Protein translation apparatus |
| EBF1      | 0.12          |               | Translation elongation factor EF-1$^\beta$ |
| EFT1      | 0.21          |               | Translation elongation factor EF-2 |
| YEF3      | 0.11          |               | Translation elongation factor EF-3 |
| IF4B      |               | $>2$          | Translation initiation factor eIF4B$^d$ |
| TIF1      | 0.42          |               | Translation initiation factor eIF4A |
| TIF51A    | 0.52          |               | Translation initiation factor eIF5A |
| RPS5      | 0.5           | Ribosomal protein RPS5 |
| YST2      | 1.9           | Ribosomal protein |
| RPA2      | 0.17          | Acridin ribosomal protein L44 |
| RPA3      | 0.65          | Acidic ribosomal protein A0 |
| RPL45     | 0.36          | Acidic ribosomal protein L45 |
| SSB1      | 0.16          | Heat shock protein family |
| SSB2      | 0.18          | Heat shock protein family |
| Not classified |
| ATP2      | 0.64          |               | ATP synthase |
| BGL2      | 2.4           | $\beta$-Glucanase |
| CDC48     | 1.5           | ATPase Family |
| DNLM1     | 6.2           | Dynamin-related protein$^c$ |
| OYE3      | 2.1           | NADPH dehydrogenase$^d$ |
| RNR4      | 2.2           | Ribonucleotide reductase small subunit |
| YBR025C   | 6.4           | Strong similarity to Yif1p |
| YKL066C   | 0.42          |               | Translationally controlled tumor protein (TCTP) |
| TFS1      | 9.7           | Suppressor of cdc25 mutation$^c$ |
| YLR179C   | 0.53          |               | Similarity to Tfs1p |
| V. Carbohydrate metabolism enzymes |
| Pentose phosphate pathway |
| TAL1      | 4.1           |               | Transaldolase$^c$ |
| TKL1      | 0.2           |               | Transketolase |
| TKL2      | 3.9           |               | Transketolase$^c$ |
| ZWF1      | 2             |               | Glucose-6-phosphate dehydrogenase |
| Glycolysis |
| ADH1      | 0.63          |               | Alcohol dehydrogenase |
| ALD5      | 2.1           |               | Aldehyde dehydrogenase |
| ALD6      | 0.52          |               | Aldehyde dehydrogenase |
| ENO1      | 1.6           |               | Enolase |
| ENO2      | 0.37          |               | Enolase |
| GLK1      | 3.8           |               | Glucokinase |
chrome c peroxidase, cytosolic catalase, superoxide dismutases, thioperoxidase (Tsa1p), thioredoxin 1 or 2, NADPH-dependent thioredoxin reductase, and NADPH-dependent glutathione reductase. Antioxidant defense properties have been reported in yeast for all these genes except cytochrome c peroxidase (23–30). We have also identified four new proteins with possible antioxidant defense properties. YDR453C is one of the other AhpC/TSA homologues discovered in the yeast genome sequencing project. YDR453C and Tsa1p are 96% similar and 86% identical. They share N-terminal and C-terminal catalytic cysteines of the AhpC/TSA homology (27). YDR453C is likely to carry the antioxidant function described for Tsa1p, which involves the reduction of peroxides with electrons donated by thioredoxin, thioredoxin reductase, and NADPH (28). YLR109W is a new antioxidant related to the peroxiredoxin AhpC/TSA protein family and is quite similar to peroxisomal membrane proteins (PMP20) A and B of Candida boidinii (31). YLR109W displays defense activity against tert-butyl hydroperoxide and represents a subclass of the AhpC/TSA protein family localized in the peroxisome. YCL035C is very homologous to glutaredoxin encoded TTR1 (32) and carries both cysteine signatures of the glutaredoxin/thioltransferase family.

The H$_2$O$_2$ Stimulon in Yeast

TABLE I—continued

| Gene name | Repression$^a$ | Induction$^b$ | Protein function |
|-----------|----------------|---------------|------------------|
| PDC1      | 0.27           | Pyruvate decarboxylase |
| SEC 53    | 0.31           | Phosphomannomutase |
| TDH 2     | 0.59           | Glyceraldehyde-3-phosphate dehydrogenase |
| TDH 3     | 0.47           | Glyceraldehyde-3-phosphate dehydrogenase |
| Trichloroacetic acid cycle | | |
| LPD1      | 0.45           | Dihydroliopasidide dehydrogenase |
| MDH1      | 0.6            | Malate dehydrogenase |
| PDB1      | 0.34           | Pyruvate dehydrogenase |
| Glycerol metabolism | | |
| DAK1      | 3              | Similarity to dihydroxyacetone kinase |
| GPD1      | 2.6            | Glycerol phosphate dehydrogenase |
| GPP1      | 0.55           | Glycerol phosphate phosphatase |
| GPP2      | 1.5            | Glycerol phosphate phosphatase$^c$ |
| YLR149W   | 1.7            | Glycerol dehydrogenase |
| Trehalose synthesis | | |
| PGM2      | 4.4            | Phosphoglucomutase |
| TPS1      | 3.8            | Trehalose-6-phosphate synthase |
| UGP1      | >2             | UDP-glucose pyrophosphorylase |
| VI. Sulfur, amino acids, and purine metabolism enzymes | | |
| Sulfur metabolism | | |
| CYS3      | 3.2            | Cystathionine $\gamma$-lyase |
| MET6      | 0.22           | Methionine synthase |
| SAM1      | 0.42           | $S$-Adenosylmethionine synthetase 1 |
| SAM2      | 0.48           | $S$-Adenosylmethionine synthetase 2 |
| Polyamine pathway | | |
| SPE2B     | 0.25           | $S$-Adenosylmethionine decarbonylase $\alpha$ chain$^c$ |
| SPE3      | 0.63           | Spermidine synthase$^c$ |
| Amino acids metabolism | | |
| ARG1      | 3.1            | Arginosuccinate synthase |
| ARO4      | 3.1            | 2-Dehydro-3-deoxypyrophosphate aldolase |
| CPA2      | 2.1            | Carbamyl phosphate synthase |
| GDH1      | 0.36           | Glutamate dehydrogenase (NADP$^+$) |
| HIS4      | 2.3            | AMP cyclohydrolase |
| ILV2 (a)  | 0.31           | Acetolactate synthase |
| ILV2 (b)  | 2              | Acetolactate synthase$^c$ |
| ILV3      | 0.6            | Dihydroxyacid dehydratase$^c$ |
| ILV5      | 0.15           | Acetoxyacid reductoisomerase |
| LYS20     | 1.8            | Probable homocitrate synthase$^c$ |
| LYS9      | 0.55           | Saccharopine dehydrogenase (NADP$^+$) |
| SHMT2     | 0.44           | Serine hydroxymethyltransferase |
| Purine and pyrimidine synthesis | | |
| GU1A      | 0.48           | GMP synthetase |
| ADE3      | 0.32           | C1-tetrahydrofolate synthase |
| ADE6      | 0.55           | 5'-Phosphoribosylformylglycinamidine synthase |
| ADE57     | 0.28           | Phosphoribosylamine-glycine ligase and phosphoribosylformylglycinamidine cyclo-ligase |
| URA1      | 0.45           | Dihydroorotate dehydrogenase$^d$ |
| Regulatory proteins | | |
| CDC37     | 1.5            | Kinase regulator |
| MPR1      | 3.1            | Similarity to S. pombe Pad1p$^c$ |
| Unknown function | | |
| YDR0929C  | 2.5            | Strong similarity to S. pombe Obr1$^c$ |
| YGL077C   | 6.8            | Ste5p-associated protein |
| YKL117W   | 2.3            | Protein with zing finger domain$^c$ |
| YLR387C   | 1.6            | |
| YNL134C   | 2.6            | |
| YNL274C   | 2.1            | Similarity to $\alpha$-ketoisocaproate reductase$^d$ |

$^a$ Repression indexes recorded here were determined in response to 0.4 mM H$_2$O$_2$.

$^b$ Stimulation indexes were determined in response to 0.2, 0.4, and 0.8 mM H$_2$O$_2$ and the highest value was recorded.

$^c$ Proteins identified during this work by the double labeling method.

$^d$ Proteins identified by mass spectrometry.

$^e$ J. Lee, unpublished results.
redox center. YOL151W is similar to NADPH dihydroflavonoid reductases involved in the plant synthesis of isoflavonoid phytoalexins. The antioxidant defense properties of these reductases were demonstrated by the isolation of an isoflavonoid reductase gene from an Arabidopsis thaliana cDNA library in a search for activities able to rescue the diamide hypersensitivity phenotype of a yeast strain deleted for the oxidative stress response regulator YAP1 (33). YBR149W is related to aldo/keto reductases and may act as an NADPH-dependent aldehyde reductase to scavenge lipid peroxidation-derived toxic aldehydes by their reduction into alcohols (34, 35). However, YBR149W could also act as an NADP⁺-dependent glycerol dehydrogenase (see below). Although its product could not be detected in our two-dimensional gels maps, GSH1 (γ-glutamyl-cysteine synthase) mRNA levels are dramatically increased by H₂O₂ (data not shown) and is therefore a part of the H₂O₂ stimulon.

**Heat Shock Proteins, Proteases, and the Translation Apparatus**—Twelve heat shock proteins (HSP) as well as proline isomerase (Cpr3p) and protein disulfide isomerase (Pdi1p) were identified as H₂O₂-inducible targets. HSPs are induced in response to a wide variety of stress conditions and are important for the protection of cells from these adverse conditions (reviewed in Refs. 21, 36, and 37). Most of the HSPs are molecular chaperones that assist abnormal proteins accumulating under stress conditions to regain their proper folding or help their proteolytic degradation. The H₂O₂ induction of several
subunits of the proteasome along with enzymes of the ubiquitin pathway, mitochondrial and lysosomal proteases is also consistent with an important proteolytic activity during the oxidative stress response. Induction of the ubiquitin pathway by oxidative stress and the specific degradation of oxidized proteins by the proteasome has been recently demonstrated (38, 39). Most of the chaperones and proteases have essential roles under nonstress conditions by assisting protein biogenesis, oligomer assembly, traffic between cellular organelles, and selective protein degradation (40, 41). Hence, in addition to their protective functions, they may help to reorchestrate the cell metabolism to the needs of the oxidative stress response. Associated with these changes, the repression of two translation initiation and four translation elongation indicates a global and nonspecific slowdown of protein translation. We could indeed demonstrate a 2.5-fold decrease of translation in response to 0.3 mM H2O2 by [14C]leucine labeling (data not shown). Taken together, the response of HSPs, proteases, and the translational apparatus to H2O2 is probably important for switching the cellular activity from biosynthetic toward protective functions.

**Carbohydrate Metabolism and NADPH Regeneration**—Twenty-five H2O2-responsive targets were identified as metabolic enzymes. Although not exhaustive, this identification provides an indication of the metabolic fluxes redistribution occurring in response to H2O2. These changes dramatically affect carbohydrate metabolism which appears to be diverted to the generation of NADPH, the most important cellular reducing power (Fig. 6). (i) The hexose monophosphate pool: Repression of phosphomannomutase (Sec53p), stimulation of phosphoglucomutase (Pgmp2p), and exclusion of glucose from glycolysis (see below) seem to redirect the hexose phosphate pool to the pentose phosphate pathway and the trehalose synthesis. (ii) Induction of trehalose synthesis: Trehalose synthesis is stimulated by oxidative stress as indicated by the induction of phosphoglucomutase (Pgmp2p), UDP-glucose pyrophosphorylase (Ugp1p), and trehalose-6-phosphate synthase (Tps1p). Trehalose synthesis is also stimulated by heat shock and osmotic stress (42–44), and its accumulation correlates with thermotolerance (45). Parrou et al. (46) also observed the induction of TPS1 by H2O2 but curiously without any trehalose accumulation. We also could not detect any change in trehalose steady state and synthesis rate levels by [14C]glucose labeling (data not shown). These data suggest the existence of an enhanced recycling of this disaccharide (46, 47). (iii) Induction of the pentose phosphate pathway: Three enzymes of the pentose phosphate pathway are induced by H2O2. Glucose-6-phosphate dehydrogenase (Zwf1p) regulates the carbon flow through this pathway by catalyzing its first step, leading to ribulose 5-phosphate, the precursor of purine biosynthesis (48). Then, pentose phosphates are interconverted to glyceraldehyde 3-phosphate or fructose 6-phosphate by transketolases (Tkl1p and Tkl2p) and transaldolase (Tal1p). Glyceraldehyde 3-phosphate can enter glycolysis and fructose 6-phosphate is converted to glucose 6-phosphate (49). However, repression of glycolysis (see below) and purine biosynthesis pathway suggest that most of the pentose phosphates are recycled to the hexose phosphate pool for NADPH production. (iv) Repression of glycolysis: H2O2 treatment results in a slowdown of glycolysis as manifested by repression of Tdh2p and Tdh3p, and the isozymes of both enolase and pyruvate decarboxylase. (v) Repression of the tricarboxylic acid cycle: The decreased expression of pyruvate decarboxylase and pyruvate dehydrogenase, the two enzymes which catalyze the alternative entries into the trichloroacetic acid cycle suggests a further slowdown of the trichloroacetic acid cycle, which is already subject to catabolite repression.
Repression of malate dehydrogenase (Mdh1p) is also consistent with this notion. (vi) Alteration of glycerol metabolism: Glycerol synthesis must be increased in response to H$_2$O$_2$ as suggested by the induction of glycerol phosphate dehydrogenase (Gpd1p) and glycerol phosphate phosphatase (Gpp2p) (Table I). In addition, Dak1p, a dihydroxyacetone kinase and YBR149Wp, a putative glycerol dehydrogenase, which have been assigned to a new salt-induced glycerol dissimilation pathway (50) are also induced by H$_2$O$_2$. This glycerol cycle composed of Gpd1p, Gpp2p, YBR149Wp, and Dak1p (Fig. 6) may function as a transhydrogenase activity to convert NADH to NADP$^+$ at the expense of one ATP (50).

In conclusion, the carbohydrate metabolism alteration seems to principally concur to the regeneration of NADPH. NADPH is composed of Gpd1p, Gpp2p, YBR149Wp, and Dak1p (Fig. 6) to principally concur to the regeneration of NADPH. NADPH is to NADPH at the expense of one ATP (50).

H$_2$O$_2$ seen here must involve several inducible control mechanisms. In addition, Dak1p, a dihydroxyacetone kinase and YBR149Wp, a putative glycerol dehydrogenase, which have been assigned to a new salt-induced glycerol dissimilation pathway (50) are also induced by H$_2$O$_2$. This glycerol cycle composed of Gpd1p, Gpp2p, YBR149Wp, and Dak1p (Fig. 6) may function as a transhydrogenase activity to convert NADH to NADP$^+$ at the expense of one ATP (50).

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