J. Microbiol. Biotechnol. (2008), **18**(2), 270–282

JOURNAL OF MICROBIOLOGY AND BIOTECHNOLOGY

Gpx3-dependent Responses Against Oxidative Stress in *Saccharomyces cerevisiae*

Kho, Chang Won[1], Phil Young Lee[1], Kwang-Hee Bae[1], Sunghyun Kang[1], Sayeon Cho[2], Do Hee Lee[1], Choong-Hyun Sun[3], Gwan-Su Yi[3], Byoung Chul Park[1], and Sung Goo Park[1]*

[1]*Translational Research Center, KRIBB, Daejeon 305-806, Korea*
[2]*College of Pharmacy, Chung-Ang University, Seoul 156-756, Korea*
[3]*School of Engineering, Information and Communications University, Daejon 305-714, Korea*

Received: April 10, 2007 / Accepted: August 6, 2007

The yeast *Saccharomyces cerevisiae* has defense mechanisms identical to higher eukaryotes. It offers the potential for genome-wide experimental approaches owing to its smaller genome size and the availability of the complete sequence. It therefore represents an ideal eukaryotic model for studying cellular redox control and oxidative stress responses. *S. cerevisiae* Yap1 is a well-known transcription factor that is required for H_2O_2-dependent stress responses. Yap1 is involved in various signaling pathways in an oxidative stress response. The Gpx3 (Orp1/PHGpx3) protein is one of the factors related to these signaling pathways. It plays the role of a transducer that transfers the hydroperoxide signal to Yap1. In this study, using extensive proteomic and bioinformatics analyses, the function of the Gpx3 protein in an adaptive response against oxidative stress was investigated in wild-type, *gpx3*-deletion mutant, and *gpx3*-deletion mutant overexpressing Gpx3 protein strains. We identified 30 proteins that are related to the Gpx3-dependent oxidative stress responses and 17 proteins that are changed in a Gpx3-dependent manner regardless of oxidative stress. As expected, H_2O_2-responsive Gpx3-dependent proteins include a number of antioxidants related with cell rescue and defense. In addition, they contain a variety of proteins related to energy and carbohydrate metabolism, transcription, and protein fate. Based upon the experimental results, it is suggested that Gpx3-dependent stress adaptive response includes the regulation of genes related to the capacity to detoxify oxidants and repair oxidative stress-induced damages affected by Yap1 as well as metabolism and protein fate independent from Yap1.

Keywords: Gpx3, oxidative stress, proteomics, *Saccharomyces cerevisiae*, Yap1

*Corresponding author
Phone: 82-42-860-4262; Fax: 82-42-860-4593;
E-mail: sgpark@kribb.re.kr

Aerobic organisms are continuously exposed to reactive oxygen species (ROS) generated during normal metabolism and mitochondria respiration. Oxidative stress induced by ROS can be defined within the context of a subtle change of redox status [40]. From this point of view, oxidative stress occurs when an imbalance exists between generation and removal of ROS [30]. Oxidative stress is damaging to all cellular constituents and is believed to play a causal role in many degenerative diseases [22]. Therefore, the cell possesses a variety of defense mechanisms to cope with oxidative stress. *Saccharomyces cerevisiae* has been widely used in the study of cellular responses to ROS as a model to understand the defense mechanisms of higher eukaryotes [6, 10].

Yeast AP-1 (Yap1) is required for an oxidative stress response in *S. cerevisiae*. Yap1 is a member of the subfamily of AP-1-like transcription factors in eukaryotes, such as mammalian c-Jun. AP-1-like proteins contain a bZip structural motif consisting of a leucine-rich zipper region and an adjacent basic region that are important for dimerization and DNA binding, respectively [5]. Yap1 is involved in the H_2O_2-induced expression of many antioxidants and related protein-encoding genes, such as TRX2, TRR1, TSA1, GSH1, SOD1, AHP1, and SSA1 [6, 29]. In other stress responses, Yap1 is also significantly involved in defense mechanisms [15].

Glutathione peroxidase 3 (Gpx3) is a typical ROS scavenger. The basal mRNA level of Gpx3 is constitutively higher than that of two other *gpx* genes. It has been reported that the *gpx3* mutant is hypersensitive to peroxides, whereas disruption of *gpx1* or *gpx2* does not show an obvious phenotype with respect to tolerance to oxidative stress [18]. Therefore, the *gpx3* gene product may be a major Gpx in *S. cerevisiae*. Recently, Delaunay *et al.* [7] identified a novel role for yeast Gpx3 as a sensor and transducer of the stress response to hydrogen peroxide. This activity occurs *via* Gpx3-mediated oxidation of Yap1. However, a detailed global analysis of Gpx3 functions against oxidative stress has not been reported.

In this study, the effects of *S. cerevisiae* Gpx3 on global protein expression states between the resting and peroxide-stressed conditions was investigated using extensive proteomic and bioinformatics tools.

MATERIALS AND METHODS

Yeast Strains and Growth Conditions

S. cerevisiae strain YPH499 (*MATa ura3-52 lys2-801 ade2-101 trp1-Δ63 his3-Δ200 leu2-Δ1*) and isogenic derivatives were used in all experiments. Cells were grown at 30°C in YPD (1% yeast extract, 2% bactopeptone, and 2% glucose), a minimal synthetic defined media (0.17% yeast nitrogen base without amino acids, 5% ammonium sulfate, 2% glucose, 0.03% adenine hemisulfate, and appropriate amino acids and bases), or galactose induction media (YPD or a minimal synthetic defined media containing 2% galactose, 1% raffinose, and 0.03% adenine hemisulfate).

Plasmids and Construction of Deletion Mutants

The DNA fragment encoding the *gpx3* ORF was amplified from the *S. cerevisiae* cDNA using two pairs of primers covering the entire coding sequence. The C-terminal Gpx3-Myc construct (pESC-LEU-Myc-Gpx3) was generated by inserting *gpx3* between the BamHI and SalI sites of pESC-LEU. A null mutant of the *gpx3* gene was constructed using a PCR-based gene deletion strategy [2, 38].

Cell Lysis

Yeast cells were grown at 30°C in YEPD or SD medium to A_{600}=0.8–1.0. Cells were harvested by centrifugation. Cells were resuspended in 1.5 cell volumes of a lysis buffer containing 50 mM Tris-Cl, pH 8.0, 1% Triton X-100, 100 mM NaCl, 1 mM EDTA, 5 mM dithiothreitol, 1× PMSF (phenylmethylsulfonyl chloride) (Sigma, Carl Roth, Germany), and a protease inhibitors cocktail (Roche, Basel, Switzerland). They were then lysed by vortexing in the presence of a single volume of glass beads. Lysates were centrifuged at 13,000 rpm for 20 min.

Determination of Tolerance Against Various Oxidants

Yeast cells were cultured to the mid-late-exponential phase either in a YEPG medium (1% yeast extract, 2% peptone, and 2% galactose) or a yeast nitrogen base medium supplemented with the appropriate amino acids or nucleic acid bases. For spotting experiments, the numbers of yeast cells were adjusted, and then spotted on YEPD agar supplemented with H_2O_2, as indicated. For the liquid test, yeast cells were grown in a YEPG medium, and cell numbers were adjusted with fresh YEPG. Growth was followed by measuring the OD_{600}. During the late-log growth phase (OD_{600} of 0.6), cultures were split into two subcultures, one of which was treated with oxidants (H_2O_2, final concentration 1 mM) [39].

Prefractionation Using Zoom-IEF Fractionator

Protein extracts samples from each strain were prefractionated using the Zoom-IEF Fractionator (Invitrogen, Vienna, Austria) [24]. To determine the protein concentration of the protein extracts, a Bradford protein assay method with BSA as a standard was used. Each sample was diluted to 0.6 mg/ml for IEF fractionation. The sample (670 μl) was loaded onto three different pH chambers in the Zoom-IEF Fractionator (pH 3.0–4.6, pH 4.6–7.0, and pH 7.0–10.0) and subsequent fractionations were processed in the order of 100 V for 20 min, 200 V for 80 min, and 600 V for 80 min. The fractionated samples were collected, desalted, concentrated, and stored at −80°C until used for 2-DE.

Two-dimensional Polyacrylamide Gel Electrophoresis Analysis

Samples prepared from each fraction were subjected to 2-DE. Two hundred g of proteins was applied on immobilized linear gradient strips (pH 4–7, 13 cm; GE Healthcare). The solubilized samples were combined with a rehydration buffer (9 M urea, 2 M thiourea, 4 M CHAPS, 16 mM DTT, 2% [w/v] Pharmalyte 4–7, and trace amounts of bromophenol blue) to a final volume of 250 μl and were rehydrated for 14 h. Isoelectric focusing was done according to the guidelines in the manual provided with the Multiphor II apparatus (GE Healthcare). The focused strips were equilibrated for 15 min at room temperature with continuous shaking in an equilibration buffer containing 6 M urea, 20% glycerol, 2% SDS, and 65 mM DTT, and subsequently for 15 min in the same buffer but containing 240 mM iodoacetamide instead of DTT. The strips were then placed on top of a 12% sodium dodecyl sulfate (SDS)-polyacrylamide gel in a PROTEAN II xi cell (Bio-Rad Laboratories) and sealed with 0.5% agarose containing bromophenol blue. Electrophoresis was carried out at 30 mA per gel with a Tris-glycine-SDS running buffer until the dye front reached the bottom of the gel. After electrophoresis, the gels were stained using a PlusOne protein silver staining kit (GE Healthcare) according to the manufacturer's specifications. Three sets of protein gels were scanned on an ImageScanner II (GE Healthcare) and images obtained were processed in the 2-D gel analysis software PDQuest (version 6.1.0; Bio-Rad Laboratories).

MALDI-TOF MS Protein Identification

Identification of gel spots after 2-D PAGE was accomplished by MS. Punches (2-mm diameter) of the 2-DE were gels containing differentially expressed protein spots were excised. In-gel trypsin digestion was performed according to the procedure published by Gharahdaghi *et al.* [12] with minor modifications [26]. Briefly, pieces of gel were destained and dried in a SpeedVac evaporator. For in-gel digestion, a gel piece was reswollen with 20 ng/ml trypsin (Promega, Madison, WI, U.S.A.) in 25 mM ammonium bicarbonate at 37°C for 14 h. After trypsin digestion, the resulting peptide mixtures were extracted by incubation with 50% ACN in 1% TFA for 15 min. Peptides were desalted using a ZipTip C18 from Millipore (Bedford, MA, U.S.A.). Peptides were dried and resuspended in a matrix solution (1 mg/ml CHCA in 50% ACN and 1% TFA) (Sigma-Aldrich, St. Louis, MO, U.S.A.) and peptides were analyzed by a MALDI-TOF Voyager DE Pro mass spectrometer (Applied Biosystems) operated in delayed extraction reflector mode at 20 kV as accelerating voltage, a pulse delay time of 90 ns, a grid voltage of 75%, and a guide wire voltage of 0.005%. Spectra were accumulated for 100 laser shots obtained over a mass range of 850–3,500 Da and were analyzed by searching in Swiss-Prot and NCBI nonredundant databases using the ProFound software (http://prowl.rockefeller.edu/profound_bin/WebProFound. exe) and Protein Prospector MSFit software (http://prospector.ucsf.edu/prospector/4.0.7/html/msfit.htm). After removal of peptide masses resulting from keratin contamination, the search program was used with the following parameters: *S. cerevisiae* species, pI range from 4 to 7 (except for one identification), monoisotopic peptide masses, 0 or 1 missed cleavage by trypsin, and a mass tolerance of a 100 ppm (0.01%) error.

Chemical partial modifications, such as oxidation of methionine and carbamidomethylation of cysteine, were taken into consideration for the queries.

Quantification of Proteins

Protein spots were outlined (first automatically and then manually) and quantified using the 2-D gel analysis software PDQuest (version 6.1.0, Bio-Rad Laboratories). The volume percentage of spots representing a certain protein was determined in comparison with the total proteins present in the 2-DE gel.

Statistical Analysis

Values are expressed as mean±standard deviation of percentage of the spot volume in each particular gel after subtraction of the background values. Between-group differences were calculated by a nonparametric Mann-Whitney U test. The level of significance was set at $p<0.05$. If the spot of a protein was not detectable, a value of 0.01 was inserted to make statistical analysis possible.

Results

Construction of *gpx3* Deletion Mutant

To investigate the exact Gpx3-dependent responses to the oxidative stress in *S. cerevisiae*, a two-dimensional electrophoresis (2-DE)/MALDI-TOF mass analysis was used to monitor the change of proteome in yeast cells (wild-type and *gpx3Δ* strains) after treatment with or without oxidative stress. As an initial step, the *gpx3*-deleted mutant of *S. cerevisiae* (YPH499) was constructed. It was confirmed that the expression of Gpx3 was completely abolished in the *gpx3Δ* mutant according to the PCR method, a Western blot analysis, and an H_2O_2-sensitive phenotype (Fig. 1).

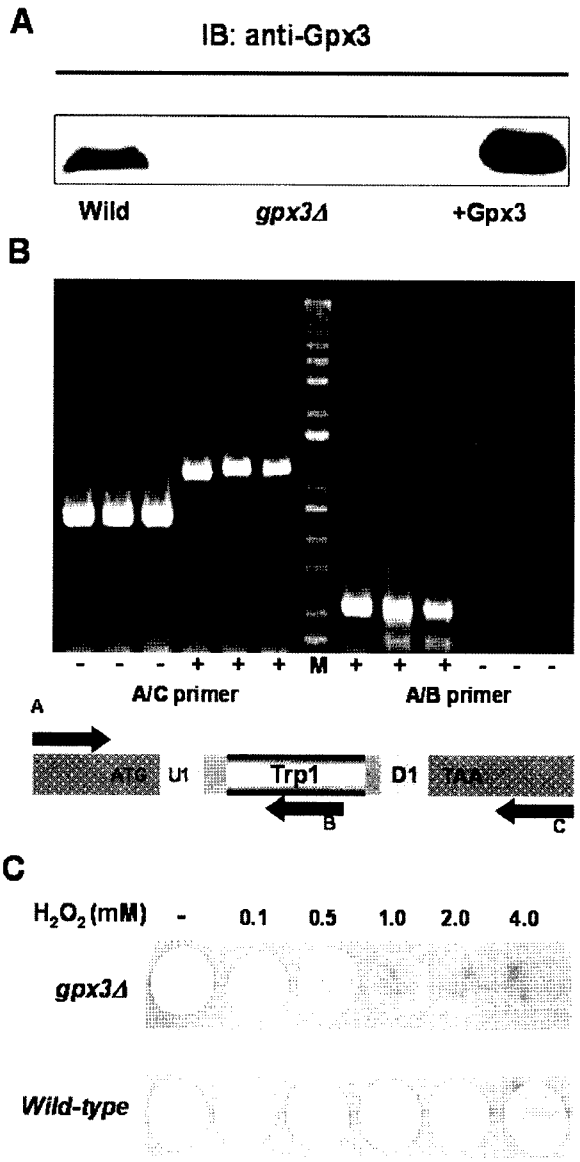

Fig. 1. Confirmation of *gpx3* gene deletion in the *gpx3Δ* mutant strain.

A. Confirmation of *gpx3* gene deletion in the *gpx3Δ* mutant. Yeast cell proteins from each strain cultured to mid/late exponential phase (OD_{600}~1.5) in liquid YEPG (1% yeast extract, 2% bactopeptone, and 2% galactose) medium were separated by 12% SDS-PAGE and analyzed by immunoblotting with an anti-Gpx3 antibody. **B**. The presence of marker cassettes in the strains used in **A** was verified by PCR from genomic DNA, using the Uptag and Downtag primers. The success of the *gpx3Δ* mutant is indicated by +. **C**. Measurement of resistance of the *gpx3Δ* mutant against the peroxide treatment. Yeasts were cultured in mid- to late exponential phase (OD_{600}~1.0) in liquid YPD medium. For spotting experiments, the cultures were diluted to OD_{600} of 0.001 and the samples from each dilution were spotted onto YPD agar supplemented with H_2O_2 (0.1–4.0 mM). Photographs were taken after 4 days of incubation at 30°C. Data are representative of three separate experiments.

Cell Growth is Affected by Gpx3

The deletion of the *gpx3* (HYR1) gene in the wild-type strain significantly increases peroxide sensitivity [1, 18]. As Gpx3 scavenges the ROS that are generated, the effect of expression of Gpx3 on H_2O_2 resistance was examined in the wild-type, *gpx3Δ*, and *gpx3Δ* overexpressing Gpx3 strains. The growth of each strain was monitored after treatment with H_2O_2 (Fig. 2). In a resting state, the growth of the *gpx3Δ* strain was similar to those of the wild type and *gpx3Δ* strain overexpressing Gpx3 (Fig. 2A). The *gpx3Δ* strain showed hypersensitivity toward H_2O_2 but the *gpx3Δ* strain overexpressing Gpx3 had an increased resistance phenotype to H_2O_2 (Fig. 2B). These data suggest that Gpx3 is significantly involved in the peroxide-induced oxidative stress response.

2-DE and MALDI-TOF Mass Analysis for H_2O_2 and Gpx3-dependent Protein Expression

Protein extracts prepared from normal or H_2O_2-treated wild-type, *gpx3Δ*, and *gpx3Δ* overexpressing Gpx3 strains were compared using IEF fractionation/2-DE in triplicates in order to allow the averaging of possible variations during the growth conditions, sample processing, and gel running. A treatment with 1.0 mM H_2O_2 for 30 min was chosen as an oxidative stress. About 600 μg of protein extracts in each pH range (pH 3–4.6, 4.6–7.0, and 7.0–

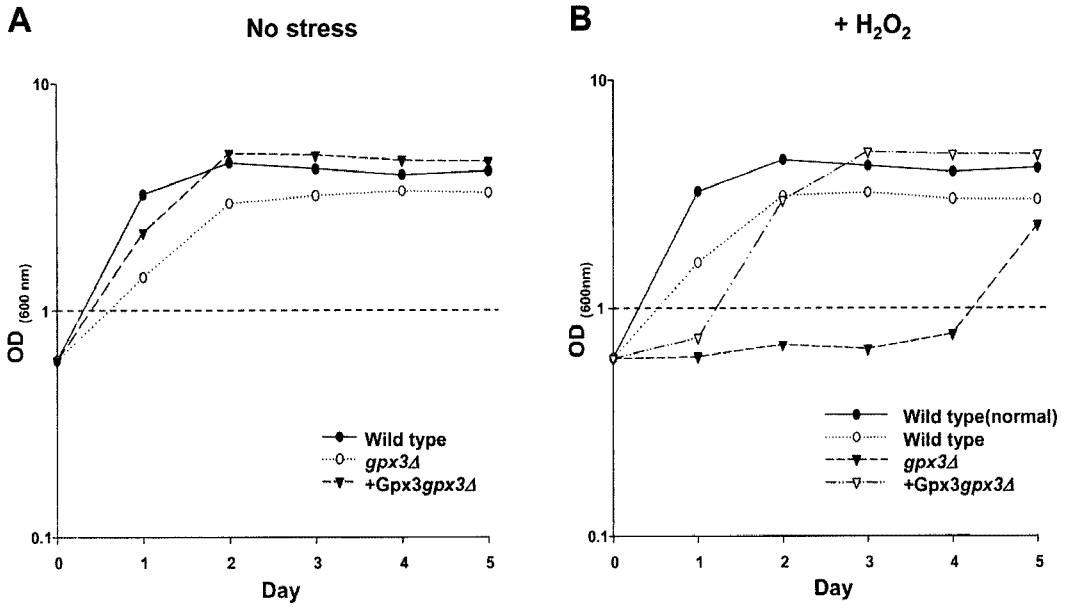

Fig. 2. Growth of the yeast wild-type and *gpx3Δ* strains under oxidative stress condition.
Cells grown overnight in YEPG medium were resuspended in fresh YEPG (OD_{600}=0.2). At the late-log growth phase (OD_{600}=0.6), the cultures were divided into two subcultures, one of which was treated with or without 1 mM H_2O_2. Their growth was monitored for 5 days (until they reached the stationary phase). **A**. Untreated; **B**. Treated with H_2O_2. Data are representative of three separate experiments.

10.0) chamber was separated according to their isoelectric points. Proteins collected from each reaction were separated according to their pI using IPG strip separation and their apparent molecular weight according to 2-DE (Fig. 3). 2-DE gel analysis software PDQuest was then used to find protein spots displaying a >1.5 average fold change in abundance in the wild-type (normal and treated with H_2O_2) and *gpx3Δ* strain (in response to H_2O_2) or between the wild-type and *gpx3Δ* strains. The *gpx3Δ* overexpressing Gpx3 was used as a positive control for comparison with the wild-type strain. Distinct protein spots through image software were digested in-gel by trypsin to generate complex peptide mixtures and then introduced into a MALDI-TOF mass spectremeter where mass-to-charge (*m/z*) ratios are measured. To identify proteins, these results were applied to Web-based PMF (peptide mass fingerprint) searching software.

A total of 260 proteins showing significant differences in expression in one or more of the comparisons (classified by pH ranges in wild-type *vs. gpx3Δ vs. gpx3Δ* overexpressing Gpx3 strain and wild-type-normal *vs.* wild-type-H_2O_2 *vs. gpx3Δ*-H_2O_2 *vs. gpx3Δ* overexpressing Gpx3 strain-H_2O_2) were identified. All identified proteins and their Protein Entry names, SwissProt Accession numbers (AC), theoretical pI and molecular weight (MW) values, number of identified spots, and Gene Ontology annotation terms for biological process and molecular function were taken from the *S. cerevisiae* gene database (http://www.yeastgenome.org/), the Swiss-Prot/TrEMBL protein database (http://us.expasy.org/sprot/), and the Munich Information Center for Protein Sequences (MIPS) (http://mips.gsf.de/genre/proj/yeast/). From these data, 135 proteins that were specifically expressed during the oxidative stress response by H_2O_2 and 95 proteins that were selectively expressed in a Gpx3-dependent manner were obtained. However, on 2-DE gels, proteins are often represented by more than one spot. Therefore, the number of expressed products is much higher than the number of the corresponding encoding genes. In this case, 33 proteins (nearly 24% of all identified proteins in the H_2O_2-dependent group) and 18 proteins (about 19% of all identified proteins in the Gpx3-dependent group) occurred as multiple isoforms, and 102 proteins and 77 proteins, respectively, were shown at a single spot. Among these results, 71 proteins (H_2O_2-dependent) and 48 proteins (Gpx3-dependent) that exceptionally displayed >2 average fold changes of expression ratios between the wild-type and *gpx3Δ* strains were selected.

Functional Classification of Identified Proteins

Identified proteins were classified into several functional groups according to their representative biological roles, whether they were H_2O_2-dependent or Gpx3-dependent. Fig. 4 represents the number of proteins changes dependent on H_2O_2 and Gpx3. The number of proteins that changed *via* H_2O_2 treatment, for each strain, and how these overlap are shown in Fig. 4A, whereas the number of proteins regulated by Gpx3-dependency in the absence and presence of oxidative stress are represented in Fig. 4B. When comparing wild-type and *gpx3* between unstressed and the H_2O_2 stress conditions, 41 proteins were observed that responded to general oxidative stress, and 30 proteins that were specifically Gpx3-dependent responded to oxidative stress (Fig. 4A). Among proteins regulated in a H_2O_2-dependent

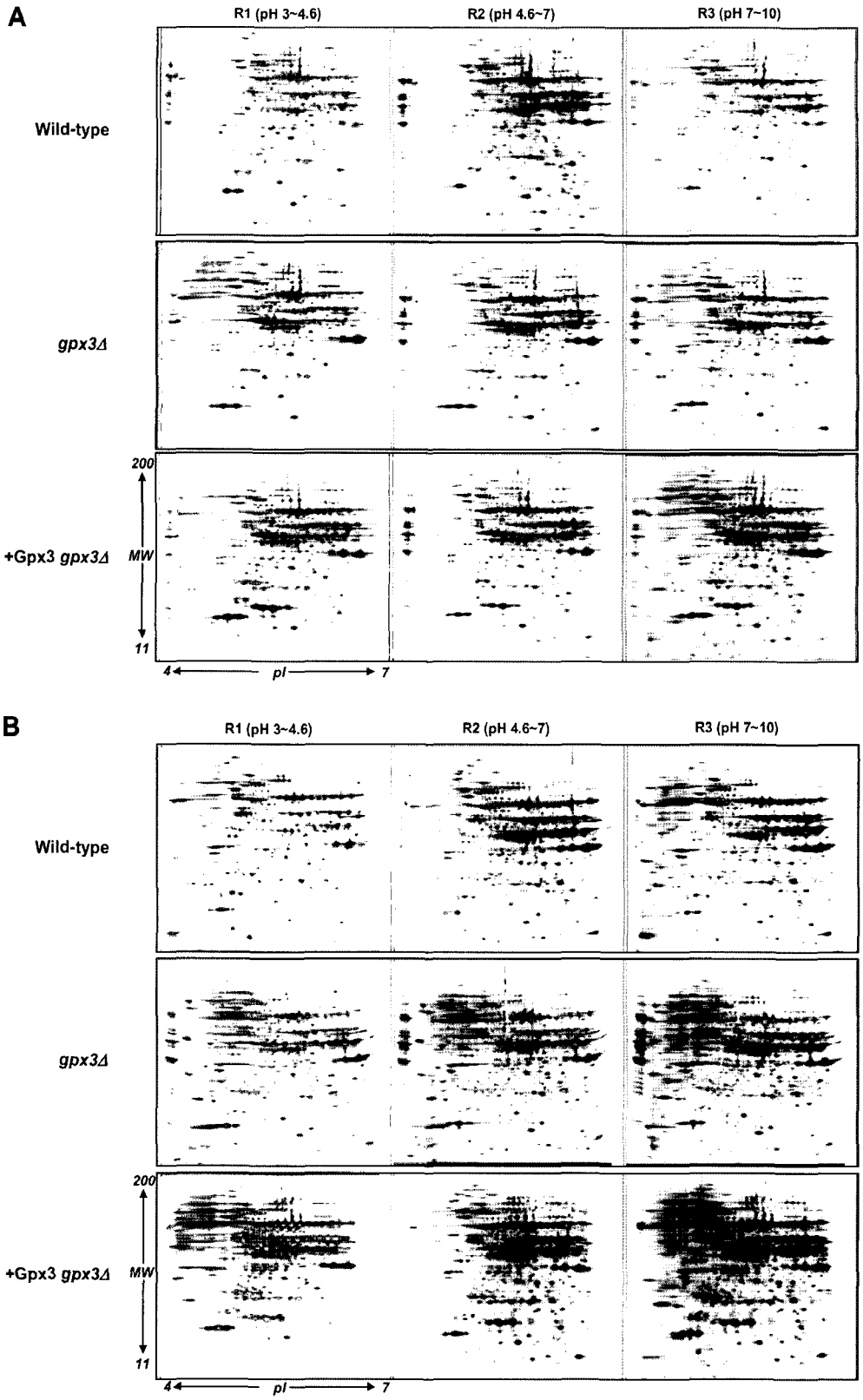

Fig. 3. Comparison of all protein extracts of *S. cerevisiae* prepared using IEF prefractionation on the 2-DE separation.
Each cell type (wild-type, *gpx3Δ* strain, *gpx3Δ* strain overexpressing Gpx3) grown overnight in a YEPG medium was resuspended in fresh YEPG (OD_{600} 0.2). At the late-log growth phase (OD_{600} 0.6), the cultures were divided into two subcultures, one of which was treated with or without 1 mM H_2O_2. **A.** Extracts prepared from untreated cells. **B.** Extracts prepared from cells exposed to H_2O_2 (1 mM) as indicated. In this case, after the prefractionation of yeast protein extracts using the Zoom-IEF fractionator, the protein fraction of each pH range (pH 3–4.6, 4.6–7.0, 7.0–10) was applied to the first-dimensional electrophoresis (on 13 cm immobilized gel strip) by IEF and then the second-dimensional electrophoresis by 12% SDS-PAGE. Data are representative of three separate experiments.

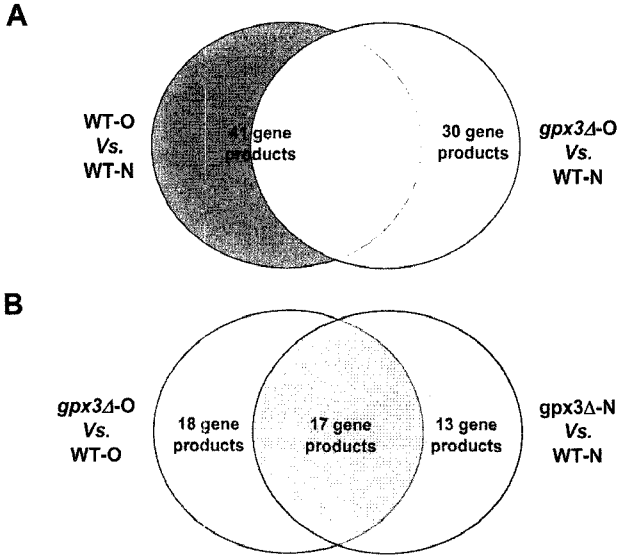

Fig. 4. Schematic representation of regulated isoform expression. **A.** Venn diagram showing the numbers of H_2O_2-dependent up- or down-regulated proteins in WT and *gpx3Δ* cells. **B.** Venn diagram showing the number of Gpx3-dependent up- or down-regulated proteins in untreated or H_2O_2-treated cells. Proteins were taken from Tables 1–3 and were included if they displayed >1.5 average fold changes in abundance.

manner, proteins previously identified with the oxidative stress response of *S. cerevisiae* were included, including Cu-Zn superoxide dismutase (SOD1), thioredoxin reductase 1 (TRR1), peroxiredoxin (TSA1), peroxiredoxin type 2 (AHP1), phosphoglycerate mutase 3 (GPM3), argininosuccinate synthase (ARG1), protein disulfide isomerase (PDI1), proteasome component (PUP3), and translational elongation factor 1B (EFB1) α [13, 17]. Specifically, oxidative stress responding proteins what are H_2O_2-dependent responsive but Gpx3-independent were found, including guanylate kinase (GUK1), fatty acid repression mutant protein 2 (FRM2), dihydroxyacetone kinase 1 (DAK1), T-complex protein 1 (CCT5), L-asparaginase I (ASP1), chromosome segregation in meiosis protein 4 (CSM4), tubulin-folding cofactor C (CIN2), and saccharopepsin precursor (PEP4) 8LA. The other proteins were related to cell rescue and defense, cellular transport, cell cycle and DNA processing, biogenesis of cellular components, metabolism, protein fate, or were unclassified proteins (Table 1).

The Gpx3 protein is an antioxidant with a function typical to a ROS scavenger. Gpx3 can also transfer hydroperoxide signals to the Yap1 transcription factor. Consequently, cells

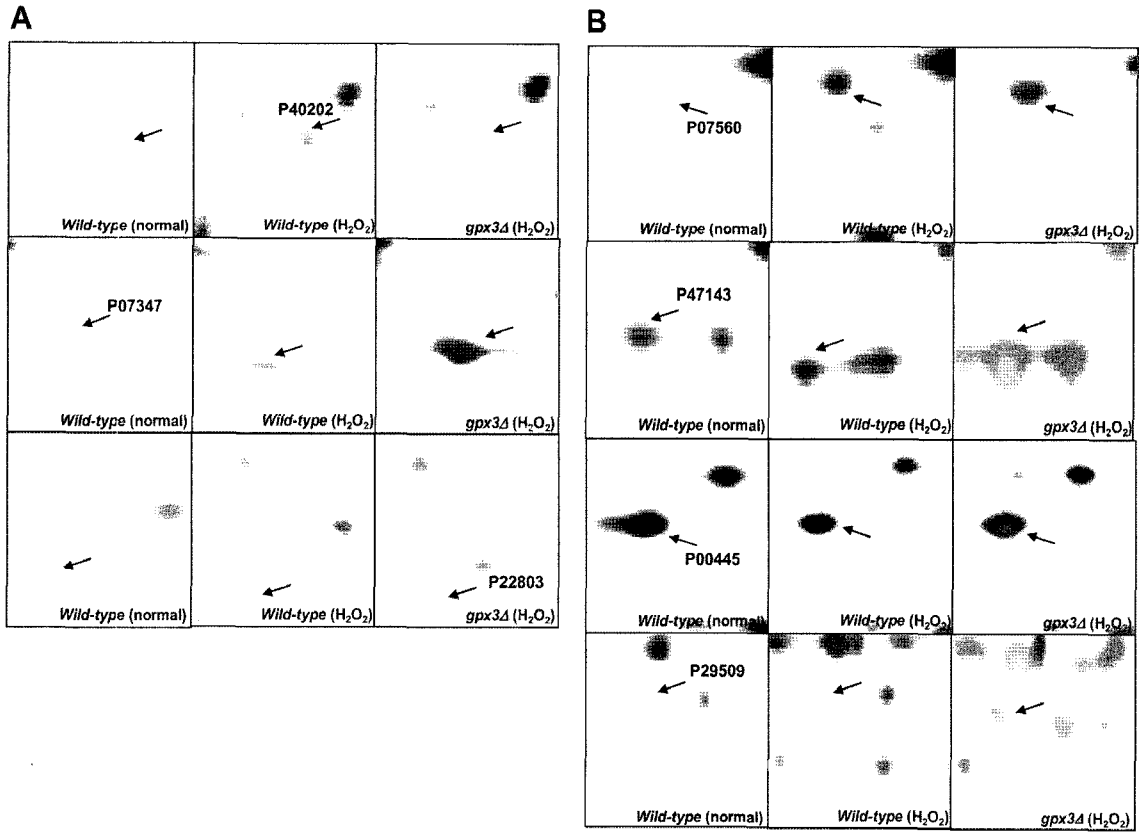

Fig. 5. Comparative analysis of proteins involved in Yap1-dependent regulated genes.
In a comprehensive analysis of proteomes regulated in H_2O_2-dependent and Gpx3-dependent in respenses *S. cerevisiae*, proteins encoding Yap1-regulated genes were analyzed with Yap1-dependent regulated genes, based on Harbison *et al.* [16] and MacIsaac *et al.* [21]: (ftp://ftpmips.gsf.de/catalogue/), (http://www.ebi.uniprot.org/uniprot-srv/index.do;jsessionid=DAA88F0A3130ECB29F9471D1F26E88D4), (http://db.yeastgenome.org), and (http://mips.gsf.de/genre/proj/yeast/searchEntryAction.do). Three proteins in Gpx3-dependent (**A**) and 4 proteins in H_2O_2-dependent (**B**), up- or down-regulated proteins under oxidative stress condition, were identified in mapping results of genes with a Yap1-binding site.

Table 1. Differentially regulated proteins by H_2O_2-dependent oxidative stress respense in *S. cerevisiae.*

Spot_No.	Protein_Name	Uniprot_ID	Log_ratio 1 (WT-N/WT-O)
I. BIOGENESIS OF CELLULAR COMPONENTS			
53	Tubulin-folding cofactor C (CIN2 protein)	P46670	1.00
II. CELL CYCLE AND DNA PROCESSING			
55	Chromosome segregation in meiosis protein 4	Q08955	0.98
III. CELL RESCUE, DEFENSE, AND VIRULENCE			
61	Superoxide dismutase [Cu-Zn]	P00445	0.87
120	Reduced viability upon starvation protein 161	P25343	0.73
17	Thioredoxin reductase 1 (E.C. 1.8.1.9)	P29509	1.05
21	Peroxiredoxin TSA1	P34760	1.38
100	Peroxisomal alkyl hydroperoxide reductase (Thioredoxin peroxidase type II)	P38013	0.86
76	Protein HMF1 (High dosage growth inhibitor)	P40037	0.71
10	T-complex protein 1, epsilon subunit (TCP-1-epsilon) (CCT-epsilon)	P40413	0.59
27	Peroxiredoxin HYR1	P40581	1.00
59	Telomere replication protein EST3 (Ever shorter telomeres protein 3)	Q03096	1.26
89	Oxidation resistance protein 1	Q08952	0.90
IV. CELLULAR TRANSPORT, TRANSPORT FACILITATION, AND TRANSPORT ROUTES			
36	SEC14 cytosolic factor (Phosphatidylinositol/phosphatidylcholine transfer protein)	P24280	1.19
40	Translocation protein SEC72 (p23)	P39742	0.80
72	Mitochondrial import inner membrane translocase subunit TIM22	Q12328	0.64
V. ENERGY			
110	Ubiquinol-cytochrome *c* reductase complex 17 kDa protein (Mitochondrial hinge protein) (Complex III polypeptide VI)	P00127	0.98
12	Fructose-bisphosphate aldolase	P14540	0.90
50	Phosphoglycerate mutase 3 (Phosphoglyceromutase 3) (PGAM 3)	Q12326	1.29
VI. METABOLISM			
66	ATP phosphoribosyltransferase (ATP-PRTase) (ATP-PRT)	P00498	1.18
101	GAL10 bifunctional protein [Includes: UDP-glucose 4-epimerase (Galactowaldenase); Aldose 1-epimerase (Mutarotase)]	P04397	0.59
79	5-Methyltetrahydropteroyltriglutamate--homocysteine methyltransferase (Methionine synthase, vitamin-B12 independent isozyme)	P05694	0.96
126	N-Terminal acetyltransferase complex ARD1 subunit (Arrest-defective protein 1)	P07347	1.48
106	Guanylate kinase (GMP kinase)	P15454	1.00
39	Siroheme biosynthesis protein MET8 [Includes: Precorrin-2 dehydrogenase; Sirohydrochlorin ferrochelatase]	P15807	1.14
47	Argininosuccinate synthase (Citrulline--aspartate ligase)	P22768	0.94
28	Fatty acid repression mutant protein 2	P37261	0.93
103	EGD2 protein (GAL4 DNA-binding enhancer protein 2)	P38879	0.62
11	L-Asparaginase I (L-asparagine amidohydrolase I) (ASP I)	P38986	1.21
86	Adenosine kinase	P47143	1.48
32	Dihydroxyacetone kinase 1 (Glycerone kinase 1) (DHA kinase 1)	P54838	0.70
24	Putative carboxymethylenebutenolidase (Dienelactone hydrolase) (DLH)	Q07505	0.55

can adapt from oxidative stress [7]. Among the 135 proteins that are specifically expressed on oxidative stress response by H_2O_2, proteins changed by Gpx3-dependent oxidative stress are categorized in Table 2. Among them, three proteins were identified in general oxidative stress response in *S. cerevisiae*. They are thioredoxin II (TRX2) [20], pyruvate decarboxylase (PDC1) [24], and *S*-adenosylmethionine synthetase 2 (SAM2) [23]. Moreover, TRX2 is known as a Yap1-dependent regulated gene [5]. In particular, superoxide dismutase 1 copper chaperone (CCS1) and glutaredoxin 2 (GRX2) were identified, implying that they are regulated in a Gpx3-dependent manner [27, 33]. In addition, the other proteins were classified into functions of cell rescue and defense, cellular transport, cell cycle and DNA processing, biogenesis of cellular components, metabolism, or protein fate. Otherwise, they were grouped as unclassified proteins (Table 2).

Seventeen proteins requiring Gpx3 for basal expression irrespectively of oxidative stress were also analyzed (Table 3). Among them, peroxisomal alkyl hydroperoxide reductase

Table 1. Continued.

Spot_No.	Protein_Name	Uniprot_ID	Log_ratio 1 (WT-N/WT-O)
VII. PROTEIN FATE (FOLDING, MODIFICATION, DESTINATION)			
113	Saccharopepsin precursor (Aspartate protease) (Proteinase YSCA)	P07267	1.43
112	Protein disulfide-isomerase precursor (PDI)	P17967	0.97
95	Proteasome component PUP3 (Macropain subunit PUP3)	P25451	1.23
18	40S ribosomal protein S0-B (Nucleic acid-binding protein NAB1B)	P46654	0.64
65	Sorting nexin 4	P47057	1.39
VIII. PROTEIN SYNTHESIS			
19	Elongation factor 1-beta (EF-1-beta) (Translation elongation factor 1B alpha) (Eukaryotic elongation factor 1B alpha) (eEF1Balpha)	P32471	1.01
IX. UNCLASSIFIED PROTEINS			
22	Hypothetical 21.8 kDa protein in IRR1-TIM44 intergenic region	P40543	0.90
38	Hypothetical 31.3 kDa protein in SQT1-MET28 intergenic region	P40570	0.78
16	Hypothetical 33.7 kDa protein in SWE1-ATP12 intergenic region	P46983	0.65
135	FAS1 domain-containing protein YDR262W precursor	Q12331	0.77

A total of 135 proteins were identified that displayed significant differences in the expression when compared (classified according to pH ranges in wild-type-normal *vs.* wild-type-H_2O_2, and wild-type-normal *vs.* *gpx3Δ*-H_2O_2). Among them, 102 proteins were individual proteins. In the Venn diagram in Fig. 4A, two groups were applied with the equation; ratio 1 (WT-N/WT-O) and ratio 2 (WT-N/*gpx3Δ*-O). With the ratio value (a<1), proteins are upregulated, and with the ratio value (a>1), proteins are downregulated. Each ratio value is substituted for the equation (absolute value (log ratio)) and only values of 0.5<absolute value (log ratio)<1.5 are selected. In the comparisons (wild-type-normal *vs.* wild-type-H_2O_2, and wild-type-normal *vs.* *gpx3Δ*-H_2O_2), 41 proteins regulated in a H_2O_2-dependent manner are represented in this table.

(AHP1) is well known as a protein regulated by Yap1-dependency in an oxidative stress condition [5]. Recently, it was reported that cytosolic thioredoxin peroxidase I (TSA 1), the protein regulated in a Yap1-dependent manner, is related to AHP1 [8]. The other proteins were not directly related with oxidative stress response, but participated in various cellular mechanisms, such as the biogenesis of cellular components, cell cycle and DNA processing, energy and each kind of metabolism, or protein fate.

Relationship of Gpx3 and Yap1 in the Adaptive Oxidative Stress Responses of *S. cerevisiae*

In *S. cerevisiae*, the bZip transcription factor Yap1 senses and transduces hydrogen peroxide (H_2O_2) and *t*-butyl hydroperoxide (*t*-BOOH) signals into cellular defense mechanisms [3, 7]. Yap1 can regulate the expression of genes encoding a variety of antioxidants and components of the cellular thiol-reducing pathways. This protein also regulates the stress tolerance to other classes of compounds including the thiol oxidant diamide, the electrophile diethylmaleate, and cadmium [3, 25, 37]. According to many reports, Yap1 in the adaptive oxidative stress response is regulated by various signaling pathways. It is known that Gpx3 is one of the signaling transducers for activating Yap1 transcription factor [7]. Whether proteins showing changes in their H_2O_2-dependent manner are related with the Yap1-dependent adaptive oxidative stress response was analyzed using methods based on Harbison *et al.* [16] and MacIsaac *et al.* [21]: (ftp://ftpmips.gsf.de/catalogue/), (http://www.ebi.uniprot.org/uniprot-srv/index.do;jsessionid=DAA88F0A3130ECB29F9471D1F26E88D4), (http://db.yeastgenome.org), and (http://mips.gsf.de/genre/proj/yeast/searchEntryAction.do.). A total of 135 proteins (Fig. 4A) that displayed significant differences in the expression (classified by pH ranges in wild-type-normal *vs.* wild-type-H_2O_2, and wild-type-normal *vs.* *gpx3Δ*-H_2O_2) were used in the analysis. Based on the references of Yap1-dependent regulated genes, the surveyed proteins were divided to three groups; a group matched with candidates at $p<0.001$ for proteins involved in both the 0.5<log ratio 1 (WT-N/WT-O)<1.5 and 0.5<log ratio 2 (WT-N/*gpx3Δ*-O)<1.5; a group matched with candidates at $p<0.005$ for proteins involved in both 0.5<log ratio 1 (WT-N/WT-O)<1.5 and 0.5<log ratio 2 (WT-N/*gpx3-ΔO*)<1.5; and a group matched with candidates of the *No threshold* for proteins independent of values of log ratio (Table 4). Seven proteins in total were analyzed. Among them, three proteins (thioredoxin II, TRX2 [20]; superoxide dismutase 1 copper chaperone, Ccs1 [34]; and N-terminal acetyltransferase A complex catalytic subunit, Ard1 [4]) were only in the Gpx3-dependent group, as indicated by the asterisk.

DISCUSSION

All organisms have evolved a number of cellular responses that allow them to cope with a variety of environmental stress conditions. These responses invariably involve the increased expression of a number of genes whose products counteract against the harmful effects of the particular conditions the cell is encountering. A stress-inducible defense

Table 2. Differentially regulated proteins by Gpx3-dependent oxidative stress response in *S. cerevisiae*.

Spot_No.	Protein_Name	Uniprot_ID	Log_ratio 2 (WT-N/*gpx3Δ*-O)
I. CELL CYCLE AND DNA PROCESSING			
131	Carboxypeptidase Y inhibitor (CPY inhibitor) (Ic) (I(C)) (DKA1 protein)	P14306	0.98
II. CELL RESCUE, DEFENSE, AND VIRULENCE			
42	Glutaredoxin-2, mitochondrial precursor (Thioltransferase)	P17695	1.37
133	Thioredoxin II (TR-II) (Thioredoxin 1)	P22803	0.66
107	Superoxide dismutase 1 copper chaperone	P40202	1.21
116	NADPH-dependent methylglyoxal reductase GRE2	Q12068	0.97
111	Ras-related protein SEC4	P07560	0.82
138	Mitochondrial import receptor subunit TOM20	P35180	0.72
III. ENERGY			
5	Cytochrome *c* oxidase polypeptide IV, mitochondrial precursor	P04037	1.16
45	Pyruvate decarboxylase isozyme 1	P06169	1.03
IV. METABOLISM			
105	Adenylate kinase cytosolic (ATP-AMP transphosphorylase)	P07170	1.27
44	Phosphoenolpyruvate carboxykinase [ATP]	P10963	0.98
4	Orotate phosphoribosyltransferase 1 (OPRT 1) (OPRTase 1)	P13298	0.58
71	Uridylate kinase (UK) (Uridine monophosphate kinase)	P15700	0.88
49	*S*-Adenosylmethionine synthetase 2 (Methionine adenosyltransferase 2)	P19358	0.70
75	Deoxyuridine 5'-triphosphate nucleotidohydrolase (dUTPase)	P33317	0.87
33	Alpha-glucosidase MAL32 (Maltase)	P38158	0.87
119	Hypothetical oxidoreductase YIR036C	P40580	0.62
3	Exosome complex exonuclease RRP45	Q05636	1.03
115	Adenylosuccinate lyase (Adenylosuccinase) (ASL) (ASASE)	Q05911	0.75
118	NADPH-dependent alpha-keto amide reductase	Q07551	0.90
102	Sorbitol dehydrogenase 2 (L-iditol 2-dehydrogenase 2)	Q07786	0.86
74	Cytosine deaminase (Cytosine aminohydrolase)	Q12178	0.84
117	Peroxisomal coenzyme A diphosphatase 1, peroxisomal [Precursor]	Q12524	1.25
80	Invertase 4 precursor (Beta-fructofuranosidase 4) (Saccharase)	P10596	0.58
V. PROTEIN FATE (FOLDING, MODIFICATION, DESTINATION)			
20	Peptidyl-prolyl cis-trans isomerase D precursor (PPIase) (Rotamase)	P35176	0.71
VI. TRANSCRIPTION			
14	MSS18 protein	P08593	0.56
30	Retrograde regulation protein 1	P32607	0.98
VII. UNCLASSIFIED PROTEINS			
136	Hypothetical 27.1 kDa protein in ACS1-GCV3 intergenic region	P39721	1.45
132	Hypothetical 12.0 kDa protein in SNP1-GPP1 intergenic region	P40524	1.13
77	Hypothetical 12.9 kDa protein in CAC2-TSL1 intergenic region	Q04201	0.55

In the comparisons (wild-type-normal *vs.* wild-type-H_2O_2, and wild-type-normal *vs.* *gpx3Δ*-H_2O_2), 30 proteins regulated by Gpx3-dependent oxidative stress response are presented in this table. In the Venn diagram shown in Fig. 4A, Gpx3-dependent proteins with values of 0.5< or absolute value (log ratio)<1.5 were selected. In this table, this indicates that proteins are upregulated at a ratio value of (a<1) and proteins are downregulated at a ratio value of (a>1).

of adaptive response mechanisms acts to protect cells from oxidative threats [13]. For instance, the exposure of bacteria or yeast to low levels of H_2O_2- or superoxide anion-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 [11, 32].

S. cerevisiae has been found to possess a protein (Yap1) that is important in mediating some aspects of stress response regulation, like the *c-jun* component of the mammalian AP-1. The Yap1 transcription factor is a major regulator of yeast in response to H_2O_2 [3, 7, 31]. The expression of many antioxidants detoxified and defended from oxidative stress is regulated by Yap1. These include the TRX2, TRR1, TSA1, GSH1, GLR1, GPX2, and AHP1 [6, 9, 14, 35]. Recently, it was reported that the activation of Yap1 is controlled by various signaling factors, such as Gpx3, Ybp1, TSA1, and TRR1 [3, 36]. According to the reports, Gpx3 senses the hydroperoxide and then reacts with Yap1 to form the Gpx3-Yap1 disulfide linkage, followed by its conversion to the intramolecular disulfide of activated Yap1. During this process, Yap1 results in a conformational

Table 3. Differentially regulated proteins by Gpx3-dependent stress response in *S. cerevisiae*.

Spot_No.	Protein_Name	Uniprot_ID	Log_ratio 3 (WT-N/*gpx3Δ*-N)	Log_ratio 4 (WT-O/*gpx3Δ*-O)
I. BIOGENESIS OF CELLULAR COMPONENTS				
112	Extracellular matrix protein 4	P36156	0.87	1.11
II. CELL CYCLE AND PROCESSING				
79	Protein TBF1 (TTAGGG repeat-binding factor 1) (TBF alpha)	Q02457	0.52	1.15
121	Telomere replication protein EST3 (Ever shorter telomeres protein 3)	Q03096	1.03	0.54
III. CELL RESCUE, DEFENSE, AND VIRULENCE				
37	Peroxisomal alkyl hydroperoxide reductase (Thioredoxin peroxidase type II)	P38013	0.89	1.32
IV. ENERGY				
8	Ubiquinol-cytochrome *c* reductase complex 14 kDa protein	P00128	0.75	0.87
81	Fructose-bisphosphate aldolase	P14540	0.69	0.58
V. METABOLISM				
32	Orotate phosphoribosyltransferase 1 (OPRT 1) (OPRTase 1)	P13298	0.63	0.53
5	Ura1p, Dihydroorotate dehydrogenase	P28272	0.93	0.69
53	1-(5-Phosphoribosyl)-5-[(5-phosphoribosylamino)methylideneamino] imidazole-4-carboxamide isomerase (5-proFAR isomerase)	P40545	0.72	0.72
29	Cytochrome *c*1 heme lyase (E.C. 4.4.1.-) (CC1HL)	Q00873	1.27	0.60
46	3,4-Dihydroxy-2-butanone 4-phosphate synthase (DHBP synthase)	Q99258	1.48	0.74
VI. PROTEIN FATE (FOLDING, MODIFICATION, DESTINATION)				
50	Peptidyl-prolyl cis-trans isomerase (PPIase) (Rotamase) (Cyclophilin)	P14832	1.46	1.10
45	Probable chaperone HSP31	Q04432	0.58	0.84
114	40S ribosomal protein S0-B (Nucleic acid-binding protein NAB1B)	P46654	0.61	0.68
69	BMH1 protein	P29311	0.60	0.61
VII. TRANSCRIPTION				
115	POS9-activating factor FAP7	Q12055	0.81	0.92
VIII. UNCLASSIFIED PROTEINS				
98	Hypothetical 27.4 kDa protein in HIT1-CDC8 intergenic region	P47115	0.85	1.31

A total of 95 proteins were identified that displayed significant differences in expression in the comparisons (classified by pH ranges wild-type-normal *vs.* *gpx3Δ*-normal, and wild-type-H_2O_2 *vs.* *gpx3Δ*-H_2O_2). T total of 77 proteins out of these 95 were individual proteins. In the Venn diagram in Fig. 4B, two groups were applied with the equation; ratio 1 (WT-N/*gpx3Δ*-N) and ratio 2 (WT-O/*gpx3Δ*-O). With a ratio value of (a<1), proteins are upregulated, and in a ratio value of (a>1), proteins are downregulated. Each ratio value is substituted for the equation (absolute value (log ratio)) and only values of 0.5< absolute value (log ratio) <1.5 are selected. Seventeen proteins (located at the intersection between wild-type-normal *vs.* *gpx3Δ*-normal and wild-type-H_2O_2 *vs.* *gpx3Δ*-H_2O_2) regulated in a Gpx3-dependent manner are presented in this table.

change and translocates into the nucleus. Yap1 translocating into the nucleus activates the target genes [7]. Moreover, it has been reported that Gpx3 has multiple functions in the adaptive oxidative stress response, such as a ROS scavenger and an efficient linker between repairing proteins and oxidative stress damage [7, 18–19].

To study the relationship between Gpx3 and Yap1 in adaptive oxidative stress response, a systematic identification of the proteins regulated in a Gpx3-dependent manner from the oxidative stress response against H_2O_2 was conducted. To overcome the relatively poor sensitivity and coverage of current proteomic methodologies, this investigation was applied to a combined system between IEF prefractionation for effective separation of complex proteomes and a 2-DE/MALDI-TOF system (Fig. 3). The results showed that 135 proteins were regulated by a specifically H_2O_2-induced oxidative stress response and that 95 proteins were selectively expressed in a Gpx3-dependent manner. Among all proteins, 102 individual proteins (H_2O_2-dependent) and 77 individual proteins (Gpx3-dependent) were selected. Seventy-one proteins (H_2O_2-dependent) and 48 proteins (Gpx3-dependent) were represented as exceptional, displaying a >2 average fold change in terms of the expression ratios between the wild type and *gpx3Δ*.

To separate proteins that were dependent on either H_2O_2 or Gpx3, they were classified into four groups (classified according to their pH range in wild-type *vs.* *gpx3Δ* *vs.* *gpx3Δ* overexpressing Gpx3 strain, and wild-type-normal *vs.* wild-type-H_2O_2 *vs.* *gpx3Δ*-H_2O_2 *vs.* *gpx3Δ* overexpressing Gpx3 strain-H_2O_2) (Fig. 4). Among the proteins, 41 proteins were observed that responded to general oxidative stress, and 30 proteins that specifically responded in a Gpx3-dependent manner to oxidative stress (Tables 1 and 2). Most patterns of these proteins including the TRX2, TRR1, TSA1, GPX2, and SOD1 were almost the same to previously identified proteins involved in an oxidative stress response.

Table 4. Proteins of Gpx3-dependent oxidative stress response are involved in Yap1-dependent regulated genes in *S. cerevisiae*.

	SwissProt ID	Protein Name	MIPS category
$p<0.001$ & $p<0.005$	P00445	Superoxide dismutase [Cu-Zn]	-32.01.01:oxidative stress response -32.07.07.07:superoxide metabolism
	*P22803	Thioredoxin II (TR-II) (Thioredoxin 1)	-32.01.01:oxidative stress response -32.07.07 :oxygen and radical detoxification
	*P40202	Superoxide dismutase 1 copper chaperone	-32.07: detoxification
	P47143	Superoxide dismutase 1 copper chaperone	-01.03.01:purine nucleotide metabolism -01.04.01:phosphate utilization
No threshold	P00445	Superoxide dismutase [Cu-Zn]	-32.01.01:oxidative stress response -32.07.07.07:superoxide metabolism
	*P07347	N-Terminal acetyltransferase complex ARD1 subunit (Arrest-defective protein 1)	-01.06.07:lipid, fatty acid, and isoprenoid utilization -10.03.01:mitotic cell cycle and cell cycle control -14.07.04:modification by acetylation, deacetylation
	P07560	Ras-related protein SEC4	-20.09.07.27:vesicle fusion -20.09.16.09.03:exocytosis -30.01.05.05.01:small GTPase-mediated signal transduction
	*P22803	Thioredoxin II (TR-II) (Thioredoxin 1)	-32.01.01:oxidative stress response -32.07.07:oxygen and radical detoxification
	P29509	Thioredoxin reductase 1 (E.C. 1.8.1.9)	-32.01.01:oxidative stress response
	*P40202	Superoxide dismutase 1 copper chaperone	-32.07: detoxification
	P47143	Superoxide dismutase 1 copper chaperone	-01.03.01:purine nucleotide metabolism -01.04.01:phosphate utilization

Among 135 proteins that displayed significant differences in expression from the comparisons (classified by pH ranges wild-type-normal *vs.* wild-type-H_2O_2, and wild-type-normal *vs.* *gpx3Δ*-H_2O_2), 7 proteins were analyzed with Yap1-dependent regulated genes based on Harbison *et al.* [16] and MacIsaac *et al.* [21]. From the references, the surveyed proteins were divided into three groups: a group matched with candidates of $p<0.001$ in proteins involved in both 0.5<log ratio1 (WT-N/WT-O)<1.5 and 0.5<log ratio 2 (WT-N/*gpx3Δ*-O)<1.5; a group matched with candidates of $p<0.005$ in proteins involved in both 0.5<log ratio1 (WT-N/WT-O)<1.5 and 0.5<log ratio 2 (WT-N/*gpx3Δ*-O)<1.5; and a group matched with candidates of *No threshold* in proteins independent of the values of the log ratio. Three proteins out of these are contained only in the Gpx3-dependent group, as indicated by an asterisk.

However, several proteins that were changed by Gpx3-dependent oxidative stress were proteins related to other cellular mechanisms. In order to investigate whether these proteins were due to Gpx3-dependency, we analyzed proteins that were changed in a Gpx3-dependent manner (Table 3). Interestingly, it was found that AHP1 is regulated in a Yap1-dependent manner under oxidative stress conditions. These data showed that Gpx3 is closely related to the Yap1-dependent signaling mechanism [7, 36]. Moreover, the other proteins, 3,4-dihydroxy-2-butanone 4-phosphate synthase (RIB3), BMH1 protein (BMH1), and dihydroorotate dehydrogenase (URA1), were not directly related to the oxidative stress response, but participated in various cellular mechanisms such as biogenesis of cellular components, cell cycle and DNA processing, and energy metabolism to defense cells. In conclusion, these results suggest that, in addition to Yap1 activation, Gpx3 has multiple functions in the adaptive oxidative stress response.

In this study, a large number of *S. cerevisiae* proteins that display H_2O_2-dependent changes have been identified. This work shows that a diverse range of cellular processes is affected by oxidative stress, and further demonstrates the role of the Gpx3-dependent responses against the adaptive oxidative stress response. Although a number of the identified protein changes were correlated with the results of previous proteomic analysis of the H_2O_2 general response in the *S. cerevisiae* [8, 13], other proteins were also identified. Thus, this work highlights the Gpx3-dependent cellular response to oxidative stress. Furthermore, putative targets of the oxidative stress response and Gpx3-dependent proteins were identified. However, the real value of the data can only be discerned in terms of the adaptive oxidative stress response. Such a systematic approach allows significantly more detail to be proposed regarding how various cellular processes are affected by oxidative stress and the Gpx3-dependent defense mechanism in addition to how regulation is achieved. It is clear that the stress response is global and has profound effects on multiple events and that Gpx3 is a critical modulator of these events - in particular during the oxidative stress response.

Acknowledgment

This research was partially supported by the Ministry of Information and Communication, Korea, under the Information Technology Research Center support program supervised by the Institute of Information Technology Assessment (IITA-2007-C1090-0701-0014) and by the Development of Next-Generation New Technology program (10024715-

2005-11) of the Korean Ministry of Commerce, Industry and Energy.

REFERENCES

1. Basu, U., J. L. Southron, J. L. Stephens, and G. J. Taylor. 2004. Reverse genetic analysis of the glutathione metabolic pathway suggests a novel role of PHGPX and URE2 genes in aluminum resistance in *Saccharomyces cerevisiae*. *Mol. Genet. Genom.* **271:** 627–637.
2. Baudin, A., O. Ozier-Kalogeropoulos, A. Denouel, F. Lacroute, and C. Cullin. 1993. A simple and efficient method for direct gene deletion in *Saccharomyces cerevisiae*. *Nucl. Acids Res.* **21:** 3329–3330.
3. Brombacher, K., B. B. Fischer, K. Rufenacht, and R. I. Eggen. 2006. The role of Yap1p and Skn7p-mediated oxidative stress response in the defense of *Saccharomyces cerevisiae* against singlet oxygen. *Yeast* **23:** 741–750.
4. Caesar, R., J. Warringer, and A. Blomberg. 2006. Physiological importance and identification of novel targets for the N-terminal acetyltransferase NatB. *Euk. Cell* **5:** 368–378.
5. Carmel-Harel, O., R. Stearman, A. P. Gasch, D. Botstein, P. O. Brown, and G. Storz. 2001. Role of thioredoxin reductase in the Yap1p-dependent response to oxidative stress in *Saccharomyces cerevisiae*. *Mol. Microbiol.* **39:** 595–605.
6. Costa, V. and P. Moradas-Ferreira. 2001. Oxidative stress and signal transduction in *Saccharomyces cerevisiae*: Insights into ageing, apoptosis and diseases. *Mol. Aspects Med.* **22:** 217–246.
7. Delaunay, A., D. Pflieger, M. B. Barrault, J. Vinh, and M. B. Toledano. 2002. A thiol peroxidase is an H_2O_2 receptor and redox-transducer in gene activation. *Cell* **111:** 471–481.
8. Demasi, A. P., G. A. Pereira, and L. E. Netto. 2006. Yeast oxidative stress response. Influences of cytosolic thioredoxin peroxidase I and of the mitochondrial functional state. *FEBS J.* **273:** 805–816.
9. Dormer, U. H., J. Westwater, D. W. Stephen, and D. J. Jamieson. 2002. Oxidant regulation of the *Saccharomyces cerevisiae* GSH1 gene. *Biochim. Biophys. Acta* **1576:** 23–29.
10. Estruch, F. 2000. Stress-controlled transcription factors, stress-induced genes and stress tolerance in budding yeast. *FEMS Microbiol. Rev.* **24:** 469–486.
11. Fedoroff, N. 2006. Redox regulatory mechanisms in cellular stress responses. *Ann. Bot.* **98:** 289–300.
12. Gharahdaghi, F., C. R. Weinberg, D. A. Meagher, B. S. Imai, and S. M. Mische. 1999. Mass spectrometric identification of proteins from silver-stained polyacrylamide gel: A method for the removal of silver ions to enhance sensitivity. *Electrophoresis* **20:** 601–605.
13. Godon, C., G. Lagniel, J. Lee, J. M. Buhler, S. Kieffer, M. Perrot, H. Boucherie, M. B. Toledano, and J. Labarre. 1998. The H_2O_2 stimulon in *Saccharomyces cerevisiae*. *J. Biol. Chem.* **273:** 22480–22489.
14. Grant, C. M., F. H. Maciver, and I. W. Dawes. 1996. Stationary-phase induction of GLR1 expression is mediated by the yAP-1 transcriptional regulatory protein in the yeast *Saccharomyces cerevisiae*. *Mol. Microbiol.* **22:** 739–746.
15. Hahn, J. S., D. W. Neef, and D. J. Thiele. 2006. A stress regulatory network for co-ordinated activation of proteasome expression mediated by yeast heat shock transcription factor. *Mol. Microbiol.* **60:** 240–251.
16. Harbison, C. T., D. B. Gordon, T. I. Lee, N. J. Rinaldi, K. D. Macisaac, T. W. Danford, N. M. Hannett, J. B. Tagne, D. B. Reynolds, *et al.* 2004. Transcriptional regulatory code of a eukaryotic genome. *Nature* **431:** 99–104.
17. Hasan, R., C. Leroy, A. D. Isnard, J. Labarre, E. Boy-Marcotte, and M. B. Toledano. 2002. The control of the yeast H_2O_2 response by the Msn2/4 transcription factors. *Mol. Microbiol.* **45:** 233–241.
18. Inoue, Y., T. Matsuda, K. Sugiyama, S. Izawa, and A. Kimura. 1999. Genetic analysis of glutathione peroxidase in oxidative stress response of *Saccharomyces cerevisiae*. *J. Biol. Chem.* **274:** 27002–27009.
19. Kho, C. W., P. Y. Lee, K. H. Bae, S. Y. Cho, Z. W. Lee, B. C. Park, S. M. Kang, D. H. Lee, and S. G. Park. 2006. Glutathione peroxidase 3 of *Saccharomyces cerevisiae* regulates the activity of methionine sulfoxide reductase in a redox state-dependent way. *Biochem. Biophys. Res. Commun.* **348:** 25–35.
20. Kuge, S. and N. Jones. 1994. YAP1 dependent activation of TRX2 is essential for the response of *Saccharomyces cerevisiae* to oxidative stress by hydroperoxides. *EMBO J.* **13:** 655–664.
21. MacIsaac, K. D., T. Wang, D. B. Gordon, D. K. Gifford, G. D. Stormo, and E. Fraenkel. 2006. An improved map of conserved regulatory sites for *Saccharomyces cerevisiae*. *BMC Bioinformatics* **7:** 113.
22. Martindale, J. L. and N. J. Holbrook. 2002. Cellular response to oxidative stress: Signaling for suicide and survival. *J. Cell Physiol.* **192:** 1–15.
23. Menant, A., P. Baudouin-Cornu, C. Peyraud, M. Tyers, and D. Thomas. 2006. Determinants of the ubiquitin-mediated degradation of the Met4 transcription factor. *J. Biol. Chem.* **281:** 11744–11754.
24. Myung, J. K. and G. Lubec. 2006. Use of solution-IEF-fractionation leads to separation of 2673 mouse brain proteins including 255 hydrophobic structures. *J. Proteome Res.* **5:** 1267–1275.
25. Nguyen, D. T., A. M. Alarco, and M. Raymond. 2001. Multiple Yap1p-binding sites mediate induction of the yeast major facilitator FLR1 gene in response to drugs, oxidants, and alkylating agents. *J. Biol. Chem.* **276:** 1138–1145.
26. Park, S. G., C. W. Kho, S. Y. Cho, D. H. Lee, S. H. Kim, and B. C. Park. 2002. A functional proteomic analysis of secreted fibrinolytic enzymes from *Bacillus subtilis* 168 using a combined method of two-dimensional gel electrophoresis and zymography. *Proteomics* **2:** 206–211.
27. Pedrajas, J. R., P. Porras, E. Martinez-Galisteo, C. A. Padilla, A. Miranda-Vizuete, and J. A. Barcena. 2002. Two isoforms of *Saccharomyces cerevisiae* glutaredoxin 2 are expressed *in vivo* and localize to different subcellular compartments. *Biochem. J.* **364:** 617–623.
28. Reverter-Branchat, G., E. Cabiscol, J. Tamarit, and J. Ros. 2004. Oxidative damage to specific proteins in replicative and chronological-aged *Saccharomyces cerevisiae*: Common targets and prevention by calorie restriction. *J. Biol. Chem.* **279:** 31983–31989.
29. Ross, S. J., V. J. Findlay, P. Malakasi, and B. A. Morgan. 2000. Thioredoxin peroxidase is required for the transcriptional response

to oxidative stress in budding yeast. *Mol. Biol. Cell* **11:** 2631–2642.

30. Sies, H. and E. Cadenas. 1985. Oxidative stress: Damage to intact cells and organs. *Philos. Trans R. Soc. Lond. B Biol. Sci.* **311:** 617–631.
31. Stephen, D. W., S. L. Rivers, and D. J. Jamieson. 1995. The role of the YAP1 and YAP2 genes in the regulation of the adaptive oxidative stress responses of *Saccharomyces cerevisiae*. *Mol. Microbiol.* **16:** 415–423.
32. Stone, J. R. and S. Yang. 2006. Hydrogen peroxide: A signaling messenger. *Antioxid. Redox Signal.* **8:** 243–270.
33. Strain, J., C. R. Lorenz, J. Bode, S. Garland, G. A. Smolen, D. T. Ta, L. E. Vickery, and V. C. Culotta. 1998. Suppressors of superoxide dismutase (SOD1) deficiency in *Saccharomyces cerevisiae*. Identification of proteins predicted to mediate iron-sulfur cluster assembly. *J. Biol. Chem.* **273:** 31138–31144.
34. Sturtz, L. A., K. Diekert, L. T. Jensen, R. Lill, and V. C. Culotta. 2001. A fraction of yeast Cu,Zn-superoxide dismutase and its metallochaperone, CCS, localize to the intermembrane space of mitochondria. A physiological role for SOD1 in guarding against mitochondrial oxidative damage. *J. Biol. Chem.* **276:** 38084–38089.
35. Tsuzi, D., K. Maeta, Y. Takatsume, S. Izawa, and Y. Inoue. 2004. Regulation of the yeast phospholipid hydroperoxide glutathione peroxidase GPX2 by oxidative stress is mediated by Yap1 and Skn7. *FEBS Lett.* **565:** 148–154.
36. Veal, E. A., S. J. Ross, P. Malakasi, E. Peacock, and B. A. Morgan. 2003. Ybp1 is required for the hydrogen peroxide-induced oxidation of the Yap1 transcription factor. *J. Biol. Chem.* **278:** 30896–30904.
37. Vido, K., D. Spector, G. Lagniel, S. Lopez, M. B. Toledano, and J. Labarre. 2001. A proteome analysis of the cadmium response in *Saccharomyces cerevisiae*. *J. Biol. Chem.* **276:** 8469–8474.
38. Wach, A., A. Brachat, R. Pohlmann, and P. Philippsen. 1994. New heterologous modules for classical or PCR-based gene disruptions in *Saccharomyces cerevisiae*. *Yeast* **10:** 1793–1808.
39. Weiss, A., J. Delproposto, and C. N. Giroux. 2004. High-throughput phenotypic profiling of gene-environment interactions by quantitative growth curve analysis in *Saccharomyces cerevisiae*. *Anal. Biochem.* **327:** 23–34.
40. Yoon, S. O., C. H. Yun, and A. S. Chung. 2002. Dose effect of oxidative stress on signal transduction in aging. *Mech. Ageing Dev.* **123:** 1597–1604.