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The Physiological Role of CPR1 in *Saccharomyces cerevisiae* KNU5377 against Menadione Stress by Proteomics

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In order to understand the functional role of CPR1 in *Saccharomyces cerevisiae* KNU5377 with regard to its multi-tolerance characteristics against high temperatures, inorganic acids, and oxidative stress conditions, whole cellular proteins were analyzed via liquid chromatography electrospray ionization tandem mass spectrometry (LC-ESI-MS/MS). This procedure was followed by two-dimensional (2-D) gel electrophoresis. Under menadione stress conditions, the 23 upregulated proteins were clearly identified only in the wild-type strain of KNU5377. Among the proteins, Sod1p, Tsa1p, Ahp1, Cpr1p, Cpr3, Ssb2p, and Hsp12p were identified as components of antioxidant systems or protein-folding related systems. The CPR1 protein could not be completely detected in the *cpr1*Δ mutant of KNU5377 and the other upregulated proteins in the wild-type strain evidenced a clear correlation with the results of immunoblot analysis. Moreover, a reduction in growth patterns (about 50%) could be observed in the *cpr1*Δ mutant, as compared with that of the wild-type strain under mild MD stress conditions. These results indicate that the upregulation of CPR1 may contribute to tolerance against MD as an inducer of oxidative stress.

Keywords: CPR1, oxidative stress, *Saccharomyces cerevisiae* KNU5377, proteome

Cyclophilin A (CypA) was isolated from both yeast and human cytosol, sharing a sequence identity of approximately 80% (Galat and Metcalfe, 1995). Although CypA is conserved to a high degree from yeast to humans, the normal biological functions of the protein remain to be thoroughly clarified. CypA is a member of a family of ubiquitous family, and it is a highly-conserved enzyme referred to as a peptidyl *cis-trans* isomerase (PPIase), or a prolyl-isomerase, which catalyzes the *cis-trans* isomerization of peptide bonds that precede proline residues (Arevalo-Rodriguez *et al.*, 2000). For all amino acids with the exception of proline, the *trans* isomer is the preferred conformer, as the result of steric clashes in the *cis* form. By way of contrast, the peptide bond that precedes proline residues is equally stable as a number of *cis* and *trans* isomers. Approximately 10% of the peptidyl-prolyl bonds of recently identified proteins exist in the *cis* form.

The prolyl-isomerase group spans three structurally-unrelated protein families: The cyclophilins, the FKBPs (FK506 binding proteins), and the parvulins. All three of these families exist in the budding yeast *Saccharomyces cerevisiae*, which expresses eight different cyclophilins (Cpr1 to Cpr8), four FKBPs (Fpr1 to Fpr4), and a single parvulin (Ess1), as the only essential prolyl-isomerase in this organism (Arevalo-Rodriguez and Heitman, 2005). The cyclophilins and FKBPs are notable because the cyclophilin A and FKBP12 members of these families mediate the effects of the immunosuppressive agents, cyclosporin A (CsA), FK506, and rapamycin (Cardenas *et al.*, 1995; Arevalo-Rodriguez *et al.*, 2000).

The yeast cyclophilin homologue, Cpr1 (cyclosporin A-sensitive proline rotamase 1), which is encoded for by the *CPR1* gene, is known to be highly conserved (Wang and Heitman, 2005). Cpr1 is localized in the cytoplasm and nucleus, and accumulates within the nucleus (Arevalo-Rodriguez *et al.*, 2004). One of the functions of Cpr1 has been reported as compromising Ess1; consequently, Cpr1 overexpression suppresses *ess1*Δ mutations. This indicates that there is a functional overlap between these two structurally-unrelated prolyl-isomerases. This provides the first known evidence regarding the activity of yeast cyclophilin A (Arevalo-Rodriguez and Heitman, 2005). Cpr1 is required for the glucose-stimulated transport of fructose-1,6-biphosphatase (FBPase) into Vid (Vacuole import and degradation) vesicles, thus resulting in the degradation of this glucolytic enzyme (Brown *et al.*, 2001). Cpr1 may also promote the appropriate subcellular localization of an essential zinc finger protein, Zpr1 (Arevalo-Rodriguez and Heitman, 2005). Finally, Cpr1 modulates the activity of two different histone-diacetylase complexes (Sin3-Rpd3 and Set3C) (Arevalo-Rodriguez *et al.*, 2000; Brown *et al.*, 2001), and this may result in transcriptional events during the switch from mitotic to meiotic cell division (Arevalo-Rodriguez and Heitman, 2005; Wang and Heitman, 2005).

Cpr1 expression is induced under stress conditions. In particular, the transcription of the genes may be regulated by a conserved heat shock response element (HSE) (Sykes *et al.*, 1993). Two stress response elements (STREs) have been

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identified within the *CPR1* promoter region. Gene expression could be also regulated by zinc-finger proteins including Msn2p/Msn4p or the MAP kinase Hog1 (Moskvina *et al.*, 1998). In the current report, *CPR1* transcription is activated by NaCl and sorbic acid, rather than by ethanol. Although there was no loss of viability in the *cpr1*Δ mutant after being exposed to high temperatures, Cpr1 may play a crucial role in providing tolerance against heat stress (Ansari *et al.*, 2002; Arevalo-Rodriguez *et al.*, 2004).

In this report, we analyzed whole cellular proteins via two major proteomic techniques; 2-D gel electrophoresis and liquid chromatography coupled with mass spectrometry (LC-MS/MS) under menadione (MD) as an oxidative stressor in KNU5377 cells. The function of one of the upregulated proteins, Cpr1, was elucidated thoroughly using the *cpr1*Δ mutant.

Materials and Methods

Strains and stress conditions

S. cerevisiae KNU5377 was isolated from sewage soil acquired from Daegu, Republic of Korea. The *cpr1*Δ mutant was constructed from haploid KNU5377 (KNY5377Y) cells in accordance with our previous study (Kim *et al.*, 2006). Yeast cells were grown aerobically in a nutrient-rich YEPD medium (1% yeast extract, 2% peptone, 2% dextrose) for 20 h at 30°C, with shaking at 160 rpm. The mid-log cultured cells ($O.D._{600}$ =1.0), following pre-cultivation at 30°C overnight, were challenged for 1 h at 30°C in YEPD liquid media, with a 0.4 mM concentration of menadione (MD).

Growth rate and cell viability

After the cultivation of wild-type and Δ*cpr1* mutants into the mid-log phase, the concentration of cells was adjusted to approximately $O.D._{600}$=0.2 and subsequently, 40 μM of MD was added to YPED media in order to monitor the growth rate at the indicated time intervals. In order to determine the cell viability, the cells were exposed to the indicated concentrations of MD for 14 h at 30°C after cultivation to the mid-log phase, after which the optical density was measured at 600 nm.

Immunoblot analysis

SDS-PAGE was conducted as reported previously (Kim *et al.*, 2006). After SDS-PAGE, the gels were transferred electrophoretically to a PVDF membrane (Bio-Rad, USA) using a transfer buffer (25 mM of Tris-base, 192 mM of glycine and 20% methanol). The PVDF membrane was blocked for 60 min at room temperature via the TTBS buffer (0.05% Tween-20, 10 mM of Tris-HCl; pH 7.6, and 150 mM of NaCl), which contained 5% non-fat skim milk and 0.02% sodium azide. The blotted membranes were then incubated overnight at 4°C with primary antibodies: anti-Sod1p (Stressgen, Canada), anti-Tsa1p (kindly provided by Prof. Park Jeen Woo of Kyungpook National University, Repubic of Korea), anti-Cpr1p (kindly provided by Dr Joseph Heitman, USA), anti-G6PDH (Sigma, USA), anti-Adhp, anti-Aldp (Rockland, USA), and anti-GAPDH and anti-actin (Abcam, UK). After four washings in TTBS, the membranes were incubated for 90 min at room temperature with a secondary antibody, either an anti-rabbit IgG (H+L) HRP conjugate (Promega, USA) or an anti-mouse IgG (Amersham Biosciences, Sweden). The membranes were washed 4 times in TTBS, left to develop via enhanced chemiluminescence (ECL kit, Amersham Biosciences), and processed.

Sample preparation for two-dimensional gel electrophoresis

After washing in PBS, the harvested cells were agitated five times for 5 min using a MicroMixer with a lysis buffer containing 80 mM of Tris-HCl (pH 8.0), 2% SDS, 1.5% β-mercaptoethanol, 10% glycerol, 1 mM of PMSF, and a protease inhibitor cocktail (Boehringer Mannheim, Germany) and an equal amount of glass beads (400-600 microns; Sigma, USA). The supernatants were immediately boiled for 5 min and cooled for 5 min on ice. Following centrifugation, proteins in the supernatants were precipitated for 1 h with trichloroacetic acid (TCA; final conc. 10%) on ice, centrifuged for 20 min at 15,000 rpm at 4°C, and then washed five times in HPLC-grade ethanol. The washed pellets were dried with a Speed Vac. The pellets were resuspended in a sample buffer containing 9.5 M of urea, 4% CHAPS, 0.1 M of DTT and 0.2% Bio-Lyte (3-10, Bio-Rad) for 1 h at room temperature and were centrifuged for 30 min at 15,000 rpm. The supernatants were then transferred to new Eppendorf tubes. The protein concentrations were measured via a modified method with Protein Assay reagent (Bio-Rad).

Two-dimensional gel electrophoresis

First-dimensional isoelectric focusing (IEF) was conducted at 20°C on commercial 17 cm, pH 4.0-7.0 immobilized pH gradient (IPG) strips, with a maximum current limitation of 50 μA/strip in PROTEAN IEF Cells (Bio-Rad). After 16 h of passive rehydration, the protein samples (1.0 mg) were loaded onto the bottoms of IPG strips. IEF was conducted via the following steps: 250 V for 1 h, 1,000-10,000 V for 6 h, and 80,000 V·h at 10,000 V. After focusing, the strips were equilibrated twice for 15 min each with the first and second equilibration buffers, which were composed of 30% (w/v) glycerol, 6 M of urea, 2% (w/v) sodium dodecyl sulfate (SDS), 50 mM of Tris-HCl (pH 8.8), 65 mM of dithiothreitol (DTT), and a trace of bromophenol blue. The second equilibration was replaced with 260 mM of iodoacetamide (IAA) rather than DTT. The gels were subjected to two-dimensional SDS-polyacrylamide gel electrophoresis (SDS-PAGE). The second dimension was conducted on 1.0 mm thick, 12.5% polyacrylamide gels at a constant current of 20 mA per gel in a PROTEAN® II xi Cell (Bio-Rad) at 14°C. After the gels were fixed for 12 h in 40% methanol and 10% acetic acid, the gels were stained for 3 h with Coomassie Brilliant Blue R-250 (Sigma), then destained. Spots of interest and those being upregulated in the wild-type strain were excised, and mass spectrometry was conducted.

LC-ESI-MS/MS

The protein spots were excised and destained with 30% methanol. Two-hundred mM ammonium bicarbonate was added to the gel-pieces and mixed for 30 min. The liquid was discarded and the washing process was repeated. The gel-pieces were shrunk via dehydration in 100% acetonitrile, removed, and dried with a Speed Vac. Enzymatic digestion

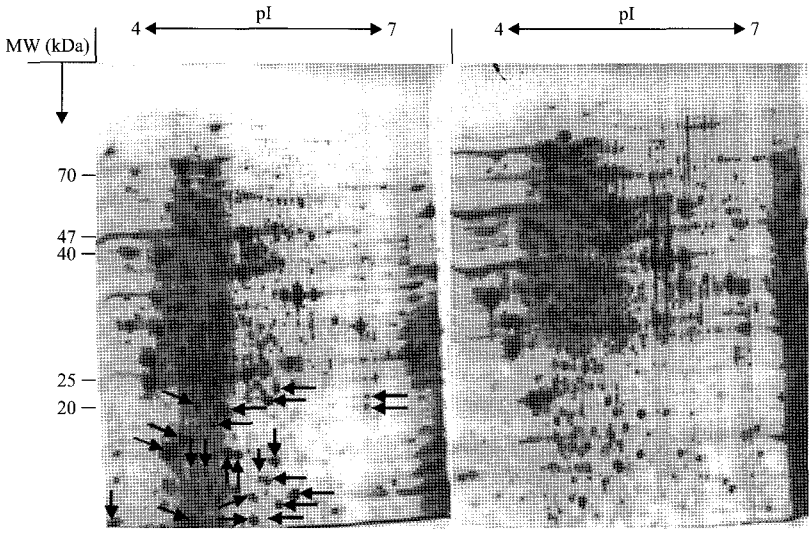

Fig. 1. A comparative analysis via 2-D gel electrophoresis of whole cellular proteins between the wild-type and *cpr1* mutant strains of *S. cerevisiae* KNU5377Y under menadione stress conditions. Total soluble protein samples were obtained from exponentially-growing cells, which were treated for 1 h with 0.4 mM of menadione. After rehydration, the first dimension was performed on a ready-made IPG strip (pH 4.0-7.0); separation distance, and 170 mm; sample application (1.0 mg protein). The running conditions are as follows: Step 1: at 250 V for 1 h, Step 2: linear ramping between 250 V and 10,000 V for 6 h, and Step 3: for 80,000 V·h at 10,000 V. After SDS-PAGE (12%), two DE images were obtained for the wild-type (left) and *cpr1* mutant (right) strains.

Table 1. Proteins identified by LC-ESI-MS/MS

No	Protein	Peptide	MW (Da)
1	Putative protein of unknown function (Hbn1p)	R.RTIYALKPELPGEITINDIQSVVQTIIK.E	20993.7
2	Fructose 1,6-bisphosphate aldolase (Fba1p)	K.FAIPAINVTSSSTAVAALEAAR.D	39620.5
3	Ubiquitous housekeeping thioredoxin peroxidase (Tsa1p)	R.KEGGLGPINIPLLADTNHSLSR.D	21589.6
4	Subunit beta1 of NAC (Egd1p)	K.VGVQVAAQHNTSVFYGLPQEK.N	17020.1
5	Protein of unknown function (Pst2p)	K.NVFAELTNM*DEVHGGSPWGAGTIAGSDGSR.S	20965.5
6	Translation initiation factor eIF-5A (Hyb2p)	K.KLEDLSPSTHNMEVPVVK.R	17114.2
7	Glyceraldehyde-3-phosphate dehydrogenase, isozyme 3 (Tdh3p)	K.VINDAFGIEEGLMTTVHSLTATQK.T	35746.4
8	Cu, Zn superoxide dismutase (Sod1p)	R.GFHIHEFGDATNGCVSAGPHFNPFK.K	15854.5
9	Thiol-specific peroxiredoxin (Ahp1p)	R.WAMVVENGIVTYAAK.E	19114.5
10	B Chain B, Yeast Guanine Nucleotide Exchange Factor	K.AIEMEGLTWGAHQFIPIGFGIK.K	10404.7
11	Mitochondrial peptidyl-prolyl cis-trans isomerase (Cpr3p)	K.HVVFGEVTKGM*DIVK.A	19918.6
12	3-phosphoglycerate kinase (Pgk1p)	K.ISHVSTGGGASLELLEGK.E	44738.1
13	Cytoplasmic peptidyl-prolyl cis-trans isomerase (Cpr1p)	R.VIPDFM*LQGGDFTAGNGTGGK.S	17390.6
14	Cofilin (Cof1p)	R.SGVAVADESLTAFNDLK.L	15900.7
15	E1 alpha subunit of the pyruvate dehydrogenase (PDH) complex (Yer178wp)	K.MHLIDLGIATEAEVK.A	46342.8
16	Ribosomal protein P2 alpha, a component of the ribosomal stalk (Rpp2ap)	K.YLAAYLLLNAAGNTPDATK.I	10745.7
17	Cytoplasmic ATPase (Ssb2p)	K.SSNITISNAVGR.L	66594.1
18	Protein component of the large (60S) ribosomal subunit (Rpl22ap)	K.VEGAVGNLGNAVTVTEDGTVVTVVSTAK.F	13693.3
19	Plasma membrane localized protein that protects membranes (Hsp12p)	K.LNDAVEYVSGR.V	11692.6
20	Ribosomal protein P2 beta, a component of the ribosomal stalk (Rpp2bp)	K.GSLEEIIAEGQK.K	11050.0
21	Subunit of the SF3a splicing factor complex (Prp9p)	K.TSSVFESHLVGKIHKK.N	63029.0
22	Glyceraldehyde-3-phosphate dehydrogenase, isozyme 2 (Tdh2p)	K.VINDAFGIEEGLMTTVHSMTATQK.T	35846.6
23	Translation initiation factor eIF-5A (Anb1p)	K.APEGELGDSM*QAAFDEGK.D	17131.2

was conducted via the addition of 20 μl of 0.0125 μg/μl of sequence grade modified trypsin (Promega) to 50 mM ammonium bicarbonate and 5 mM calcium chloride. The mixtures were then incubated for 16 h at 37°C, and then dried with a Speed Vac. The pellets containing a peptide mixture were utilized for LC-ESI-MS/MS. The LC-ESI-MS/MS analyses were conducted by the Yonsei Proteome Research Center (www.proteomix.org) at Yonsei University.

Results

Proteomic analysis

We performed comparative analyses of protein expression between the wild-type and the *cpr1*Δ mutant of *S. cerevisiae* KNU5377Y during MD stress, via LC-ESI-MS/MS followed by 2-D PAGE. As is shown in Fig. 1 and Table 1, the 23 upregulated proteins in the wild-type strain under MD stress were identified by LC-ESI-MS/MS. Among the proteins, Cu, Zn superoxide dismutase (Sod1p), ubiquitous housekeeping thioredoxin peroxidase (Tsa1p), thiol-specific peroxiredoxin (Ahp1), cytoplasmic peptidyl-proryl *cis-trans* isomerase (Cpr1p), mitochondrial peptidyl-proryl *cis-trans* isomerase (Cpr3), cytoplasmic ATPase (Ssb2p) and plasma membrane-localized protein which protects the membrane (Hsp12p), were classified as either antioxidant systems or protein-folding related systems. These proteins comprise an important antioxidant system in the oxidative stress response.

Immunoblot analysis

In order to confirm the upregulated proteins in the wild-type strain, we performed translational expression changes of Sod1p, Tsa1p, glucose-6-phosphate dehydrogenase (G6PDH), alcohol dehydrogenase (Adhp) and aldehyde dehydrogenase (Aldp) via immunoblot analysis between the wild-type and *cpr1*Δ mutant. As is shown in Fig. 2, we observed Cpr1 expression in the *cpr1*Δ mutant strain. After the administration of MD treatment, the expressions of Cpr1, Sod1, and Tsa1 were upregulated significantly in the wild-type strain; however, the expressions of Sod1 and Tsa1 were downregulated in the mutant strain (Figs. 2A and 2B). Although the expressions of glucose-6-phosphate dehydrogenase (G6PDH) and aldehyde dehydrogenase (Aldp) were upregulated in

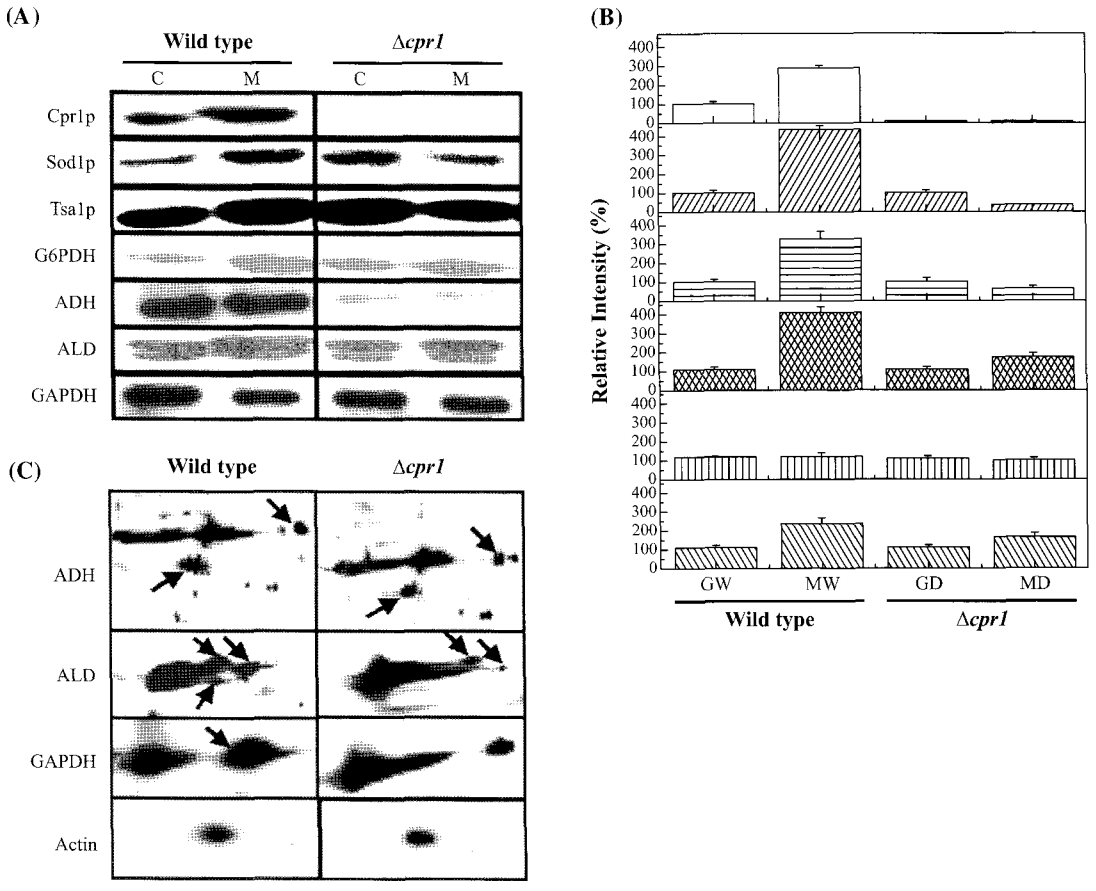

Fig. 2. Western-blot detection of antioxidant enzymes under menadione stress. Mid-log cultured *S. cerevisiae* KNU5377Y cells were treated for 1 h with 0.4 mM of menadione at 30°C. After the preparation of the cell lysates, 30 μg of proteins were separated via SDS-PAGE and transferred to PVDF membranes, which were probed with each antibody. Immunodetection was conducted via chemiluminescence. The PVDF membranes were exposed to Hybond-ECL films, and the films were developed. The immunoblot detection of non-treated cells (C) and MD-treated cells (M)(A). Relative intensity corresponding with Immunoblot detection (B). After 2-D gel electrophoresis, the separated proteins were transferred to PVDF membranes, which were probed with each specific antibody. ADH, alcohol dehydrogenase; ALD, aldehyde dehydrogenase; G6PDH, glucose-6-phosphate dehydrogenase. Actin was used as a housekeeping control (C).

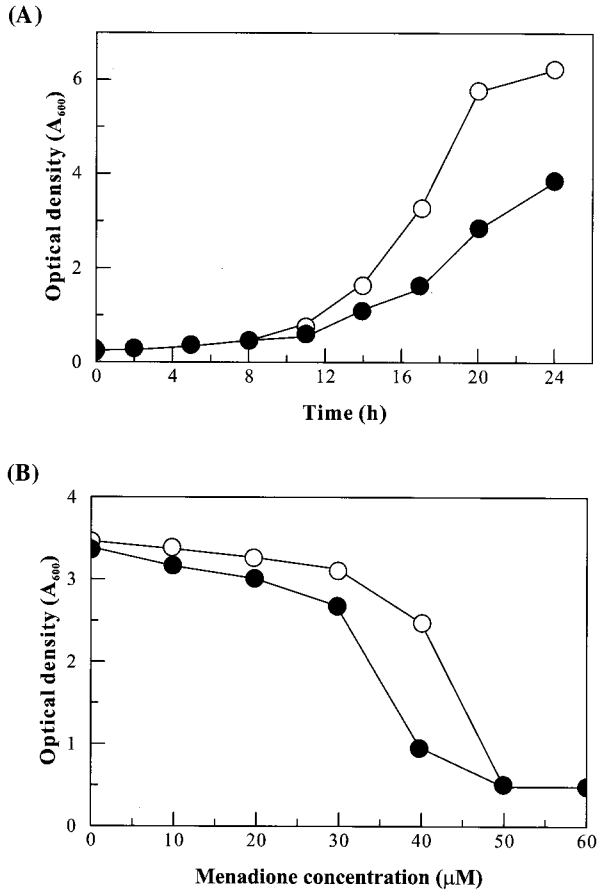

Fig. 3. The effect of menadione on the growth rate of the wild-type strain and the Δ*cpr1* mutant strains of *S. cerevisiae* KNU5377Y. After the pre-cultivation of the wild-type and Δ*cpr1* mutant strains overnight at 30°C, the concentration of cells was adjusted to approximately $O.D._{600}$=0.1 and subsequently, 40 μM of MD was added to the YPED media to monitor the growth rate (A). In order to measure cell viability, the cells were exposed to the indicated concentration of MD for 14 h at 30°C, after which the optical density was measured at 600 nm (B). Values represent the means of at least three independent experiments. ○, wild-type strain; ●, Δ*cpr1* mutant strain.

the wild-type and mutant strains, an expression of more than two-fold could be observed only in the wild-type strain. Moreover, we confirmed the expressions via immunoblot analysis after 2-DE (Fig. 2C).

Cell viability and growth rate

The growth rate of the wild-type and Δ*cpr1* mutant strains were compared under 40 μM of MD for 24 h. After 12 h, reduced growth in the mutant strain was observed. This reduction growth was approximately 60% as compared with that of the wild-type strain during the early stationary phase (Fig. 3A). After developing to the mid-log phase, two strains were challenged with various concentrations (0-60 μM) of MD for 14 h. Although there were no significant differences in viability between the two strains under 20 μM of MD, the viability of the Δ*cpr1* mutant strain evidenced a greater reduction, up to 30%, as compared with that of the wild-type strain (Fig. 3B).

Discussion

In this report, upregulated proteins including Cpr1, Sod1, and Tsa1 were identified under MD stress conditions. This was done in order to determine the level of tolerance against oxidative stress evidenced by the KNU5377Y cells (Fig. 1 and Table 1). By mutation of the *cpr1* gene, it could be confirmed that Cpr1 might contribute to the level of tolerance against MD (Fig. 3) and it was regulated in a HSF1-dependent manner under MD stress conditions in the KNU5377Y cells (data not shown). The *cpr1*Δ mutant was more sensitive to various types of stressors, including high temperature, hydrogen peroxide, high ethanol concentrations, osmotic shock, sulfuric acid, and lactic acid, than those of the wild-type strains in our previous study (Kim *et al.*, 2006).

Cytoplasmic ATPase (Ssbp) and heat-shock protein 12 (Hsp12p) were overexpressed in the wild-type strain, but not in the *cpr1*Δ mutant during MD stress. Many heat-shock proteins (Hsps) that function as molecular chaperones could also function as an excellent stress indicator in laboratory strains of *S. cerevisiae* (Estrruch, 2000), and they may perform a pivotal function in cellular resistance against a variety of stresses, with the exception of Hsp104 (Davidson *et al.*, 1996). Although the cellular functions of HSPs in stress responses have yet to be well established in the *S. cerevisiae*, it is clear that the Hsp104 and Hsp70 families, and small Hsps (sHsps), such as Hsp12p, might contribute to stress tolerance, in order to prevent protein aggregation and to promote the proteolysis of aberrant stress-damaged proteins (Dijck and Walker, 2006).

A study of deficient mutants of antioxidant systems harboring Sod1 and Tsa1 showed that only the mutant strain was sensitive to hydrogen peroxide and MD as oxidative stressors (Christian *et al.*, 1998; Tucker and Fields, 2004). However, the wild-type strain evidenced tolerance to stress and performed a protective function for cellular proteins against stressors, via the overexpression of Sod (Davidson *et al.*, 1996; Estrruch, 2000; Lushckak and Gospodaryov, 2005). The mitochondrial cyclophilin, Cpr3, catalyzes the *cis-trans* isomerization of the peptide bonds at the N-terminal to proline residues, and accelerates protein refolding following the importation of mitochondria, particularly at elevated temperatures. Upregulated proteins were observed under MD stress in the wild-type yeast, but not in the *cpr1*Δ mutant cells. Egd1p (subunit beta 1 of the nascent polypeptide-associated complex) is associated with cytoplasmic ribosomes, and enhances the DNA binding of the Gal4p activator (George *et al.*, 1998; Reimann *et al.*, 1999), Hyp2p. The translation initiation factor, eIF-5A, binds to translational machinery components. The putative translation factor eIF5A is highly conserved from the archaeobacteria to mammals, and is essential for cell viability via interaction with the structural components of the 80S ribosome, as well as with the translation elongation factor 2 (eEF2). The eIF5A mutant cells evidence altered polysome profiles, and are sensitive to translation inhibitors (Zanelli *et al.*, 2006). Nitroreductases comprise a family of eubacterial conserved sequences that catalyze the reduction of nitrosubstituted compounds which

generate reactive nitrogen oxide species (RNOxS), using FMN or FAD as a prosthetic group and NADH or NADPH as reducing power agents (de Oliveria *et al.*, 2007). Pst2p is an unknown protein, which is similar to a family of flavodoxin-like proteins induced by oxidative stress in a Yap1p-dependent manner (Lee *et al.*, 1999).

Due to the fact that the genetic background of the wild-type and industrial strain has yet to be fully elucidated, the selection of a transformed strain is difficult to select. Therefore, we conducted an experiment in order to confirm the mutation effects in a laboratory strain of *S. cerevisiae* BY4741 (MATa; *his3Δ1*; *leu2Δ0*; *met15Δ0*; *ura3Δ0*; *YDR155c::kanMX4*, http://web.uni-frankfurt.de/fb15/mikro/euroscarf/), and constructed a strain expressing CPR1 with the yeast expression vector, pVTU260, and determined that the CPR1-expressing strain is more resistant to oxidative stress than the Δ*cpr1* mutant (data not shown).

In this study, Tdh2p and Tdh3p coding for glyceraldehyde-3-phosphate dehydrogenase and Pgk1p coding for 3-phosphoglycerate kinase were induced during MD stress. Tdh2p and Tdh3p have been detected in exponentially-growing cells (Delgado *et al.*, 2001). *PGK1* is expressed abundantly in cells growing in glucose, and their transcription levels are increased as the result of heat shock (Piper *et al.*, 1986). Recently, cyclophilin has been suggested to facilitate the operation of a variety of reducing systems, such as thioredoxin or glutaredoxin systems, in eukaryotic organisms containing yeast, or AhpF and AhpD in bacteria as disulfide reducers, and this has been correlated with the levels of peroxidase expression (Rouhier and Jacquot, 2005; Bona *et al.*, 2007).

In conclusion, we utilized a proteomic technique to identify upregulated proteins under MD stress in KNU5377Y cells, that evidence tolerance against multi-stress, as was previously reported. Although the functions of Cpr1 in yeast strains remain to be clarifed, it was determined that the protein might contribute to the tolerance of KNU5377Y against MD under oxidative stress conditions.

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