Available online at www.sciencedirect.com

SCIENCE DIRECT®

Biochimica et Biophysica Acta 1642 (2003) 9–16

BIOCHIMICA ET BIOPHYSICA ACTA

BBA

www.bba-direct.com

Acquisition of heat shock tolerance by regulation of intracellular redox states

Jeonghoon Ueom, Soonhyun Kwon, Sunhee Kim, Youngkee Chae, Kyunghee Lee*

Department of Applied Chemistry and Recombinant Protein Expression Center, Sejong University, 98 Gunja-Dong, Gwangjin-Gu, Seoul 143-747, South Korea

Received 14 January 2003; received in revised form 5 May 2003; accepted 4 June 2003

Abstract

In the yeast *Saccharomyces cerevisiae*, a mild heat treatment strongly induces Hsp104p which provides acquisition of thermotolerance. The mechanism by which Hsp104p protects cells from the severe heat shock has not yet been completely elucidated. In this study, a pivotal role of Hsp104p as an efficient scavenger of the reactive oxygen species (ROS) is investigated. In our previous study, we reported that Hsp104p acted as a regulator in the mitochondrial respiration pathway. In this report, the recombinant wild-type and hypersensitive *ras* mutants (*ira2Δ*) with the extrachromosomal plasmids wild-type and mutant hsp104 genes were studied. The resulting strains successfully expressed both wild-type and mutant Hsp104p and showed the thermotolerance phenotype in the strain with the functional wild-type Hsp104p expressed. Upon treatment with H_2O_2 and menadione, the strains with the functional Hsp104p expressed showed higher survival rates than the other mutants, indicating the protective role of Hsp104p from the oxidative stress. Fluorescence measurement of the oxidation-dependent probe, 2′,7′-dichlorofluoroscein diacetate (H_2DCFDA), also indicated that Hsp104p significantly reduced the amount of ROS. Resistance to the oxidative stress was independent of the amount of the glutathione in the hyperactivated *ras* mutants. Taken all together, this study confirms that Hsp104p plays a crucial role in keeping cells from being damaged by the oxidative stress, thus acting as a modulator of the intracellular redox state.

Keywords: Hsp104p; Thermotolerance; Oxidative stress; Reactive oxygen species (ROS); Ras/cAMP pathway

1. Introduction

Hsp104p plays a crucial role in providing thermotolerance in the budding yeast *Saccharomyces cerevisiae* [1]. When yeast cells are incubated at a sublethal temperature (37 °C for 30 min), Hsp104p is strongly induced and helps cells survive short exposures to extreme temperatures, otherwise lethal. The level of Hsp104p expression is also enhanced by a variety of other stresses, including ethanol, arsenate, and cadmium stresses [2].

It is not well understood how Hsp104p makes yeast cells resistant against extreme stress at a molecular level. It does not function as a molecular chaperon to ensure proper folding of nascent proteins, but helps cells resolubilize aggregated proteins formed by a severe stress [3]. Hsp104p contains two ATP binding domains and exhibits ATPase activities. In the absence of any of these two ATP binding sites, yeast cells lose not only thermotolerance but also resolubilization activity of aggregated proteins in vitro [4].

Interestingly, Hsp104p is constitutively expressed when grown in the acetate or galactose medium [5], implying that expression of Hsp104p may be controlled by respiratory pathway. As predicted, it was observed that Hsp104p functions as a regulator in the mitochondrial respiration pathway judging from the ^{13}C-NMR spectroscopy for quantification of the intermediates in the TCA cycle [6]. From the ^{13}C–^{13}C homonuclear data between the wild-type and the hsp104 deletion mutant (*hsp104Δ*), the turnover rate of the TCA cycle was found to be higher in the absence of Hsp104. The integrity of mitochondrial membrane was also impaired and the intracellular redox state consequently became imbalanced in *hsp104Δ* [7].

Most aerobic cells are very susceptible to reactive oxygen species (ROS) as by-products of respiration pathways. They have therefore developed multiple defense mechanisms where either the cellular antioxidant molecules or the antioxidant enzymes remove ROS to rescue cells from oxidative stress. ROS have been previously implicated as

* Corresponding author. Tel.: +82-2-3408-3214; fax: +82-2-462-9954.
E-mail address: khlee@sejong.ac.kr (K. Lee).

0167-4889/$ - see front matter © 2003 Elsevier B.V. All rights reserved.
doi:10.1016/S0167-4889(03)00081-8

being involved in mediating many signal transduction pathways such as the Ras–cAMP pathway [8]. In *S. cerevisiae*, the activity of adenylate cyclase is influenced by the Ras1 and Ras2 proteins which are positively regulated by the guanine nucleotide exchange factor (GEF) and negatively modulated by the GTPase activating proteins (GAPs), IRA-1 and IRA-2 [9–11]. With the *ira2* gene deleted, the mutant strain (*ira2Δ*) accumulates the activated Ras–GTP and causes an increased level of the intracellular cAMP [7]. Consequently, *ira2Δ* exhibits hypersensitive phenotypes when cells were submitted to a severe heat shock, nitrogen starvation, or sporulation defects. In our previous study, the hyperactivated *ras* mutant with *ira2* deleted became more sensitive in response to several oxidants including H_2O_2 and the superoxide anion generator, menadione [12].

To address the importance of functional Hsp104p on the protection from the oxidative stress, we have expressed Hsp104p wild-type and mutant proteins in the hypersensitive *ras* mutant. The phenotypes of transformed strains were verified by Western blotting and thermotolerance. To understand the metabolic regulation of ROS by Hsp104, the recombinant strains were treated with H_2O_2 and menadione to examine the survival rates. Fluorescence measurement of the oxidation-dependent probe, 2′,7′-dichlorofluoroscein diacetate (H_2DCFDA), indicated that Hsp104p significantly reduced the amount of ROS. Because the reduced glutathione (GSH) is also an important intracellular antioxidant, we have also attempted to correlate between the intracellular GSH level and the protection against the oxidative stress. Based on these investigations, we conclude that the presence of Hsp104p directly confers the cross protection against the heat shock and the oxidative stress.

2. Materials and methods

2.1. Yeast strains and plasmids

The yeast strains and their genotypes used in this study are as follows: SP1 (*MATa his3 leu2 ura3 trp1 ade8*) and *ira2Δ* (*MATa his3 leu2 ura3 trp1 ade8 ira2::ADE8*) [13] (Table 1). The YPD medium containing 1% (w/v) yeast extract, 2% (w/v) Bacto-peptone, and 2% (w/v) dextrose was used for cell growth. The Do-Ura medium contains 0.67% Bacto-yeast nitrogen base without amino acid, 2% Dextrose, 0.2% drop-out amino acids. All the chemicals used for the oxidative stress and the enzymatic analysis were purchased from either Sigma (St. Louis, MO, USA) or Aldrich (Milwaukee, WI, USA). Plasmids used to transform yeast strains are pRS316 as an empty vector, pYS104 containing wild-type hsp104 gene, pKT620 containing hsp104 with the second ATP binding site mutated, and pKT218 + pKT620 containing hsp104 with the first and second ATP binding sites mutated (Table 2). Plasmids were purified by the plasmid spin kit (GENENMED, Seoul, Korea). The antibody against Hsp104p was purchased from Stressgen (Victoria, BC, Canada). ECL (Enhanced Chemiluminescence, Pierce, Rockford, IL, USA) assay was used for detection in Western blot.

Table 1
Yeast strains used in this study

Strains	Genotype	Source
SP1	*MATa his3 leu2 ura3 trp1ade8*	R. Ballester
Ira2Δ	*MATa his3 leu2 ura3 trp1ade8 ira2::ADE8*	R. Ballester
WpRS316	*MATa his3 leu2 ura3trp1ade8* (pRS316 = *URA3 CEN6/ARS4*)	This study
WpYS104	*MATa his3 leu2 ura3trp1ade8* (pYS104 = *URA3 CEN6/ARS4*)	This study
WpKT620	*MATa his3 leu2 ura3trp1ade8* (pKT620 = *URA3 CEN6/ARS4*)	This study
WpKT218/620	*MATa his3 leu2 ura3trp1ade8* (pKT218/620 = *URA3 CEN6/ARS4*)	This study
IRpRS316	*MATa his3 leu2 ura3 trp1ade8 ira2::ADE8 ura3trp1ade8* (pRS316 = *URA3 CEN6/ARS4*)	This study
IRpYS104	*MATa his3 leu2 ura3 trp1ade8 ira2::ADE8 ura3trp1ade8* (pYS104 = *URA3 CEN6/ARS4*)	This study
IRpKT620	*MATa his3 leu2 ura3 trp1ade8 ira2::ADE8 ura3trp1ade8* (pKT620 = *URA3 CEN6/ARS4*)	This study
IRpKT218/620	*MATa his3 leu2 ura3 trp1ade8 ira2::ADE8 ura3trp1ade8* (PKT218/620 = *URA3CEN6/ARS4*)	This study

2.2. Yeast transformation

Both wild-type and *ira2Δ* mutant strains were grown up to OD 1.0 at 600 nm of 1.0 in 10 ml of YPD medium. Cells were collected by centrifugation and washed once with 0.1 M lithium acetate and incubated at 25 °C for 30 min. Cells were resuspended in 2 ml of 0.1 M lithium acetate, and 5 μl of the desired plasmid was added along with 10 μl of salmon sperm DNA (5 mg/ml) and 700 μl of 40% PEG3300, and the mixture was incubated at 30 °C for 30 min. Heat shock was given at 42 °C for 8 min so that the plasmid was transformed into the cell. Selections were made in the Do-Ura plates to screen colonies containing pRS316-based plasmids.

2.3. Western blotting

Log-phase grown cells were harvested by centrifugation and resuspended in 1 ml media and 200 μl ethanol (with 2 mM PMSF). Cells were disrupted by vigorous vortexing for 5 s 10 times at 4 °C in the presence of 200 μl of glass beads. After incubation at −20 °C for 1 h, cell crude extracts were dried by speed vacuum and suspended in the sample buffer containing 50 mM Tris (pH 6.8), 100 mM DTT, 2% SDS, 0.1% bromophenol blue, 10% glycerol for 10% SDS-PAGE analysis. Proteins were blotted onto a PVDF membrane, which were then incubated with polyclonal anti-rabbit antibody of Hsp104p (Stressgen) and developed with an ECL detection kit (Pierce).

Table 2
Characteristics of plasmids used in this study

Plasmids	Type of Hsp104p	Genotype
PRS316	no copy	gdp promoter cen6/ars4, URA3
PYS104	wild type	gdp promoter cen6/ars4, URA3
PKT620	second ATP binding site mutated	gdp promoter cen6/ars4, URA3
PKT218/620	both ATP binding sites mutated	gdp promoter cen6/ars4, URA3

2.4. Thermotolerance test

Induction of the Hsp104p was carried out by galactose instead of a mild heat treatment. Cells were grown to reach a log phase in Do-Ura with 2% glucose or 2% galactose for a control or an induction, respectively. Cells were then harvested and heat shocked at 50 °C for 15 min in a pyrex tube using a heating block to measure survival rates. Aliquots were spread onto the YPD plates and the number of colonies was counted after 48 h incubation at 30 °C.

2.5. Survival against oxidative stress

Cells were grown to an early log phase (OD of 0.15–0.2) at 25 °C in 20 ml Do-Ura medium containing 2% galactose and harvested by centrifugation. Various amounts of H_2O_2 were added to 10 ml of cells resuspended in potassium phosphate buffer (pH 7.4) which were then incubated for 30 min at 25 °C. Ten microliters of cells was taken every 10 min and plated on the YPD plates to monitor the cell viability after 48 h incubation at 30 °C. The number of colonies was counted to measure the survival rate.

2.6. Fluorimetric measurement of ROS production

Yeast cells grown up to a log phase in 10 ml Do-Ura were diluted in 10 ml of 100 mM potassium phosphate buffer (pH 7.4) and 20 μl of 5 mM stock solution of 2′,7′-H_2DCFDA dissolved in 100% ethanol was added to each culture [14]. Cells were incubated for 30 min at 25 °C with shaking and placed on ice, centrifuged, washed twice with ice-cold distilled water, and resuspended in 300 μl of water. After being vortexed with 300 μl glass beads (Sigma), the supernatant was retained after centrifugation at 3000 rpm for 10 min. Total protein amount was measured by using the Bradford method [15]. Fluorescence data were recorded with the excitation wavelength of 504 nm and the emission wavelength of 524 nm.

2.7. Preparation of cell-free extracts

Yeast cells cultured in 5 ml Do-Ura treated with two oxidants were resuspended in 5 ml of 0.1 M potassium phosphate (pH 7.4) containing 2 mM EDTA, and the same volume of 2 M $HClO_4$ and glass beads were added. Cells were disrupted by vigorous vortexing for 30 s 10 times at 4 °C. After incubation on ice for 1 h, cell debris was removed by centrifugation and the supernatant was neutralized to pH 7.0 by gradually adding 2 M KOH/0.3 M MOPS. After another centrifugation, the supernatant was collected in a 15-ml tube and frozen immediately with liquid nitrogen.

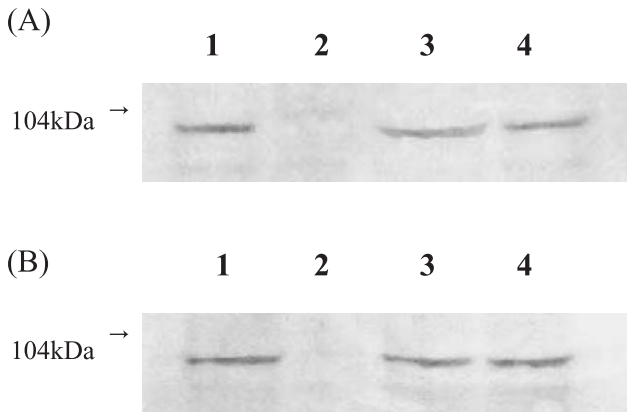

Fig. 1. Western blot of the expressed Hsp104p in wild type (A) and *ira2Δ* mutant (B) with four different plasmids, pYS104 (lane 1), pRS316 (lane 2), pKT620 (lane 3), and pKT218+pKT620 (lane 4). The crude extract from each recombinant strain was separated on a 10% SDS-PAGE, transferred to a PVDF membrane, and probed with the Hsp104.

2.8. Measurement of the cellular GSH and GSSG ratio

The total amount of glutathione (GSH+GSSG) in cell extracts was measured by adding 5 μl of glutathione reductase (50 units/ml) into a reaction mixture containing 700 μl of 0.3 mM NADPH and 100 μl of 5,5′-dithiobis(2-nitrobenzoic acid) (DTNB) and 200 μl of the cell extract [16]. The increase of the absorbance at 412 nm was monitored over 20 min. The individual amount of GSSG or GSH was measured by incubating 100 μl of cell extract with 2-vinyl pyridine for 60 min at room temperature, followed by the same procedure described above. The actual concentration of either GSH or

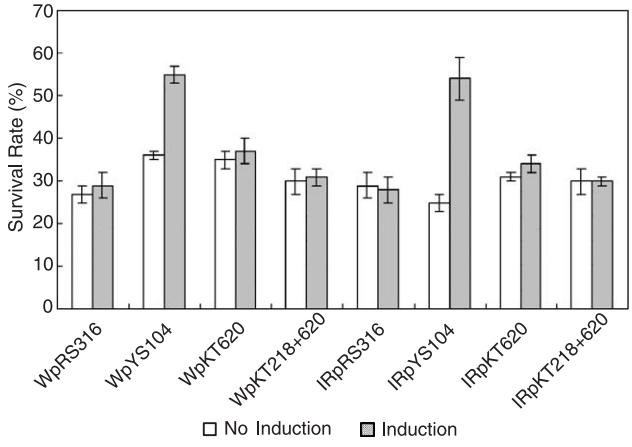

Fig. 2. Thermotolerance phenotype. Each recombinant strain was grown in a medium containing the galactose to express the plasmid copy of hsp104 genes, and examined for the thermotolerance. Survival rates were measured after a heat shock (HS) at 50 °C for 15 min and compared with a control.

GSSG was calculated from the calibration curve obtained from the known concentrations of GSH or GSSG.

3. Results

3.1. Expression of Hsp104 wild type and mutants

We have previously reported that yeast cells were more sensitive to the oxidative stress in the absence of Hsp104p than in its presence [7]. To address the importance of Hsp104p in protecting against the oxidative stress, it is necessary to establish that the more sensitive response of *hsp104Δ* strain upon oxidative stress is directly attributed to the function of Hsp104. Since we have earlier shown that the hyperactivated Ras mutant whose *ira2* gene was deleted is more sensitive to H_2O_2 and menadione [12], we attempted to construct recombinant strains in which different types of Hsp104p were expressed. For this purpose, the wild-type (SP1) and the hyperactive *ira2Δ* mutant (IR2.5) were trans-

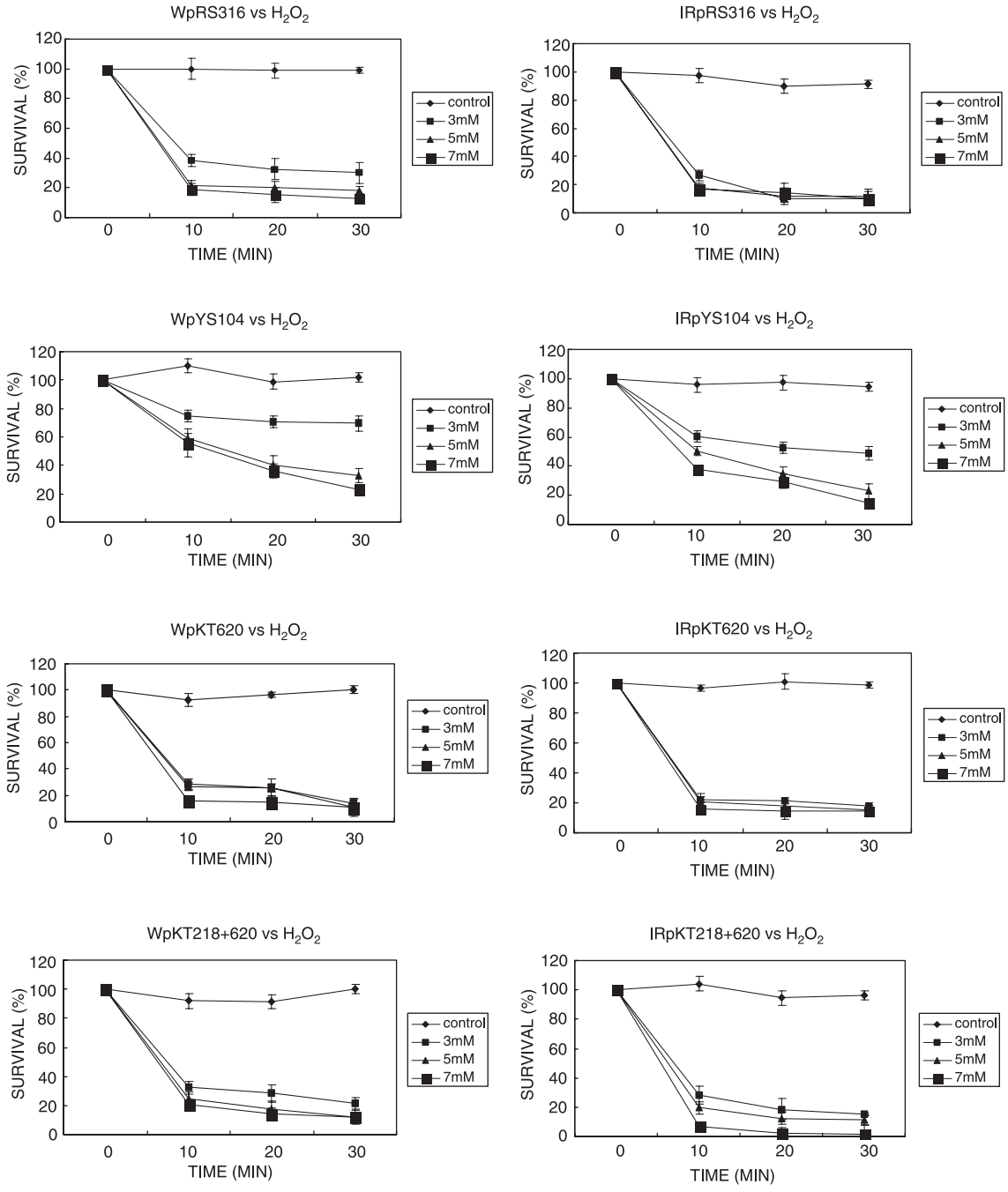

Fig. 3. Survival rates for the recombinant strains upon H_2O_2 addition. Cells were treated with H_2O_2 at various concentrations and the number of colonies was counted and presented as a ratio to that of untreated cells.

formed with four different plasmids. The plasmids used here are listed in Table 2. All the recombinant cells showed a normal growth at 37 °C, indicating that they undergo normal metabolic pathways. The hsp104 gene was under the control of the gpd (glyceraldehyde 3-phosphate dehydrogenase) promoter and the expression of Hsp104p was confirmed by Western blot analysis (Fig. 1). Expression of Hsp104p was observed in the recombinant strains with pYS104, pKT620, and pKT218 + pKT620, but not those with pRS316 as expected.

3.2. Thermotolerance phenotype in recombinant strains

Once expression of the recombinant Hsp104p from the plasmids was confirmed, we asked if the expressed Hsp104p was functional in vivo by examining the thermotolerance phenotype. If pretreated at 37 °C, not only Hsp104p but also other heat shock proteins, such as Hsp70, were known to be coexpressed to provide thermotolerance [17]. On the other hand, if the recombinant cells are grown in the presence of galactose to activate the gpd

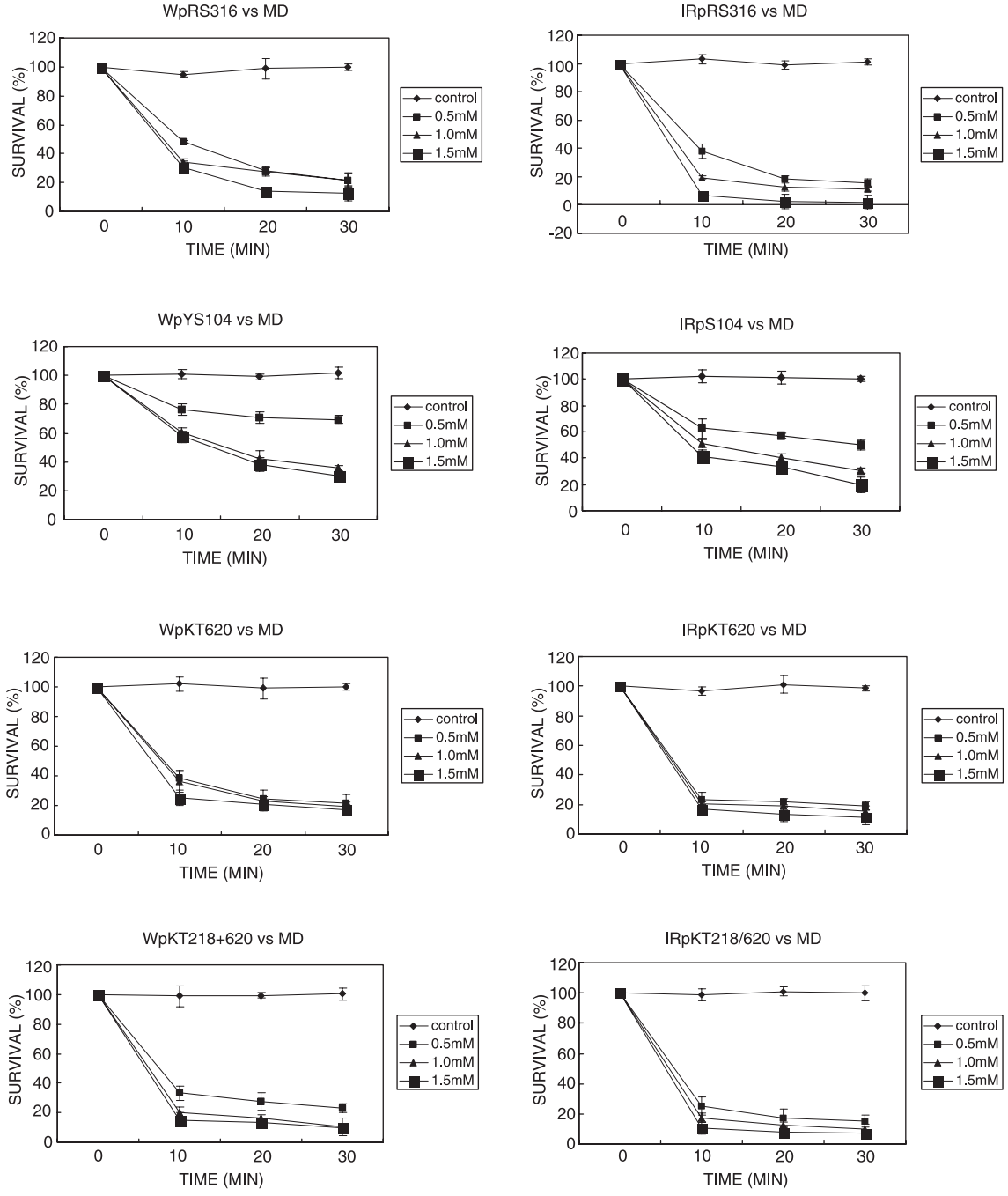

Fig. 4. Survival rates for the recombinant strains upon menadione exposure. Cells were incubated with various concentrations of menadione in a time-dependent manner and counted for survival.

promoter in the extrachromosomal plasmid, only Hsp104p is expected to be produced. For this purpose, the recombinant strains were grown to reach a log phase in a Do-Ura medium containing galactose and were given the heat shock at 50 °C for 15 min to examine the thermotolerance effect (Fig. 2). By counting the colonies on the YPD plates after 2 days, we have found that only the cells with the wild-type Hsp104p showed the enhanced survival rates in response to the high-temperature exposure in both wild-type and *ira2Δ* backgrounds as demonstrated in WpYS104 and IRpYS104. Neither did any Hsp104p mutant show this effect. These results demonstrated that only the functional Hsp104p was capable of providing the thermotolerance phenotypes in these recombinant cells.

3.3. Sensitivity of ira2Δ mutant to hydrogen peroxide (H_2O_2) and menadione (MD)

We have raised the possibility that the thermoprotective effect of Hsp104p may be mainly due to the regulation of the redox metabolism in the aerobic yeast cells since Hsp104p functions not only as a protector against a heat shock but also as a regulator in the mitochondrial respiration pathway. To directly investigate the role of Hsp104p on the protection against the oxidative stress, we have studied the survival rates of the *ira2Δ* mutant cells when the oxidative stress was given. The recombinant cells were exposed to various concentrations of H_2O_2 and menadione, and aliquots were taken every 10 min and spread on the YPD plates to measure the survival rates. Only the cells harboring the wild-type Hsp104p were protected significantly against the oxidative stress introduced by the H_2O_2 and menadione exposure (Figs. 3 and 4). When either ATP binding site was mutated, cells remained susceptible to the oxidative stress. Keeping both ATP binding sites functional was an absolute requirement for providing protection not only against the heat shock (Fig. 2) but also against the oxidative stress (Figs. 3 and 4). The same protection patterns were observed for both the wild-type and the *ira2Δ* mutant. This result shows that the hypersensitive response of the Ras mutants against oxidative stress can be overcome by the presence of Hsp104p.

3.4. Fluorimetric measurement of ROS production

Since the functional Hsp104p protects cells against oxidative stress as shown in Figs. 3 and 4, it was envisioned that the ROS level in the recombinant strains would decrease with the presence of the functional hsp104. Upon incubation with 3.0 M H_2O_2, the level of intracellular ROS is expected to increase, although the exact nature of ROS remains unknown. This possibility was supported by a report in which Hsp104, a regulator of the mitochondrial respiratory pathway, may directly function as a protector against lethal effects of free radicals [18].

To measure the level of ROS in the recombinant cells, we have carried out a fluorescence study with 2′,7′-H_2DCFDA which is converted to a fluorescent analog only in the presence of ROS. Exposure of cells to H_2O_2 has increased the level of ROS by 68% in WpRS316 and 83% in IRpRS316 compared to those of the control untreated cells. Only the functional Hsp104p lowered the level of ROS (Fig. 5A), which correlated well with the survival rates dependence upon oxidative stress (Figs. 3 and 4). As a comparison, we have measured the ROS levels following treatment of cells with heat shock (50 °C for 15 min). A similar dependence of functional Hsp104p on reduction of ROS was found in WpYS104 as well as IRpYS104 (Fig. 5B). A decreased level of ROS directly implies a role as an antioxidant and consequently a protective role of Hsp104p against the oxidative stress as well as heat shock.

3.5. Intracellular levels of GSH/GSSG

To examine the relationship between the levels of GSH acting as an intracellular antioxidant and the levels of protection against oxidative stress by Hsp104, we have measured the ratios of GSH and GSSG for the recombinant strains (Fig. 6). In the case of the wild-type background, the ratio of GSH/GSSG was the highest when the functional

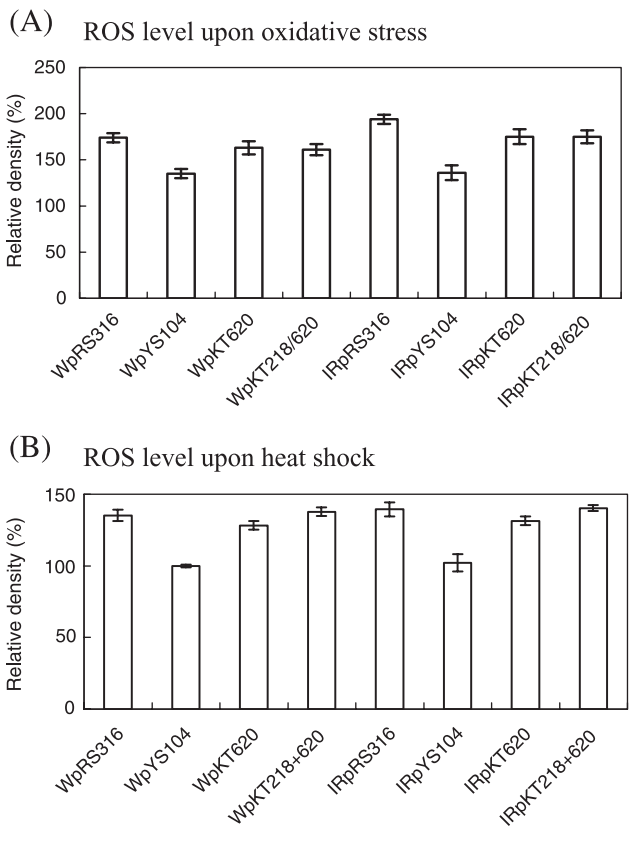

Fig. 5. Redox states determined by the measurement of the cellular ROS levels. The responses to the oxidative stress (A) as well as heat shock (B) were monitored by measuring intracellular ROS levels by H_2DCFDA fluorescence for cells exposed to 3.0 mM H_2O_2 and heat treatment at 50 °C for 15 min. Fluorescence was recorded with the excitation wavelength of 504 nm and the emission wavelength of 524 nm.

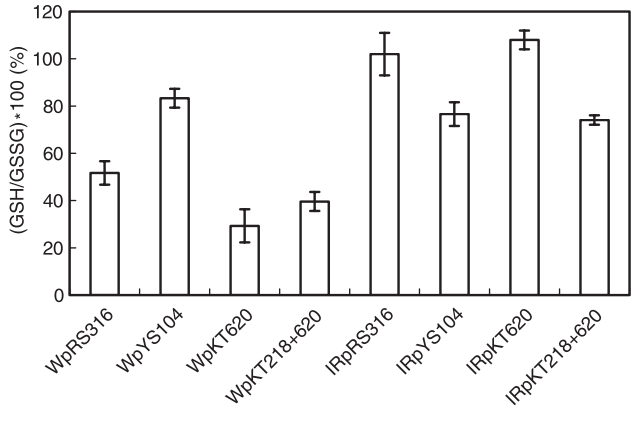

Fig. 6. Intracellular levels of the glutathione. The ratio between cellular GSH and GSSG was compared as an indicator of cellular redox states. Total amount of the glutathione (GSH + GSSG) was measured by the glutathione reductase in the presence of NADPH and DTNB. Following the blocking procedure, the amount of free GSH in the cell extract was measured separately. The cellular capacity for antioxidation was compared by the ratio of GSH to GSSG.

Hsp104p was expressed. The two recombinant strains with mutated hsp104 genes showed a lower ratio. On the other hand, very intriguingly, there was no correlation found in the case of *ira2Δ* background. Therefore, it can be interpreted that the antioxidation pathway by GSH in the wild-type was influenced by the presence of the functional Hsp104, implying that the two pathways may overlap in the regulation of ROS. On the contrary, the protection against the oxidative stress by Hsp104 is totally independent of the levels of GSH/GSSG in *ira2Δ*. This observation suggests that once the Ras/cAMP pathway is hyperactivated, GSH does not play a significant role in removing ROS, and instead the presence of the functional Hsp104p becomes crucial in keeping cells from being damaged by the oxidative stress.

4. Discussion

It has been proposed that the oxidative stress and the antioxidant enzymes play a major role in heat-induced cell death [19]. If the antioxidant genes are deleted, the resulting mutant strains were more sensitive to the heat shock. The same group (Dr. Schiest at Harvard School of Public Health) has recently reported that yeast cells are more resistant to the oxidative stress following lethal heat shock at 50 °C in anaerobic conditions than in aerobic conditions, in a fashion independent of the presence of Hsp104. They suggested that the toxicity of the lethal heat shock results mainly from the oxidative stress [20]. We have also reported that the cadmium stress was considerably alleviated by the presence of the functional Hsp104p which decreased the intracellular ROS level [21]. However, the localization studies with immunoelectron microscopy have produced conflicting results in relation to this question. They reported that Hsp104p was localized near the protein aggregates in the cells given a mild heat shock, while it was distributed over the entire cytoplasm and no protein aggregation was found when the oxidative stress was given [22]. This result implies that the mechanism by which yeast cells acquire thermotolerance is distinct from that of the oxidative stress. Whether Hsp104p functions as a thermoprotector through the reduction of ROS continues to be a matter of ongoing controversy. But it is widely accepted that the heat shock is another type of oxidative stress because it generates ROS.

For the purpose of investigating a protective role of Hsp104p against the oxidative stress, we have exploited *ira2Δ* mutant where Ras was hyperactivated because the Ras/cAMP pathway negatively regulates Hsp104p via stress response elements (STREs). Expression of Hsp104p from the chromosome is suppressed at a low level in such mutants [23]. As shown in Fig. 1, Hsp104p was detected in such mutants only in the presence of the extrachromosomal copy of the hsp104 gene.

In our previous studies, we have observed that Hsp104p functions as a respiratory regulator in yeast, judging from the data of the metabolic turnover rates in the TCA cycle for the wild-type and *hsp104Δ* using ^{13}C-NMR spectroscopy [6]. Hsp104p helps mitochondria keep their integrity, and efficiently maintain metabolic homeostasis within cells, thus preventing the formation of ROS, which can mediate intracellular signal cascades. We therefore addressed the possibility that ROS are involved in heat shock cascade by showing that hyperactivated Ras mutants produced lower levels of ROS upon H_2O_2 and menadione exposure in the presence of the functional Hsp104. Moreover, the presence of two ATP binding sites plays a significant role on rescuing cells from the oxidative stress. Throughout this study, a substantial amount of evidence was accumulated to support a direct relationship between the heat shock and the oxidative stress.

In measuring the level of ROS, the fluorescence study with carboxy-H(2)-DCFDA could be inaccurate and unreliable if yeast cells were shifted to anoxia [24]. The error comes from the leakage of fluorescent analog into the supernatant through the membrane and causes an inaccuracy in quantitative measurements. It could be possible that the difference in the level of ROS found among various strains might be due to the differential leakage of the fluorescent analog since we found that mitochondrial membrane integrity could be altered depending on the presence of the Hsp104p. However, when we tested this possibility with the wild-type and *hsp104Δ*, there was no difference in the level of ROS in the culture medium.

We have shown that Hsp104p regulates redox states within cells and provide thermotolerance. In the absence of Hsp104, mitochondria lose their membrane integrity as well as the control of the electron transport, resulting in a higher level of intracellular ROS. This observation is of special interest for the development of a new therapy to cure degenerative diseases resulting from mitochondrial dysfunctions. Parsell et al. have reported that Hsp104p helped cells resolubilize certain protein aggregates resulting from severe

heat shocks [25]. Recently, we were able to demonstrate that the functional Hsp104p protects Aβ (1–42) aggregation in an ATP-independent fashion [26], while resolubilization of Aβ fibrils by functional Hsp104p occurs in an ATP concentration-dependent fashion. Studies on the antioxidant effect of Hsp104p in other types of amyloid-related diseases are in progress.

In conclusion, significant overlap exists between the mechanisms for providing thermotolerance and ROS removal. Hsp104p plays a crucial role in keeping cells viable against heat shock and the subsequent oxidative stress by suppressing the level of ROS through a mechanism distinct from the GSH-mediated ROS removal.

Acknowledgements

We thank Dr. R. Ballester (University of California at Santa Barbara) for providing the wild-type and *ira2Δ* mutant strains, and Dr. S. Lindquist (MIT) for providing plasmids containing hsp104 genes. This work was supported by a grant (R04-2000-000-00014-0) to K. Lee from the Korea Science and Engineering Foundation.

References

[1] Y. Sanchez, S.L. Lindquist, Science 248 (1990) 1112–1115.
[2] D.A. Parsell, J. Taulien, S. Lindquist, Philos. Trans. R. Soc. Lond., Ser. B: Biol. Sci. 339 (1993) 279–285.
[3] D.A. Parsell, Y. Sanchez, J.D. Stitzel, S. Lindquist, Nature 353 (1991) 270–273.
[4] E.C. Schirmer, C. Queitsch, A.S. Kowal, D.A. Parsell, S. Lindquist, J. Biol. Chem. 273 (1998) 15546–15552.
[5] Y. Sanchez, J. Taulien, K.A. Borkovich, S. Lindquist, EMBO J. 11 (1992) 2357–2364.
[6] K. Lee, S. Kang, S. Lindquist, Bull. Korean Chem. Soc. 19 (1998) 295–299.
[7] K. Lee, Y. Cho, J. Ueom, S. Na, J.E. Park, I. Jo, J. Kang, Bull. Korean Chem. Soc. 22 (2001) 1175–1176.
[8] H.M. Lander, J.S. Ogiste, K.K. Teng, A. Novogrodsky, J. Biol. Chem. 270 (1995) 21195–21198.
[9] S. Powers, K. O'Neill, M. Wigler, Mol. Cell. Biol. 9 (1989) 390–395.
[10] K. Tanaka, K. Matsumoto, A. Toh-e, Mol. Cell. Biol. 9 (1989) 757–768.
[11] K. Tanaka, M. Nakafuku, F. Tamanoi, Y. Kaziro, K. Matsumoto, A. Toh-e, Mol. Cell. Biol. 10 (1990) 4303–4313.
[12] K. Chae, K. Lee, Bull. Korean Chem. Soc. 19 (1998) 1202–1206.
[13] D.H. Gutmann, M. Boguski, D. Marchuk, F. Wiegler, F.S. Collins, R. Ballester, Oncogene 8 (1993) 761–769.
[14] M.V. Miceli, M.R. Liles, D.A. Newsome, Exp. Cell Res. 214 (1994) 242–249.
[15] M.M. Bradford, Anal. Biochem. 71 (1976) 248.
[16] O.W. Griffith, Anal. Biochem. 106 (1980) 207–212.
[17] Y. Sanchez, D.A. Parsell, J. Taulien, J.L. Vogel, E.A. Craig, S. Lindquist, J. Bacteriol. 175 (1993) 6484–6491.
[18] D. Davermann, M. Martinez, J. McKoy, N. Patel, D. Averbeck, C.W. Moore, Free Radic. Biol. Med. 33 (2002) 1209–1220.
[19] J.F. Davidson, B. Whyte, P.H. Bissinger, R.H. Schiestl, Proc. Natl. Acad. Sci. U. S. A. 93 (1996) 5116–5121.
[20] J.F. Davidson, R.H. Schiestl, J. Bacteriol. 183 (2001) 4580–4587.
[21] K. Lee, J. Ueom, Bull. Korean Chem. Soc. 22 (2001) 514–518.
[22] K. Fujita, R. Kawai, H. Iwahashi, Y. Komatsu, Biochem. Biophys. Res. Commun. 248 (1998) 542–547.
[23] A. Stanhill, N. Schick, D. Engelberg, Mol. Cell. Biol. 19 (1999) 7129–7538.
[24] R. Dirmeier, K.M. O'Brien, M. Engle, A. Dodd, E. Spears, R.O.J. Biol. Chem. 277 (2002) 34773–34784.
[25] D.A. Parsell, A.S. Kowal, M.A. Singer, S. Lindquist, Nature 372 (1994) 475–478.
[26] B. Kong, J. Ueom, I. Kim, D. Lim, J. Kang, K. Lee, Bull. Korean Chem. Soc. 23 (2002) 1773–1777.