The Journal of Microbiology (2012) Vol. 50, No. 5, pp. 882–887
DOI 10.1007/s12275-012-2411-z

NOTE

Identification of Chaperones in Freeze Tolerance in *Saccharomyces cerevisiae*

Mahendran Chinnamara Naicker, I Seul Jo, and Hana Im*

Department of Molecular Biology, Sejong University, Seoul 143-747, Republic of Korea

(Received August 3, 2012 / Accepted September 18, 2012)

Exposure to low temperatures reduces protein folding rates and induces the cold denaturation of proteins. Considering the roles played by chaperones in facilitating protein folding and preventing protein aggregation, chaperones must exist that confer tolerance to cold stress. Here, yeast strains lacking individual chaperones were screened for reduced freezing tolerance. In total, 19 of 82 chaperone-deleted strains tested were more sensitive to freeze-thaw treatment than wild-type cells. The reintroduction of the respective chaperone genes into the deletion mutants recovered the freeze tolerance. The freeze sensitivity of the chaperone-knockout strains was also retained in the presence of 20% glycerol.

Keywords: chaperone, yeast, freeze tolerance, *Saccharomyces cerevisiae*, cold shock response

Low temperatures can cause several serious problems in living organisms, including decreased membrane fluidity, the formation of stable secondary structures in DNA and RNA, slow protein folding, and reduced enzymatic reaction rates (Jones *et al.*, 1987; Phadtare, 2004; D'Amico *et al.*, 2006). The cold denaturation of proteins leads to the loss of important functions (Privalov, 1990). If denatured proteins do not refold to their native form with the help of cellular chaperones, they may eventually form aggregates. Molecular chaperones such as DnaK and trigger factor have been reported to support the growth of bacteria at low temperatures (Kandror and Goldberg, 1997; D'Amico *et al.*, 2006). Under adverse environmental conditions, the rate of misfolding and aggregation is high enough to arrest the cell cycle (Trotter *et al.*, 2001). In contrast to heat shock responses, which are well documented at the molecular level, cold shock responses are not well understood, especially in eukaryotes.

An international consortium carried out a systematic deletion of all open reading frames in the yeast *Saccharomyces cerevisiae* using a polymerase chain reaction (PCR)-mediated gene deletion strategy (Giaever *et al.*, 2002). The wealth of available biochemical and genetic knowledge and existence of systemic deletion mutant collections of all nonessential genes makes *S. cerevisiae* an excellent eukaryotic model system for studying gene function (Fernandez-Ricaud *et al.*, 2005). Analyses using these deletion mutant collections can target genes whose mutation affects components of important pathways leading to an altered phenotype (Birrell *et al.*, 2002). If a yeast strain lacks a particular chaperone that has a critical role in cold tolerance, it would be more sensitive to freezing conditions. First, 104 yeast chaperone genes involved in protein folding, chaperones, and heat shock proteins were extracted from the functional annotations in the Saccharomyces Genome Database and Yeast Protein Database. Among these 104 genes, 22 encode essential genes, the knockout of which is lethal. Thus, the freeze tolerance of the remaining 82 deletion strains was tested. *Saccharomyces cerevisiae* BY4741 and its chaperone-deleted derivatives were purchased from Open Biosystems (USA). The yeast strains were grown in YPD (1% yeast extract, 2% peptone, and 2% glucose) medium at 30°C with shaking at 180 rpm. When the OD_{600} of the culture reached ~0.5–0.6, the effects of simple freezing-and-thawing on the yeast cells was evaluated as described previously (Kandror *et al.*, 2004). In brief, 1 ml of each culture was frozen at -20°C for 1 h, and then thawed at 30°C for 30 min. All experiments were independently repeated at least four times in duplicate. The number of colony forming units (CFU) per culture before and after freeze–thaw treatments was determined on YPD plates. The survival rate was calculated as the ratio of the CFU after freeze-thaw treatment to that before freezing. The relative survival rates of the mutant yeast strains were calculated as the survival rates of the mutant strains compared to that of wild-type yeast. As shown in Table 1, 19 (*hsc82Δ*, *hsp82Δ*, *phb1Δ*, *phb2Δ*, *atp11Δ*, *atp12Δ*, *cpr1Δ*, *cpr3Δ*, *cpr7Δ*, *fpr2Δ*, *scj1Δ*, *sse1Δ*, *alf1Δ*, *zuo1Δ*, *ssb1Δ*, *mcx1Δ*, *ssq1Δ*, *fmo1Δ*, and *sym1Δ*) of 82 chaperone-knockout mutants tested were more susceptible to freeze-thaw stress compared to the wild-type strain. The reduced viability of these deletion mutants suggests the importance of these chaperones in freeze tolerance. Only one knockout strain out of nineteen selected, *atp11Δ*, showed a reduced growth rate under normal growth condition (Giaever *et al.*, 2002; data not shown). However, the result in which the other five slow growing knockout strains among the tested

*For correspondence. E-mail: hanaim@sejong.ac.kr; Tel.: +82-2-3408-3643; Fax: +82-2-3408-3661

Table 1. Relative survival rates of chaperone-knockout yeast mutants

Deleted gene	Relative survival rate (%)	Deleted gene	Relative survival rate (%)
cne1 (CalNExin and calreticulin homolog 1)	82.60	*cin4* (Chromosome Instability 4)	90.00
ssa1 (Stress-Seventy subfamily A 1)	90.59	*sno4* (SNZ proximal Open reading frame 4)	120.00
ssa2 (Stress-Seventy subfamily A 2)	88.88	***atp11* (ATP synthase 11)**	**55.34**[a]
hsp104 (Heat Shock Protein 104)	100.00	*rbl2* (Rescues Beta-tubulin Lethality)	107.69
xdj1 (Homolog of *E. coli* DnaJ 1)	96.80	*lhp1* (La-Homologous Protein 1)	81.08
***hsc82* (Member of Hsp90 family)**	**57.78**[a]	*pex19* (PEroXisome related 19)	120.58
***scj1* (*S. Cerevisiae* DnaJ 1)**	**67.59**[a]	*hsp31* (Heat Shock Protein 31)	93.75
mdj2 (Mitochondrial DnaJ 2)	94.62	*ssa4* (Stress-Seventy subfamily A 4)	104.54
cus2 (Cold sensitive U2 snRNA Suppressor 2)	97.89	*ssq1* (Stress-Seventy subfamily Q 1)	81.61
hch1 (High-Copy Hsp90 suppressor 1)	130.52	***cpr7* (Cyclosporin-sensitive Proline Rotamase 7)**	**59.20**[a]
cin1 (Chromosome Instability1)	112.35	*mdj1* (Mitochondrial DnaJ 1)	100.00
***sse1* (Member of (HSP70) family)**	**71.33**[a]	***cpr3* (Cyclosporin-sensitive Proline Rotamase 3)**	**64.89**[a]
***mcx1* (Mitochondrial ClpX 1)**	**72.50**[a]	*sil1* (Subtilisin inhibitor-like 1)	99.43
pet100 (PETite colonies 100)	114.63	*cin2* (Chromosome INstability 2)	97.17
bcs1 (ubiquinol-cytochrome c reductase (bc1) Synthesis 1)	260.60	***hsp82* (Heat shock protein 82)**	**66.06**[a]
caj1 (Nuclear type II J heat shock protein of the *E. coli* dnaJ family)	71.42	*yar1* (Yeast Ankyrin Repeat 1)	102.25
hmf1 (Homologous Mmf1p Factor 1)	94.00	*ecm10* (ExtraCellular Mutant 10)	96.04
***phb1* (ProHiBitin 1)**	**50.91**[a]	*pih2* (Pregnancy-induced hypertension 2)	93.22
hsp30 (Heat Shock Protein 30)	110.78	*cpr2* (Cyclosporin-sensitive Proline Rotamase 2)	101.69
yek2 (Yeast ortholog of mouse KE2)	118.91	***fmo1* (flavin containing monooxygenase1)**	**71.46**[a]
cpr6 (Cyclosporin-sensitive Proline Rotamase 6)	100.00	*lhs1* (Lumenal Hsp Seventy 1)	98.30
pac10 (Protein required in the Absence of Cin8p)	95.18	*gac1* (Glycogen Accumulation 1)	101.12
shy1 (SURF Homolog of Yeast 1)	105.26	*mpd1* (Member of the Protein Disulfide isomerase 1)	89.83
***phb2* (ProHiBitin 2)**	**63.00**[a]	***atp12* (Adenosine triphosphate 12)**	**57.74**[a]
mga1 (Protein similar to hsf1)	112.19	*hsp150* (Heat shock protein 150)	101.69
ssa3 (Stress-Seventy subfamily A 3)	89.32	***sym1* (Stress-inducible Yeast MPV17 protein 1)**	**52.59***
***alf1* (ALpha-tubulin Foldin 1)**	**66.47**[a]	***cpr1* (Cyclosporin-sensitive Proline Rotamase 1)**	**66.55**[a]
hsp78 (Heat Shock Protein 78)	82.85	*hsp42* (Heat shock protein 42)	90.39
cpr5 (Cyclosporin-sensitive Proline Rotamase 5)	96.66	*plp1* (Proteolipid protein 1)	99.43
pex3 (PEroXin 3)	123.33	*iny1* (Interacting with Vps33p and Ypt7p 1)	100.00
snl1 (Suppressor of Nup116-C Lethal 1)	86.66	*fpr4* (FKBP Proline Rotamase (isomerase) 4)	96.66
***zuo1* (zuotin 1)**	**67.77**[a]	***ssb1* (Stress-Seventy subfamily B 1)**	**70.30**[a]
mpd2 (Member of the Protein Disulfide isomerase (PDI) Family 2)	91.17	*tcm62* (TriChoderMin resistance 62)	112.77
fpr3 (Fk 506-sensitive Proline Rotamase 3)	111.12	*hsp26* (Heat shock protein 26)	102.22
jem1 (DnaJ-like protein of the ER Membrane)	112.12	*fpr1* (FKBP Proline Rotamase (isomerase) 1)	102.22
hms2 (High-copy Mep Suppressor 2)	102.32	*apj1* (Anti-Prion DnaJ 1)	86.66
srp40 (Serine Rich Protein 40)	90.00	*ydj1* (Yeast DnaJ 1)	132.77
mrh1 (Membrane protein Related to Hsp30p 1)	98.07	*eug1* (ER protein Unnecessary for Growth under standard laboratory conditions 1)	100.55
cpr8 (Cyclosporin-sensitive Proline Rotamase 8)	90.00	***fpr2* (FKBP Proline Rotamase (isomerase) 2)**	**65.76**[a]
sse2 (Member of (HSP70) family)	128.57	*ccs1* (Copper Chaperone for SOD1 1)	125.00
cpr4 (Cyclosporin-sensitive Proline Rotamase 4)	100.00	*ump1* (Ubiquitin-Mediated Proteolysis 1)	93.33

[a] Chaperones-knockout strains which show statistically significant reduction ($p<0.05$) in freeze-tolerance are denoted in bold.

chaperone pool, including *eug1Δ* and *mdjΔ*, were not sensitive to freezing treatment (Table 1), suggests that the sensitivity of the selected chaperone knockout strains to freeze stress is not due to the growth defects of the mutant strains.

To confirm that the reduced freeze tolerance of the chaperone-knockout strains was due to the loss of a chaperone gene, the missing genes were cloned from wild-type yeast genomic DNA and reintroduced into the corresponding deletion mutants. Each pair of primers used to amplify the 19 chaperone genes is shown in Table 2. The chaperone genes were amplified by PCR using *Pfu* polymerase (Promega, USA). The products were digested with the appropriate restriction endonucleases and cloned into pACT2AD (Clontech, USA), which was modified to remove the nuclear localization signal and activation domain. The resulting individual clones were confirmed by DNA sequencing and transformed into the corresponding chaperone-knockout strains using the lithium acetate method (Schiestl and Gietz, 1989). The transformants were selected based on auxotrophic complementation using complete synthetic medium lacking leucine (MP

Table 2. PCR primers used for cloning of chaperone genes

Gene	Primers
hsc82	Sense: 5′ GGA TCC ATG GCT GGT GAA ACT TTT GAA TTT CAA GC 3′ Antisense: 5′ CTC GAG TTA ATC AAC TTC TTC CAT CTC GGT GTC AGC 3′
hsp82	Sense: 5′ GAC AAG CTT ATG GCT AGT GAA ACT TTG AAT TTC AAG 3′ Antisense: 5′ GAC AAG CTT CTA ATC TAC CTC TTC CAT TTC GGT GTC 3′
phb1	Sense: 5′ ACT AGT AAAA ATG TCT AAT TCT GCC AAA CTT ATC GAT GTC ATC 3′ Antisense: 5′ TTC GAA ACG GCC AAT GTT CAA AAG CAA GGA 3′
phb2	Sense: 5′ ACT AGT AAAA ATG AAT AGA TCA CCT GGT GAG TTC CAA AGG TAC 3′ Antisense: 5′ TTC GAA TTT GCC CCT TCC ATC GAT TCT TGC 3′
atp11	Sense: 5′ ACT AGT AAAA ATG TGG AGA CTA ACC AGA AAA ATC GGC ACT 3′ Antisense: 5′ GCT AGC ATT TTC CAT GGA CTG TGA TAG CGA AAT CAA 3′
ayp12	Sense: 5′ GAC AAG CTT ATG CTGCCA TCA TTA AGG AAG GG 3′ Antisense: 5′ GTC AAG CTT TTA TTG CTT AAA AGC AAT CGC 3′
cpr1	Sense: 5′ GAC AAG CTT ATG TCC CAA GTC TAT TTT GAT GTC GAA G 3′ Antisense: 5′ GTC AAG CTT TTA TAA TTC ACC GGA CTT GGC AAC AAC 3′
cpr3	Sense: 5′ GAC AAG CTT ATG TTT AAA CGT TCC ATC AAT CAA CAG TCC C 3′ Antisense: 5′ GTC AAG CTT TCA TAA CTC ACC AGC TTC TTC GAT AAC 3′
cpr7	Sense: 5′ GGA TCC ATG ATT CAA GAT CCC CTT GTA TAT TTA GAC ATC TCC 3′ Antisense: 5′ CTC GAG TTA GGA GAA AAA CTT TGA TAT ATT CTT TCT TGT CTT TTC C 3′
fpr2	Sense: 5′ GAC AAG CTT ATG ATG TTT AAT ATT TAC CTT TTC GTC ACT TTT TTT TCC 3′ Antisense: 5′ GTC AAG CTT CTA GGC GGC TGA TTT CAC GT 3′
scj1	Sense: 5′ ACT AGT AAAA ATG ATT CCA AAA TTA TAT ATA CAT TTG ATA CTA TCT TTA TTG TTG TTG 3′ Antisense: 5′ GCT AGC CAA CTC ATC TTT GAG CAT ATT TTG CCC AG 3′
sse1	Sense: 5′ GGA TCC ATG AGT ACT CCA TTT GGT TTA GAT TTA GGT AAC AAT AAC TCT G 3′ Antisense: 5′ CTC GAG TTA GTC CAT GTC AAC ATC ACC TTC AGT GTC C 3′
alf1	Sense: 5′ GGA TCC ATG GTT AGA GTT GTC ATA GAG AGT GAA TTG GTC C 3′ Antisense: 5′ CTC GAG TCA AAT TTC ATC ATC GCT CTC CAC GTC 3′
zuo1	Sense: 5′ GGA TCC ATG TTT TCT TTA CCT ACC CTA ACC TCA GAC ATC ACT G 3′ Antisense: 5′ CTC GAG TCA CAC GAA GTA GGA CAA CAA GCT GGA TG 3′
ssb1	Sense: 5′ GGA CTC GAG ATG GCT GAA GGT GTT TTC CAA GGT G 3′ Antisense: 5′ GGA CTC GAG TTA ACG AGA CAT GGC CTT GGT G 3′
mcx1	Sense: 5′ ACT AGT AAA AAT GTT GAA ATC TGC AAG CAA AAA CTT TTT TAG 3′ Antisense: 5′ GCT AGC TGT TAA GCT TCT CTT GGG AAT TGT TGG G 3′
ssq1	Sense: 5′ ACT AGT AAAA ATG CTT AAA TCT GGT AGA CTC AAC TTT CTC 3′ Antisense: 5′ GCT AGC TTT ACC TTG ATT CTG CTG GTT TTT TGT TGC 3′
fmo1	Sense: 5′ GGA AAG CTT ATG ACA GTG AAT GAC AAA AAA AGA TTG GC 3′ Antisense: 5′ GGA AAG CCT TTA AGT ATG TGG CGC CGG AAG GA 3′
sym1	Sense: 5′ GAC AAG CTT ATG AAG TTA TTG CAT TTA TAT GAA GCG AG 3′ Antisense: 5′ GTC AAG CTT TTA TTC GAC CAC GGG TGG ATA ATG AAC 3′

Biomedicals, USA). Expression of the introduced chaperone genes from the P_{ADH1} promoter of pACT2AD was confirmed by real-time quantitative PCR (qPCR). The yeast strains were cultured at 30°C to the mid-log phase (OD_{600}=~0.5–0.6), and total RNA was extracted using the hot phenol method (Kohrer and Domdey, 1991). cDNAs were synthesized using a ReverTra Ace® qPCR RT Kit (Toyobo, Japan) and quantified by real-time qPCR. All of the introduced chaperone genes were expressed in their host strains (data not shown). When the freeze tolerance of the strains was assessed based on the CFU after freeze-thaw treatment, the survival rates of the freeze-sensitive mutants were recovered to the level of the wild-type strain (Fig. 1). In particular, the overexpression of Sse1p, Phb2p, Atp11p, Atp12p, or Cpr3p increased the freeze tolerance of the mutants to a higher level than that in the wild type. These results confirm that the lack of a particular chaperone in the knockout yeast strains caused the freeze sensitivity of those strains.

Several factors during both the freezing and thawing processes affect cell survival. Since there was an exponential loss of cell viability during prolonged storage at -20°C (Kandror *et al.*, 2004), the frozen state also affects cell survival. Freezing induces the formation of intracellular ice crystals and dehydration, and it can damage the structure of the cell wall, plasma membrane, and organelles (Seki *et al.*, 2009). Cryoprotectants such as glycerol and trehalose promote the excretion of water, decrease the formation of ice crystals, and thus prevent some of the aforementioned damage (Kandror *et al.*, 2004). To address whether the effects of the identified chaperones are based on chaperone-mediated protein folding rather than general protection from ice formation, the survival rates of the chaperone-knockout mutants upon freeze-thaw treatment was measured in the presence of 20% glycerol. The presence of 20% glycerol greatly increased the freeze tolerance of the wild-type and mutant strains, suggesting that ice crystal formation during freezing may be one of the major causes of cell death. However, the relative freeze sensitivity of the chaperone-deleted mutants compared to the wild-type strain was still maintained (Fig. 2). Thus, the reduced viability of the deletion mutants was likely due to more rigorous protein denaturation and aggregation in the absence of particular chaperones during freezing and thawing.

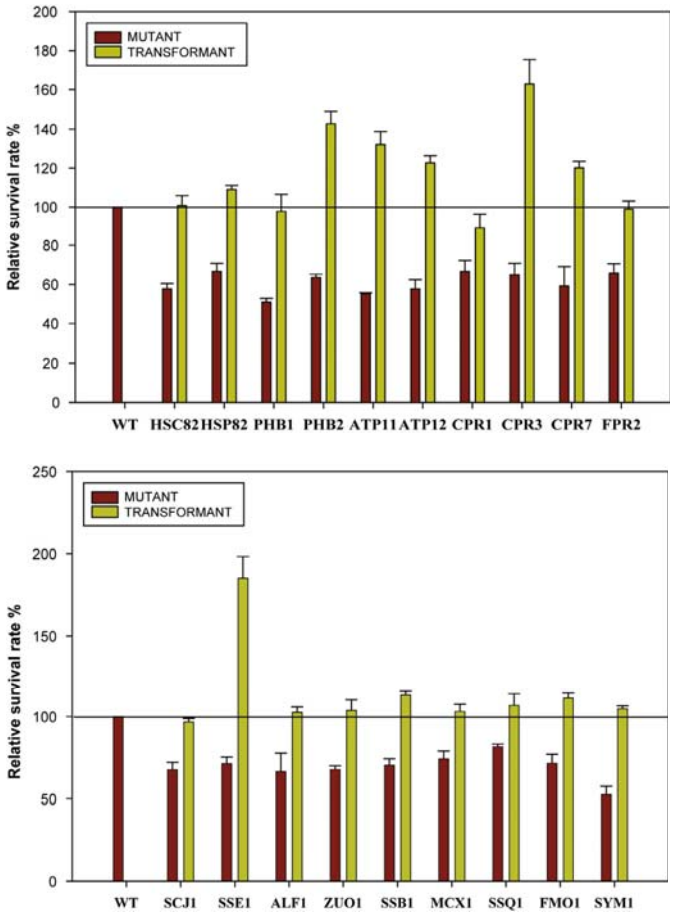

Fig. 1. Relative survival rates of wild-type cells, chaperone-knockout strains, and chaperone transformants upon freeze-thaw treatment. After freeze-thaw treatment, the viability of the cells was assessed as the CFU on YPD plates. The experiments were repeated four times; a bar graph of the mean relative survival rates and standard deviations is shown. The relative survival rates of the chaperone mutants are presented as brown bars; those of the chaperone transformants are shown as green bars.

Chaperones play vital roles in many cellular processes, including protein folding, targeting, transport, degradation, and signal transduction. When the chaperones involved in freeze tolerance were classified based on functional categories, Hsp90, Hsp70, Hsp40, peptidyl-prolyl *cis/trans* isomerases (PPIases), prohibitins, and ATP synthase assembly proteins were identified (Table 3). Note that mutants lacking PPIases were identified most frequently as being freeze-thaw-sensitive, suggesting the roles of PPIases in protection against freeze-induced protein denaturation. All of the tested

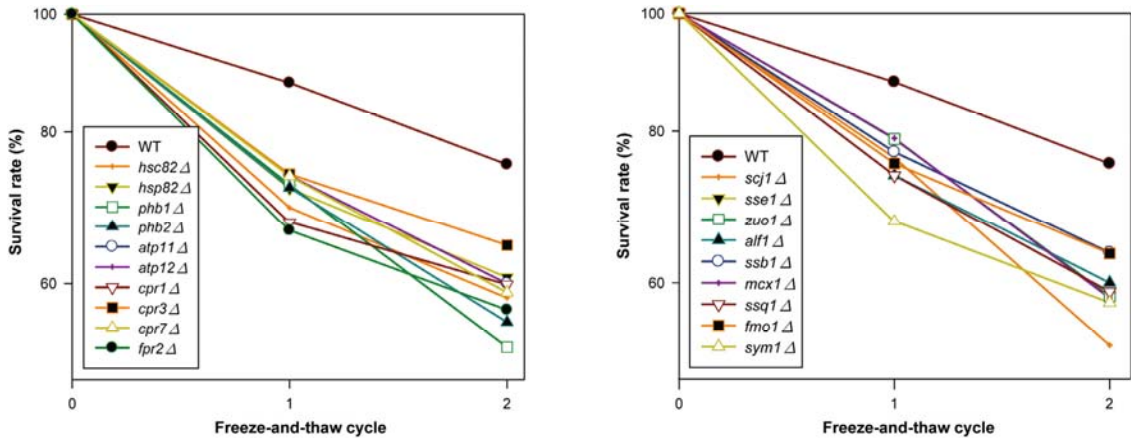

Fig. 2. Survival rates of the wild-type cells and chaperone-deleted mutants upon freeze-thaw treatment in the presence of 20% glycerol. Mid-log cultures of wild-type and chaperone-knockout yeast cells were subjected to freeze–thaw cycles in the presence of 20% glycerol. The survival rates were calculated as the CFU after freeze-thaw treatment divided by the CFU before treatment.

Table 3. Functional categories of chaperones involved in freeze tolerance

Family	Proportion in test pool	Selected/Tested
Hsp90	2/82	2/2 (100.00%)
Hsp70	9/82	3/9 (33.33%)
Hsp 40	8/82	2/8 (25.00%)
PPIase	12/82	4/12 (33.33%)
ATP synthase	2/82	2/2 (100.00%)
Prohibitins	2/82	2/2 (100.00%)
Other chaperones	47/82	4/47 (8.51%)

Hsp90, ATP synthase assembly proteins, and prohibitins were involved in freeze tolerance. Hsp90 family members are required for folding a specific set of nascent difficult-to-fold polypeptides as well as for refolding denatured proteins into their native conformations (Nathan *et al.*, 1997). Not only Hsp90 family chaperones but also co-chaperones such as Cpr7p and Sse1p (Liu *et al.*, 1999; Zuehlke and Johnson, 2012) were selected. Yeast Cpr7p, a PPIase, functions with the Hsp90 chaperone complex, and the mutation or inhibition of Hsp90 causes a severe growth defect in *cpr7* mutant cells (Marsh *et al.*, 1998). Sse1p expression, which is required for cell wall integrity and morphogenesis in yeast, is induced by several stress conditions, including heat shock (Shaner *et al.*, 2008). This suggests that Hsp90 chaperones and their co-chaperones function together to protect cells from freeze-induced protein denaturation and aggregation.

Hsp40/DnaJ regulates Hsp70 chaperone activity by stimulating the intrinsically weak ATPase activity of Hsp70 proteins, and it facilitates the interaction of Hsp70 with polypeptide substrates (Bukau and Horwich, 1998; Douglas *et al.*, 2009). Ssb1p is a component of the ribosome-nascent protein complex, which assists in the folding and translocation of newly synthesized proteins (Werner-Washburne *et al.*, 1987). Zuotin encoded by *zuo1* is a ribosome-associated DnaJ-like protein that may act in conjunction with the Hsp70 proteins Ssb1p and Ssb2p in the folding of nascent polypeptide chains. Both the Hsp70 chaperone Ssb1p and its Hsp40 partner Zuo1p were identified here as freeze-thaw tolerance genes. The role(s) played by *ssb2* in freeze-tolerance could not be tested in this study, because the knock-out strain of *ssb2* was unavailable from the yeast deletion library (Open Biosystems, USA). Since the high homology between Ssb1p and Ssb2p proteins suggests, but does not guarantee, functional overlap, it is likely that *ssb2* contributes to freeze-tolerance in yeast. However, the possibility that certain isoforms may perform better than others in specific tasks, such as the refolding/degradation of subsets of cold-denatured proteins, should not be ruled out. For example, it has been shown that the constitutively expressed cytosolic Hsp70 proteins, Ssa1p and Ssa2p, are 98% identical, but they display clearly distinct functions with regard to the propagation of yeast [URE3] prions (Sharma and Masison, 2008) and the Vid pathway (the vacuolar-mediated degradation of gluconeogenesis enzymes; Brown *et al.*, 2000). Furthermore, a single amino acid substitution for Gly of Ala at residue #83 was sufficient to interconvert the specificity of the distinct functions of these two Hsp70 chaperones (Sharma and Masison, 2011). Scj1p, another member of the Hsp40 family, was also identified as a freezing tolerance-related chaperone. Scj1p cooperates with Kar2p to mediate the maturation of proteins in the endoplasmic reticulum (ER) lumen (Schlenstedt *et al.*, 1995), and a lack of Scj1p induces the unfolded protein response pathway and cell wall defects (Silberstein *et al.*, 1998). Yeast Phb1p and Phb2p form a prohibitin complex (Tatsuta *et al.*, 2005), which helps in the stabilization of nascent polypeptides in mitochondria (Nijtmans *et al.*, 2000). Thus, the deletion of *phb1* or *phb2* not unexpectedly results in freeze-thaw sensitivity. The yeast mitochondrial ATP synthase (F_1F_0) complex consists of two oligomeric units: an integral membrane component (F_0) and a peripherally bound catalytic unit (F_1). Assembly of the F_1 unit requires the assistance of the chaperones Atp11p and Atp12p (Ackerman and Tzagoloff, 1990). These two chaperones interact with beta and alpha subunits, respectively, and prevent self-aggregation (Wang and Ackerman, 2000; Wang *et al.*, 2000). Decreased cell viability in the absence of *atp11* or *atp12* upon freezing may be due to a decrease in functional ATP synthase. The resulting decrease in ATP levels in *atp11* and *atp12* mutant cells would also hamper the function of other chaperones that require ATP for their activity.

Cyclophilins and FKBPs are enzymes that catalyze peptidyl-prolyl *cis/trans* isomerization, a rate-limiting step in protein folding. Cyclophilins and FKBPs are highly conserved in unicellular and multicellular organisms, with various subcellular distributions. Eight cyclophilins and four FKBPs have been identified in *S. cerevisiae* (Dolinski *et al.*, 1997). As peptidyl-prolyl isomerization becomes tremendously slow at low temperatures, PPIases would be important for coping with low temperatures by facilitating the folding of functionally significant proteins. Yeast mutants lacking Cpr1p, Cpr3p, Cpr7p, or Fpr2p are sensitive to freeze-thaw stress. Yeast Cpr1p is required for the glucose-stimulated transport of fructose-1,6-bisphosphatase into vacuole import and degradation vesicles, leading to the degradation of this glucolytic enzyme. It also promotes the proper subcellular localization of an essential zinc finger protein, Zpr1p. Cpr1p is involved in the response to several stresses, including cadmium, cobalt, copper, hydrogen peroxide, sodium dodecyl sulfate, tert-butyl hydroperoxide (Kim *et al.*, 2010), and menadione (Kim *et al.*, 2011). Yeast Cpr3p, a cyclophilin in the mitochondrial matrix, assists in the folding of imported proteins in mitochondria (Matouschek *et al.*, 1995). Fpr2p is localized in the ER membrane and is regulated in response to the accumulation of unfolded proteins in the ER (Partaledis and Berlin, 1993). The frequent selection of PPIases in our screen suggests that the promotion of peptidyl-prolyl *cis/trans* isomerization facilitates protein refolding from the cold-denatured conformation.

High degrees of conservation in the chaperone machinery between yeast and mammals, including Hsp70-Hsp90 chaperones and their cofactor requirements, illustrate the value of using *S. cerevisiae* as a model organism to study protein folding and adaptation to environmental stress in eukaryotic cells. As the molecular chaperones and their co-chaperones involved in freeze–thaw tolerance have not been addressed previously, the information obtained in this study provides important insight into the roles played by chaperones in cell survival at low temperatures.

This research was supported by Basic Science Research Program through the National Research Foundation of Korea (NRF) funded by the Ministry of Education, Science and Technology (KRF-2012-0002875).

References

Ackerman, S.H. and Tzagoloff, A. 1990. Identification of two nuclear genes (ATP11, ATP12) required for assembly of the yeast F1-ATPase. *Proc. Natl. Acad. Sci. USA* **87**, 4986–4990.

Birrell, G.W., Brown, J.A., Wu, H.I., Giaever, G., Chu, A.M., Davis, R.W., and Brown, J.M. 2002. Transcriptional response of *Saccharomyces cerevisiae* to DNA-damaging agents does not identify the genes that protect against these agents. *Proc. Natl. Acad. Sci. USA* **99**, 8778–8783.

Brown, C.R., McCann, J.A., and Chiang, H.L. 2000. The heat shock protein Ssa2p is required for import of fructose-1, 6-bisphosphatase into Vid vesicles. *J. Cell Biol.* **150**, 65–76.

Bukau, B. and Horwich, A.L. 1998. The Hsp70 and Hsp60 chaperone machines. *Cell* **92**, 351–366.

D'Amico, S., Collins, T., Marx, J.-C., Feller, G., and Gerday, C. 2006. Psychrophilic microorganisms: challenges for life. *EMBO Reports* **7**, 385–389.

Dolinski, K., Muir, S., Cardenas, M., and Heitman, J. 1997. All cyclophilins and FK506 binding proteins are, individually and collectively, dispensable for viability in *Saccharomyces cerevisiae*. *Proc. Natl. Acad. Sci. USA* **94**, 13093–13098.

Douglas, P.M., Summers, D.W., and Cyr, D.M. 2009. Molecular chaperones antagonize proteotoxicity by differentially modulating protein aggregation pathways. *Prion* **3**, 51–58.

Fernandez-Ricaud, L., Warringer, J., Ericson, E., Pylvanainen, I., Kemp, G.J., Nerman, O., and Blomberg, A. 2005. PROPHECY – a database for high-resolution phenomics. *Nucleic Acids Res.* **33**, D369–D373.

Giaever, G., Chu, A.M., Ni, L., Connelly, C., Riles, L., Véronneau, S., Dow, S., Lucau-Danila, A., Anderson, K., André, B., and *et al.* 2002. Functional profiling of the *Saccharomyces cerevisiae* genome. *Nature* **418**, 387–391.

Jones, P.G., VanBogelen, R.A., and Neidhardt, F.C. 1987. Induction of proteins in response to low temperature in *Escherichia coli*. *J. Bacteriol.* **169**, 2092–2095.

Kandror, O., Bretschneider, N., Kreydin, E., Cavalieri, D., and Goldberg, A.L. 2004. Yeast adapt to near-freezing temperatures by STRE/Msn2,4-dependent induction of trehalose synthesis and certain molecular chaperones. *Mol. Cell* **13**, 771–781.

Kandror, O. and Goldberg, A.L. 1997. Trigger factor is induced upon cold shock and enhances viability of *Escherichia coli* at low temperatures. *Proc. Natl. Acad. Sci. USA* **94**, 4978–4981.

Kim, I.S., Jin, I., and Yoon, H.S. 2011. Decarbonylated cyclophilin A Cpr1 protein protects *Saccharomyces cerevisiae* KNU5377Y when exposed to stress induced by menadione. *Cell Stress Chaperones* **16**, 1–14.

Kim, I.S., Kim, H.Y., Shin, S.Y., Kim, Y.S., Lee, D.H., Park, K.M., and Yoon, H.S. 2010. A cyclophilin A CPR1 over-expression enhances stress acquisition in *Saccharomyces cerevisiae*. *Mol. Cells* **29**, 567–574.

Kohrer, K. and Domdey, H. 1991. Preparation of high molecular weight RNA. *Meth. Enzymol.* **194**, 398–405.

Liu, X.D., Morano, K.A., and Thiele, D.J. 1999. The yeast Hsp110 family member, Sse1, is an Hsp90 cochaperone. *J. Biol. Chem.* **274**, 26654–26660.

Marsh, J.A., Kalton, H.M., and Gaber, R.F. 1998. Cns1 is an essential protein associated with the hsp90 chaperone complex in *Saccharomyces cerevisiae* that can restore cyclophilin 40-dependent functions in cpr7Δ cells. *Mol. Cell. Biol.* **18**, 7353–7359.

Matouschek, A., Rospert, S., Schmid, K., Glick, B.S., and Schatz, G. 1995. Cyclophilin catalyzes protein folding in yeast mitochondria. *Proc. Natl. Acad. Sci. USA* **92**, 6319–6323.

Nathan, D.F., Harju-Vos, M., and Lindquist, S. 1997. *In vivo* functions of the *Saccharomyces cerevisiae* Hsp90 chaperone. *Proc. Natl. Acad. Sci. USA* **94**, 12949–12956.

Nijtmans, L.G.J., de-Jong, L., Sanz, M.A., Coates, P.J., Berden, J.A., Back, J.W., Muijsers, A.O., Van der-Speck, H., and Grivell, L.A. 2000. Prohibitins act as a membrane-bound chaperone for the stabilization of mitochondrial proteins. *EMBO J.* **19**, 2444–2451.

Partaledis, J.A. and Berlin, V. 1993. The FKB2 gene of *Saccharomyces cerevisiae*, encoding the immunosuppressant-binding protein FKBP-13, is regulated in response to accumulation of unfolded proteins in the endoplasmic reticulum. *Proc. Natl. Acad. Sci. USA* **90**, 5450–5454.

Phadtare, S. 2004. Recent developments in bacterial cold-shock response. *Curr. Issues Mol. Biol.* **6**, 125–136.

Privalov, P.L. 1990. Cold denaturation of proteins. *Crit. Rev. Biochem. Mol. Biol.* **25**, 281-305.

Schiestl, R.H. and Gietz, R.D. 1989. High efficiency transformation of intact yeast cells using single stranded nucleic acid as carrier. *Curr. Genet.* **16**, 339–346.

Schlenstedt, G., Harris, S., Risse, B., Lill, R., and Silver, P.A. 1995. A yeast DnaJ homologue, Scj1p, can function in the endoplasmic reticulum with BiP/Kar2p via a conserved domain that specifies interactions with Hsp70s. *J. Cell Biol.* **129**, 979–988.

Seki, S., Kleinhans, F.W., and Mazur, P. 2009. Intracellular ice formation in yeast cells *vs.* cooling rate: predictions from modeling *vs.* experimental observations by differential scanning calorimetry. *Cryobiology* **58**, 157–165.

Shaner, L., Gibney, P.A., and Morano, K.A. 2008. The Hsp110 protein chaperone Sse1 is required for yeast cell wall integrity and morphogenesis. *Curr. Genet.* **54**, 1–11.

Sharma, D. and Masison, D.C. 2008. Functionally redundant isoforms of a yeast Hsp70 chaperone subfamily have different antiprion effects. *Genetics* **179**, 1301–1311.

Sharma, D. and Masison, D.C. 2011. Single methyl group determines prion propagation and protein degradation activities of yeast heat shock protein (Hsp)-70 chaperones Ssa1p and Ssa2p. *Proc. Natl. Acad. Sci. USA* **33**, 13655–13670.

Silberstein, S., Schlenstedt, G., Silver, P.A., and Gilmore, R. 1998. A role for the DnaJ homologue Scj1p in protein folding in the yeast endoplasmic reticulum. *J. Cell Biol.* **143**, 921–933.

Tatsuta, T., Model, K., and Langer, T. 2005. Formation of membrane-bound ring complexes by prohibitins in mitochondria. *Mol. Biol. Cell* **16**, 248–259.

Trotter, E.W., Berenfeld, L., Krause, S.A., Petsko, G.A., and Gray, J.V. 2001. Protein misfolding and temperature up-shift cause G_1 arrest via a common mechanism dependent on heat shock factor in *Saccharomyces cerevisiae*. *Proc. Natl. Acad. Sci. USA* **98**, 7313–7318.

Wang, Z.G. and Ackerman, S.H. 2000. The assembly factor Atp11p binds to the beta-subunit of the mitochondrial F(1)–ATPase. *J. Biol. Chem.* **275**, 5767–5772.

Wang, Z.G., Sheluho, D., Gatti, D.L., and Ackerman, S.H. 2000. The alpha-subunit of the mitochondrial F(1)–ATPase interacts directly with the assembly factor Atp12p. *EMBO J.* **19**, 1486–1493.

Werner-Washburne, M., Stone, D.E., and Craig, E.A. 1987. Complex interactions among members of an essential subfamily of hsp70 genes in *Saccharomyces cerevisiae*. *Mol. Cell. Biol.* **7**, 2568–2577.

Zuehlke, A.D. and Johnson, J.L. 2012. Chaperoning the chaperone: A role for the co-chaperone Cpr7 in modulating Hsp90 function in *Saccharomyces cerevisiae*. *Genetics* **191**, 805–814.