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Contribution of Hsc70 to barotolerance in the yeast *Saccharomyces cerevisiae*

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Abstract The contribution of Hsc70 to barotolerance in logarithmic-phase cells of the *HSC70* (*ssb1* and *ssb2*) deletion mutant and in strains expressing the *HSC70* gene on either a low- or a high-copy-number plasmid was studied. The deletion-mutant strain had higher thermotolerance and a slightly lower barotolerance than the control strain. The strain that expresses the *HSC70* gene in high copy number had a higher barotolerance than the strain that expresses the gene in low copy number. These results suggest that Hsc70 contributes to barotolerance during exponentially growing conditions as does Hsp104 during heat-shock treatment.

Key words *Saccharomyces cerevisiae* · Barotolerance · Thermotolerance · Heat-shock protein · Molecular chaperone

Introduction

Hydrostatic pressure affects almost all physiological activities in living cells. Bett and Cappi (1965) studied the viscosity of water as a function of pressure up to 10,000 kg cm^{-2}. They found that relative and absolute viscosity decreases as pressure increases from 0 to 2,000 kg cm^{-2} at ambient temperature (Bett and Cappi 1965). Decreased viscosity caused by high pressure results in the destruction of hydrogen bonding, as does an increase in temperature (Bett and Cappi 1965). Thus, the effects of high temperature and high hydrostatic pressure may be analogous for organisms. On the basis of this consideration, new technology that uses high pressure instead of high temperature has been developed for use in the food industry (Hayashi 1989). Corresponding to the new technology, research has focused on understanding how organisms grow or survive under conditions of pressure. In the range of pressure that inhibits growth, Abe and Horikoshi (1995) found that hydrostatic pressure from 40 to 60 MPa promoted acidification of vacuoles in yeast cells, and Tamura et al. (1998) demonstrated the induction of a yeast heat-shock protein (HSP) in the same range of pressure. Organisms can be sterilized by pressure greater than 100 MPa. Sonoike et al. (1993) demonstrated that hydrostatic pressure sterilization is more effective at cold temperatures (about 0°C) than at room temperature. For example, *Lactobacillus casei* is killed by hydrostatic pressure (300 MPa) with a death rate of 0.32 at 0°C, 0.1 at 20°C, and 0.32 at 60°C (Sonoike et al. 1993). Iwahashi et al. (1991) demonstrated that pressure treatment of more than 100 MPa decreased the colony-forming units (CFU), but a mild heat-shock treatment of 43°C for 30 min increased barotolerance (resistance to hydrostatic pressure). This result was shown to be due to the accumulation of trehalose and Hsp104 (Iwahashi et al. 1997a).

Hsp104 was shown to contribute to barotolerance and to maintain trehalose-metabolizing enzymes at higher temperature (Iwahashi et al. 1997b, 1998). On the other hand, yeast cells have other molecular chaperones, such as Hsp90, Hsc90, Hsp70, and Hsc70 (Piper 1993). It was demonstrated that these molecular chaperones make no direct contribution to thermotolerance (Piper 1993). However, it was shown that overproduction of Hsp70 suppresses the decreased thermotolerance by deletion of the *HSP104* gene (Sanchez et al. 1993). This finding suggests that Hsps have a similar function in the role of molecular chaperone.

In this report, we estimated the contribution of Hsp70 and Hsc70 to trehalose-metabolizing enzyme activities and the contribution of Hsc70 to barotolerance. We found evi-

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dence that Hsc70 contributes both trehalose-metabolizing enzyme activity and barotolerance during exponentially growing conditions, as does Hsp104 do during heat-shock treatment.

Materials and methods

Strains and growing conditions

Strains and plasmids used in this work are listed in Table 1. The strains of JN54 and JN212 and the plasmids of pRS316K and pRS426 were a gift from E. Craig. The pRS426ssb– and pRS316Kssb– plasmids carry deletion of the coding region of *SSB1*. The W303aLEU2+ and ΔHSP104 LEU2+ strains were a gift from S. Lindquist. Cells were grown on YPD medium (2% polypeptone, 1% yeast extract, and 2% glucose) or SD medium (0.67% yeast nitrogen base without amino acid) at 30°C as previously described (Iwahashi et al. 1995).

Barotolerance and thermotolerance

Hydrostatic pressure treatments of cells were performed at 140 or 160 MPa at 25°C using a pressure-generating system (Iwahashi et al. 2000), and high-temperature treatments were performed at 44° and 46°C in cryotubes. Barotolerance and thermotolerance were estimated from the CFU value as previously described (Iwahashi et al. 1991). Barotolerance was expressed as a percentage of the CFU of the high-pressure- and high-temperature-treated cells, relative to the untreated control.

Immunoblotting and enzyme activities

Ssb1p was detected in cell extracts using its specific antibody (no. 799), which was originally isolated as human Hsp70 antibody (Iwahashi et al. 1995). Enzyme activities were measured in crude extracts with or without heat-shock treatment (43°C for 2 h) and are shown as the mean values of three independent experiments, as described previously. Neutral trehalase activity was measured at 37°C according to App and Holzer (1989) using a glucose test kit purchased from Wako (Tokyo, Japan). One unit is the amount of enzyme that produces 1 μmol trehalose in 1 min. Trehalose-6-phosphate (T6P) synthase activity was measured spectrophotometrically, as reported by Vandercammen et al. (1989). One unit is the amount of enzyme that produces 1 μmol uridine diphosphate (UDP) in 1 min. Trehalose-6-phosphate (T6P) phosphatase activity was measured according to Vandercammen et al. (1989), except that glucose was measured using the glucose test kit from Wako. Again, one unit (U) is the amount of enzyme that produces 1 μmol trehalose in 1 min.

Results and discussion

Contribution of Hsc70 to trehalose metabolism

The molecular chaperone Hsp104 was shown to contribute to trehalose-metabolizing enzyme activities (Iwahashi et al. 1998). Yeast cells have several kinds of molecular chaperones that may also contribute to the activity of trehalose-metabolizing enzymes. To examine this possibility, we measured neutral trehalase, T6P synthase, and T6P phosphatase activities in the strains that have the deletion in *HSP70* (*SSA1* and *SSA2*) or *HSC70* (*SSB1* and *SSB2*) genes. *SSA1* and *SSA2* belong to one of the subfamilies of *HSP70*, and the *HSC70* subfamily is constituted of *SSB1* and *SSB2*. Table 2 summarizes the activities of the three enzymes in the strains. The deletion of *HSP104* affected the activities of the three enzymes, especially in the heat-shocked cells. The deletions of *SSA*s decreased neutral trehalase activity but increased T6P synthase and T6P phosphatase activities. As it has been shown that the deletion of *SSA*s increased the amount of heat-shock protein (Craig and Jacobson 1985), the induction of two enzyme activities possibly reflects this

Table 1. Strains and plasmids

Strain or plasmid	Genotype	Source
JN54	*MATa his3 leu2 ura3 trp1 lys2*	E.A. Craig
JN14	*MATa his3,3 leu2 ura3 trp1 lys2 ssa1–3::HIS3 ssa2–2::Ura3*	E.A. Craig
JN212	*MATa his3, leu2 ura3 trp1 ssb1::LEU2 ssb2::HIS3*	E.A. Craig
JN54-pRS316K	*MATa his3, leu2 ura3 trp1 ssb1::LEU2 ssb2::HIS3*, pRS316K-SSB1	This work
JN54-pRS316K ssb–	*MATa his3, leu2 ura3 trp1 ssb1::LEU2 ssb2::HIS3*, pRS316K-ssb–	This work
JN54-pRS426	*MATa his3, leu2 ura3 trp1 ssb1::LEU2 ssb2:: HIS3,* pRS426-SSB1	This work
JN54-pRS426 ssb–	*MATa his3, leu2 ura3 trp1 ssb1::LEU2 ssb2:: HIS3,* pRS426-ssb–	This work
W303aLEU2+	*MATa can1 ade2 his3 LEU2+ trp1 ura3*	S. Lindquist
ΔHSP104 LEU2+	*MATa can1 ade2 his3 leu2 trp1 ura3 hsp104::LEU2*	S. Lindquist
pRS316K	*URA3 SSB1 AmpR* (YCp-type, low copy)	E.A. Craig
pRS316Kssb–	*URA3 ΔSSB1 AmpR* (YCp-type, low copy)	This work
pRS426	*URA3 SSB1 AmpR* (YEp-type, high copy)	E.A. Craig
pRS426ssb–	*URA3 ΔSSB1 AmpR* (YEp-type, high copy)	This work

effect. On the other hand, the deletion of *SSB*s decreased all activities, especially in logarithmic-phase cells. This result possibly reflects the similar contribution of Hsp104 to these enzyme activities.

The *HSC70* deletion strain has lower barotolerance and high thermotolerance

One of the most important roles of Hsp104 is its contribution to thermotolerance. The deletion of the *HSP104* gene decreased thermotolerance (Sanchez and Lindquist 1990). In contrast, Hsc70 was considered not to contribute to thermotolerance, but its deletion in yeast caused cold sensitivity (Craig and Jacobson 1985). The contribution of Hsp104 and Hsc70 to trehalose metabolism is similar in some respects (Table 2), because deletion of the *HSP104* gene decreased neutral trehalase, T6P synthase, and T6P phosphatase activity under heat-shock conditions, and deletion of the *HSC70* gene also decreased these enzyme activities, especially during exponential growth. However, their roles in thermotolerance are believed to be different or opposite. On the other hand, Hsp104 was shown to contribute to barotolerance (Iwahashi et al. 1997a, b), but the role of Hsc70 has not been investigated.

As a first step, we measured the time course of barotolerance (140 MPa, 25°C) and thermotolerance (44° and 46°C) in logarithmic-phase cells of the *HSC70* (*ssb1* and *ssb2*) deletion mutant and the control strain (Fig. 1). Logarithmic-phase cells were selected for the following reasons: the deletion of *SSB*s decreased trehalose-metabolizing enzyme activity, especially in logarithmic phase, and Hsc70 was expressed in logarithmic phase but was repressed under heat-shock conditions (Iwahashi et al. 1995). The mutant strain that has the deletion in the genes of *SSB1* and *SSB2* had higher thermotolerance and slightly lower barotolerance than those in the control strain (Fig. 1). Higher thermotolerance suggests that the deletion of the *ssb1* and *ssb2* genes induced other unknown factors that increase thermotolerance without the heat-shock treatment. It has been shown that growth rate strongly affected basal stress tolerance because the lower growth rate suggests growth under stress conditions (Arguelles 1994). Under stress conditions, yeast cells induced many kinds of genes of concern to stress tolerance (Iwahashi 2000). Basal stress tolerance can be understood as stress tolerance without the stress treatment, as in heat-shock treatment. The deletion of *ssb1* and *ssb2* caused a decrease in growth rate, especially at lower temperatures (Craig and Jacobson 1985). Thus, higher thermotolerance could be caused by low growth rate. However, the *HSC70* deletion strains showed relatively lower barotolerance under stress conditions, as shown by a lower growth rate (Fig. 1). This finding could be understood as a consequence of the effect of the deletion of *HSC70* combined with higher basal stress tolerance.

Table 2. Effect of the deletion of molecular chaperones on the activities of trehalose-metabolizing enzymes

Strain	Neutral trehalase		T6P synthase		T6P phosphatase	
	LP	HS	LP	HS	LP	HS
Wild type	100[a]	100	100	100	100	100
HSP104- (ΔHSP104 LEU2+)	110[b]	83	118	56	111	47
SSA- (JN14)	28	34	130	181	242	147
SSB- (JN212)	34	48	58	70	50	66

LP, logarithmic-phase cells; HS, heat-shocked cells; T6P, trehalose-6-phosphate

[a] The wild-type strain to the *HSP104* strain (W303aLEU2+) showed specific activity of 5.3 mU/mg protein in LP and 15.7 mU/mg protein in HS (neutral trehalase), that of 0.72 U/mg protein in LP and 1.0 U/mg protein in HS (T6P synthase), and that of 0.071 U/mg protein in LP and 0.11 U/mg protein in HS (T6P phosphatase); the wild type to *SSA* and *SSB* (JN54) showed 18, 34 (neutral trehalase), 0.78, 1.1 (T6P synthase), and 0.057, 0.136 (T6P phosphatase), respectively

[b] Relative activity to that of the activity of the wild type as 100

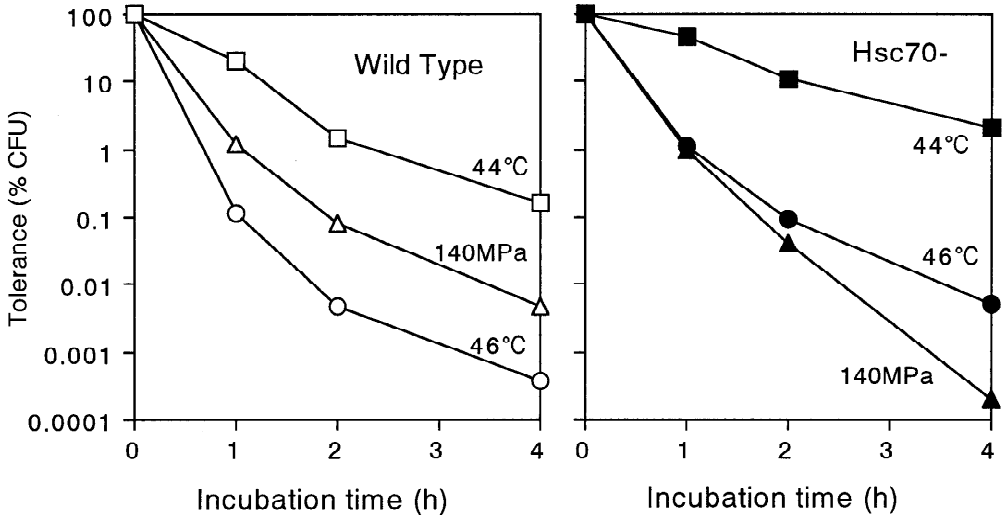

Fig. 1. Barotolerance and thermotolerance of the strain that has a deletion in the *SSB1* and *SSB2* (*HSC70*) genes. Exponentially growing yeast cells (the wild type of JN54 [*left*] and the *HSC70* strain of JN212 [*right*]) were treated at 44° or 46°C and 140 MPa. The pressure test was conducted at 25°C. After treatment, yeast cells were diluted and spread on a YPD (2% polypeptone, 1% yeast extract, 2% glucose) plate. Mean values shown were obtained from three to six independent experiments

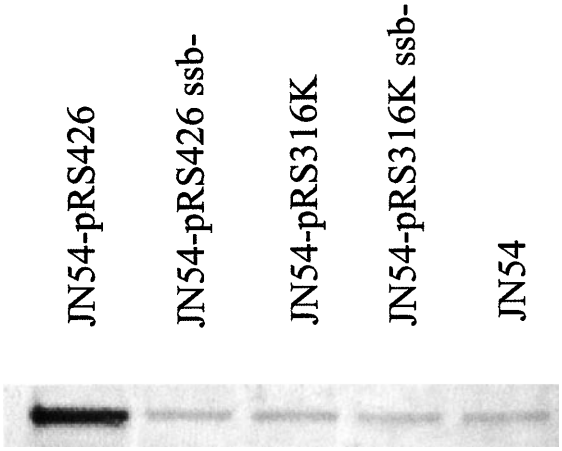

Fig. 2. Relative cellular content of Hsc70 in the strains carrying the *HSC70* gene on the plasmids shown. Ssb1p (Hsc70) was visualized using a specific antibody to Ssb1p in the extract of exponentially growing yeast cells as described in the text. The names of the strains are indicated *above* the lanes

Barotolerance of the strains having various amounts of Hsc70

In the absence of Hsc70, yeast cells seem to exhibit higher basal stress tolerance. To avoid this effect, we estimated the contribution of Hsc70 to barotolerance by varying the levels of Hsc70 (*SSB1*) expression in the cell. We constructed different Hsc70-expressing strains by transforming the wild-type strain JN54 with each of the following plasmids: pRS316K, pRS316Kssb–, pRS426, and pRS426ssb– (Table 1). The Hsc70 content of the cells is shown in Fig. 2. As expected, the strain JN54-pRS426 had the highest Hsc70 content. Barotolerance of the constructed strains is summarized in Table 3. JN54-pRS426 had the highest barotolerance, and its control strain, JN54-pRS426ssb1–, had high barotolerance, but it was much lower than that of JN54-pRS426. The JN54-pRS316K strain had a higher barotolerance than that of JN54-pRS316Kssb1– (control) and JN54 (parent).

The increased barotolerance caused by the introduction of the *SSB1* gene is much higher with pRS426 (2,000-fold) than with pRS316K (10-fold). Thus, the amount of Hsc70 correlated with barotolerance. The high barotolerance of JN54-pRS316Kssb– and JN54-pRS426ssb– is possibly a result of the lower growth rate, which is often observed under selective pressure growth conditions (Arguelles 1994). As the growth rates of JN54-pRS426 and JN54-pRS426ssb– or those of JN54-pRS316K and JN54-pRS316K are almost of the same degree (data not shown), the differences in barotolerance can be considered to be a result of different Hsc70 concentrations.

How Hsc70 contributes to barotolerance

We could confirm the contribution of Hsc70 to barotolerance. Because Hsc70 was believed not to contribute to thermotolerance, this protein was ignored in the aspect of stress tolerance and its physiological function is not shown. Our results demonstrated the role of Hsc70 as baroprotectant. However, it remains to be shown how Hsc70 contributes to barotolerance. One possibility is contribution through the trehalose metabolisms, because Hsc70 affects trehalose-metabolizing enzyme activities. Trehalose and neutral trehalase are important factors for barotolerance (Iwahashi et al. 1997a, 2000). Neutral trehalase contributes to barotolerance after the accumulation of trehalose, and the contribution of trehalose is dependent on the concentration in the cells. Thus, the roles of trehalose and neutral trehalase are significant during stationary phase or after heat-shock treatment. We confirmed that the contribution of Hsc70 occurs in the logarithmic phase. The accumulation of trehalose was generally observed in stationary phase or after heat-shock treatment (Piper 1993). Thus, the main role of Hsc70 to barotolerance is not through trehalose metabolism.

Iwahashi et al. (in press) recently showed the induction of many kinds of molecular chaperones and proteolytic factors after pressure treatment using yeast DNA microarray technology. They monitored about 6,000 open reading frames after pressure treatment (corresponding to LD_{50}) and recovery for 1 h. Their results suggested that yeast cells require the activation of protein metabolism after pressure treatment (during recovery conditions). They did not list *HSC70* as the significantly induced ORF, but we may accept the idea that the amount of Hsc70 affects the capacity of recovery for survival. We would like to estimate the role of molecular chaperones, especially Hsc70, during recovery after pressure shock treatment.

Table 3. Barotolerance of the strains that have *SSB1* on plasmids

Strain	Barotolerance (%)[a]
Wild type (JN54)	$3.5 \pm 2 \times 10^{-5}$
+Plasmid (JN54-pRS316Kssb1–)	$2.4 \pm 3 \times 10^{-3}$
+*SSB1* (JN54-pRS316K)	$2.2 \pm 1 \times 10^{-2}$
+Plasmid (JN54-pRS426ssb1–)	$5.2 \pm 3 \times 10^{-4}$
+*SSB1* (JN54-pRS426)	$1.2 \pm 0.7 \times 10^{0}$

[a] The pressure treatment was carried out under 160 MPa for 2 h at 25°C

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