APPLIED AND ENVIRONMENTAL MICROBIOLOGY, Nov. 1998, p. 4614–4617 Vol. 64, No. 11
0099-2240/98/$04.00+0

Evidence for the Interplay between Trehalose Metabolism and Hsp104 in Yeast

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Received 29 June 1998/Accepted 7 September 1998

Disruption of the *HSP104* gene in a mutant which cannot accumulate trehalose during heat shock treatment caused trehalose accumulation (H. Iwahashi, K. Obuchi, S. Fujii, and Y. Komatsu, Lett. Appl. Microbiol 25: 43–47, 1997). This implies that Hsp104 affects trehalose metabolism. Thus, we measured the activities of enzymes involved in trehalose metabolism. The activities of trehalose-synthesizing and -hydrolyzing enzymes are low in the *HSP104* disruption mutant during heat shock. This data is correlated with intracellular trehalose and glucose levels observed in the *HSP104* disruption mutant. These results suggest that during heat shock, Hsp104 contributes to the simultaneous increase in both accumulation and degradation of trehalose.

In cells of yeasts and some other organisms, exposure to temperatures higher than the optimum for growth results in enhancement of the synthesis of heat shock proteins and the metabolism of trehalose (17). These cells then acquire the ability to survive under more extreme conditions, a phenomenon referred to as transitory thermotolerance (17). Trehalose and neutral trehalase (Nth1 and Nth2) are suggested to be protectants against thermal and other stress conditions. Trehalose biosynthesis is catalyzed by the sequential action of trehalose-6-phosphate synthase (Tps1) and trehalose-6-phosphate phosphatase (Tps2) activities (called the trehalose synthase complex) (12), with UDP-glucose and glucose-6-phosphate used as substrates. These activities are increased by heat shock treatment, and yeast accumulates trehalose (12, 17). It has been observed that trehalose prevents protein denaturation (4) and stabilizes the membrane (5, 13). Neutral trehalase catalyzes the hydrolysis of trehalose to glucose, and its expression is induced by heat shock treatment (12). Mutants deficient in neutral trehalase show decreased thermotolerance in spite of high trehalose levels in cells (9–11). Thus, neutral trehalase activity possibly supplies energy to rescue certain cellular systems during and after exposure to supraoptimal temperatures (12). It has also been reported that trehalose inhibits disaggregation of proteins by Hsp104 (20), thus suggesting that neutral trehalase is an important factor in thermotolerance. In *Saccharomyces cerevisiae*, Hsp104, Hsp90, Hsp70, and Hsp26 are the major heat shock proteins (17). However, only Hsp104 has been genetically confirmed to be an important factor in transitory thermotolerance (14, 17). Hsp104 is considered to be in the same family as the ClpA and ClpB proteins of *Escherichia coli*, which are believed to assist in protein degradation (15). Hsp104 forms an oligomeric structure in the presence of ATP in a way similar to that of the ClpA protein (16). Thus, Hsp104 may regulate proteases or be involved in preventing or resolving the aggregation of vital cellular proteins during exposure to supraoptimal temperatures (8, 15, 16). Much work has centered on the role of heat shock proteins and trehalose metabolism in the heat shock response, but no direct relationship or interplay between the two systems has been clearly demonstrated. With respect to Hsp104 and trehalose, at least three studies (3, 7, 22) have shown no effect of Hsp104 on trehalose accumulation in yeast. Thus, the function of Hsp104 is considered to be independent of trehalose metabolism (8). However, Hottiger et al. observed about 30% slower trehalose degradation during recovery from heat shock in an *HSP104* disruption mutant (*hsp104* mutant) (3). Recently, synergy between Hsp104 and trehalose was documented based on studies with mutants carrying disruptions of *HSP104* and *TPS1*, encoding trehalose-6-phosphate synthase (Tps1) (20). In these studies, the activities of trehalose-metabolizing enzymes (which regulate trehalose concentration) were not determined.

Recently, we studied mutants which are not able to accumulate trehalose and/or Hsp104, in order to examine the contributions of these factors to barotolerance (7). The double mutant could neither synthesize Hsp104 nor accumulate trehalose in the stationary phase; however, it accumulated trehalose during heat shock treatment (7). This suggested the important control of Hsp104 over trehalose metabolism. In this study, we show the effect of Hsp104 on trehalose metabolism by using mutants carrying a disruption of *HSP104*. Data on the cellular contents of trehalose and glucose as well as on the activities of neutral trehalase, trehalose-6-phosphate synthase, and trehalose-6-phosphate phosphatase are presented.

Strains and growth conditions. *S. cerevisiae* strains used in this work are listed in Table 1. Cells were grown on yeast extract-peptone-dextrose medium at 30°C, as previously described (6). Heat shock was performed on logarithmic-phase cells by shifting them from 30°C to 43°C for 0 to 120 min.

Enzyme activities. Enzyme activities were measured in crude extracts. Data presented are averages and standard deviations (SD) of at least three independent experiments. The crude extracts were prepared by breaking the cells (30 s each for 10 times) with glass beads in an equal volume of 50 mM imidazole hydrochloride buffer, pH 7.0, containing protease inhibitors and Complete TM (Boehringer, Mannheim, Germany). Broken cells were centrifuged at 12,000 × *g* for 20 min, and the supernatants were used as the crude extracts. Neutral trehalase activity was measured at 37°C, according to App and Holzer (1), by using a glucose test kit purchased from Wako Co., Osaka, Japan. One unit of neutral trehalase is the amount of enzyme that hydrolyzes 1 μmol of trehalose in 1 min. Trehalose-6-phosphate synthase activity was measured spectrophotometrically, as reported by Vandercammen et al. (21). A reaction mixture containing 2 mM UDP-glucose, 10 mM glucose-6-phosphate, 1 mM EDTA, 50 mM KCl, and 10 mM

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TABLE 1. Yeast strains

Strain	Genotype (phenotype)	Source or reference
W303aLEU2+	*MATα can1 ade2 his3 LEU2*$^+$ *trp1 ura3*	S. Lindquist
Δhsp104 LEU2	*MATα can1 ade2 his3 leu2 trp1 ura3 hsp104*::*LEU2*$^+$	S. Lindquist
224A-12D	*MATα his4 leu2 ura3* (trehalose deficient)	C. De Virgilio
CWG12	*MATα ade2 his3* (and/or *his4*) *leu2 ura3 hsp104*::*LEU2*$^+$ (trehalose deficient)	7
CWG13	*MATα ade2 his3* (and/or *his4*) *leu2 ura3*	7
CWG14	*MATα ade2 his3* (and/or *his4*) *leu2 ura3* (trehalose deficient)	7
CWG15	*MATα ade2 his3* (and/or *his4*) *leu2 ura3 hsp104*::*LEU2*$^+$	7

Mg acetate was incubated at 42°C for 20 min. The reaction was stopped by heating, and the solution was then centrifuged. UDP was measured in the supernatant by the decrease in absorbance at 340 nm in a mixture containing 0.15 mM NADH, 0.25 mM phosphoenolpyruvate, 100 mM KCl, 5 mM $MgCl_2$, 10 μg of pyruvate kinase per ml, 10 μg of lactate dehydrogenase per ml, and 25 mM HEPES (pH 7.1) (18). One unit of trehalose-6-phosphate synthase is the amount of enzyme that produces 1 μmol of UDP in 1 min. Trehalose-6-phosphate phosphatase activity was measured according to Vandercammen et al. (21). One unit of trehalose-6-phosphate phosphatase is the amount of enzyme that produces 1 μmol of trehalose in 1 min. Trehalose in the reaction mixture was measured as glucose after hydrolysis by acid trehalase (5). The reaction mixture contained 25 mM sodium phosphate buffer (pH 6.0), 0.5 mM trehalose-6-phosphate, 10 mM $MgCl_2$, and 50 mM KCl, and the crude extract was incubated at 30°C.

Trehalose, glucose, and protein measurements. Cellular trehalose was measured by liquid chromatography after extraction in a boiling-water bath, as previously described (5). Cellular glucose was measured in the crude extracts by using the glucose test kit from Wako Co. Protein was measured with a DC protein assay kit (Bio-Rad Japan). Data presented are averages and SD values of at least three independent experiments.

A double mutant deficient in *HSP104* and trehalose accumulates trehalose during heat shock treatment. In the course of studying the contribution of trehalose and Hsp104 to barotolerance, we isolated a double mutant unable to accumulate trehalose (in the logarithmic and stationary phases) and unable to synthesize Hsp104 (Table 1) (7). However, this mutant accumulates trehalose during heat shock. This showed a correlation between trehalose metabolism and Hsp104 (7). To understand the accumulation of trehalose in the double mutant, we estimated the cellular contents of trehalose in CWG13 (wild type), CWG14 (trehalose deficient), CWG15 (*HSP104* disruption), and CWG12 (trehalose and *HSP104* deficient) under heat shock conditions (Fig. 1). The wild type and the *hsp104* mutant accumulated trehalose within 30 min of heat shock and gradually increased the amount. The *hsp104* mutant accumulated more trehalose than the wild-type strain after 30 min. As expected, the trehalose-deficient strain was unable to accumulate trehalose during the entire heat shock period. In the double mutant, trehalose accumulation started after 30 min of heat shock, but the level was much lower than in the single *hsp104* mutant and the wild type.

Glucose levels in the *hsp104* mutant. Trehalose is synthesized from UDP-glucose and glucose-6-phosphate and is broken down to glucose (12). Thus, we also estimated the cellular content of glucose in the *hsp104* mutant during the heat shock conditions. As shown in Fig. 2, the glucose level in the wild type increased within 60 min of heat shock and remained fairly constant thereafter. Similar patterns of glucose accumulation were observed in the *hsp104* mutant. However, the levels were lower in both logarithmic-phase cells and heat-shocked cells than in the wild-type strain. These results suggest that Hsp104 affects glucose levels especially during heat shock.

Disruption of *HSP104* affects neutral trehalase activity. The levels of trehalose and glucose observed in the various mutants

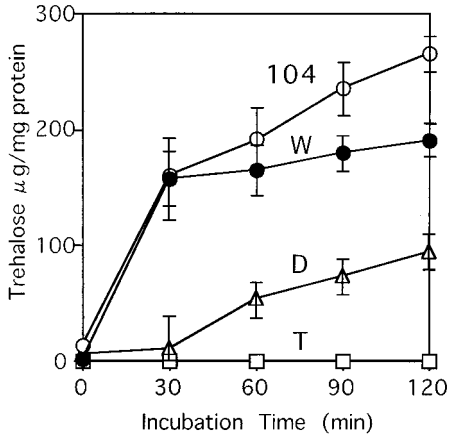

FIG. 1. Accumulation of trehalose in trehalose-deficient and/or *hsp104* mutant cells during heat shock treatment. Logarithmic-phase cells of the wild-type strain (CWG13 [W]), the trehalose-deficient mutant (CWG14 [T]), the *hsp104* mutant (CWG15 [104]), and the double mutant (CWG12 [D]), were incubated at 43°C, and trehalose accumulation was estimated at the indicated times. The error bar for each sample represents the SD of at least three independent experiments.

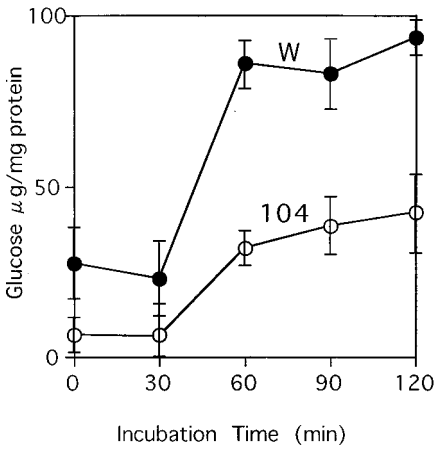

FIG. 2. Accumulation of glucose in *hsp104* mutant cells during heat shock treatment. Logarithmic-phase cells of the wild type (CWG13 [W]) and the *hsp104* mutant (CWG15 [104]) were incubated at 43°C, and intracellular glucose levels were estimated at the indicated times. The error bar for each sample represents the SD of at least three independent experiments.

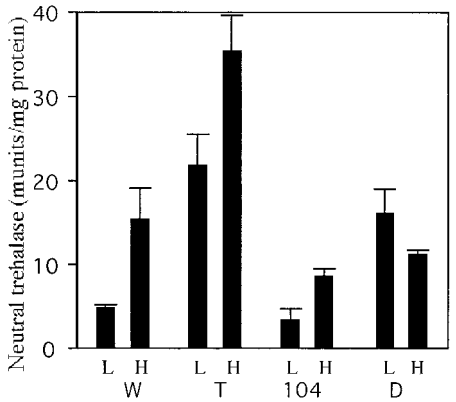

FIG. 3. Neutral trehalase activities in trehalose-deficient and/or *hsp104* mutant cells. Logarithmically growing (L) and heat-shocked (H) cells of the wild type (CWG13 [W]), the trehalose-deficient mutant (CWG14 [T]), the *hsp104* mutant (CWG15 [104]), and the double mutant (CWG12 [D]) were harvested, and crude extracts were prepared. The enzyme activities of neutral trehalase were measured in the crude extracts as described in the text. The error bar for each sample represents the SD of at least three independent experiments.

during heat shock imply that disruption of *HSP104* reduced the ability to degrade trehalose to glucose. To examine this possibility, we measured neutral trehalase activities in the mutants in logarithmic phase and after heat shock treatment for 60 min (Fig. 3). The trehalose-deficient mutant showed higher neutral trehalase activities in the logarithmic phase and during heat shock than did the wild-type strain (Fig. 3). In contrast, the *hsp104* mutant clearly showed lower neutral trehalase activity after heat shock treatment than did the wild-type strain. This suggests that Hsp104 positively contributes to neutral trehalase activity. The trehalase activities of the double mutants were higher than that of the wild-type strain in logarithmic-phase cells but lower in the heat-shocked cells. This result explains why the double mutant accumulates trehalose during heat shock.

Hsp104 contributes to trehalose metabolism during heat shock. The neutral trehalase activities, trehalose content, and glucose content of the mutants during heat shock suggest that Hsp104 contributes to trehalose metabolism. However, the trehalose-deficient strain is not genetically well characterized. Thus, we examined trehalose-metabolizing enzyme activities in a different *HSP104* disruption background. Figure 4A shows neutral trehalase activity in the logarithmic phase and after heat shock of the *hsp104* mutant. As expected, the *hsp104* mutant shows lower neutral trehalase activity after heat shock treatment. We confirmed the contribution of Hsp104 to neutral trehalase in the *hsp104* mutant and its isogenic strain system. In addition, the *hsp104* mutant showed lower trehalose-6-phosphate synthase and phosphatase activities after heat shock treatment than did the wild type (Fig. 4B and C). This suggests that Hsp104 also contributes to trehalose biosynthesis during heat shock conditions. In our experiments, heat shock treatment did not increase trehalose-6-phosphate synthase activity up to three- to fivefold, as reported previously by Hottiger et al. (2) and Ribeiro et al. (19). This is due probably to the different heat shock conditions (43°C) and assay methods used for the present study.

We have defined the effects of Hsp104 on neutral trehalase, trehalose-6-phosphate synthase, and trehalose-6-phosphate phosphatase activities. Disruption of *HSP104* caused a decrease in the activities of these enzymes (Fig. 3 and 4). Earlier reports suggested that Hsp104 has no effect on the accumulation of trehalose because the *hsp104* mutant accumulates a normal level of trehalose under heat shock conditions (3, 7, 22). Our data do not contradict those observations but explain the reasons for the normal accumulation of trehalose, i.e., the decreased synthesizing and degrading abilities of the Hsp104 mutant. It seems that the contribution of Hsp104 to trehalose metabolism is not the only target of Hsp104, as the *hsp104* mutant showed low levels of glucose in the logarithmic phase (Fig. 2). Our data show an interaction between trehalose metabolism and Hsp104; however, we are not certain how this happens. Recently, data have accumulated which show that not only trehalose accumulation but also trehalose degradation is important in order for yeast cells to acquire thermotolerance (9–12). It has been suggested that disruption of *NTH1*, encoding neutral trehalase, reduces the intracellular glucose level of yeast during heat shock (12) and that the glucose supply is important for thermotolerance (4). Hsp104 seems to contribute to the supply of glucose in the cells through its effect on neutral trehalase, because glucose (Fig. 2) and neutral trehalase activity (Fig. 4) are low in the *hsp104* mutant. We suggest that the molecular chaperone, Hsp104, interacts directly or

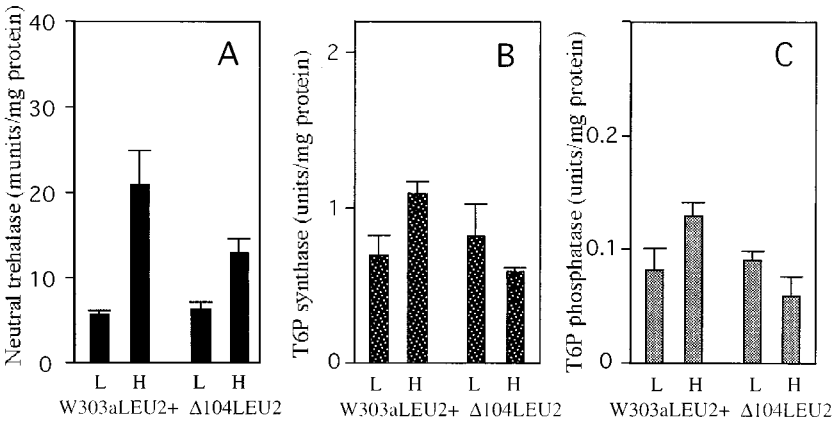

FIG. 4. Activities of enzymes of trehalose metabolism in *hsp104* mutant cells. Logarithmically growing (L) and heat-shocked (H) cells of the wild-type strain (W303aLEU2+) and the *hsp104* mutant (Δ104LEU2) were harvested, and crude extracts were prepared. The enzyme activities of neutral trehalase (A), trehalose-6-phosphate (T6P) synthase (B), and trehalose-6-phosphate phosphatase (C) were measured in crude extracts as described in the text. The error bar for each sample represents the SD of at least three independent experiments.

indirectly (e.g., cyclic AMP-dependent protein kinase) with neutral trehalase, trehalose-6-phosphate synthase, and trehalose-6-phosphate phosphatase under different temperature conditions to bring about the increased stability. This speculation is based on results showing that Hsp104 contributes to trehalose-metabolizing enzymes under heat shock conditions (Fig. 4). Several proteins in yeast cells are induced to function at high temperatures; such proteins may need Hsp104 for expressing or maintaining their activities at high temperatures.

We thank S. Lindquist, C. De Virgilio, and A. Wiemken for strains and Tomiko Kazama for expert technical assistance.

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