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EXPERIMENTAL ARTICLES

Effect of Calcium Ions on the Hsp104 Synthesis and Heat Tolerance of *Saccharomyces cerevisiae*

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Abstract—Effect of calcium ions on heat tolerance of *Saccharomyces cerevisiae* and on the induction of Hsp104 synthesis by this microorganism was studied. Short-term (30 min) treatment with $CaCl_2$ at 30°C enhanced the heat tolerance to the lethal heat shock (50°C); the synthesis of Hsp104 was induced as well. The effect of Ca^{2+} on the heat tolerance and Hsp104 synthesis was shown to be ion-specific and was inhibited by $LaCl_3$, which is known to block calcium ion channels on the cytoplasmic membrane. The effect of Ca^{2+} depended on the potential of the inner mitochondrial membrane. When the cells were treated with sodium azide, which reduced the electrochemical potential, the effect of calcium both on heat tolerance and Hsp104 synthesis was suppressed. Depending on the concentration of exogenous Ca^{2+} and the ambient conditions, calcium ions may either induce or inhibit the expression of the stress genes and cell viability.

Key words: Saccharomyces cerevisiae, heat tolerance, calcium, Hsp104, mitochondria.

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Under optimal conditions, the level of calcium in the cytosol of yeast cells is 100–200 nM. Maintaining the low calcium concentration is extremely important for normal cell functions. A short-term increase in Ca^{2+} concentration under various physiological stimuli is necessary for the regulation of Ca^{2+}-dependent enzymes, for the cell growth cycle and activation of the stress genes expression. Long-term incubation with the sexual pheromone (α factor), hypotonic shock, addition of glucose to the starving cells, amiodarone treatment, etc., were shown to cause an increase of calcium concentration in the yeast cytoplasm. This increase may due either to calcium inflow from the external medium or to its release from the inner stores such as vacuole and endoplasmic reticulum [1]. Long-term increase of calcium level in the cytosol may lead to programmed cell death (PCD) [2].

The microarray method was used to demonstrate that incubation of yeast cells with calcium induced the expression of more than 160 genes [3]. The proteins encoded by these genes participate in the regulation of a set of metabolic pathways and in the transport of ions or small molecules that regulate the ion homeostasis: *PMC1* coding for Ca^{2+} ATPase responsible for Ca^{2+} transport of into the vacuole; *PMR1* coding for the carrier of Ca^{2+} and Mn^{2+} to the Golgi apparatus; *ENA1* coding for Na^+/Li ATPase from the cytoplasmic membrane, etc. Transcription of these genes is necessary for the growth of yeast at high concentrations of calcium in the medium. Their expression is controlled by Ca^{2+}/calmodulin-dependent phosphatase (calcineurin) [1] participating in activation of the Crz1 transcriptional factor [1, 3].

Synthesis of the heat shock proteins (HSPs) is induced under heat shock thus promoting the development of induced heat tolerance to the subsequent lethal heat treatment. The role of the Hsp104 protein from *S. cerevisiae* in the process is well enough studied. In yeast cells, Hsp104 causes dissociation of the proteins aggregated by the heat shock. This results in enhanced cell viability after the lethal heat shock [4]. Transcription of the heat shock genes in eukaryotes is regulated by binding of the heat shock transcriptional factor (Hsf) to the heat shock elements (*HSE*) located in promoters of the corresponding genes [5]. In *S. cerevisiae*, heat shock and other stresses activate an Hsf-independent mechanism for activation of HSP genes' expression. Two highly homologous transcriptional factors, Msn2 and Msn4, participate in this process via association with the stress-sensitive element *STRE* [6].

The nature of the signal activating transcriptional factors involved in the heat shock response is still unknown. Denaturation of intracellular proteins [7] and intensified generation of reactive oxygen species (ROS) are suggested to play the role of a signal [8]. Fluctuations in the level of intracellular calcium concentration in plant [9] and animal cells [5, 10] under heat shock are also considered as possible signals affecting Hsf transcriptional activity. Heat shock in plant and animal cells was found to cause an increase of calcium concentration in the cytosol followed by

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induction of heat shock proteins. Introduction of calcium chelators or calcium channels blockers suppressed the induction [11, 12].

Data regarding the changes in calcium concentration in the cytosol of yeast cells in response to heat shock are not presently available. The role of calcium ions in heat stress tolerance of the yeast and in the regulation of Hsp104 synthesis is unknown as well. Thus, the objective of this study was to elucidate the role of calcium in heat tolerance and Hsp104 biosynthesis regulation in *S. cerevisiae*.

MATERIALS AND METHODS

Saccharomyces cerevisiae, strain Ψ-74-D694, kindly supplied by Prof. S. Lindquist (Whitehead Institute for Biomedical Research, United States), was used throughout the study. The yeast were maintained on YEPD culture medium (yeast extract, 5 g/l; peptone, 10 g/l; glucose, 20 g/l; agar, 15 g/l) at 30°C.

Yeast were cultivated at 30°C in 100-ml flasks containing 25-ml liquid YEPD medium for 14–16 h. In the experiments, the necessary volume of the 12-h culture was added to the fresh culture medium and incubated to the concentration of 2×10^7 cell/ml.

To elucidate the role of Ca^{2+}, Mg^{2+}, and Mn^{2+} in heat tolerance, the cells were harvested by centrifugation (5000 *g*, 5 min), suspended in fresh YEPD medium containing 100–400 mM of $CaCl_2$, $MgCl_2$ or $MnCl_2$ (pH 5.5), and incubated at 30 or 39°C for the specified time intervals. To block calcium channels and mitochondrial activity, 0.2–1.0 mM of $LaCl_3$ and 0.15 mM of NaN_3 were used, respectively. To remove the blockers and ions, the cells were precipitated by centrifugation and resuspended in fresh YEPD medium. The suspension was distributed into test tubes (1 ml per tube) and subjected to 50°C for 0–8 min on a thermostatic rotary shaker (110 rpm). After heat shock, the suspension was cooled, diluted, and plated on solid YEPD medium. The number of colony-forming units (CFU) was counted after incubation for 24–48 h at 30°C. The survival rate of the cells was determined as the number of colonies from the heat-treated cells to the number of colonies from nontreated cells (expressed in percent).

To study the effect of Ca^{2+}, Mg^{2+}, and Mn^{2+} on the induction of Hsp104 and Ssa2 synthesis, the cells were treated as described above, washed and kept at –70°C prior to protein isolation. Then the cells were defrozen, resuspended in the buffer for protein purification (0.1 M of Tris–HCl, 3 mM of SDS, 1 mM of β-mercaptoethanol, pH 7.4–7.6), frozen with liquid nitrogen, and triturated with quartz sand. The crude cell components were removed by centrifugation (15000 *g*, 15 min), the protein was precipitated with a triple volume of cooled acetone. The pellet was washed three times with acetone and diluted in the buffer (0.625 M of Tris–HCl, 8 mM of SDS, 0.1 M of β-mercaptoethanol, 10% glycerol, 0.001% bromophenol blue, pH 6.8). The concentration of protein was determined according to Lowry et al. [13]. Separation of proteins by SDS-Na electrophoresis in 12% PAAG was followed by immunoblotting with antibodies against Hsp104 (SPA-8040, StressGen, United States) and Ssa2 (kindly provided by Prof. E. Craig, University of Wisconsin, United States), in accordance with the method described earlier [14].

All experiments were performed in triplicate. The results obtained were statistically treated: the mean values and P values were calculated.

RESULTS AND DISCUSSION

To elucidate the role of calcium in formation of the heat shock response in yeast cell, the effect of $CaCl_2$ on the synthesis of Hsp104 and heat tolerance at 50°C (lethal heat shock) was studied. The yeast cells grown at 30°C were pretreated with 200 mM of $CaCl_2$ for 30, 60, and 120 min, washed, and subjected to heat shock (50°C). The heat shock at 50°C had a pronounced lethal effect on the yeast cells (Fig. 1a). Treatment with 200 mM of $CaCl_2$ for 30 min resulted in an increased survival rate of the cells. However, if the calcium treatment was prolonged (120 min), the protecting effect decreased significantly.

PAAG electrophoresis and immunoblotting against Hsp104 (Fig. 1b) demonstrated the absence of Hsp104 in the control (0 min). In accordance with the literature [4], heat shock (39°C) caused dramatic activation of this protein synthesis. The treatment with 200 mM of $CaCl_2$ at 30°C for 30 min also led to an increase in Hsp104 synthesis, though in a lesser extent than the treatment at 39°C. However, prolonged incubation (120 min) of the cells with calcium inhibited the synthesis of this protein. No increase in Ssa2 synthesis (one of the homologues of the yeast Hsp70 protein) was recorded in the cells of *S. cerevisiae* at 39°C if compared with the control (Fig. 1b). The amount of this protein did not change after the treatment with 200 mM of $CaCl_2$ as well. The level of Hsp104 in the analyzed samples corresponded completely to the ability of yeast to tolerate the heat shock. By varying calcium concentrations, we found 400 mM of $CaCl_2$ to be optimal for Hsp104 synthesis and heat tolerance (Fig. 1c, Fig. 1d). Therefore, in further experiments we used treatment of the cells with 400 mM of $CaCl_2$ for 30 min.

To demonstrate that calcium ions are responsible for the tolerance to lethal heat shock (50°C) and for the induction of Hsp104 synthesis, as well as to show the specificity of this effect, magnesium and manganese ions were used in the following study. The yeast grown at 30°C were treated with $CaCl_2$, $MgCl_2$ and $MnCl_2$ in concentrations of 400 mM for 30 min, washed, and subjected to the heat shock (50°C). When the heat shock duration was increased, the percentage of viable cells in the control variant decreased drastically (Fig. 2a, curve *1*) while the cells treated with cal-

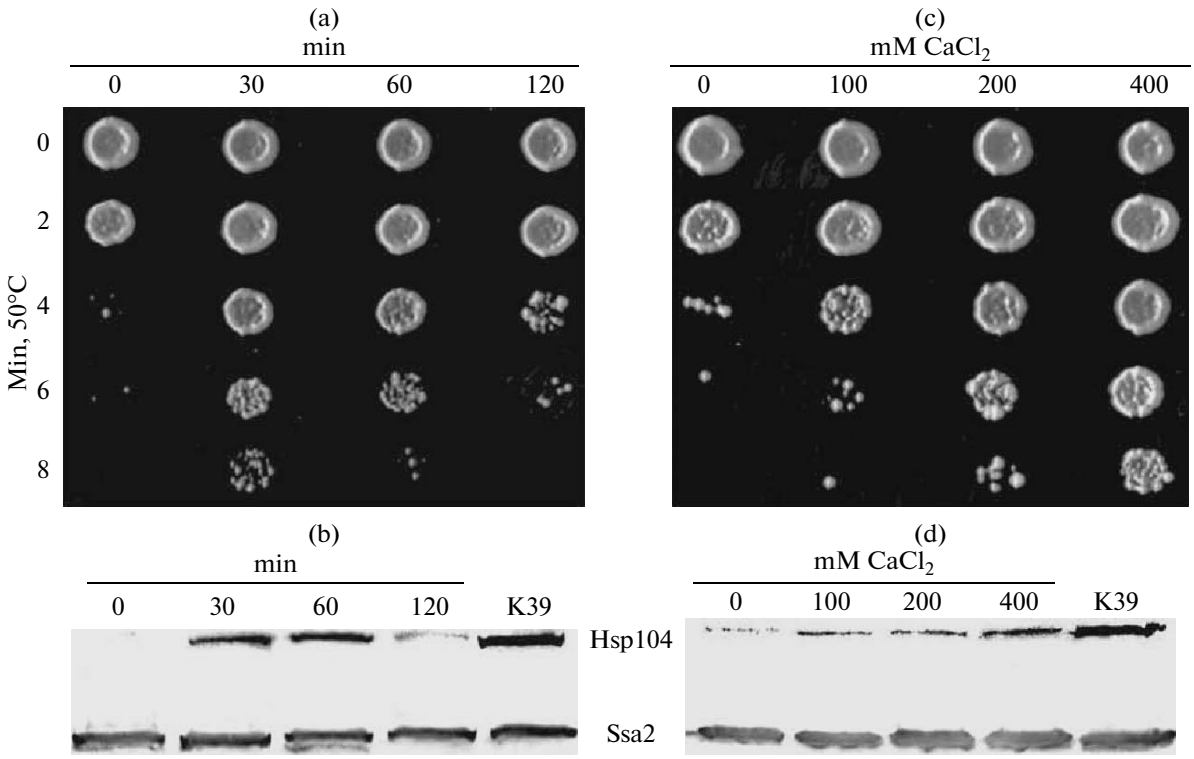

Fig. 1. Effect of Ca^{2+} on heat tolerance and Hsp104 synthesis in *S. cerevisiae*. The cells were treated either with 200 mM of $CaCl_2$ at 30°C for 0, 30, 60, and 120 min (a, b) or with 100, 200, and 400 mM of $CaCl_2$ at 30°C for 30 min (c, d); survival rate after the heat shock (50°C) (a, c); Hsp104 and Ssa2 synthesis in calcium-treated cells (b, d). At the right, synthesis of Hsp104 and Ssa2 at 39°C for 30 min is shown (K39).

cium acquired enhanced heat tolerance at 50°C (Fig. 2a, curve *2*). Magnesium and manganese exhibited a significantly less pronounced effect (Fig. 2a, curves *3*, *4*). Immunoblotting results showed that, unlike calcium ions, magnesium and manganese ions did not cause significant induction of Hsp104 synthesis. Specific effect of calcium on induction of Hsp104 was confirmed in experiments with the blocker of calcium channels, $LaCl_3$ (Fig. 3). Varying $LaCl_3$ concentrations, we found that induction of Hsp104 synthesis after calcium treatment was effectively inhibited by 1 mM of $LaCl_3$.

Short-term treatment with moderately high temperatures is known to induce the tolerance of yeast to the subsequent severe heat shock. This effect is named acquired or induced heat tolerance, correlates with Hsp104 synthesis and trehalose accumulation [15] suggesting the protective function of Hsp104 [4]. In order to study the effect of calcium treatment on induced heat tolerance and heat-induced Hsp104, the yeast grown at 30°C were incubated at 30 or 39°C for 30 min with or without 400 mM of $CaCl_2$. As expected, calcium treatment at 30°C induced Hsp104 synthesis and enhanced the survival rates under the lethal heat shock (50°C) (Fig. 4). On the contrary, in the case of the 39°C heat shock, calcium-treated cells exhibited suppression of the heat-induced Hsp104 synthesis and induction of heat tolerance if compared with non-treated cells (Fig. 4). Therefore, depending on temperature conditions, calcium may either induce or inhibit the synthesis of Hsp104.

We have shown previously that induction of Hsp104 synthesis by heat shock and development of induced heat tolerance at 39°C were suppressed in *S. cerevisiae* in the presence of mitochondrial blockers and uncouplers [15, 17]. To find out the relation between the mitochondrial dysfunctions and the ability of calcium to induce heat tolerance and Hsp104 synthesis, the yeast grown at 30°C were treated with 400 mM of $CaCl_2$ with or without 0.15 mM of NaN_3 (NaN_3 inhibits cytochrome oxidase activity and the hydrolytic activity of F_1 ATPase) [17]. The results obtained indicated that NaN_3 inhibited the positive effect of calcium treatment (400 mM of $CaCl_2$), an increase in survival rate following severe heat shock and induction of Hsp104 synthesis (Fig. 5). Obviously, impairment of the mitochondrial functions suppressed the calcium effect on heat tolerance and Hsp104 production.

We established earlier that the heat shock caused an increase of the potential on the inner mitochondrial membrane ($mt\Delta\Psi$) in yeast cells and *Arabidopsis thaliana* cell cultures. This reaction plays the role of a signal activating HSP synthesis. The presence of

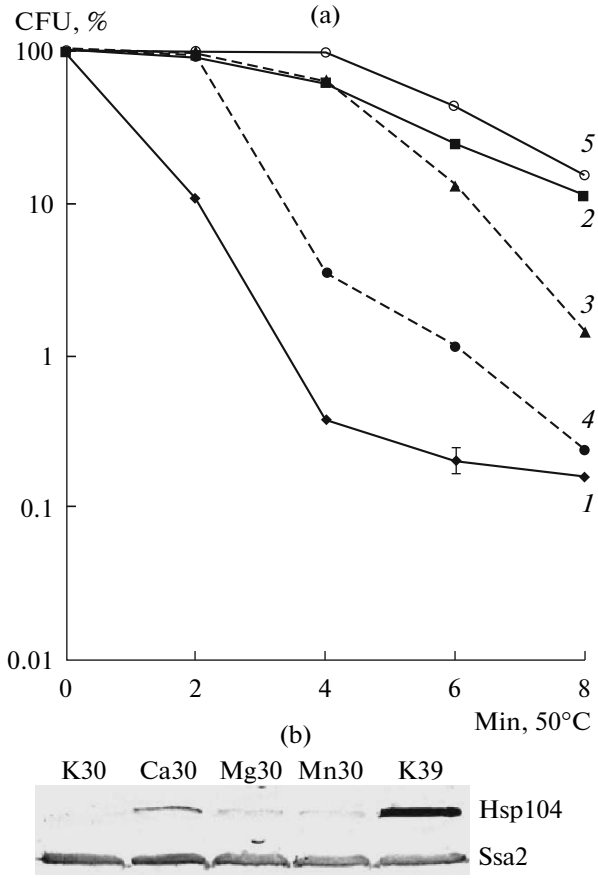

Fig. 2. Effect of Ca^{2+}, Mg^{2+}, and Mn^{2+} on heat tolerance and Hsp104 synthesis in *S. cerevisiae*. The cells were incubated for 30 min in the absence (*1*) or presence of 400 mM of $CaCl_2$ (*2*), $MgCl_2$ (*3*), or $MnCl_2$ (*4*) at 30 or 39° (*5*). Survival rate after heat shock (50°C) determined as number of colony-forming units (CFU) (a); Hsp104 and Ssa2 synthesis in the cells in control (K30) and after treatment with Ca^{2+} (Ca30), Mg^{2+} (Mg30), and Mn^{2+} (Mn30) (b). At the right, synthesis of Hsp104 and Ssa2 at 39°C for 30 min is shown (K39).

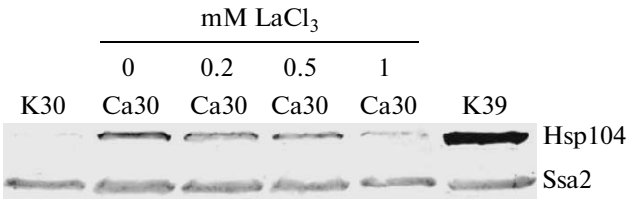

Fig. 3. Effect of various $LaCl_3$ concentrations on Hsp104 synthesis in *S. cerevisiae*. The cells were incubated at 30°C for 30 min with addition of 0, 0.2, 0.5, and 1 mM of $LaCl_3$ and then incubated at 30°C for 30 min in the absence (K30) or in the presence of 400 mM of $CaCl_2$ (Ca30). At the right, synthesis of Hsp104 and Ssa2 at 39°C for 30 min is shown (K39).

sodium azide and other mitochondrial blockers and uncouplers in the medium during heat shock suppressed the increase of the potential on the inner mitochondrial membrane, induction of HSP synthesis, and induced heat tolerance [17–19]. This phenomenon was confirmed by other studies [20], which demonstrated that heat shock leads to hyperpolarization of the inner mitochondrial membrane in animal cells and to be accompanied by activation of Hsp70 expression. Pozniakovsky et al. [2] found mtΔΨ to be enhanced in the yeast cells treated with amiodarone which disturbs calcium homeostasis of the cell. The authors proposed the enhancement of mtΔΨ to result from the increase in the level of cytosol calcium and its subsequent transport to mitochondria. To prove that an increase of mtΔΨ under mild heat shock resulted from the enhanced level of mitochondrial calcium, investigation of the effect of extracellular calcium on Hsp104 synthesis and *S. cerevisiae* heat tolerance was required.

The results obtained demonstrated that short-term calcium treatment (30 min, 30°C) led to the increased resistance of yeast cells to lethal heat shock (Figs. 1a, 1c). Exogenous calcium also enhanced the tolerance of *S. cerevisiae* to the effect of amiodarone [21] and α factor [22]. At the same time, calcium treatment increased heat resistance of tobacco plants [23].

Calcium is known to induce HSP synthesis in plants and animals. Exogenous calcium caused the increase in the activity of the β-glucuronidase gene, which was expressed in *Arabidopsis* cells under the *AtHSP18.2* promoter [11]. Treatment with exogenous $CaCl_2$ activated *HSP26* and *HSP70* gene expression in wheat, while calcium channels blockers (verapamil and $LaCl_3$) and EGTA suppressed it [24]. We obtained the similar results: calcium treatment (30 min, 30°C) caused induction of Hsp104 synthesis in *S. cerevisiae* (Figs. 1b, d) whereas $LaCl_3$ led to its suppression (Fig. 3). The effect on heat tolerance and Hsp104 synthesis was found to be calcium-specific, since other bivalent ions (manganese and magnesium) did not manifest similar effects (Fig. 2). The data discussed allowed us to suggest that calcium treatment-induced Hsp104 synthesis resulted in enhanced heat tolerance of the yeast. Nevertheless, calcium may be involved in modulating of other heat tolerance mechanisms including the increase in trehalose level.

Calcium effect on Hsp104 synthesis in *S. cerevisiae* may be linked with the ability of Ca^{2+} to activate Hsf (heat shock factor). In animal and plant cells, calcium was shown to activate Hsf by initiating its binding to HSE (heat shock element) [12, 25]. This phenomenon may be due to the capability of human Ca^{2+}/calmodulin-dependent protein kinase (CaMK II) to phosphorylate Ser in the 230th position [26]. No data exist regarding phosphorylation of Hsf of *S. cerevisiae* by Ca^{2+}/calmodulin-dependent protein kinase. However, deletion of the gene coding for this enzyme suppressed the ability of yeast to obtain induced heat tolerance [27]. Calcium may also activate transcriptional activity of the Msn2 and Msn4 factors. Activity of

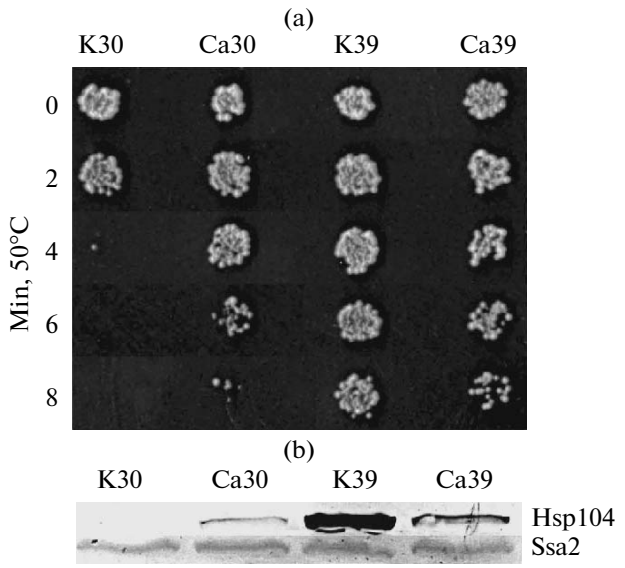

Fig. 4. Effect of calcium on heat tolerance and Hsp104 synthesis at 30 and 39°C in *S. cerevisiae*. The cells were incubated at 30 or 39°C for 30 min in the absence (K30, K39) or in the presence of 400 mM of $CaCl_2$ (Ca30, Ca39). Survival rate after the heat shock (50°C) (a); Hsp104 and Ssa2 synthesis (b).

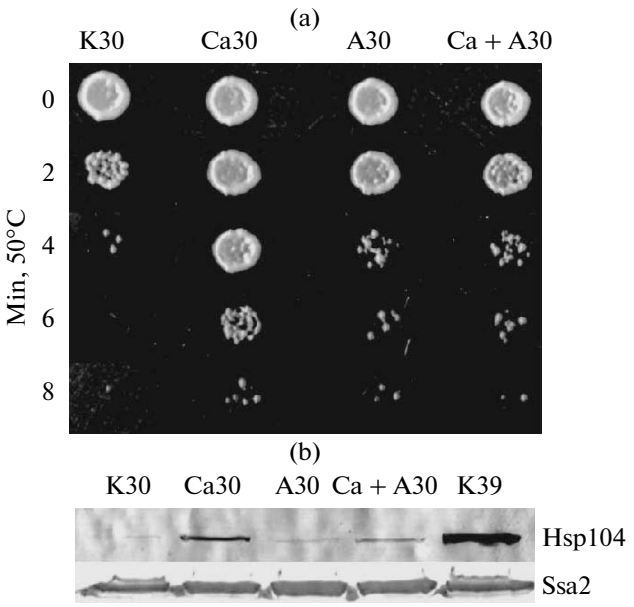

Fig. 5. Effect of sodium azide on heat tolerance and Hsp104 synthesis after calcium treatment of *S. cerevisiae*. The cells were incubated at 30°C for 30 min in the absence (K30) or in the presence of 400 mM of $CaCl_2$ (Ca30), 0.15 mM of NaN_3(A30), 400 mM of $CaCl_2$, and 0.15 mM of NaN_3 (Ca + A30). Survival rate after the heat shock (50°C) (a); Hsp104 and Ssa2 synthesis (b). At the right, synthesis of Hsp104 and Ssa2 at 39°C for 30 min is shown (K39).

Msn2 and Msn4 is negatively controlled by cAMP-dependent protein kinase A—cAMP-PK [6].The level of cAMP in the yeast cells is known to be affected by heat shock [28], while the *bcy1* with cAMP-PK hyperactivity did not demonstrate Ca^{2+}/calmodulin-dependent expression of the *ENA1* gene [29].

Accumulation of calcium in the cytosol under stress conditions may switch on the protective program and, consequently, to the activation of stress genes expression [1]; however, the excess of calcium may be harmful to the cell [2]. Actually, short-term calcium treatment (30 min) induced heat tolerance and Hsp104 synthesis in the yeast cells, while longer exposure to calcium (120 min) suppressed both functions (Figs. 1a, b).

Extrapolation of the results obtained for yeast subjected to other growth-limiting stresses [1] and the results regarding the plant and animal objects including heat shock response [5, 23] suggests that elevated calcium level in the cytosol under heat shock occurs in yeast cells as well. This is in accordance with the fact that under heat shock (39°C), when HSP and heat tolerance are induced, the presence of exogenous calcium suppressed Hsp104 synthesis and induced heat tolerance (Fig. 4). We suggest that the natural increase in calcium concentration under heat shock, together with the introduction of calcium to cytosol from exogenous $CaCl_2$ has an additive effect, and the increase of calcium concentration above a certain level suppresses HSP genes' expression.

It is likely that the effect of mitochondrial dysfunction on heat tolerance and Hsp104 synthesis may be explained the same way. Our experiments demonstrated that the stimulating calcium effect on Hsp104 production and heat tolerance in yeast was suppressed by addition of sodium azide, an mt$\Delta\Psi$ inhibiting agent. This substance also suppressed induction of HSP synthesis and heat tolerance under mild heat shock in yeast [15, 17, 18] and plant [19] cells. Energized animal mitochondria absorb Ca^{2+} depending on the electrochemical potential of the inner mitochondrial membrane. If the electrochemical potential of the inner mitochondrial membrane is depolarized, Ca^{2+} influx into the mitochondrion of plant or animal cell is reduced, resulting in the increase in the level of calcium in the cytosol [30]. We suggest sodium azide treatment in the presence of exogenous calcium to inhibit the inflow of Ca^{2+} into the mitochondria and cause an increase of its level in the cytosol. This implies that the transport of calcium ions from the cytosol to the mitochondria acts as a signal for HSP genes expression. In fact, in plant and animal cells, an increase of the level of Ca^{2+} in the cytosol under various stresses was accompanied by an increased calcium level in the mitochondria [30, 31]. The same process may occur in yeast mitochondria under heat shock. However, the mechanism of the transport of calcium to the yeast mitochondria is unclear. Unlike mitochondria of the plant and animal cells, mitochondria of the yeast *in organello* do not possess an active cal-

cium transport system [32]. However, since calcium treatment of permeabilized yeast cells under oxidative stress was shown to cause the opening of a mitochondrial pore, yeast mitochondria are probably able to accumulate calcium in vivo [33].

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REFERENCES

1. Cyert, M.S., Calcineurin Signaling in *Saccharomyces cerevisiae*: How Yeast Go Crazy in Response to Stress, *Biohem. Biophys. Res. Commun.*, 2003, vol. 311, pp. 1143–1150.
2. Pozniakovsky, A.I., Knorre, D.A., Markova, O.V., Hyman, A.A., Skulachev, V.P., and Severin, F.F., Role of Mitochondria in the Pheromone- and Amiodarone-Induced Programmed Death of Yeast, *J. Cell Biol.*, 2005, vol. 168, no. 2, pp. 257–269.
3. Yoshimoto, H., Saltsman, K., Gasch, A.P., Li, H.X., Ogawa, N., Botstein, D., Brown, P.O., and Cyert, M.S., Genome-Wide Analysis of Gene Expression Regulated by the Calcineurin/Crz1p Signaling Pathway in *Saccharomyces cerevisiae, J. Biol. Chem.*, 2002, vol. 277, no. 34, pp. 31079–31088.
4. Doyle, S.M. and Wickner, S., Hsp104 and ClpB: Protein Disaggregating Machines, *Trends Biochem. Sci.*, 2009, vol. 34, no. 1, pp. 40–48.
5. Pirkkala, L., Nykanen, P., and Sistonen, L., Roles of the Heat Shock Transcription Factors in Regulation of the Heat Shock Response and Beyond, *FASEB J.*, 2001, vol. 15, no. 7, pp. 1118–1131.
6. Trott, A. and Morano, K.A., The Yeast Response to Heat Shock, in *Topics in Current Genetics: Yeast Stress Responses,* Hohmann, S. and Mager, P.W.H., Eds., New York: Springer, 2003, pp. 71–119.
7. Ananthan, J., Goldberg, A.L., and Voelmy, R., Abnormal Proteins Serve as Eukaryotic Stress Signals and Trigger the Activation of Heat Shock Genes, *Science*, 1986, vol. 232, pp. 522–524.
8. Moraitis, C. and Curran, B.P., Reactive Oxygen Species May Influence the Heat Shock Response and Stress Tolerance in the Yeast *Saccharomyces cerevisiae, Yeast*, 2004, vol. 21, pp. 313–323.
9. Kotak, S., Larkindale, J., Lee, U., von Koskull-Döring, P., Vierling, E., and Scharf, K.D., Complexity of the Heat Stress Response in Plants, *Curr. Opin. Plant Biol.*, 2007, vol. 10, no. 3, pp. 310–316.
10. Kultz, D., Evolution of the Cellular Stress Proteome: from Monophyletic Origin to Ubiquitous Function, *J. Exp. Biol.*, 2003, vol. 206, no. 18, pp. 3119–3124.
11. Liu, H.T., Sun, D.Y., and Zhou, R.G., Ca^{2+} and AtCaM3 Are Involved in the Expression of Heat Shock Protein Gene in *Arabidopsis, Plant Cell Environ.*, 2005, vol. 28, pp. 1276–1284.
12. Price, B.D. and Calderwood, S.K., Ca^{2+} Is Essential for Multistep Activation of the Heat Shock Factor in Permeabilized Cells, *Mol Cell Biol.*, 1991, vol. 11, no. 6, pp. 3365–3368.
13. Lowry, O.H., Rosebrough, N.I., Farr, A.L., and Rendall, R.J., Protein Measurement with the Folin Phenol Reagent, *J. Biol. Chem.*, 1951, vol. 193, pp. 265–275.
14. Timmons, T.M. and Dunbar, B.S., Protein Blotting and Immunodetection, *Meth. Enzymol.*, 1990, vol. 182, pp. 679–701.
15. Tereshina, V.M., Thermotolerance in Fungi: The Role of Heat Shock Proteins and Trehalose, *Mikrobiologiya*, 2005, vol. 74, no. 3, pp. 293–304 [*Microbiology* (Engl. Transl.), vol. 74, no. 3, pp. 247–257].
16. Rikhvanov, E.G., Varakina, N.N., Rusaleva, T.M., Rachenko, E.I., Borovskii, G.B., and Voinikov, V.K., The Induction of *Saccharomyces cerevisiae* Hsp104 Synthesis by Heat Shock Is Controlled by Mitochondria, *Genetika*, 2004, vol. 40, no. 4, pp. 341–347 [*Russ. J. Genetics* (Engl. Transl.), vol. 40, no. 4, pp. 341–347].
17. Rikhvanov, E.G., Varakina, N.N., Rusaleva, T.M., Rachenko, E.I., Knorre, D.A., and Voinikov, V.K., Do Mitochondria Regulate the Heat-Shock Response in *Saccharomyces cerevisiae?, Curr. Genet.*, 2005, vol. 48, pp. 44–59.
18. Rikhvanov, E.G., Lukina, E.A., Varakina, N.N., Rusaleva, T.M., Gamburg, K.Z., Knorre, D.A., Borovskii, G.B., and Voinikov, V.K., Mitochondria as a Critical Element of Heat Shock Response in Yeast with Different Types of Energy Metabolism, *Fiziol. Rastenii*, 2006, vol. 53, no. 5, pp. 695–702 [*Russ. J. Plant Physiol.* (Engl. Transl.), vol. 53, no. 5, pp. 615–621].
19. Rikhvanov, E.G., Gamburg, K.Z., Varakina, N.N., Rusaleva, T.M., Fedoseeva, I.V., Tauson, E.L., Stupnikova, I.V., Stepanov, A.V., Borovskii, G.B., and Voinikov, V.K., Nuclear-Mitochondrial Cross-Talk During Heat Shock in *Arabidopsis* Cell Culture, *Plant J.*, 2007, vol. 52, no. 4, pp. 763–778.
20. Balogh, G., Horvath, I., Nagy, E., Hoyk, Z., Benko, S., Bensaude, O., and Vigh, L., The Hyperfluidization of Mammalian Cell Membranes Acts as a Signal to Initiate the Heat Shock Protein Response, *FEBS J.*, 2005, vol. 272, no. 23, pp. 6077–6086.
21. Courchesne, W.E. and Ozturk, S., Amiodarone Induces a Caffeine-Inhibited, *MID-1* Depedent Rise in Free Cytoplasmic Calcium in *Saccharomyces cerevisiae, Mol. Microbiol.*, 2003, vol. 47, no. 1, pp. 223–234.
22. Iida, H., Nakamura, H., Ono, T., Okumura, M.S., and Anraku, Y., *MID1*, a Novel *Saccharomyces cerevisiae* Gene Encoding a Plasma Membrane Protein, Is Required for Ca^{2+} Influx and Mating, *Mol. Cell. Biol.*, 1994, vol. 14, no. 12, pp. 8259–8271.
23. Gong, M., van der Luit, A., Knight, M., and Trewavas, A., Heat-Shock-Induced Changes in Intracellular Ca^{2+} Level in Tobacco Seedlings in Relation to Thermotolerance, *Plant Physiol.*, 1998, vol. 116, pp. 429–437.
24. Liu, H.T., Li, B., Shang, Z.L., Li, X.Z., Mu, R.L., Sun, D.Y., and Zhou, R.G., Calmodulin Is Involved in Heat Shock Signal Transduction in Wheat, *Plant Physiol.*, 2003, vol. 132, no. 3, pp. 1186–1195.
25. Li, B., Liu, H.T., Sun, D.Y., and Zhou, R.G., Ca^{2+} and Calmodulin Modulate DNA-Binding Activity of Maize Heat Shock Transcription Factor *in vitro*, *Plant Cell Physiol.*, 2004.

26. Holmberg, C.I., Hietakangas, V., Mikhailov, A., Rantanen, J.O., Kallio, M., Meinander, A., Hellman, J., and Morrice, N., MacKintosh C., Morimoto R.I., Phosphorylation of Serine 230 Promotes Inducible Transcriptional Activity of Heat Shock Factor 1, *EMBO J.*, 2001, vol. 20, no. 14, pp. 3800–3810.

27. Iida, H., Ohya, Y., and Anraku, Y., Calmodulin-Dependent Protein Kinase II and Calmodulin Are Required for Induced Thermotolerance in *Saccharomyces cerevisiae, Curr. Genet.*, 1995, vol. 27, no. 2, pp. 190–193.

28. Biryukova, E.N., Medentsev, A.G., Arinbasarova, A.Yu., and Akimenko, V.K., Adaptation of the Yeast *Yarrowia lipolytica* to Heat Shock, *Mikrobiologiya*, 2007, vol. 76, no. 2, pp. 184–190 [*Microbiology* (Engl. Transl.), vol. 76, no. 2, pp. 158–163].

29. Hirata, D., Harada, S., Namba, H., and Miyakawa, T., Adaptation to High-Salt Stress in *Saccharomyces cerevisiae* Is Regulated by Ca^{2+}/Calmodulin-Dependent Phosphoprotein Phosphatase (Calcineurin) and CAMP-Dependent Protein Kinase, *Mol. Gen. Genet.*, 1995, vol. 249, pp. 257–264.

30. Jacobson, J. and Duchen, M.R., Interplay between Mitochondria and Cellular Calcium Signaling, *Mol. Cell Biochem*, 2004, vol. 256–257, nos. 1–2, pp. 209–218.

31. Logan, D. and Knight, M., Mitochondrial and Cytosolic Calcium Dynamics Are Differentially Regulated in Plants, *Plant Physiol.*, 2003, vol. 133, pp. 21–24.

32. Jung, D.W., Bradshaw, P.C., and Pfeiffer, D.R., Properties of a Cyclosporin-Insensitive Permeability Transition Pore in Yeast Mitochondria, *J. Biol. Chem.*, 1997, vol. 272, no. 34, pp. 21104–21112.

33. Kowaltowski, A.J., Vercesi, A.E., Rhee, S.G., and Netto, L.E., Catalases and Thioredoxin Peroxidase Protect *Saccharomyces cerevisiae* against Ca^{2+}-Induced Mitochondrial Membrane Permeabilization and Cell Death, *FEBS Lett.*, 2000, vol. 473, no. 2, pp. 177–182.