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EXPERIMENTAL ARTICLES

The Role of Trehalose and Glycogen in the Survival of Aging *Saccharomyces cerevisiae* Cells

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Abstract—The role of the storage carbohydrates trehalose and glycogen in the survival of aging *Saccharomyces cerevisiae* cells was studied. Culture aging for one week did not reduce cell viability. During this period, the cells accumulated the storage carbohydrates and showed increased activity of the glycolytic enzymes hexokinase and phosphofructokinase. However, further aging led to a drastic drop in cell viability and to a decrease in the cellular content of trehalose and glycogen and in the activity of hexokinase and phosphofructokinase. The possible reasons for these changes are discussed.

Key words: aging, glycogen, trehalose, glycolytic enzymes, *Saccharomyces cerevisiae.*

Recent investigations showed that metabolic control and dysfunctions play an important part in yeast aging [1] and that there are genes that control aging processes [1] and are responsible for the stationary-phase adaptation of yeast cells [2]. These genes are involved in the regulation of many metabolic reactions, which suggests that the aging of *Saccharomyces cerevisiae* is a complex process resembling that of mammals [3].

There are two major mechanisms of yeast aging, replicative and chronological. The replicative aging of a mother yeast cell, defined as its ability to produce buds, is measured by the number of buds produced. In the case of animal cells, the analogue is their proliferative lifespan. The chronological aging of yeasts is closely related to their postmitotic life and resembles the aging of mitotically inactive fibroblasts in multicellular organisms. The study of the chronological aging of a cell population provides more adequate results than the study of their replicative aging since, in the former case, the investigated cell population does not become heterogeneous because of bud formation.

The metabolisms of trehalose and glycogen in *S. cerevisiae* are closely related to many cellular functions [4], including the regulation of the cell cycle [5] and cell growth [6].

The aim of this work was to study the dynamics of trehalose and glycogen in chronologically aging *S. cerevisiae* cells.

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MATERIALS AND METHODS

The strain *Saccharomyces cerevisiae* Y-1173 was kindly provided by V.M. Vagabov from the Skryabin Institute of Biochemistry and Physiology of Microorganisms in Pushchino. The strain was cultivated at 28°C on a shaker (200 rpm) in 700-ml flasks with 200 ml of Reader medium. Yeast growth was evaluated spectrophotometrically (SF-46 spectrophotometer; 600 nm). Spectrophotometric data were recalculated for dry biomass by using a calibration curve.

Cell aging was induced by incubating stationary-phase cells under nonstarvation conditions. Preliminarily, we determined the threshold (minimum) optical density of cell populations at which the cells do not produce buds in spite of the presence of all essential nutrients in the medium (this density was found to be 22 OD units). The postmitotic state of cells in such populations is determined by the so-called crowd-sensing effect. In aging experiments, *S. cerevisiae* cells grown to the late exponential phase were harvested by centrifugation, suspended to give an optical density of 22 units, and incubated for 12 h. This point was arbitrarily chosen as zero aging time. Two times a week, the cells were allowed to settle onto the flask bottom at 5°C and the depleted medium was replaced by an equivalent volume of fresh nutrient medium. We did not observe any cell growth after such medium replacement. Cells were sampled once a week by centrifugation at 3000 *g*. The precipitated cells were washed twice with cold distilled water. The viability of the cells was estimated by plating them onto malt extract agar [8]. The results were expressed in colony-forming units (CFU). The number of CFU at zero time was taken to be 100%.

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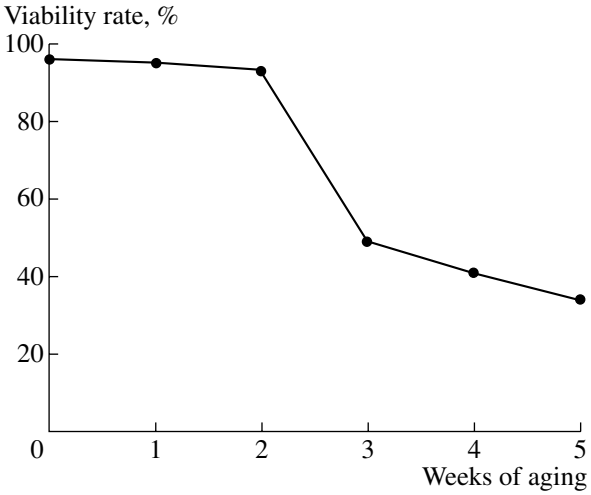

Fig. 1. The viability of cells in an aging *S. cerevisiae* culture. The viability rate at zero time was taken to be 100%.

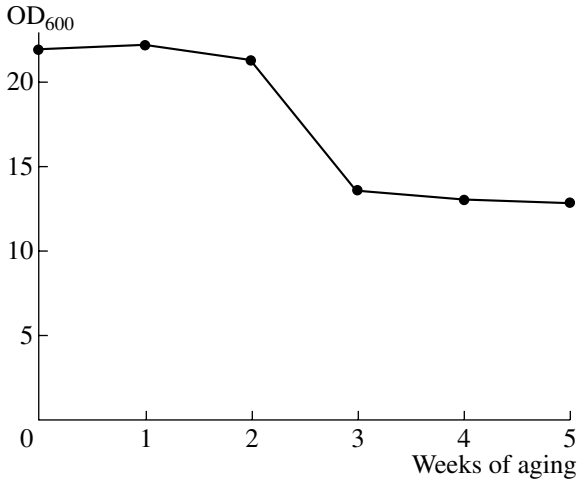

Fig. 2. Changes in the optical density of the aging *S. cerevisiae* culture.

Trehalose and glycogen were determined by the method of Parrou and Francois [7]. Hexokinase (EC 2.7.1.1) and phosphofructokinase (EC 2.7.1.11) were assayed as described by Maitra and Lobo [9]. The lipid peroxidation rate was estimated by analyzing 2-thiobarbituric acid–reactive (TBA-reactive) products [10]. To determine the number of carbonyl groups in cellular proteins, cells were washed three times in cold 50 mM potassium phosphate buffer (pH 7.4) and resuspended in the same buffer to a concentration of 20 mg absolutely dry biomass per milliliter. Two aliquots (0.5 ml) of this suspension were mixed with 0.5 ml of 10% trichloroacetic acid each. The mixtures were centrifuged at 10000 *g* for 15 min, and the supernatants were discarded. One pellet (the control) was suspended in 1 ml of 2 M HCl, and the other (the sample) was suspended in 1 ml of 0.2% 2,4-dinitrophenylhydrazine in 2 M HCl. The control and the sample mixtures were incubated at 37°C for 1 h, supplemented with 600 mg guanidine chloride each, and centrifuged at 10000 *g* for 15 min. Then the optical densities of the supernatants were measured at 540 nm. The number of protein carbonyl groups was calculated by assuming that the molar extinction coefficient of hydrazone produced from 2,4-dinitrophenylhydrazine is 21.0 $mmol^{-1}$ cm^{-1}.

Protein was quantified by the Lowry method. The reagents used in the work were purchased from Sigma. All the experiments were performed in triplicate.

RESULTS

Preliminary experiments with dense cell suspensions showed that the regular replacement of depleted nutrient medium with fresh medium had no appreciable influence on the dynamics of the reserve carbohydrates in the cells and the activity of the glycolytic enzymes hexokinase and phosphofructokinase. Nor did the medium replacement influence cell budding in the aging *S. cerevisiae* culture. This is readily explicable by the fact that cells in such suspensions are subject to the crowd-sensing effect and undergo true postmitotic aging. Another important finding in these experiments was that the depletion of nutrient medium and the accumulation of toxic metabolic products exert negligible effects on the chronological aging of *S. cerevisiae* cells.

As can be seen from Fig. 1, the relative number of viable cells in the aging culture decreased insignificantly up to the second week of the experiment. During the third week, cell viability dropped to a level of 44% and then decreased insignificantly (to 36% of the original level). A similar dynamics was observed for the optical density of the aging culture (Fig. 2). These changes were accompanied by an increase in the average cell size. Budding cells were not observed (data not presented).

The cellular content of trehalose and glycogen increased during the first week of the experiment, virtually did not change during the second week, and gradually decreased to zero during the third and fourth weeks (Fig. 3). In this case, as mentioned above, cell viability did not decrease below 36%.

Bearing in mind that glycolysis and the metabolism of the reserve carbohydrates in yeast cells are closely related [11], we assayed the key glycolytic enzymes hexokinase and phosphofructokinase in the aging cells (Table 1). The activity of these enzymes increased during the first week of the experiment, virtually did not change during the second week, and gradually decreased about twofold during the third and fourth weeks. These data suggest that, in aging cells, the activity of the upper glycolytic pathway (and, hence, glycolysis as a whole) decreases in time.

Peroxidation is considered to be a major factor that causes cell aging [12, 13], which poses the problem of its involvement in the aging of yeasts in our experi-

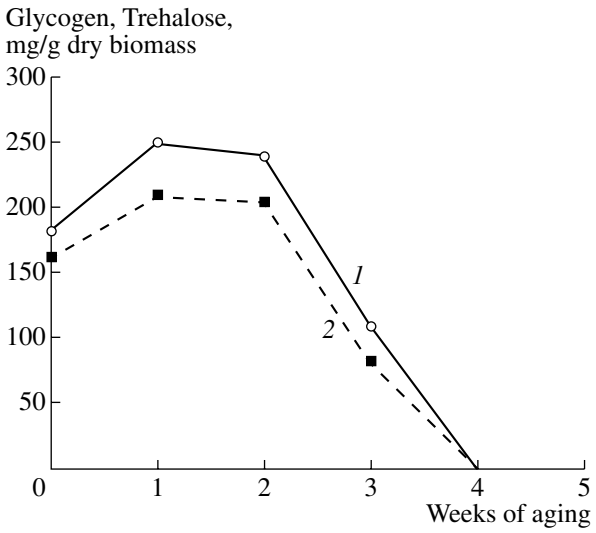

Fig. 3. The content of (*1*) glycogen and (*2*) trehalose in aging *S. cerevisiae* cells.

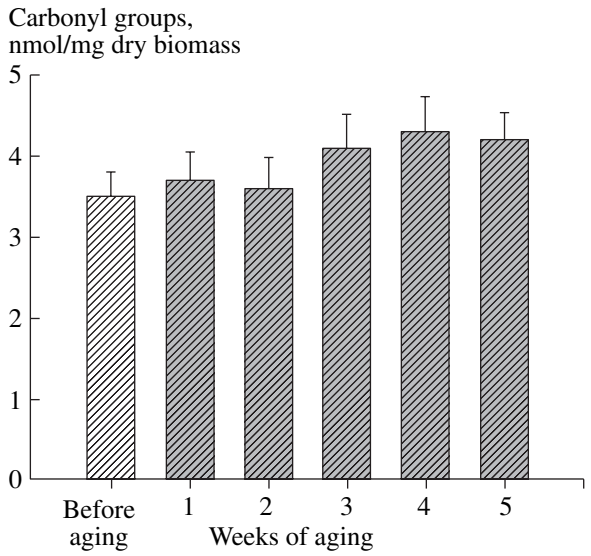

Fig. 4. The content of the carbonyl groups of proteins in aging *S. cerevisiae* cells.

ments. The measurement of TBA-reactive products (TBA is a marker of peroxidation intensity [14]) showed that the level of these products in the aging cells changed insignificantly (Table 2), suggesting that peroxidation does not contribute significantly to cell aging. This suggestion is in agreement with the determination of the content of protein carbonyl groups (Fig. 4), which serves as a marker of oxidative damage to biopolymers.

The experiments showed that all of the measured parameters (cell viability, the cellular content of the reserve carbohydrates, and the activity of the glycolytic enzymes) exhibited marked changes during the first and third weeks of the experiment, suggesting basic metabolic alterations in aging cells during these periods.

DISCUSSION

The data obtained suggest that the drastic increase in the activity of the glycolytic enzymes with the concurrent rise in the accumulation rate of the reserve carbohydrates during the first week of cell aging may be an adaptive mechanism to enhance viability. The increased activity of hexokinase and phosphofructokinase (the enzymes of the upper glycolytic pathway) advances the utilization of glucose and promotes the supply of glycolytic intermediates to various biosynthetic reactions. This results in the accumulation of reserve carbohydrates in aging cells, allowing them to enhance survival. During the further incubation of the aging cells, the activity of the glycolytic enzymes and the accumulation rate of the reserve carbohydrates fall, which leads to a decrease in the intensity of adaptive processes and in the survival rate of the yeast population. It should be noted that Kandor *et al.* [15] observed a similar dynamics of trehalose in *Escherichia coli* cells subjected to cold shock.

Our data may seem to contradict the findings of Lillie and Pringle [16], who reported that *S. cerevisiae* cells with a lost ability to accumulate reserve carbohydrates are poorly viable. However, this contradiction is only illusory, since Lillie and Pringle experimented with carbon-starved stationary-phase cells, whereas we experimented with nonstarved stationary-phase cells incubated in a regularly refreshed nutrient medium. This approach not only removed the unfavorable effects of nutrient medium exhaustion and the accumulation of toxic metabolic products but also probably promoted the development of adaptive processes in aging cells.

Furthermore, the aging of a cell population is likely to be associated with the appearance of cells that are more adapted to the environment. The least adapted

Table 1. The dynamics of hexokinase and phosphofructokinase activities (nmol NADH/(min mg protein)) in aging *S. cerevisiae* cells

Enzyme	Before aging	After 1 week of aging	After 2 weeks of aging	After 3 weeks of aging	After 4 weeks of aging	After 5 weeks of aging
Hexokinase	245.1 ± 17.1	335.6 ± 19.7	324.7 ± 16.5	162.3 ± 14.2	158.8 ± 12.7	141.3 ± 12.3
Phosphofructokinase	168.7 ± 9.7	219.6 ± 12.8	215.8 ± 15.3	132.4 ± 8.4	128.7 ± 7.9	115.8 ± 11.5

Table 2. The dynamics of TBA-reactive products in aging *S. cerevisiae* cells

Conditions	TBA-reactive products, nmol/mg dry biomass
Before aging	14.7 ± 0.44
Aging, weeks:	
1	16.8 ± 0.68
2	17.3 ± 0.49
3	16.6 ± 0.85
4	17.4 ± 0.76
5	18.2 ± 0.65

cells obviously die, whereas the most adapted cells survive due to their metabolic advantages. It can be suggested that it is the spontaneous selection of metabolically advanced cells that allows an aging cell population to survive without accumulating reserve carbohydrates.

It should be noted that the death of a fraction of the studied aging cell population must decrease its density and thus abolish the crowd-sensing effect, allowing the remaining live cells to bud. However, we were unable to detect budding cells in the aging yeast population. This can be explained by the accumulation of a hypothetical aging factor that suppresses the replicative and the general functional activity of *S. cerevisiae* cells [17].

The important role of glycogen and especially trehalose in the adaptation and survival of cells exposed to various stress factors is well known [18, 19]. The loss of the ability of aging cells to accumulate glycogen and trehalose can hardly be accounted for by the development of peroxidation processes or by oxidative damage to biopolymers, as is evident from the normal content of TBA-reactive products and the carbonyl groups of proteins in such cells. Nor can nutritional starvation be responsible for the loss of the ability of aging cells to accumulate reserve carbohydrates, since the nutrient medium was regularly refreshed during the experiments. The decreased activity of the key glycolytic enzymes hexokinase and phosphofructokinase is also unlikely to be responsible for the loss of the ability of aging cells to accumulate trehalose and glycogen since the activity of these enzymes decreased only twofold by the end of the experiment and the remaining activity is quite sufficient to maintain the necessary level of reserve carbohydrates in the cells.

Alterations in the regulation of enzymes involved in the metabolism of reserve carbohydrates (particularly, glycogen and trehalose synthetases) seem to be the most probable reason for the inability of aging yeast cells to accumulate trehalose and glycogen. This regulation is a complex, multistep, and multicomponent process, involving, in particular, the intracellular signaling system [20]. It would be of interest to study the expression of the enzymes of trehalose and glycogen metabolism in aging yeast cells along with a comparative study of the viability of yeast cells greatly differing in the accumulation rate of reserve carbohydrates. Of interest also is the study of the survival mechanisms of aging yeast cells with an impaired ability to accumulate trehalose and glycogen. This line of research is to be performed in our laboratory.

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