BIOLOGIJA. 2005. Nr. 1. P. 38–40

Study of cell wall permeability properties of synchronous *Saccharomyces cerevisiae* cells in different phases of cell cycle

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This report presents the results obtained during investigation of the relationship between permeability properties of the yeast *Saccharomyces cerevisiae* cells which were synchronized to the **S**- or **M**-phases by treating them with hydroxyurea (HU, 10 mM) and colchicin (1 μg/ml), respectively. Permeability properties of the yeast strain p63-DC5 as the accumulation of the tetraphenylphosphonium cation (TPP^+) were measured by a TPP-selective electrode. The results obtained have shown that the amount of lipophilic cations accumulated by yeast *Saccharomyces cerevisiae* cells was different and depended on the phase of cell cycle. The results indicate existence of a subpopulation of budded cells with a higher permeability of cells to tetraphenylphosphonium cations, and this increased rate of TPP^+ accumulation was associated with a particular **S**-phase, which exhibits physiological and permeability properties different from those of other cells.

Key words: *Saccharomyces cerevisiae*, cell cycle, permeability, lipid-soluble ions (LSI), lipophilic cations (LC), tetraphenylphosphonium (TPP^+)

INTRODUCTION

Cell cycle population is one of the important factors that should be considered while studying yeast cells metabolism. Several studies have shown that the yeast cell wall is not a static shield, but a highly dynamic structure which can change according to the physiological needs of the cell. During the cell cycle, the cell wall has to be remodeled to be more plastic in the point of bud emergence where the growth takes place [1, 2]. It was indicated that the expression of over 50% of all cell wall proteins is cell cycle related [3]. There are several reports on the cell cycle phase dependency of native and foreign protein production and secretion in *S. cerevisiae* [4–6]. Recently it has been determined that the transformation efficiency and permeability properties of yeast *S. cerevisiae* cells depend on the cell wall structure of different strains. The subpopulation of budded cells in exponentially growing culture has shown a higher transformation efficiency [7]. However, up to now little has been known about the dependence of the cell wall permeability properties on cell cycle.

The aim of the present study was to determine how changes in cell wall architecture modify the permeability properties of yeast cells during cell cycle. We examined the effect of cell cycle position on the permeability of *S. cerevisiae* cells to lipophilic cations of tetraphenylphosphonium (TPP^+) in synchronous culture.

The results obtained by us demonstrate that the population of budded cells was more permeable to TPP^+ cations than cells in other morphological stages of the cell cycle. We suggest that the high rate of TPP^+ accumulation is associated with **S**-phase, which exhibits physiological and permeability properties different from those of other cells.

MATERIALS AND METHODS

***Yeast Saccharomyces cerevisiae strain* p63-DC5** *(MATa, ade1, leu2-3, leu2-112, his3-11, his3-15)* a gift from Habil. Dr. K. Sasnauskas, Vilnius) was used. Yeast cells were grown in YEPD medium (1% yeast extract, 2% peptone (Difco, USA), 2% glucose) at 30 °C on a reciprocal shaker at 150 rpm.

Cell-cycle synchronization Cells that were growing exponentially (this type of asynchronized cell population was used as the experimental control) were divided into subcultures which were treated in order to have cells synchronized at different phases of cell cycle. To arrested culture of p63-DC5 in the **S** and **M** phases, 10 mM hydroxyurea or 1 μg/ml of colchicin were added, respectively [8, 9]. After incu-

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bation with HU (2.5 h) and colchicin (4 h) at 30 °C the cells were harvested by centrifugation at 3000 × g for 5 min at room temperature. Yeast cells after incubation with HU and colchicin were washed in TE buffer (10 mM Tris-HCl, 1 mM EDTA, pH 7.8) two times for recovery from chemicals.

Tetraphenilphosphonium accumulation measurements. Cells were concentrated 200 times in TE buffer, and TPPBr was added to the final concentration 3×10^{-7} M. After incubation, at an indicated time, yeast cells were precipitated and the supernatant was used for measuring the residual TPP^+ concentration. 200 µl of the supernatant were added to 400 µl of the TE buffer (with 3×10^{-7} M TPPBr) in which a TPP^+ selective combination electrode was immersed. The electrode potential drift was estimated with a Hanna pH213 ion meter connected with a recorder, and the yeast absorbed quantity of TPP^+ was calculated. The protein concentration was determined by Lowry method [10].

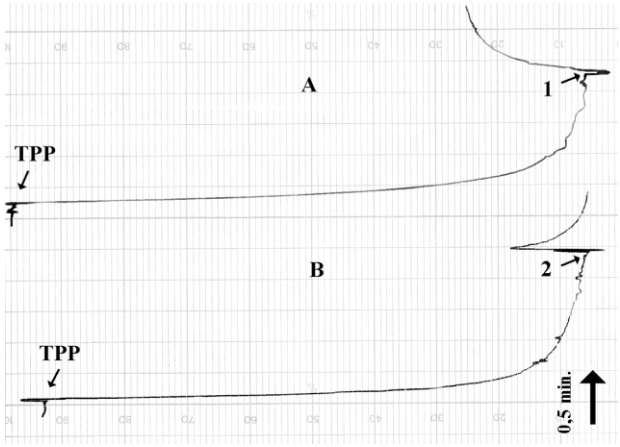

Fig. 1. Principle of TPP^+ uptake by yeast *S. cerevisiae.* Decrease in TPP^+ concentration recorded after addition of cell incubation medium. Arrows 1 and 2 indicate the time when 200 µl of samples were added. A: supernatant of yeast cells after incubation with TPP^+; B: control aliquot of the medium with TPP^+. Calibration was performed by increasing TPP^+ concentration from 2×10^{-7} M to 3×10^{-7} M

RESULTS AND DISCUSSION

Interaction of lipophilic cations with microbial and yeast cells has become one of the most widely used methods to investigate the principles of membrane permeability. Lipophilic cations such as tetraphenylphosphonium (TPP^+) have been applied for estimation of the membrane potential and permeability properties of organisms, cells, organelles, membrane vesicles and liposomes [11–13]. Transfer of the particular lipophilic-soluble ion species across the membrane is passive and not influenced by either primary or secondary active processes. Moreover, entry of LSI does not alter the membrane voltage [14].

In this work, permeability of yeast cells in different phases of the cell cycle was studied. The results demonstrated a relationship between the permeability properties of yeast cells and cell cycle phases. Yeast cells were synchronized to **S**- or **M**-phase by treatment with hydroxyurea (10 mM) as an inhibitor of DNA replication and colchicin (1 µg/ml) as a microtubule-disruption agent, respectively. The permeability properties were evaluated by the rate of TPP^+ accumulation by yeast cells in **S**- and **M**-phase and compared with the rate of TPP^+ accumulation by asynchronous culture. The accumulation of permeate cations TPP^+ in yeast cells was measured with a TPP^+-selective electrode (Fig. 1).

We determined the kinetics of TPP^+ accumulation for cells of p63-DC5 strain in **S**- and **M**-phases. The yeast cells in both phases and control reached the steady state in the distribution of TPP^+ within 30 min at 30 °C. However, the amount of TPP^+ accumulated at various time was quite different. The rate of TPP^+ accumulation by cells in **S**-phase was mach higher in the first minutes of incubation and achieved the steady state after 15 minutes. The amount of accumulated TPP^+ cations of cells treated with colchicin was slightly higher than in control, but it did not reach the amount of TPP absorbed by yeast cells in **S**-phases after 15 min of incubation. The accumulation of TPP^+ by yeast cells in **S**- phase of the growth was about two times higher than in **M**-phase after 15 min of incubation (Fig. 2).

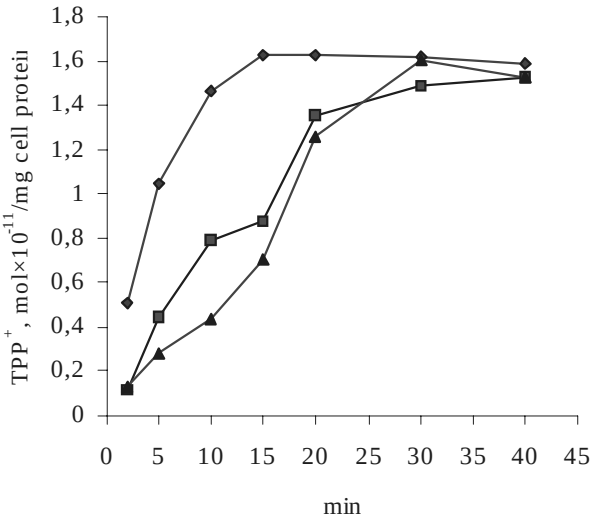

Fig. 2. Kinetics of tetraphenylphosphonium cations (TPP^+) accumulation by yeast *S. cerevisiae* p63-DC5 strain. ♦ – synchronous yeast cells in **S**-phase, ■ – synchronous yeast cells in **M**-phase, ▲ – asynchronous yeast cells.
Cells were prepared and estimation was done according to description in Materials and Methods. Means of the data of 3 experiments are presented

Differences in the lipophilic fluorescent dye probe uptake curves are known to reflect a number of morphological dissimilarities (structure and / or thickness of cell wall) [12]. Thus, it is possible to ima-

gine that yeast cells in **S**-phase possess the highest permeability to lipophilic cations, and this increase is associated with the morphogenetic properties of the cells in **S**-phase of cell cycle. It has been noted that during bud growth in **S**-phase increase the synthesis and assembly of cell wall components. The rapid growth of buds causes a certain flexibility, at least in growing areas, without altering the protective function of the cell wall [2, 5]. Moreover, the cell wall of *S. cerevisiae* exhibits variations in porosity during growth and cell division. Maximum porosity is observed during bud growth where the wall is in a more plastic, expanded state as compared to stationary phase cells [1, 3, 15].

Based on the results presented, we note the permeability properties of *S. cerevisiae* cells are influenced by and change during the cell cycle. Our results are in good agreement with the data mentioned above, thus, it is possible to imagine that changes in the cell envelop occurring in **S**-phase of cell cycle condition the highest permeability of yeast cells to lipophilic cations.

Received 26 January 2004
Accepted 18 November 2004

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SINCHRONIZUOTØ MIELIØ *SACCHAROMYCES CEREVISIAE* LÀSTELIØ, ESANÈIØ SKIRTINGOSE CIKLO FAZËSE, LAIDUMO SÀVYBIØ TYRIMAS

Santrauka

Buvo tirtas mieliø *Saccharomyces cerevisiae* p63-DC5 làsteliø, esanèiø skirtingose ciklo fazëse, sugebëjimas akumuliuoti lipofilinius tetrafenilfosfonio (TPP^+) jonus. Làstelës iki **S** fazës buvo sinchronizuotos panaudojus hidroksikarbamidà (10 mM), iki M stadijos – kolchicinà (1 μg/ml). Akumuliacija buvo registruojama TPP jonø selektyviu elektrodu. Nustatyta, kad **S** fazëje esanèios làstelës greièiau akumuliavo ir per 15 minuèiø sukaupë maksimalø lipofilinio katijono kiekį. **M** fazëje esanèios làstelës pasiþymëjo maþesniu akumuliacijos greièiu (lyginant su **S** faze), bet pastebimai didesniu nei nesinchronizuotø làsteliø. Manome, kad mieliø làsteliø ciklo metu pasikeitusi sienelës struktûra turëjo átakos lipofiliniø katijonø laidumo pokyèiams.