The role of ATP as a regulator of effective plasmid transformation in *Saccharomyces cerevisiae*

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A relationship between the energetic stage of the recipient yeast *Saccharomyces cerevisiae* cells and the efficiency of plasmid DNA transformation was studied. The depletion of the intracellular ATP pool was done by treating the cells with inhibitors that specifically decrease the intracellular ATP pool. The intracellular level of ATP was reduced in gradual manner and was concentration-dependent. The level of ATP of the strain with the impaired mitochondrial system was higher than in the parental strain, however, the same effect of the inhibitors on the intracellular level of ATP was observed. The results confirmed our earlier suggestion that the glycolytic system of ATP synthesis is energy-productive and seems to be an indispensable prerequisite of effective transformation of yeast *Saccharomyces cerevisiae.* The obtained results allow to suggest that the transformation efficiency is sensitive to the inhibition of substrate phosphorylation of the recipient cells.

Key words: ATP, *rho*^{o} mutants, transformation, *Saccharomyces cerevisiae*

INTRODUCTION

Plasmid transformation of yeast cells has become a widely used procedure in genetic and molecular biology studies, although it is not known how high-molecular DNA enters the yeast cells [1–3]. Is the input of metabolic energy coupled to plasmid DNA transport during transformation? The energy requirement for plasmid DNA transfer through the plasma membranes has not been evaluated experimentally. Very few studies have been performed to investigate the effect of different situations of cell culture and energy requirement on plasmid transformation through the plasma or nuclear membranes [4–6]. The energy pool of the cell can be manipulated independently with inhibitors that influence the phosphorylated intermediates, such as intracellular ATP, whose concentration indicates the vitality and energy charge of the cell. In yeast, ATP is the source of the free energy to be invested in metabolism. It was shown that yeast growing on glucose, galactose or ethanol consumed more than 50% of ATP produced in catabolism to drive processes other than the production of biomass [7, 8].

Data will be presented suggesting that by blocking the glycolytic pathway the plasmid DNA uptake was also affected.

MATERIALS AND METHODS

Yeast strain and plasmid. *S.cerevisiae* p63-DC5 (*MATa, ade1, leu2-3, leu2-112, his3-11, his3-15*) was a gift from Dr. Habil. K. Sasnauskas, Vilnius). Plasmid pL3 (7,9 kb, multicopy, containing the bacterial plasmid pBR327 sequences, yeast gene *LEU2* and part of the yeast 2 μm plasmid) was employed in the transformation experiments. Plasmid DNA was prepared according to Maniatis et al. [9].

Cultivation, transformation and treatment. Yeast cells were grown in complete YEPD medium (1% yeast extract, 2% peptone, 2% glucose) at 30 °C on a reciprocal shaker at 150 rpm. The solidified medium contained 2% agar (Difco, USA). The transformation of yeast cells was carried out according to Ito et al. [1]. SD (0.67% yeast nitrogen base without amino acids, 2% glucose) agar with appropriate supplements was employed as a selective medium. For the selection of *LEU*$^{+}$ yeast transformants, SD medium was supplemented with 10 μg/ml of histidine and 50 μg/ml of adenine. All inhibitors were added before plasmid DNA. Cell viability was determined by appropriate dilution of the cells on YEPD plates.

Determination of ATP. Cells for ATP measuring were prepared as follows. Cells from the exponen-

tial phase were harvested, suspended in TE (10 mM-Tris/HCl, 1 mM-EDTA, pH 7.8). Then they were disrupted using glass beads. Unbroken cells were removed by centrifugation, and a cell-free extract was used for ATP assay. All procedures were done at 0 °C. The ATP content of the cells was measured using an ATP Bioluminescence Assay kit (Sigma) based on the luciferin-luciferase reaction [10]. Luminescence measurements were made using a Fluoroscan Ascent FL (Labsystems) luminometer.

Mutagenesis. Mutagenesis was performed by ethidium bromide (EthBr) according to Mahler and Perlman [11]. Mitochondrial mutants were selected on the criterion that they are unable to grow on a non-fermentable carbon source such as glycerol, but able to grow on glucose medium.

RESULTS AND DISCUSSION

The aim of this study was to determine the factor that mediates the penetration of plasmid DNA during transformation. Attention was paid to the energy requirement in this process. Previously it has been reported that sodium arsenate at 2–5 mM concentration significantly diminishes the level of ATP and causes a decrease in the number of transformants [12].

In this study, *S. cerevisiae* strains p63-DC5 and a mitochondrial mutants (*rho*^{o}) of this strain were used. The results confirmed and expanded the experimental observation of our earlier study. Results presented in Figs. 1 and 2 show that arsenate and fluoride caused a gradual and concentration-dependent decrease in the internal ATP level in both strains studied. ATP inhibition and a simultaneous decrease in its level was fast, occurring after only 3–5 min of preincubation. Sodium fluoride is a well-known inhibitor of glycolysis, therefore, the inhibition at the level of enolase might be involved [13, 14]. The level of intracellular ATP decreased from 0.0756 to 0.0095 U/mg/ml protein as compared with untreated cells for p63-DC5 strain and from 0.1161 to 0.0029 U/mg/ml protein for p63-DC5(*rho*^{o}) strain. The inhibitory effect of potassium fluoride was more pronounced than that of sodium arsenate. The observed effect was expected, because yeast cells used for transformation were cultivated under conditions of glucose repression, therefore cells were highly dependent on glycolytic energy metabolism.

Cells of *S. cerevisiae (rho*^{o}*)* strain maintained a higher intracellular ATP level (Table 1), higher (approximately 153.6%) than in the parental strain. The resulting mutants displayed a slow growth phenotype and were unable to grow anaerobically. It was suggested [15, 16] that it might be due to the inability of the inhibitor of ATPase to control efficiently ATP hydrolysis; as a result, the intracellular ATP level increased. Moreover, it may be related to the increase of the rate of glycolysis after inhibition of oxidative phosphorylation of the mitochondria. This effect was observed with BNK cells after treatment with oligomycin and a subsequent exposure to light [17]. However, an efficient depletion of the intracellular ATP pool was determined after treatment of yeast p63-DC5(*rho*^{o}) cells with arsenate and sodium fluoride as well. The results have shown that the strain with the impaired mitochondrial synthesis system was more sensitive to the treatment by both inhibitors, especially by sodium fluoride.

Table 1. **Effect of sodium arsenate and sodium fluoride on the intracellular ATP level of *S. cerevisiae* cells**

Strains and inhibitors	Intracellular ATP, U/mg/ml protein	%
p63-DC5	0.0756	100 %
Sodium arsenate, mM		
2	0.0640	84.66
5	0.0327	43.25
Sodium fluoride, mM		
25	0.0129	17.03
50	0.0095	12.60
p63-DC5(*rho*^{o})	0.1161	100%
Sodiumn arsenate, mM		
2	0.0714	61.50
5	0.0413	35.60
Sodium fluoride, mM		
25	0.0160	13.78
50	0.0029	2.50

Cells in exponential growth phase were prepared and ATP determination procedure as noted in Materials and Methods.

In this study, a relationship between de-energization of the yeast cells by depletion of the intracellular ATP pool and transformation efficiency was observed. At a 2 mM concentration of sodium arsenate, a significant inhibition of transformation was observed (Table 2). The incubation of yeast cells with 5 mM sodium arsenate caused a significant decrease of ATP level (about 50%) and severely reduced the number of transformants (from 2333 to 853) (Fig. 1, Tables 1 and 2). The number of viable cells in experiments and in control remained about 1.0×10^8 cells/ml. Only at a concentration of 5 mM a decrease by about 25% in the cell number was observed (Table 2).

Transformation efficiency was sensitive to sodium fluoride; the number of transformants decreased already at a 25 mM concentration of NaF (from 2903 to 323) and a significant effect of the treatment at 50 mM of NaF was observed (Table 2). The presence of fluoride in the transformation medium did

Table 2. **Transformation efficiency of *S. cerevisiae* strains p63-DC5**

Inhibitors, mM	Transformation efficiency per 1 μg of DNA	Cell viability, %
Control	2333 ± 125	1.0×10^8
Sodium arsenate		
1 mM	1563 ± 116	1.0×10^8
2 mM	1173 ± 161	1.0×10^8
5 mM	853 ± 31	9.6×10^7
Control	2038 ± 109	1.2×10^8
Sodium fluoride		
10 mM	1630 ± 88	1.2×10^8
20 mM	1045 ± 136	1.2×10^8
50 mM	312 ± 24	1.0×10^8

Cells in exponential growth phase were prepared and the transformation procedure was done as described in Materials and Methods. Transformation efficiency was presented as the number of transformants per 1mg of plasmid DNA. Mean of the data of 4-5 experiments are presented.

not significantly decrease the viability of cells under the used concentration range of inhibitors; the number of viable cells slowly decreased at 50 mM of NaF (Table 2). These results allow to suggest that the transformation efficiency was reduced due to the lack of ATP production by glycolysis.

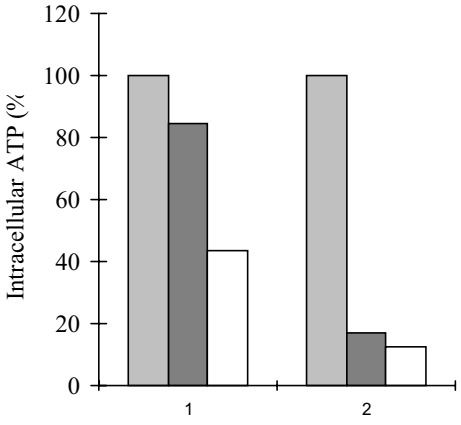

Fig. 1. Influence of inhibitors on intracellular ATP content in *Saccharomyces cerevisiae* p63-DC5 cells.
Here 100% corresponds to the ATP content per mg/ml protein.
1 – sodium arsenate 2 and 5 mM, 2 – sodium fluoride 25 and 50 mM

The reduction of the ATP pool and a correlated decrease of the transformation efficiency suggested that the yeast transformation process which includes the interaction of plasmid DNA with cytoplasm membrane, penetration into cells and replication of the transformed DNA depended on the energetic state of the recipient cells. As the viability of treated cells

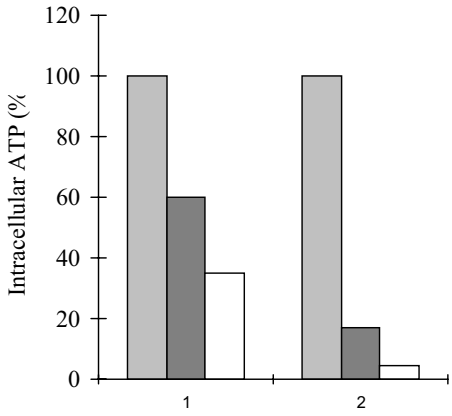

Fig. 2. Influence of inhibitors on intracellular ATP content in *Saccharomyces cerevisiae* p63-DC5 (*rho°*) cells.
Here 100% corresponds to the ATP content per mg/ml protein.
1 – sodium arsenate 2 and 5 mM, 2 – sodium fluoride 25 and 50 mM

was not as significantly decreased as the transformation efficiency, it is possible to suppose that a certain level of the glycolytic ATP could be a limiting factor rather for the effective plasmid DNA penetration than for the recovery of the transformed cells. These results corresponded to the results obtained in experiments with ethidium-bromide-induced mitochondrial (*rho°*) mutants which were respiratory-incompetent and unable to synthesise the mitochondrial cytochrome [18]. A comparable efficiency of the mitochondrial (*rho°*) mutant transformation to the strain with the intact mitochondrial energy transformation system (parental strain) indicated that the mitochondrial function impaired by induced mutation did not seem to be significant [19].

Thus, the genetic transformation of *Saccharomyces cerevisiae* by plasmid DNA as a complex of consequent events depended on the energetic state of the recipient cells. The results suggest that the glycolytic system of ATP synthesis is energy-productive and seems to be an indispensable prerequisite for the effective transformation of yeast *Saccharomyces cerevisiae.* Thus, the study has shown that ATP is a potential member of this regulatory process. However, it remained unclear at which step(s) ATP was required.

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ATP VAIDMUO PLAZMIDINĖJE MIELIŲ *SACCHAROMYCES CEREVISIAE* TRANSFORMACIJOJE

S a n t r a u k a

Buvo tiriama mielių *Saccharomyces cerevisiae* transformacijos efektyvumo priklausomybė nuo viduląstelinio ATP kiekio. Viduląstelinio ATP ištekliams išsekinti buvo panaudotas arsenatas ir effektyvus glikolizės inhibitorius – fluoridas. Viduląstelinis ATP mažėjo laipsniškai ir priklausė nuo inhibitoriaus koncentracijos. Mitochondriniai DNR rho° mutantai pasižymėjo didesniu ATP kiekiu, bet naudotų inhibitorių poveikis buvo panašus kaip ir su motininiu kamienu. Pateikti rezultatai patvirtino ankstesnę hipotezę, kad glikolizės metu sintezuojamas ATP yra naudojamas mielių *Saccharomyces cerevisiae* transformacijai.