BIOLOGIJA. 2004. Nr. 2. P. 29–32

Relationship between the efficiency of yeast *Saccharomyces cerevisiae* transformation and cell cycle

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The efficiency of yeast *Saccharomyces cerevisiae* cell transformation during cell cycle was investigated. The results showed considerable changes in transformation efficiency during the synchronized growth of yeast culture and suggest that the process is greatly influenced by certain changes in the phase of cell cycle. The maximum of transformation efficiency was achieved when cells in **S**-phase were transformed and the budded cells were observed (after their treatment cells with 12 mM hydroxyurea) and, on the contrary, the minimum was noticed when the cells were transformed in **M**-phase after their treatment with 1 μg/ml colchicin. The results lead to the conclusion that yeast cells in the **S**-phase of cell cycle show an enhanced perception or competence to exogenous DNA. The phenomenon of competence is supposed to occur in yeast cells.

Key words: *Saccharomyces cerevisiae*, transformation, cell cycle, competence

INTRODUCTION

Cell cycle is a process by which a cell ensures its correct reproduction and is central to the understanding of all life; moreover, many processes are related to the cell cycle events. Cell transfection and transformation are procedures extensively used to introduce DNA into cells. Different delivery systems, such as liposomes, viral, special vectors have been developed [1–4]. In order to move towards *in vivo* gene cloning, great efforts have been made to improve these procedures *in vitro*. An *in vitro* biochemical research performed in order to introduce important genes into cells is usually carried out using the cells that grow exponentially, and surprisingly little is known about the dependence on cell cycle of transformed gene expression. Up to now, only a few data [3, 5, 6] described the dependence of yeast transformation on the physiological state of the recipient cells, but not on the cell cycle.

Earlier we have shown that the transformation efficiency of yeast cells was 3-fold higher when recultivation was done and cells were allowed to grow for other 1–2 generations [7]. The aim of this study was to estimate what phase of cell cycle is more sensitive or competent to DNA.

In the present work, hydroxyurea and colchicin were used to synchronize yeast cells and the relationship of transformation efficiency with cell cycle phases was analysed. The phenomenon of competence is supposed to occur in growing yeast cells.

MATERIALS AND METHODS

Strain and plasmid. Yeast *Saccharomyces cerevisiae* strain p63-DC5 *(MATa, ade1, leu2-3, leu2-112, his3-11, his3-15)*, a gift from Dr. Habil. K. Sasnauskas, was used for transformation. The plasmid pL3 (7.9 kD, multicopy, containing the bacterial plasmid pBR327 sequences, yeast gene *LEU2* and part of yeast 2 μm plasmid) was employed in the transformation experiments. The plasmid was prepared according to Maniatis et al. [8].

Cultivation, transformation and synchronization procedure. Yeast cells were grown in complete YEPD medium (1% yeast extract, 2% peptone (Difco, USA), 2% glucose) or minimal SD medium (0.67% yeast nitrogen base without amino acids (Difco, USA), 2% glucose) at 30 °C. Transformation of the yeast cells was carried out according to Ito et al. [5]. Cells were grown overnight at 30 °C in YEPD medium to the early exponential growth phase (OD_{590} 0.12–0.14). For synchronisation to **S**-phase, the early log culture cells were treated with hydroxyurea at a final concentration of 12 mM [9]. After incubation at 30 °C for 120 min with shaking, the majority of the cells (approximately 90%) were arrested in **S**-phase as the budded cells. To effect an arrest in **M**-phase colchicin was added to the final concentration 1 μg/ml, and cells were incubated for 4 h [10]. Cells of the synchronous culture for transformation were collected by centrifugation for 10 min, washed three times with water, and resuspended in 0.1 M lithium acetate buffer to a final concentration of $2 \cdot 10^8$ cells per ml. The following transformation procedure was done according to Ito et al. For selection of *LEU*$^+$ yeast transformants, SD medium was supplemented with 10 μg/ml of histidine and 50 μg/ml of adenine. Cell cycle phases were controlled morphologically under the light microscope. The viability of cells was determined after a corresponding treatment, and cells were counted by spreading dilutions of cell suspension on YEPD agar medium.

RESULTS AND DISCUSSION

In the present study, yeast *S. cerevisiae* cells were synchronized to the **S**- or **M**-phase by treatment with hydroxyurea and colchicin, respectively. The efficiency of transformation was investigated with cells in **S**- and **M**-phases of the cell cycle and compared with the transformation efficiency of the asynchronized culture.

The results presented in this study show that hydroxyurea blocked the cell cycle of *S. cerevisiae* cells at the **S**-phase; about 90% of the cells were budded. The number of transformants obtained with yeast cell culture at this phase of the growth was higher than with asynchronous culture and reached up to 14426 transformants per μg DNA (268.2% compared with control) (Table 1). It is well-known that the microtubule-disruption agent colchicin induces the mitotic arrest in eucaryotic cells and cells are accumulated in the **M**-phase of cell cycle [10, 13]. The number of transformants with cells treated with colchicin was considerably lower and reached only 2410 transformants (44.8%). A more pronounced increase in the number of transformed cells can be determined when the number of transformants of two phases is compared. The transformation efficiency at the **S**-phase of the growth was about 6 times higher than in **M**-phase. Thus, it is possible to conclude that the yeast cells in the **S**-phase of growth have shown the highest capability of taking up the exogenous DNA. It should be noted that the concentrations of the inhibitors did not decrease the viability of cells, which was determined by spreading of the cell suspension on the YEPD agar medium and was observed also by increasing the optical density of the cells (Table 2). The results presented in Table 2 were determined in experiments carried out without the transformation procedure. Moreover, the concentration of the HU in our study was lower than the one used in other studies [11, 12].

The experimental data confirmed the results obtained in our previous study that the efficiency of yeast *S. cerevisiae* transformation undergoes significant changes in the period of growth of the synchronous culture. We have shown that the transformation efficiency is not a simple function of cellular growth; the phenomenon may be characterised as being undulatory, with the maximum and the minimum of transformation achieved during a cell cycle [7]. The relation between cell cycle and some cell functions is well known. There are several reports on the cell cycle phase dependency on native and foreign protein production and secretion in *S. cerevisiae* [12, 14]. The results presented in this study corresponded to the results obtained with synchronised L929 mouse fibroblast cells. The high activity of gene expression was seen when cells in **S**-phase were used to transfect synchronised fibroblast cells [12].

Based on the results presented, the efficiency of *S. cerevisiae* plasmid transformation was influen-

Table 1. **Comparison of the transformation efficiency of asynchronous cells of *Saccharomyces cerevisiae* p63 strain and cells of the strain after synchronization**

Phase of cell growth	Number of transformants per 1 μg of plasmid DNA	%
Asynchronous (control)	5380 ± 170.1	100
S-phase	14426 ± 422.1	268.2
M-phase	2410 ± 116.2	44.8

Cells for transformation procedure were prepared as described in the Materials and Methods. Means of the data of 3–4 experiments are presented.

Table 2. **Effect of hydroxyurea and colchicin on viability of *Saccharomyces cerevisiae* cells**

Treatment	Viable cells, 10^6 cells/ml	Viable cells, 10^7cells/ml
Control	12.7. ± 0.8	
Hydroxyurea, 12 mM	12.0 ± 0.6	
Control		227.3 ± 8.9
Colchicin, 1 μg/ml		227.4 ± 11.6

Cells were grown at 30 °C in YEPD medium to early exponential growth phase. Hydroxyurea (HU) was added at a final concentration of 12 mM and colchicin 1 μg/ml). Cultures then were incubated with shaking at 30 °C for 2.5 and 4 h, respectively. In all cases, cell density remained constant for the duration of the arrest. Means of the data of 3 experiments are presented.

ced and changed during the cell cycle. One of the reasons for the observed phenomenon may be related to the properties of the cell wall and the morphogenetic processes of yeast on the **S**-phase of the cell cycle which include: localisation of secretion of other materials to the surface of the bud; localisation of new cell-wall growth to the tip of the bud during much of the period of the bud growth [15]. Several studies have shown that the yeast cell wall is not a static shield but a highly dynamic structure that can change according to the physiological needs of the cell. During a cell cycle, the cell wall has to be remodelled to be more plastic in the point of bud emergence where the growth takes place. The rapid growth of buds suggests that the events ruling the development of such an apparently rigid structure may allow a certain flexibility, at least in growing areas, without altering the protective function of the cell wall. In this regard, it has been revealed that the cell wall of *S. cerevisiae* exhibits variations in porosity during growth and cell division. Maximum porosity is observed during the bud growth where the wall is in a more plastic, expanded state as compared with stationary phase cells [16, 17]. Furthermore, yeast cells subjected to a heat shock may become weaker, and this leads to plasma membrane stretching. All the mentioned and other concomitant events facilitate the penetration of the plasmid DNA by increasing the permeability of the cell envelope to large molecules, including exogenous DNA.

A variety of methodologies exist for the transport of exogenous DNA into the cell interior, and usually it is achieved in two ways: natural transport and that induced with various specific treatment. A minor subpopulation (never exceeding 20%) of bacterial cells in a given culture develops competence under specific growth and developmental process. The best characterised naturally transformable bacteria are the Gram-negative species *Haemophillus influenza* [18] and the Gram-positive *Streptococcus pneumoniae* and *Bac. subtilis* [19, 20]. However, it should be noted that the competence of prokaryotic cells appears as a physiological state during the cell growth.

The problem of the possibilities of the phenomenon of competence in yeast cells is not discussed on the same level as with bacterial cells. It has been noted that cells growing exponentially are competent to take up DNA [6, 21, 22], whereas the results presented in this study indicate that yeast cells in **S**-phase are more sensitive to DNA and the efficiency of yeast transformation seems to be comparable with the efficiency of genetic transformation of *Bac. subtilis* in the state of competence [23]. Direct insertion and integration of the functionally active genes into eukaryotic cells is now a commonplace procedure which has a wide application in the molecular biology and genetics. To achieve effective production, a number of factors should be taken into account, including selection of suitable methods, gene expression system, process optimization, etc. The cell cycle of a yeast cell population is an important factor that ought to be considered.

Received 17 October 2002
Accepted 19 September 2003

References

1. Brzobohaty B, Kovac L. FEMS Microbiol Lett 1986; 35: 29–31.
2. Faber KN, Haima P, Harder W, Veenhuis M, AB G. Curr Genet 1994; 25: 305–10.
3. Gietz RD, Schiestl RH. Yeast 1991; 7: 253–63.
4. Hourioux Ch, Touze A, Coursaget P, Roingeard Ph. J Gen Virol 2000; 81: 1099–101.
5. Ito H, Fukuda J, Murata K, Kimura A. J Bacteriol 1983; 153: 163–8.
6. Suga M, Isobe M, Hatakeyama T. Yeast 2001; 18: 1015–21.
7. Chaustova L, Jasaitis A. Biologija 1994; 1: 8–13.
8. Maniatis T, Fritsch EE, Sambrook J. Molecular cloning: A laboratory manual. Cold Spring Harbor Lab., Cold Spring Harbor, N. Y. 1982: 545.
9. Rhind N, Russel P. Mol Cell Biol 1998; 18: 3782–7.
10. Cohen E, Gitler C, Ben-Shaul Y. Cell Differ 1981; 10: 333–342.
11. Venturi ChB, Erkine AM, Gross DS. Mol Cell Biol 2000; 20 (17): 6435–48.
12. Marenzi S, Adams RLP, Zardo G, Lenti L, Reale A, Caiafa P. Mol Biol Reports 1999; 26: 261–7.
13. Kamei H. Exp Cell Res 1995; 218: 155–65.
14. Rodriguez-Pena JM, Cid VJ, Arroyo J, Nombela C. Mol Cell Biol 2000; 20: 3245–55.
15. Pringle JR, Lillie SH, Adams AEM, Jacobs CW, Haarer BK, Coleman JS, Robinson L, Bloom R, Preston RA. Yeast cell biology. Alan R. Liss Inc, New York, ed. Hicks J. 1986; 33: 47–80.
16. De Nobel JG, Klis FM, Ram A, Van Unen H, Priem J, Munnik T, Van Den Ende H. Yeast 1991; 7: 589–98.
17. Popolo L, Gualtieri T, Ragni E. Med Mycol 2001; 39(1): 11–121.
18. Elkins C, Thomas CE, Seifert HS, Sparling PF. J Bacteriol 1991; 173: 3911–5.
19. Tortosa P, Dubnau D. Curr Opin Microbiol 1999; 2: 588–92.
20. Hakenbeck R. Res Microbiol 2000; 151(6): 453–6.
21. Meilhoc E, Masson JM, Teissie J. Biotecnhol 1990; 8: 223–7.
22. Suga M, Isobe M, Hatakeyama T. Yeast 2000; 16: 889–96.
23. Chaustova LP, Grinius LL, Griniuviene BB, Jasaitis AA, Kadziauskas JP, Kiausinyte RJ. Europ J Biochem 1980; 103: 349–57.

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RYŠYS TARP MIELIŲ *SACCHAROMYCES CEREVISIAE* TRANSFORMACIJOS EFEKTYVUMO IR LĄSTELĖS CIKLO FAZĖS

Santrauka

Tirta mielių *Saccharomyces cerevisiae* transformacijos efektyvumo priklausomybė nuo ląstelės ciklo fazės. Rezultatai rodo ryškius transformacijos efektyvumo skirtumus mielių augimo metu. Manoma, kad efektyvumą nulemia mielių ląstelių augimo fazė. Maksimalus transformacijos efektyvumas buvo pasiektas dirbant su **S** augimo fazėje esančiomis ląstelėmis (po inkubacijos su hidroksikarbamidu (12 mM) dauguma ląstelių buvo su pumpurais). Ląstelių, esančių **M** ciklo fazėje (po inkubacijos su kolhicinu, 1mg/ml), transformacijos efektyvumas buvo labai žemas. Rezultatai leidžia daryti prielaidą, kad **S** ciklo fazėje esančios mielių ląstelės egzogeninės DNR atžvilgiu yra palankesnės.