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High-Efficiency Transformation of *Saccharomyces cerevisiae* Cells by Bacterial Minicell Protoplast Fusion

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After a new transformation procedure, 10% of *Saccharomyces cerevisiae* cells were found to contain transforming DNA sequences. We used direct transfer of plasmid molecules by fusing bacterial minicell protoplasts to yeast protoplasts. Since the procedure significantly reduces the toxic effect of procaryotic protoplasm on the eucaryotic organism, it might be generally applicable in other systems in which transformation is inefficient or impossible.

A variety of methods have been developed for the introduction of DNA into eucaryotic cells, and each technique has had different results and applications. Widely used techniques like the calcium phosphate coprecipitation method (5) or capillary microinjection of nucleic acids into cells (3) seem to result in either low-efficiency transformation of a large number of cells or in high-efficiency transformation of a rather limited number of cells.

Several groups (2, 9) have reported the direct transfer of plasmids from bacterial cells to yeast or mammalian cells by converting bacterial cells to protoplasts via lysozyme treatment and then fusing the protoplasts with tissue-cultured mammalian cells or yeast protoplasts by polyethylene glycol (PEG) treatment. As can be seen in Table 1, the efficiency of this method is similar to that of the widely used transformation method for yeast protoplasts described by Beggs (1). As the ratio of bacterial protoplasts to yeast protoplasts increases, increases in the number of transformed colonies are progressively smaller and finally, in accordance with data reported by others, fusion of the eucaryotic partner with too many bacterial protoplasts decreases the viability of the cells, lowering the number of surviving transformed colonies. Clearly, large amounts of procaryotic cell components seem to be toxic for eucaryotic cells. This toxicity and the resultant depression of transformation efficiency may be due to the excess of bacterial DNA (perhaps poison sequences) or to a component(s) of the bacterial cytoplasm.

We thought a simple and straightforward way to eliminate chromosomal DNA and to reduce the load of procaryotic protoplasm was to use anucleate bacterial minicells (8) in the protoplast fusion technique. Minicells are produced during the growth of certain *Escherichia coli* strains and are known to harbor no chromosomal DNA; they do, however, contain plasmid molecules if the parental strain does. To test the hypothesis we tried to use minicell protoplasts for the transformation of *Saccharomyces cerevisiae* and mammalian cells, and here we report our results with yeast cells.

We used a yeast 2μm plasmid-based shuttle vector (pGY14, Fig. 1) that carries the yeast gene *LEU2* for the transformation of Leu$^-$ yeast cells. (GY787, *leu2-3 leu2-112* Can^r, the plasmid, and the cells were kindly supplied by G. B. Kiss, Institute of Genetics, Biological Research Center, Szeged, Hungary.) Yeast cell protoplasts were produced enzymatically from exponentially growing cells according to standard methods (1).

Minicell-producing *E. coli* DS410 (from S. Erdei, Institute of Biochemistry, Biological Research Center, Szeged) was transformed by standard procedures (7), and plasmid-containing minicells were purified from the cultures by the repeated-gradient centrifugation method described by Reeve (8). (Minicells can be stored at −70°C for long periods without loss of transforming ability.)

Minicell protoplasts were produced by lysozyme treatment as follows. Cells were suspended in 100 μl of 50 mM Tris hydrochloride (pH 8.0) containing 20% sucrose and incubated for 10 min with 20 μl of lysozyme solution (10 mg/ml)–50 μl of 250 mM EDTA. This treatment converted 90 to 95% of the minicells (or bacterial cells) to protoplasts. The protoplasts were diluted with 50 mM Tris hydrochloride (pH 8.0) containing 9% sucrose, if needed.

Yeast protoplasts were produced from exponentially growing cultures after centrifugation. The cells were suspended in 1 M sorbitol–25 mM EDTA–66 mM beta-mercaptoethanol and incubated for 30 min at 30°C. After another centrifugation, the cells were suspended in 1 M sorbitol–10 mM EDTA–100 mM sodium citrate (pH 5.8) containing 3 mg of Helicase per ml and incubated with careful shaking for 60 to 100 min. Over 90% of the cells were converted to protoplasts by this treatment.

Yeast protoplasts were washed with 1 M sorbitol–10 mM $CaCl_2$–10 mM Tris hydrochloride (pH 7.5) and pelleted at room temperature, and the supernatant was discarded and replaced with a suspension of minicell or bacterial protoplasts. After another centrifugation, the pelleted cells were cautiously suspended in PEG solution (in 10 mM $CaCl_2$ and 10 mM Tris hydrochloride [pH 7.5]), with occasional shaking during the incubation period.

After this step, the cells were pelleted again and suspended in a 1:1 mixture of complete yeast medium and 1.2 M sorbitol. Approximately 20 min later, regenerated yeast cells were plated on yeast-nitrogen base minimal medium to select for Leu$^+$ transformants.

Optimal results were achieved by using 10^4 to 10^6 minicell protoplasts per 10^5 yeast protoplasts and treating them in a fusion mixture containing 30 to 35% PEG for 25 to 30 min (Fig. 2).

The results of the different transformation procedures are summarized in Table 1. The transformation of yeast protoplasts with naked DNA in the presence of Ca^{2+} ions (1) and the direct transfer of plasmids by using bacterial

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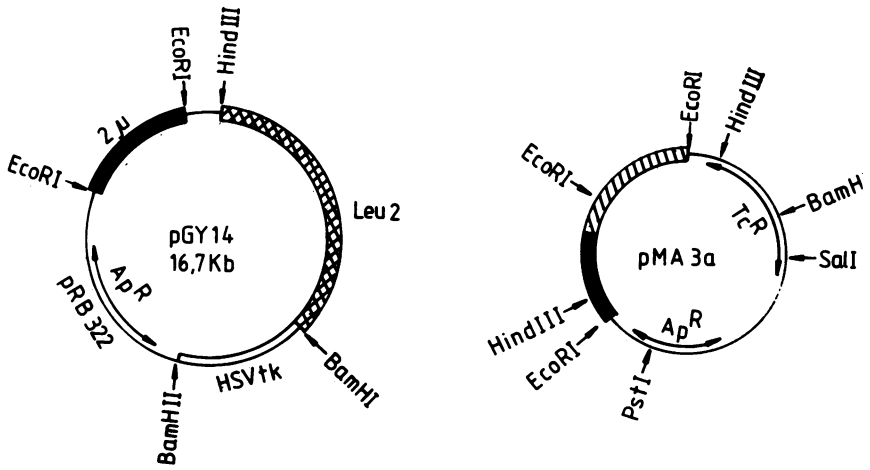

FIG. 1. Structure of plasmids pMA3a (4) and pGY14 (6) used for the transformation of yeast cells. ■, 2μm plasmid sequences (including replication origin); ▨, ▨. *LEU2* gene; □, herpes simplex virus thymidine kinase (HSVtk) gene; ——, pBR322 sequences.

protoplasts (2, 9) yielded very similar results, whereas minicell fusion-mediated transformation proved to be much more efficient; up to 2.4 to 2.7% of the treated yeast cells grew to transformed colonies.

It is well known that only 10 to 20% of protoplasted yeast cells regenerate into colony-forming yeast cells. In this case, it means that approximately 9 to 13% of the surviving cells expressed the transforming gene(s). Thus, the high efficiency

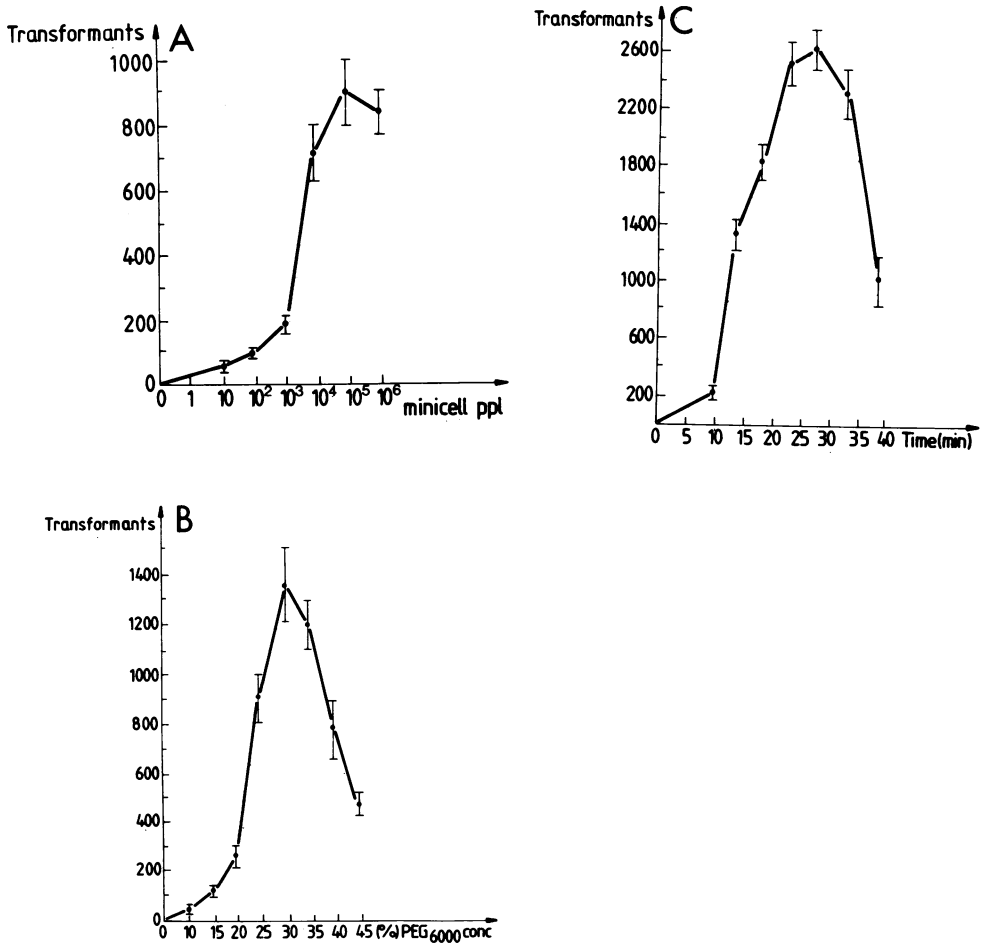

FIG. 2. Influence of protoplast ratio, PEG concentration, and length of PEG treatment on the efficiency of transformation. (A) Increasing amounts of bacterial minicell protoplasts were mixed and fused with 10^5 yeast protoplasts with 20% PEG for 15 min. (B) A mixture of yeast and minicell protoplasts was fused with different concentrations of PEG for 15 min. (C) A mixture of yeast and minicell protoplasts was fused with 30% PEG for different times.

TABLE 1. Comparison of different yeast transformation procedures

Method	No. of transformed cells per μg of plasmid DNA	Transformed colonies as % of regenerating colonies
DNA mediated (μg of DNA per 10^7 cells)[a]		
0.5	3.0×10^3–5.4×10^3	0.015–0.03
1.0	4.0×10^3–7.0×10^3	0.04–0.07
2.5	3.0×10^3–3.3×10^3	0.07–0.08
Fusion of protoplasts (no. of bacteria/10^5 yeast cells)[b]		
10^2	1×10^5–2×10^5	0.07–0.08
10^4	2×10^3–4×10^3	0.13–0.17
10^5	1×10^2–2×10^2	0.05–0.07
Fusion with minicells (no. of minicells/10^5 yeast cells)[c]		
10^2	10^7	0.03–0.05[d]
10^4	10^6–10^7	0.65–0.8[d]
10^5	10^6	2.4–2.7[e]

[a] DNA-mediated transformation of protoplasts with naked DNA, PEG, and Ca^{2+} ions (1).
[b] Fusion of yeast and bacterial protoplasts (2,9) in 30% PEG for 30 min.
[c] Fusion of yeast and bacterial minicell protoplasts.
[d] In 30% PEG for 30 min.
[e] In 20% PEG for 15 min.

of our method raises the possibility of performing experiments with nonselectable DNA sequences without the use of cotransforming marker genes.

To prove this point, we transformed Leu$^-$ yeast cells with pMA3a (another 2μm-plasmid-based construction which carries the yeast *LEU2* gene and the entire pBR322, kindly given by M. J. Dobson, Department of Biochemistry, Oxford University, Oxford, England). No selective pressure was applied, and all regenerating colonies were screened by hybridization for the presence of transforming sequences and by plating on minimal medium to prove the expression of the *LEU2* gene carried by the transforming plasmid. From 9 to 14% of the colonies harbored DNA sequences hybridizing with the probe (pBR322 DNA), and 7 to 11% of the colonies were able to grow without leucine in the medium.

In conclusion, we proved that elimination of bacterial chromosomal DNA (and significant reduction of fused procaryotic cytoplasm) during the fusion-mediated transformation of yeast protoplasts increased the transformation efficiencyapproximately 10 to 20 times. The method is simple and reproducible. We think that this approach could also be used to improve transformation in other organisms when it is otherwise inefficient or impossible.

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LITERATURE CITED

1. **Beggs, J. D.** 1978. Transformation of yeast by a replicating hybrid plasmid. Nature (London) **275**:104–109.
2. **Broach, J. R., J. N. Strathern, and J. B. Hicks.** 1979. Transformation in yeast: development of a hybrid cloning vector and isolation of the CAN1 gene. Gene **8**:121–133.
3. **Diacumakos, E. G.** 1973. Methods of micromanipulation of human somatic cells in culture. Methods Cell Biol. **7**:287–311.
4. **Dobson, M. J., M. F. Tuite, N. A. Roberts, A. J. Kingsman, S. M. Kingsman, R. E. Perkins, S. C. Conroy, B. Dunbar, and S. M. Fothergill.** 1982. Conservation of high efficiency promoter sequences in *Saccharomyces cerevisiae*. Nucleic Acids Res. **10**:2625–2637.
5. **Graham, F. L., and A. J. van der Eb.** 1973. New technique for the assay of infectivity of human Ad5 DNA. Virology **52**:456–467.
6. **Kiss, G. B., R. E. Pearlman, K. V. Cornish, J. D. Friesen, and V. L. Chan.** 1982. The herpes simplex virus thymidine kinase gene is not transcribed in *Saccharomyces cerevisiae*. J. Bacteriol. **149**:542–547.
7. **Maniatis, T., E. F. Fritsch, and J. Sambrook.** 1983. Molecular cloning: a laboratory manual, p. 250–251. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
8. **Reeve, J.** 1979. Use of minicells for bacteriophage-directed polypeptide synthesis. Methods Enzymol. **68**:493–503.
9. **Schaffner, W.** 1980. Direct transfer of cloned genes from bacteria to mammalian cells. Proc. Natl. Acad. Sci. USA **77**: 2163–2167.
10. **Sherman, F., G. R. Fink, and C. W. Lawrence.** 1982. Methods in yeast genetics. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.