Transformation of *Saccharomyces cerevisiae* spheroplasts by high electric pulse

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An electric pulse was applied to transformation of yeast cell. *Saccharomyces cerevisiae* D13-1A *(a his3-532 trpl gal2)* was used as a recipient strain for plasmid YRp7. Spheroplasts of *S.cerevisiae* D13-1A were placed under an electric pulse in the presence of YRp7. Transformants were observed when the pulse height was above 5 kV/cm. The number of transformants increased with increasing pulse height and 945 transformants per μg DNA were obtained at 10 kV/cm. Introduction of DNA might be caused by breakdown of the cell membrane under high electric voltage.

Transformation Saccharomyces cerevisiae *Spheroplast* *Plasmid* *Electric pulse*

1. INTRODUCTION

DNA-mediated transformation of yeasts provides a new aspect of research on eukaryote cells. A key step of the transformation is the injection of DNA fragments through the cell membrane. Since Hinnen et al. [1] found that the use of polyethylene glycol (PEG) and calcium chloride caused transformation of yeast cells, similar procedures have been performed by many researchers.

Recently, the application of an electric pulse was reported by Neumann et al. [2], who succeeded in the transformation of mammalian cells. Disadvantages linked to external reagents such as PEG could be avoided by this method. Under an electric field above a certain threshold level, a short period of breakdown occurred in the mammalian cell membrane, and DNA outside the cells flowed in the cells. Incorporation of protein or polystyrene latex particles was also demonstrated under an electric pulse application [3,4]. These reports show that the cell membrane may be repaired after a puff of electric breakdown.

Yeast cells are smaller than mammalian cells as mentioned above. Therefore, the threshold level is theoretically higher than that for mammalian cells. However, the electric breakdown and its instant repair are also expected for yeast cells if the electric conditions are controlled.

Here, an electric pulse was applied to the spheroplasts of yeast cells. Optimum conditions of electric pulse were surveyed for the DNA-mediated transformation of yeast.

2. MATERIALS AND METHODS

2.1. *Yeast strain plasmid*

Saccharomyces cerevisiae D13-1A *(a his3-532 trpl gal2)* was used as the recipient strain for DNA YRp7. YRp7 is the shuttle vector which consists of pBR322 and TRP1 yeast gene [5]. YRp7 was prepared from cleared lysate of *Escherichia coli* JA221 (YRp7). The cleared lysates were prepared by the method of Birnboim and Doly [6]. *E. coli* JA221 *(F^- leuB6 Δ trpE5 lacY hsdR recA)* was also used for plasmid analysis.

2.2. *Preparation of spheroplasts*

S. cerevisiae D13-1A was cultured in YPD medium (1% yeast extract, 2% polypeptone, 2% glucose) with reciprocal shaking at 30°C. The fresh logarithmic cells were harvested by centrifugation at $1000 \times g$. The collected cells were suspended in 1.2 M sorbitol solution and treated with Zymolase

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6000 (final concentration 200 μg/ml; Kirin Brewery, Tokyo) and 2-mercaptoethanol (final concentration 20 mM) for 1 h at 30°C. The suspension was then centrifuged for 5 min at 1000 × g and the supernatant discarded. The resulting pellet was washed twice with 1.2 M sorbitol solution and finally suspended in a solution (0.5 ml) containing 1.2 M sorbitol, 10 mM $CaCl_2$ and 10 mM Tris-HCl (pH 7.5).

2.3. *Transformation conditions*

DNA YRp7 (final concentration 10 μg/ml) was added to a spheroplast suspension, and the mixture incubated for 5 min at room temperature. A portion of the mixture (0.1 ml) was placed between a couple of parallel platinum electrodes (distance 1 mm), then electric pulses were applied to the electrodes. The electric pulse was supplied with a discharging circuit described below. The mixture was spread onto the plates containing selective medium (0.7% Difco yeast nitrogen base, 2% glucose, 50 μg/ml histidine, 1.2 M sorbitol, 3% agar) to obtain transformants growing without tryptophan. The plates were covered with the same medium and incubated at 30°C for 4–5 days. Colonies appearing on the plates were counted.

2.4. *Discharging circuit*

A simple circuit for electric pulse supply was composed of a capacitor ($C = 1$ μF), a switch and a power unit. Initially the capacitor was charged until the terminal voltage reached 1 kV. Then the power was turned off, and the discharging circuit connecting with the electrodes was turned on. The voltage between the electrodes decreased exponentially. The time constant of the exponential decay under the given ionic condition was about 50 μs.

2.5. *Detection of DNA*

DNAs were extracted from the transformants and recipient cells by the method of Davis et al. [7]. Electrophoresis in agarose gel (slab type; 0.8 or 1.8%) was carried out with the Tris-borate buffer described by Maniatis et al. [8].

2.6. *Digestion of plasmid DNA*

Plasmid DNAs were digested with restriction endonuclease, *Eco*RI, *Sal*I, *Hae*III and *Sau*3AI, following the method of Davis et al. [9].

3. RESULTS AND DISCUSSION

The effect of pulse height on transformation was investigated. As shown in fig.1, transformants appeared when the pulse height was higher than 5 kV/cm. Above this threshold level, the number of transformants increased with pulse height and reached 945 per μg DNA at 10 kV/cm.

When pulses were applied repeatedly to the spheroplasts, however, the number of transformants decreased markedly, as shown in fig.2. The maximum number of transformants was obtained when a unique pulse was applied. A decrease in the number of viable cells was observed with increasing number of pulses (fig.3). These results showed that repeated pulses caused irreversible breakdown of the cell membrane. Therefore, the pulse height was fixed at 10 kV/cm and unique pulse was applied to the cell.

The effect of DNA concentration on transformation was examined. As shown in fig.4, the transformation efficiency increased with increasing concentration of plasmid DNA.

Fig.5 shows electrophoretic patterns of DNA extracted from recipient cells and transformants. The transformants gave two more bands than those obtained for the recipient cells. These two bands were

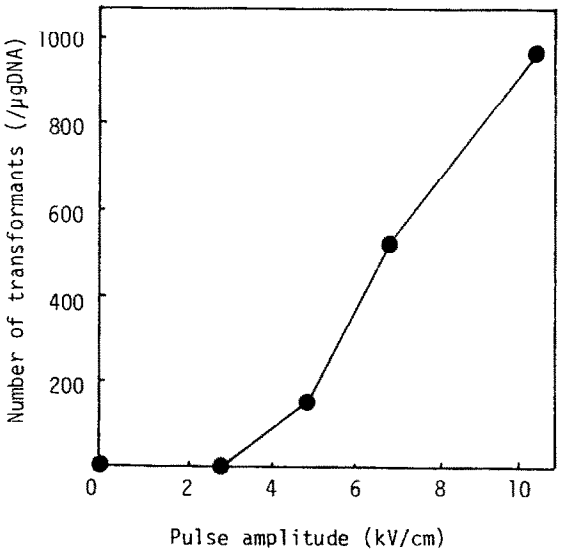

Fig.1. Effect of pulse amplitude on transformation. A unique pulse with various amplitudes was applied to the spheroplast suspension. Other transformation conditions were as described in the text.

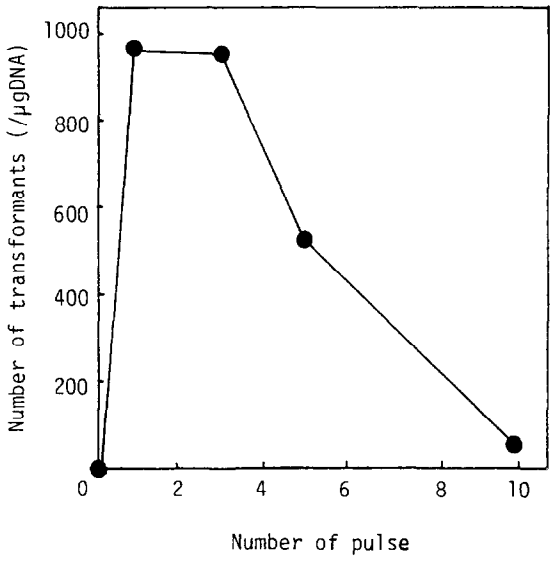

Fig.2. Effect of the number of pulses on transformation. A pulse of 10 kV/cm was applied to the spheroplast suspension. Other transformation conditions were as described in the text.

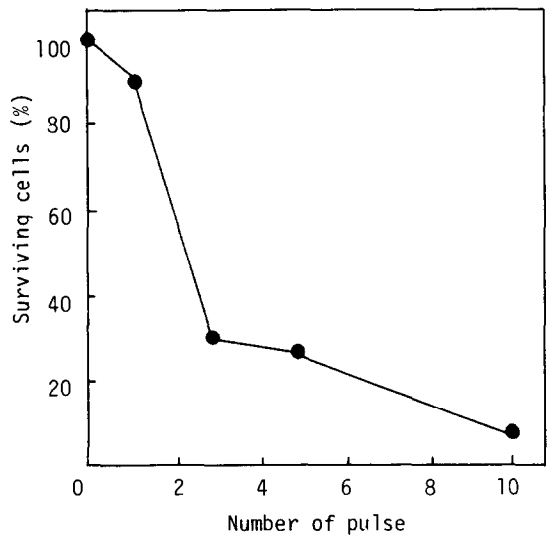

Fig.3. Effect of the number of pulses on cell viability. A pulse of 10 kV/cm was applied to the spheroplast suspension. Viable cells were counted after 3-5-day incubation in the agar medium containing 0.7% yeast nitrogen base, 2% glucose, 50 μg/ml Trp and His, 1.2 M sorbitol and 3% agar.

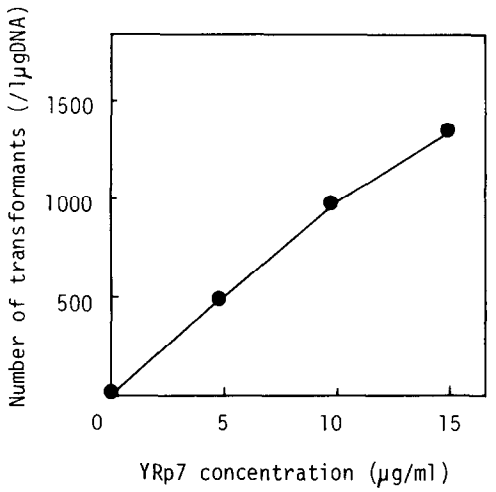

Fig.4. Effect of DNA concentration on transformation. A unique pulse of 10 kV/cm was applied to the spheroplast suspension. Other transformation conditions were as described in the text.

estimated as closed circular and open circular forms of YRp7. Restriction analysis of the plasmid in *E. coli* JA221 transformed with cleared lysates of Trp^+ transformants was performed using *Eco*RI, *Sal*I, *Hae*III and *Sau*3AI. The electrophoretic pattern of restriction endonuclease digests showed that the plasmid recovered from the Trp^+ transformant was similar to YRp7 plasmid (fig.6). From 945 colonies carrying the Trp^+ phenotype, 25 were picked and examined for plasmid analysis. All colonies showed the same electrophoretic pattern of DNAs as that of YRp7.

According to the theoretical works by Zimmermann et al. [10] or Kinoshita and Tsong [11], the apparent membrane voltage (V_m) is estimated as $V_m = frE$, where f is the shape factor, r is the radius of the cell, and E is the external electric field. In the present case, V_m was estimated as 0.9–1.5 V, since r was 1.2–2.0 μm, f was 1.5 for spherical cells, and E was 5 kV/cm. This value was considered high enough to cause the pore formation, since most cell membranes were broken by about 1 V of V_m as reported previously [12–15]. Therefore, DNA could flow in the recipient cells of *S. cerevisiae* during pore formation.

In addition to the effect of a high electric field on membrane structure described above, another effect on plasmid DNA is considered below. Since DNA is a polar molecule, orientation occurs in

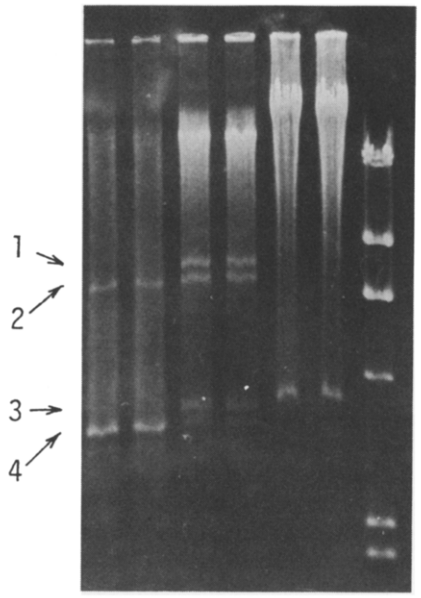

Fig.5. Electrophoretic patterns of DNAs in transformants obtained by the electric pulse method. DNAs extracted from yeasts and YRp7 plasmid were prepared as described in the text. Electrophoretic conditions are also described in the text. Lanes A,B:YRP plasmid; C,D:transformants; E,F:recipient cells; G:DNA. Arrows show open circular form of 2 μm DNA (1), open circular form of YRp7 (2), closed circular form of 2 μm DNA (3), and closed circular form of YRp7 (4).

high electric fields. The orientation of DNA has been followed by electrodichroism measurement [16]. On the other hand, Diekmann et al. [17] suggested that the DNA strand was extended from a weakly bent to a straight form in high electric fields. Changes in the orientation and conformation of DNA as described above might make it easy for molecular DNA to pass through the pores of cell membrane.

In conclusion, the present method has the following advantages. (i) Chemical reagents such as PEG, which are often lethal for cells, are not required to induce transformation. (ii) Transformation efficiency is comparable with that of the conventional method. (iii) Transformation conditions are controlled easily by means of electric power. Therefore, this method is simple, easy and very efficient technology to introduce plasmid DNA into *S. cerevisiae*.

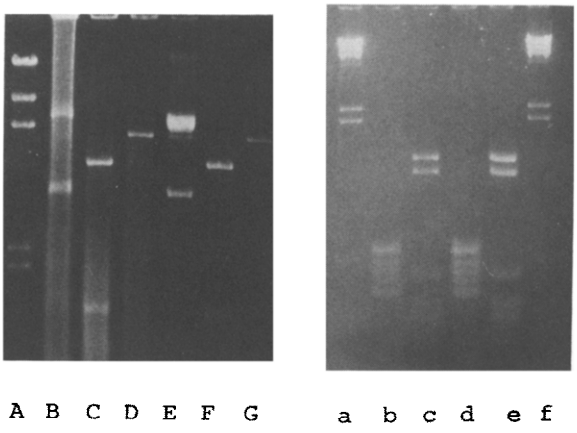

Fig.6. Plasmid analysis in *E. coli* JA221 transformed with the DNA extracted from Trp$^+$ transformant. 0.8% (A–G) or 1.8% (a–e) of agarose was used in gel electrophoresis. Other conditions were as described in the text. A, *Hin*dIII digests of λphage DNA; B, YRp7 plasmid; C, *Eco*RI digests of (B); D, *Sal*I digest of (B); E, DNA recovered from Trp$^+$ transformant; F, *Eco*RI digests of (E); G, *Sal*I digest of (E). b, *Hae*III digests of (B); c, *Sau*3AI digests of (B); d, *Hae*III digests of (E); e, *Sau*3AI digests of (E); a, e, *Hin*dIII digests of λphage DNA.

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