18 Transformation of Intact Cells of *Saccharomyces cerevisiae*: Lithium Methods and Possible Underlying Mechanism

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18.1 Introduction

Before the initial publication of the lithium method in 1983 (Ito et al. 1983), it is hard to imagine that how intact cells of *Saccharomyces cerevisiae* could be transformed, which are surrounded by rigid and thick cell walls. However, the developed lithium method allowed for the first time the successful transformation of intact *S. cerevisiae* cells using plasmid DNA (Ito et al. 1983). This monumental method represented a significant advance in genetic and biological studies of yeast, and has also contributed to the rapid analysis of the genes of higher animals and plants. Subsequently, the method has been modified in several ways. Here, we describe the principles of the original and modified lithium methods, a possible mechanism underlying transformation of intact cells, and finally the modified lithium method that we currently use.

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18.1.1 Original Lithium Method

In the original lithium method (Ito et al. 1983), a most important factor that increased the transformation efficiency (number of transformants per μg plasmid DNA) of intact *S. cerevisiae* cells was the presence of monovalent cations such as Na^+, K^+, Rb^+, Cs^+, and especially Li^+. It is surprising that divalent cations such as Ca^{2+}, which is effective in *Escherichia coli* transformation, are not effective for transformation of intact *S. cerevisiae* cells. Lithium was tested because it is effective in separation of inorganic polyphosphate, a negatively charged macromolecule similar to DNA, from anion-exchange columns (Kawai et al. 2010). In addition to the use of lithium, the original lithium method has several important features: (i) incubation of intact cells with both polyethylene glycol (PEG) and plasmid DNA is essential for transformation; (ii) short-term incubation of intact cells with PEG and plasmid DNA at 42 °C (heat shock) increases the transformation efficiency; and (iii) transformation of cells harvested at mid-log phase is most efficient (Ito et al. 1983). PEG was tested in the original lithium method because this reagent is used in the spheroplast method (Hinnen et al. 1978). The original lithium method yielded about 450 transformants/μg of plasmid DNA (Ito et al. 1983).

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18.1.2 Modified Lithium Methods

Based on the principles established in the original lithium method (Ito et al. 1983), Gietz and co-workers succeeded in improving the efficiency to 5×10^6–1×10^7/μg of plasmid DNA from 10^8 cells by immediately mixing washed intact cells with PEG, lithium acetate (LiAc), plasmid DNA, and single-stranded carrier DNA (ssDNA), and then incubating them at 42 °C for 40–60 min (Gietz et al. 1992, Gietz et al. 1995, Gietz and Woods 2002, Schiestl and Gietz 1989). Their protocol has been referred to as the LiAc/ssDNA/PEG method (Gietz and Woods 2002).

In contrast to the approach of Gietz et al., who added components to the original lithium method, we removed LiAc from the original method, and found that intact cells could be transformed by incubating cells with only PEG and plasmid DNA at 30 °C and then at 42 °C (heat shock) (Hayama et al. 2002). This method was tentatively called the "natural transformation method" (Hayama et al. 2002).

18.1.3 Possible Mechanism Underlying Transformation of Intact Cells

We evaluated the effect of ssDNA and LiAc on transformation efficiency in the LiAc/ssDNA/PEG method, and observed that LiAc and ssDNA synergistically improved the transformation efficiency of intact *S. cerevisiae* cells (Table 18.1) (Pham et al. 2011b). Using transmission electron microscopy (TEM), we observed *S. cerevisiae* cells incubated with PEG and negatively charged Nanogold (in this context, a mimic of DNA) in the presence of no additional reagents (Fig. 18.1a), ssDNA (Fig. 18.1b), LiAc (Fig. 18.1c), and both LiAc and ssDNA (Fig. 18.1d). Together, LiAc and ssDNA made the cell wall form extremely protruded, loose, and porous structures (Fig. 18.1d); LiAc alone caused the cell wall to protrude slightly (Fig. 18.1c) (Pham et al. 2011b). Taken together the synergistic effect of LiAc and ssDNA on transformation efficiency, we attributed the high transformation efficiency achieved with LiAc and ssDNA to this altered cell wall structure that was synergistically caused by LiAc and ssDNA (Pham et al. 2011b).

Using the natural transformation method (Hayama et al. 2002), we transformed approximately 5,000 strains in each of which a nonessential gene was deleted. Several deletion mutants had high transformation efficiency (e.g. *spf1*) whereas others had low transformation efficiency (e.g. *arc18* and *she4*), and the findings provided evidence that DNA enters the cell via endocytotic membrane invagination (Kawai et al. 2004). Using fluorescence microscopy, we visualized the process of transformation achieved by the

Table 18.1 ssDNA and LiAc synergistically enhance transformation efficiency and frequency

	Transformation efficiency cfu/μg		Viable cells cfu		Transformation efficiency (A/B)
Composition[a]	pRS415 (A)	Fold	(× 10^4) (B)	Fold	Fold
None[b]	2,008 ± 1,606	1	1,592 ± 8	1.00	1
ssDNA[c]	13,613 ± 8,226	7	1,821 ± 141	1.14	6
LiAc[d]	84,888 ± 37,692	42	1,128 ± 400	0.71	60
LiAc + ssDNA[e]	1,007,500 ± 657,319	501	1,428 ± 76	0.90	560

[a]The cells were incubated with 36 % PEG and 0.2 μg pRS415 at 42 °C for 20 min in 42 μl transformation mix containing no additional reagents[b], 0.29 mg/ml ssDNA[c], 10.7 mM LiAc[d], or both 10.7 mM LiAc and 0.29 mg/ml ssDNA[e]
(From Pham, T.A., S. Kawai, and K. Murata. 2011b. Visualization of the synergistic effect of lithium acetate and single-stranded carrier DNA on *Saccharomyces cerevisiae* transformation. Curr. Genet. 57: 233-239 with permission.)

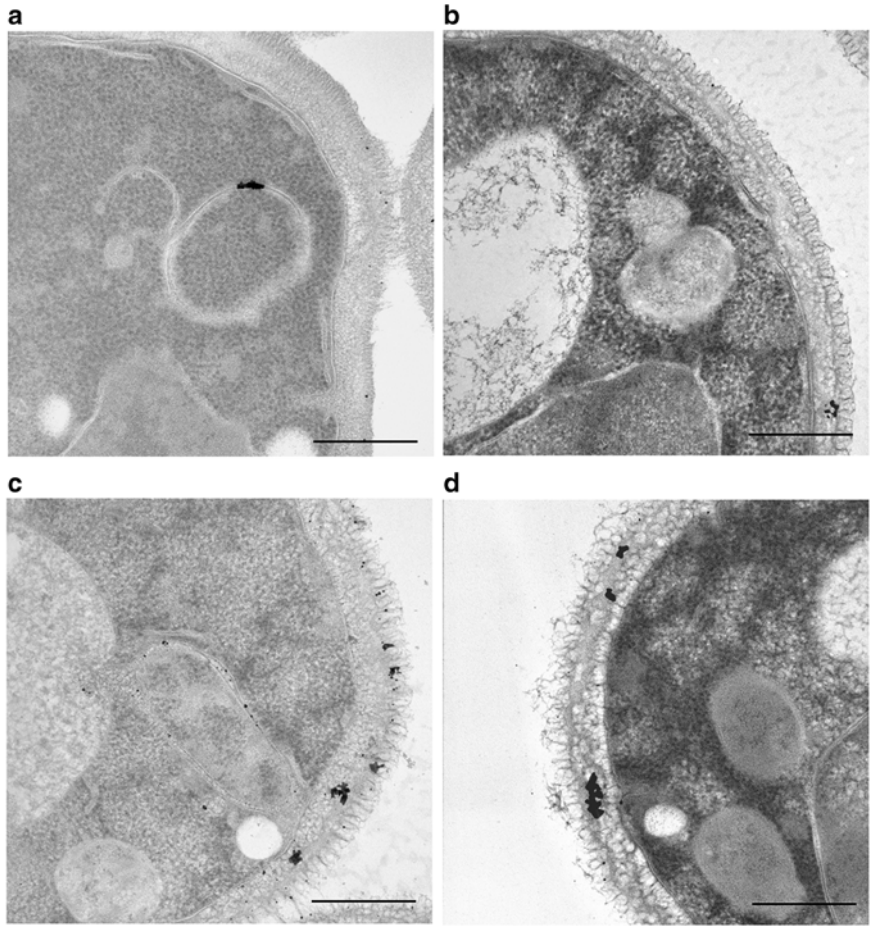

Fig. 18.1 Visualizing the effects of ssDNA and LiAc. Cells were incubated at 42 °C for 20 min with PEG and negatively charged Nanogold, in the presence of no additional reagents (**a**), ssDNA (**b**), LiAc (**c**), both LiAc and ssDNA (**d**) as described in Table 18.1, treated, and observed by transmission electron microscopy. Panels show images at 47,800-fold magnification (scale bar is 0.50 μm). Signals from Nanogold are observed as dots. For more detail, see the reference (From Pham, T.A., S. Kawai, and K. Murata. 2011b. Visualization of the synergistic effect of lithium acetate and single-stranded carrier DNA on *Saccharomyces cerevisiae* transformation. Curr. Genet. 57: 233-239 with permission.)

natural transformation method with YOYO-1-labelled plasmid DNA (YOYO-1/YEp13) (Pham et al. 2011a). YOYO-1 is a widely used cell-impermeable fluorescent DNA probe (Gurrieri et al. 1997). We observed that YOYO-1/YEp13 attaches to the region around intact cells incubated with PEG, and PEG was required for the attachment of YOYO-1/YEp13 onto cells and their successful transformation. Moreover, the fluorescence intensity of *spf1* cells was higher than that of wild type (WT) cells, and the intensity of unwashed cells was much higher than that of washed cells (Fig. 18.2). The transformation efficiency of unwashed cells was 14.8-fold (*spf1* cell) and 2.3-fold (WT cell) greater than that of washed cells, suggesting that washing the cells removes attached DNA from the cells and thereby decreases transformation efficiency. Based on these observations, we concluded that (i) the DNA absorbed on the cell surface is taken up by the cell; (ii) delivery of DNA into the nucleus mainly occur in cells spread on solid selective medium, and (iii) the high capacity of *spf1* cells to absorb DNA is at least partially responsible for

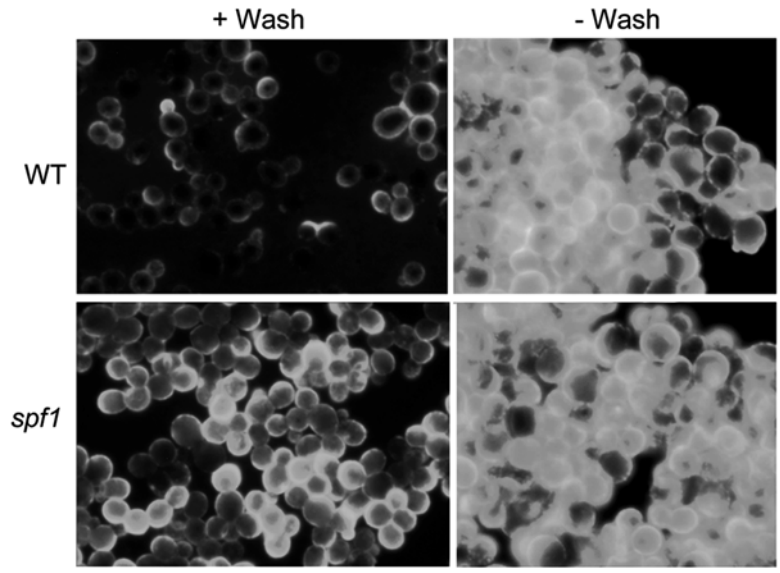

Fig. 18.2 Behaviour of YOYO-1/YEp13 during transformation. Intact WT (*upper*) and *spf1* (*lower*) cells were incubated at 30 °C for 1 h in 80 µl suspension containing 34 % (w/v) PEG and YOYO-1/YEp13 equivalent to 1.8 µg YEp13. The incubated cells were observed after washing (*left*: + Wash) or without washing (*right*: - Wash). (Note: LiAc and ssDNA were not included in the mixture). (From Pham, T.A., S. Kawai, E. Kono, and K. Murata. 2011a. The role of cell wall revealed by the visualization of *Saccharomyces cerevisiae* transformation. Curr. Microbiol. 62: 956-961 with permission.)

the high-transformation phenotype of *spf1* cells (Kawai et al. 2004, Pham et al. 2011a).

18.1.4 The Modified Lithium Method (LiAc/ssDNA/PEG Method)

We have recognized the high efficiency of LiAc/ssDNA/PEG method, and are now using a method that is essentially the same as the reported one (Gietz and Woods 2002). Below, we describe the practical procedure and provide some additional comments.

18.2 Materials

1. Pure water: prepared using an Elix Advantage 3 (Millipore). All reagents and media are prepared using pure water. Sterilized distilled water (SDW) is prepared by autoclaving (121 °C, 20 min).
2. Liquid YPD medium: 1.0 % yeast extract, 2.0 % tryptone, and 2.0 % glucose in pure water (pH 5.6). For solid medium, add 2.0 % agar. Sterilize medium by autoclaving (121 °C, 20 min). The antibiotic stock solution (e.g. geneticin, 50 mg/ml in pure water; hygromycin B, 50 mg/ml in pure water) is sterilized by filtration (Advantec, Dismic-25cs, Cellulose Acetate, pore size 0.20 µm) and added to YPD medium after autoclaving (final concentrations: geneticin, 100 µg/ml; hygromycin B, 300 µg/ml).
3. Synthetic complete (SC) medium: 0.67 % yeast nitrogen base without amino acids (Becton, Dickinson and Company), 2.0 % glucose, 690 mg/l-Leu Do supplement (Clontech), 100 mg/l leucine. For SC medium without nutrients, use the appropriate Do supplement (Clontech). Liquid medium is solidified by addition of 2 % agar.
4. 50 % PEG: Add 10 g PEG (Sigma, P-3640), 200 µl 1.0 M Tris-HCl (pH 8.0), 100 µl

200 mM EDTA (pH 8.0) to pure water. Dissolve the PEG, and add pure water to a final volume of 20 ml. Sterilize by autoclaving. Store at room temperature.

5. 1.0 M LiAc: Dissolve LiAc (Nacalai Tesque, Extra pure grade) in pure water to reach 1.0 M; pH is not adjusted. Sterilize by autoclaving. Store at room temperature.
6. TE: 10 mM Tris-HCl (pH 8.0), 1.0 mM EDTA (pH 8.0), in pure water. Sterilize by autoclaving.
7. 2.0 mg/ml ssDNA: Dissolve salmon sperm DNA (Sigma, D-1626) in TE at 2.0 mg/ml with gentle stirring at room temperature for 2–3 h. Store at −30 °C. Just before use, thaw a small portion, incubate the aliquot in boiling water for 5 min, and immediately cool in ice/water. After the transformation, discard any remaining boiled and cooled ssDNA.
8. Transformation mix: Mix 240 µl 50 % PEG, 36 µl 1.0 M LiAc, and 50 µl 2.0 mg/ml ssDNA.

18.3 Methods

1. Inoculate the *S. cerevisiae* strain into 1.0 ml of liquid YPD medium, and cultivate the strain aerobically at 30 °C to reach saturation.
2. Transfer 30 µl of the saturated preculture to 1.5 ml liquid YPD medium and further cultivate the cells for 4–5 h.
3. Collect the cells by centrifugation at 14,000 *g* for 1 min. Remove the supernatant completely.
4. Add 40 µl of transformation mix to the cells, and resuspend the cells in the transformation mix by vigorous vortex mixing.
5. Add less than 4 µl of plasmid DNA (or DNA fragment) and mix by vigorous vortex mixing. *Comments*: PCR reaction mixture can be used without purification.
6. Incubate the tubes in a 42 °C water bath for 40 min.
7. For selective solid SC medium without nutrients: Place 100 µl SDW on solid selective medium. Collect the cells in the suspension by centrifugation at 14,000 *g* for 1 min, and discard the supernatant completely. Resuspend the cells in SDW (44 µl). Put the 2 µl suspension and the remaining suspension (−42 µl) onto 100 µl SDW on the solid selective media (Mix the suspension with the 100 µl SDW on the solid media). Spread the cells onto the solid media. When counting viable cells, add SDW to the cell suspension to reach 1.0 ml, suspend very gently by pipetting, dilute the suspension in SDW, and spread the dilutions onto solid YPD medium to count cells. With the remaining suspension, spread 50 µl onto the selective solid medium; then, collect the cells in the remaining suspension by centrifugation at 14,000 *g* for 1 min, and discard the supernatant. Resuspend the cells in SDW (less than 100 µl) and spread on the selective solid medium. *Comments*: If washing of cells is required to remove PEG, wash gently to avoid removing the DNA attached to the cells (Fig. 18.2).
8. For selective solid YPD medium with antibiotics: Collect the cells by centrifugation at 14,000 *g* for 1 min, and completely remove the supernatant. Suspend the cells in 1.0 ml SC liquid medium and cultivate the cells aerobically overnight at 30 °C. Spread 100 µl of the culture on the selective solid medium. Collect the cells in the remaining culture by centrifugation at 14,000 *g* for 1 min, and discard the supernatant. Resuspend the cells in the SDW (less than 100 µl) and spread on the selective solid medium.
9. Incubate solid medium at 30 °C for 3–4 days.

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