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Establishment of "Highly Efficient" Transformation Method of *Saccharomyces cerevisiae* Based on Molecular Basis for Yeast's Competence

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Research aims

Transformation of a yeast *Saccharomyces cerevisiae* is an indispensable method for the study of this good model of the eucaryotic cell. An improvement of the transformability of the yeast cell has been required, since a transformability of yeast cell is lower by approximately 10^{-3} fold than that of *Escherichia coli*. Understanding of the molecular mechanism for transformation of *S. cerevisiae* would help to improve the transformation methods of not only *S. cerevisiae* but also other eukaryotic cells. To understand the mechanism, transformability of all nonessential gene mutants (about 5,000 strains) of *S. cerevisiae* was examined by natural transformation[1] and the three mutants, *pde2*, *spf1*, and *pmr1* were found to show high transformability.[2] The Pde2p is high-affinity cAMP phosphodiesterase. In the *pde2*, intracellular level of cAMP is elevated. Therefore it has been predicted that elevated basal cAMP level enhances transformability. Tpk1p, Tpk2p, and Tpk3p are redundant catalytic subunits of protein kinase A (PKA), which is a major cAMP target in the Ras-cAMP pathway. PKA is a tetramer consisting of hetero- or homodimer of the catalytic subunits and homodimer of regulatory subunits encoded by *BCY1*. Taken together, it has been presumed that enhanced intracellular cAMP level increases transformability through PKA.

This study was aimed to construct the *S. cerevisiae* strains having the "highly efficient" transformability and to find the transformation conditions giving "highly efficient" transformability.

Methods

The natural transformation method and lithium acetate (LiAc)/single-stranded carrier DNA (SS-DNA)/polyethylene glycol (PEG) method were conducted as described.[1,3] Yeast cells were precultured overnight, transferred to fresh YPD medium to reach A_{600} of 0.1, cultured to reach A_{600} of 0.3 to 0.6, and then collected by centrifugation. In natural transformation method, the collected cells were suspended in 20 μl TE (10 mM Tris-HCl, pH 8.0, and 1 mM EDTA), and incubated with 20 μl plasmid DNA (0.5 μg YEp13 in TE) and 120 μl PEG4,000 (47% w/v in TE) for 1 h at 30°C, and then for 5 min at 42°C. In LiAc/SS-DNA/PEG method, the collected cells were suspended in LiAc/SS-DNA/PEG solution (20 μl 0.5 μg YEp13, 36 μl 1 M LiAc in water, 50 μl 2 mg/ml SS-DNA in TE, and 240 μl 50% w/v PEG3,350 in TE) and incubated for 40 min at 42°C. In both methods, after the cells were incubated, the cells were collected, washed once by 1 ml TE, and spread onto selective SD solid medium (conventional method) or spread directly onto selective SD solid medium without washing (direct method). Just before spreading the cells onto SD selective solid medium, numbers of viable cells were counted by spreading the diluted suspensions of the cells onto YPD solid medium. Transformability was judged from the number of transformants and from the transformation frequency [the number of transformants/the number of viable cells]. Transformability is presented as an average value of more than three independent determinations.

Gene disruption was performed by PCR-targeting as described.[4] Strains used in this study are listed in Table 1.

Results

The first approach to construct the *S. cerevisiae* strains having the "highly efficient" transformability was to identify the catalytic subunit of PKA concerning transformability. If one or two subunits inhibit transformability, deleting of the gene for the subunit was supposed to enhance transformability. If one or two subunits enhance transformability, an overexpression of the gene for the subunit was supposed to greatly enhance transformability. In order to validate this supposition, we examined the transformability of the following strains: (i) strains overexpressing each of *TPK1*, *TPK2*, *TPK3* (MK793, 794, 795), (ii) strains overexpressing *BCY1* (the gene for regulatory subunit of PKA) plus each of *TPK1*, *TPK2*, *TPK3* (MK973, 974, 975), (iii) single mutants lacking each of *BCY1*, *TPK1*, *TPK2*, *TPK3* (XPY1α, MK450, 451, 452), and (iv) double mutants lacking two of *TPK1*, *TPK2*, *TPK3* (MB13, 23, 12) (Table 1).

However, transformability of these strains exhibited no obvious difference from that of isogenic wild type strain (WT) when transformability was examined by natural transformation method and LiAc/SS-DNA/PEG method (data not shown). We assumed that some effects of overexpressing of *BCY1*, *TPK1*, *TPK2*, *TPK3* or deletions of *BCY1*, *TPK1*, *TPK2*, *TPK3* on transformability might be detected through deletion of *PDE2* from the tested strains. Hence, we examined the transformability of the following

strains: (v) *pde2* overexpressing each of *TPK1*, *TPK2*, *TPK3* (MK979, 980, 981), (vi) *pde2* overexpressing *BCY1* plus each of *TPK1*, *TPK2*, *TPK3* (MK940, 941, 942), (vii) *pde2* lacking each of *TPK1*, *TPK2*, *TPK3* (MK871, 872,

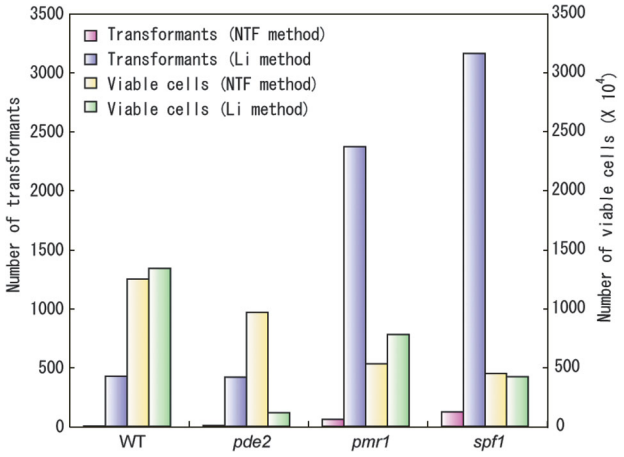

Fig. 1. Number of transformants and viable cells obtained by LiAc/SS-DNA/PEG method (Li method) and natural transformation method (NTF method).
Averages of five (Li method) and three (NTF method) independent experiments are shown. YEp13 (0.5 μg) was used.

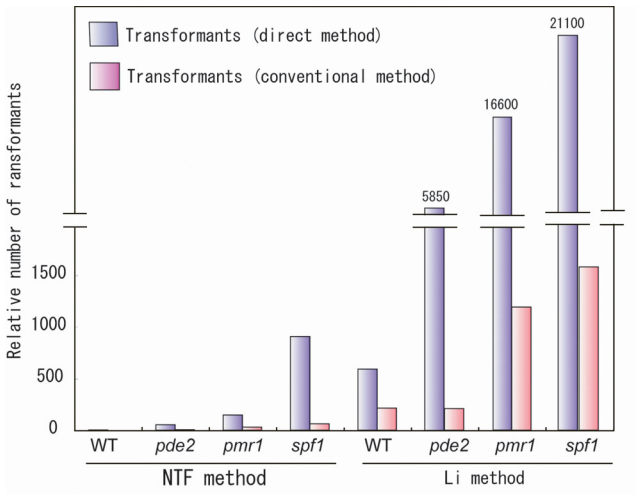

Fig. 2. Relative number of transformants obtained by conventional method and direct method..
LiAc/SS-DNA/PEG method (Li method) and natural transformation method (NTF method) were conducted. YEp13 (0.5 μg) was used. In each method, the cells were spread on the selective medium and washing the cells once by 1 ml TE (conventional method) or without washing (direct method). Averages of five (Li method) and three (NT method) independent experiments are shown. Number of transformants ontained by NTF method plus conventional method was relatively taken as 1.

Table 1. *S. cerevisiae* strains used in this study.

Strain	Genotype	Source
BY4742	*MATα leu2Δ0 lys2Δ0 ura3Δ0 his3Δ1*	EUROSCARF
MK351	*MATα leu2Δ0 lys2Δ0 ura3Δ0 his3Δ1 spf1Δ::kanMX4*	EUROSCARF
MK355	*MATα leu2Δ0 lys2Δ0 ura3Δ0 his3Δ1 pde2Δ::kanMX4*	EUROSCARF
MK480	*MATα leu2Δ0 lys2Δ0 ura3Δ0 his3Δ1 pmr1Δ::kanMX4*	EUROSCARF
MK793	*MATα leu2Δ0 lys2Δ0 ura3Δ0 his3Δ1* [*TPK1* in YEplac195]	This study
MK794	*MATα leu2Δ0 lys2Δ0 ura3Δ0 his3Δ1* [*TPK2* in YEplac195]	This study
MK795	*MATα leu2Δ0 lys2Δ0 ura3Δ0 his3Δ1* [*TPK3* in YEplac195]	This study
MK973	*MATα leu2Δ0 lys2Δ0 ura3Δ0 his3Δ1* [*TPK1* in YEplac195] [*BCY1* in V056]	This study
MK974	*MATα leu2Δ0 lys2Δ0 ura3Δ0 his3Δ1* [*TPK2* in YEplac195] [*BCY1* in V056]	This study
MK975	*MATα leu2Δ0 lys2Δ0 ura3Δ0 his3Δ1* [*TPK3* in YEplac195] [*BCY1* in V056]	This study
MK979	*MATα leu2Δ0 lys2Δ0 ura3Δ0 his3Δ1 pde2Δ::kanMX4* [*TPK1* in YEplac195]	This study
MK980	*MATα leu2Δ0 lys2Δ0 ura3Δ0 his3Δ1 pde2Δ::kanMX4* [*TPK2* in YEplac195]	This study
MK981	*MATα leu2Δ0 lys2Δ0 ura3Δ0 his3Δ1 pde2Δ::kanMX4* [*TPK3* in YEplac195]	This study
MK940	*MATα leu2Δ0 lys2Δ0 ura3Δ0 his3Δ1 pde2Δ::kanMX4* [*TPK1* in YEplac195] [*BCY1* in V056]	This study
MK941	*MATα leu2Δ0 lys2Δ0 ura3Δ0 his3Δ1 pde2Δ::kanMX4* [*TPK2* in YEplac195] [*BCY1* in V056]	This study
MK942	*MATα leu2Δ0 lys2Δ0 ura3Δ0 his3Δ1 pde2Δ::kanMX4* [*TPK3* in YEplac195] [*BCY1* in V056]	This study
MK342	*MATα leu2Δ0 lys2Δ0 ura3Δ0 his3Δ1* [YEplac195]	This study
MK959	*MATα leu2Δ0 lys2Δ0 ura3Δ0 his3Δ1* [YEplac195] [V056]	This study
MK325	*MATα leu2Δ0 lys2Δ0 ura3Δ0 his3Δ1 pde2Δ::kanMX4* [YEplac195]	This study
MK960	*MATα leu2Δ0 lys2Δ0 ura3Δ0 his3Δ1 pde2Δ::kanMX4* [YEplac195] [V056]	This study
MK450	*MATα leu2Δ0 lys2Δ0 ura3Δ0 his3Δ1 tpk1Δ::kanMX4*	EUROSCARF
MK451	*MATα leu2Δ0 lys2Δ0 ura3Δ0 his3Δ1 tpk2Δ::kanMX4*	EUROSCARF
MK452	*MATα leu2Δ0 lys2Δ0 ura3Δ0 his3Δ1 tpk3Δ::kanMX4*	EUROSCARF
MK871	*MATα leu2Δ0 lys2Δ0 ura3Δ0 his3Δ1 tpk1Δ::kanMX4 pde2::URA3*	This study
MK872	*MATα leu2Δ0 lys2Δ0 ura3Δ0 his3Δ1 tpk2Δ::kanMX4 pde2::URA3*	This study
MK873	*MATα leu2Δ0 lys2Δ0 ura3Δ0 his3Δ1 tpk3Δ::kanMX4 pde2::URA3*	This study
MK870	*MATα leu2Δ0 lys2Δ0 ura3Δ0 his3Δ1 pde2::URA3 spf1Δ::kanMX4*	This study
MK1341	*MATα leu2Δ0 lys2Δ0 ura3Δ0 his3Δ1 pde2::URA3 pmr1Δ::kanMX4*	This study
MK1342	*MATα leu2Δ0 lys2Δ0 ura3Δ0 his3Δ1 pmr1Δ::kanMX4 spf1Δ::HIS3MX6*	This study
MK1343	*MATα leu2Δ0 lys2Δ0 ura3Δ0 his3Δ1 pde2::URA3 pmr1ΔkanMX4 spf1Δ::HIS3MX6*	This study
W303-1A	*MATa ade2-1 his3-11,15 lue2-3,112 trp1-1 ura3-1 can1-100*	Dr. Mazon
MB13	*MATa ade2-1 his3-11,15 lue2-3,112 trp1-1 ura3-1 can1-100 tpk1::LUE2 tpk3::URA3*	Dr. Mazon
MB23	*MATa ade2-1 his3-11,15 lue2-3,112 trp1-1 ura3-1 can1-100 tpk2::HIS3 tpk3::URA3*	Dr. Mazon
MB12	*MATa ade2-1 his3-11,15 lue2-3,112 trp1-1 ura3-1 can1-100 tpk1::LUE2 tpk2::HIS3*	Dr. Mazon
MK943	*MATa ade2-1 his3-11,15 lue2-3,112 trp1-1 ura3-1 can1-100 pde2Δ::kanMX6*	Dr. Mazon
MK944	*MATa ade2-1 his3-11,15 lue2-3,112 trp1-1 ura3-1 can1-100 pde2Δ::kanMX6 tpk1::LUE2 tpk3::URA3*	This study
MK945	*MATa ade2-1 his3-11,15 lue2-3,112 trp1-1 ura3-1 can1-100 pde2Δ::kanMX6 tpk2::HIS3 tpk3::URA3*	This study
MK946	*MATa ade2-1 his3-11,15 lue2-3,112 trp1-1 ura3-1 can1-100 pde2Δ::kanMX6 tpk1::LUE2 tpk2::HIS3*	This study
MYL40α	*MATα ura3-52*	Dr. Heitman
XPY1α	*MATα ura3-52 bcy1::kanMX2*	Dr. Heitman

873), and (viii) *pde2* lacking two of *TPK1*, *TPK2*, *TPK3* (MK944, 945, 946). However, transformability of these strains exhibited no obvious difference from that of *pde2*.

Then, we decided to examine the transformation conditions giving "highly efficient" transformability by using mutants with the high transformability, i.e., *pde2*, *spf1*, and *pmr1*. LiAc/SS-DNA/PEG method gave higher transformability of the mutants than natural transformation method (Fig. 1).

The high transformability mutants showed higher transformability than WT. We have recently found that (i) plasmid DNA is bound to the region around cell surface during transformation process and (ii) washing the cells, on which plasmid DNA is bound, results in removal of the bound plasmid DNA from the cell and in decrease of transformability (Tuan *et al.*, unpublished data). The yeast cells are washed just before spreading on the selective solid medium in conventional methods.[1,3] Based on our recent finding, transformability obtained by the conventional method was compared with that obtained without washing, i.e. that obtained by spreading the cell directly (by the direct method) (Fig. 2).

Transformability of the cells tested (WT, *pde2*, *spf1*, *pmr1*) was enhanced by the direct method. Accordingly, before spreading the cells on the selective solid medium to obtain suitable number of colonies for counting, dilution of the cells in 35% PEG4,000 gave higher transformability than that in usual buffer (e.g. TE) (data not shown).

We expected that the double mutants for *PDE2*, *SPF1*, and *PMR1* show higher transformability than single mutants and that the triple mutant exhibits the much higher transformability. Hence the mutants were constructed (MK870, MK1341, 1342, 1343; Table 1) and the transformability of the mutants was examined. However, transformability was not enhanced in the double and triple mutants.

Conclusion

In order to obtain the highly efficient transformability, we point out the followings. First, disruption of "one", but not two or three of *PDE2*, *SPF1*, and *PMR1* in the yeast cell would improve the transformability. Second, LiAc/SS-DNA/PEG method gives higher transformability of *pde2*, *spf1*, and *pmr1* than WT and those obtained by natural transformation method. Third, the direct method, in which the cells are not washed before spreading on the selective solid medium, gives higher transformability than that obtained by conventional method in which the cells are "washed". Finally, when the cells are required to be diluted before spreading the cells on the selective solid medium, the cells should be diluted in 35% PEG4,000, but not in usual buffer (e.g. TE).

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