Karyoduction in *Saccharomyces cerevisiae*

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We introduced the nuclei isolated from the respiration-sufficient killer strain of the yeast *Saccharomyces cerevisiae* into the yeast protoplasts prepared from the respiration-deficient non-killer strain with the aid of polyethylene glycol. The resulting karyoductants were respiration-deficient non-killers. Nuclear staining with ethidium bromide or DAPI and tetrad analysis of the karyoductants presented evidence that the nuclei introduced into protoplasts were fused to the resident nuclei, leading to stable diploids. This technique termed karyoduction will be useful in the study of nucleo–cytoplasmic relationship in yeast and other organisms.

Yeast *Fusion* *Karyoduction*

1. INTRODUCTION

It has been reported that the transfer of organella, such as spinach chloroplasts [1] and yeast mitochondria [2] into protoplasts was effectively induced by the presence of polyethylene glycol (PEG). Here, we have introduced nuclei isolated from the yeast *Saccharomyces cerevisiae* into yeast protoplasts with the aid of PEG. This technique termed 'karyoduction' will be useful in the study of nucleo–cytoplasmic relationship in yeast and other organisms.

2. MATERIALS AND METHODS

2.1. Saccharomyces cerevisiae *strains*

Nuclei were isolated from the cells of strain A364A (*a ade1 ade2 lys2 tyr1 his7 ura1* [ϱ^+ KIL-k]). Recipient protoplasts were prepared from the cells of strain YIY6A-1-1 (*a leu2–3,112 his7 ura1* [α° KIL-0]). YTM1 (*α/α ade1/ade1 trp1/trp1 his2/his2*) was used as a mating partner in tetrad analysis.

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2.2. *Preparation of protoplasts*

Protoplasts were prepared as in [3]. In brief, cells were cultured in YEPD medium (1% yeast extract, 2% peptone and 2% dextrose) to log phase, harvested and washed once with deionized water. The cells were treated with 2-mercaptoethanol (final conc. 2.5%) and then converted to protoplasts with a lytic enzyme Zymolyase 60000 (Kirin Brewery, final conc. 0.2 mg/ml). The protoplasts were suspended in a stabilizing medium containing 1.2 M sorbitol, 7% Ficoll (Pharmacia Fine Chemicals), 10% glycerol, 20 mM *N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid (HEPES)–KOH (pH 7.5) and 1 mM $MgCl_2$, and kept on ice until use. 10–25% of the protoplasts from A364A and 2–6% of those from YIY6A-1-1 were viable; able to regenerate in YEPD medium containing 1.2 M sorbitol.

2.3. *Isolation of nuclei*

Nuclei were isolated as in [4]. The protoplasts of A364A in the stabilizing medium were disrupted by French press (Aminco) at 3000 lb/in^2, followed by homogenization with a glass homogenizer (Wheaton). The homogenate was centrifuged at 3000 × *g* for 5 min to remove the undisrupted pro-

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toplasts as precipitate. The supernatant fraction was centrifuged at 15000 × *g* for 20 min to collect nuclei as pellets. The pellets (crude nuclei) were resuspended in the stabilizing medium, if needed, followed by the successive centrifugation as above (3000 × *g* and 15000 × *g*). The crude nuclei in the stabilizing medium were then mixed with an equal volume of 30% Ficoll [in 20 mM Hepes (pH 7.5) and 1 mM $MgCl_2$] and centrifuged at 30000 × *g* for 30 min. The precipitated nuclei were resuspended in the stabilizing medium, and kept on ice until use. The isolated nuclei were highly intact as organella, shown by electron microscopic observation and biochemical studies in [4].

2.4. *Transfer of nuclei into protoplasts*

Nuclei (4×10^8) from A364A and viable protoplasts ($4–9 \times 10^7$) from YIY6A-1-1 were mixed and pelleted by centrifugation at 10000 × *g* for 10 min. The pellet was suspended in 1 ml solution containing 20% PEG, 7% Ficoll, 50 mM $CaCl_2$ and 10 mM Tris–HCl (pH 7.5) followed by incubation at 25°C for 30 min. The nuclei–protoplasts aggregates were sedimented by centrifugation at 1500 × *g* for 10 min, washed with a solution containing 1.2 M sorbitol and 50 mM $CaCl_2$, and resuspended in 1 ml of the same solution. Aliquots (0.1 ml) of the suspension were added to 7 ml selection medium containing 3% agar (Difco), prewarmed at 45°C, and poured onto the selection medium solidified by 2% agar (selection plate). The plates were incubated at 28°C for 5–7 days. The selection medium was SD (synthetic dextrose) minimal medium [5] supplemented with 1.2 M sorbitol, histidine (20 mg/l) and uracil (20 mg/l).

2.5. *Genetic analysis*

Conditions for crosses and sporulation were standard [5]. Genetic techniques including tetrad analysis have been described [6]. Diagnoses of respiration ability (ϱ^+ and ϱ°) and killer phenotype (KIL-k and KIL-0) were carried out as in [2,7].

3. RESULTS AND DISCUSSION

Cells of A364A and YIY6A-1-1 could not revert to grow on the selection plates, since these strains carried more than 2 mutations on requirements in addition to *his7* and *ura1*. When protoplasts from A364A were fused with protoplasts from YIY6A-1-1 in the presence of PEG (by transferring nuclei into protoplasts), fusants appeared on the selection plates at 10^{-4}/viable protoplast. All of 50 fusants, randomly selected, were respiration-sufficient killers. On the contrary, when the mixture of nuclei from A364A and protoplasts from YIY6A-1-1 was treated with PEG and plated, fusants appeared on the selection plates at 10^{-6}/viable protoplast. About 90% of the fusants were respiration-deficient non-killers. These fusants were considered to be transformants made by the transfer of nuclei into the recipient protoplasts, and were termed 'karyoductants'. The remaining fusants were respiration-sufficient killers. It seemed likely that these fusants were resulted from the fusion between protoplasts of YIY6A-1-1 and protoplasts of A364A contaminating in the nuclei preparation.

Karyoductants showed mating type *a* and required histidine and uracil for growth. The mutations on requirements were allelic to *his7* and *ura1*. Most of the karyoductants were diploids estimated in terms of cell size and DNA content/cell [2]. Each of 6 karyoductants, randomly selected, was crossed with the standard diploid strain YTM1, and the tetraploids were subjected to tetrad analysis after sporulation. Segregation patterns of auxotrophic mutations in 1 of 6 crosses were shown in table 1.

Table 1

Segregation of auxotrophic mutations[a] in asci of the hybrid constructed by crossing a karyoductant with a standard diploid YTM1

Segregation in asci	Auxotrophic mutations				
	lys2	*tyr1*	*ura1*	*leu2*	*trp1*
$4^+:0^-$	8	10	7	12	9
$3^+:1^-$	4	2	3	0	0
$2^+:2^-$	0	0	2	0	3

[a] Diagnoses of auxotrophic mutations and their allelisms were carried out by omission and complementation tests [5]

Segregation ratio in the markers mating type (*a* or α), *ade1*, *ade2*, *his2* and *his7* could not be obtained, since prototrophic or non-mater segregants appeared in most of the tetrads

The same results were obtained from the other 5 crosses. Segregation patterns of the mutations *lys2*, *tyr1*, *leu2* and *trp1* indicated that the karyoductant was disomic in at least 3 chromosomes II, III and IV, carrying the genotypes *LYS2/lys2*, *TYR1/tyr1*, *LEU2/leu2* and *TRP1/TRP1*. In some of tetrads, mating type segregated as follows: $a:\alpha$: non-matter = 1:1:2 or 0:0:4. These segregations are characteristic in the tetraploid carrying the genotype of mating type $a/a/\alpha/\alpha$, indicating that the genotype of mating type of the karyoductant was *a/a*. *ade2* mutation was detected in the tetrad segregants. All of the results were consistent with the conclusion that at least 4 chromosomes II (*lys2 tyr1*), III (*a LEU2*), IV (*TRP1*) and XV (*ade2*) in the donor nuclei were simultaneously introduced into the recipient protoplasts. It may be suggested that intact nuclear organella were directly introduced or transferred into the recipient protoplasts, since all of 6 karyoductants, randomly selected, contained the same at least 4 chromosomes from the donor nuclei, although the recipient protoplasts could be transformed to grow on the selection plates if they received only chromosome III or *LEU2* gene on it from the donor nuclei. The donor nuclei, thus introduced into the recipient protoplasts, fused with the resident nuclei, since karyoductants contained single nucleus/cell when stained with ethidium bromide or 4′,6-diamidino-2-phenylindole (DAPI).

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