Transfer of isolated nuclei into protoplasts of *Aspergillus nidulans*

Cs. Vágvölgyi and L. Ferenczy

Department of Microbiology, Attila József University, P.O. Box 533, H-6701 Szeged (Hungary)
Received 1 July 1991; accepted 12 September 1991

Abstract. Nuclei were isolated from protoplasts of a haploid auxotrophic *Aspergillus nidulans* strain. Transformation of protoplasts prepared from a complementary haploid auxotrophic strain with these purified nuclei resulted in both heterokaryotic and diploid colonies. The nutritionally-complementing colonies appeared at a frequency of 5×10^{-7} to 10^{-8}.
Key words. Protoplast; nuclear transfer; heterokaryotic colonies; nutritional complementation; *Aspergillus nidulans.*

Gene transfer via fusion of fungal protoplasts is of special importance when there is no other way to construct new genetic combinations [1]. For the successful selection of fusion products, the partner strains must carry genetic markers, the presence of which may be disadvantageous in certain cases [2]. To eliminate this problem, isolated nuclei of a wild-type strain can be transplanted [3]. In the few successful studies of this kind, *Saccharomyces cerevisiae* [4–6] and *Trichoderma viride* [7] were the species used. In a series of model experiments, we tried to achieve the transfer of nuclei of *Aspergillus nidulans* via the method of polyethylene glycol(PEG)-induced protoplast fusion [8]. Haploid auxotrophic strains of *Aspergillus nidulans* (SZMC 1156: y, $PABA^-$, ade^-; SZMC 1157: y, $PABA^-$, lys^-) produced by UV-irradiation from *A. nidulans* R21 (y, $PABA^-$, ts6; Hebrew University, Jerusalem) were used. These stable mutants had already been successfully used in both intraspecific and interspecific protoplast fusion experiments [9]. The methods of protoplast formation and the preparation of nuclei were as described earlier [10]. For checking nuclear integrity following the PEG treatment, the isolated nuclei were stained either with mithramycin or with 4′,6-diamidino-2-phenylindole (DAPI) [11].

The process of nuclear transfer involved treating the nuclei with PEG. The procedure was as follows. A nuclear fraction prepared from strain SZMC 1157 was diluted tenfold with nucleus isolation buffer (7.5% Ficoll, 10% glycerol, 30 mM N-2-hydroxyethylpiperazine-N′-2-ethanesulphonic acid (HEPES)-KOH, pH 7.0, 2 mM $MgCl_2$, 1.5 mM phenylmethylsulphonyl fluoride (PMSF), and 1 M sorbitol) and centrifuged at 14,500 g for 25 min. The resulting pellet was suspended in 100 µl of the same buffer and mixed with freshly prepared protoplasts of strain SZMC 1156, at a ratio of 1 nucleus per 5 protoplasts. Following centrifugation at 14,500 g for 20 min, the supernatant was removed and 1 ml of fusion solution (30% PEG 4000, 100 mM $CaCl_2$, and 5% DMSO) was added. After standing on ice for 5 min, the fusion mixture was incubated at 20 °C for 15 min. Aliquots were mixed with osmotically stabilized minimal agar medium at 42 °C and poured as an overlay onto osmotically stabilized nutritionally supplemented minimal agar plates, then incubated at 35 °C for 7 days.

Regenerating mycelia could be detected microscopically on osmotically stabilized minimal agar plates after one day (fig. 1). After a longer incubation period, these mycelia developed into slow-growing colonies with yellow

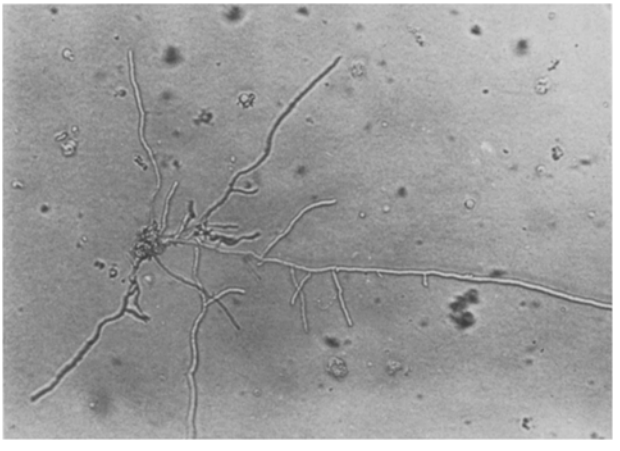

Figure 1. Regenerating colony after karyoduction based on protoplast fusion.

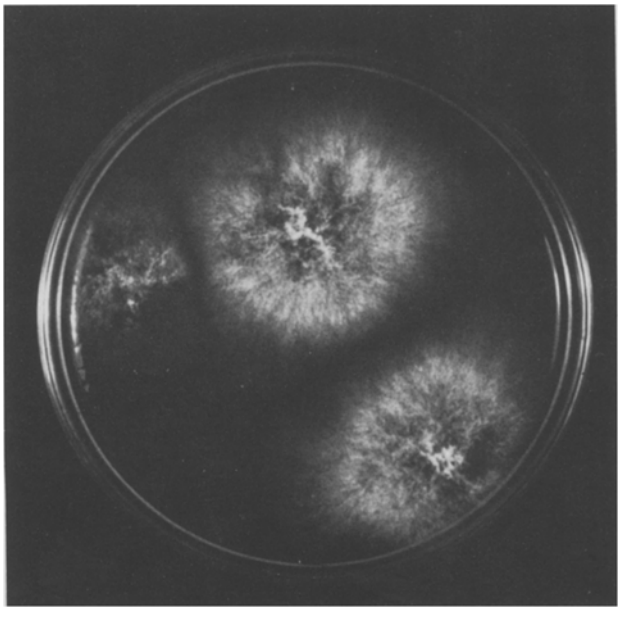

Figure 2. Heterokaryotic colonies after transfer of isolated nuclei.

Frequencies of complementation of recipient protoplasts by isolated *Aspergillus nidulans* nuclei in three separate experiments.

Experiment no.	1	2	3
Number of recipient protoplasts	10^9	10^9	$3 \cdot 10^9$
Frequency of complementation due to karyoduction	$5.0 \cdot 10^{-7}$	$5.3 \cdot 10^{-7}$	$8.1 \cdot 10^{-7}$
Number of colonies from karyoduction	5	6	11

conidia and irregular growth (fig. 2). They exhibited a typical heterokaryotic morphology, the character of which was confirmed by biochemical and genetic analyses. The frequency of diploid formation from these heterokaryons was similar to the frequency seen with the products of protoplast fusion.

With a PEG-Ca^{2+} system for induction, nuclear uptake may have occurred as a result of an ordinary protoplast fusion. In such a case, entrapment of nuclei by the aggregates of the recipient protoplasts could provide a way for nuclear uptake to occur without damage to the isolated nuclei. Experiments using fluorescent-stained nuclei for karyoduction also indicated that this was the mechanism. Following PEG treatment, brightly stained nuclei with good morphological characteristics could be observed inside the aggregates of the recipient protoplasts.

It may be interesting to note that recent observations indicated that bacterial cells could be taken up into *Aspergillus* protoplasts by using PEG-mediated protoplast fusion [12].

The crucial condition for karyoduction proved to be the quality of the nuclear preparation. Isolated nuclei had to be free of cytoplasmic contamination, which caused nuclear clumping and especially of viable protoplasts. As a control, in each experiment the same amount of nuclear fraction as had been used for karyoduction was spread in a top layer onto osmotically stabilized complete medium to check for the presence of protoplasts able to regenerate. In all cases, these control experiments indicated that the complemented colonies had indeed originated from the transfer of isolated nuclei.

The frequency of nuclear uptake was low, but reproducible (table). The maximum complementation achieved was one nutritionally-complementing colony from 5×10^7 isolated nuclei.

Acknowledgments. The authors are grateful to Miss Erzsébet Petrina and Mr Gergely L. Nagy for technical assistance.

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0014-4754/92/030271-02$1.50 + 0.20/0

In vitro protein synthesis and α amylase activity in F cells from hepatopancreas of *Palaemon serratus* (Crustacea; Decapoda)

J. Y. Toullec, M. Chikhi and A. van Wormhoudt

Laboratoire de Biologie Marine du Collège de France, F-29110 Concarneau (France)
Received 17 June 1991; accepted 2 September 1991

Abstract. In crustaceans, all the steps in the assimilation of food take place in the hepatopancreas. To facilitate the study of this organ, a method for the dissociation of cell types was developed. The hepatopancreas of the prawn *Palaemon serratus* was mechanically dissociated and the cells separated by Percoll density-gradient centrifugation. The E and R cells had similar densities of around 1.05 g/ml. The F cells were separated into two distinct fractions with densities of 1.075 and 1.082 g/ml. The B cells sedimented at a density of 1.12 g/ml. The ratio between the two populations of F cells was found to vary during the intermolt cycle while B cells disappeared after the molt. When the density gradient fractions were incubated with ^{3}H-leucine, incorporation was highest in the F cell fractions. Measurements of α-amylase activity, indicated that the two populations of F cells may be derived from the same cell type.

Key words. Crustacean; prawn; hepatopancreas; cell suspension; protein synthesis; α-amylase activity.