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ELECTROFUSION OF ORIENTED SCHIZOSACCHAROMYCES POMBE CELLS THROUGH APICAL PROTOPLAST-PROTUBERANCES

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SUMMARY: The electrofusion of oriented Schizosaccharomyces pombe cells through apical protoplast-protuberances was demonstrated. The protuberances arose after an exposure of early-exponential phase cells to digestive enzymes from hepatopancreas of Helix pomatia. The orientation of cylindric cells within pearl chains was produced by the application of inhomogenous alternating electric fields.

In addition to the genetic engineering techniques, induced protoplast fusion in yeasts can be used to circumvent restrictions imposed by natural mating systems. The technique of fusion permits production of hybrid cells, which are of great importance not only for the understanding of fundamental questions of gene expression and cell cycle regulation, but also for practical applications in industry.

Fusion of protoplasts can be achieved with the aid of chemicals (such as polyethylene glycol) or by the application of a sequence of direct current pulses of high intensity ($kV.cm^{-1}$ range) (1). With the electrofusion technique close membrane contact is established between two protoplasts by the application of a weak inhomogenous alternating field ($kV.cm^{-1}$, MHz range). The field brings about the generation of dipoles within the protoplasts which results in movement of protoplasts into the region of highest field intensity, and in the formation of pearl chains. Cell fusion is induced by a short electrical pulse which causes reversible breakdown of the plasma membranes at the sites of contact of the collected protoplasts. The chains are formed in parallel with field lines, however, the orientation of individual

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spherical protoplasts is very likely random. Therefore, in contrary to mating, the fusing pairs of protoplasts have not any specific orientation.

The results in this communication demonstrate that the electrofusion technique provides the possibility also for fusion of oriented cells through apical protoplast - protuberances. Cylindric cells of Schizosaccharomyces pombe were used in our experiments because it was observed that the orientation with their longest axis parallel to the field lines is produced for relatively broad range of frequencies and low conductivities of the external media (2), and the cell wall at the poles is in a readiness for vegetative growth, lysis and formation of conjugation protuberances (3,4,5).

MATERIAL AND METHODS

Yeast strain: Schizosaccharomyces pombe HAL3 was kindly supplied by M.Sipicki (L.K.University, Debrecen, Hungary).

Preparation of cells with protoplast protuberances: The yeasts were grown in YEPG medium (2 % glucose, 2 % pepton, 1 % yeast extract) with shaking at 30°C. During the early-log phase, the cell suspension containing 5.10^8 cells was withdrawn and centrifuged. The pellet was washed twice with distilled water and then once with 1% 2-mercaptoethanol. The cells were finally resuspended in 5 ml of 1.5 % solution of lytic enzymes (crude preparation from digestive tract of Helix pomatia) in 0.8 M KCl to which 5 µl of 2-mercaptoethanol was added. The suspension was incubated about 120 min at 28°C and shaken gently during this interval. Only a small area of the cell wall at one pole in almost each cell was lysed under these conditions. The resulting cells were then washed three times with 1.2 M sorbitol and the pellet was resuspended in 5 ml of the same solution. Aliquots containing 1.10^7 cells were withdrawn and centrifuged. The pellets were resuspended in selected sorbitol solutions (in the range from 0.5 M to 1.2 M) in order to release a small part of each protoplast from its cell wall - ghost. This was the way how to obtain local protoplasts of different sizes.

Fusion conditions: The electrofusion was studied in a setup consisting of two parallel platinum electrodes (0,2 mm apart) sealed directly onto the surface of microscope slide. A small drop (50 µl) of the suspension of cells with protoplast-protuberances in 0.8 M sorbitol to be examined was placed between electrodes and covered with cover slip. The chamber was supplied electrically with a combined frequency and pulse generator EF-1 (from Kothera – Plasek, UK, Prague) which provides the peak to peak voltage from 0 to 90 V and frequencies from 5 KHz to 5 MHz for dielectrophoresis, and square field pulses (60 to 300 V) from 1 µs to 1.10^4 µs duration to induce fusion experiments. The protoplast suspension was adjusted in all experiments to about 10^7 protoplast.ml^{-1}. The field strength 500 V.cm^{-1}, the frequency 1,5 MHz and the collection time about 2 min were used. Square field pulses (5 kV.cm^{-1}, 10 µs) were applied in order to induce fusion of local protoplasts. The behavior of the cells in the

electrical field was monitored using television set. The television screen was photographed in order to obtain the illustrations of our results presented in this paper.

RESULTS

The cells with protoplast protuberances mostly at one pole were prepared as described in "Methods". The effect of hypotonic conditions on the sizes of protuberances is demonstrated in Fig.1. As can be seen, the volumes of protuberances increase with decreasing concentration of sorbitol in the cell suspensions.

Our next step was to compare the behavior of asymmetric cells with local protoplasts of different sizes under alternating nonuniform electric fields. Under conditions chosen (collection time less than 2 min, voltage 0,25-2 $kV.cm^{-1}$, concentration of sorbitol from 0,5 to 1 M and cell concentration about 1.10^7 cells ml^{-1}) there were no stirring problems. An almost identical response of cells with and without protuberances was produced: (a) All cells oriented parallel with their longest axis to the field lines, (b) the formation of chains of variable length occurred. Three configurations of adjacent cells with apical protuberances were observed within the chains (Fig.2): (a) Head to head, (b) head to tail and (c) tail to tail. Only the first configuration mentioned above is convenient from the point of view of subsequent fusion. It should be emphasized that some protoplasts, namely those which were produced at low sorbitol concentrations (0,5 and 0,6 M) were completely released from ghosts during manipulations with the cell suspensions, and in the

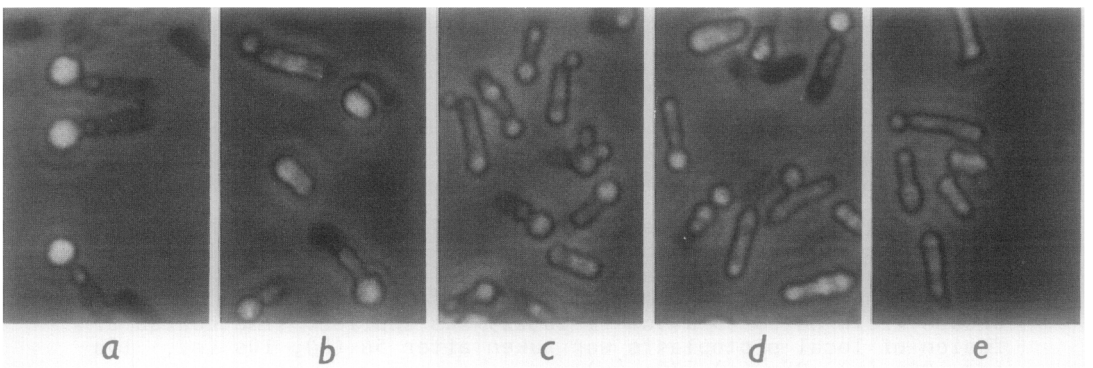

Fig.1. Effects of various sorbitol concentrations on sizes of protoplast protuberances. (a) 0.5 M, (b) 0.6 M, (c) 0.7 M, (d) 0.8 M and (e) 1.0 M.

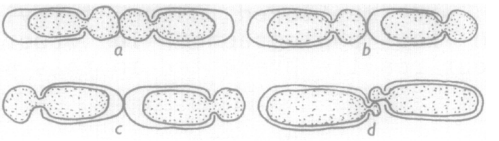

Fig.2. Configurations of oriented cells with apical protoplast-protuberances. (a)"Head to head", (b)"head to tail", (c)"tail to tail" and (d)"side by side-head to head".

experiment with the 1M sorbitol side by side-head to head configuration of local protoplasts was observed (Fig.2d). For these reasons 0.8 M sorbitol was selected for the following electrofusion experiments.

When close membrane contacts of protuberances were achieved by chain formation under the conditions described in "Methods", the fusion process was triggered by injecting direct current pulse (5 kV.cm $^{-1}$, 10 μs). 30 s after the application of this pulse the amplitude of alternating field was gradually reduced to zero (during about 10 μs). Among many local protoplast fusions observed in parallel experiments one was selected in order to illustrate this event (Fig.3). By rising the strength of the alternating field 30 s after the application of pulse, the pairs

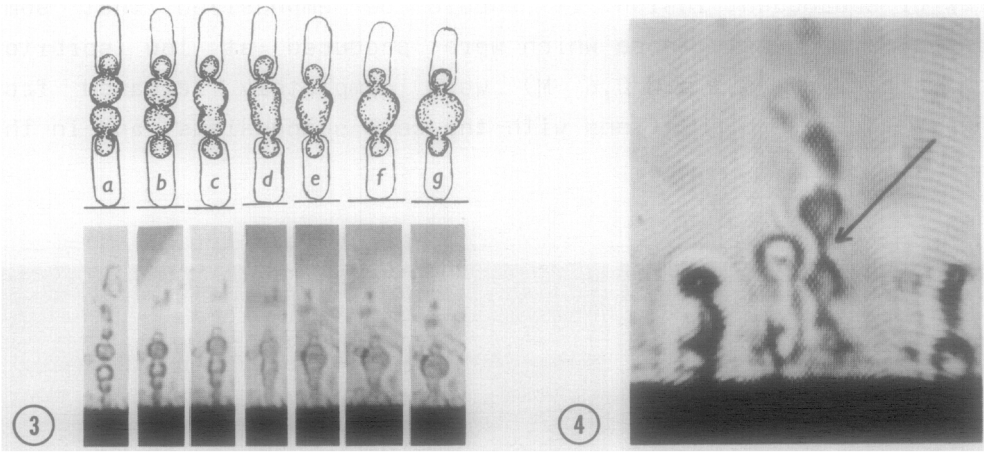

Fig.3. Sequential micrographs of electrofusion of S.pombe cells through protoplast-protuberances. (a) The cells with protuberances in 0.8 M sorbitol were collected by means of nonuniform alternating electric field (1.5 MHz, 500 V.cm^{-1}). Square field pulse (5 kV.cm^{-1}, 10 μs) was applied. Time course of fusion of local protoplasts was taken after 5s (b), 10s (c), 15s (d), 20s (e), 25s (f), 30s (g).

Fig.4. The stretching of the cell-cell bridge between the protuberances of protoplasts under nonuniform alternating field (1.5 MHz, 2 kV.cm^{-1}).

of local protoplasts with a continuous connection established can be distinguished from others because cell-cell bridges formed under these conditions can undergo appreciable stretching (Fig.4). If the field was removed the tubes shrank, but they can be stretched again by reincreasing the amplitude of alternating field.

DISCUSSION

The method of induced protoplast fusion is of ever increasing importance for the construction of hybrid cells in yeast because it allows cell hybridization regardless of mating type, ploidy and species affiliation of the cells.

Owing to high fusion efficiency electrofusion is becoming the most attractive technique among the fusion methods in studying the transport of genetic material and its expression. The yield of hybrids obtained by intraspecific fusion of S.cerevisiae protoplasts (10^{-4} range), however, does not stand comparison with the efficiency of the mating process (10^{-1} range) in which cells of opposite mating types synchronized by pheromones are taking part. Some increase in hybridization frequency after fusion (about 10 fold) was observed when cells of a-mating type (S.cerevisiae) were synchronized by α-factor treatment before protoplasting (6).

The further step in imitation of the mating process is fusion of properly oriented protoplasts. The results summarized in this paper demonstrate that the dielectrophoresis is the technique which can be used for this purpose when combined with electrofusion of cylindric cells with apical protoplast-protuberances. The possibility of production of these protuberances is based on the following assumptions: (a) There are conditions under which the lysis of cell walls occurs preferentially inside of zone of growth which is more sensitive to lytic enzymes than the rest of the wall. (b) There are growth conditions under which most of the cells in culture can form protoplast protuberances localized at one pole. Both of these assumptions were fulfilled in our experiments with S.pombe. In addition, it was shown that the sizes of protoplast-protuberances which are exposed to fusion can be approximately settled by selecting the proper concentration of sorbitol in external medium.

Our results on the behavior of cells with protuberances in alternating field are in agreement with observations described by others (2). It should be emphasized, that after fusion of local protoplasts the hybrid protoplast can be released from both parental ghosts by shaking the suspension and/or by decreasing the sorbitol concentration. Another advantage of local protoplast -fusion is that only two adjacent protoplasts (and never more) can fuse.

We believe that oriented fusion of cells with apical protoplast-protuberances might also be useful in genetic manipulation of species other than S.pombe.

REFERENCES

1. Zimmermann,U. (1982) Biochim.Biophys.Acta 694, 227-277
2. Iglesias,F.J.,Lopez,M.C.,Santamaria,C., and Dominguez,A. (1985) Biophys.J. 48, 721-726
3. Johnson,B.F. 1968) J.Bacteriol. 95, 1169 - 1172
4. Streiblová,E., Wolf,A. (1972) Z.Allg.Mikrobiol. 12, 673-684
5. Streiblová,E., Wolf,A. (1975) Can.J.Microbiol. 16, 827-831
6. Curran,B.P.G., and Carter,B.L.A. (1986) Curr.Genet. 10, 943-945