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DIRECT EXPERIMENTAL EVIDENCE OF THE VECTORIAL CHARACTER OF THE INTERACTION BETWEEN ELECTRIC PULSES AND CELLS IN CELL ELECTROFUSION

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Fusion is induced between cultured mammalian cells growing on culture flasks by electric pulses. The yield of fusion and the level of polynucleation are dependent on the relative direction of the applied pulses. The effect of the pulse on the cells is thus proved to be vectorial and not scalar.

Cultured mammalian cell fusion is the most effective way to transfer genetic information between eucaryotic species. In the 60's and 70's fusing properties of virus and of polyethyleneglycol [1] were used to induce those fusions. Due to the empiric character of these experiments the molecular mechanism of the processes remained unknown and the yield of viable hybrids was always very low may be as a consequence of the presence of exogeneous reagents known to be lethal for the cells [2]. The new 'electrofusion' method [3–6] appears very promising for in some cases [3,6] no exogeneous agents (polyethyleneglycol, pronase) are added. Hybridization may then be obtained with a high efficiency [4,7]. The technique consists in applying high voltage pulses with a short duration to a cell culture. Up to now no direct experimental evidence of the molecular mechanism responsible in inducing the fusion has been obtained. More important the very trigger of the process remained unknown and is the object of speculations [8]: the electric field-induced membrane potential appearing as a good candidate.

The 'electrofusion' method we are using in our laboratory [6,7,9] is original because the electric pulse is applied on cells growing in monolayers in culture dishes (Nunc, Denmark). This method is described in great detail in Ref. 9. The cells are Chinese hamsters ovaries (CHO). The major point is that the electric field is homogeneous being generated by two thin parallel stainless steel electrodes (distance: 6 mm, length: 30 mm). The field lines are thus parallel. As the cells are attached to the dish, the direction of the field lines relative to one particular cell can be modified simply by a rotation of the culture flask. In that case all the cells rotated but the field lines, being associated to the electrodes, keep the same direction. Such a rotation is easy and fast to operate with our experimental set-up. In the present work, repetitive pulses (1.5 kV/cm, 100 μs duration, 1 s delay) were applied to the cell culture either in parallel or in crossed directions. The extend of fusion was monitored either by computing the fusion index R

$$R = \frac{\sum_{n=2}^{\infty} nC_n}{\sum_{n=1}^{\infty} nC_n}$$

(expressed in % where C_n is the number of cells

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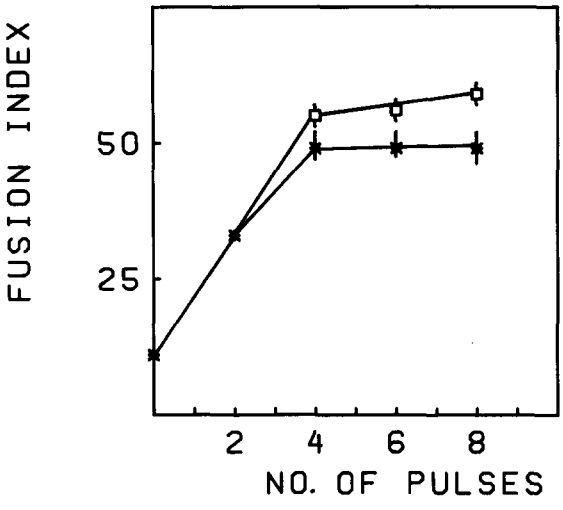

Fig. 1. Changes in the fusion index R as a function of the number of applied repetitive pulses. The direction of the associated electric fields is either parallel (*) or crossed (□). In the later case the culture flask was rotated after half of the pulses were applied.

containing n nuclei), or by plotting the polynucleation histogram as a function of the number of nuclei per cell.

As shown in Fig. 1, the fusion index R increased if the repetitive pulses are applied in crossed directions. As already described [9], this index reached a maximum value after only three or four pulses applied in the same direction. It can be seen on Fig. 1 that the fusion index R is still increasing if eight pulses are applied along crossed directions (4×4). Another difference between fusions obtained by applying repetitive pulses which are either parallel or perpendicular is the change in the polynucleation histogram. Increasing the number of pulses under non saturating conditions (i.e. less than four if they are parallel) (Fig. 2) induces a larger number of multifusion events (comparison between △ and * on the curve). Such an effect is larger if the pulses are applied under crossed directions (* and □). This last observation indicates that the increase in the fusion index R which is shown in Fig. 1 is in fact due to the occurrence of a larger number of multifusion events giving polynucleated cells with a great number of nuclei.

The major conclusion of this study is the demonstration of the vectorial character of the electrofusion. The extend of the fusion may be increased by altering the position of the cells as referred to the electrodes. This means that the trigger is vectorial in nature and that any cause which is only scalar can be excluded. This gives a definitive evidence that Joule heating can be rejected; fur-

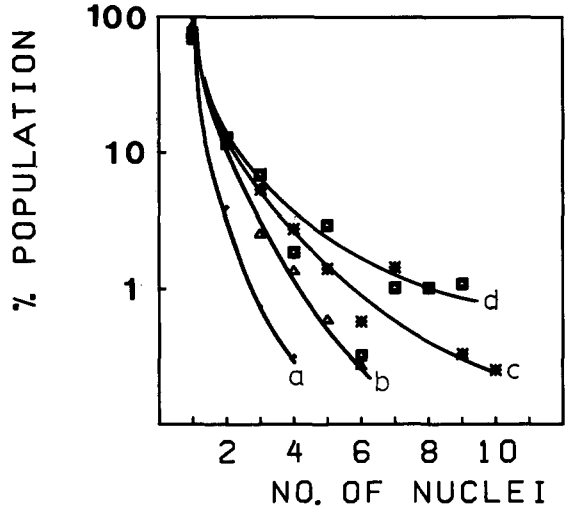

Fig. 2. Histogram of polynucleation under different experimental conditions: a, control (a spontaneous polynucleation is present) (●); b, two parallel pulses have been applied (△); c, four parallel pulses have been applied (*); d, four crossed pulses (2×2) have been applied (□).

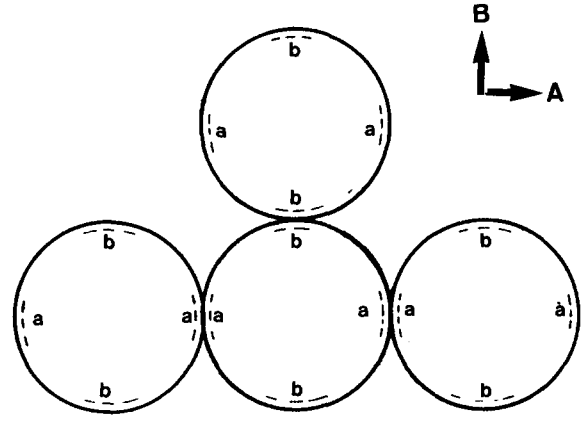

Fig. 3. Schematic picture of the fusion experiment. The cells are supposed to be circular. The field is either in direction A or in direction B. If the field is parallel to A (B) the induced potential is the largest in areas 'a' ('b'). Fusion occurs in areas 'a' ('b'). On the picture the fusion index R would be 0.75 if the field is parallel to A, 0.5 if it is parallel to B and to 1 if crossed pulses are applied. In the last condition, a cell with four nuclei is observed.

thermore, taking into account the power of the generator and the volume which is heated, the temperature increase is computed to be 6 deg. C (with the additional restricting assumption that no heat exchange with external medium occurs between the pulses). As the initial temperature was 20 °C, the sample never reached the temperature 37 °C which is present in the culture oven. Any electrochemical effect may be rejected because it is either homogeneous in the bulk or located close to the electrodes and we observed that on the contrary the fusion effect is homogeneous in the pulsed sample.

Electric field linked effects appeared as good candidates as was already speculated but never experimentally demonstrated [5,8,9]. Nevertheless ionization effects may be rejected on kinetics reasons. In our previous study [9] we observed that the electric pulses must be longer than 20 μs in order to induce the fusion; ionization, being a very fast process (in the nanosecond range), does not require such a time lag. As suggested in Refs. 5–9, an electroperforation triggered by the electric field induced membrane potential [10] can now be considered as the initial step in the fusion. This potential is known to be dependent on the angle θ between the radius where is the point M of interest and the direction of the field (as an approximation it is given by the relationship

$$V(M) = 1.5rE\cos\theta$$

if the cell is supposed to be a sphere). Perforation is induced by large values of $V(M)$ i.e. in the regions where θ is close either to 0 or π thus possessing the vectorial character demonstrated in the present work. The reaction process which is suggested is given on Fig. 3. When the field is applied in direction A, the induced potential is very large in areas 'a', where the perforation is going to occur triggering the fusion, nothing occurred in areas 'b' where the induced potential is zero ($\theta = \pi/2$). If now the field is applied in direction B (crossed direction), the perforation and the associated fusion happened in areas 'b' only. New fusions are added to the already multifused cells, the fusion index would increase and the number of cells with a large number of nuclei in them would be larger as observed in our experiments.

As a general conclusion this study provides the first direct experimental evidence of the vectorial character of the interaction between electric pulses and the associated cell fusion. Other methods of 'electrofusion' were irrelevant of this observation, the cells being either randomly distributed [3,4] or aligned along the field lines after pronase treatment [5,8]; in this last case the contact areas were specifically in the region where the induced potential was going to be the larger; no definitive conclusion on the vectoriality of the interaction electric pulse-cell was thus possible; pseudo lateral fusion was observed only under very high field intensities [11] which are very damaging for the cells. This study gives a strong experimental support to the hypothesis where the 'electrofusion' is triggered by the perforation associated to the electric field. What are the following events occurring thereafter is still an open question which is under investigation in our laboratory.

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