Short Communication Plant Tissue Culture Letters, **5**(2), 90-92 (1988)

Interkingdom Electrofusion between Tobacco Mesophyll Protoplasts and Cultured Butterfly Cells

Hiromichi MORIKAWA, Jun MITSUHASHI,* and Yasuyuki YAMADA

Research Center for Cell and Tissue Culture, Faculty of Agriculture, Kyoto University, Kyoto 606, Japan
* *Division of Forest Protection, Forestry and Forest Products Research Institute, Kukizaki, Ibaraki 305, Japan*

(Received December 11, 1987)
(Accepted February 10, 1988)

Interkingdom cell fusion between plant protoplasts and animal cells is interesting in relation to the fundamental studies of cell membrane properties and to the transfer of genes from animal to plant cells or vice versa.[1,2] Here we report an electrofusion between tobacco mesophyll protoplasts and cultured butterfly cells.

Tobacco mesophyll protoplasts were isolated from *Nicotiana tabacum* var. Samsun plants grown in a greenhouse according to Thanutong et al.[3] Cultured butterfly cells, NIAS-PX-58, have been obtained from pupal ovaries of the swallow tail butterfly (*Papilo xuthus*)[4] and maintained by a weekly subculture in Mitsuhashi and Maramorosch medium (MM medium).[5]

Seven days after subculture, cultured butterfly cells were harvested by centrifugation at $40\times g$ for 5 min and first washed three times with 0.5 M sorbitol containing 2.5 mM $MgCl_2$ (chamber medium). The washed animal cells were then mixed with plant protoplasts at a ratio of 10 : 1 or 1 : 1 in the same medium and centrifuged. The resulting supernatant was removed and deionized Dispase (Godo Susei Co., Tokyo) solution was added so that the final concentration of Dispase was 2,000 units/ml/5×10^6 cells dissolved in the chamber medium. About 40 μl of this mixture was placed in an electrofuion chamber ($2\times 2\times 10$ mm) equipped with two platinum plate electrodes.[6] After standing for about 5 min to allow the cells and protoplasts to sediment to the bottom, then a single exponentially-decaying fusion pulse (initial field intensity = 0.5 to 0.7 kV/cm; time constant = 0.1 to 0.2 msec) was applied to the mixture by the capacitor-discharge method.[6] No AC electric field was applied.

Figure 1 shows typical interkingdom electrofusion products. The cultured butterfly cells were morphologically heterogeneous[7] and appeared round- or longitudinally-shaped[6] (indicated by arrowheads in A). Upon electrofusion, variously shaped interkingdom fusion products were observed. Round-shaped or longitudinally-shaped (arrows in A and B) butterfly cells fused with the mesophyll protoplasts. Also, a clump of animal cells or a large animal cell body (arrows in C or D, respectively) fused with the mesophyll protoplasts. The latter cell body might have been formed by fusion of the animal cells. In round-shaped interkingdom heterokaryons, the animal cell part (arrow in E) generally tended to locate at the periphery or cytoplasm of the plant protoplasts.

Pretreatment of the butterfly cells with Dispase and the presence of this enzyme during electrofusion treatment were found to be essential for successful interkingdom electrofusion. This effect of the enzyme is attributable to the proteolytic activity of the enzyme and can be interpreted, that modification of the surface membrane of animal cells and plant protoplasts is necessary for mutual fusion. Cocking[1] also reported that the treatment with a protease made *Xenopus laevis* cells or carrot protoplasts more prone to fuse in response to high pH/high Ca^{2+}/high temperature treatment.

In the case of interkingdom electrofusion between barley mesophyll protoplasts and cultured mouse

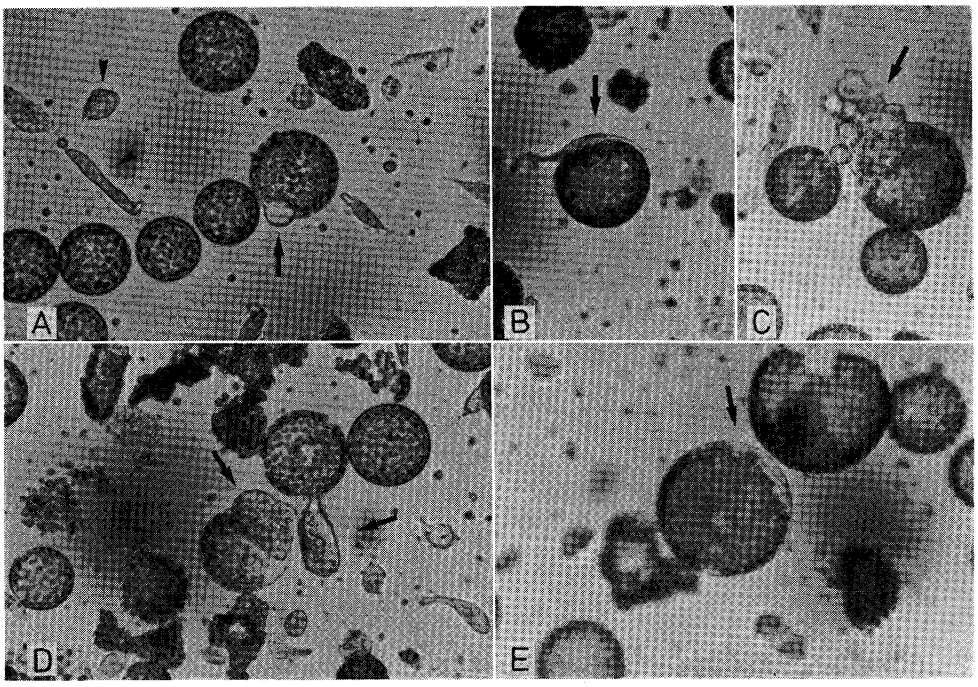

Fig. 1. Typical interkingdom electrofusion products between tobacco mesophyll protoplasts and cultured butterfly cells. The protoplast-animal cell mixture suspended in the chamber medium was electrofused with an exponentially-decaying pulse (0.7 kV/cm at time constant of 0.2 msec) in the presence of about 2,000 units/ml of Dispase. Pictures were taken about 30 min after pulsation. See text for details.

lymphoblast cells, L 5178 Y, pretreatment of the mouse cells with 1.3% Cellulase Onozuka R 10 and 1.3% Macerozyme R 10 for 90 min at 36℃ was found to be effective (Morikawa, Okada and Senda, unpublished results). This effect of these cell wall degrading enzyme preparations may also be attributable to proteolytic activities of these preparations.

So far we could not detect any synkaryon formation in these cases. Preliminary experiments indicated that modifications of culture conditions such as the osmotic potential or salt concentration of the medium are necessary for the culture of these heterokaryons.

We are indebted to Messrs. Y. Sugimoto and Y. Hayashi for their help in this study. This work was in part supported by Grants-in-Aid from the Ministry of Education, Science and Culture of Japan.

References

1) Cocking, E. C., 1984. In "Cell Fusion" (ed. by Evered, D., J. Whelan), p. 119-128, Pitman, London.
2) Davey, M. R., R. H. Clothier, A. R. Underwood, M. Ballis, E. C. Cocking, 1987. In "Abstracts of VI International Congress of Plant Tissue and Cell Culture" (Minnesota, 1986), p. 419.
3) Thanutong, P., I. Furusawa, M. Yamamoto. 1983. Theor. Appl. Genet., **66**: 209-215.
4) Mitsuhashi, J., 1973. Appl. Ent. Zool. **8**: 64-72.
5) Mitsuhashi, J., K. Maramorosch, 1964. Contrib. Boyce Thompson Inst., **22**: 435-460.
6) Morikawa, H., K. Sugino, Y. Hayashi, J. Takeda, M. Senda, A. Hirai, Y. Yamada, 1986. Biotechnology, **4**: 57-60.
7) Mitsuhashi, J., 1976. In "Invertebrate Tissue Culture, Research Applications" (ed. by Maramorosch, K.), p. 13-38, Academic Press, New York.

≪和文要約≫

タバコ葉肉プロトプラストとナミアゲハ培養細胞の電気融合

森川弘道，三橋　淳*，山田康之

京都大学農学部生物細胞生産制御実験センター
* 林業試験場保護部

タバコ (*Nicotiana tabacum* var. Samsun) 葉肉プロトプラストおよびナミアゲハ (*Papilo xuthus*) 培養細胞を用い，動植物間細胞電気融合について研究した．その結果，ディスパーゼ存在下で電気パルス (0.5〜0.7 kV/cm，時定数＝0.1〜0.2 msec の減衰パルス1回) を印加すると効率よく動植物ヘテロカリオンが形成されることがわかった．