Automatic Insertion of a Multifunctional Microelectrode Aimed at Automatic Microinjection

Soichiro SHIMODA[1] , Mikako SAITO[1,2] ,
Ikuko KOZONE[1,2] and Hideaki MATSUOKA[1,2]
[1]*Department of Biotechnology and Life Science, Tokyo University of Agriculture and Technology, Koganei, Tokyo 184-8588, Japan*
[2]*CREST, Japan Science and Technology Agency, Kawaguchi, Saitama 332-0012, Japan.*
bio-func@cc.tuat.ac.jp

Microinjection is potentially a useful method for the introduction of multiple genes and molecular probes in the same target cell at any prescribed time. Practically, however, microinjection is inconvenient because it requires a great deal of skill. In order to conquer this problem, we intend to develop an automatic high throughput microinjection machine. One of the key technologies for such a machine is an automatic insertion system for a microelectrode.

At first, we measured the membrane potential of the target cell with a multi-channel microelectrode (MME). The cells were rice protoplasts prepared from calli of *Oryza sativa* L. japonica cv. Nipponbare [1] and feeder free mouse ES cells culture [2]. The MME was driven by the control of the pulsing motor. When the MME tip was inserted into a target cell, the potential sharply decreased to become a steady level that was more negative. The steady level of rice cells ranged from –10 to –74 mV, while that of mouse ES cells ranged from –8 to –32 mV, respectively. The intracellular potentials of respective cells were so varied that it could hardly be used as an indicator of the microelectrode position.

In the second place, we intended to use the spatial derivative of the membrane potential. The control circuit was composed of a low-pass filter, a differentiation circuit, a peak amplifier, and a programmable controller for a stepping motor (Fig.1). The minimum step of manipulator was 0.25 □m and its driving speed was 6.25 □m/sec. Just when the MME passed through the cell membrane, a pulsing output signal was sent to the stepping motor drive controller in order to stop the MME movement immediately. Then lucifer yellow dye was introduced into a cell by electrophoresis. From the diffusion pattern of the lucifer yellow, it was determined whether the position of MME tip was in the cytosol, in the vacuole, or outside of the cell. Figure 2 shows the successful automatic insertion of a MME into a rice cell and a mouse ES cell. The success rate of automatic insertion was 30/37=81% and 71/108=66% for rice cells and mouse ES cells, respectively.

In the present study, fluorescent dye could be introduced into single-cells. The next target is the plasmid DNA. According to preliminary experiment, however, DNA could hardly been introduced in the absence of phospholipids. Moreover, DNA in the cells was not active. Based on the present results, we intend to investigate optimum conditions for the successful introduction of DNA without losing its activity.

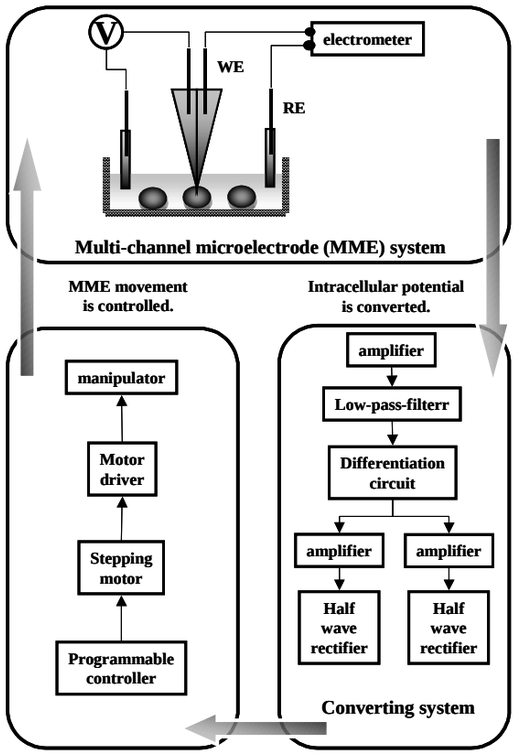

Fig.1 Automatic Microinjection System

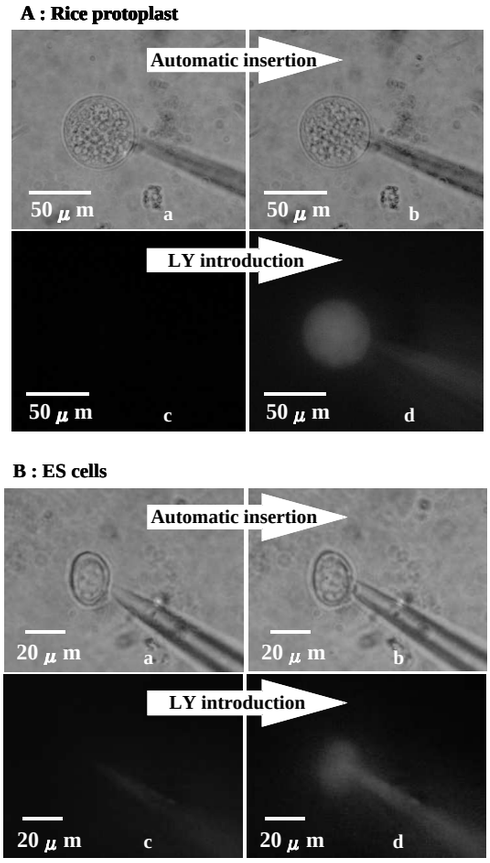

Fig.2 LY introduction after automatic insertion of a MME into a rice protplast (A) and a ES cell (B)
a : before insertion of a MME b : after insertion of a MME
c : before LY introduction d : after LY introduction

References

[1] M.Saito et al. *J. Biotechnol.* **105**, 41-49 (2003).
[2] A.G.Smith, *J. Tissue Culture Methods* **13**, 89-94 (1991).