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Electrofusion of Plant Protoplasts and Three Dimensional Network of Actin Filaments Observed with Confocal Scanning Laser Microscopy

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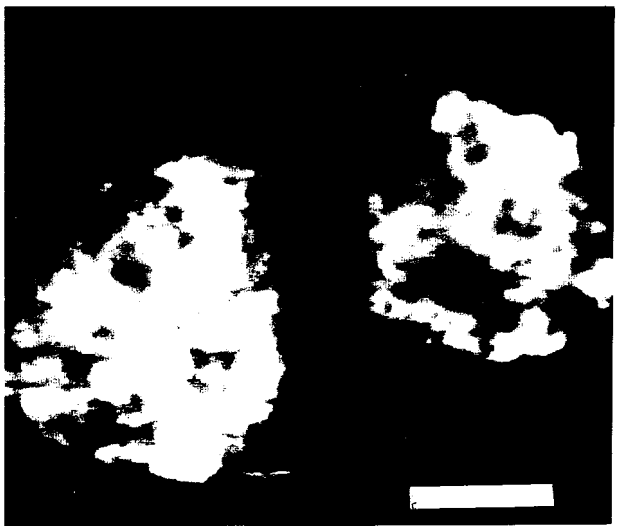
Electrofusion is a powerful tool for studying membrane properties, organization of cytoskeleton and cell motility on the early process of cell fusion. Recently, confocal microscopy have become available for the detection of actin filaments and aggregates in protoplasts (Kobori et al. 1989). The purpose of the present study is to report on the three dimentional structure and dynamical aspects of plant protoplasts during early process of electrofusion. Protoplasts from soybean *(Glycin Max)* and lettuce cotyledons *(Lactuca sativa)* were isolated by using the enzyme solution (Phansiri et al. 1991, Taniguchi and Maeda 1991). Protoplasts aligned in an alternating electric field at frequency of 1 MHz and a field strength of 200 V/cm for 30 s. Fusion was induced by DC pulses of 1,600 V/cm of 50 μs duration using Shimadzu Somatic Hybridizer. Control and AC and /or DC field applied protoplasts were stained by rhodamine-123 for mitochondria (Wu 1987, Chen 1989, Taniguchi, Maeda 1991)· or rhodamine-phalloidin for actin (Taniguchi et al. 1993). The specimens were viewed with a confocal scanning laser microsocpe (Noran Instruments) equipped with a Nikon oil-immersion objective lens with x 100 objective magnification and numerical aperture of 1.3. An argon laser with 488 nm or 529 nm was used to excite for rhodamine- 123 or rhodamine-phalloidin, respectively. Protoplasts were photographed with 20 to 40 parallel optical slices 0.2 to 0.5 μm apart. These images were projected to create a single image in which the specimen was in focus. A series of images was collected at different fcus focus levels (step size 0.05 to 0.5 μm).

When protoplasts from soybean were treated with AC field and stained with rhodamine-123, many yellow-green fluorescence spots (mitochondoria) were observed in the areas of cytoplasm (Fig. 1). In this figure, two protoplasts contact with wide surface areas but in one of two protoplasts mitochondrias are seen to localize in other side. We have also examined the distribution of actin in lettuce and soybean protoplasts. The actin filaments in the dark and light grown protoplasts from lettuce were stained with rhodamine-phalloidin. In control protoplasts, actin located mostly at the periphery of the cells. In AC-treated protoplasts, on the other hand, actin was located at the contact area and at the periphery of nucleus and chloroplasts. After electrofusion we analyzed the



Fg. 1. Confocal microscopic image of soybean protoplasts stained with rhodamine-123, Scale bar, 10 μm.

changes of F-actin organization by time sequential images of confocal micrographs.

Three dimensional observations of protoplasts were also made by a high voltage electron microscope (1 MeV) equipped with a revolving stage to get a pair of stereo-micrographs. Minimum dose and low temperature techniques were used. We discuss these images in detail.

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