Plant Physiol. (1991) 97, 1585–1587
0032-0889/91/97/1585/03/$01.00/0

Received for publication July 15, 1991
Accepted September 5, 1991

*Communication*

# Efficiency of Particle-Bombardment-Mediated Transformation Is Influenced by Cell Cycle Stage in Synchronized Cultured Cells of Tobacco[1]

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## ABSTRACT

**Plasmid DNA pBI221 harboring β-glucuronidase gene was delivered to synchronized cultured tobacco (*Nicotiana tabacum* L. cv Bright Yellow-2) cells of different cell cycle stages by a pneumatic particle gun. The cells bombarded at M and $G_2$ phases gave 4 to 6 times higher transformation efficiency than those bombarded at the S and $G_1$ phases.**

We have been studying transformation of plant cells and tissues by using gas-pressure-driven (1, 3) and pneumatic (2, 7) particle gun devices. In the present study, using the pneumatic particle gun, we introduced the GUS[2] gene into cultured tobacco (*Nicotiana tabacum* L. cv Bright Yellow-2) cells and detected GUS activity by a histochemical assay. Synchronized cells at different stages of the cell cycle were used as target cells to determine the effect of the cell cycle on the transformation efficiency.

## MATERIALS AND METHODS

### Plant Material and Plasmid DNA

Suspension-cultured cells of tobacco (*Nicotiana tabacum* L. cv Bright Yellow-2) were subcultured weekly as reported previously (3). Chimeric plasmid DNA, pBI221 (Clontech, Palo Alto, CA), which has the GUS gene under the control of the cauliflower mosaic virus 35S promoter and nopaline synthetase polyadenylation region, were used.

### Synchronization of Tobacco Cells

Synchronization of suspension-cultured cells of tobacco was carried out by the treatment with aphidicolin, a specific inhibitor of DNA polymerase alpha (5). Aphidicolin was added at a concentration of 5 μg/mL to the cell culture at stationary phase (7 d after subculture) and culture was continued for another 24 h. The treated cells were then washed with 10 volumes of fresh culture medium and cultured for 12 h in fresh medium for synchronous growth. Synchrony of mitosis was assessed by measuring the mitotic index after staining the cells with propionic orcein. One thousand to 1500 cells were observed at each time interval.

### Bombardment of Synchronized Cells

One hundred milligrams of synchronized tobacco cells ($5 \times 10^5$ cells), harvested at 2, 5, 9, and 12 h after the release from aphidicolin solution, were bombarded once with DNA-coated gold particles using a pneumatic particle gun device at an accelerating pressure of 150 kg/cm$^2$ as described elsewhere (2). Other bombardment conditions are the same as described previously (2).

### Assay for GUS Expression

After the bombardment, the filter paper with the cells was transferred to a plastic net in a plastic container (Toyobo Co., Plantex ccp-102) containing 17 mL of culture medium, and cultured for 2 d at 26°C in the dark. The cells were then transferred to fresh liquid medium and cultured at 26°C in the dark with shaking at 100 rpm for another 8 d, after which the cells were collected by vacuum filtration. During this culture period (10 d), the fresh weight of the cells increased by 40- to 60-fold.

The collected cells were transferred to 15 mL of filter-sterilized 5-bromo-4-chloro-3-indolyl-β-D-glucuronic acid solution (2). After incubation for 24 h at 37°C, 30 mL of 70% (v/v) ethanol was added to stop the reaction and to maintain aseptic conditions. GUS-expressing cells were counted under a binocular microscope (×6.6, Nikon, SMZ-10).

## RESULTS AND DISCUSSION

The degree of synchrony of tobacco cells treated with aphidicolin is shown in Figure 1A. Few dividing cells were observed during the initial 5 h after the cells had been released

[1] This research was supported by a grant from the Yamada Science Foundation and by Grants-in-Aid from the Ministry of Education, Science and Culture of Japan.

[2] Abbreviation: GUS, β-glucuronidase.

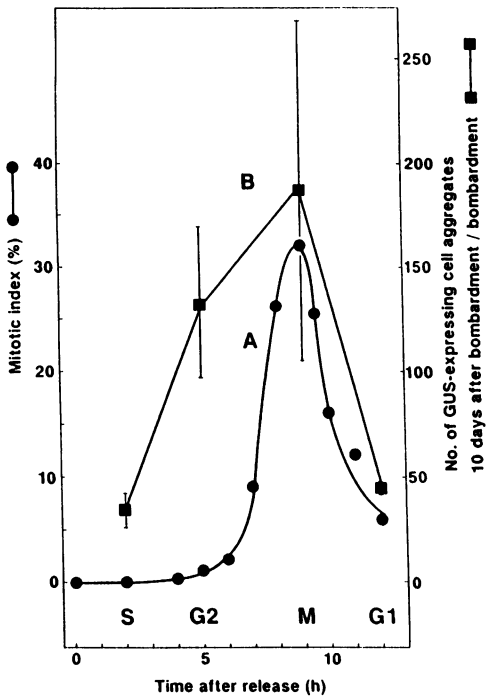


**Figure 1.** Mitotic index (curve A) and number of GUS-expressing cell aggregates (curve B) plotted against time after release of tobacco cells from aphidicolin block. The vertical bar indicates the deviation of three countings. See text for detail.

from aphidicolin block. At 5 h, only 1% of the cells were dividing. Thereafter, mitotic index increased sharply and peaked at 9 h, when 32% of the cells were dividing. Then mitotic index drastically decreased and about 6% of the cells were dividing at 12 h after release. This profile of the synchrony of the cell cycle was similar to that obtained with the same cell line by the previous authors (6), and we considered that times of 2, 5, 9, and 12 h after the release from aphidicolin solution corresponded to S, $G_2$, M, and $G_1$ phases, respectively, of the cell cycle (see Fig. 1A).

The number of the GUS-expressing cells obtained from cells that had been bombarded at the S, $G_2$, M, and $G_1$ phases of the cell cycle was determined with a GUS expression assay after 10 d of postbombardment culture. The activity of cauliflower mosaic virus 35S promoter is reported to change as a function of the cell cycle stage (4). We observed, however, that the synchrony of the cells was much decreased after the second cell cycle following release from aphidicolin block (our unpublished results). Thus, we performed the GUS assay when the cells had attained the stationary phase to minimize the effects, if any, of the cell cycle stage on the GUS expression in the bombarded cells.

The number of GUS-expressing cell aggregates drastically changed as a function of the time after release from aphidicolin block (or the phase of the cell cycle) as shown in Figure 1B. The highest number was obtained at M phase (187 ± 82) and the second highest at $G_2$ phase (136 ± 36), whereas the numbers at S and $G_1$ phases were at low level (34 ± 8 and 45

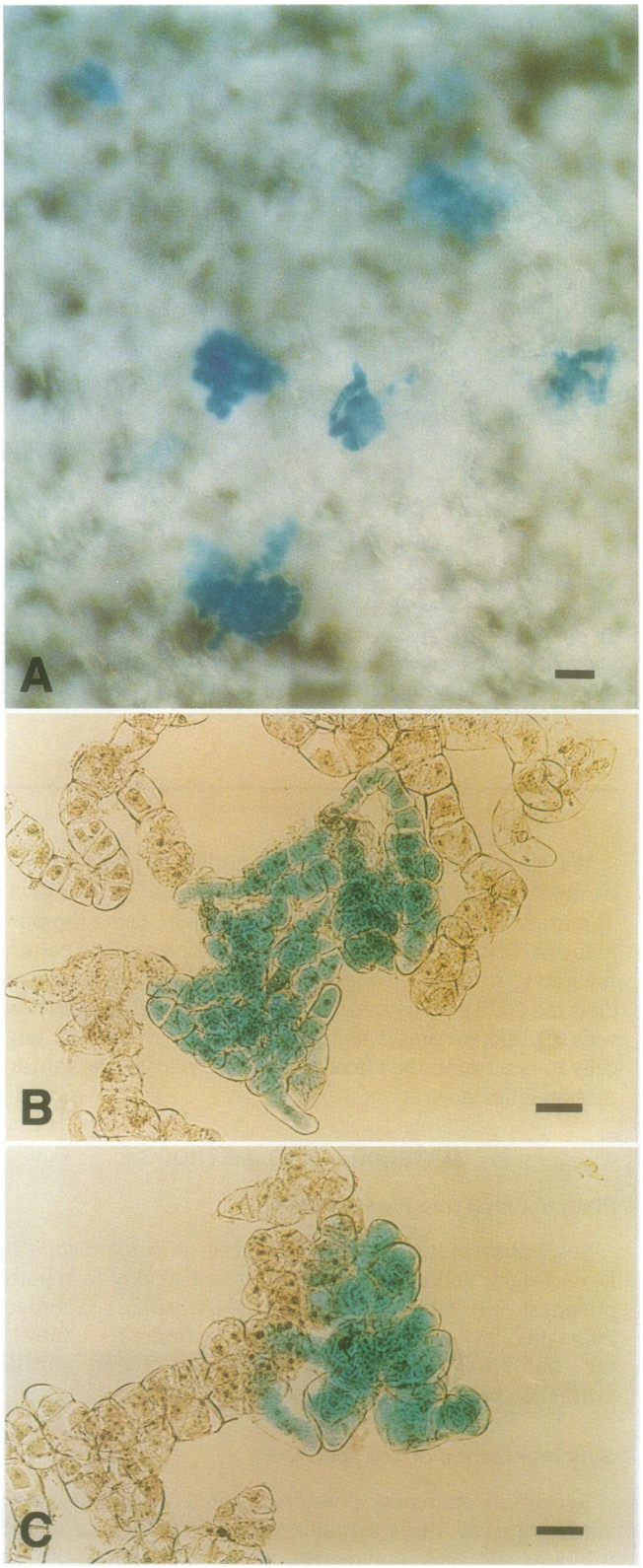


**Figure 2.** Microphotographs of GUS-expressing cell aggregates. GUS-expressing cell aggregates differed in size when observed under a binocular microscope (A). At higher magnification, some GUS-expressing cells were observed to be uniformly stained (B) and others were chimeric (C). Bar = 100 μm (A) and 50 μm (B and C).

± 3, average of three countings ± SD), respectively. We repeated this experiment two more times and similar results were obtained.

The GUS-expressing cells were present in the form of cell aggregates consisting of 1 to 40 cells that were stained in blue color (Fig. 2). At higher magnification, some of GUS-expressing cells were observed to be uniformly stained (Fig. 2B) and the others were stained partly dark and partly less dark (Fig. 2C). Control cells that were bombarded with noncoated gold particles and cultured for 10 d gave no blue cells in the histochemical GUS assay (data not shown). In conclusion, the present results clearly show that the efficiency of particle-bombardment-mediated transformation is influenced by the stage of the cell cycle.

Because the GUS assay used was destructive, further analysis of GUS-expressing cells was hampered. Whether the GUS-expressing cells contain those cells having residual activity of transiently expressed GUS enzymes and/or those that were stably transformed with the GUS gene is an open question. In future studies, the use of nondestructive substrates for the GUS enzyme in combination with the use of inhibitors that arrest the cells at specific cell cycle stage, or the use of selectable marker genes, will give a more mechanistic understanding of the influence of cell cycle on these processes. We are currently investigating along this line of study and results will be published elsewhere.

## ACKNOWLEDGMENTS

We wish to thank Dr. John Fitchen for his critical reading of the manuscript and correcting our English. We also thank Professor Akio Kobayashi of Okayama University for his invaluable discussion throughout this study.

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