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Introduction of *Escherichia coli* Cells and Spheroplasts into *Vinca* Protoplasts

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ABSTRACT

In the presence of 10% polyvinyl alcohol (PVA), Escherichia coli cells or spheroplasts can be easily introduced into Vinca protoplasts by endocytosis. Uptake proceeded quite rapidly; bacterial cells or spheroplasts were found within the cytoplasm of Vinca protoplasts after 10 min of incubation with PVA.

INTRODUCTION

Successful introduction into plant cells of foreign genes cloned into appropriate vectors may provide a new approach for improvement of higher plants. Simple and effective methods of introducing cloned foreign genes into plant cells have not been established, however.

Hasezawa et al. (1981) have reported the successful transformation of Vinca rosea protoplasts by Ti plasmid-harbouring spheroplasts of Agrobacterium tumefaciens with the aid of polyethylene glycol (PEG) or polyvinyl alcohol (PVA). Lebeurier et al. (1982) have shown that cloned recombinant plasmids consisting of tandem dimers of cauliflower mosaic virus DNA and pBR322 were infectious in plant leaves without removal of pBR322. These results suggest the possibility of transferring recombinant plasmids from bacterial hosts directly into plant protoplasts.

Escherichia coli K12 strain has served as one of the most popular hosts for cloning recombinant plasmids. In addition, E. coli spheroplast-mediated transfer of cloned recombinant plasmids has already been achieved in yeast (Broach et al. 1979; Kingsman et al. 1979) and animal cells (Rassoulzadegan et al. 1982; Sandri-Goldin et al. 1981; Schaffner 1980).

In the hope of extending spheroplast-mediated gene transfer systems to plant protoplasts, we examined the process of uptake of E. coli cells and spheroplasts by Vinca rosea protoplasts using a transmission electron microscope.

MATERIALS AND METHODS

E. coli C600 was grown overnight at 35° C in liquid Luria broth (Miller 1972), and spheroplasts were prepared by lysozyme-EDTA treatment (Birdsell and Costa-Robles 1967).

Protoplasts were isolated from suspension cultures of Vinca rosea L. using an enzyme mixture consisting of 0.1% Pectolyase, 1% Cellulase RS and 0.4 M D-mannitol (Hasezawa et al. 1981).

E. coli cells or spheroplasts (1×10^9 cells) suspended in a small volume of 0.4 M mannitol solution were added to a pellet of Vinca protoplasts (1×10^6 cells). The mixture was diluted with 0.4 M mannitol solution to a total volume of 0.5 ml, and 0.5 ml of 20% (W/V) PVA (degree of polymerization 300) was added.

After incubation for 10 min at room temperature, samples were fixed with 0.1 M cacodylate buffer (pH 7.2) containing 2% glutaraldehyde for 2 hr at room temperature and were post-fixed with the same buffer containing 2% osmium tetroxide for 12 hr at 5° C. The fixed protoplasts were observed following thin sectioning. E. coli spheroplasts were also prepared for ultrastructural studies.

RESULTS AND DISCUSSION

When E. coli cells were incubated with Vinca protoplasts in the presence of PVA, almost all the protoplasts contained the bacterial cells within the cytoplasm as well as on the surface (Fig. 1). The presence of various degrees of invagination of the plasma membrane at the sites of bacterial attachment and cytoplasmic vesicles containing bacterial cells (Fig. 2) indicate that the uptake of bacterial cells into protoplasts occurred by an endocytic process. This endocytic uptake appears to proceed quite rapidly, for the cytoplasmic vesicles containing bacteria were found after 10 min incubation. Most of the vesicles contained single bacterial cells, and only a minor fraction had more than two. Ultrastructural evidence is already available for endocytic uptake of small particles by isolated plant protoplasts (Davey and Power 1975; Davey et al. 1976; Suzuki et al. 1977; Takebe 1977; Willison et al. 1971).

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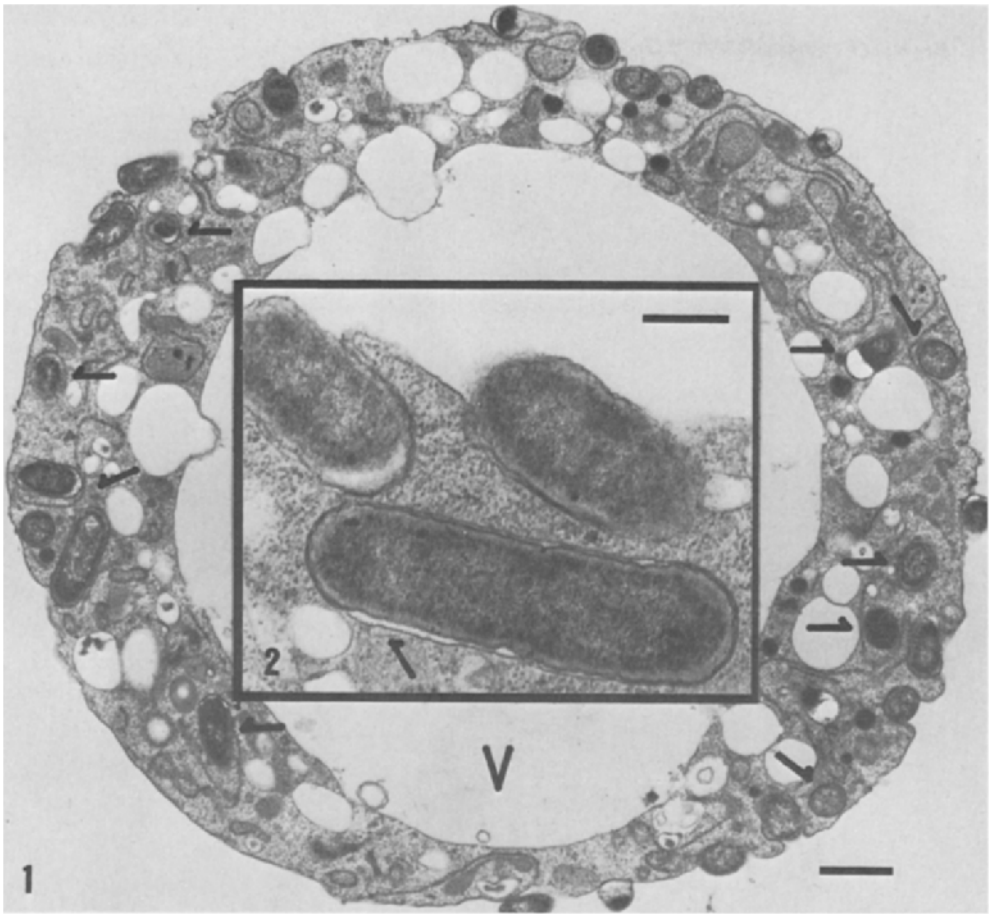


Fig. 1. A whole view of a sectioned Vinca protoplast. Note a large central vacuole (V) and E. coli cells within the cytoplasm (arrows). Protoplasts were fixed after 10 min treatment with PVA. The bar is 2 μm.

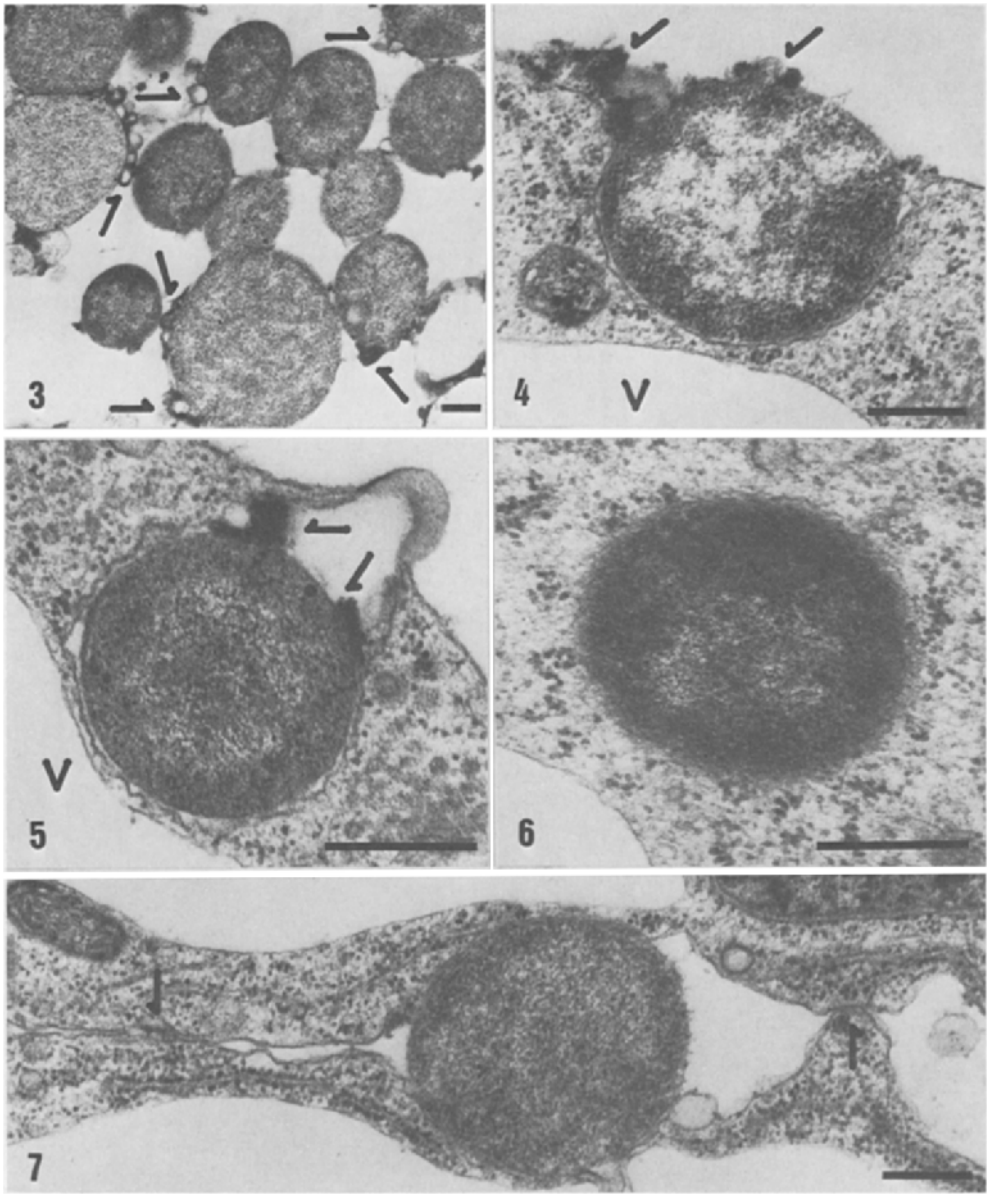
Fig. 2. An E. coli cell enclosed within a vesicle (an arrow) and others in the invaginating plasma membrane. The bar is 0.5 μm.

Davey and Cocking (1972) have reported that uptake of Rhizobium leguminosarum by pea mesophyll cells occurs only during the formation of protoplasts by enzymatic digestion, while isolated protoplasts are incapable of taking up bacteria. In our preliminary studies (Hasezawa et al. 1982), the method employed in the present study was shown to be effective in introducing R. leguminosarum into previously prepared plant protoplasts.

Electron microscopic observation of E.coli spheroplasts indicated that they were spherical in shape, filled with fine granules, and devoid of the cell wall. Frequently, spheroplasts retained cell wall remnants on their surface (Fig. 3). When incubated with Vinca protoplasts in the presence of PVA, E. coli spheroplasts were found on the smooth surface of protoplasts as well as within invaginations of the plasma membrane (Fig. 4). In addition, bacterial spheroplasts enclosed within vesicles were observed in the protoplast cytoplasm (Fig. 5). The process of incorporation of E. coli spheroplasts in Vinca protoplasts was thus indistinguishable from that of the bacterial cells, suggesting that both were taken up in an essentially similar fashion. The apparent intact fine structure of E. coli spheroplasts and Vinca protoplasts after incorporation (Fig. 5) indicates that their structural, and possibly physiological, integrity was kept through out the uptake process. The presence within the protoplast cytoplasm of free spheroplasts (Fig. 6) suggests that the engulfed spheroplasts were eventually freed of the surrounding vesicles. On occasion, as can be seen in Fig. 7, bacterial spheroplasts were also observed sandwiched between adhering Vinca protoplasts. Presumably, these spheroplasts represent those trapped between the fusing protoplasts.

Without any cytological evidence, Rassoulzadegan et al. (1982), Sandri-Goldin et al. (1981) and Schaffner (1980) have reasoned that fusion between bacterial protoplasts and recipient cells is responsible for PEG-induced direct transfer of cloned recombinant plasmids from E. coli protoplasts into animal cells. In the present study, however, no evidence of fusion between E. coli spheroplasts and Vinca protoplasts was obtained, despite careful examinations. In yeast and animal cells (Broach et al. 1979; Kingsman et al. 1979; Rassoulzadegan et al. 1982; Sandri-Goldin et al. 1981; Schaffner 1980), therefore, it seems more likely that the cloned recombinant plasmids were released from engulfed bacterial protoplasts into the recipient cells. The endocytic uptake of E. coli spheroplasts by Neurospora crassa protoplasts was also confirmed by electron microscopy (unpublished observations).

Endocytosis of E. coli cells and spheroplasts also occurred when 20% PEG was used in place of 10% PVA. As is typical of mature Vinca cells, the cytoplasm of Vinca protoplasts exist as a thin layer around a large central vacuole (Fig. 1). Due to drastic dehydration, the cytoplasm of PEG-treated protoplasts was frequently thinner than the diameter of E. coli spheroplasts. This may reduce the extent of



Figs. 3-7. The bar is 0.5 μm.

Fig. 3. A general view of freshly isolated E. coli spheroplasts. Note the cell wall remnants adhering to the surface of some spheroplasts (arrows).

Fig. 4. Plasma membrane invagination at the site of E. coli spheroplast attachment. The cell wall remnants (arrow) and part of a central vacuole (V) are also seen.

Fig. 5. An intracytoplasmic E. coli spheroplast enclosed within a vesicle. The cell wall remnants (arrow) and part of a central vacuole (V) are also seen.

Fig. 6. A naked, intracytoplasmic E. coli spheroplast.

Fig. 7. An E. coli spheroplast entrapped between the fusing protoplasts (arrows).

endocytic uptake of the spheroplasts. In contrast, such dehydration of the cytoplasm was absent in PVA-treated protoplasts. Vinca protoplasts into which E. coli cells or spheroplasts had been introduced by PVA treatment are presently being cultured.

In conclusion, the introduction of E. coli cells and spheroplasts into plant protoplasts described in this report suggests that as with yeast and animal cells, bacterial cells or spheroplasts can be used as vehicles for direct transfer of foreign genes into plant cells.

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