Induced Transfer of Higher Plant Chloroplasts into Fungal Protoplasts

Abstract. *Chloroplasts isolated from spinach leaves have been transferred in large numbers into protoplasts of* Neurospora crassa *with the help of polyethylene glycol. The chloroplasts show high photosynthetic activity—at least until the time of uptake—and the protoplasts continue to show active cytoplasmic streaming after chloroplast uptake.*

From reports of induced chloroplast uptake by isolated plant protoplasts (*1*), it is difficult to assess the potential of this method of information transfer in attempts to improve agronomically important species. Knowledge of the physiological condition and structural nature of the cell liable to give maximum uptake of plastids, the activity of the plastids before and after uptake, and conclusive electron micrographic evidence showing uptake are all presently lacking. We used a model system to investigate some of these parameters and report the induced uptake of chloroplasts into protoplasts, both of which remain viable and active at least until the time of uptake.

The slime strain of *Neurospora crassa* is due to three recessive genes that give rise to free-living colonies of fungal protoplasts that can be grown easily under sterile conditions on nutrient media (*2*). The protoplasts range from 10 to 80 μm in diameter and have one or more prominent vacuoles surrounded by granular cytoplasm (Fig. 1a); they lack chloroplasts or proplastids that might confuse the interpretation of the results, and they show pronounced and rapid cytoplasmic streaming.

The chloroplasts, from hydroponically grown 2- to 3-week-old spinach plants (*Spinacea oleracea*), were isolated by a 4-second disruption of the young leaves with a VirTis blender into an isolation medium (*3*). They were filtered through two layers of Miracloth and collected by centrifugation (1000*g* for 2 minutes). Immediately after isolation the chloroplasts were assayed with an oxygen electrode (*4*) in the isolation medium. The buffer HEPES (50 mmole/liter; *p*H 8.0) was substituted for the MES buffer. The chloroplasts released 70 to 100 μmole of oxygen per milligram of chlorophyll per hour.

Uptake of freshly isolated chloroplasts into fungal protoplasts was induced by adding to a mixture of the plastids and protoplasts (1 : 1, by volume) an equal volume of either 40 or 60 percent polyethylene glycol (molecular weight, 6000) (*5*). The addition of polyethylene glycol at either concentration caused immediate adherence of chloroplasts to the plasmalemmas of the protoplasts, but at *p*H 7.5 it caused little or no fusion of fungal protoplasts. After 5 or 10 minutes the protoplasts were removed from the polyethylene glycol solution by centrifuging them for 5 minutes at 100*g* and resuspending the pellet in the same medium without polyethylene glycol.

Some protoplasts would take up only two or three chloroplasts but in several cases as many as 40 chloroplasts were taken up (Fig. 1, d to f). About 50 percent of the protoplasts took up one or more chloroplasts. Uptake appeared to proceed by means of several well-defined, albeit rapid, stages. First the plastid adhered to the surface of the plasmalemma (Fig. 1b) and then immediately appeared under a slight protrusion of the plasmalemma (Fig. 1c). Attachment of the chloroplast to the plasmalemma occurs in the presence of polyethylene glycol, but actual uptake appears to take place only after the polyethylene glycol has been washed off. In cases where very large numbers of chloroplasts are taken up, after about 60 to 90 minutes they arrange themselves in a closely appressed single layer just inside the plasmalemma, which gives the fungal protoplasts the appearance of typical leaf mesophyll protoplasts (Fig. 1, e and f). Chloroplasts (which do not fuse with each other under the conditions used) and protoplasts appear to be normal in ultrastructure after uptake and the chloroplasts do not appear to be surrounded by any extra membrane (Fig. 1g). Treatment with polyethylene glycol does no visible damage to the fungal protoplasts, which continue to show active cytoplasmic streaming for 4 to 6 hours.

This model system indicates that polyethylene glycol is highly effective in inducing rapid uptake of large numbers of plastids into a high percentage of isolated protoplasts, that treatment with polyethylene glycol is apparently not injurious to chloroplasts or protoplasts, and rapid cytoplasmic streaming probably is responsible for the subsequent arrangement of the plastids in association with the plasmalemma.

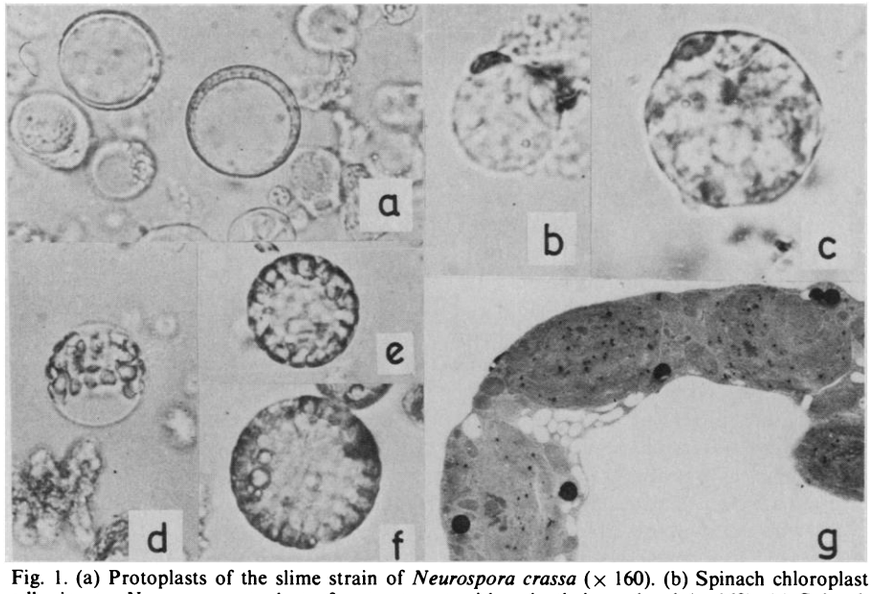

Fig. 1. (a) Protoplasts of the slime strain of *Neurospora crassa* (× 160). (b) Spinach chloroplast adhering to *N. crassa* protoplast after treatment with polyethylene glycol (× 160). (c) Spinach chloroplast taken into the protoplast cytoplasm and causing a slight protrusion of the plasmalemma after treatment with polyethylene glycol (× 325). (d) Many chloroplasts in the cytoplasm of a fungal protoplast 30 minutes after treatment with polyethylene glycol (× 200). (e and f) Spinach chloroplasts 90 minutes after treatment with polyethylene glycol, having arranged themselves around the plasmalemma in a single layer (× 200). (g) Electron micrograph of a portion of a *N. crassa* protoplast with included spinach chloroplasts 90 minutes after treatment with polyethylene glycol (× 8000).

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References and Notes

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2. S. Emerson, *Genetica* **36**, 162 (1963).
3. The isolation medium included ethylenediaminetetraacetic acid (2 mmole/liter), manganese chloride (1 mmole/liter), magnesium sulfate (1 mmole/liter), sodium chloride (10 mmole/liter), MES (50 mmole/liter, *p*H 6.2), and sorbitol (0.3 mole/liter) at 0°C.
4. T. Delieu and D. A. Walker, *New Phytol.* **71**, 201 (1972).
5. The polyethylene glycol was prepared in Vogel's medium (*2*), which contained the following additives for each 100 ml of medium: sucrose (2 g), glucose (1.8 g), yeast extract (750 mg), Difco nutrient broth (750 mg), KH_2PO_4 (9.5 mg), $CaCl_2$ $2H_2O$ (147 mg) at *p*H 7.5.

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