Proc. Natl. Acad. Sci. USA
Vol. 85, pp. 4305–4309, June 1988
Botany

Transfer of foreign genes into intact maize cells with high-velocity microprojectiles

(gene transfer/cell culture)

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Communicated by William L. Brown, November 2, 1987

ABSTRACT **This report describes a process for delivering foreign genes into maize cells that does not require the removal of cell walls and is capable of delivering DNA into embryogenic and nonembryogenic tissues. Plasmid harboring a chimeric chloramphenicol acetyltransferase (CAT) gene was adsorbed to the surface of microscopic tungsten particles (microprojectiles). These microprojectiles were then accelerated to velocities sufficient for penetrating the cell walls and membranes of maize cells in suspension culture. High levels of CAT activity were consistently observed after bombardment of cell cultures of the cultivar Black Mexican Sweet, which were comparable to CAT levels observed after electroporation of protoplasts. Measurable increases in CAT levels were also observed in two embryogenic cell lines after bombardment. Gene expression was observed only when an intron from the alcohol dehydrogenase 1 gene of maize was ligated between the 35S promoter and the CAT coding region. CAT activity was detected in cell cultures bombarded with microprojectiles with an average diameter of 1.2 μm, but not after bombardment with microprojectiles 0.6 or 2.4 μm in diameter. Bombarding the same sample several times was found to markedly enhance CAT activity. These results demonstrate that the particle bombardment process can be used to deliver foreign DNA into intact cells of maize. Because this process circumvents the difficulties associated with regenerating whole plants from protoplasts, the particle bombardment process may provide significant advantages over existing DNA delivery methods for the production of transgenic maize plants. In addition, the process should be of value for studying transient and stable gene expression within intact cells and tissues.**

Methods for transferring foreign nucleic acids into plant cells have helped advance basic studies of gene expression (1) and have permitted the introduction of agriculturally important traits into some crop species (2). Although the production of transgenic plants has become routine for some plant species (3), the genetic transformation of most cereal crops has proven to be difficult. Many of the methods available for delivery of exogenous DNA into cells of higher plants [i.e., electroporation (4) or calcium phosphate coprecipitation (5)] currently require removal of the cell wall as a necessary part of the transformation system. Although progress has been made in regenerating plants from protoplasts of rice (6, 7), the regeneration of cereal protoplasts remains difficult and time consuming at best. Isolated cells of tobacco with intact walls have been microinjected and regenerated to produce transgenic plants (8) but microinjection is likely to be limited to single cell systems in which the potential for regeneration of the injected cells is very high. Transfer of DNA mediated by *Agrobacterium tumefaciens* provides a desirable alternative to protoplast-dependent systems in many dicotyledonous species but use of the bacterium for transforming important cereal crops has thus far been hindered by the limited host range of the bacterium (3). For these reasons, an efficient system for delivering genetic material directly into intact and regenerable tissues might generally aid in the recovery of genetically transformed plants and might specifically enhance our ability to genetically engineer cereal crop species.

Sanford and coworkers (9) have developed a method whereby substances can be delivered into cells of intact tissues via a particle bombardment process. Small high-density particles (microprojectiles) are accelerated to high velocity by a particle gun apparatus. These microprojectiles have sufficient momentum to penetrate plant cell walls and membranes and can carry DNA or other substances into the interior of bombarded cells. It has been demonstrated that such microprojectiles can enter cells without causing cell death and that they can effectively deliver foreign genes into intact epidermal tissue of *Allium cepa* (10). However, it has not previously been shown that the particle bombardment process could be extended beyond the very large-celled *Allium* model system to economically important species that have cells of a more typical size.

We specifically wished to determine the feasibility of using the particle bombardment process for delivering exogenous DNA into intact cells of maize and to examine some of the variables that may influence the efficiency of such delivery. The capacity of maize to produce embryogenic cell cultures (11) made this species a particularly attractive candidate for genetic transformation by the particle bombardment process. Suspension cultures derived from the cultivar Black Mexican Sweet (BMS) and two embryogenic suspensions were bombarded with microprojectiles coated with plasmid harboring a chimeric chloramphenicol acetyltransferase (CAT) gene. We have found that high velocity microprojectiles are able to carry DNA into intact maize cells and that foreign DNA can then be expressed at high levels.

MATERIALS AND METHODS

Plasmids. The plasmids used in this study have been described (12) and their structures are shown in Fig. 1. The plasmid pCaMVI$_1$CN consists of the 35S promoter from cauliflower mosaic virus, a fragment (*Bcl* I/*Bam*HI) from the alcohol dehydrogenase intron 1 (Adh1), a CAT coding region, and the nopaline synthase polyadenylylation region. The plasmid pCaMVCN is identical to pCaMVI$_1$CN but lacks the Adh1 intron fragment.

Plant Materials. Embryogenic suspension cultures 3-86-17 and 13-217 were derived from type II embryogenic callus

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Abbreviations: CAT, chloramphenicol acetyltransferase; BMS, Black Mexican Sweet maize cultivar.

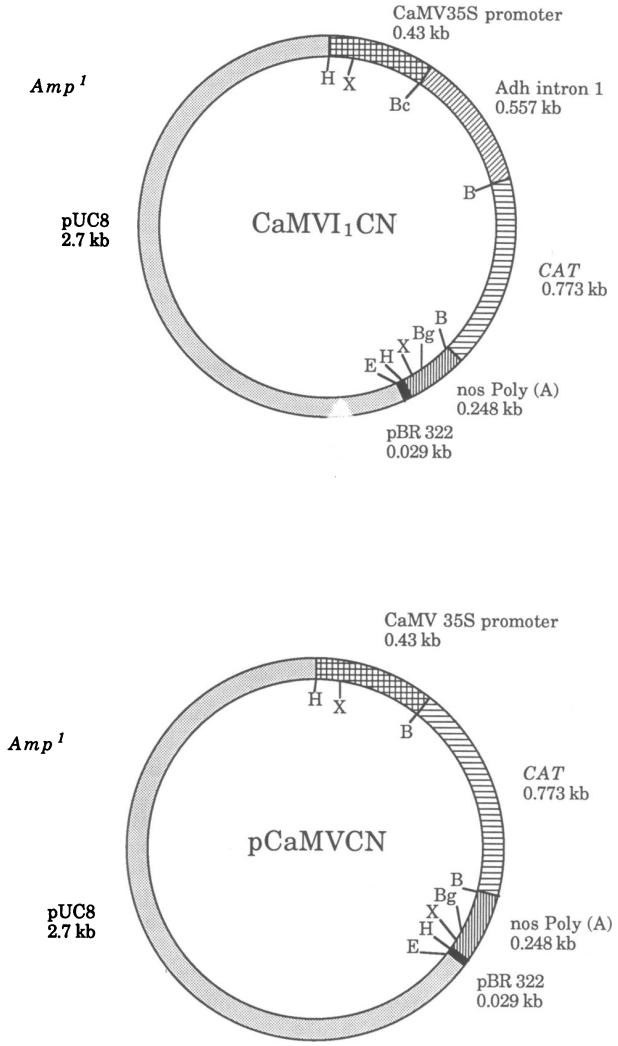

FIG. 1. Diagram of pCaMVCN and $CaMVI_1CN$ showing the source of the DNA fragments used in construction of the plasmid. pCaMVN was derived from pCAMVneo (4) by replacement of the neomycin phosphotransferase coding region with that of the CAT coding region. The addition of the *Bcl* I/*Bam*HI Adh1 intron fragment to the *Bam*HI site at the 5′ end of the CAT coding region yielded $pCaMVI_1CN$ (12). B, *Bam*HI; Bc, *Bcl* I; Bg, *Bgl* II; E, *Eco*RI; H, *Hin*dIII; X, *Xba* I. Amp, ampicillin; kb, kilobase(s).

(13). The callus was initiated from a maize inbred designated R21 (for line 3-86-17) or B73 × G35 (for line 13-217). Inbred R21 was derived from a regenerated plant from a long-term callus culture of public inbred B73 and is very similar to B73. Both R21 and G35 are proprietary inbred lines of Pioneer Hi Bred International (Johnston, IA). Suspension cultures of the cultivar BMS were obtained from V. Walbot (Stanford University). Suspension cultures were maintained in Murashige and Skoog (MS) medium (14) supplemented with 2,4-dinitrophenol (2 mg/liter) and sucrose (30 g/liter). The suspension cultures were passed through a 710-μm sieve 7 days prior to the experiment, and the filtrate was maintained in MS medium. In preparation for bombardment with the microprojectiles, cells were harvested from suspension culture by vacuum filtration on a Buchner funnel (Whatman no. 614). The same cell batch from each genotype was used within each experiment.

Particle Bombardment of Suspension Cultures. The particle gun device and general methods for bombardment of cells with microprojectiles have been described (9, 10). Before bombardment, cells (100 mg fresh weight) were placed in a 3.3-cm Petri dish. The cells were dispersed in 0.5 ml of fresh culture medium to form a thin layer of cells covered by a film of medium. The uncovered Petri dish was placed in the sample chamber and a vacuum pump was used to decrease the pressure in the chamber to 0.1 atm (1 atm = 101.3 kPa) (operation in a partial vacuum allows the microprojectiles to maintain their velocity over a longer distance, since air resistance is an important factor in their deceleration).

Unless otherwise noted, the cells were bombarded with tungsten particles with an average diameter of 1.2 μm (GTE Sylvania). In one experiment, cells were also bombarded with microprojectiles with an average diameter of 0.6 μm (GTE Sylvania) or 2.4 μm (General Electric). Plasmid DNA was adsorbed to the microprojectiles by adding 5 μl of DNA (1 μg per μl of TE buffer, pH 7.7; 0.1 M) to 25 μl of a suspension of tungsten particles (0.05 g per ml of distilled water) in a 1.5-ml Eppendorf tube. $CaCl_2$ (25 μl of a 2.5 M solution) and spermidine free base (10 μl of a 0.1 M solution) were then added to the suspension. Particles became agglomerated and settled to the bottom of the Eppendorf tube ≈10 min after addition of $CaCl_2$ and spermidine. Most of the supernatant (45 μl) was then removed and the particles were deagglomerated by briefly (1 sec) touching the outside of the Eppendorf tube to the probe (horn type) of a sonicator (Heat System/Ultrasonics, Plainview, NY). Five microliters of the resulting suspension of microprojectiles was then placed on the front surface of a cylindrical polyethylene macroprojectile. The macroprojectile was then placed into the barrel of the particle gun device and a blank gun powder charge (no. 1 gray extra light; Speed Fasteners, Saint Louis) was loaded into the barrel behind the macroprojectile. A firing pin device was used to detonate the gun powder charge, accelerating the macroprojectile down the barrel of the device where it impacts with a stopping plate. Upon impact, the microprojectiles are propelled from the front surface of the macroprojectile and continue toward the cells through a small aperture in the stopping plate. The cells were positioned 15 cm from the end of the barrel of the particle gun. After bombardment, the Petri dish was removed from the apparatus and the cells were transferred to 5 ml of fresh medium in a 15-ml polypropylene tube. The cells were then maintained in this tube with agitation at 27°C until harvested for analysis. Controls consisted of cells bombarded with microprojectiles lacking DNA.

In one set of experiments, the cells were treated either with a medium of high osmotic potential or with a mixture of medium and mineral oil during bombardment. In the first case, 100 mg of BMS cells was dispersed in 0.5 ml of MS medium supplemented with mannitol (0.4 M), 30 min prior to bombardment. After particle bombardment, the cells were left in the mannitol-containing medium for an additional 30 min, after which they were resuspended in 5 ml of standard MS medium. In the second case, the cells were dispersed in an emulsion of either 0.1 ml of sterile mineral oil and 0.5 ml of MS medium, 0.2 ml of mineral oil and 0.4 ml of MS medium, or 0.6 ml of MS medium lacking mineral oil. After bombardment, the cells were suspended in 5 ml of MS medium.

CAT Assays. Analyses of CAT activity were performed 96 hr after bombardment. Tissue extracts were prepared by sedimenting the cells at ≈13,000 × *g* for 10 min in a 1.5-ml Microfuge tube. The supernatant was removed and 100 μl of buffer (0.25 M Tris·HCl, pH 7.8) was added to the pellet. The sample was homogenized on ice for ≈2 min with a disposable polypropylene pestle (Kontes) driven at 300 rpm by an electric motor. After grinding, the sample was Vortex mixed briefly to complete the extraction of soluble protein. Cell debris was removed by centrifugation at 13,000 × *g* in a Microfuge at 4°C for 10 min. The supernatant was decanted and normalized to a volume of 200 μl with the Tris·HCl buffer.

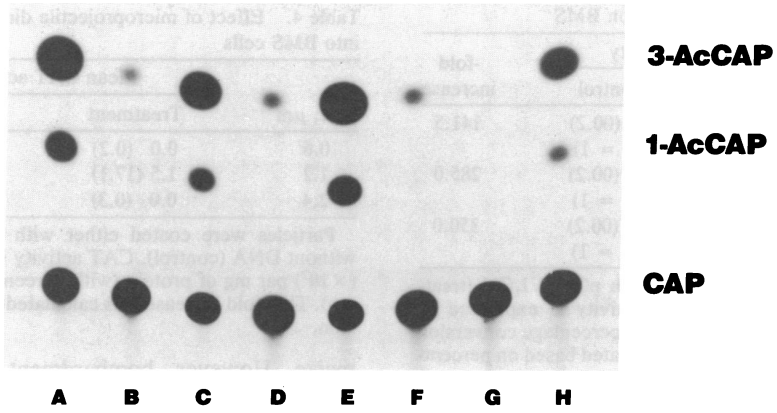

FIG. 2. Detection of CAT reaction products by TLC and autoradiography 4 days after bombardment of BMS suspension cultures. Lanes: a, c, and e, bombardment with 1.2-μm projectiles coated with pCaMVI$_1$CN; b, d, and f, bombardment with naked projectiles; g, unreacted chloramphenicol substrate; h, positive control—products produced by purified bacterial CAT enzyme (0.010 unit). The positions of unreacted chloramphenicol (CAP) and the enzymatic products 1-acetylchloramphenicol (1-AcCAP) and 3-acetylchloramphenicol (3-AcCAP) are shown.

The CAT activity in the extracts was determined as reported (15) except the samples were heated at 60°C for 12 min before addition of substrates. The reaction mixture was incubated for 1.5 hr at 37°C, and reaction products were extracted from the mixture with 300 μl of cold ethyl acetate, air dried, and resuspended in 20 μl of ethyl acetate for spotting on TLC plates (Baker, Phillipsburg, NJ). After TLC resolution of chloramphenicol and its acetylated derivatives by using chloroform/methanol (95:5), autoradiograms of the TLC plates were made (60-hr exposure at 22°C; DuPont Cronex film).

Quantitative results were obtained by scintillation counting of separated spots of chloramphenicol and its acetylated derivatives, and the percentage conversion to acetylated products was calculated. CAT activity (1 unit of CAT catalyzes the acetylation of 1 ng of chloramphenicol per min at 37°C) was determined by comparison with a standard curve of acetylation conversions obtained with purified bacterial CAT (P-L Biochemicals). Protein in the cell extracts was determined according to Bradford (16). CAT activity was standardized on the basis of units of CAT activity per mg of soluble protein.

RESULTS

CAT activity was compared in suspension cell cultures of BMS after bombardment with naked microprojectiles and microprojectiles coated with pCaMVI$_1$CN. As shown in Fig. 2 and Table 1, increases in CAT activity were consistently observed in the treatments involving the pCaMVI$_1$CN plasmid. The induced levels of CAT activity were typically 20- to 200-fold greater than the CAT activity in the controls. CAT activity in BMS was monitored over a 4-day period following bombardment with microprojectiles coated with pCaMVI$_1$CN. Expression of the introduced CAT gene was detectable 24 hr after bombardment and was still high after 96 hr of incubation (data not shown). CAT activity was also measured after 96 hr in BMS cells bombarded with pCaMVCN (no intron). Expression in these cells was not greater than background levels found in cells bombarded with uncoated microprojectiles. Consequently, all subsequent experiments were performed with the pCaMVI$_1$CN plasmid.

The postbombardment CAT activity of two embryogenic cell suspensions clearly revealed that DNA was being delivered into these cell lines, but generally at lower rates than observed in BMS (Table 2).

Samples of BMS were subjected to repeated bombardments with plasmid-coated microprojectiles. Multiple bombardments of the same sample clearly produced higher levels of CAT activity than did single bombardments (Table 3, Fig. 3). Triple bombardment was seen to increase CAT activity in a similar manner in the embryogenic line 3-86-17 (Fig. 3).

The effect of microprojectile diameter was examined by monitoring CAT expression after bombardment with different particle sizes (Table 4). BMS suspensions were bombarded with microprojectiles that were 0.6, 1.2, or 2.4 μm in diameter. Significant levels of CAT activity were only found in cells bombarded with microprojectiles with an average diameter of 1.2 μm.

Table 1. Introduction of DNA into BMS maize suspension cells by using high-velocity microprojectiles

Exp.	Mean CAT activity		-fold increase	*F* test*
	Treatment	Control		
1	5.7 (36.1) (n = 10)	0.1 (1.8)	20.1	0.00 (0.00)
2	1.8 (19.3) (n = 4)	0.0 (0.9)	21.4	0.17 (0.11)
3	4.3 (34.7) (n = 3)	0.0 (0.2)	173.5	0.10 (0.06)
4	1.5 (17.1) (n = 2)	0.0 (0.2)	85.5	0.10 (0.26)

In each experiment, BMS cells were bombarded with 1.2-μm tungsten particles that were coated with pCaMVI$_1$CN (treatment) or left uncoated (control). n, Number of replicates in each experiment. CAT activity is expressed as enzyme units ($\times 10^2$) per mg of protein (percentage chloramphenicol converted to its acetylated derivatives shown in parentheses). Calculation of the -fold increase of treatment over control was based on percentage conversion.

*Probability that the difference between treatment and control is due only to chance, based on analysis of variance by *F* test.

Table 2. Delivery of DNA into cell suspensions of embryogenic (3-217, 3-86-17) and nonembryogenic (BMS) cell lines

Line	Mean CAT activity		-fold increase
	Treatment	Control	
BMS	1.6 (7.7) (n = 3)	0.1 (0.5) (n = 1)	15.4
13-217	0.1 (0.7) (n = 2)	0.0 (0.3) (n = 1)	2.3
3-86-17	0.2 (1.7) (n = 2)	0.0 (0.4) (n = 1)	4.3

Particles were either coated with pCaMVI$_1$CN (treatment) or without DNA (control). CAT activity is expressed as enzyme units ($\times 10^2$) per mg of protein (with percentage conversion in parentheses), as measured 96 hr after bombardment. The -fold increase was calculated based on conversion rates.

Table 3. Effect of multiple bombardments on BMS

Bombardments	Mean CAT activity		-fold increase
	Treatment	Control	
1	3.5 (28.3) (n = 4)	0.0 (00.2) (n = 1)	141.5
2	11.0 (57.0) (n = 2)	0.0 (00.2) (n = 1)	285.0
3	13.5 (70.0) (n = 2)	0.0 (00.2) (n = 1)	350.0

Particles (1.2 μm) were coated either with pCaMVI$_1$CN (treatment) or without DNA (control). CAT activity is expressed as enzyme units ($\times 10^2$) per mg of protein (with percentage conversion in parentheses). The -fold increase was calculated based on percentage conversion.

Two treatments that could potentially limit the damage to cells caused by microprojectile penetration were evaluated. To reduce turgor pressure, cells were incubated in MS medium containing 0.4 M mannitol prior to bombardment. It was observed that the osmotically adjusted cells exhibited a substantially lower level of CAT activity than cells maintained in the normal MS medium during bombardment (data not shown). In another experiment, sterile mineral oil was added to the cell suspensions such that many cells became coated with a layer of oil. Addition of different amounts of mineral oil to cell suspensions did not enhance CAT activity in relation to cells bombarded in normal culture medium (data not shown).

DISCUSSION

Consistently high levels of CAT activity were found in cell cultures of BMS after bombardment with microprojectiles coated with plasmid bearing a chimeric CAT gene (pCaMVI$_1$-CN). The levels of CAT expression in cells bombarded with pCaMVI$_1$CN-coated microprojectiles were often 200-fold greater than in controls and were comparable to CAT levels previously observed in bombarded onion tissue (10). The dramatic increase in CAT activity after bombardment was not unlike that previously seen in protoplasts of BMS electroporated with this plasmid (13, 17). These results unambiguously demonstrate that high-velocity microprojectiles can be used to carry foreign genes into intact cells of maize. However, bombardment with pCaMVCN-coated microprojectiles (lacking the Adh1 intron) failed to induce obvious increases in CAT activity. In electroporation experiments with BMS protoplasts, this same plasmid induces detectable levels of CAT activity, although at only one-tenth the level induced by the same construct with the Adh1 intron placed between the CAT coding region and 35S promoter (13). The fact that the intron-containing construct was necessary to detect expression after particle bombardment may suggest that DNA delivery by microprojectiles to intact cells by the present technology is still less efficient than DNA delivery to protoplasts by electroporation.

Table 4. Effect of microprojectile diameter on DNA delivery into BMS cells

Size, μm	Mean CAT activity		-fold increase
	Treatment	Control	
0.6	0.0 (0.2)	0.0 (0.2)	1.0
1.2	1.5 (17.1)	0.0 (0.2)	85.8
2.4	0.0 (0.3)	0.0 (0.2)	2.5

Particles were coated either with pCaMVI$_1$CN (treatment) or without DNA (control). CAT activity is expressed as enzyme units ($\times 10^2$) per mg of protein (with percentage conversion in parentheses). The -fold increase was calculated based on percentage conversion.

The fact that CAT activity was readily detectable in BMS and two embryogenic suspension cultures suggests that the particle bombardment process may be used in diverse genotypes or tissue types. While the experiments summarized in Table 2 indicate that particle bombardment may be less efficient in embryogenic cell lines than in BMS, more recent experiments indicate that bombardment parameters can be adjusted so that CAT levels can be achieved in embryogenic cell lines that are equal to those observed in BMS.

Numerous variables can be further optimized to increase the efficiency of delivery by the particle bombardment process. For example, repeated bombardment of the same sample led to marked increases in CAT expression. This suggests that modifications in the number and spatial distribution of particles that impact the target tissue may increase the number of cells that are penetrated and that receive DNA. Optimization of these factors should be possible by design changes in the macroprojectile and stopping plate of the particle gun apparatus.

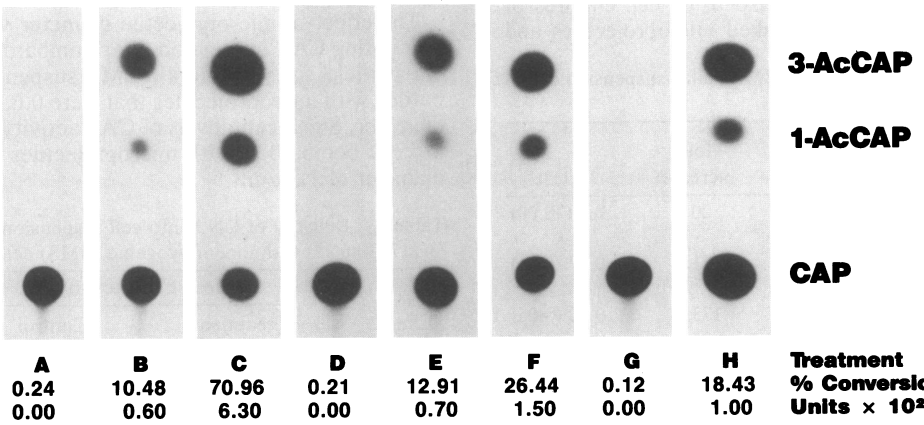

FIG. 3. Effects of multiple bombardment on CAT activities, as revealed by TLC and autoradiography. Lanes: a, single bombardment of a BMS cell suspension with microprojectiles lacking DNA; b, single bombardment of a BMS cell suspension with pCaMVI$_1$CN-coated microprojectiles; c, triple bombardment of a BMS cell suspension with pCaMVI$_1$CN-coated particles; d, single bombardment of a 3-86-17 embryogenic cell suspension with microprojectiles lacking DNA; e, single bombardment of a 3-86-17 embryogenic cell suspension with pCaMVI$_1$CN-coated particles; f, triple bombardment of a 3-86-17 embryogenic cell suspension with pCaMVI$_1$CN-coated microprojectiles; g, unreacted chloramphenicol substrate; h, positive control—purified bacterial CAT enzyme (0.010 unit). Percentage conversion represents proportion of total radioactivity recovered as products—units of CAT activity are defined in the text. The positions of unreacted chloramphenicol (CAP) and the enzymatic products 1-acetylchloramphenicol (1-AcCAP) and 3-acetylchloramphenicol (3-AcCAP) are shown.

The size of the microprojectiles used for bombardment clearly influenced levels of CAT activity. Although tungsten particles with an average diameter of 1.2 μm proved superior to the other two sizes tested in this study, optimal particle size has yet to be determined and may differ for specific cell types. Particles that are too small may fail to penetrate certain cells, while particles that are too large will be lethal to others. Factors other than cell size, such as thickness of the cell wall or abundance of intracellular nucleases, may also affect the efficiency of transformation by microprojectiles.

One factor we felt would be important to cell survival was osmotic pressure. We assumed that it would be desirable to reduce turgor pressure and thereby limit the loss of cell cytoplasm through lesions in the cell wall formed by microprojectile penetration. Such osmotic adjustment is often essential in microinjection experiments. Experiments in which mannitol was used to increase the osmotic pressure of the surrounding medium indicate that this was not beneficial but was actually deleterious. Reductions in turgor pressure may have decreased the efficiency of microprojectile penetration.

It was also thought that a layer of oil over the cells might improve viability by helping to seal lesions after penetration. Mineral oil did not change CAT expression in bombarded cells. It is possible that the viscosity of the oil reduced the velocity of the microprojectiles, thereby reducing penetration efficiency and counteracting any beneficial effect.

Numerous bombardment and culture variables remain to be determined and optimized. These variables include the distance that the cells are placed from the aperture of the stopping plate, the velocity at which the microprojectiles impact the cells, the degree of vacuum within the sample chamber during bombardment, and the size and shape of the microprojectiles. The optimal mode of DNA absorption to the projectiles and better ways to optimally distribute and anchor cells before bombardment also need to be determined.

The particle bombardment process should offer several significant advantages for delivering nucleic acids to plant cells and may be of particular value for species in which efficient transformation and protoplast regeneration systems do not presently exist. One appealing feature of the particle bombardment process is that it allows treatment of cells whose walls are intact. Because the obstacle of regenerating whole plants from protoplasts may be circumvented, the genetic engineering of important grain species may be facilitated. Transient assays of genes introduced into cells by the particle bombardment process should also aid in studies of gene expression in economically important monocots, particularly since it may be possible to deliver genes to cells within intact tissues. The particle bombardment process may thus make investigations of tissue-specific gene expression possible without the need for regenerating whole plants.

We thank Nelson Allen and Edward Wolf for their efforts in the continuing evolution of the particle gun apparatus and Sheila Maddock for supplying the embryogenic cell cultures. This work was supported by grants from the U.S. Department of Agriculture and the Cornell Biotechnology Program.

1. Kuhlemeier, C., Green, P. J. & Chau, N.-H. (1987) *Annu. Rev. Plant Physiol.* **38**, 221–257.
2. Goodman, R. M., Hauptli, H., Crossway, A. & Knauf, V. C. (1987) *Science* **236**, 48–54.
3. Fraley, R. T., Rogers, R. B. & Horsch, R. B. (1986) *CRC Crit. Rev. Plant Sci.* **4**, 1–46.
4. Fromm, M., Taylor, L. P. & Walbot, V. (1986) *Nature (London)* **319**, 791–793.
5. Lörz, H., Baker, B. & Schell, J. (1985) *Mol. Gen. Genet.* **199**, 178–182.
6. Fujimura, T., Sakurai, M., Akagi, H., Negishi, T. & Hirose, A. (1985) *Plant Tissue Cult. Lett.* **2**, 74–75.
7. Abdullah, R., Cocking, E. C. & Thompson, J. A. (1986) *Bio/Technology* **4**, 1087–1090.
8. Crossway, A., Oakes, J. V., Irvine, J. M., Ward, B., Knauf, V. C. & Shewmaker, C. K. (1986) *Mol. Gen. Genet.* **202**, 179–185.
9. Sanford, J. C., Klein, T. M., Wolf, E. D. & Allen, N. (1987) *Particle Sci. Technol.* **5**, 27–37.
10. Klein, T. M., Wolf, E. D., Wu, R. & Sanford, J. C. (1987) *Nature (London)* **327**, 70–73.
11. Kamo, K. K. & Hodges, T. K. (1986) *Plant Sci. Lett.* **45**, 111–117.
12. Callis, J., Fromm, M. & Walbot, V. (1987) *Genes Dev.* **1**, 1183–1200.
13. Green, C. E., Armstrong, C. L. & Anderson, P. C. (1983) in *Molecular Genetics of Plants and Animals*, eds. Downey, K., Voellmy, R. M., Fazelahmad, A. & Schultz, J. (Academic, New York), Vol. 20, pp. 147.
14. Murashige, T. & Skoog, F. (1962) *Physiol. Plant.* **15**, 473–497.
15. Gorman, C. M., Moffat, L. F. & Howard, B. H. (1982) *Mol. Cell. Biol.* **2**, 1044–1051.
16. Bradford, M. M. (1976) *Anal. Biochem.* **72**, 248–254.
17. Fromm, M., Taylor, L. P. & Walbot, V. (1985) *Proc. Natl. Acad. Sci. USA* **82**, 5824–5828.