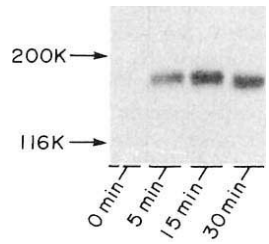


Fig. 3 Time course of phosphorylation of adenylate cyclase by protein kinase C. Size markers, M_r.

Methods. Bovine brain (caudate) adenylate cyclase was purified by modification[11] of the procedures described in Fig. 1 legend. The final material was >95% pure as judged by its specific activity (1.5 μmol min^{-1} mg^{-1}) and SDS-PAGE[11]. The 160K peptide of adenylate cyclase obtained by this procedure was incubated with protein kinase C in the presence of 10 mM Tris-HCl, *p*H 7.2, 100 mM NaCl, 6 mM $MgCl_2$, 0.1 mM $CaCl_2$, 0.1 mg ml^{-1} phosphatidylserine, 25 mM NaH_2PO_4, 0.1 mM TPA and 0.025 mM [γ-^{32}P]ATP (~2,500 c.p.m. $pmol^{-1}$) at 25 °C for the indicated periods. The protein kinase C was prepared, as described by Uchida *et al.*, from porcine brain[27]. Because the adenylate cyclase was stored in 5 mM Tween 60, phosphorylation reactions were conducted in mixed micelles (phosphatidylserine/Tween 60) and the TPA concentration (0.1 mM) was chosen to give >1 molecule of TPA per micelle. The kinase activity was >95% dependent on $CaCl_2$, phosphatidylserine and TPA and the preparation was free of cAMP-, cGMP- or Ca^{2+}/calmodulin-dependent protein kinase activity. Reactions were stopped by dilution with ice-cold 100 mM NaCl, 10 mM Tris-HCl, *p*H 7.4 and phosphorylated samples were further purified on a 0.5 ml wheat-germ agglutinin–agarose column to remove contaminating phosphoproteins. The samples were then electrophoresed on a 6% SDS-polyacrylamide gel. After drying and autoradiography the gels were cut and counted to determine the stoichiometry.

other hand, Nabika *et al.*[23] have found that in vascular smooth muscle cells, angiotensin II also enhances cyclic AMP accumulation stimulated by isoprenaline and vasoactive intestinal peptide. To probe the possible physiological significance of the phosphorylation mechanism proposed here, such cell systems might be useful models. They could be used to examine directly whether effects on responsiveness of adenylate cyclase mediated by receptors coupled to phosphatidylinositol breakdown can be correlated with phosphorylation of the enzyme. Together with previous findings on the regulation of receptor function by phosphorylation[24], the results presented in this paper delineate further the complexity and variety of mechanisms that have evolved to regulate cellular sensitivity to hormone and drug actions.

We thank Donna Addison for help with the manuscript and Dr K. B. Seamon, NIH, for the 1,9-dideoxyforskolin.

Received 16 January; accepted 2 March 1987.

1. Nishizuka, Y. *Nature* **308**, 693-698 (1984).
2. M. Berridge & Irvine, R. F. *Nature* **312**, 315-321 (1984).
3. Castagna, M. *et al. J. biol. Chem.* **257**, 7849-7857 (1982).
4. Kikkawa, U., Takai, Y., Tanaka, Y., Miyake, R. & Nishizuka, Y. *J. biol. Chem.* **258**, 11442-11445 (1983).
5. Sibley, D. R., Nambi, P., Peters, J. R. & Lefkowitz, R. J. *biochem., Biophys. Res. Commun.* **121**, 973-979 (1984).
6. Kelleher, D. J., Pessin, J. E., Ruoho, A. & Johnson, G. L. *Proc. natn. Acad. Sci. U.S.A.* **81**, 4316-4320 (1984).
7. Sibley, D. R., Jeffs, R. A., Daniel, K., Nambi, P., & Lefkowitz, R. J. *Archs Biochem. Biophys.* **244**, 373-381 (1986).
8. Bell, J. D., Buxton, I. L. & Brunton, L. L. *J. biol. Chem.* **260**, 2625-2628 (1985).
9. Seamon, K. B., Padgett, W. & Daly, J. W. *Proc. natn. Acad. Sci. U.S.A.* **78**, 3363-3367 (1981).
10. Bender, J. L. & Neer, E. J. *J. biol. Chem.* **258**, 2432-2439 (1983).
11. Yoshimasa, T. *et al. J. biol. Chem.* (submitted).
12. Pfeuffer, E., Drehev, R.-M., Metzfer, H. & Pfeuffer, T. *Proc. natn. Acad. Sci. U.S.A.* **82**, 3086-3090 (1985).
13. Cousson, F., Haiech, J., D'Alayer, J. & Monneron, A. *Proc. natn. Acad. Sci. U.S.A.* **82**, 6736-6740 (1985).
14. Pfeuffer, E., Mollner, S. & Pfeuffer, T. *EMBO J.* **4**, 3675-3679.
15. Yeager, R. E., Heideman, W., Rosenberg, G. B. & Storm, D. R. *Biochemistry* **24**, 3776-3783 (1985).
16. Smigel, M. D. *J. biol. Chem.* **261**, 1976-1982 (1986).
17. Seamon, K. B., Vaillancourt, R., Edwards, M. & Daly, J. W. *Proc. natn. Acad. Sci. U.S.A.* **81**, 5081-5085 (1984).
18. Sibley, D. R., Strasser, R. H., Caron, M. G. & Lefkowitz, R. J. *J. biol. Chem.* **260**, 3883-3886 (1985).
19. Jakobs, K. H., Bauer, S. & Watanabe, Y. *Eur. J. Biochem.* **151**, 425-430 (1985).
20. Katada, T., Gilman, A. G., Watanabe, Y., Bauder, S. & Jakobs, K. H. *Eur. J. Biochem.* **151**, 431-437 (1985).
21. Olianas, M. C. & Onali, P. *J. Neurochem.* **47**, 890-897 (1986).
22. Sugden, D., Vanecek, J., Klein, D. C., Thomas, T. P. & Anderson, W. B. *Nature* **314**, 359-361 (1985).
23. Nabika, T., Nara, Y., Yamori, Y., Lovenberg, W. & Endo, J. *Biochem. biophys. Res. Commun.* **131**, 30-36 (1985).
24. Sibley, D. R. & Lefkowitz, R. J. *Nature* **317**, 124-129 (1985).
25. Laemmli, U. K. *Nature* **227**, 680-685 (1970).
26. Bolton, A. E. & Hunter, W. M. *Biochem. J.* **133**, 529-530 (1973).
27. Uchida, T. & Filburn, C. R. *J. biol. Chem.* **258**, 12311-12314 (1984).

High-velocity microprojectiles for delivering nucleic acids into living cells

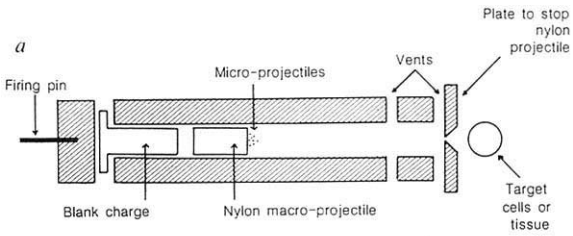
T. M. Klein*, E. D. Wolf†, R. Wu‡ & J. C. Sanford*

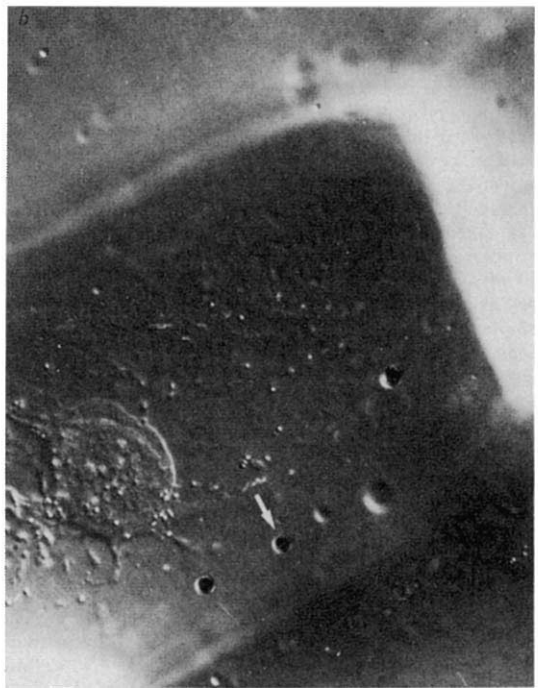
* Department of Horticultural Sciences, New York State Agricultural Experiment Station, Cornell Univesity, Geneva, New York 14456, USA
Departments of † Electrical Engineering and ‡ Biochemistry, Cornell University, Ithaca, New York 14453, USA

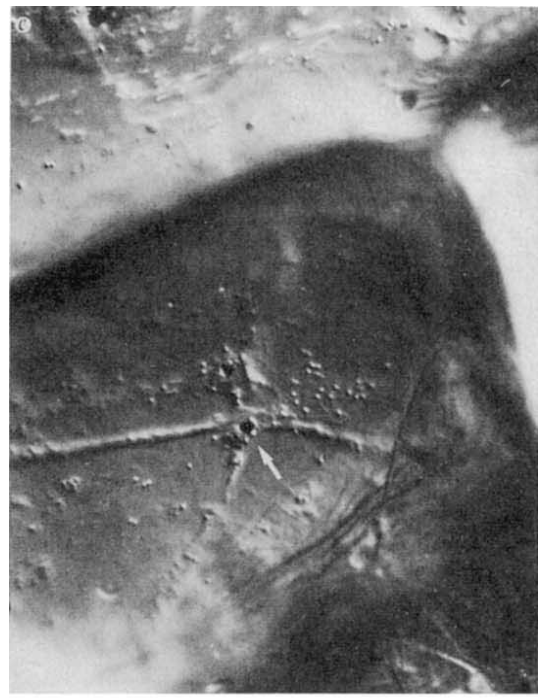
We report here a novel phenomenon, namely that nucleic acids can be delivered into plant cells using high-velocity microprojectiles. This research was conducted in the hope of circumventing some of the inherent limitations of existing methods for delivering DNA into plant cells[1–6]. After being accelerated, small tungsten particles (microprojectiles) pierce cell walls and membranes and enter intact plant cells without killing them. Microprojectiles were used to carry RNA or DNA into epidermal tissue of onion and these molecules were subsequently expressed genetically. This approach can therefore be used to study the transient expression of foreign genes in an intact tissue. It remains to be shown that smaller cell types, as are found in regenerable plant tissues, can be stably transformed by this method. If this proves possible, it would appear to provide a broadly applicable transformation mechanism capable of circumventing the host-range restrictions of *Agrobacterium tumefaciens*[1], and the regeneration problems of protoplast transformation[2–5].

Several devices for accelerating small particles to high velocities have been designed and tested[7]. The device (particle gun) illustrated in Fig. 1*a* was used to accelerate tungsten microprojectiles (spherical particles 4 μm in diameter) into intact epidermal cells of *Allium cepa* (onion) (Fig. 1*b* and *c*). Many cells can be bombarded simultaneously and about 90% of the cells in a 1-cm^2 of *A. cepa* epidermal tissue (~2,000 cells) typically contain microprojectiles following bombardment. Cells from *A. cepa* can survive penetration by many microprojectiles (Fig. 1*d*). However, the viability of cells (as determined by the maintenance of cytoplasmic streaming for at least 24 h after bombardment) is adversely affected by penetration by a large number of microprojectiles (Fig. 2).

To demonstrate that nucleic acid can be delivered into cells by this method, RNA isolated from tobacco mosaic virus (TMV) strain U_1 was adsorbed to the surface of 4-μm tungsten particles before their acceleration into *A. cepa* cells. Expression of the viral RNA was monitored by examining the bombarded cells microscopically for the presence of viral inclusion bodies (crystallized virus particles[8]) 3 days after treatment. Crystalline material, in the form of hexagonal plates, round plates and needles, was observed in the cytoplasm and vacuole of 30–40% of the cells that contained microprojectiles (Table 1). These distinctive crystalline inclusions were never observed in unbombarded tissue or in tissue bombarded with microprojectiles containing no nucleic acid. The observed percentage of cells expressing TMV following bombardment was comparable to







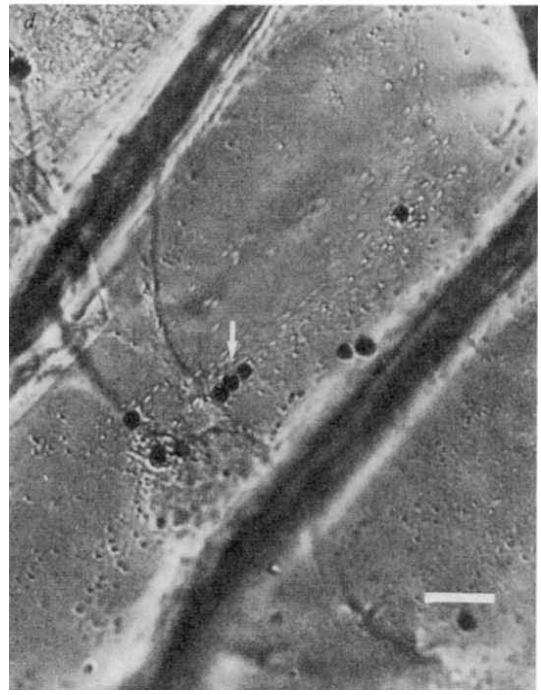


Fig. 1 *a*, Schematic diagram of the particle gun. About 0.05 mg of tungsten particles (average diameter 4 μm, General Electric Corp., Cleveland, Ohio) is placed on the front surface of a cylindrical nylon projectile (diameter, 5 mm; length, 8 mm) as a suspension in 1–2 μl of water. A gun powder charge (GY22AC, gray extra light, No. 1, Speed Fasteners Inc., St Louis, Missouri), detonated with a firing pin, is used to accelerate the nylon projectile down the barrel of the device. The tungsten particles continue toward the target cells through a small 1-mm aperture in a steel plate designed to stop the nylon projectile. The tungsten microprojectiles leave the particle gun with an initial velocity of about 430 m s^{-1}. This value was determined by allowing the nylon macroprojectile to leave the barrel of the device and estimating its velocity in-flight with a chronograph (Ohler Research, Austin, Texas). The target cells are placed ~10–15 cm from the end of the device. *b–d*, Nomarski micrographs of *A. cepa* epidermal cells following bombardment with tungsten microprojectiles 4 μm in diameter. The epidermal layer was stripped from the underlying bulb tissue before bombardment. *b*, Cell with 3 microprojectiles on its surface (one arrowed); *c*, microprojectile (arrowed) within the same cell as revealed by focusing 40 μm below the surface of the cell; *d*, Eight microprojectiles (one arrowed) in the interior of a living cell. Scale bar, 20 μm.

that reported for the delivery of TMV RNA into protoplasts by liposome uptake[9,10] or electroporation[11].

Using the particle gun, DNA can be delivered into intact plant cells, and this can result in transient expression of a foreign gene. Tungsten microprojectiles were coated with plasmid (p35S-CAT)[12] containing a gene that encodes chloramphenicol acetyltransferase (CAT). These microprojectiles were used to bombard 1-cm^2 sections of *A. cepa* epidermal tissue. Extracts from epidermal tissue bombarded with microprojectiles coated with p35S-CAT showed very high levels of CAT activity with most of the chloramphenicol being converted to its acetylated derivatives during the assay (Fig. 3). Strong CAT activity was detected in extracts from as little as a single 1-cm^2 piece of bombarded epidermal tissue (~2,000 cells) whereas control tissue showed negligible CAT activity.

These findings indicate that particle bombardment can be used to deliver RNA or DNA into large numbers of intact plant cells simultaneously and that the foreign nucleic acids introduced by this process can subsequently be expressed. The introduction of DNA into *A. cepa* epidermal tissue by the particle bombardment process should be a useful tool for the study of transient expression of foreign nucleic acids in plant cells. The process does not require cell culture or the pretreatment of the recipient tissue in any way and only small quantities of DNA are needed (about 0.1 μg per treatment). Because the preparation of particles and the bombardment of tissue can be rapid, many nucleic acid samples can be tested in a short period of time.

Particle bombardment may eventually prove useful for the transformation of other plant species and may be of particular

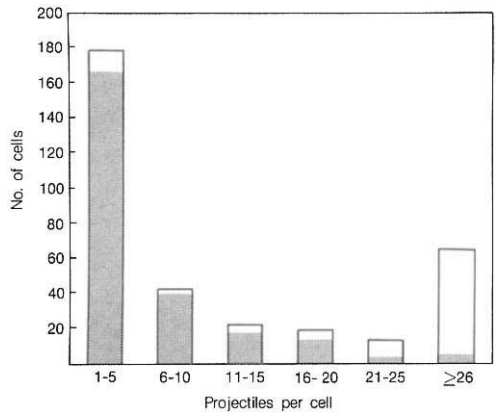


Fig. 2 Relationship between cell survival and the number of microprojectiles within a cell. Bars (solid lines), total cells; shaded part of bar, living cells. Three 1-cm^2 sections of *A. cepa* epidermal tissue were bombarded as described in Fig. 1. About 100 cells from each section were analysed microscopically for viability (based on the maintenance of cytoplasmic streaming) and the number of microprojectiles in these cells were counted. Data from the three sections of tissue were pooled.

Table 1 The delivery of TMV RNA to *A. cepa* cells by accelerated microprojectiles

Trial	Cells containing microprojectiles: without inclusions	with inclusions	Proportion penetrated cells with inclusion bodies (%)
1	44	34	43.6
2	85	35	29.2
3	168	98	36.8
4	257	76	22.8
5	147	52	26.1

A. cepa epidermal cells were scored for presence of TMV inclusion bodies following bombardment with microprojectiles to which TMV RNA has previously been adsorbed. About 50 microscope fields (×500) were observed within a 1-cm^2 area of tissue for each trial.

Methods. For adsorption of RNA to the tungsten microprojectiles, 2 μl of a TMV RNA[21] solution (2 μg of RNA per μl of distilled water) were added to 18 μl of a suspension of tungsten microprojectiles (10 mg of tungsten per ml of distilled water). The RNA was precipitated by the addition of 7.5 μl of 0.25 M $CaCl_2$ solution and the resulting suspension was centrifuged for 30 min at 13,000*g*. The particles were resuspended and 2 μl of the suspension was placed on the front surface of the nylon projectile. Centrifugation brings the RNA precipitate into close association with the tungsten surfaces and results in uniform adsorption which can be visualized by staining with the fluorescent dye DAPI[22]. Following bombardment the tissue was incubated for 3 days at 21 °C.

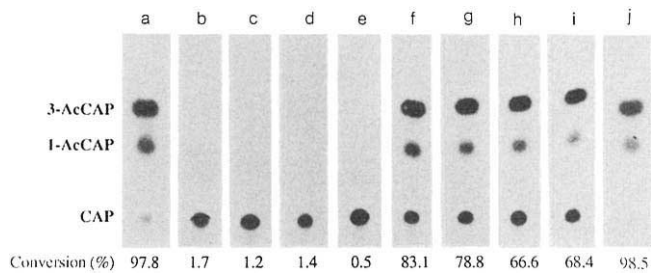


Fig. 3 CAT activity from *A. cepa* epidermal tissue bombarded with microprojectiles coated with plasmid harbouring the *CAT* gene. Lane a, positive control: a colony of *E. coli* carrying pBR325 was sonicated in 1 ml of buffer and centrifuged at 10,000*g* for 5 min. The supernatant (10 μl) was used for the CAT assay. Lane b, untreated epidermal tissue (10 pooled sections); lane c, epidermal tissue (10 pooled sections) bombarded with naked microprojectiles; lane d, epidermal tissue (10 pooled sections) bombarded with microprojectiles coated with pUC13. Lane e, microprojectiles (30 μl of a suspension prepared as described in the text) were rubbed over the surface of each of 10 sections of epidermal tissue which were pooled for the assay. Lane f–i, epidermal tissue (10 sections pooled for each assay) bombarded with microprojectiles coated with p35S-CAT; lane j: epidermal tissue (one section for the assay) bombarded with p35S-CAT. CAP, chloramphenicol; 3-AcCAP and 1-AcCAP, monoacetylated forms of chloramphenicol. The plasmid (p35S-CAT), which has been previously described[12], includes the following components: a 941-basepair (bp) region including the 35S promoter from cauliflower mosaic virus[23]; a 900-bp region encoding the CAT activity[24]; a 700-bp region carrying the 3′ noncoding sequences and the transcription termination region of the ribulose bisphosphate carboxylase gene and a derivative of the pUC13 plasmid in which the *Hin*cII site was changed to a *Cla*I site. The resulting construct is 5.3 kilobases (kb) long. The plasmid p35S-CAT was grown in *Escherichia coli*, isolated by phenol extraction, and purified by CsCl/ethidium bromide density-gradient centrifugation[25]. The microprojectiles were coated by adding 10 μl of the plasmid DNA (1.0 μg of DNA per μl of TE buffer, *p*H 7.7) to 100 μl of a suspension of tungsten microprojectiles (0.1 g of tungsten per ml of distilled water). The DNA was precipitated by the addition of 100 μl of a $CaCl_2$ solution (2.5 M) and 40 μl of a spermidine solution (free base, 0.1 M). The resulting suspension was centrifuged for 30 min at 13,000*g*. *A. cepa* epidermal tissue (1-cm^2 sections) was then bombarded with the DNA-coated microprojectiles (2 μl of the suspension placed in the middle of the front surface of the nylon macroprojectile). Assays of CAT activity were performed on extracts prepared from tissue that had been incubating for 3 days following bombardment with the microprojectiles. Tissue extracts were prepared by placing the epidermal sections in an Eppendorf tube (1.5 ml) with 100 μl Tris-HCl (0.25 M, *p*H 7.8) and grinding the tissue with a pellet pestle mixer (Kontes). CAT activity in the extracts was determined in 30-min assays as previously reported[26], except that the amount of ^{14}C-chloramphenicol was decreased to 0.1 μCi per assay[18]. All the radioactivity in the Eppendorf tube (~170,000 c.p.m. in each assay) was spotted on the thin-layer chromatography (TLC) plate (Chromagram, Kodak). After resolution of chloramphenicol and its acetylated derivatives by chromatography, an autoradiogram (12-h exposure) was made. Quantitative results were obtained by scintillation counting of separated spots of chloramphenicol and its acetylated derivatives, and the conversion calculated.

value for those species that cannot be genetically engineered successfully with existing techniques. Although several delivery systems have been used to transfer genes into various dicotyledonous plant species[1,2,13,14], these techniques have not yet been useful for the production of whole, transformed plants of graminaceous species such as rice, wheat, or corn. Although there is evidence that *Agrobacterium* can transfer DNA to *Zea mays*[15], *Asparagus*[16], *Chlorophytum* and *Narcissus*[17], the efficient use of this agent for the transfer of genes to monocots may be hampered by the restricted host range of the bacterium[1]. Genes have been transferred to protoplasts of the monocots *Triticum monococcum* and *Lolium multiflorum* by incubation with plasmid DNA[3,4] and to *Zea mays*[5] and *Oryza sativa*[18] by electroporation, but these techniques are restricted by the difficulties of regenerating whole plants from protoplasts of most monocot species[19,20]. The particle bombardment process should not suffer from the host-range limitations of infectious agents such as *Agrobacterium*, and the problems associated with systems that require regeneration of plants from protoplasts might be circumvented by bombarding regenerable tissues such as meristems or embryogenic callus with DNA-bearing microprojectiles. To accomplish this, the particle bombardment process must be refined so that cells much smaller (10–20 μm) than those of *A. cepa* epidermal tissue can be penetrated by microprojectiles.

This work was supported by grants from the Cornell Biotechnology Program, the US Department of Agriculture, and the Rockefeller Foundation. We thank N. Allen for input on the

design of the particle gun and its construction, M. Zaitlin and M. Nishiguchi for TMV RNA, and M. McCann for technical assistance with the CAT assays.

Received 7 January; accepted 2 March 1987.

1. Fraley, R. T., Rogers, S. G. & Horsch, R. B. *CRC crit. Rev. Pl. Sci.* **4**, 1-46 (1986).
2. Steinbiss, H. H. & Broughton, W. *Int. Rev. Cytol. (Suppl.)* **16**, 191-207 (1983).
3. Lörz, H., Baker, B. & Schell, J. *Molec. gen. Genet.* **199**, 178-182 (1985).
4. Potrykus, I., Saul, M. V., Petruska, J., Paszkowski, J. & Shillito, J. R. D. *Molec. gen. Genet.* **199**, 183-188 (1985).
5. Fromm, M., Taylor, L. P. & Walbot, V. *Proc. natn. Acad. Sci. U.S.A.* **82**, 5824-5828 (1985).
6. De la Peña, A., Lörz, H. & Schell, J. *Nature* **325**, 274-276 (1987).
7. Sanford, J. C., Klein, T. M., Wolf, E. D. & Allen, N. *Particle Sci. Technol.* (in the press).
8. Christie, R. G. & Edwardson, J. R. *Light and Electron Microscopy of Plant Virus Inclusions, Florida Agric. Exp. Station Monograph Ser. 9* (University of Florida, Gainesville, 1977).
9. Fukunaga, Y., Nagata, T. & Takebe, I. *Virology* **113**, 752-760 (1981).
10. Fraley, R. T., Dellaporta, S. L. & Papahadjopoulos, D. *Proc. natn. Acad. Sci. U.S.A.* **79**, 1859-1863 (1982).
11. Nishiguchi, M., Langridge, W. H. R., Szalay, A. A. & Zaitlin, M. *Pl. Cell Rep.* **5**, 57-60 (1986).
12. Morelli, G., Nagy, F., Fraley, R. T., Rogers, S. G. & Chua, N.-H. *Nature* **315**, 200-204 (1985).
13. Crossway, A. *et al. Molec. gen. Genet.* **202**, 179-185 (1986).
14. Brisson, N. & Hohn, T. *Meth. Enzym.* **118**, 659-668 (1986).
15. Grimsley, N., Hohn, T., Davies, J. W. & Hohn, B. *Nature* **325**, 177-179 (1987).
16. Hernalsteens, J.-P., Thia-Toong, L., Schell, J. & Van Montagu, M. *EMBO J.* **3**, 3039-3041 (1984).
17. Hooykaas-Van Slogteren, G. M. S., Hooykaas, P. J. J. & Schilperoort, R. A. *Nature* **311**, 763-764 (1984).
18. Ou-Lee, T. M., Turgeon, R. & Wu, R. *Proc. natn. Acad. Sci. U.S.A.* **83**, 6815-6819 (1986).
19. Vasil, I. K. *Int. Rev. Cytol. (Suppl.)* **16**, 79-88 (1983).
20. Potrykus, I. & Shillito, R. F. *Meth. Enzym.* **118**, 549-578 (1986).
21. Zaitlin, M. in *Nucleic Acids in Plants* Vol. 2 (eds Hall, T. C. & Davis, J. W.) 31-64 (CRC Press, Boca Raton, Florida, 1979).
22. Coleman, A. W. & Goff, J. L. *Stain Technol.* **60**, 145-154 (1985).
23. Hohn, T., Richards, K. & Lebeurier, G. *Current Topics Microbiol. Immun.* **96**, 193-236 (1982).
24. Alton, N. K. & Vapnek, D. *Nature* **282**, 864-869 (1979).
25. Maniatis, T., Fritsch, E. F. & Sambrook, J. *Molecular Cloning, a Laboratory Manual* (Cold Spring Harbor Laboratory, New York, 1982).
26. Gorman, C. M., Moffat, L. F. & Howard, B. H. *Molec. cell. Biol.* **2**, 1044-1051 (1982).

Determination of bacteriophage λ tail length by a protein ruler

Isao Katsura

Department of Biophysics and Biochemistry, Faculty of Science, University of Tokyo, Hongo, Tokyo, Japan

How the size and shape of living structures are determined by genetic information is one of the fundamental problems in biology. Here I describe a study in which the size of a biological supramolecular structure was changed in a predictable way by *in vitro* genetics, with the size both before and after manipulation being exactly determined. I have studied the tail of bacteriophage λ, whose length is determined by the length of the 'ruler protein', the product of gene *H*. The length of the tail can be decreased or increased by deleting the middle part of gene *H* or by forming a small duplication there, and the length of the tail is proportional to the size of the protein. These results can be regarded as a special case of protein engineering[1], namely supramolecular protein engineering.

Previous work[2] has shown that some λ mutants *in vivo* that have small in-frame deletions in gene *H* produce viable phage particles with a tail slightly shorter than wild-type. This proves that gene *H* determines the tail length and gives strong evidence for a scheme in which its product (abbreviated as gpH) is a ruler that measures length during tail assembly. The question arose from that work regarding the structure-function relationship of gpH, which has at least three functions: tail assembly, tail-length determination and DNA injection[2]. It also led to interest in the relationship between the length of gpH and the length of the tail, and whether it is possible to change the tail length over a much wider range of sizes.

In this study, deletion and duplication mutants in gene *H* of λ phage were isolated in the following way. First, a fragment of λ DNA containing gene *H* was cloned in the plasmid pBR322 (ref. 3). Then, either a deletion (of one of a number of sizes)

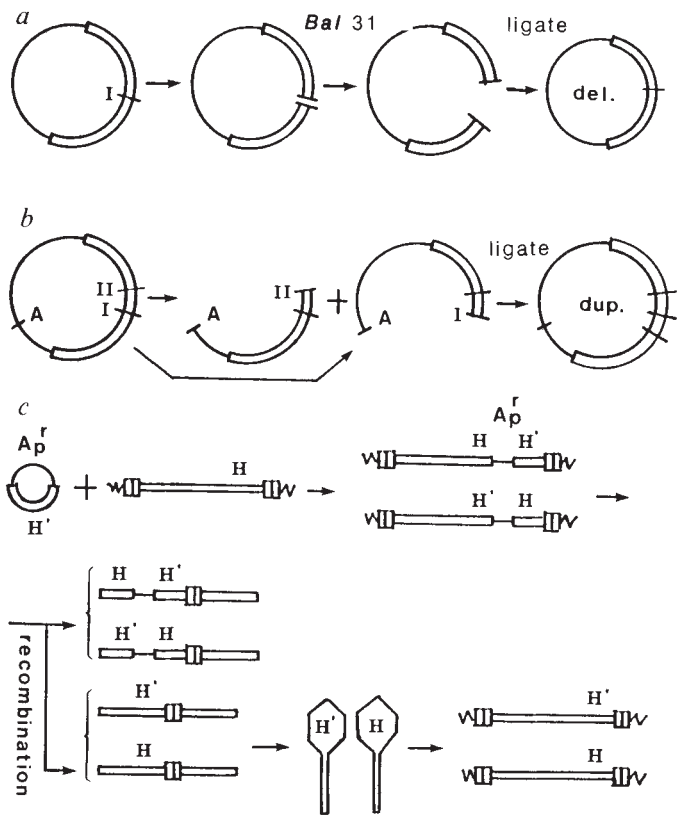


Fig. 1 Production of deletion (del.) and duplication (dup.) mutants in gene *H* of λ phage by *in vitro* mutagenesis. *a*, Production of deletions in cloned gene *H*. The *Bcl*I fragment (base numbers 9,361-13,820[9]) of λ DNA containing gene *H* was inserted into the *Bam*HI site of pBR322 (ref. 3), where the direction of the λ sequence in the hybrid plasmid was counter-clockwise on the conventional map of pBR322. Then it was cleaved at the unique *Hpa*I site (I in the figure), digested to various extents with *Bal*31 exonuclease, and self-ligated with T4 DNA ligase. *b*, Production of a duplication in cloned gene *H*. The 5.5-kilobase (kb) fragment from the same hybrid plasmid cleaved with *Hpa*I (I) and *Ava*I (A) and the 3.8-kb fragment from cleavage with *Pvu*II (II) and *Ava*I were purified by electrophoresis in an 0.7% agarose gel and joined together with T4 DNA ligase. *c*, Introduction of cloned gene *H* having a deletion or duplication mutation (*H'*) into the λ genome. Cells of *E. coli* HI97 *polA supF* (λ*c*Its857 *S*am7) were transformed with the plasmids shown in *a* and *b* using selection for ampicillin resistance (*Ap*^{r}). (They were plated with tryptone top agar on LA plates with magnesium[5] containing also 50 μg ml^{-1} ampicillin and incubated for 24 h at 32 °C.) Because these plasmids, having the replication origin of ColE1[3], cannot replicate in *E. coli polA* (ref. 11), the transformants were those in which the plasmids were inserted by homologous recombination into gene *H* of the λ prophage (right-hand top row of *c*). The efficiency of transformation was about 10 colonies per μg DNA. The ampicillin-resistant lysogens were cultured in M9A medium[5] and subjected to heat-induction of the prophage. The λ DNA having the plasmid insertions (at top left in the second row of *c*) was too long to be packaged into λ phage particles. The packaged DNA molecules were those from which the inserted plasmids had been excised by homologous recombination. Through insertion and excision the wild-type gene *H* of the original λ genome was replaced by the mutated gene *H* of the plasmids with a certain probability. Therefore, the lysates contained both wild-type and *H* deletion (or duplication) phages (bottom left on the second row of *c*). *E. coli* W3350 Su$^-$ was lysogenized by these phages. Lysogens having the *H* deletion or *H* duplication prophage were distinguished from those having wild-type prophage by their inability to produce active phage after heat induction. It is not completely excluded that phages having a deletion or duplication in gene *H* may form plaques, but none of the phages of this study did so. (See ref. 12 for detailed methods of genetic engineering and ref. 5 for the test of defective prophage mutants.)