by CsCl-isodensity centrifugation. After growth on C600 r_k^- m_k^- new progeny from the B1 monomer clone were again analyzed by centrifugation on CsCl gradients. Only monomers were observed, indicating that the monomer population was stable and that amplification did not occur. In contrast, the B1 trimer clone contained, after growth on C600 r_k^- m_k^-, a mixture of monomers and trimers. Monomers were thus obtained from trimers by excision and recombination. We have, in addition, observed that the size of the plaques from monomer and trimer clones vary depending on the bacterial strain used for plating (Table 1). When plated on strain C600 r_k^- m_k^- Rec B^-C^-, monomers always give minute plaques, an observation that can be used for cloning monomer fragments. Since transfection with the hybrid DNA was initially carried out on this strain (*1*) it is not unexpected that multimers of the inserted fragment were isolated.

We may conclude from these observations that insertion of multimers of DNA fragments into the genome of λ leads to hybrids that can recombine within the foreign fragment. This recombination can be inter- or intramolecular (*5*). The rapidity of the conversion toward a population in which monomers are predominant suggests that intramolecular recombination is favored. Since the phage is Red^-, the bacterial Rec system is probably responsible for the recombination. Thus the bacterial recombination system works normally for DNA from a eukaryotic virus. This mechanism, if it exists, may yield adenovirus DNA circles. Our failure to observe circular DNA can be explained by the absence of a replication origin in the fragment which, in the present case, eliminates plasmid multiplications.

The experiments to construct the hybrid DNA were performed in a P3 laboratory in 1975 according to the Asilomar guidelines. Biohazards associated with the experiments described in this report have been examined previously by the French National control committee. The ultracentrifuge is located inside the P3 facility.

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References and Notes

1. P. Tiollais *et al.*, *Gene* **1**, 49 (1976).
2. A. Rambach and P. Tiollais, *Proc. Natl. Acad. Sci. U.S.A.* **71**, 3927 (1974).
3. J. Sambrook, J. Williams, P. A. Sharp, T. Grodzicker, *J. Mol. Biol.* **97**, 369 (1975).
4. M. Perricaudet and P. Tiollais, *FEBS Lett.* **56**, 7 (1975).
5. A. J. D. Bellett, H. G. Busse, R. L. Baldwin, in *The Bacteriophage Lambda*, A. D. Hershey, Ed. (Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y., 1971), pp. 501–513.
6. P. A. Sharp, P. H. Gallimore, J. Flint, *Cold Spring Harbor Symp. Quant. Biol.* **39**, 457 (1974).

28 February 1977

Cloning of Cauliflower Mosaic Virus (CLMV) DNA in *Escherichia coli*

Abstract. *A plasmid containing cauliflower mosaic virus DNA can be faithfully cloned in* Escherichia coli, *but proved to be noninfective in test plants.*

The selection of novel plants having beauty or utility may be one of man's oldest activities (*1*). A major limitation to the formation of novel plant genomes is the fact that only related species can be crossed. While it is unreasonable to expect that the removal of the mating barrier will lead directly to the survival of plants containing arbitrarily constructed genomes, this barrier must be circumvented if these novel genetic systems are to be further explored.

One possible way to avoid the mating barrier is by the fusion of protoplasts and the subsequent growth of adult plants from the fused cells (*2*). Another is by the introduction of foreign genes directly. This route presents many attractive possibilities, but the frequency of genetic transformation in plants is low and the results up to now are highly variable (*3*). The introduction of genes into plant cells would be greatly facilitated if these DNA segments were included as part of a replicon that had the ability to replicate within the recipient cell. Such transduction experiments have been done by using a specifically deleted SV40 DNA into which bacterial or phage genes have been inserted (*4*). It is not unreasonable to expect that desirable genes can be introduced into plants by this route. Indeed, the only known beneficial virus to date infects tulips to produce variegated flowers of great beauty (*5*).

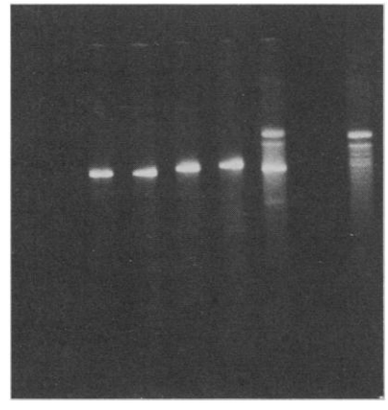

Fig. 1. Sal I cleavage pattern of CLMV DNA. About 1 μg of DNA was incubated with different levels of Sal I in 50-μl reaction volumes containing 60 m*M* tris, *p*H 7.6, 50 μg of BSA per milliliter, 5 m*M* $MgCl_2$, and 0.14*M* NaCl at 37°C for 3 hours. The reaction was terminated by the addition of 5 μl of bromphenol blue in 80 percent glycerol containing 0.1*M* EDTA. The sample was subjected to electrophoresis through 1 percent agarose gels in tubes, treated with fluorochrome, and photographed as described (*10*). The first four gels, from left to right, are CLMV DNA cleaved with different amounts of Sal I: gel 1, 1 unit; gel 2, 3 units; gel 3, 5 units; gel 4, 10 units; gel 5, uncleaved CLMV; gel 6, uncleaved CLMV—a fresh preparation.

The caulimoviruses, of which cauliflower mosaic virus (CLMV) is a prominent example, are the only plant viruses known to contain DNA (*6*). Therefore, in spite of its limited host range, the lack of a local lesion assay, and relatively weak infectivity, this viral DNA represents one of the few possible transducing vehicles for the introduction of foreign DNA segments into plant cells.

As a first step in developing this viral DNA as a transducing vehicle, we have formed a recombinant plasmid and cloned it in *Escherichia coli*. In a reversal of this experiment, we attempted to see whether the recombinant plasmid was replicated in plants. The results indicate that the first experiment was successful (although the cloned DNA could not be demonstrated to be infective to plants). The second experiment gave inconclusive results to be mentioned later. Since this work has now been halted for nonscientific reasons, we report on progress to date.

CLMV virus particles were isolated by the method of Shepard (*7*). Two milliliters of virus containing 0.2 to 0.3 μg of virus were mixed with 0.8 ml of proteinase K (600 μg/ml) (EM Laboratories) plus 6 percent sarkosyl in 0.25*M* EDTA, *p*H 8.0, which had been incubated at 37°C for 1 hour before use. After 16 hours at 37°C, the solution was extracted with one volume of redistilled phenol that was neu-

tralized with 1.0*M* tris, *p*H 9.0. The aqueous phase was extracted with an equal volume of $CHCl_3$, then dialyzed against 10 m*M* tris, 1 m*M* EDTA, *p*H 8.0. When examined in the electron microscope, mostly relaxed rings about 7500 base pairs in length were seen along with some linear and twisted structures. In 1.0 percent agarose gels a variety of bands with the major components corresponding to relaxed rings and linear molecules were observed. This distribution varies from preparation to preparation and changes with storage. However, when the preparations were treated with Sal or Bam endonucleases, a single band appeared having the mobility of a linear molecule about 7500 base pairs long (Fig. 1). In addition, there were some fainter bands in much less than molar yields, and these probably arose from variants purified with the predominant species. The mobility of the Sal and Bam products appear identical. The linearity of these molecules was confirmed by electron microscopy. We conclude that there is a single Sal and a single Bam site, but cannot rule out the unlikely possibility that both Sal and Bam have two sites equally spaced less than a few hundred base pairs apart.

In order to clone CLMV/Sal we used a composite plasmid, pGM706 (*8*), carrying both ampicillin and tetracycline resistance and a single Sal site. Insertion of DNA at this site inactivates the plasmid-specific tetracycline resistance. The CLMV/Sal was ligated to pGM706/Sal, and the reaction mixture was used to transform Ca^{2+}-treated GM4 (*E. coli* K12 r_B^- m_B^- *rec* A^-). The transformation mixtures were distributed to a large number of tubes from which multiple plates con-

Table 1. The length (in kilobase pairs) of various restriction segments of CLMV/Sal I and CL DNA based on the known lengths of λvir/RI and λvir/Hae (*10*). The bands in parentheses are considered minor because they are present in substantially less than molar yields.

Segments	CLMV/Sal I	CLS4	CLS8
Eco RI (1.4 percent agarose)			
1	5.0	5.0	5.0
1a (minor)	(4.9)		
2	2.4	2.4	2.4
2a (minor)	(1.8)		
3	0.5	0.5	0.5
	7.9	7.9	7.9
Bam (1.4 percent agarose)			
1	5.5	5.5	5.5
1a (minor)	(4.2)		
2	2.4	2.4	2.4
7.9	7.9	7.9	7.9

Segments	CLMV/Sal I	CLS4	CLS8
Hind II + III (2.4 percent agarose)			
1	1.69	1.69	1.69
2	1.25	1.25	1.25
3	1.14	1.14	1.14
4	0.86	0.86	0.86
5	0.61*	0.60*	0.60*
6	0.59	0.59	0.59
7	0.51*	0.48*	0.48*
8	0.39	0.39	0.39
	7.04	7.00	7.00
(Some small fragments have run off the gel.)			

Segments	CLMV/Sal I	CLS4	CLS8
Hae III (3.4 percent agarose)			
1	1.30	1.30	1.30
2	1.24*	1.23*	1.23*
3	1.13	1.13	1.13
4	0.99*	0.98*	0.98*
5	0.92	0.92	0.92
6	0.88	0.88	0.88
6a	(0.67)		
7	0.58	0.58	0.58
8	0.53	0.53	0.53
	7.57	7.55	7.55
(Perhaps some small segments cannot be seen.)			

*The CLS4 and CLS8 bands have slightly different mobilities from asterisked CLMV/Sal I bands.

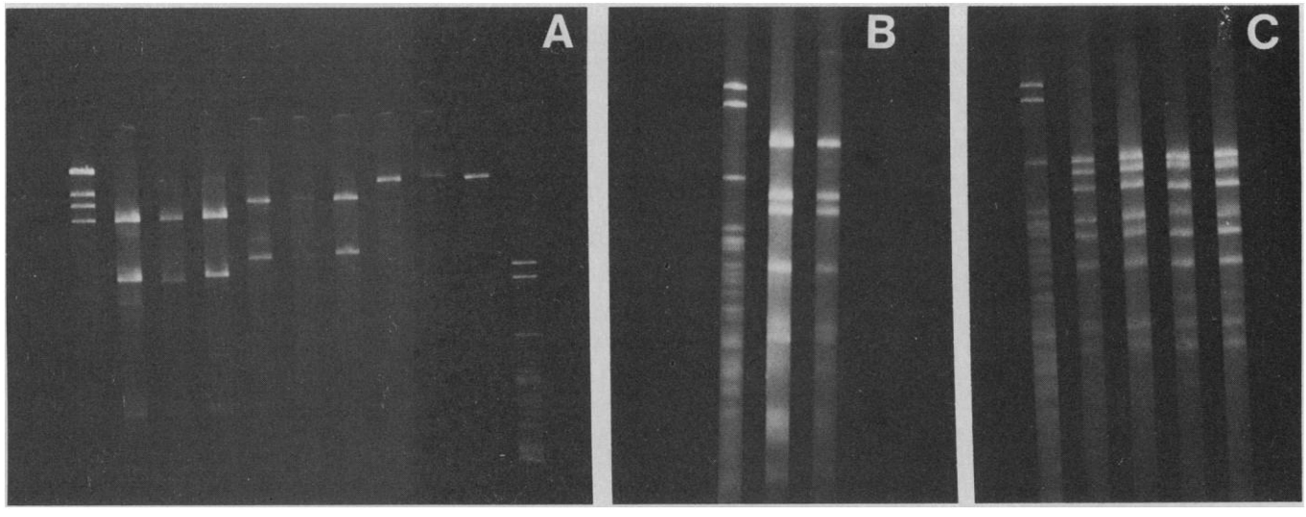

Fig. 2. Agarose gel electrophoresis of restriction segments obtained from cauliflower mosaic virus DNA and from two clones of this same DNA amplified in *E. coli* K. (A) Eco RI, prepared from *E. coli* RY13 by the procedure of Greene *et al.* (*11*), and Bam, prepared from *Bacillus amyloliquefaciens* by the procedure of Wilson and Young (*12*), was allowed to act on 500 ng of purified DNA's in 50-μl reaction volumes containing 50 m*M* NaCl, 10 m*M* $MgCl_2$, 50 m*M* tris, *p*H 7.6, at the level of 1 unit of enzyme per 1 μg of DNA for 3 hours at 37°C. At the end of this incubation, 5 μl of bromphenol blue in 80 percent glycerol and 0.1*M* EDTA was added and the sample was subjected to electrophoresis through 1.4 percent agarose gels in tubes, treated with fluorochrome, and photographed as described (*13*). The gels from left to right are: gel 1, λvir/Eco RI; gel 2, CLMV/Eco RI; gel 3, CLS4/Eco RI; gel 4, CLS8/Eco RI; gel 5, CLMV/Bam; gel 6, CLS4/Bam; gel 7, CLS8/Bam; gel 8, CLMV/Sal I; gel 9, CLS4; gel 10, CLS8; gel 11, λvir/Hae. The lettering scheme here refers to the origin of the DNA or the type of restriction endonuclease. For example, CLMV or λvir refers to DNA isolated from virus particles; CLS4 refers to the DNA segment obtained from the purified plasmid pCLS4, after treatment with Bam and recovery of the CL segment from agarose gels. The restriction endonucleases are abbreviated as RI (for Eco RI) Hind (for Hind II + III) and Hae (for Hae III). (B) Hind II + III, prepared from *Haemophilus influenzae* by the procedure of Danna *et al.* (*13*) was incubated under the conditions described in (A) in 50-μl mixtures containing 50 m*M* NaCl, 6 m*M* $MgCl_2$, 6 m*M* tris, *p*H 7.6, BSA (100 μg/ml), and DNA (50 μg/ml). Gel 1, λvir/Hae; gel 2, CLMV/Hind; gel 3, CLS4/Hind. CLS8 responds in an identical manner to CLS4. (C) Hae III, prepared from *Haemophilus aegypticus* (*14*), was incubated as described in (A) and (B) in mixtures containing 7 m*M* $MgCl_2$, 7 m*M* tris, *p*H 7.4, and 7 m*M* β-mercaptoethanol. Gel 1, λvir/Hae, gel 2, CLS4/Hae; gel 3, CLS8/Hae; gel 4, CLMV/Hae; gel 5, CLS4/Hae + CLMV/Hae.

Table 2. Infectivity of DNA. The source of DNA is abbreviated as described in the legend to Fig. 2A. In the case of pCLS4, we mean covalently closed rings. The pCLS4/Sal I refers to this DNA treated with Sal I to produce a mixture of two linear molecules: the plasmid vehicle, p, and the cloned segment CLS4. In the case of lines 6 and 7 these linear segments were religated with T4 DNA ligase (gift of Dr. F. Asubel) to produce 30 percent and 60 percent rings, some of which may be nicked. These were assayed by scanning gels stained with ethidium bromide. Leaves of *Brassica* plants (*Brassica campestris*, "Tendergreen mustard" c.v. Burpee's seed) were washed with sterile 0.15*M* NaCl containing 0.015*M* sodium citrate, *p*H 7.0 (SSC), and then treated with DNA in SSC. Two young leaves were inoculated with carborundum on each plant, each with about half the amount of DNA shown. After 1 hour, the treated leaves were washed with sterile distilled water. The affected plants show an altered pattern of pigmentation and other symptoms that generally allow unambiguous scoring after 1 month (*7*).

DNA source	Amount (μg)	Plants infected/ inoculated
1. CLMV	10.0	6/9
	0.1	4/9
2. CLMV/Sal I	3.0	0/3
	1.0	0/6
3. pCLS4	10.0	0/6
	1.0	0/6
4. pCLS4/Sal I	10.0	0/3
	1.0	0/3
5. pML21	20.0	0/2
	10.0	0/4
6. CLS4/Sal I/religated (30 percent rings)	10.0	0/6
	1.0	0/6
7. CLMV/Sal I/religated (60 percent rings)	10	0/4
	1	0/9

taining ampicillin (30μg/ml) (Wyeth) were prepared. Many ampicillin resistant colonies were selected and tested on plates containing tetracycline (25 μg/ml) (Lederle). In this way, 13 amp^R tet^S clones which were derived from five separate tubes were selected, and therefore must contain at least five independent transformants. Plasmid DNA was prepared from all 13 strains by the Brij-lysis procedure (*9*); gel analysis before and after cleavage by Sal showed that each plasmid contained a long insert that proved to be the same length as CLMV/Sal. Further digestion by Bam and Eco RI gave identical patterns. Two strains, pCLS4 and pCLS8, were selected and plasmid DNA was purified by phenol extraction, ethanol precipitation, ethidium bromide–CsCl banding, and dialysis.

We attempted to clone CLMV/Bam by an equivalent procedure involving pGM 439 (*8*). However, the plasmid DNA's showed that none contained inserts characteristic of intact CLMV.

The plasmid DNA's pCLS4 and pCLS8 were each cleaved by Sal and subjected to electrophoresis in agarose tubes (1 cm in diameter); the zone containing the inserted viral segment, now called CL, was dissolved in saturated KI and the DNA was recovered on hydroxyapatite. These DNA's were cut by Eco RI, Bam, Hind, and Hae endonucleases. The results (Fig. 2 and Table 1) indicate that CLS4 and CLS8 have identical restriction segments and that these are nearly identical, with the same restriction segments formed from CLMV/Sal. Some possible differences are marked by the asterisks in Table 1.

Infectivity studies on "Tendergreen" mustard plants were performed as described (Table 2). In each case, CLMV DNA itself displayed infectivity. In contrast, CLMV DNA that was cleaved by Sal showed no infectivity even after religation. Likewise, the clonally amplified CLS4 showed no infectivity even after religation. Although the reason for this lack of infectivity is not known, some possibilities might be mentioned. (i) Perhaps there are closely spaced Sal sites, and the small segment between them is lost. (ii) Perhaps CLMV, like certain other plant viruses, has a bipartite genome, in which case two virus particles would be required to establish infection. Perhaps the minor bands mentioned above are derived from some helper virus. (iii) Perhaps the native CLMV DNA has some crucial secondary structure that is lost on cleavage or state of modification that is not present in the cloned material.

Passage experiments were initiated in leaf homogenates in an effort to determine whether the pCLS4 was replicated in the plant or acquired the ability to cause symptoms. Again no special symptoms could be observed, even after three and four passages.

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References

1. A. Leroi-Gourhan, *Science* **190**, 562 (1975); C. D. Darlington, *The Evolution of Man and Society* (Simon and Schuster, New York, 1969).
2. J. B. Power *et al.*, *Nature* **263**, 500 (1976); G. Melchers and G. Labib, *Mol. Gen. Genet.* **135**, 277 (1974); P. S. Carlson, H. H. Smith, R. Dearing, *Proc. Natl. Acad. Sci. U.S.A.* **69**, 229 (1972).
3. L. Ledoux, *Genetic Manipulations with Plant Material* (Plenum, New York, 1975).
4. D. H. Hamer, D. Davoli, C. A. Thomas, Jr., G. C. Fareed, *J. Mol. Biol.*, in press; S. P. Goff and P. Berg, in preparation.
5. R. J. Dubos, in *Perspectives in Virology*, M. Pollard, Ed. (Wiley, New York, 1959), pp. 291–299.
6. R. J. Shepherd, *Adv. Virus Res.* **20**, 305 (1976).
7. P. P. Pirone, G. S. Pound, R. J. Shepherd, *Phytopathology* **51**, 540 (1961); R. J. Shepherd, R. J. Wakeman, R. R. Romanko, *Virology* **36**, 150 (1968); R. J. Shepherd, G. E. Bruenning, R. J. Wakeman, *ibid.* **4**, 339 (1970).
8. D. H. Hamer and C. A. Thomas, Jr., *Proc. Natl. Acad. Sci. U.S.A.* **73**, 1537 (1976).
9. D. B. Clewell and D. R. Helinski, *ibid.* **62**, 1159 (1969).
10. D. H. Hamer and C. A. Thomas, Jr., *Chromosoma* **49**, 243 (1975).
11. P. J. Greene, M. C. Betlack, H. W. Boyer, H. M. Goodman, *Methods Mol. Biol.* **9**, 87 (1974).
12. G. A. Wilson and F. E. Young, *J. Mol. Biol.* **97**, 123 (1974).
13. K. J. Danna, G. H. Sack, D. Nathans, *ibid.* **78**, 363 (1973).
14. J. H. Middleton, M. H. Endgell, C. A. Hutchinson, *J. Virol.* **10**, 42 (1972).

14 February 1977

The Effects of *Escherichia coli* and Yeast DNA Insertions on the Growth of Lambda Bacteriophage

Abstract. *The effects of Eco RI endonuclease-cleaved* Escherichia coli *and yeast (*Saccharomyces cerevisiae*) DNA fragments on the propagation of the lambda bacteriophage vectors containing them were determined on a nonmutated and a PolA* E. coli *K12 host. Observable alterations in the growth of hybrids containing yeast DNA insertions were less frequent and less extreme than those seen in hybrids containing* E. coli *DNA. A lambda–*E. coli *hybrid was selected after extensive growth on the PolA (deficient in polymerase I) host which also grew very well on the PolA$^+$ host and may have resulted from some alteration in the hybrid. Hybrids selected on the PolA host gave no evidence for the expression of polymerase I activity. No lambda-yeast hybrid made from the λgt vector lacking lambda-specific recombination (*red$^-$*) had a yield of viable bacteriophage on infection greater than two-thirds that of "wild-type" lambda.*

Recent work has focused on the preparation in vitro of viable recombinant DNA molecules containing eukaryotic or prokaryotic DNA joined to a self-replicating prokaryotic vector DNA. An important concern in these experiments is the effect of the inserted DNA on the propagation of the vector in which it is inserted. A detrimental effect is of interest from the experimental standpoint because this may lead to difficulty in preparation, complete loss of the hybrid, or selection of a sequence alteration in the hybrid DNA which allows more efficient propagation. Any effect on growth of the hybrid is of importance since it might be indicative of expression of some function by the inserted DNA in the bacterial cell.