Xenopus. Their data suggest a model in which identical tRNA genes are arranged in tandem, interspersed by spacer DNA. Our results show a different principal of organization in yeast.

Our data also suggest that the frequency of occurrence of the different tRNA genes on the yeast genome may vary extensively from one tRNA to another. Table 2 shows that, whereas nine clones hybridize with $tRNA_3^{Leu}$, only two hybridize with $tRNA^{Phe}$.

It is obviously of importance to analyze and compare the DNA from various of our clones. In doing so, we may be able to answer the following questions:

1) For a set of clones carrying genes for a particular tRNA, what is the conserved sequence—is it confined to the structural gene or does it contain auxiliary information?

2) Do iso- or heteroclusters of tRNA genes exist, and if so what is their organization and mode of transcription?

JACQUES S. BECKMANN
PETER F. JOHNSON
JOHN ABELSON
Department of Chemistry, University of California, San Diego, La Jolla, 92093

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28. We would like to thank our colleagues listed in Table 2 for sending us numerous pure species of tRNA. Drs. P. Piper and H. Feldman supplied us with valuable information used in identifying yeast tRNA fingerprints. We thank Dr. Peter Geiduschek for his continuous interest in our work. Supported by NCI grant CA 10984.

24 February 1977

Excision and Recombination of Adenovirus DNA Fragments in *Escherichia coli*

A hybrid between the bacteriophage λ genome and the adenovirus type 2 (Ad2) Eco RI-B DNA fragment has been constructed in vitro (*1*). The Ad2-Eco RI-B fragment was inserted as monomers in two directions in the clones analyzed; but in some cases we observed multimers of the inserted fragment in the λ phage vector (*1*). We now report that during growth in *Escherichia coli* recombination may occur within multiple adenovirus DNA fragments of the λ vector.

The vector used, obtained from λ2Pam (*1*, *2*) contains all the essential genes of the phage and has a 26 percent deletion in the middle of the genome. The adenovirus Eco RI-B DNA fragment was purified by electrophoresis in polyacrylamide slab gels. Since the Eco RI endonuclease cleavage sites are symmetrical, a DNA fragment can be inserted in two directions. The resulting two hybrids containing the Ad2-Eco RI-B DNA fragment can be distinguished since there is a

Table 1. Plaque morphology of λ-Ad2-Eco RI-B hybrids plated on different strains.

Clone	C600 $r_k^- m_k^-$	C600 $r_k^- m_k^-$ Rec $B^- C^-$	C600 $r_k^- m_k^-$ Rec A^-
λ-Ad2-Eco RI-B4			
Monomer	Normal	Minute	Normal
Trimer	Normal	Normal	Normal
λ-Ad2-Eco RI-B7			
Monomer	Normal	Minute	Normal
Trimer	Normal	Normal	Normal
λ-Ad2-Eco RI-B1			
Monomer	Normal	Minute	Normal
Trimer	Normal	Normal	Normal

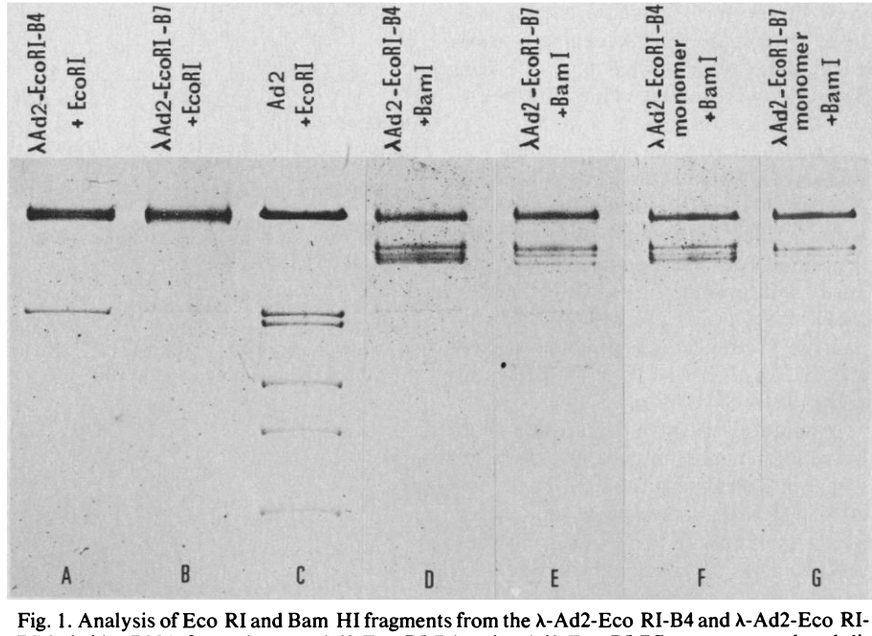

Fig. 1. Analysis of Eco RI and Bam HI fragments from the λ-Ad2-Eco RI-B4 and λ-Ad2-Eco RI-B7 hybrids. DNA from clones λ-Ad2-Eco RI-B4 and λ-Ad2-Eco RI-B7 was extracted and digested with endonucleases Eco RI and Bam HI (*1*). The DNA fragments were analyzed by electrophoresis in a slab gel containing a linear gradient of polyacrylamide from 2.5 to 7.5 percent (*1*). The Eco RI fragments of Ad2 DNA were used as size markers.

Bam HI endonuclease cleavage site close to one end of the Ad2-Eco RI-B fragment (*3*). There is also a Bam HI cleavage site close to the left end of the right arm of the λ2Pam vector (*4*). Consequently, after digestion of the hybrid DNA with Bam HI endonuclease two different cleavage patterns were observed after electrophoresis (*1*). When several clones were analyzed, an additional fragment was observed in some clones; this fragment comigrates with the Ad2-Eco RI-B fragment (in Fig. 1, compare D and E with C, and with A and B). This pattern suggests that some hybrids contain several Ad2-Eco RI-B fragments and that, at least, two adjacent fragments were inserted in the same direction.

The electrophoretic analysis does not allow us to determine the number of inserted fragments, or to establish whether the DNA analyzed comes from a homogenous or heterogenous phage population.

The hybrid phage λ-Ad2-Eco RI-B4 was grown on strain C600 r_k^- m_k^- and the phage preparation was analyzed by preparative isopycnic centrifugation in CsCl gradients. Three phage populations that varied in buoyant densities were observed (Fig. 2A). The buoyant density difference between the central and the two extreme phage bands are equal and correspond (*5*) to a difference in DNA content equal to one Ad2-Eco RI-B fragment. Similarly, the absolute amount of DNA in the hybrid phages was determined by comparing (*5*) their buoyant density with the density of phage λ2Pam (data not shown). Finally, both sets of data indicate that the three phage types correspond to the insertion of one, two, and three Ad2-Eco RI-B fragments, respectively. These classes of hybrid phages are therefore referred to as monomers, dimers, and trimers, respectively. A second clone of hybrid phage λ-Ad2-Eco RI-B7, which has a different Bam HI electrophoretic pattern (Fig. 1E), was also analyzed by CsCl centrifugation. The progeny were apparently composed of monomers, dimers, and trimers. The phage progeny of the third clone λ-Ad2-Eco RI-BA were composed only of monomers and trimers (Fig. 2B). The monomers of clones B1, B4, and B7 were purified by CsCl isopycnic centrifugation. Electrophoretic analysis of their Bam HI DNA fragments revealed that the fragment which comigrated with the Eco RI-B fragment had now disappeared (Fig. 1, F and G).

The genotypes of the B1, B4, and B7 clones could now be established by comparison of the electrophoretic pattern of the DNA's and the buoyant density analysis of the phages (Fig. 3). The λ vector has a 26 percent deletion. Therefore insertion of three Eco RI-B DNA fragments each of which contains 2.8 megadaltons of DNA yields a phage with a genome length slightly above that of the intact λ genome.

The observed multimers of the Ad2-Eco RI-B fragment may be due either to multiple insertions of the fragment when the hybrid DNA was constructed or to specific duplications during phage multiplication. In order to distinguish between these alternatives, monomers and trimers from clone B1 were extensively purified

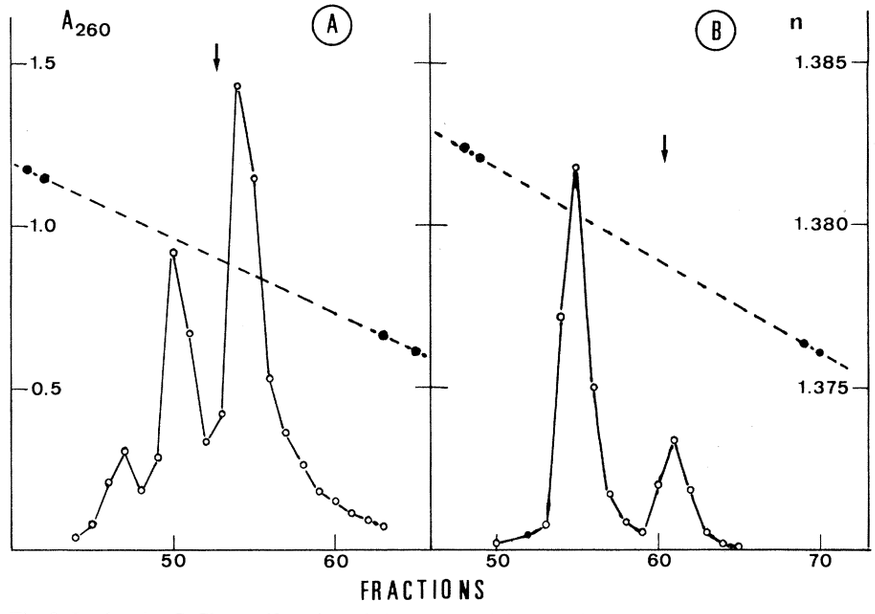

Fig. 2. Isodensity CsCl centrifugation of phages containing hybrid DNA. Clones λ-Ad2-Eco RI-B4 (A) and λ-Ad2-Eco RI-B1 (B) were grown in C 600 r_k^- m_k^-, and the progeny were purified on CsCl gradients. Centrifugation was performed at 20,000 rev/min for 72 hours in an SW41 rotor.

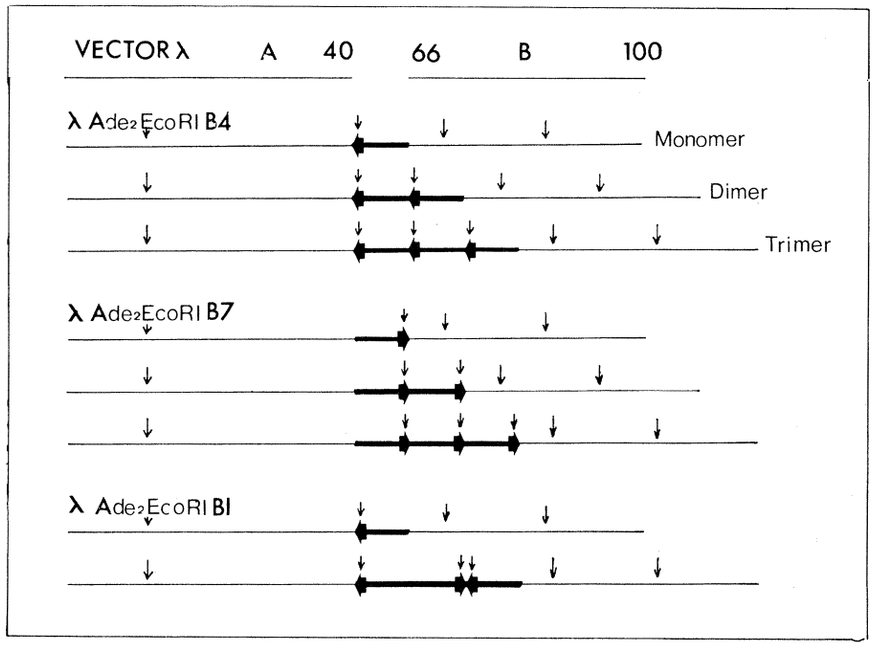

Fig. 3. Genotypes of the λ vector and the λ-Ad2-Eco RI-B hybrids. The λ vector consists of a left arm (A) (0 to 40 percent) and a right arm (B) (66 to 100 percent) which contains the sRI_4^+, sRI_5^+, and a Pam mutation. The Ad1-Eco RI-B DNA fragments of the dimer and the trimer of the clone B4 are all inserted in the same direction. The l-strand (*1, 6*) of the adenovirus fragment corresponds to the l-strand of λ. The Ad2-Eco RI-B DNA fragments of the dimer and the trimer of the clone B7 are all inserted in the same direction. The l-strand (*1, 6*) of the adenovirus fragment corresponds to the r-strand of λ. Two of the three fragments of the trimer of the clone B1 are inserted in opposite directions. The genotype of the monomer of the clone B1 is identical to the genotype of the clone B4.

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.

MICHAEL PERRICAUDET
ALEXANDRE FRITSCH
Unité de Génie Génétique, Institut Pasteur, 25, rue du Dr. Roux, 750 15 Paris, France
ULF PETTERSSON
LENNART PHILIPSON
Department of Microbiology, Biomedical Center, Uppsala University, Uppsala, Sweden
PIERRE TIOLLAIS
Unité de Génie Génétique, Institut Pasteur

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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*).

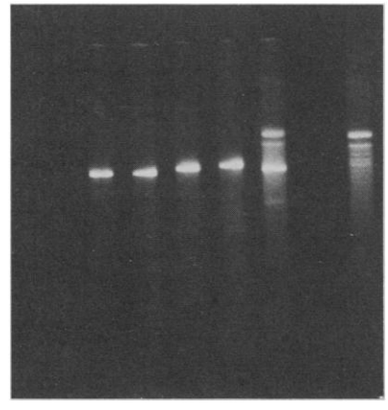

Fig. 1. Sal I cleavage pattern of CLMV DNA. About 1 μg of DNA was incubated with different levels of Sal I in 50-μl reaction volumes containing 60 m*M* tris, *p*H 7.6, 50 μg of BSA per milliliter, 5 m*M* $MgCl_2$, and 0.14*M* NaCl at 37°C for 3 hours. The reaction was terminated by the addition of 5 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-