flect the presence of cistrons for either the 23*S* or the 16*S* rRNA, or both. We also found in hybridization studies with an Eco RI digest of total Chl-DNA that at least two other fragments (A and D or E) of Chl-DNA contain rRNA sequences. These fragments can now be used to construct a physical map of the chloroplast genome and to locate precisely the position and orientation of the rRNA cistrons. Furthermore, other genes, such as those for chloroplast transfer RNA cistrons, can be identified by similar hybridization experiments.

MARGARET I. LOMAX
ROBERT B. HELLING
Division of Biological Sciences, University of Michigan, Ann Arbor 48109

LANNY I. HECKER
STEVEN D. SCHWARTZBACH
W. EDGAR BARNETT
Division of Biology, Oak Ridge National Laboratory, Oak Ridge, Tennessee 37830

References and Notes

1. J. E. Manning and O. C. Richards, *Biochim. Biophys. Acta* **259**, 285 (1972); N. Slavik and C. L. Hershberger, *FEBS Lett.* **52**, 171 (1975).
2. S. D. Schwartzbach, G. Freysinnet, J. A. Schiff, *Plant Physiol.* **53**, 533 (1974).; D. Cohen and J. A. Schiff, *Arch. Biochem. Biophys.* **177**, 201 (1976).
3. D. J. Gruol and R. Haselkorn, *Biochim. Biophys. Acta* **447**, 82 (1976).
4. E. Stutz, E. J. Crouse, L. Graf, B. Jenni, H. Kopecka, in *Proceedings of Genetics and Biogenesis of Chloroplasts and Mitochondria*, T. Bucher, Ed. (North-Holland, Amsterdam, in press).
5. J. Hedgepeth, H. M. Goodman, H. W. Boyer, *Proc. Natl. Acad. Sci. U.S.A.* **69**, 3448 (1972).
6. R. B. Helling, H. M. Goodman, H. W. Boyer, *J. Virol.* **14**, 1235 (1974).
7. R. B. Hallick, C. Lipper, O. C. Richards, W. J. Rutter, *Biochemistry* **15**, 3039 (1976).
8. E. M. Southern, *J. Mol. Biol.* **98**, 503 (1975).
9. S. N. Cohen, A. C. Y. Chang, H. W. Boyer, R. B. Helling, *Proc. Natl. Acad. Sci. U.S.A.* **70**, 3240 (1973); M. So, R. Gill, S. Falkow, *Mol. Gen. Genet.* **142**, 234 (1975).
10. E. M. Lederberg and S. N. Cohen, *J. Bacteriol.* **119**, 1072 (1974).
11. V. Hershfield, H. W. Boyer, C. Yanofsky, M. A. Lovett, D. R. Helinski, *Proc. Natl. Acad. Sci. U.S.A.* **71**, 3455 (1974).
12. M. Melli and J. O. Bishop, *Biochem. J.* **120**, 225 (1970).
13. S. D. Schwartzbach, L. I. Hecker, W. E. Barnett, *Proc. Natl. Acad. Sci. U.S.A.* **73**, 1984 (1976).
14. L. Stolarsky, N. Slavik, C. L. Hershberger, *Biochim. Biophys. Acta* **335**, 30 (1973).
15. P. Guerry, D. J. LeBlanc, S. Falkow, *J. Bacteriol.* **116**, 1064 (1973).
16. R. Dean and E. N. Jackson, unpublished work.
17. M. Mandel and J. Marmur, *Methods Enzymol.* **12B**, 195 (1968).
18. D. E. Kohne, *Biophys. J.* **8**, 1104 (1968); E. H. Davidson and B. R. Hough, *J. Mol. Biol.* **56**, 491 (1971); R. A. Firtel and J. Bonner, *ibid.* **66**, 339 (1972).
19. All experiments involving recombinant DNA molecules were carried out under P2-level containment conditions, as specified in "Guidelines for research involving recombinant DNA molecules" [*Fed. Regist.* **41** (No. 131), 27913 (1967)].
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Cloning of Yeast Transfer RNA Genes in *Escherichia coli*

Abstract. *Four thousand* Escherichia coli *clones containing yeast DNA inserted into the plasmid pBR313 have been isolated. Of these, 175 clones were identified as carrying yeast transfer RNA genes. The initial analysis of the inserted transfer RNA genes via the colony hybridization technique with individual radioactive transfer RNA species is reported. The data indicate that yeast transfer RNA genes are not highly clustered, although some clustering exists. In addition, it was observed that the reiteration number of different transfer RNA genes may vary extensively.*

In order to study the principles of gene organization and expression in yeast we have undertaken a project to isolate and characterize yeast transfer RNA (tRNA) genes. There are several reasons for choosing yeast tRNA genes as a system for studying gene organization and expression. The gene products have been well characterized; most yeast tRNA's have been purified, and many sequenced (*1*). As in *Escherichia coli*, nonsense suppressors have been isolated (*2*), and it has been demonstrated that these suppressor genes are the structural genes of tRNA (*3*, *4*). Thus, there is extensive information available on the genes and gene products for this class of molecules. Recombinant DNA technology now supplies us with a method for isolating the tRNA genes in pure form. We report here our initial efforts to collect and catalog the yeast tRNA genes.

The first step was to obtain an adequate number of *E. coli* clones carrying random fragments of yeast DNA (the "shotgun experiment"). These clones were then screened by hybridization to identify those containing yeast tRNA genes. This

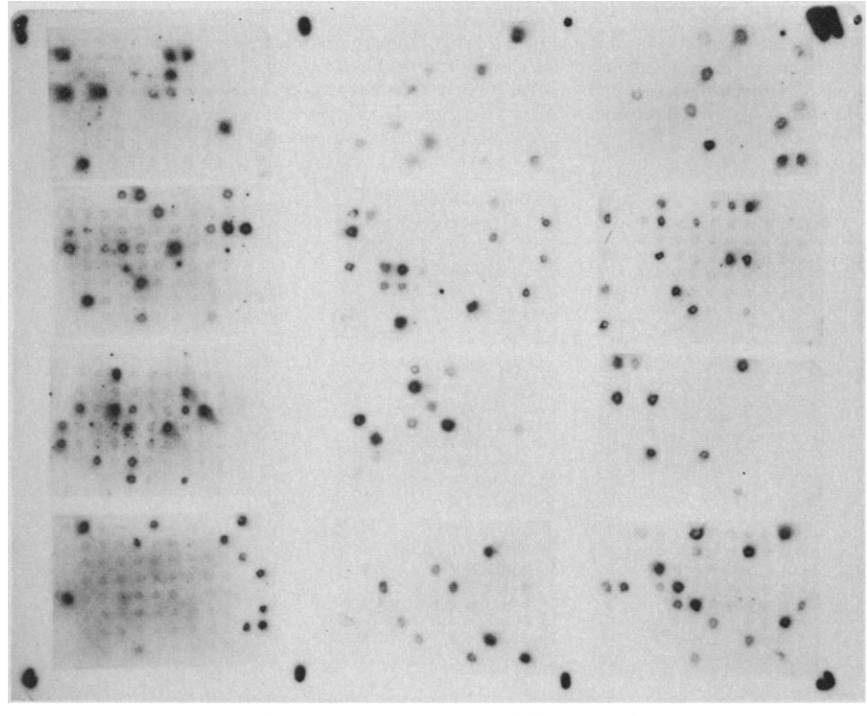

Fig. 1. Screening for recombinant clones carrying yeast tRNA genes. One thousand one hundred and forty clones carrying endo R Hind III restricted fragments of the yeast genome inserted into pBR313 were transferred to 12 filters (95 clones per filter) and assayed for yeast tRNA genes by hybridization with in vivo ^{32}P-labeled 4*S* RNA. Labeling and extraction of stable RNA's were performed according to Rubin (*12*). The RNA was subsequently separated on a 10 percent polyacrylamide gel in 4*M* urea. The positions of 4*S*, 5*S*, and 5.8*S* RNA were revealed by autoradiography. Appropriate bands were cut out, and the RNA's were eluted in 0.3*M* NaCl and purified by DEAE-cellulose column chromatography. We have modified the colony hybridization procedure developed by Grunstein and Hogness (*11*). The candidate clones were replica-plated in an 8 by 12 cm matrix onto 15-cm (diameter) petri plates (95 clones per dish). After an overnight growth period, a sheet of Whatman 540 filter paper was pressed onto the plate and removed (most of the bacteria adhere to the filter). The filter was then treated with 0.5*M* NaOH, neutralized, dried, and used in the hybridization test. We have routinely used 10^5 to 10^6 count/min of purified labeled RNA per filter. However, when total 4*S* RNA was used the hybridization solution contained 10^6 to 10^7 count/min per filter. Hybridization was for 12 to 18 hours at 37°C in a solution of 50 percent formamide, 0.6*M* NaCl, 0.06*M* sodium citrate, *p*H 7.0, and 0.5 percent sodium dodecyl sulfate. The filters were then washed, treated with ribonuclease (optional), and autoradiographed.

subcollection of clones was then further screened with pure tRNA species to identify clones carrying particular tRNA genes.

The vehicle used in these experiments was the plasmid pBR313 (*5*). This plasmid is a derivative of Col E1 carrying antibiotic resistance genes for tetracycline (*tet*^{r}) and ampicillin (*amp*^{r}). Foreign DNA can be inserted by ligation into this plasmid (*6*) at a number of single restriction sites. In our experiments we used endo R Hind III (*7*) restriction endonuclease to cleave the plasmid DNA. This enzyme cleaves the circular plasmid DNA at a single site in the *tet*^{r} gene (*5*). Yeast DNA was purified from *Saccharomyces cerevisiae* strain X2180 1A (*8*) and cleaved with endo R Hind III. The yeast DNA fragments were annealed and ligated to Hind III cleaved pBR313 DNA to produce a random collection of hybrid DNA molecules (*6*). These recombinant plasmids were introduced (*9*) into *E. coli*

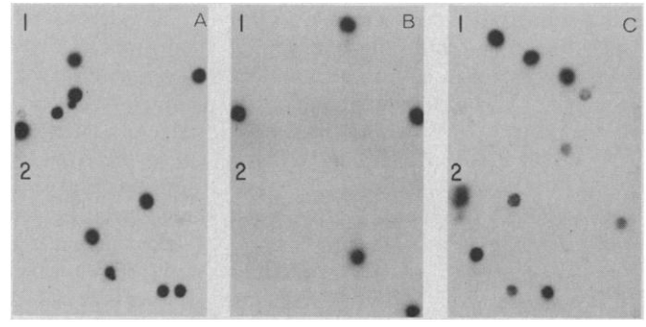

Fig. 2. Screening for specific tRNA genes among 4*S*-containing clones. The 175 clones that were shown to hybridize with ^{32}P-labeled 4*S* RNA (see Fig. 1) were tested for their ability to anneal different purified tRNA species. The in vivo labeled 4*S* RNA's were separated by two-dimensional polyacrylamide gel electrophoresis (*3*). Individual spots were selected by autoradiography, eluted, and used in our hybridization screening. The hybridization to three individual tRNA samples (T1, panel A; T2, panel B; T3, panel C) is shown here. T1, T2, and T3 were later identified by their ribonuclease T1 two-dimensional oligonucleotide maps as $tRNA_3^{Leu}$ (*14*), $tRNA_{UUA}^{Leu}$ (*15*), and $tRNA_2^{Ser}$ (*16*), respectively.

Table 1. Catalog of the 4*S* clones. All 175 clones containing 4*S* genes obtained by a series of 32 independent transformations are listed. The identity of the various purified tRNA species found to hybridize with each clone is indicated. Screening for the various tRNA species is described in Fig. 2, with the use of either individual 4*S* spots obtained by two-dimensional separation of in vivo labeled tRNA (*3*) or by incubating purified tRNA species with bacteriophage T4 polynucleotide kinase and [γ-^{32}P]ATP (*13*). These tRNA's were the gifts from several laboratories (Table 2). In several cases, the identity of the in vivo labeled tRNA samples eluted from two-dimensional gels could be ascertained by their ribonuclease T1 patterns or by the coincidence of their hybridization patterns to the 175 clones with a known purified tRNA species. The spots T1, T2, and T3 and R8, R10, and R11 were shown by oligonucleotide mapping, respectively, to be tRNA's specific for Leu_3, Leu_{UUA}, Ser_2, Ala, Tyr, and Phe; T9 was not analyzed but did cohybridize with the same clones as $tRNA^{Lys}$. Abbreviations for the tRNA species are as follows: Asp, aspartic acid; Arg, arginine; Gly, glycine; Leu, leucine; Phe, phenylalanine; Ser, serine; Thr, threonine; Trp, tryptophan; Tyr, tyrosine; subscripts indicate individual tRNA species.

Clone	tRNA species	Clone	tRNA species	Clone	tRNA species	Clone	tRNA species	Clone	tRNA species
1-i	Thr	7-j	T2(Leu_{UUA})	l		n		d	
2-b	Val_2	m	Gly	n		q	Val_2	k	
c	Asp	8-a	Thr	o	T3(Ser_2)	s		o	
e	Thr	c	Thr	p		u	Arg_3, Tyr, Asp	25-l	
f	Thr	e	T2(Leu_{UUA}), T9	q	T8	19-b		p	Val_2
j		f	Asp	s	Phe, R8(Ala)	f	Arg_3, Asp	26-d	R8(Ala)
k	Leu_3	j		12-a		g	T9	f	Val_2
m	Leu_3	k		d	Leu_3	i	T15	h	T9
p	Thr	l		e	Asp	k	T15	27-a	
r	Val_2	n		o		m	Leu_3	b	T3(Ser_2)
3-b		p	Asp	p		n	T8	d	
d	Gly, T9	q		q	Asp	r		g	
h		s	T8	13-a		t		j	
i	Val_2	u	Leu_3	14-c	T9	20-b	Trp	28-b	
p	R8 (Ala)	9-d	T9	d		f	T9	c	T9
q	T15	e	T9	e	T15	g		e	
4-a	Leu_3	g	T3(Ser_2)	g		m	Trp	i	Asp
d		k	T15	k	R8(Ala)	n	T3(Ser_2)	29-e	
e		l		15-a		o		i	T15
g		n		c		21-d	Arg_3, Asp	l	Val_2, T16
i		10-b		d		k		n	Arg_3
j		e		g	T2(Leu_{UUA})	n	Arg_3, Asp	p	
k	R8 (Ala)	f		h	T9	22-c		q	T2(Leu_{UUA})
l		g		16-h		f		r	Arg_3, T9
o		h	Tyr	17-a		g		30-e	
5-a	Asp	i		c	Leu_3	h		g	T15
e	Phe	l		d		i		h	Gly
f	Gly	m	Arg_3, Asp	f	Asp	23-a	T9	i	R6
i	R8 (Ala), T16	p	Arg_3	h	T9	f	Leu_3	l	T9
j		q	T2(Leu_{UUA})	p	T9	g		31-l	Asp
6-a	Leu_3	11-d	Thr	18-c		h	Gly	32-e	
c		f	Leu_3	f		k	Tyr	f	T16
g		g	T3(Ser_2)	g		l		g	T16
h	Tyr	h		k		24-b	Asp	h	T16
l	Gly	j		l		c	T15		
		k							

strain C600 SF8 (*10*) by transformation (*6*). After addition of the DNA the transformation mixture was divided into a number of separate growth tubes in order to minimize the chances of obtaining sibling clones. The transformation mixtures were plated on ampicillin medium to select for clones carrying the plasmid. From 32 independent transformations, 12,000 ampicillin-resistant clones were isolated. Four thousand of these clones were tetracycline sensitive, indicating that foreign DNA had been inserted into the *tet*^{r} gene.

This collection of 4000 clones containing yeast DNA was screened by the colony hybridization technique [(*11*) and legend to Fig. 1] to detect those containing sequences complementary to 4*S*, 5*S*, or 5.8*S* RNA. In these experiments the RNA probes were labeled in vivo by growing yeast in medium containing [^{32}P]orthophosphate (*12*). The labeled RNA was purified by polyacrylamide gel electrophoresis (*3*). One hundred and seventy-five clones hybridized specifically with 4*S* RNA, 122 hybridized with 5*S* RNA, and 121 with 5.8*S* RNA. The 5*S* and 5.8*S* clones also hybridized with 4*S* RNA, but this hybridization was due to the presence of contaminating ribosomal RNA sequences in our 4*S* RNA and was not due to tRNA. Figure 1 illustrates the detection of clones hybridizing with 4*S* RNA.

The collection of 175 clones hybridizing to 4*S* RNA was then screened with purified tRNA's. This process is not yet complete, but the results obtained with 18 different species of tRNA give us a preview of the form the final catalog will take. Prior to the final screening process each clone was purified and retested. Two grids were formed containing the entire 4*S* collection (Fig. 2).

Pure yeast tRNA's were obtained in two ways. The tRNA was labeled with ^{32}P in vivo (*12*), and the individual species were purified by two-dimensional polyacrylamide gel electrophoresis (*3*). The purity and, in some cases, the identity of these tRNA's was ascertained by mapping the T1 ribonuclease products of each species. In this way 12 pure species of yeast tRNA have been isolated.

In addition, we have been fortunate in obtaining a number of pure yeast tRNA's from our colleagues (Table 2). These unlabeled tRNA's can be made radioactive by incubation with [γ-^{32}P]ATP and bacteriophage T4 polynucleotide kinase (*13*). In this way we have obtained ten pure tRNA species. In several cases these proved to be identical to members of the first set so that in all we have screened our set of 4*S* clones with 18 different species of pure yeast tRNA. The acceptor activity of six of these is unknown. Figure 2 gives an example of the detection of 4*S* clones hybridizing to a pure species of tRNA. In this case the tRNA's were purified by two-dimensional polyacrylamide gel electrophoresis (*3*). The results with three pure species of tRNA are shown. These were called T1, T2, and T3, but further analysis showed that they were, respectively, $tRNA_3^{Leu}$ (*14*), $tRNA_{UUA}^{Leu}$ (*15*), and $tRNA_2^{Ser}$ (*16*). Figure 2 shows that there are ten clones hybridizing with $tRNA_3^{Leu}$ (panel A), 5 clones hybridizing with $tRNA_{UUA}^{Leu}$ (panel B), and 11 clones hybridizing with $tRNA_2^{Ser}$ (panel C). In panel C there are five dark spots and six light ones. We assume that the light spots are due to a contaminating RNA species in the $tRNA_2^{Ser}$ preparation, but there are several other possible explanations that must be ruled out.

The results of hybridization tests with all 18 pure tRNA probes are given in Tables 1 and 2. Of the 175 clones in the 4*S* collection, 97 hybridize to at least one of the tRNA's. Ten of the clones hybridize to two tRNA's and one clone to three different tRNA species (18u, Table 1). Note that a possible cluster of Arg_3, and Asp tRNA genes occurs four times (Table 2).

These results suggest some tentative conclusions regarding the organization of tRNA genes in the yeast genome. The average molecular size of an endo R Hind III fragment is about 2.5×10^6 daltons. Thus each fragment represents $^1/_{4000}$ of the yeast haploid genome (about 10^{10} daltons) (*17*). We have isolated 4000 clones at random. Hybridization studies have shown that there are 360 tRNA genes per yeast haploid genome (*18*). If these genes are widely spaced we would have expected to isolate by chance about 360 clones containing one tRNA species each (although in our collection the chance of obtaining a particular tRNA gene is $1\text{-}e^{-1}$ or 0.63). On the other hand, if tRNA genes are tightly clustered we would have detected far fewer 4*S* clones. Our results suggest that the tRNA genes in yeast are not tightly clustered. If our collection of 175 clones contains 360 tRNA genes, on the average, there will be two genes per clone. The possibility that yeast tRNA genes are widely spaced had already been suggested from the genetic mapping of tyrosine inserting suppressors. These suppressors are unlinked and often map on separate chromosomes (*19*). Clarkson and collaborators (*20*) have studied the organization of tRNA genes in

Table 2. Frequency of occurrence of the different tRNA genes. The data reported under Table 1 are summarized: column 1 lists the total number of clones hybridizing to each tRNA. Column 2 represents the minimum number of independently obtained clones for each tRNA, computed by subtracting any possible siblings. Also listed are the clones that react with more than one of the tRNA's tested, and their frequency of occurrence. We gratefully acknowledge the various donors of the different purified tRNA species as indicated for each tRNA. Abbreviations for the tRNA species are as follows: Ala, alanine; Asp, aspartic acid; Arg, arginine; Gly, glycine, Leu, leucine; Phe, phenylalanine; Ser, serine; Thr, threonine; Trp, tryptophan; Tyr, tyrosine; Val, valine.

Item	Number of clones found	Minimum independent number
A. tRNA species		
Ala(R8) (*21*) (G. Keith)	6	6
Asp (*22*) (G. Keith; P. Bolton and D. Kearns)	15	12
Arg_3 (*23*) (J. Weissenbach and G. Keith)	8	7
Gly (P. Bolton and D. Kearns)	6	6
Leu_{UUA}(T2) (*15*)	5	5
Leu_3 (*14*) (S. H. Chang; G. Pixa and G. Keith)	10	9
Phe(R11) (*24*) (B. Reid)	2	2
Ser_2(T3) (*16*)	5	4
Thr (J. Weissenbach and G. Keith)	7	4
Trp (*25*) (G. Keith; B. Reid)	2	1
Tyr(R10) (*26*) (B. Reid; P. Bolton and D. Kearns)	4	4
Val_2 (*27*) (S. Montasser and G. Keith)	7	6
R6	2	2
T8	3	3
T9	14	12
T15	8	7
T16	5	3
B. Possible clusters of two or more tRNA genes		
Gly, T9	1	1
Ala(R8), T16	1	1
Leu_{UUA}(T2), T9	1	1
Ala(R8), Phe	1	1
Arg_3, Asp	4	3
Arg_3, Asp, Tyr(R10)	1	1
Arg_3, T9	1	1
Val_2, T16	1	1

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

References and Notes

1. A. Rich and U. L. RajBhandary, *Annu. Rev. Biochem.* **45**, 805 (1976); B. G. Barrell and B. F. C. Clark, *Handbook of Nucleic Acid Sequences* (Joynson-Bruvvers, Oxford, 1974).
2. D. C. Hawthorne and U. Leupold, *Microbiol. Immun.* **64**, 1 (1974).
3. P. W. Piper, M. Wasserstein, F. Engbaek, K. Kaltoft, J. E. Celis, J. Zeuthen, S. Liebman, F. Sherman, *Nature (London)* **262**, 757 (1976).
4. M. R. Capecchi, S. H. Hughes, G. M. Wahl, *Cell* **6**, 269 (1975); M. C. Brandiss, J. W. Stewart, F. Sherman, D. Botstein, *J. Mol. Biol.* **102**, 467 (1976); R. F. Gesteland, M. Wolfner, P. Grisafi, G. Fink, D. Botstein, J. R. Roth, *Cell* **7**, 381 (1976).
5. F. Bolivar, R. L. Rodriguez, M. C. Betlach, H. W. Boyer, in preparation.
6. J. Velten, K. Fukada, J. Abelson, *Gene* **1**, 93 (1976).
7. H. O. Smith, *Methods Mol. Biol.* **7**, 71 (1975).
8. This strain, originally from Dr. Mortimer, was described by W. Duntze, V. MacKay, T. R. Manney [*Science* **168**, 1472 (1970)].
9. This work was done under P2 conditions according to the guidelines as defined by the National Institutes of Health.
10. K. Struhl, J. R. Cameron, R. W. Davis, *Proc. Natl. Acad. Sci. U.S.A.* **73**, 1471 (1976).
11. M. Grunstein and D. S. Hogness, *ibid.* **72**, 3961 (1975).
12. G. M. Rubin, *Methods Cell Biol.* **12**, 45 (1975).
13. A. M. Maxam and W. Gilbert, *Proc. Natl Acad. Sci. U.S.A.* **74**, 560 (1977).
14. S. H. Chang, S. Kuo, E. Hawkins, N. R. Miller, *Biochem. Biophys. Res. Commun.* **51**, 951 (1973).
15. P. W. Piper and M. Wasserstein, in preparation.
16. H. G. Zachau, D. Dutting, H. Feldmann, *Angew. Chem. Int. Ed. Engl.* **5**, 422 (1966).
17. M. M. Bhargava and H. O. Halverson, *J. Cell Biol.* **49**, 423 (1971).
18. E. Schweizer, C. MacKechnie, H. O. Halvorson, *J. Mol. Biol.* **40**, 261 (1969); H. Feldman, *Nucleic Acids Res.* **3**, 2379 (1976).
19. R. A. Gilmore, J. W. Stewart, F. Sherman, *J. Mol. Biol.* **61**, 157 (1971).
20. S. G. Clarkson and M. L. Birnstiel, *Cold Spring Harbor Symp. Quant. Biol.* **38**, 451 (1973).
21. R. W. Holley, J. Apgar, G. A. Everett, J. T. Madison, M. Marquisee, S. H. Merrill, J. R. Penswick, A. Zamir, *Science* **147**, 1462 (1965).
22. J. Gangloff, G. Keith, J. P. Ebel, G. Dirheimer, *Biochim. Biophys. Acta* **259**, 198 (1972).
23. B. Kuntzel, J. Weissenbach, G. Dirheimer, *FEBS Lett.* **25**, 189 (1972).
24. U. L. RajBhandary, S. H. Chang, A. Stuart, R. D. Faulkner, R. M. Hoskinson, H. G. Khorana, *Proc. Natl. Acad. Sci. U.S.A.* **57**, 751 (1967).
25. G. Keith, A. Roy, J. P. Ebel, G. Dirheimer, *FEBS Lett.* **17**, 306 (1971).
26. J. T. Madison, G. A. Everett, H. Kung, *Science* **153**, 531 (1966).
27. V. D. Axelrod, V. M. Kryukov, S. N. Isaenko, A. A. Bayev, *FEBS Lett.* **45**, 333 (1974).

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

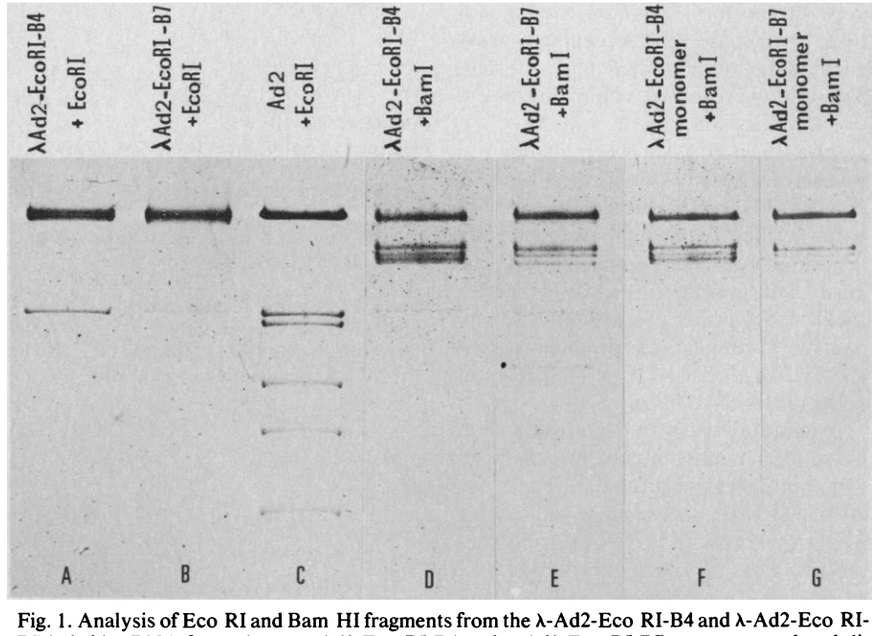

Fig. 1. Analysis of Eco RI and Bam HI fragments from the λ-Ad2-Eco RI-B4 and λ-Ad2-Eco RI-B7 hybrids. DNA from clones λ-Ad2-Eco RI-B4 and λ-Ad2-Eco RI-B7 was extracted and digested with endonucleases Eco RI and Bam HI (*1*). The DNA fragments were analyzed by electrophoresis in a slab gel containing 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.