Bacteriophage T4 Transfer RNA

Thesis by

John H. Wilson

In Partial Fulfillment of the Requirement

for the Degree of

Doctor of Philosophy

California Institute of Technology

Pasadena, California

1972

(Submitted November 19, 1971)

Acknowledgments

I want to acknowledge in this small and inadequate way a few of the very large debts I've accumulated in these past five years.

Bill Wood allowed me the independence and freedom to explore and define my own limitations, to suffer my own mistakes (often), and to enjoy my own successes (rare, but sweet). I have valued enormously his advice and criticism but most of all his strong personal support. Let me record here that our Great Bet has ended in a draw---I have not beaten him at tennis nor has he beaten me at golf.

I miss Bob Edgar. His insights into and viewpoints on science and people were genuinely refreshing and unique.

Sam Ward and Steve Beckendorf have been delightful accomplices. Their enthusiasm for life, their sense of humor, their pointed scientific criticism (exceeded only by the sharpness of their elbows under the basket), and their friendship will be sorely missed.

The Phage Group with its ever-changing membership, its wide range and free flow of ideas, and its general care and concern has been a constant stimulation and joy.

I thank Norman, Don, Willie, Ken, Harry, and Bill for many hours of fun and frustration on the tennis court. I thank Mitch, the Alchemists, and the Tuesday-Thursday gang for countless hours of entertainment and exasperation on the basketball court. I thank John, the Tree and the Talk for many late-night debauches. Do you wonder that my degree took so long?

I thank the National Science Foundation, the National Institutes of Health, Earle C. Anthony, and my wife for financial support.

Most important I wish to dedicate this thesis to my wife Linda who understands me thoroughly and still loves me.

ABSTRACT

Two T4-coded nonsense suppressors, $\underline{psu}_a^+$ and $\underline{psu}_b^+$, have been isolated and characterized. Both were isolated as pseudo-wild type revertants of phage strains which carry multiple amber mutations. $\underline{psu}_a^+$ is an amber suppressor which occurs at a frequency of 10^{-11} to 10^{-12} and is indistinguishable from wild type phage in its growth on both B and K strains of E. coli bacteria. $\underline{psu}_b^+$ may be either an amber or an ochre suppressor which occurs at a frequency of 10^{-7} - 10^{-10} and makes small plaques on B strains but grows very poorly or not at all on K strains. Phage with the characteristics of $\underline{psu}_a^+$ occur in populations of $\underline{psu}_b^+$ phage at a frequency of 10^{-4}. Both suppressors insert serine in response to the amber codon at an efficiency of about 45%. $\underline{psu}_a^+$ and $\underline{psu}_b^+$ map less than 0.3 map units apart and are located between genes e and 57 about 8 map units from gene e. On the basis of their initial frequencies of appearance and the frequency of $\underline{psu}_b^+$ mutation to $\underline{psu}_a^+$, we speculate that $\underline{psu}_a^+$ is derived from the wild type ser-tRNA by two base changes in the anticodon and that $\underline{psu}_b^+$ is a one-base-change intermediate.

151 independent suppressor-negative derivatives of $\underline{psu}_b^+$ phage have been isolated and characterized. They fall into two complementation groups. One, designated mb (modifier of $\underline{psu}_b^+$ phenotype), is unlinked to to $\underline{psu}_b^+$ and has been located about 10 map units from rII. The second group, designated $\underline{psu}_b^-$, is made up of deletions and single base changes which affect sites within 0.2 map units of the original mutation. Those $\underline{psu}_b^-$ mutants which still contain the original mutation, psu_b, have been

mapped relative to psu_b and each other by a series of two-factor and intragenic three-factor crosses. ^{32}P-labeled tRNA from mb, psu_b^- and wild type infected cells have been compared by polyacrylamide gel electrophoresis. In mb-infected cells several of the tRNA species are missing, while in psu_b^--infected cells only the ser-tRNA is clearly absent. These studies suggest there are only two phage genes which are essential for the production of functional ser-tRNA. One is the structural gene for the ser-tRNA and the second plays an undefined role which affects several tRNAs.

E. coli cells infected with phage strains carrying a large deletion of gene e or gene psu_b^+, are missing most if not all of the phage tRNAs normally present in wild type infected cells. By DNA-RNA hybridization we have demonstrated that the DNA corresponding to the missing tRNAs is absent. Thus the genes for these tRNAs must be clustered in the same region of the genome as the ser-tRNA gene. We have been able to locate and to define a maximum size for the cluster by physically mapping the deletions of genes e and psu_b^+ by examination of heteroduplex DNA in the electron microscope. That such deletions can be isolated indicates that the phage-specific tRNAs from this cluster are dispensable.

TABLE OF CONTENTS

General Introduction

For each of the common amino acids there is generally more than one tRNA. A certain multiplicity of tRNAs is expected as a result of the degeneracy of the genetic code. Since there is "wobble" in anticodon-codon pairing, one might predict that there would be considerably fewer tRNA species than codons. However, as column chromatographic techniques have improved, investigations on several different organisms have demonstrated a multiplicity much greater than anticipated (Gauss, van der Haar, Maelicke & Cramer, 1971). The reason for this degree of multiplicity is not understood. Even less clear is why these different tRNAs vary so widely in amount. These two mysteries of unexpectedly large numbers and widely varying levels of tRNA species have led many to postulate roles for tRNAs other than simple adaptors in protein biosynthesis (Sueoka & Kano-Sueoka, 1970). Particularly intriguing is the possibility that tRNAs may play a regulatory role in cell differentiation (Sueoka & Kano-Sueoka, 1970).

In circumstantial support of this hypothesis are the observations that there are changes in the relative amounts and/or kinds of tRNA species during some developmental events (Gauss, et al., 1971). Such widely varying processes as embryogenesis, regeneration, change of a normal cell to a malignant cell, sporulation and viral invasion have all been shown to be accompanied by changes in the pattern of isoaccepting tRNAs (Gauss, et al., 1971; Sueoka& Kano-Sueoka, 1970). The physiological significance of the multiplicities and amounts of tRNAs and the relation of their altered patterns to particular developmental events are unknown.

The bacteriophage T4 is one of the viruses which upon infection alters the pattern of tRNAs within the host cell. This alteration is the result of two effects. One is the modification of preexisting host tRNA--demonstrated for the leucine tRNA (Sueoka & Kano-Sueoka, 1970). The second is the production of 5 to 10 phage-specific tRNAs (Daniel, Sarid & Littauer, 1970). It is the latter phenomenon which is the subject of this thesis.

Why does the virus synthesize its own species of tRNA in apparent partial duplication of the host tRNA species? Though there has been much speculation no reason for their presence has been demonstrated (Sueoka & Kano-Sueoka, 1970; Daniel, et al., 1970). Are these tRNAs capable of reading codons which are commonly used by the virus but never or infrequently by some of its hosts? Are they involved in the turn-off of host protein synthesis or the turn-on or turn-off of any viral protein? Do they in some manner regulate the relative amounts of viral proteins? Are they essential in some hosts T4 encounters naturally but not in the common laboratory strains of E. coli?

We have begun to investigate the function of the T4 tRNAs by the isolation and characterization of genetically altered phage strains which differ from wild type in the number or nature of their tRNAs.

References

Daniel, V., Sarid S. & Littauer,U.Z. (1970). Science, 167, 1682.

Gauss, D. H., von der Haar, F., Maelicke, A. & Cramer F. (1971) Ann. Rev. Biochem. 39, 227.

Sueoka, N. & Kano-Sueoka, T. (1970). Prog. in Nucleic Acid Res. and Mol. Biol. 10, 23.

Bacteriophage T4 Transfer RNA

I. Isolation and Characterization of Two Phage-Coded Nonsense Suppressors

John H. Wilson* and Sandra Kells†

Division of Biology, California Institute of Technology

Pasadena, Calif. 91109, U.S.A.

Running title: T4-Coded Nonsense Suppressors

*Present address: Department of Biochemistry, Stanford University Medical Center, Stanford, California 94305.

†Present address: Department of Biophysics, University of Chicago, Chicago, Illinois 60637.

Abstract

Two phage-coded nonsense suppressors, $\underline{psu}_a^+$ and $\underline{psu}_b^+$, have been isolated and characterized. Both were isolated as pseudo-wild type revertants of phage strains which carry multiple amber mutations. $\underline{psu}_a^+$ is an amber suppressor which occurs at a frequency of 10^{-11} to 10^{-12} and is indistinguishable from wild type phage in its growth on both B and K strains of E. coli bacteria. $\underline{psu}_b^+$ may be either an amber or an ochre suppressor which occurs at a frequency of 10^{-7} to 10^{-10} and makes small plaques on B strains but grows very poorly or not at all on K strains. Phage with the characteristics of $\underline{psu}_a^+$ occur in populations of $\underline{psu}_b^+$ phage at a frequency of 10^{-4}. Both suppressors insert serine in response to the amber codon at an efficiency of about 45%.

$\underline{psu}_a^+$ and $\underline{psu}_b^+$ map less than 0.3 map units apart and are located between genes e and 57 about 8 map units from gene e. That $\underline{psu}_a^+$ and $\underline{psu}_b^+$ insert the same amino acid and map so close together suggests that they result from different mutations within the same gene. On the basis of their initial frequencies of appearance and the frequency of $\underline{psu}_b^+$ mutation to $\underline{psu}_a^+$, we speculate that $\underline{psu}_a^+$ is derived from wild type by two base changes and that $\underline{psu}_b^+$ is a one-base-change intermediate.

1. Introduction

Upon infection of its host bacterium the bacteriophage T4 causes the synthesis of several phage specific tRNAs (Daniel, Sarid & Littauer, 1970). Fingerprint analysis of the individual low molecular weight RNA bands from polyacrylamide gel fractionation of the post-infection RNA suggests that T4 produces 5 to 10 tRNAs (W. H. McClain, personal communication; J. N. Abelson, personal communication). tRNAs specific for arginine, glycine, isoleucine, proline, leucine, and serine have been identified among the phage-coded species (Scherberg & Weiss, 1970; W. H. McClain, personal communication). Though there has been much speculation, no reason for their presence has been demonstrated (Daniel *et al.*, 1970; Sueoka & Kano-Sueoka, 1970).

We have begun to investigate the function of the T4 tRNAs by the isolation and characterization of genetically altered phage strains which differ from wild type phage in the number or nature of their tRNAs. The first report describes the properties of two phage-coded nonsense suppressors. One of these, psu_a^+, appears to be identical to psu_1^+, a phage-coded amber suppressor described by McClain (1970).

2. Materials and Methods

(a) Phage strains

Phage strains derived from the wild type T4D were obtained from the collection of Dr. R. S. Edgar. Most of the amber (*am*) and temperature sensitive (*ts*) mutants used have been described elsewhere (Epstein *et al.*, 1963; Edgar & Lielausis, 1965; Wood & Henninger, 1969). Those

am mutants designated NG were isolated by Edgar and Lielausis (unpublished) from wild type T4D after mutagenesis with N-methyl-N'-nitro-N-nitrosoguanidine. K6 is an *am* mutant selected to grow on H12R8A (*su-3*$^+$) but not CR63 or S/6/5 (Edgar & Lielausis, unpublished). The *rII* mutant *rdf41* is a deletion in T4D which encompasses both the A and B cistrons. The *rII* ambers, ochres (*oc*) and opals (*op*) derived from the wild type T4B were obtained from Dr. S. Champe. The gene *e* ochres and opals and the deletion eG223 derived from the standard type T4B were obtained from the collection of Dr. G. Streisinger via Dr. W. Salser. Phage stocks of gene *e* mutants were prepared on citrate lysozyme plates by the confluent lysis technique as described by Epstein *et al.* (1963). Multiply mutant phage strains are described in Table 1.

(b) *Escherichia coli* host strains

CR63 was used as the permissive host for *am* mutants and as the non-permissive host for psu_b strains. S/6/5 was used as the non-permissive host for psu_b strains and the permissive host for *am*$^+$ mutants. The *am* suppressor strains S26R1e (*su-1*$^+$), S26R1d (*su-2*$^+$), H12R8A (*su-3*$^+$) which insert serine, glutamine, and tyrosine respectively in response to the *am* codon, and their parent strain S26 (*su*$^-$) (Garen, Garen & Wilhelm, 1965) were obtained from Dr. A. Garen. 594 is a streptomycin resistant K strain. CAJ70, obtained from Dr. S. Brenner, is permissive for *op* mutants. The B strains of *E. coli*, B/5, G(λ) and Bb, which are non-permissive for *am* mutants, have been described by Wilson, Luftig & Wood (1970). BB from the

Table 1

T^4 multiple mutants

Strain designation	Mutations	Defective genes
X4E	amB25, amA455, amB252, amN52, amB280, amB262	34, 35, 37, 38
X74	amB20, amN133	14, 15
SX4	amE18, amB17, amN120 amB252, rdf41	18, 23, 27, 35, rII
X174	amB252, amN128	10, 11
X372	amN52, amB280, amM69	37, 63

Caltech collection is a B strain which is permissive for am mutants. It was originally obtained from Berkeley as a non-permissive strain. The origin of the difference is unclear (Russell, 1967).

(c) Media and buffers

H broth used for phage and bacterial growth, and EHA top and bottom agar, used for plating assays, were prepared as described by Steinberg & Edgar (1962). Synthetic growth medium contained per liter 7 gm Na_2HPO_4, 3 gm KH_2PO_4, 6 gm NaCl, 1 gm NH_4Cl, 0.12 gm $MgSO_4$, 0.01 gm $CaCl_2$, 4 gm glucose, and 0.6 gm Casamino acids. Dilution buffer was prepared as described by King (1968). Synthetic medium minus Casamino acids was used for preparation of radioactively labeled infected-cell lysates.

(d) Radioactively labeled lysates of phage-infected cells

Strain Bb was grown to a concentration of 5×10^7 cells/ml. in synthetic medium lacking Casamino acids. The cells were collected by centrifugation at 10,000 g for 10 min and resuspended at $2\text{-}4 \times 10^8$ cells/ml. One ml. aliquots of this suspension were warmed to 37°C, infected with phage at a multiplicity of 5-10, and aerated. 15 min after infection, 2.5 to 10 μc of a uniformly labeled ^{14}C amino acid mixture (New England Nuclear) were added. For pulse-labeled lysates an equal volume of 10% TCA was added 2.5 min after addition of the ^{14}C amino acids. Nonpulse-labeled cultures were lysed by addition of $CHCl_3$ (Mallinckrodt) 45 min after infection. All lysates were dialyzed against 0.065 M-Tris, pH 6.8, 1% SDS.

(e) Electrophoresis on polyacrylamide gels containing SDS

Procedures for the preparation and running of discontinuous polyacrylamide gels containing SDS were as described by Laemmli (1970). Dialyzed lysates were made 1-2% in 2-mercaptoethanol. Proteins were dissociated by heating the sample for 2 min in boiling water. Samples containing 300,000 cts/min in a volume of 0.3 ml. were run on 7.5% polyacrylamide gels containing 0.1% SDS. Gels were stained by the method of Weber & Osborn (1969) with Coomassie brilliant blue R250 (Mann) and destained by the method of Ward (1970). Gels were sliced vertically, dried on filter paper and autoradiographed for 72 hr on Kodak no-screen X-ray film (Fairbanks, Levinthal & Reeder, 1965). Resulting autoradiographs were traced with a Joyce-Loebl densitometer and the area under curves determined with a planimeter. Over the range of exposure times used the area under all measured bands was a linear function of the exposure time, indicating that the area under the tracing of a band is a measure of the amount of radioactivity in the band.

(f) Other methods

Standard phage crosses were a modification of the procedure of Steinberg & Edgar (1962). A stationary phase culture of CR63 was diluted 1/1000 in H broth and grown for 3 hr at 30°C. Cells were collected by centrifugation and resuspended at 4×10^8 cells/ml. Parent strains at a multiplicity of 7.5 each were added to the cells in the presence of 0.004 M KCN at 30°C. After 5 min, anti-T4 serum

was added to kill all unadsorbed phage. 10 min after infection the cells were diluted 10^4 into H broth at 30°C. 90 min after infection $CHCl_3$ was added.

Serum blocking assays were as described by Ward et al. (1970). All assays were done with hyperimmune rabbit antisera prepared directly by injection of a purified antigenic component. Tail fiberless particles were prepared from B/5 cells infected with the multiple am mutant X4E as described by Edgar & Wood (1966).

3. Results

(a) Isolation of psu_a^+ and psu_b^+

Our initial discovery of a phage-coded nonsense suppressor grew out of an unrelated attempt to find variants of T4 which could adsorb solely by their baseplates. We anticipated that such mutants, if they exist, would appear as pseudo-wild type revertants of the multiple am mutant strain X4E which carries six am mutations in four tail fiber genes. In such a strain revertants to true wild type were expected to occur at a frequency of less than 10^{-30}. No phenotypic am^+ revertants ($<10^{-10}$) could be detected in phage stocks of X4E. In order to detect revertants at frequencies as low as 10^{-13} on a single plate, we prepared tail fiberless particles using the X4E strain and plated them directly on an su^- host, S/6/5. Plaques appeared at a frequency of about 10^{-11} relative to the titer of the tail fiberless particles. Revertants from two different preparations of tail fiberless particles each divided into two classes. Neither class contained the type of

revertant in which we were originally interested.

None of the five revertants in the first group yielded *am* progeny when crossed to wild type phage. In addition, they appeared to produce normal tail fibers, they made the products of genes *34* and *37* as analyzed by SDS gel electrophoresis and blocked both A- and BC'-specific sera (Ward *et al.*, 1970) equally as well as wild type phage. These observations suggest that all the original *am* lesions have been repaired. Since these apparent revertants to true wild type are present at least 10^{19} times more frequently than expected, they may contain an additional mutation which drastically alters the frequency of reversion. In line with this suggestion is the observation that several of these revertants gave rise to a high proportion of progeny with altered plaque morphology. This group has not been investigated further.

All six of the revertants in the second group plated like wild type phage, but when crossed to wild type phage yielded progeny carrying *am* mutations. By complementation tests it was shown that these progeny carried in various recombinant combinations all the *am* mutations present in X4E. Even though these revertants still retained all six *am* mutations, they have normal tail fibers by the criteria applied above. Thus these phage must contain an additional mutation which in some manner suppresses the X4E *am* mutations. One of the revertants, X4ER2, was designated as carrying phage suppressor a (psu^+_a) and renamed psu^+_a:X4E. $psu^+_a{}^{:X4E}$/was then crossed to the other five revertants in this group. If any of the other X4E revertants contained a

suppressor at a site other than $\underline{psu}_a^+$, $\underline{am}$ mutants would appear in the progeny of such a cross at a frequency related to the distance between it and $\underline{psu}_a^+$. None of the approximately 200 progeny examined for each cross was of the $\underline{am}$ phenotype. Thus all these revertants of X4E must contain suppressor mutations which map very close to $\underline{psu}_a^+$ and may be identical to it.

Two additional pseudo-wild type revertants which contain suppressor mutations have been isolated from phage stocks of the multiple $\underline{am}$ mutants X74 and SX4. Though not tested extensively both these revertants show suppression characteristics in common with $\underline{psu}_a^+$, suggesting they may also contain $\underline{psu}_a^+$. In summary, we find that pseudo-wild type revertants which carry a suppressor mutation, possibly $\underline{psu}_a^+$ in all cases, occur in a population of tail fiberless particles or phage at a frequency of 10^{-11} to 10^{-12}.

Among the phenotypically $\underline{am}^+$ revertants of the multiply mutant strains X74, X174 and X372 and the single mutant $\underline{am}$N133 (gene $\underline{15}$) we have found an additional class of revertants distinct from either of the above two classes. These revertants arise at a frequency of 10^{-7} to 10^{-10} and all make very small plaques on S/6/5 and no plaques on CR63. When crossed to wild type, they yield progeny which carry the $\underline{am}$ mutations present in the original strains, suggesting that they carry a second site suppressor mutation. One of the revertants, X74R7, was designated as carrying phage suppressor b, $\underline{psu}_b^+$, and renamed $\underline{psu}_b^+$:X74. To investigate the plating characteristics of phage carrying $\underline{psu}_b^+$ we plated $\underline{psu}_b^+$:X74 and the single mutant $\underline{psu}_b^+$ (see legend to

Table 2) on many of the K and B strains in common use in our laboratory. The results are shown in Table 2. It appears that the peculiar plating phenotype of strains which carry $\underline{psu}_b^+$ reflects a general difference in the ability of B and K bacterial strains to support $\underline{psu}_b^+$ growth.

During these preliminary characterizations of $\underline{psu}_b^+$, it was noted that crosses involving $\underline{psu}_b^+$ strains showed a significantly reduced yield of progeny. Since all crosses were done in CR63 on which $\underline{psu}_b^+$ does not make a plaque, this observation suggested that the poor growth properties of $\underline{psu}_b^+$ might be partially dominant. To investigate this dominance effect we measured the burst size of CR63 cells mixedly infected with $\underline{psu}_b^+$ and wild type as a function of the ratio of $\underline{psu}_b^+$ to total input phage as shown in Figure 1. The burst size is almost directly proportional to the fraction of wild type phage in the input. One interpretation of these results is that the wild type product of the gene corresponding to $\underline{psu}_b^+$ is required, approximately stoichiometrically, for phage growth in CR63. However, since strains which carry deletions encompassing the entire gene grow like wild type on CR63 (Wilson, Kim & Abelson, 1972) we interpret the partial dominance of $\underline{psu}_b^+$ to indicate that the altered product of the gene defined by $\underline{psu}_b^+$ has a deleterious effect on phage growth in CR63. The basis for this effect remains unclear. One possibility is considered in the discussion.

Because of the poor growth of strains which carry $\underline{psu}_b^+$ we have found this suppressor difficult to analyze. Consequently, its

Table 2

Plating characteristics of $\underline{psu}_o^+$:X74 and $\underline{psu}_o^+$

Bacterial strains		$\underline{am}$ suppression	$\underline{psu}_o^+$:X74	$\underline{psu}_o^+$
K strains	S26	su^-	±	±
	S26R1e	su^+	±	±
	H12R8A	su^+	±	±
	594	su^-	0	
	CR63	su^+	0	0
	CAJ70	su^-	0	
B strains	B/5	su^-	+	
	S/6/5	su^-	+	+
	G(λ)	su^-	+	
	BB	su^+	+	+
	Bb	su^-	+	

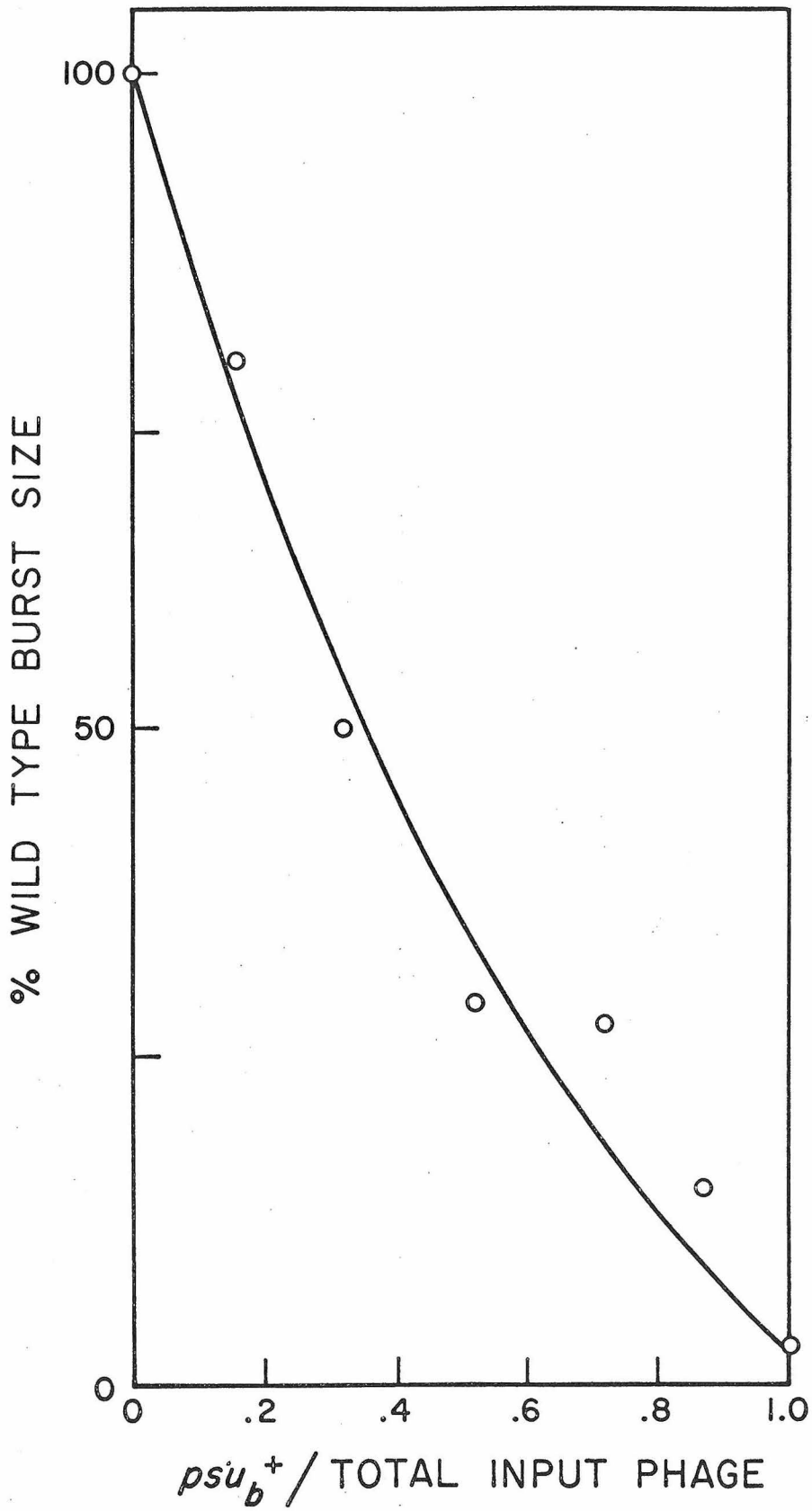
$\underline{psu}_o^+$ was isolated from the progeny of a cross of $\underline{psu}_o^+$:X74 by wild type phage. That it contains none of the $\underline{am}$ mutations present in X74 is based on two criteria: 1) No $\underline{am}$ mutants, to a level of 1%, are found among the progeny of a cross of $\underline{psu}_o^+$ by wild type. 2) Greater than 10% wild type phage are present in the progeny of a cross of $\underline{psu}_o^+$ by X74. $\underline{psu}_o^+$ makes a slightly larger plaque on S/6/5 than does $\underline{psu}_o^+$:X74, though still much smaller than wild type phage. In the

Table 2 (cont.)

table the plaque size of $\underline{psu}_b^+$:X74 and $\underline{psu}_b^+$ on S/6/5 is represented by +. $\pm$ indicates plaques that are just barely visible. 0 indicates no plaques can be seen.

Fig. 1. Gene dosage effects of $\underline{psu}^{+}_{b}$.

CR63 bacteria were grown to 5 x 10^{7} cells/ml. in H broth, collected by centrifugation, and resuspended at a concentration of 4 x 10^{8} cells/ml. Phage at a total multiplicity of 13 to 15 were added in the ratios indicated in the presence of 0.004 M KCN at 30°C. After 10 min anti-T^{4} serum was added to kill all unadsorbed phage. 15 min after infection the cells were diluted 10^{4} into H broth at 30°C. Infective centers were assayed on S/6/5 at 30°C 5 min after dilution. Total progeny were assayed on CR63 65 min after dilution. The burst size for each was calculated as total progeny/infective center.


100
50
0
% WILD TYPE BURST SIZE
0
.2
.4
.6
.8
1.0
psu$_b^+$ / TOTAL INPUT PHAGE

characterization is not as extensive as that of psu_a^+. However, it is exactly its peculiar growth properties which have made it so invaluable in the studies described in the following paper by Wilson & Abelson (1972).

(b) Suppression pattern of psu_a^+ and psu_b^+

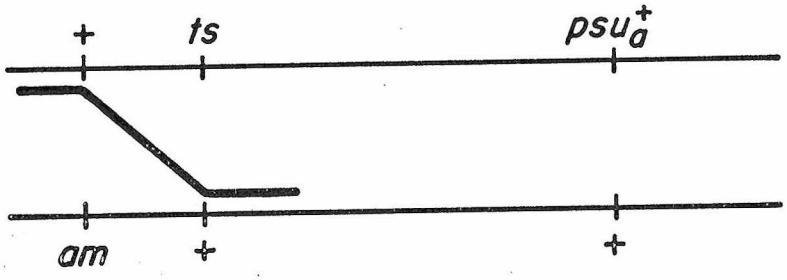
The isolation of phage suppressors apparently identical to psu_a^+ and psu_b^+ from different multiply mutant strains, suggests that psu_a^+ and psu_b^+ can suppress a number of *am* mutations in several different late genes. Because of clear differences in the quality of psu_a^+ suppression of *am* mutations in early and late genes, their suppression patterns are discussed separately.

(i) psu_a^+ and psu_b^+ suppression of nonsense mutations in late genes

To determine the pattern of psu_a^+ suppression we devised a test which would allow us to easily test a large number of mutations. It is described in detail below for mutations in gene *37*. We first constructed strains which carry psu_a^+ and a *ts* mutation in gene *37* (*tsB78*) as follows. The progeny of a cross of psu_a^+:X4E by the triple mutant *amB25*:*tsB78*:*rdf41* were plated on S/6/5 at 25°C. Since all the progeny must contain *amB25* (X4E contains *amB25*), only those that also contain psu_a^+ will make plaques on S/6/5. By testing *r* plaques for temperature sensitivity we isolated psu_a^+ strains which have the following genotype:

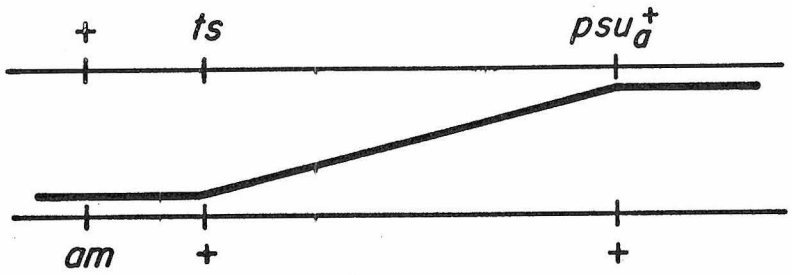
$$psu_a^+ : tsB78 : rdf41 : amB25 + \ldots$$

where +... indicates there may be present additional am mutations contained in X4E. rdf41 was included for reasons not relevant to the experiment described and is an unselected marker in the crosses described in Table 3. To determine whether a particular mutation in gene 37 is suppressed by psu_a^+, we cross it to the psu_a^+ strain just described and measure the per cent of the progeny which plate under conditions (S/6/5 at 42°C) which are non-permissive for both parents. There are two types of ts^+ recombinants which may plate under these conditions: 1) those $ts^+ am^+$ or $ts^+ op^+$ recombinants which have arisen from a crossover in the interval between tsB78 and the mutation being tested,



and 2) those which arise in the interval between tsB78 and psu_a^+, if

the test mutation is suppressed at 42°C.



The presence of other am mutations in the psu_a^+ strain tends to decrease the observed recombination in the first interval, but does not affect recombination in the second interval. If the test mutation is not suppressed by psu_a^+, the per cent of progeny which plate under non-permissive conditions will be less than that expected for the control cross of the test mutation by tsB78 alone. Mutations which are suppressed will yield a higher per cent of progeny which will plate under non-permissive conditions in the test cross against the psu_a^+ strain than in the control cross against tsB78 alone. The difference in per cent recombination between the psu_a^+ strain and tsB78 is a measure of

the linkage between psu_a^+ and gene 37.

The results of test crosses of the kind described for gene 37 are shown in Table 3. psu_a^+ suppresses all except one am mutation but does not suppress any op mutation in gene 37. Due to the high recombination in gene 34, the test crosses with various gene 34 am and op mutations are not so clear-cut as with gene 37 mutations and only the results with amA455 (closest to tsA44 of mutations tested) have been included in Table 3. The results for the crosses against amH26 in gene e suggest that psu_a^+ suppresses the am mutation and is closely linked to gene e. The linkage to gene e is verified in the mapping crosses described in section 3(d).

Since the only oc mutations in T4 late genes are in gene e and psu_a^+ is closely linked to gene e, we used an alternative method for testing psu_a^+ suppression of these mutations. We constructed a strain containing psu_a^+ and a deletion of gene e, eG223, which overlaps the sites of the nonsense mutants tested. In a cross of psu_a^+:eG223 by a gene e nonsense mutant only those mutations which can be suppressed by psu_a^+ will give rise to recombinant progeny capable of making a plaque on S/6/5 in the absence of added egg white lysozyme.

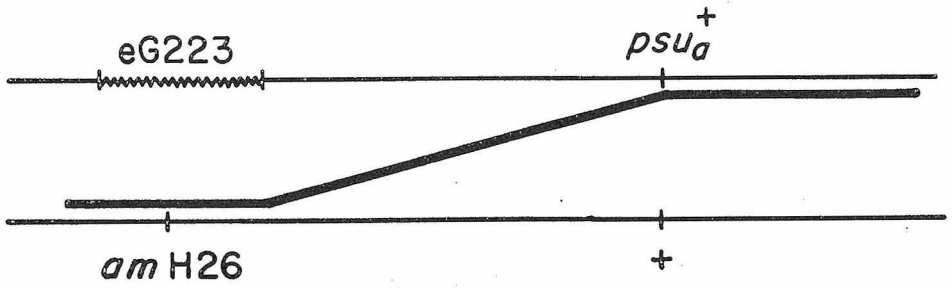


Table 3

psu_a^+ suppression of nonsense mutations in late genes

Gene	Mutant	% R in crosses against		Suppression of mutation at 42°C
		tsB78	psu_a^+:tsB78:rdf41:amB25+...	
37	amA481	*	32	+
	amNG182	*	42	+
	amN52	*	31	+
	amNG220	*	40	+
	amNG187	*	3.3	-
	amNG475	*	41	+
	opC23	*	2.4	-
	opC91	3.4	2.4	-
	opC121	*	3.9	-
		tsA44	psu_a^+:tsA44:rdf41:amN52+...	
34	amA455	6.0	36	+
		tseC3	psu_a^+:tseC3:rdf41:amB25+...	
e	amH26	2.9	7.5	+

Crosses are of the standard type. The total progeny were measured by plaque assay on CR63 at 25°C and recombinant progeny were titered by plaque assay on S/6/5 at 42°C. The per cent recombination (% R) is calculated as 200% (recombinant progeny/total progeny). *tsB78 is located near the middle of gene 37 and is expected to give less than 6% recombination with any of the mutants listed.

Of the eight nonsense mutants tested (amH26, oceL1, oceL2, oceL3, oceL4, oceL5, opeL1P12 derived from oceL1, opeL4P41 derived from oceL4) amH26 yields about 3% viable recombinant progeny, while the other nonsense mutants do not yield viable recombinants at a frequency of greater than 0.1%. In an analogous manner we had hoped to define the suppressor capabilities of psu_b^+. However, we have not been able to isolate the double mutant psu_b^+:eG223, presumably because the combined growth limiting properties of the two mutations prevent the double mutant from making a plaque.

To compare the pattern of am mutant suppression by psu_a^+ and psu_b^+ with that of bacterial suppressors we isolated several psu_a^+:am, psu_a^+:op, and psu_b^+:am double mutants as described in the legend of Table 4. In Table 4 the growth of these double mutants on S/6/5 is compared with that of the nonsense mutant alone on the bacterial suppressors, $su\text{-}1^+$, $su\text{-}2^+$, and $su\text{-}3^+$. It should be noted that the occurrence of an am mutation which is temperature sensitive on a particular suppressor host seems to be relatively rare. Of the 20

Table 4

Suppression patterns of bacterial and phage nonsense suppressors

Gene	Mutant	S26R1e *su-1*$^+$	S26R1d *su-2*$^+$	H12R8A *su-3*$^+$	*psu*$_a^+$: mutant S/6/5 *su*$^-$	*psu*$_b^+$: mutant S/6/5 *su*$^-$
37	*am*A481	+	+	+	+	+
	*am*NG182	+	+	+	+	+
	*am*N52	+	+	+	+	+
	*am*NG187	ts	0	+	ts	ts
	*am*NG475	+	+	0	+	
	*am*B280	+			+	
	*op*C121	0	0	0	0	
34	*am*H34	+	+	+	+	+
	*am*B25	+	+	+	+	+
	*am*B265	+	+	0	+	+
	*am*N58	+	+	0	+	
	*am*A459	+	+	0	+	+
	*am*B258	+	0	+	+	
	*am*K6	0	0	ts	0	
18	*am*NG197	ts	0	+	ts	

Table 4 (cont.)

All the $\underline{psu}_a^+$ double mutants were isolated from the crosses described in Table 3. With the exception of $\underline{psu}_a^+$:amNG187, $\underline{psu}_a^+$:amK6, and $\underline{psu}_a^+$:opC121 the doubles were isolated from the progeny plated on S/6/5 at 42°C. $\underline{psu}_a^+$:amNG187 was isolated from progeny plated on S/6/5 at 30°C. $\underline{psu}_a^+$:amK6 was isolated from progeny plated on H12R8A at 30°C. $\underline{psu}_a^+$:opC121 was isolated from progeny plated on CAJ70 at 42°C. Those listed as double mutants yield only the indicated mutant, as judged by complementation, in the progeny of a cross to wild type phage. The presence of $\underline{psu}_a^+$ in $\underline{psu}_a^+$:amK6 and $\underline{psu}_a^+$:opC121 was verified by crossing them to amNG187 and testing for progeny which are temperature sensitive on S/6/5.

$\underline{psu}_b^+$:am doubles were generated in crosses of $\underline{psu}_b^+$ or $\underline{psu}_b^+$:amN133 by the respective am. The criteria for the double mutants are that they have the $\underline{psu}_b^+$ plating phenotype and that the suppressor-negative revertants of them (see section 3(e)) contain only the am of interest. The $\underline{psu}_b^+$ strain which contains amN52 also carries amN133.

Plaque formation at both temperatures is designated as +. ts indicates plaque formation at 30°C but not at 42°C. 0 means no plaques can be seen.

gene 34 and 37 am mutations tested only one, NG187, is temperature sensitive on su-1$^+$. In an attempt to find another one we screened 71 am mutations in genes 6, 7, 18, and 23 before we found amNG197. The identity of the suppression patterns of psu$_a^+$ and su-1$^+$ indicates that psu$_a^+$ probably inserts serine in response to the am codon. The results with psu$_b^+$:am double mutants, though not compelling, suggest that psu$_b^+$ also inserts serine.

Taken together the results on suppression of nonsense mutations in late genes suggest that psu$_a^+$ is a classical amber suppressor. The results do not define the range of mutations which psu$_b^+$ can suppress but only extend the observation that psu$_b^+$ can suppress a variety of am mutations.

That both these suppressors appear to insert serine raises a question as to their origin. The original stocks of the multiple am mutants from which psu$_a^+$ and psu$_b^+$ were isolated were grown on CR63 which carries su-1$^+$ (Stretton & Brenner, 1965). Though there is no precedent for the incorporation of host DNA into the T4 genome it is theoretically possible that either psu$_a^+$ or psu$_b^+$ or both were picked up from the host bacterium and are not alterations of phage genes. To eliminate this possibility we isolated suppressor mutations with the plating characteristics of psu$_a^+$ and psu$_b^+$ from a stock of X74 phage which had been grown from a single plaque on H12R8A (su-3$^+$). The revertant with psu$_a^+$ plating characteristics, when crossed to amNG187, yielded progeny which were temperature sensitive on S/6/5. Isolation of suppressors with properties like psu$_a^+$ and psu$_b^+$ from stocks grown on an su-3$^+$ host

argues that $\underline{psu}_a^+$ and $\underline{psu}_b^+$ were not picked up from the host bacterium.

(ii) $\underline{psu}_a^+$ suppression of nonsense mutations in early genes

$\underline{psu}_a^+$ suppression of nonsense mutations in early genes was tested in crosses with strains carrying $\underline{psu}_a^+$ and a ts mutation as described above for late genes (Table 3) and the results are shown in Table 5.

Since $\underline{psu}_a^+$ is unlinked to all the genes tested, we were surprised to find such generally low recombination values in these crosses. As shown in Table 3, analogous crosses involving am mutations in unlinked late genes yield a much higher per cent recombination. The reason for these reduced recombination values in crosses against am mutations in early genes is not clear.

In most of the crosses between am mutants and strains carrying $\underline{psu}_a^+$ there were two different-sized plaques among the recombinant progeny. From the relative frequency of the large (wild type) and small plaques we anticipated that the small plaques represented phage carrying $\underline{psu}_a^+$ and the test am mutation. To test this idea we picked small plaques from among the recombinant progeny of crosses involving amE114, amN55, amE117, and amNG411. Each of these small plaques, when crossed to wild type phage, yielded the test am, verifying the notion that the small plaques are made by phage which carry both $\underline{psu}_a^+$ and the test am mutation. Thus in addition to the criterion of an increase in recombination frequency as described in Table 3, we have used the criterion of a smaller plaque type among the recombinant progeny to decide whether a particular mutation is suppressed by $\underline{psu}_a^+$. Using

Table 5

psu^+_a suppression of nonsense mutations in early genes

Gene	Mutant	% R in crosses against		Difference in % R	% R in *psu*^+_a crosses		Size of smaller plaque	*psu*^+_a suppression of mutation
		(1)	(2)	(2) - (1)	wild type	small		
		*ts*L66(42)	*psu*^+_a:*ts*L66:*am*N52+...					
56	amE114†	14.7	22	+7.3	16.4	5.3	2	+
41	amN81	13.9	36	+22	9.8	26	2	+
42	amN122	2.8	2.8	0			-	-
	amN55†	2.1	1.7	-	1.5	.2	1	+
	amE117†	.96	7.1	+6.1	1.2	5.9	1	+
	amNG352	3.7	31	+27	3.4	28	2	+
	amNG411†	3.4	12.9	+9.5	2.5	10.4	1	+
43	amB22	7.0	32	+25	2.8	29	2	+
	amNG305	7.2	31	+24	4.2	27	2	+
	amNG493	7.5	33	+26	4.9	28	3	+
	amNG562	8.7	43	+34	8.4	35	3	+

Table 5 (cont.)

psu_a^+ suppression of nonsense mutations in early genes

Gene	Mutant	% R in crosses against (1)	% R in crosses against (2)	Difference in % R (2) - (1)	% R in psu_a^+ crosses: wild type	% R in psu_a^+ crosses: small	Size of smaller plaque	psu_a^+ suppression of mutation
	amE4305	7.9	23	+15.1	5.0	18	2	+
		rdf41	psu_a^+:rdf41:amN52+...					
52	amE38	6.3	28	+22	4.3	24	1	+
	amE118	7.5	28	+20	3.7	24	1	+
	amE663	7.8	2.0	-			-	-
39	amN116	6.6	21	+14.4	3.1	17.8	1	+
	amC237	5.0	37	+32	2.7	34	1	+
	amE29	5.9	28	+22	3.5	25	1	+
	amE142	5.9	19.7	+13.8	2.6	17.1	2	+
rIIA	amHB118	*	<0.1					-
	amHB122		<0.1					-
	amEM640		<0.1					-

Table 5 (cont.)

psu^+_a suppression of nonsense mutations in early genes

Gene	Mutant	% R in crosses against (1)	% R in crosses against (2)	Difference in % R (2) - (1)	% R in *psu*^+_a crosses: wild type	% R in *psu*^+_a crosses: small	Size of smaller plaque	*psu*^+_a suppression of mutation
	*am*HB35		<0.1					-
	*am*N11		<0.1					-
	*am*C204		<0.1					-
	*am*HB32		<0.1					-
	*oc*N21		<0.1					-
	*op*X665		<0.1					-
rIIB	*am*HB232		15.6	+15.6			1	+
	*am*EM84		4.2	+4.2			1	+
	*am*NT332		7.3	+7.3			1	+
	*oc*UV375		<0.1					-
	*op*X655		<0.1					-
		*ts*CB53(*45*)	*psu*^+_a:*ts*CB53:*am*N52+...					

Table 5 (cont.)

psu^+_a suppression of nonsense mutations in early genes

Gene	Mutant	% R in crosses against		Difference in % R	% R in *psu*^+_a crosses		Size of smaller plaque	*psu*^+_a suppression of mutation
		(1)	(2)	(2) - (1)	wild type	small		
44	amN82	2.4	3.6	+1.2			-	-
45	amNG18	1.2	1.0	-			-	-
46	amB3	2.8	24	+21			2	+
	amN130	8.0	31	+23			3	+
	amNG209	3.6	31	+27			2	+
	amNG371	4.9	27	+22			2	+
	amNG454	4.7	27	+22			2	+
47	amA456	12.1	10.4	-			-	-
	amNG106	6.3	34	+28			2	+
	amNG163	8.3	32	+24	6.2	26	2	+
	amNG277	10.2	26	+15.8	3.6	22	2	+
	amNG583	7.1	6.9	-			-	-

Table 5 (cont.)

psu_a^+ suppression of nonsense mutations in early genes

Gene	Mutant	% R in crosses against (1)	% R in crosses against (2)	Difference in % R (2) - (1)	% R in psu_a^+ crosses: wild type	% R in psu_a^+ crosses: small	Size of smaller plaque	psu_a^+ suppression of mutation
		tsCB106(31)	psu_a^+:tsCB106:amB25+...					
55	amB1292	16.3	26	+9.7			2	+
	amNG372	13.0	28	+15			2	+
30	amH39	16.3	18.6	+2.3			2	+
	amC104	16.1	5.6	-			-	-
	amNG83	9.7	15.7	+6			3	+
31	amN54	1.3	9.2	+7.9	.45	8.8	2	+
	amS54	.9	8.5	+7.6	.77	7.7	1	+
	amNG71	2.9	35	+32			-	+
		tsP7(32)	psu_a^+:rdf41:tsP7:amN52+...					
32	amE315	1.9	18.7	+16.6			1	+
	amNG461	1.5	18	+16.5			1	+

Table 5 (cont.)

Crosses are of the standard type. The per cent recombination (% R) is determined as in the legend to Table 3. Crosses involving genes 52, 39, and rII were plaque assayed on CR63 at 30°C for total progeny and on G(λ) at 30°C for recombinant progeny. *The rdf41 crosses for the rII test mutants were not done since rdf41 encompasses the entire rIIA and rIIB cistrons. Two additional % R's are calculated for crosses against psu_a^+ strains which yield two easily distinguishable plaque types among the recombinant progeny. The larger plaques are assumed to be wild type and the % R is tabulated under that heading. The % R for the smaller class of plaques is listed under small. The size of the smaller class of plaques when the progeny are plated on S26 (su^-) relative to the am mutant alone on S26Rle($su\text{-}1^+$) is denoted by a number from 1-3. 3 indicates the plaques are approximately the same size. 1 and 2 denote two arbitrary classes of intermediate sized plaques. - indicates that a class of smaller plaques is not detectable. All test am mutants are suppressed in bacterial hosts which carry $su\text{-}1^+$. psu_a^+ suppression or non-suppression of the test mutant is designated by + and - respectively. †Suppression of these mutations was determined directly by isolation of the double mutant (see text).

these two criteria we conclude that most am mutations in early genes are suppressed by psu_a^+. However psu_a^+ suppression of am mutations in early genes is qualitatively different from its suppression of am mutations in late genes, since strains which carry psu_a^+ and an am mutation in a late gene make plaques on S26 (su^-) which are indistinguishable from the am mutant alone on S26Rle.

One striking result is the lack of suppression of all seven am mutations tested in the rIIA cistron. psu_a^+ shows suppression of at least one am mutation in all other early genes for which more than one am mutation has been tested. The meaning of this result is unclear.

(c) Efficiency of psu_a^+ and psu_b^+ suppression

(i) Burst size of psu_a^+:am double mutants

We had assumed from the reduced plaque size (and reduced number of phage in the plaque) of strains which carry psu_a^+ and an am mutation in an early gene that psu_a^+ suppressed these mutations very poorly. To get a rough measure of the efficiency of psu_a^+ suppression and an idea of the relationship of plaque size to degree of suppression, we compared the burst sizes of various psu_a^+:am double mutants on S26 (su^-) with that of the am alone on S26Rle ($su\text{-}1^+$). The results are shown in Table 6. To our surprise the burst sizes of the psu_a^+:am double mutants defective in early and late genes on S26 are indistinguishable and equal to about 60% of the burst of wild type phage. The reason for the small plaque phenotype of psu_a^+:am doubles in early genes is not clear, but may simply reflect the difference in

Table 6

Burst sizes of psu_a^+:*am* double mutants

Gene	Mutant	% wild type burst size	
		Single mutant S26Rle(*su-1*$^+$)	psu_a^+:*am* double mutant S26(*su*$^-$)
56	*am*E114	119	55
42	*am*N55	98	55
	*am*E117	90	49
	*am*NG411	95	51
31	*am*NG71	116	95
	average (early genes)	104	61
34	*am*H34	110	71
	*am*B25	109	73
	*am*B265	131	44
	*am*A459	125	78
37	*am*A481	100	62
	*am*NG182	115	48
	*am*N52	126	55
	*am*NG187		60
	average (late genes)	117	61
	psu_a^+	117	101

S26 and S26Rle bacteria were grown to 5 x 10^7 cells/ml. in H broth, collected by centrifugation, and resuspended at 4 x 10^8 cells/ml.

Table 6 (cont.)

Phage at a multiplicity of 0.3 were added to the cells in the presence of 0.004 M KCN at 30°C. After 5 min anti-T4 serum was added to kill all unadsorbed phage. 10 min after infection the cells were diluted 10^4 into H broth at 30°C. Infective centers were assayed on CR63 5 min after dilution. Total progeny were assayed on CR63 60 min after dilution. The burst size for each was calculated as total progeny/infective center.

physiological state of bacteria in liquid culture and in agar.

(ii) Gene product synthesis in psu_a^+:*am* and psu_b^+:*am* double mutants

The above results suggest that psu_a^+ suppresses *am* mutations reasonably efficiently. The poor growth characteristics of the single mutant psu_b^+ preclude any such simple assessment of its efficiency. To determine accurately the efficiency of suppression of psu_a^+ and psu_b^+ we measured the amount of gene *34* or *37* product (P34 or P37) produced in doubly mutant strains which carried an *am* mutation in one of those genes as well as psu_a^+ or psu_b^+. The bands corresponding to P34 and P37 have been identified on SDS polyacrylamide gels (Laemmli, 1970; Ward & Dickson, 1971). P7 has been identified as the band which is located between P34 and P37 (King & Laemmli, in preparation). As a measure of the absolute amount of these gene products present in individual ^{14}C-labeled lysates, we used P7 as an internal standard and normalized the amount of P34 or P37 to the amount of P7 on the same gel. The per cent of P34 or P37 present in the suppressor strain as compared with wild type is taken as the efficiency of suppression. The results are shown in Table 7, and typical densitometer tracings from which the data were derived are shown in Figure 2. Both suppressors show an efficiency of about 45%.

(d) Map positions of psu_a^+ and psu_b^+

The experiments on suppression of a gene *e* *am* mutation (shown in Table 3) suggested that psu_a^+ is closely linked to gene *e*. To determine

Table 7

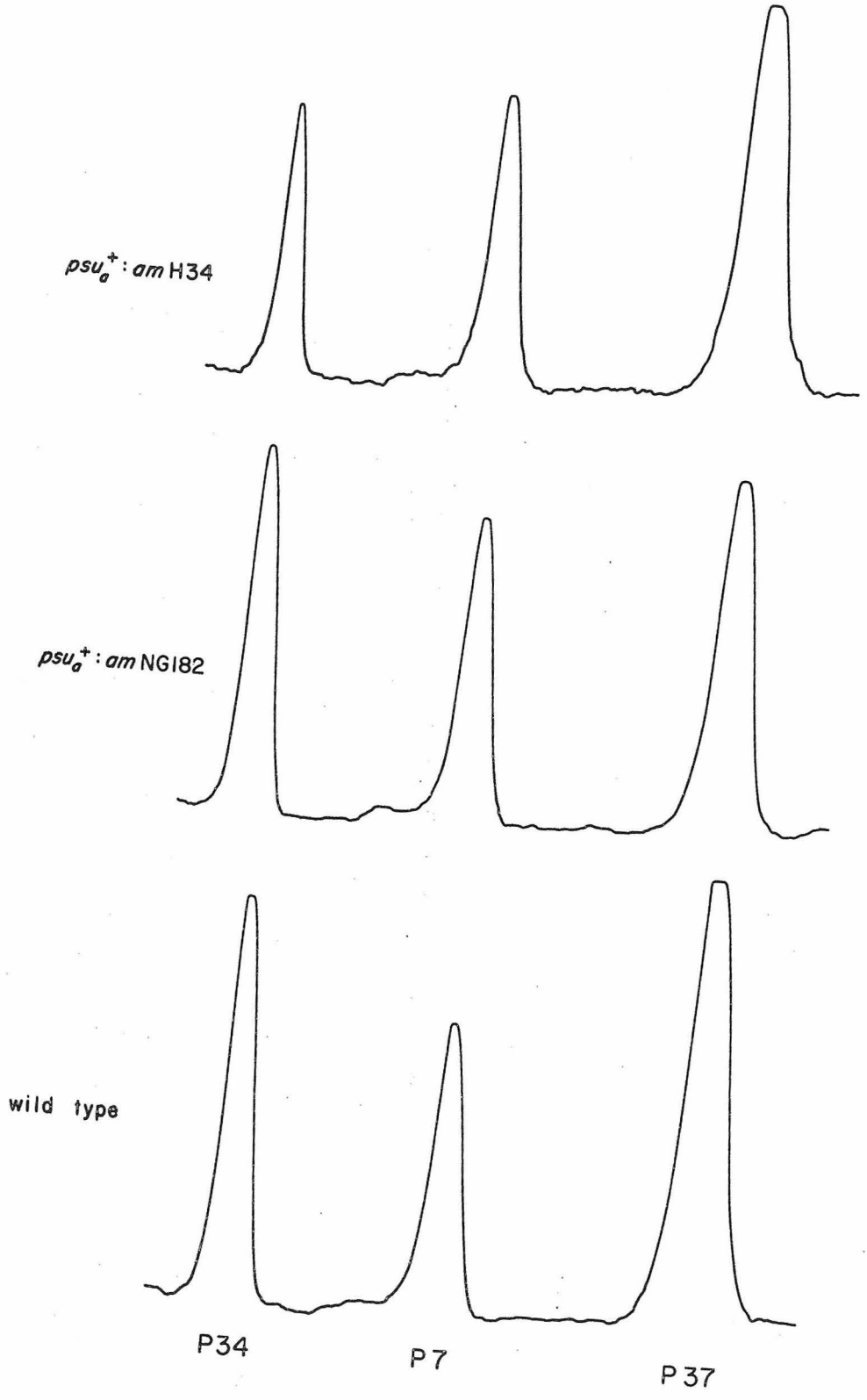
Efficiency of $\underline{psu}_a^+$ and $\underline{psu}_b^+$ suppression

Gene	Strain	% wild type P34	% wild type P37
34	$\underline{psu}_a^+$:amH34	45	
	$\underline{psu}_a^+$:amB25	42	
	$\underline{psu}_a^+$:amB265	52	
	$\underline{psu}_b^+$:amB265	34	
37	$\underline{psu}_a^+$:amA481		48
	$\underline{psu}_a^+$:amNG182		52
	$\underline{psu}_a^+$:amN52		42
	$\underline{psu}_a^+$:opC121		0
	$\underline{psu}_b^+$:amA481		56

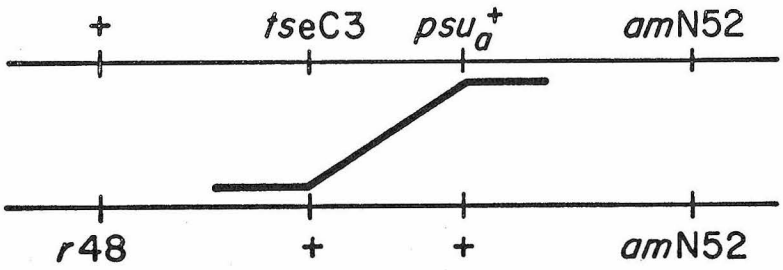
Preparation of lysates, electrophoresis, autoradiography, and analysis of the densitometer tracings of the autoradiographs are described in Materials and Methods. All values for P34 or P37 are normalized to the amount of P7 present in the same gel. The values shown represent a single determination for $\underline{psu}_b^+$ strains and an average of two determinations for the $\underline{psu}_a^+$ strains.

Fig. 2. Densitometer tracings of autoradiographs.

Preparation of lysates, electrophoresis, and autoradiography are as described in Materials and Methods. The 2 cm of the autoradiograph containing the bands which correspond to P34, P7, and P37 were traced with a Joyce-Loebl densitometer at an expansion ratio of 20:1. Typical tracings are shown.


psu$_a^+$: am H34
psu$_a^+$: am NG182
wild type
P34
P7
P37

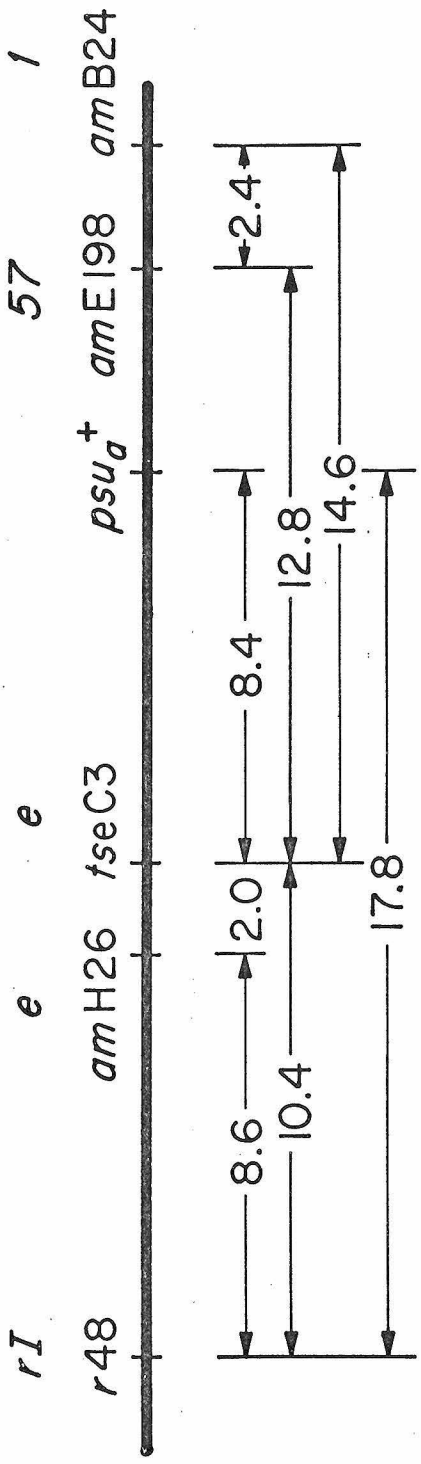
its map position more accurately we constructed the strain $\underline{psu}_a^+$:$\underline{tse}$C3:$\underline{am}$N52 and crossed to $\underline{r}^{48}$:$\underline{am}$N52.



Since both parental strains contain $\underline{am}$N52, only those progeny which have undergone a crossover as indicated and carry $\underline{psu}_a^+$ but not $\underline{tse}$C3 will plate on S/6/5 at 42°C. The ratio of $\underline{r}^{48}$ to $\underline{r}^+$ plaques among these recombinant progeny is 2.8, indicating that $\underline{psu}_a^+$ and $\underline{r}^{48}$ are on opposite sides of $\underline{tse}$C3. If the progeny of this cross are assayed at 25°C on S/6/5 so that $\underline{tse}$C3 is an unselected marker, the titer of $\underline{r}^{48}$ plaques becomes a measure of the distance from $\underline{psu}_a^+$ to $\underline{r}^{48}$. The results of this and other crosses between markers in the same region are summarized in Figure 3.

Fig. 3. Map position of $\underline{psu}_a^+$.

Crosses are of the standard type. The crosses involving $\underline{psu}_a^+$ are described in the text. Total progeny for all crosses were plaque assayed on CR63 at 25°C. Except as noted in the text recombinant progeny were plaque assayed on S/6/5 at 42°C. The numbers below the map are %R and were calculated as 200% (recombinant progeny/total progeny). Recombinant progeny from crosses involving $\underline{r}^{48}$ were pre-adsorbed on S/6/5 at room temperature for 5 min before plating to insure uniform plaque morphology. A three-factor cross between $\underline{r}^{48}$:amH26 and tseC3 shows a ratio of $\underline{r}^{48}$ to $\underline{r}^+$ in the recombinant progeny of 0.5, indicating that tseC3 maps to the right of amH26. The strain $\underline{psu}_a^+$:tseC3:amN52, used to position $\underline{psu}_a^+$, was isolated as a temperature sensitive plaque from the progeny of the cross $\underline{psu}_a^+$:amN52 by tseC3:amN52 plated at 25°C on S/6/5. The map is drawn so that right to left corresponds to the clockwise direction on the circular T4 map as it is customarily represented (Edgar, 1968). The order shown for genes 1 and 57 is the currently accepted one (Edgar, 1968; S. K. Beckendorf, R. D. K. Josslin & R. L. Russell, personal communications).


gene
mutant
rI
r48
e
amH26
e
tseC3
e
57
psu$_a^+$
amE198
1
amB24
8.6
2.0
8.4
2.4
10.4
12.8
14.6
17.8

The inability of psu_b^+ phage to form plaques on CR63 makes this suppressor much simpler to map. Recombinant progeny from a cross between psu_b^+ and a ts mutant can be assayed by plating on CR63 at 42°C. psu_b^+ was mapped with respect to ts mutations in genes e, 31, 42 and 45 as shown in Table 8.

The results suggest that psu_b^+ also is linked to gene e. The reason for the depressed recombination values in the psu_b^+ crosses is unclear. Control crosses between amA455 in gene 34 and each of the ts mutants show an average of 35% recombination. The low recombination in psu_b^+ crosses may be an effect of the reduced burst size due to the partial dominance of the psu_b^+ growth properties.

That psu_b^+ is linked to gene e is confirmed by the cross of psu_b^+ to psu_a^+:amN52 (Table 8). amN52 single mutants should appear in the progeny of this cross at a frequency dependent on the distance between psu_a^+ and psu_b^+. Since no suppressor-negative recombinants were found, psu_b^+ must map very near psu_a^+, and is therefore linked to gene e.

(e) Are psu_a^+ and psu_b^+ in the same gene?

Several observations suggest that psu_a^+ and psu_b^+ may result from different mutations within the same gene. As already described, they are located within a few tenths of a map unit of each other and both appear to insert serine in response to the am codon. A third piece of suggestive evidence comes from an analysis of the pseudo-wild type revertants of psu_b^+. When psu_b^+:X74 is plated at high concentration on CR63 two distinctive plaque types appear. At a frequency about 10^{-3} of

Table 8

Mapping data for $\underline{psu}_b^+$

Strain	% R in crosses against $\underline{psu}_b^+$
*ts*eC3 (2)	7.2
*ts*CB106 (31)	17.3
*ts*L66 (42)	25
*ts*CB53 (45)	19.1
$\underline{psu}_a^+$:*am*N52	<0.3

Crosses are of the standard type. Total progeny from the crosses of the temperature sensitive mutants and $\underline{psu}_b^+$ were measured by plaque assay on S/6/5 at 25°C. Recombinant progeny were titered by plaque assay on CR63 at 42°C. % R is calculated as 200% (recombinant progeny/total progeny). The total progeny from the cross of $\underline{psu}_b^+$ by $\underline{psu}_a^+$:*am*N52 were measured by plaque assay on CR63. None of 664 plaques tested were *am* in phenotype. Since only about one-half the suppressor-negative recombinants would be expected to contain *am*N52 and only one of the two parents plate on CR63, the % R is calculated as <0.3.

the $\underline{psu}_b^+$:X74 titer is a class of small heterogeneous plaques, which when picked and replated make normal plaques on CR63 and no plaques on S/6/5. The basis for the change in the nature of the plaques upon replating is not known. These phage are suppressor-negative revertants to the _am_ phenotype and are characterized in detail by Wilson & Abelson (1972). At a frequency of about 10^{-4} is a class of normal plaques. Nine independent plaques of this type have been picked, characterized, and shown to have properties in common with $\underline{psu}_a^+$. They make normal plaques on both CR63 and S/6/5, but when crossed to wild type phage yield the _am_ mutations contained in X74. One of the nine has been crossed to _am_NG187. The progeny of this cross contain phage which are temperature sensitive on S/6/5, suggesting that these pseudo-wild type phage insert serine at the site of the _am_ codon. The high frequency of appearance of these pseudo-wild type phage suggests they are a single step change from $\underline{psu}_b^+$. To determine the distance from $\underline{psu}_b^+$ to the site of the change we crossed each of the pseudo-wild type phage to wild type phage and screened more than 53,000 total progeny for the $\underline{psu}_b^+$ plating phenotype. Only three of the progeny make small plaques on S/6/5 and no plaques on CR63. These plaques have not been characterized further to determine whether they are $\underline{psu}_b^+$. Nevertheless this result indicates a maximum recombination frequency between $\underline{psu}_b^+$ and the second site of 0.01%. If the pseudo-wild type derivatives of $\underline{psu}_b^+$ are in fact $\underline{psu}_a^+$, then these results suggest that both suppressors do affect the same gene.

4. Discussion

We have isolated and characterized two phage-coded nonsense suppressors, $\underline{psu}_a^+$ and $\underline{psu}_b^+$. Both suppressors seem to insert serine in response to the am codon and map no more than a few tenths of a map unit apart. On the basis of their initial frequencies of appearance (10^{-7} to 10^{-10} for $\underline{psu}_b^+$ and 10^{-11} to 10^{-12} for $\underline{psu}_a^+$) and the frequency of $\underline{psu}_b^+$ reversion to what seems to be $\underline{psu}_a^+$ (10^{-4}), we speculate that 1) $\underline{psu}_a^+$ is an amber suppressor with the anticodon -CUA- which is derived by two mutations from a serine tRNA with the anticodon -UGA-, and 2) $\underline{psu}_b^+$ is a one-base-change intermediate, an ochre suppressor with the anticodon -UUA-. This interpretation of the relationship of $\underline{psu}_a^+$ and $\underline{psu}_b^+$ is consistent with all the observations we have made and in addition suggests a reason for the partial dominance of $\underline{psu}_b^+$. Bacteria which carry an ochre suppressor generally grow very poorly. One explanation for this, as yet unproven, is that the oc codon is normally used in the chain termination signal for protein synthesis and that the ochre suppressor tRNA in some manner interferes with proper termination, causing the poor growth phenotype. By analogy we might expect that a phage-coded ochre suppressor would lead to a reduced phage burst size. We might also expect that the amount of the reduction would depend upon the concentration of the ochre suppressor within the cell. As shown in Figure 1, $\underline{psu}_b^+$ meets both expectations.

W. H. McClain (1970) has isolated and characterized another T4-coded nonsense suppressor, $\underline{psu}_1^+$. $\underline{psu}_1^+$ and $\underline{psu}_a^+$ appear to be identical.

He has demonstrated that psu_1^+ is an amber suppressor which inserts serine in response to the *am* codon with an efficiency of about 50% and is located about eight map units from gene *e*. The nucleotide sequences of the suppressor and wild type tRNAs have been determined (W. H. McClain & B. Barrell, personal communication). The suppressor tRNA has the anticodon -CUA- and was derived from the wild type by two base changes in the anticodon. As is shown by Wilson & Abelson (1972), the mutation which results in psu_b^+ activity maps at or near the anticodon as defined by psu_1^+.

Though the circumstantial evidence is quite strong that psu_b^+ is a one-base-change intermediate between the wild type and the amber suppressor, we have been unable to demonstrate the expected ochre suppressor phenotype. In an attempt to determine whether psu_b^+ does suppress *oc* mutations, we crossed psu_b^+ to an *oc* mutation in gene *e*, *oce*L1. The input ratio in the mixedly infected cell in this cross was 7.5 *oce*L1 to 1 psu_b^+. From this input ratio and a value of 8% for the recombination in equal input crosses, we would expect the psu_b^+:*oce*L1 double mutant to constitute about 15% of the progeny with the psu_b^+ phenotype (Visconti & Delbrück, 1953). We isolated 26 phage with the psu_b^+ phenotype from among the progeny of the cross plated on S/6/5 without added egg white lysozyme. None of these 26 yielded *oce*L1 when crossed to wild type phage. Since, if psu_b^+ were an ochre suppressor, we would have expected to find 4 psu_b^+:*oc* double mutants, these results suggest tentatively that psu_b^+ does not suppress *oc* mutations. The *am* mutation at the same site, *ame*L1/a, grows on $su\text{-}1^+$. Consequently, the

relationship between $\underline{psu}_a^+$ and $\underline{psu}_b^+$ remains unclear, and its final elucidation will probably require nucleotide sequence analysis of the $\underline{psu}_b^+$ suppressor tRNA.

We are grateful to Dr. R. L. Russell and Dr. W. B. Wood for many helpful discussions. We are especially indebted to Dr. W. H. McClain and Dr. B. G. Barrell for generously offering us their unpublished results upon which depend, directly and indirectly, many of the arguments and conclusions presented in this paper. This research was supported by grants (GM-06965 and AI-09238) from the U.S. Public Health Service.

REFERENCES

Daniel, V., Sarid, S. & Littauer, U. V. (1970). Science, 167, 1682.

Edgar, R. S. & Lielausis, I. (1965). Genetics, 52, 1187.

Edgar, R. S. & Wood, W. B. (1966). Proc. Nat. Acad. Sci., Wash. 55, 498.

Edgar, R. S. (1968). Handbook of Biochemistry, ed. by Herbert A. Sober, p. I-25. The Chemical Rubber Company, Cleveland.

Emrich, J. (1968). Virology, 35, 158.

Epstein, R. H., Bolle, A., Steinberg, C. M., Kellenberger, E., Boy de la Tour, E., Chevalley, R., Edgar, R. S., Susman, M., Denhardt, G. H. & Lielausis, A. (1963). Cold Spr. Harb. Symp. Quant. Biol. 28, 375.

Fairbanks, G., Jr., Levinthal, C. & Reeder, R. H. (1965). Biochem. Biophys. Res. Comm. 20, 393.

Garen, A., Garen, S. & Wilhelm, R. C. (1965). J. Mol. Biol. 14, 167.

King, J. (1968). J. Mol. Biol. 32, 231.

Laemmli, U. K. (1970). Nature, 227, 680.

McClain, W. H. (1970). FEBS Lett. 6, 99.

Russell, R. L. (1967). PhD. Thesis, California Institute of Technology, Pasadena, California.

Scherberg, N. H. & Weiss, S. B. (1970). Proc. Nat. Acad. Sci., Wash. 67, 1164.

Steinberg, C. M. & Edgar, R. S. (1962). Genetics, 47, 187.

Stretton, A. O. W. & Brenner, S. (1965). J. Mol. Biol. 12, 456.

Sueoka, N. & Kano-Sueoka, T. (1970). Prog. in Nucleic Acid Res. and Mol. Biol. 10, 23.

Visconti, N. & Delbrück, M. (1953). Genetics, 38, 5.

Ward, S. (1970). Anal. Biochem. 33, 259.

Ward, S., Luftig, R. B., Wilson, J. H., Eddleman, H., Lyle, H. & Wood, W. B. (1970). J. Mol. Biol. 54, 15.

Ward, W. & Dickson, R. C. (1971). J. Mol. Biol., in the press.

Weber, K. & Osborn, M. (1969). J. Biol. Chem. 244, 4406.

Wilson, J. H., Luftig, R. B. & Wood, W. B. (1970). J. Mol. Biol. 51, 423.

Wilson, J. H. & Abelson, J. N. (1972). J. Mol. Biol.

Wilson, J. H., Kim, J. S. & Abelson, J. N. (1972). J. Mol. Biol.

Wood, W. B. & Henninger, M. (1969). J. Mol. Biol. 39, 603.

Bacteriophage T_4 Transfer RNA

II. Mutants Defective in the Formation of Functional Suppressor tRNA

John H. Wilson*

Division of Biology, California Institute of Technology

Pasadena, Calif. 91109, U.S.A.

John N. Abelson

Department of Chemistry, University of California (San Diego)

La Jolla, Calif. 92037, U.S.A.

Running title: Mutants Defective in tRNA Formation

*Present address: Department of Biochemistry, Stanford University Medical School, Stanford, California 94305.

Abstract

151 independent suppressor-negative derivatives of a T4-coded nonsense suppressor, $\underline{psu}_b^+$, have been isolated and characterized. They fall into two complementation groups. One, designated mb (modifier of $\underline{psu}_b^+$ phenotype), is unlinked to $\underline{psu}_b^+$ and has been located about 10 map units from rII. The second group, designated $\underline{psu}_b^-$, is made up of deletions and single base changes which affect sites within 0.2 map units of the original mutation. Those $\underline{psu}_b^-$ mutants which still contain the original mutation, psu_b, have been mapped relative to psu_b and each other by a series of two- and three-factor crosses. ^{32}P-labeled tRNA from mb, $\underline{psu}_b^-$ and wild type infected cells has been compared by polyacrylamide gel electrophoresis. In mb-infected cells several of the tRNA species are missing, while in $\underline{psu}_b^-$-infected cells only the ser-tRNA is clearly absent.

These studies suggest there are only two phage genes which are essential for the production of functional ser-tRNA. One is the structural gene for the ser-tRNA and the second plays an undefined role which affects several tRNAs.

1. Introduction

The formation of a functional tRNA is a complex and not well understood process involving transcription of the structural gene, possible cleavage of a precursor (Altman & Smith, 1971; W. H. McClain, personal communication), and modification of individual nucleotides. The resultant tRNA must be able to recognize its amino acyl-tRNA synthetase, the tRNA binding site on the ribosomes, the appropriate ribosomal factors, and its matching codon in the messenger RNA. A block in the maturation process or a structural alteration within the tRNA which causes the loss of a recognition site will lead to the loss of tRNA activity. The isolation of strains which cannot make functional tRNA could be extremely valuable both in defining the steps in the maturation of tRNA and in elucidating structure-function relationships within the tRNA molecule.

The bacteriophage T4 offers a unique system for the easy isolation and genetic characterization of strains which are unable to produce a functional tRNA. T4 makes several new species of tRNA upon infection of Escherichia coli bacteria (Daniel, Sarid & Littauer,1970). Normally, none of these phage-specific tRNAs is essential (Wilson, Kim & Abelson, 1972). However we can artificially make the ser-tRNA essential by altering it to read the amber codon and then coupling it with an amber mutation. This procedure allows us to identify phage strains which have lost the capacity to make functional ser-tRNA. The peculiar plating phenotype of strains which carry psu_b^+ (Wilson & Kells, 1972)

simplifies the isolation of these strains. Suppressor-negative derivatives of $\underline{psu}_0^+$:amN133 will grow on an $\underline{su}^+$ K host strain while $\underline{psu}_0^+$:amN133 itself will not. Since the presence of $\underline{psu}_0^+$ is essential for phage growth in one host and lethal in another, we can select either for or against $\underline{psu}_0^+$. The non-essential nature of the phage tRNAs, the potential for bidirectional selection and the simplicity of genetic analysis in T4 combine to make a unique system for the exploration of general questions about tRNA formation and function.

In this paper we have exploited this system to isolate and characterize a number of suppressor-negative mutants. The evidence presented suggests that there are only two phage genes which are essential for the production of functional ser-tRNA.

2. Material and Methods

(a) Phage and bacterial strains

Phage and bacterial strains are described in Wilson & Kells (1972). CR63 was used as the non-permissive host and S/6/5 as the permissive host for strains carrying $\underline{psu}_0^+$. $\underline{psu}_1^+$:amB20:amN022 and the suppressor-negative derivative $\underline{psu}_1^-$1:ameL1/a were kindly given to us by W. H. McClain. The suppressor strain $\underline{n}_1$:oceL3 was kindly provided by H. Kneser.

(b) Media and buffers

Media and buffers are described in Wilson & Kells (1972). Phosphate free buffer, pH 7.4, contains per liter 5.85 gm NaCl, 1.21 gm Tris-HCl, and 1.2 gm $MgSO_4$. Phage stocks were dialyzed against phosphate free buffer prior to use in experiments to analyze phage tRNAs.

Low phosphate medium is described by Landy, Abelson, Goodman & Smith (1967).

(c) Transfer RNA from phage-infected cells

W3110 was grown at 37°C in low phosphate medium to a concentration of 3×10^8 cells/ml. At time 0 the bacteria were infected with phage at a multiplicity of 10-15. After 2 min, 1 mC [^{32}P] orthophosphate was added. 8 min after infection chloramphenicol was added to a final concentration of 50 µg/ml. 70 min after infection the cells were collected by centrifugation. The bulk RNA was extracted with phenol (Brubaker & McCorquodale, 1963) and precipitated with ethanol from 0.2 M sodium acetate, pH 5. tRNA was separated by electrophoresis in 10% polyacrylamide gel slabs with 0.1 M Tris-acetate pH 8.5 as running buffer (Peacock & Dingman, 1967). The autoradiography of the gel slabs was as described by Adams, Jeppeson, Sanger & Barrell (1969).

(d) Phage crosses

Standard phage crosses are as described by Wilson & Kells (1972). UV phage crosses are a modification of the procedure described by Barnett, Brenner, Crick, Shulman & Watts-Tobin (1967). CR63 bacteria were grown to about 5×10^7 cells/ml. in H broth, collected by centrifugation and resuspended in H broth at 4×10^8 cells/ml. 0.5 ml. of bacteria were added to 0.5 ml. of a mixture of the two parental phage each at a multiplicity of 7.5. After 15 min incubation at 30°C, the infected cells were diluted 1/20 into synthetic growth medium and UV irradiated at a dose corresponding to about 40 phage-lethal hits. The

infected cells were then diluted 1/20 into H broth and incubated at 30°C. After 90 min $CHCl_3$ (Mallinckrodt) was added to insure lysis. This UV procedure increased recombination 5- to 20-fold over the standard crosses.

(e) Nomenclature

We have used a nomenclature similar to those of McClain (1970) and Abelson et al. (1970). Specific usage is described in the text. We use the superscripts + and - to indicate the potential of the serine-tRNA gene to make suppressor tRNA.

3. Results

(a) Isolation of suppressor-negative derivatives of psu_b^+:amN133

Since strains which carry psu_b^+ do not grow on CR63 (Wilson & Kells, 1972), we tried to isolate suppressor-negative derivatives by isolating from a population of psu_b^+ phage those rare phage which can make plaques on CR63. Central to this strategy is the assumption that the presence of functional psu_b^+ causes the lack of growth on CR63. If this assumption is correct, as is suggested by the observation that the poor growth properties of psu_b^+ strains are partially dominant (Wilson & Kells, 1972), suppressor-negative derivatives isolated in this manner will carry mutations in phage genes specifically required for the production of functional psu_b^+.

When psu_b^+:X74 (X74 is the strain designation for the double mutant amN133:amB20) is plated at high concentration on CR63, two types of

plaques are visible as described in Wilson & Kells (1972). At a frequency about 10^{-4} of the $\underline{psu}_b^+$:X74 phage are normal sized plaques containing phage which appear to be $\underline{psu}_a^+$:X74. The second type of plaque appears at a frequency of about 10^{-3} and is rather small and irregular. Upon retesting, the phage in these plaques plate like amber (am) mutants, making normal sized plaques on CR63 but none at all on S/6/5. The reason why these suppressor-negative mutants make such poor plaques initially is not clear, but the characteristic plaque type makes them easy to identify.

For this investigation of suppressor-negative mutants we began with the strain $\underline{psu}_b^+$:amN133. This double mutant was isolated from a cross of $\underline{psu}_b^+$:X74 by wild type and was used because it makes slightly larger plaques on S/6/5 than $\underline{psu}_b^+$:X74. To isolate independent suppressor-negative mutants we first plated a stock of $\underline{psu}_b^+$:amN133 on BB ($\underline{su}^+$) bacteria and picked 130 separate plaques. Since BB is $\underline{su}^+$, there is little or no selection against the rare suppressor-negative mutants which arise in the predominantly $\underline{psu}_b^+$:amN133 plaque. We replated these plaque suspensions on CR63, and for each picked two of the small, irregular plaques and verified their am phenotype by replating on CR63 and S/6/5. We purified these suppressor-negative mutants from contaminating $\underline{psu}_b^+$:amN133 phage (generally at a frequency of 10^{-2} to 10^{-4}) by replating them on CR63 and again picking a single plaque. Suppressor-negative mutants purified in this manner contained a level of $\underline{psu}_b^+$ phage which further purification by the same method did not significantly change. We conclude that the $\underline{psu}_b^+$ phage in the

purified plaques represent true revertants to the $\underline{psu}_b^+$ phenotype.

To determine the number of genes among which the mutations responsible for the suppressor-negative phenotype are distributed, we examined phage production in $\underline{su}^-$ (S/6/5) cells mixedly infected with X74 and one of the suppressor-negative mutants. Only those strains which carry a recessive mutation in a gene other than the suppressor gene will produce phage in mixed infection with X74; i.e. complement X74. On the basis of this complementation test, we separated the suppressor-negative mutants into two classes with the properties shown in Table 1. Class 1 contains mutants of a single type since it cannot be subdivided by complementation against one of its members. Since class 1 mutants seemed to supply functional $\underline{psu}_b^+$ in mixed infections, we inferred the presence of additional mutations in these strains in a gene which we will call mb (modifier of the $\underline{psu}_b^+$ phenotype). Class 2 mutants will be referred to as $\underline{psu}_b^-$.

To insure that each suppressor-negative mutant is independent, we kept no more than one mutant of each complementation group from each initial $\underline{psu}_b^+$:amN133 plaque. This procedure left us with 27 independent class 1 mutants and 124 independent class 2 mutants.

(b) Characterization of mb mutants

(i) Genotype of class 1 suppressor-negative mutants

The class 2 mutant which we have characterized most extensively was isolated in a preliminary study as a derivative of $\underline{psu}_b^+$:X74. We performed two kinds of crosses to determine the genotype of this

Table 1

Classification of suppressor-negative derivatives of $\underline{psu}_b^+$:$\underline{am}$N133

	-	Class 1	Class 2
-		0	0
X74	0	+ sm	0
X62	0	+	+
Class 1	0	0	+ sm

Complementation tests were performed in trays by mixing 1 drop of tester phage (listed in the first column) at a concentration of 4×10^7 phage/ml. with 2 drops of a suspension of suppressor-negative phage (1 plaque in 2 ml. dilution buffer). To this mixture was added 1 drop of S/6/5 plating bacteria and 1 ml. melted EHA top agar. The agar was allowed to harden for 15 min and then the trays were incubated overnight at 30°C. At these concentrations of phage, full complementation, as represented by X62 ($\underline{am}$B25:$\underline{am}$A455, both in gene $\underline{34}$), shows not as a clear spot but as many individual plaques. + indicates that there is complementation and the plaques are wild type size. + sm indicates there is complementation but the plaques are smaller than wild type. For the tests in the bottom line, one class 1 mutant was grown into a stock, adjusted to 4×10^7 phage/ml. and used as a tester strain.

class 1 mutant. The first cross was between the mutant strain and X74. If the class 1 mutant contained at least one of the X74 *am* mutations, the cross could not yield wild type progeny. When the progeny of this cross were plated on su^- bacteria/(S/6/5) (neither parent makes a plaque under these conditions), no wild type plaques were observed. Those 15 to 20% of the total progeny which did grow made small plaques characteristic of phage which carry functional psu_b^+. These results verify that this class 1 mutant carries at least one of the original *am* mutations, psu_b^+, and an additional mutation (designated *mbl* as the first *mb* mutation isolated) which is recombined away from psu_b^+ with a frequency of 30 to 40%. Thus *mbl* must be unlinked to psu_b^+.

The second cross was between the class 1 mutant, *mbl*:psu_b^+:X74, and wild type. Such a cross would be expected to yield 3 different types of progeny with the *am* phenotype: *mbl*:psu_b^+:X74 (type 1), X74 (type 2), and *mbl*:X74 (type 3). The progeny from this cross were found to contain *am* mutants which can be separated by complementation into 3 groups with the properties shown in Table 2. Complementation by strains carrying the single *am* mutations present in X74 showed that both were represented among the *am* progeny, demonstrating that the original class 1 mutant contains both *am* mutations.

Since the parent strain *mbl*:psu_b^+:X74 and the strain *mbl*:X74 both made small plaques on CR63, we anticipated that *mbl* and *mbl*:psu_b^+ might make small plaques on both S/6/5 and CR63. In hopes of finding these strains, we plated the progeny from the second cross on S/6/5, picked small plaques and replated them on S/6/5 and CR63. The majority of

Table 2

Amber progeny from a cross of wild type by a class 1 mutant

	-	Type 1 (*mbl*:psu_b^+:X74)	Type 2 (X74)	Type 3 (*mbl*:X74)
-		0	0	0
X74	0	+ sm	0	0
X62	0	+	+	+
Class 1 (*mbl*:psu_b^+:X74)	0	0	+ sm	0

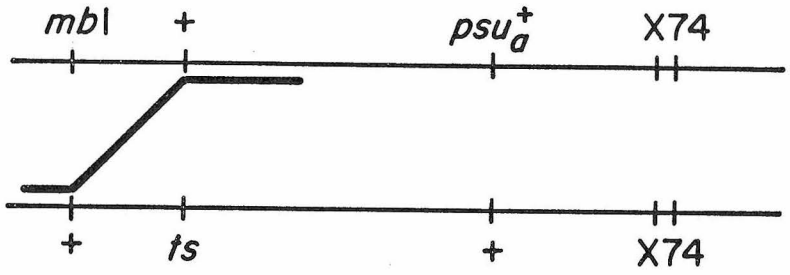
Complementation tests were performed as indicated in Table 1. The three patterns of complementation are designated type 1, 2, and 3. The inferred genotypes are listed in parentheses below each type. Though each type can be separated into three subgroups by complementation against the strains which carry the single *am* mutations present in X74, only the inferred genotype of the majority species is shown.

the small plaques upon retesting plated like $\underline{psu}_b^+$ (small plaques on S/6/5 and none at all on CR63), or like wild type (large plaques on both). However, two made small plaques with equal efficiency on both CR63 and S/6/5. Stocks of each of these were grown and then crossed to strain X74. Crosses with one of the strains yielded type 2 and 3 am progeny, but no phage with the $\underline{psu}_b^+$ plating phenotype, suggesting that it contained mbl but not $\underline{psu}_b^+$. The cross with the other strain yielded am progeny of all three types, as well as phage with the $\underline{psu}_b^+$ plating phenotype, demonstrating that it contained both mbl and $\underline{psu}_b^+$.

It subsequently became apparent that the small plaque phenotype associated with mbl is anomalous. Other mb mutants, including one shown to carry an mb deletion, made normal size plaques and gave wild type burst sizes on CR63 as compared with only 20% of wild type for mbl. These results suggest that the gene defined by the mb mutations is non-essential and that the mbl strains described above may all carry another mutation responsible for the small plaque phenotype. As will be shown in section 3(d), the effect of mb5, which gave a wild type burst, and mbl on the population of phage tRNAs is identical. Strains carrying mbl have been used to position the mb gene on the T4 genetic map. Since the mapping is based on the function of the mb gene and not the small plaque phenotype, we have disregarded the possible presence of the additional mutation.

(ii) Map position of mbl

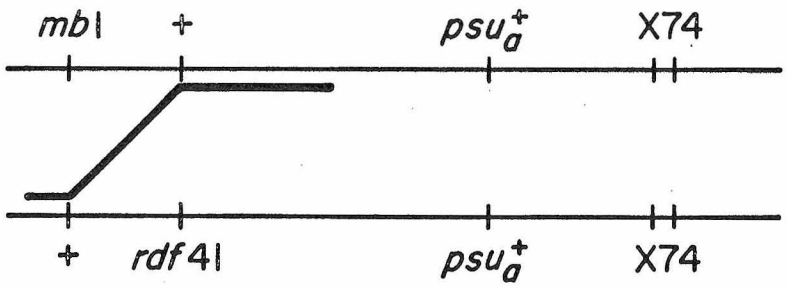
The position of mbl in the T4 genetic map was determined roughly by the following kind of cross:



Because strains which carry $\underline{psu}_b^+$ make extremely tiny plaques at 42°C, we used strains carrying $\underline{psu}_a^+$ in these crosses. mbl has an identical effect on both suppressors as is described in section 3(b)(iv). Since both parents contain the X74 am mutations, the only progeny which will plate on S/6/5 bacteria are those that can make functional $\underline{psu}_a^+$; i.e. those that contain $\underline{psu}_a^+$ but not mbl. By plating on S/6/5 at 42°C (or on G(λ) in crosses involving an rII marker) we select for $\underline{ts}^+$ (or $\underline{rII}^+$). Thus we demand a crossover between mbl and the ts (or rII) mutation. Since only one of the parents in these preliminary crosses contains the unlinked marker $\underline{psu}_a^+$, only about half the appropriate crossovers between mbl and the test marker will yield recombinants which plate under restrictive conditions. We calculate the per cent recombination between mbl and the test marker as %R = 400% ($\underline{mb}^+$:$\underline{ts}^+$ ($\underline{rII}^+$):$\underline{psu}_a^+$:X74/total progeny). By these crosses we determined that mbl is unlinked to temperature sensitive mutations in genes e, 10, 31, 42, and 45 and is located about 11 map units from rII.

To verify these results and more precisely position mbl relative to rII we constructed the strain $\underline{psu}_a^+$:rdf41:X74 and repeated the

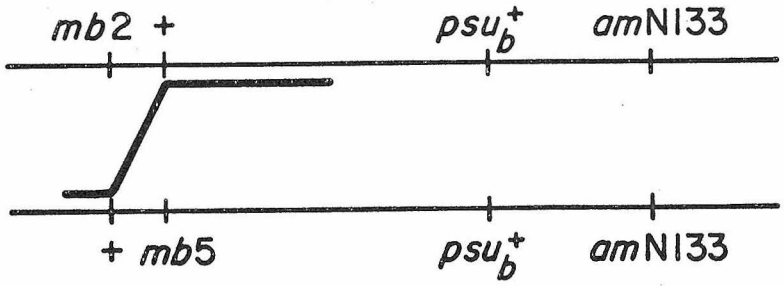
cross as shown:



Since all progeny now contain $\underline{psu}_a^+$:X74, all $\underline{mb}^+$:$\underline{rII}^+$ recombinants will plate on G(λ) and we can calculate the recombination frequency as %R = 200% ($\underline{mb}^+$:$\underline{rII}^+$:$\underline{psu}_a^+$:X74/total progeny). This cross indicates that mb1 is located 9.3 map units from one end of the rII deletion, rdf41. We have not yet determined on which side of rII the gene defined by mb1 lies.

(iii) Recombination between mb mutants

To determine whether the various mb mutations all represent a specific mutation within the gene defined by mb1 or are part of a class of mutations which prevent the function of the gene, we mapped five different mb mutations relative to one another as shown below:



Only those progeny which have undergone a crossover between the two mb mutations as indicated will plate on S/6/5. The results of two-factor crosses, shown in Figure 1, demonstrate that these mutations can occur at several different points and, therefore, probably represent a class of mutations which prevent the function of the mb gene.

(iv) Effect of mbl on other phage-coded nonsense suppressors

If the effect of a mutation in mb is really to prevent the formation of functional psu_b^+ rather than simply to alter peculiar psu_b^+ plating phenotype in some trivial way, then it ought also to prevent the formation of functional psu_a^+, since psu_a^+ and psu_b^+ seem to be alternate forms of the ser-tRNA (Wilson & Kells, 1972). To determine the effect of mbl on psu_a^+, we crossed psu_a^+:X74 by mbl:X74 and examined am progeny by complementation as shown in Table 3. As would be predicted if mbl affects both suppressors identically, there is a class of am progeny which complements in a manner analogous to mbl:psu_b^+:X74. The one difference is that the plaques which form when X74 complements mbl:psu_a^+:X74 are normal size, a characteristic of psu_a^+, as opposed to the small plaques characteristic of psu_b^+ formed when X74 complements mbl:psu_b^+:X74.

Wilson & Kells (1972) have suggested from several common properties that psu_1^+ (McClain, 1970) and psu_a^+ are identical. In support of this notion we have demonstrated in a manner similar to that described for psu_a^+ that mbl also prevents the formation of functional psu_1^+. The strain mbl:psu_1^+:amN022 (amN022 is in gene 48) has an am phenotype.

Fig. 1. Recombination between mb mutants. mb1 was isolated as a suppressor-negative mutant of psu_b^+:X74. The other four mb mutant strains were isolated as independent suppressor-negative mutants of psu_b^+:amN133. The crosses are of the standard type. The per cent recombination (%R) shown below the map was calculated as %R = 200% (mb^+ progeny/total progeny). mb6 shows no recombination with any other mb mutation and psu_b^+ revertants of it have not been found. These two observations suggest that mb6 is a deletion which overlaps all the mb markers shown.

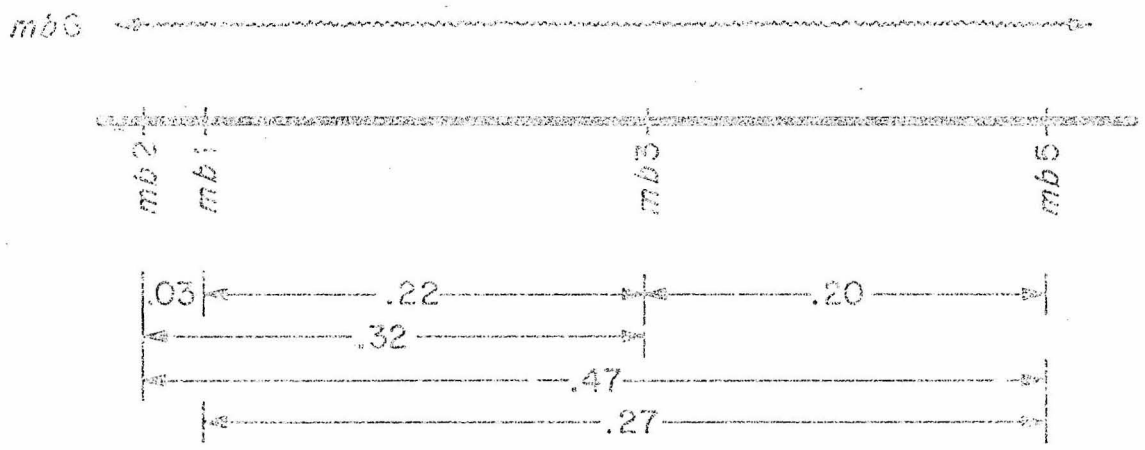

mb6
mb2
mb1
mb3
mb5
.03
.22
.20
.32
.47
.27

Table 3

Amber progeny from a cross of $\underline{psu}_a^+$:X74 by mbl:X74

		X74 and	
	-	mbl:X74	mbl:$\underline{psu}_a^+$:X74
-		0	0
X74	0	0	+
X62	0	+	+
mbl:X74	0	0	0

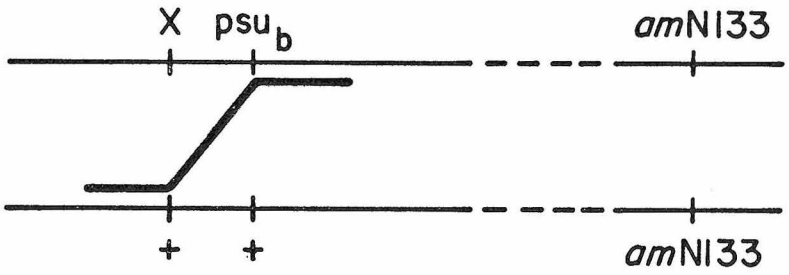
Complementation tests were performed as described in Table 1. The inferred genotypes of the am progeny are indicated above the appropriate columns.

We have also examined the effect of mbl on a phage-coded ochre suppressor, n_1 (Kneser, personal communication). That n_1 is an ochre suppressor and grows on CR63 distinguishes it from both psu_a^+ and psu_b^+. From among the progeny of a cross of mbl by n_1:oceL3 we isolated the strain mbl:n_1:oceL3. The strain was verified to contain oceL3 by crossing it to wild type and demonstrating by complementation the presence of gene e mutants in the progeny. This strain makes plaques on S/6/5 and, after addition of $CHCl_3$ to the top of the plate, displays the small lysozyme halo characteristic of the parent strain nl:oceL3. The strain was shown to contain mbl by crossing it to X74 and demonstrating by complementation the presence of mbl:X74 in the progeny. The functional n_1 is therefore formed in the presence of mbl. Assuming that n_1 represents a tRNA alteration, these results suggest that mb function is not required for production of all of the phage tRNAs.

(c) Characterization of psu_b^- mutants

(i) Subclassification of psu_b^- mutants

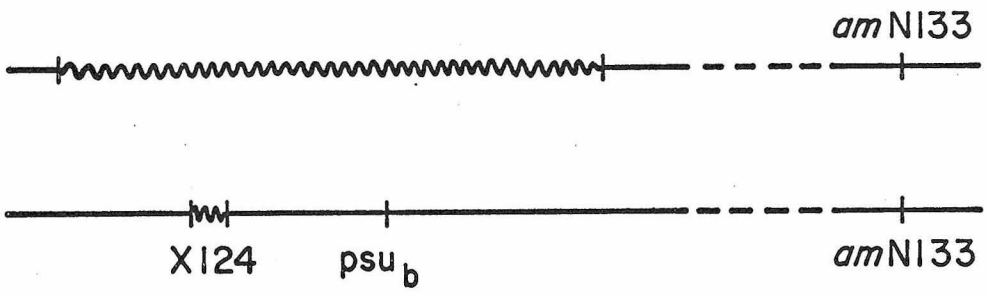
The data in Table 1 show that the psu_b^- mutants do not complement X74. These results are consistent with either a second alteration within the gene defined by psu_b^+ or with a dominant mutation in another gene, either of which must prevent the formation of functional psu_b^+. The location of the secondary mutation, X, was determined by measuring its distance to the primary mutation, psu_b, by the type of cross shown below:



Since all progeny will contain amN133, only those in which the secondary mutation has been separated from the original mutation will plate on S/6/5. Except for 10 which remain untested because of their high frequency of reversion to psu_b^+, all psu_b^- mutations affect sites within 0.2 map units of psu_b. Thus, if there are genes in which dominant mutations can prevent the formation of functional psu_b^+, they must be very close to the gene defined by psu_b^+, or else have not been mutated in any of the psu_b^- strains.

The cross described divides the psu_b^- strains into two groups. The first includes those that can generate the psu_b^+ phenotype by recombination. These mutants still contain the original mutation and in addition a mutation at a second site, called X, as shown in the above diagram. We have designated this group psu_b^-X mutants. We will refer to the site of the original mutation as psu_b and to the second site mutation as X followed by the isolation number. The second group is comprised of those psu_b^- strains which cannot generate the psu_b^+ phenotype by recombination. There are at least two kinds of mutations which would have this property: deletions which encompass psu_b and

single base changes at the site of psu_b. To distinguish these possibilities we crossed the psu_b^- mutants of this group to a psu_b^-X mutant of the first group, psu_b^-X124:amN133 (X124 map location shown in Figure 3). In such a cross, psu_b^- strains carrying a deletion which includes the wild type sequence opposite X124 will not be able to generate psu_b^+ progeny.



Those mutants which failed to generate psu_b^+ progeny in crosses to amN133 and psu_b^-X124:amN133 have been designated $psu_b^-\Delta$ (Δ for deletion). Consistent with the idea that these strains carry deletions is the observation that none of these mutants, when plated on S/6/5 in large numbers, gave any psu_b^+ revertants (frequency $<10^{-7}$). Several of these strains have been more directly demonstrated to carry deletions by examination of heteroduplex DNA in the electron microscope as described in Wilson et al. (1972).

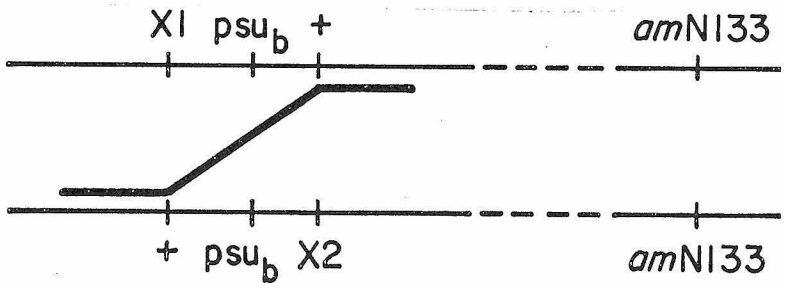
The two strains which yielded psu_b^+ recombinants in crosses with psu_b^-X124:amN133 but not with amN133 were crossed to psu_b^-X5:amN133 (X5 and X124 are on opposite sides of psu_b; see Figure 3). Both these strains yielded psu_b^+ recombinants indicating they also contain the wild type sequence opposite X5. These two strains may carry either a single

base change at psu_b or a small deletion encompassing psu_b but not the wild type sequences opposite X5 or X124. These two strains, designated $\underline{psu_b^-}$W (W to suggest they may be revertants to true wild type), have not been characterized further.

Table 4 shows the distribution of suppressor-negative strains among the two complementation groups and the subclasses of $\underline{psu_b^-}$ defined above.

(ii) Genetic mapping of second site mutations

The various second site mutations can be positioned relative to one another and psu_b by two kinds of crosses. The per cent $\underline{psu_b^+}$ recombinants in crosses between $\underline{psu_b^-}$X:amN133 and amN133 is a measure of the distance from psu_b to the second site mutation as shown in the diagram in the previous section. Similarly, a cross between two $\underline{psu_b^-}$X strains measures the relative positions of the two second site mutations as shown below:



Since all progeny will contain amN133, only those which have undergone a recombination between the two second sites, as indicated, will plate on S/6/5.

Table 4

Distribution of suppressor-negative derivatives

mb		27
psu^-_b		
	psu^-_b (unclassified)	10
	psu^-_bX	61
	psu^-_bΔ	51
	psu^-_bW	2
		151

The isolation and classification of suppressor-negative revertants is described in the text.

From these crosses it became apparent that the $\underline{psu}_b^-X$ strains were of two types. One kind, presumably resulting from single base changes, mapped as a classical point mutant. The other kind, presumably deletions, failed to generate $\underline{psu}_b^+$ recombinants in crosses with more than one unique $\underline{psu}_b^-X$ strain of the first type. As another way of determining whether the $\underline{psu}_b^-X$ strains carry second site single base changes or deletions, we screened revertants which plate on S/6/5 for those with the $\underline{psu}_b^+$ phenotype. All but four of the apparent single-base-change mutants yielded revertants with the $\underline{psu}_b^+$ phenotype, while none of the apparent deletions did so. The four exceptions, $\underline{psu}_b^-X18$, $\underline{psu}_b^-X10$, $\underline{psu}_b^-X46$ and $\underline{psu}_b^-X73$ have been arbitrarily grouped with those which carry single base changes.

The preliminary mapping of the $\underline{psu}_b^-X$ strains showed 11 different second site single base changes with several apparent repeats of some of them. To determine if these apparent repeats were actually at the same site or simply too close to be separated by standard crosses, we crossed them to one another in UV crosses. Several sites were separated by these crosses and mapped relative to one another as shown in Figure 2. The relative map positions of the unique sites are shown in Figure 3.

The order shown in Figure 3 is deduced solely from two-factor crosses of the standard and UV type. The lack of complete additivity of the two-factor crosses reflects the fact that these crosses were done over a number of days and that there were day-to-day variations in recombination frequency. The additivity in crosses carried out with

Fig. 2. Separating and ordering of second site clusters. All crosses are of the UV type described in Materials and Methods. The per cent recombination shown below the maps is calculated as %R = 200% ($\underline{psu}_b^+$ recombinants/total progeny). In all crosses only the small plaques characteristic of $\underline{psu}_b^+$ were counted on the S/6/5 plates; large plaques were assumed to be $\underline{am}^+$ revertants at the site of amN133. Self crosses of the individual $\underline{psu}_b^+$X strains yielded $\underline{psu}_b^+$ progeny at a frequency at least 8-fold lower than the biparental crosses. (a) X28 and X38 were originally assigned to the group defined by X5. (b) X59 was initially grouped with X6, and X25 with X1. X13 shows no detectable recombination with X6, X59, X25, or X1. (c) X45 and X92 showed no detectable recombination in standard crosses. X10 was initially grouped with X20. X124 shows no detectable recombination with X10 and X20. The position of X10 has been verified by three-factor UV crosses with $\underline{psu}_1^-$1 as described in section 3(c)(iv) (data not shown).

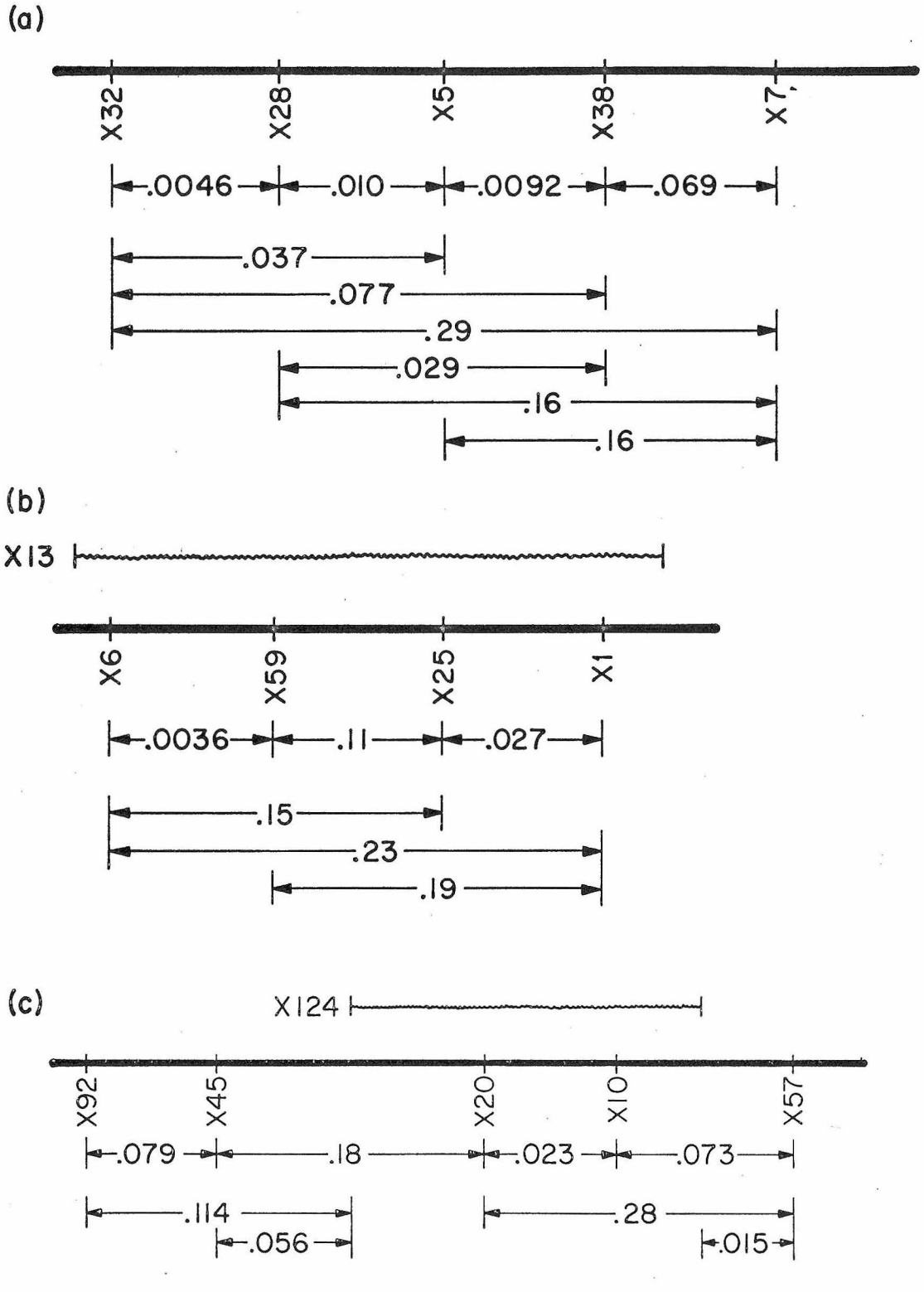
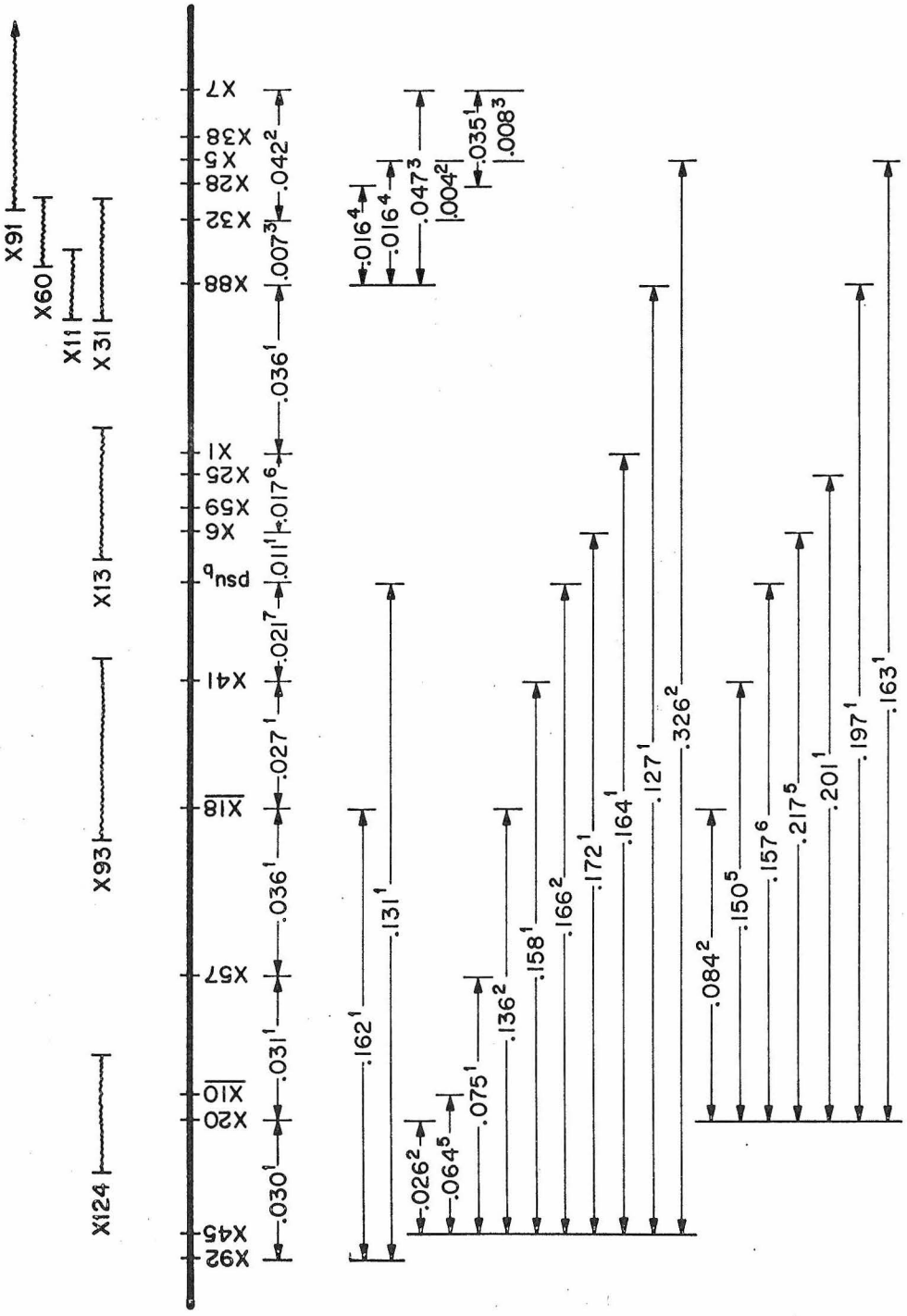
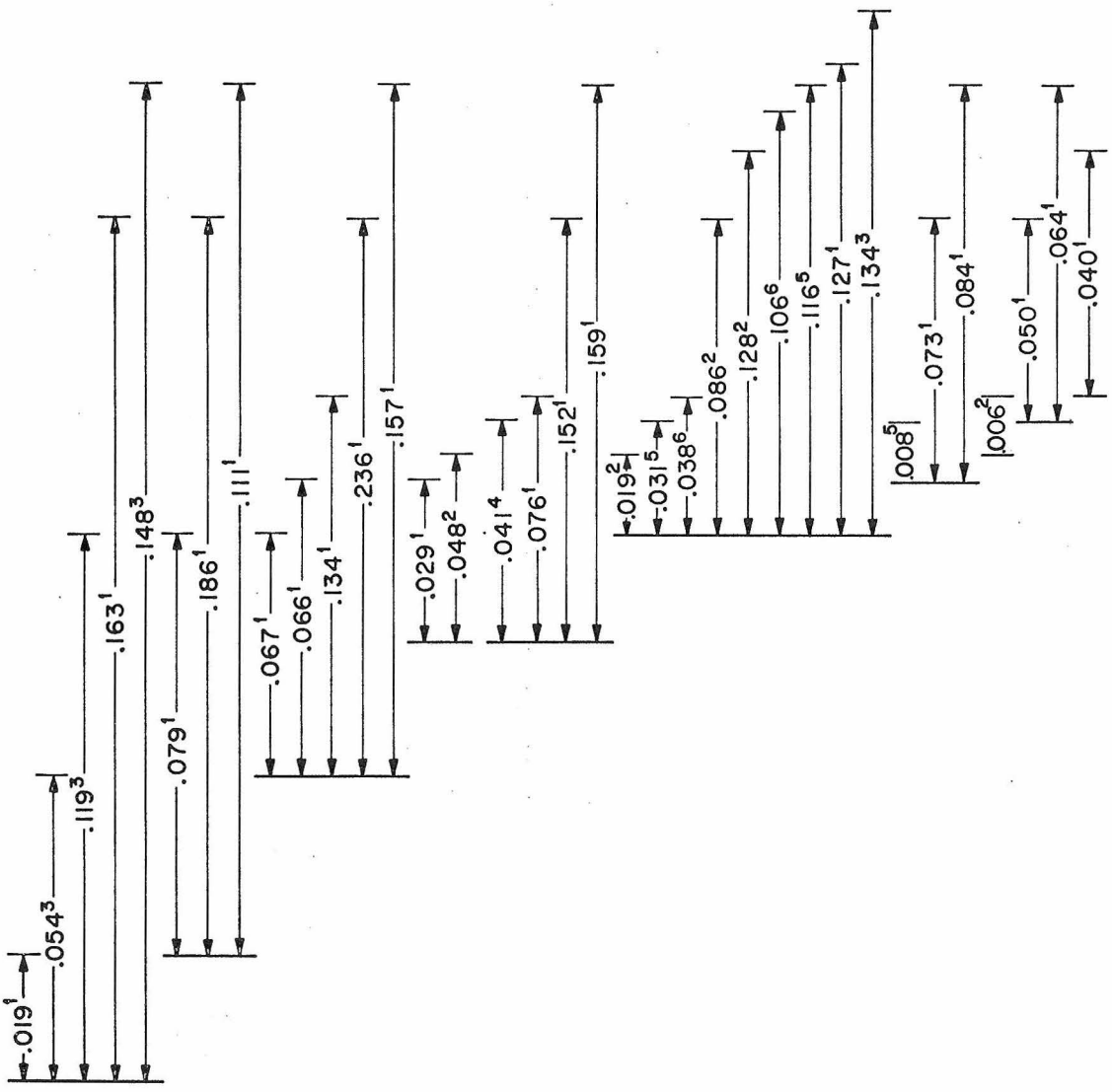

(a)
X32
X28
X5
X38
X7'
.0046
.010
.0092
.069
.037
.077
.29
.029
.16
.16
(b)
X13
X6
X59
X25
X1
.0036
.11
.027
.15
.23
.19
(c)
X124
X92
X45
X20
X10
X57
.079
.18
.023
.073
.114
.056
.28
.015

Fig. 3. Genetic map of second site mutations. The order of sites is deduced from two-factor standard and UV crosses. The presumed second-site deletions are shown above the map. Their ends are located arbitrarily between two point mutants with which they do and do not recombine. None of the recombination data used to position the deletions is shown. The per cent recombination for the point mutants is shown below the map. The data presented are solely from standard crosses and were calculated as %R = 200% ($\underline{psu}^{+}_{b}$ recombinants/total progeny). The superscript indicates the number of crosses used to determine the %R shown. X10 and X18 are underlined to indicate they may be deletions (see text). We have classified as repeats those markers which fail to recombine in UV crosses. Lack of recombination does not constitute a rigorous proof these mutants are at the same site (Ronen & Salts, 1971; Tessman, 1965). In the following list the number of repeats/is listed in parentheses after the site designation. X7(2), X5(4), X28(5), X32(1), X88(1), X1(5), X25(4), X59(1), X41(6), X10(2; X46, X73; see text), X13(5).


X91
X60
X11
X31
X124
X93
X13
X92
X45
X10
X20
X57
X18
X41
psub
X6
X59
X25
X1
X88
X32
X28
X5
X38
X7
.030¹
.031¹
.036¹
.027¹
.021⁷
.011¹
.017⁶
.036¹
.007³¹
.042²
.162¹
.131¹
.026²
.064⁵
.075¹
.136²
.158¹
.166²
.172¹
.164¹
.127¹
.326²
.016⁴
.016⁴
.047³
.004²
.035¹
.008³
.084²
.150⁵
.157⁶
.217⁵
.201¹
.197¹
.163¹


.019¹
.054³
.119³
.163¹
.148³
.079¹
.186¹
.111¹
.067¹
.066¹
.134¹
.236¹
.157¹
.029¹
.048²
.041⁴
.076¹
.152¹
.159¹
.019²
.031⁵
.038⁶
.086²
.128²
.106⁶
.116⁵
.127¹
.134³
.008⁵
.073¹
.084¹
.006²
.050¹
.064¹
.040¹

the same preparation of bacteria is much better. The order deduced from the two-factor crosses is supported by the overlap pattern of the deletions and by three-factor crosses with psu$^-$1 as described in section 3(c)(iv).

The sum of the %R's shown along the base of the map is 0.258. From this number and several independent estimates of the per cent recombination per nucleotide, 0.005 to 0.01 (Stahl, Edgar & Steinberg, 1964; Goldberg, 1966) we can estimate the number of nucleotides between the outermost sites, X92 and X7, as 25-50.

Analysis of the per cent recombination across the two most saturated regions of the map, X6 to X1 and X32 to X7, gives a similar estimate. If we assume that the smaller recombination frequencies represent exchanges between adjacent nucleotides and that the relatively higher %R in the intervals X59 to X25 and X38 to X7 (Fig. 2) indicates the presence of another nucleotide in these intervals, then there are 5 nucleotides in the X6 cluster and 6 in the X32 cluster. Over these small distances it is appropriate to calculate %R per nucleotide interval (internucleotide bond) rather than per nucleotide. Thus, for the X6 cluster (4 intervals and 0.017% R) and the X32 cluster (5 intervals and 0.042% R), we calculate %R's per nucleotide interval of 0.004 and 0.008. These estimates suggest the number of nucleotides from X92 to X7 is between 30 and 60. Both these rough estimates agree and are within the limits of the general size expected for a tRNA gene, suggesting that these mutations are distributed over a single tRNA gene.

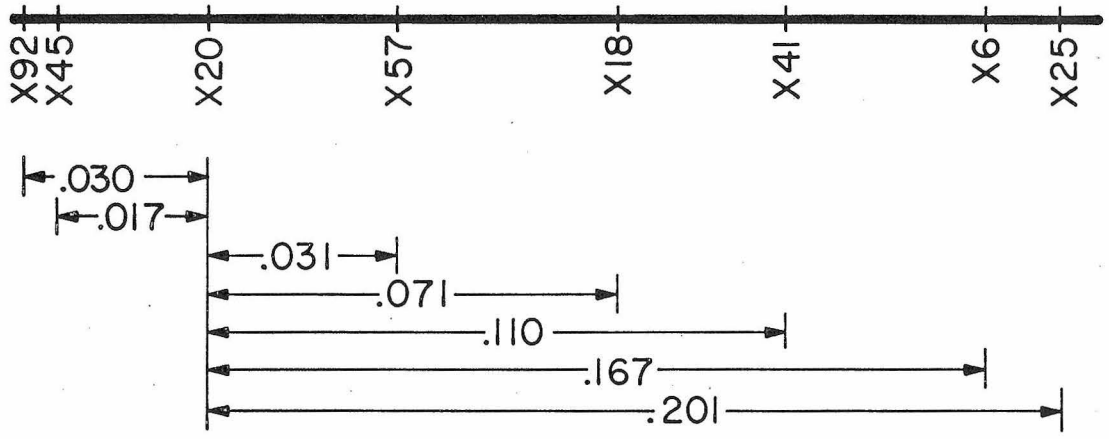
(iii) Position of psu_b relative to the anticodon

Wilson & Kells (1972) have cited circumstantial evidence which suggests that $\underline{psu}_b^+$ results from a nucleotide change in the anticodon. The central position of psu_b in the map in Figure 3 also suggests that it is located near the middle of the gene; i.e. near the anticodon. We have attempted to genetically position psu_b relative to the anticodon by making use of a suppressor-negative derivative of $\underline{psu}_1^+$, $\underline{psu}_1^-1$ (isolated, characterized, and generously given to us by W. H. McClain). $\underline{psu}_1^+$ results from the alteration of two adjacent nucleotides in the anticodon of the ser-tRNA (W. H. McClain & B. Barrell, personal communication). For simplicity we will refer to the site of this double mutation as psu_1.

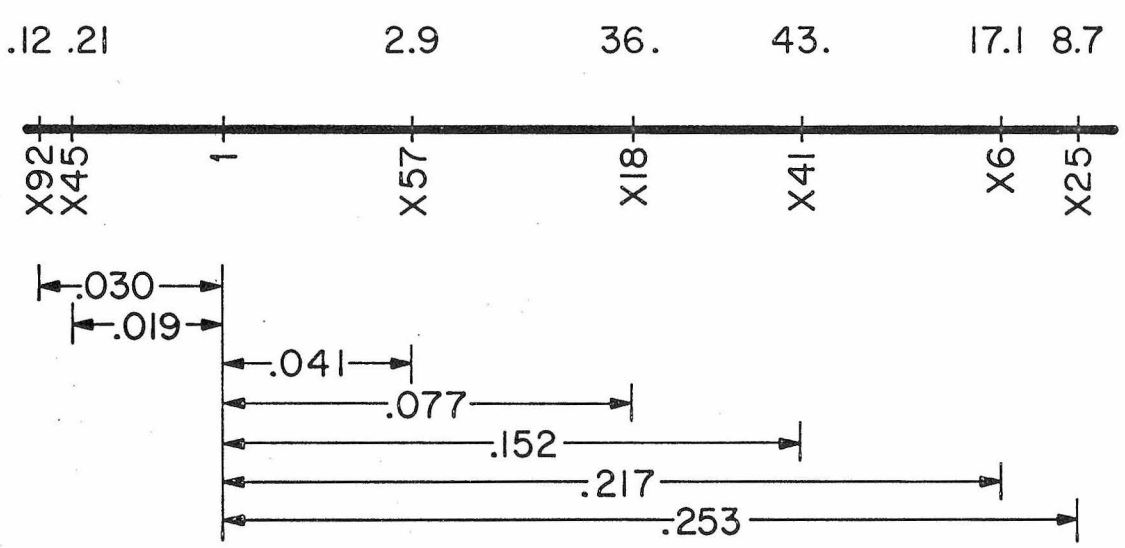
Neither $\underline{psu}_b^+$ nor $\underline{psu}_1^+$ recombinants are generated in standard or UV crosses of $\underline{psu}_1^-1$:X74 by $\underline{psu}_b^-$X20:amN133, indicating that X20 and 1 (the second site mutation in $\underline{psu}_1^-1$) are at the same site. To determine whether psu_1 and psu_b are on the same or opposite sides of X20, we compared the results of crosses between $\underline{psu}_1^-1$:X74 or $\underline{psu}_b^-$X20:amN133 and several $\underline{psu}_b^-$X strains as shown in Figure 4. The general agreement of the recombination frequency in comparable crosses and the changes in the ratio of $\underline{psu}_1^+$ to $\underline{psu}_b^+$ recombinants from much less than 1 to much greater than 1 for secondary mutations on the left and right of X20, demonstrate that psu_1 is on the same side of X20 as psu_b. To more precisely determine the relative positions of psu_1 and psu_b, we crossed $\underline{psu}_1^-1$:X74 and $\underline{psu}_b^-$X20:amN133 to $\underline{psu}_b^-$X41:amN133, $\underline{psu}_b^-$X6:amN133 and

Fig. 4. Comparison of distances from second site mutants to X20 and 1. Crosses are of the standard type. All crosses were performed at the same time on the same preparation of bacteria. The positions of the second site mutations are taken directly from Fig. 3. (a) $\underline{psu}_b^-$X20:$\underline{am}$N133 was crossed by the strains carrying the second site mutations shown. %R shown below the map was calculated as %R = 200% ($\underline{psu}_b^+$ recombinants/total progeny). (b) $\underline{psu}_1^-$1:X74 was crossed by the strains carrying the second site mutations shown. %R shown below the map was calculated as %R = 200% ($\underline{psu}_b^+$ + $\underline{psu}_1^+$ recombinants/total progeny). Since $\underline{psu}_1^+$ and $\underline{psu}_b^+$ recombinants make easily distinguishable plaques, we can count them separately and calculate their ratio. The ratio of $\underline{psu}_1^+$ to $\underline{psu}_b^+$ recombinants is indicated above the map. $\underline{psu}_1^-$1:X74 was isolated from a cross of $\underline{psu}_1^-$:$\underline{am}$eL1/a by psu$_a$:X74.

(a)


X92
X45
X20
X57
X18
X41
X6
X25
.030
.017
.031
.071
.110
.167
.201

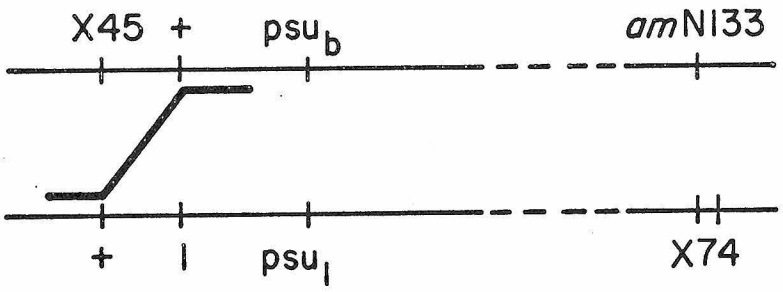
(b)


.12 .21
2.9
36.
43.
17.1 8.7
X92
X45
1
X57
X18
X41
X6
X25
.030
.019
.041
.077
.152
.217
.253

amN133 alone. The crosses against the psu_b^-X strains are repeats of those described in Figure 4. The crosses against amN133 give the distance of psu_b and psu_1 to the common site of X20 and 1. The results of these crosses, shown in Figure 5, suggest that psu_1, like psu_b, is located between X41 and X6. Thus psu_b must be located in or quite near the anticodon. These results also confirm the conclusion of Wilson & Kells (1972) that psu_b affects the ser-tRNA.

(iv) Intragenic three-factor crosses

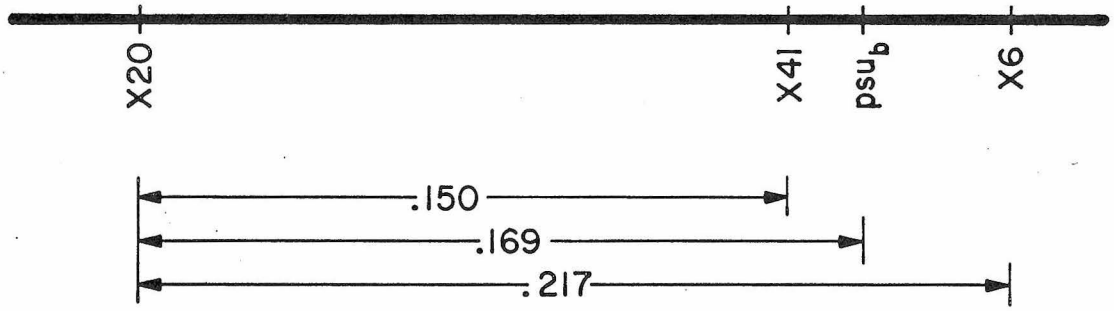
Since psu_b and psu_1 are very close together and give rise to different sized plaques, we can use the anticodon region of the gene as an outside marker to order second site mutations which are both on the same side of the anticodon as illustrated below:



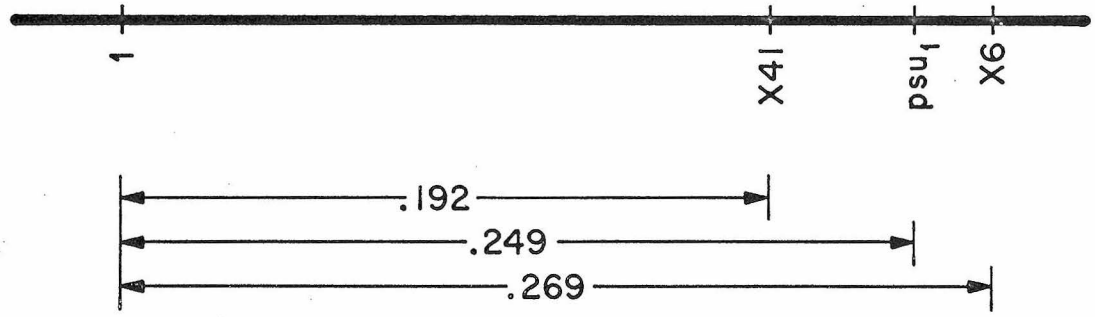
If the order of X45 and 1 is as shown, the ratio of psu_1^+ to psu_b^+ recombinants (the ratio of large to small plaques) will be less than 1.0. The ratio for this cross was / 0.21. As shown in Figure 4 these intragenic three-factor crosses support the order deduced from the two-factor crosses shown in Figure 3.

Fig. 5. Relative positions of psu_b and psu_1. Crosses are of the standard type. This set of crosses was performed five times on three different preparations of bacteria. The %R's shown below the maps were calculated as in Fig. 4 and represent the average of all five crosses. The positions of X20, X41 and X6 are taken from Fig. 3. The position of psu_b and psu_1 relative to X41 and X6 is determined by a comparison of the %R's for the three intervals measured. $(psu_b\text{-}X41)/(X6\text{-}X41)$ gives the fraction of the distance from X41 to X6 at which psu_b is located. A similar calculation gives the position of psu_1. We think that the difference in the absolute values of %R for corresponding intervals in these crosses reflects a slightly lower efficiency of plating of $\underline{psu}_b^+$ strains.

(a)



(b)



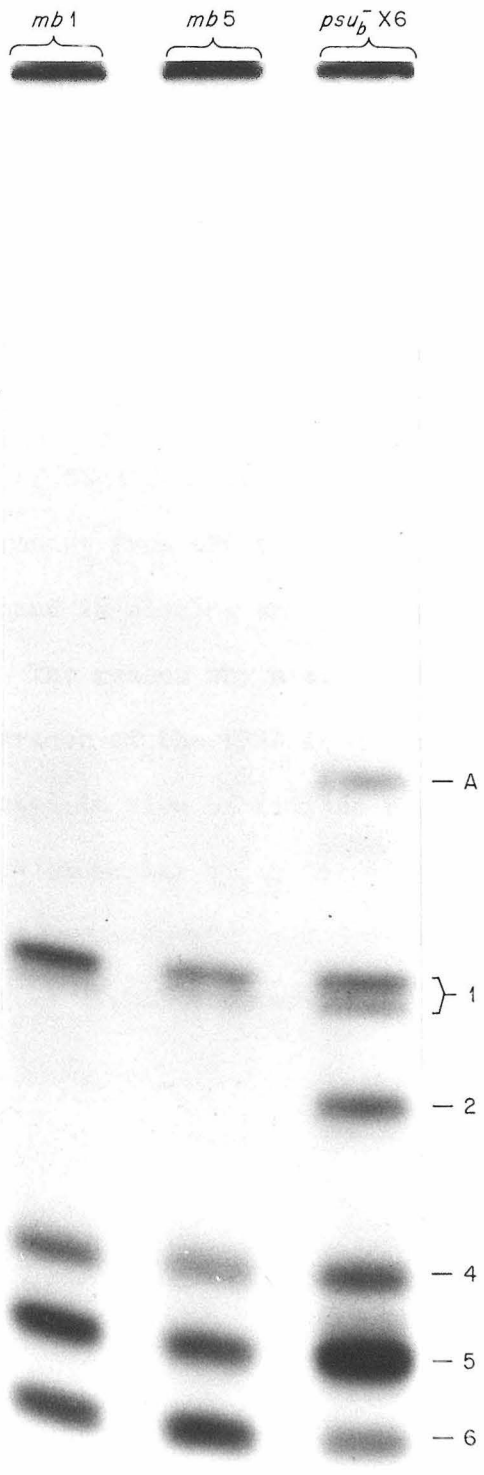
(d) Production of phage tRNAs in suppressor-negative strains

All suppressor-negative derivatives of psu_b^+:amN133 were selected to prevent the formation of functional psu_b^+. To determine their specific effect on the ser-tRNA and general effect on the population of phage tRNAs, we labeled cells infected with three different suppressor-negative mutants with [^{32}P] orthophosphate, extracted the bulk RNA and electrophoresed it in 10% polyacrylamide gel slabs as described in Materials and Methods.

Plate 1 shows the separation of low molecular weight RNA from infected cells obtained by electrophoresis in 10% polyacrylamide gels. The RNA from wild type T4 infected cells separates into six distinct bands. Five of these bands (1, 2, 3, 4, and 6) contain distinct species of RNA as judged by their oligonucleotide maps and one, band 5, contains a mixture of some 3 to 5 unique species (J. Abelson, G. Paddock & T. Pinkerton, unpublished experiments; W. H. McClain & B. Barrell, personal communication). The T4 RNA pattern is clearly distinct from that of the uninfected host, indicating that most E. coli tRNA synthesis is halted following T4 infection.

We do know something about the identity of the various T4 RNA bands. Bands 1 and 2 and additional high molecular weight bands probably contain tRNA precursors (W. H. McClain & B. Barrell, personal communication; G. Paddock & J. Abelson, unpublished results). Band 3 contains a ser-tRNA (W. H. McClain & B. Barrell, personal communication) and band 4 contains a leu-tRNA (Pinkerton, Paddock & Abelson, 1971). Bands 5 and 6 contain respectively 3-5 and 1 unidentified tRNA species (J. Abelson, G. Paddock & T. Pinkerton, unpublished experiments).

Plate 1. Separation of ^{32}P-labeled tRNAs from T4-infected cells. ^{32}P-labeled RNA was extracted from cells and electrophoresed on 10% polyacrylamide gel slabs as described in Materials and Methods.


mb1
mb5
psu$_b^-$X6
A
1
2
4
5
6

mb1 and mb5:psu_b^+:amN133 both have identical effects on the population of phage RNAs. In addition to eliminating band 3, the ser-tRNA, they eliminate band 2 and reduce the amount of label in band 5, suggesting they may also affect some species of tRNA included in band 5. That these two strains give identical results indicates that mb mutations have the same effect in the presence or absence of psu_b^+.

The band pattern of RNAs from cells infected with psu_b^-X6:amN133 shows two clear differences from the pattern of wild-type infected cells. The ser-tRNA band is missing and a new slower running band, band A, has appeared. The reason why a single base change should result in the disappearance of the tRNA is not understood but the result was not unexpected in view of similar results with suppressor-negative mutants of the bacterial su_{III}^+ tyr-tRNA (Abelson et al., 1970). Band A may be a precursor to band 3 which accumulates because of the second site change within the ser-tRNA.

4. Discussion

151 independent suppressor-negative derivatives of $\underline{psu}_b^+$ phage have been classified into two complementation groups. Since none of the phage tRNAs is essential (Wilson *et al.*, 1972), there is no selection against mutations in genes which affect more than one tRNA. Thus the number of different complementation groups should define the number of different phage genes essential to the production of the phage ser-tRNA. For the ser-tRNA there seem to be only two essential phage genes. One is the structural gene for the tRNA and the second, defined by *mb* mutations, plays an undefined role which affects the production of several tRNAs. It should be noted that the effect of the *mb* mutations on the tRNAs may be an indirect result of the absence of the wild type function. Several genes in the region of the map near *rII* affect membrane function. It is conceivable that in the absence of wild type *mb* function the infected cell membrane is altered, leading to a change in the cell's internal environment which in some ways affects the production of several of the phage tRNAs. In any case, the implication of only two phage genes in the maturation of the ser-tRNA suggests that there are very few phage genes other than the tRNA structural genes which are essential for phage tRNA production. Thus precursor cleavage if it occurs, nucleotide modification and amino acid charging can be carried out by host enzymes, implying a great deal of similarity in the maturation and structure of host and phage-induced tRNAs.

ACKNOWLEDGMENTS

We are grateful to Dr. R. L. Russell, Dr. W. B. Wood, Dr. A. Sarabhai, and Dr. W. H. McClain for many helpful discussions. We are especially indebted to Dr. W. H. McClain and Dr. B. G. Barrell for generously offering us their unpublished results, upon which depend directly and indirectly many of the arguments and conclusions presented in this paper. This research was supported by grants (GM-06965, AI-09238, and CA-10984) from the U.S. Public Health Service. One of us (J.N.A.) is a Faculty Research Associate of the American Cancer Society.

REFERENCES

Abelson, J. N., Gefter, M. L., Barnett, L., Landy, A., Russell, R. & Smith, J. D. (1970). J. Mol. Biol. 47, 15.

Adams, J. M., Jeppeson, P. G. N., Sanger, F. & Barrell, B. G. (1969). Nature, 223, 1009.

Altman, S. & Smith, J. D. (1971). Nature New Biology, 223, 35.

Barnett, L., Brenner, S., Crick, F. H. C., Shulman, R. G. & Watts-Tobin, R. J. (1967). Roy. Soc. London, Philos. Trans. 252, 487.

Brubaker, L. H. & McCorquodale, D. J. (1963). Biochim. biophys. Acta, 76, 48.

Daniel, V., Sarid, S. & Littauer,U. V. (1970). Science, 167, 1682.

Goldberg, E. B. (1966). Proc. Nat. Acad. Sci., Wash. 56, 1457.

Landy, A., Abelson, J. N., Goodman, H. J. & Smith, J. D. (1967). J. Mol. Biol. 29, 457.

McClain, W. H. (1970). FEBS Lett. 6, 99.

Peacock, A. C. & Dingman, C. W. (1967). Biochemistry, 6, 1818.

Pinkerton, T. C., Paddock, G. & Abelson, J. N. (1971). Fed. Proc. 30, 1218.

Ronen, A. & Salts, Y. (1971). Virology 45, 496.

Stahl, F. W., Edgar, R. S. & Steinberg, J. (1964). Genetics 50, 539.

Tessman, I. (1965). Genetics 51, 63.

Wilson, J. H. & Kells, S. (1972). J. Mol. Biol.

Wilson, J. H., Kim, J. S. & Abelson, J. N. (1972). J. Mol. Biol.

Bacteriophage T4 Transfer RNA

III. Clustering of the Genes for the T4 Transfer RNAs.

John H. Wilson*

Division of Biology, California Institute of Technology

Pasadena, California, U.S.A. 91109

Jung Suh Kim

Division of Chemistry and Chemical Engineering

California Institute of Technology

Pasadena, California, U.S.A. 91109

John N. Abelson

Department of Chemistry, University of California (San Diego)

La Jolla, California, U.S.A. 92037

Running title: Clustering of T4 tRNA Genes

*Present address: Department of Biochemistry, Stanford University Medical School, Stanford, California, U.S.A. 94305

Abstract

E. coli cells infected with phage strains carrying a large deletion of gene *e* or gene psu_b^+, a nonsense suppressor resulting from a change in the phage ser-tRNA, are missing most if not all of the phage tRNAs normally present in wild type infected cells. By DNA-RNA hybridization we have demonstrated that the DNA corresponding to the missing tRNAs is absent. Thus the genes for these tRNAs must be clustered in the same region of the genome as the ser-tRNA gene. We have been able to locate and to define a maximum size for the cluster by physically mapping the deletions of genes *e* and psu_b^+ by examination of heteroduplex DNA in the electron microscope. That such deletions can be isolated indicates that the phage-specific tRNAs from this cluster are dispensable.

1. Introduction

The T4-coded nonsense suppressors, $\underline{psu}_a^+$, $\underline{psu}_b^+$ and $\underline{psu}_1^+$, all result from mutations within the T4 ser-tRNA and map about 8 map units clockwise from gene e on the standard T4 genetic map (McClain, 1970; Wilson & Kells, 1972). An ochre suppressor, n_1, identified and characterized by H. Kneser (personal communication), is located about 7 map units from gene e, suggesting that it might be close to the site of the gene for the ser-tRNA (J. Wilson, unpublished experiments). Since two different tRNA genes seem to map close together and many other genes in T4 are clustered into functionally related groups, we anticipated that genes for some of the other phage tRNAs might be located in the region of the genome between genes e and 57.

To test this notion we assayed the low molecular weight RNA produced in cells infected with phage strains carrying various deletions of genes e and $\underline{psu}_b^+$. These experiments suggested that most if not all the genes for the phage tRNAs are clustered around the site of the ser-tRNA gene. By correlating the physical map position of the deletions as determined by examination of heteroduplex DNA in the electron microscope (Davis, Simon & Davidson, 1971) with the presence or absence of tRNAs, we have been able to locate this cluster and to define its maximum size.

2. Materials and Methods

(a) Phage and bacterial strains

Deletion mutants of gene e, derived from wild type T4B, were ob-

tained from the collection of Dr. G. Streisinger through Dr. W. Salser and Dr. J. Owen. Those obtained from Dr. W. Salser also carry the spackle mutation, S12, which permits growth of the deletion mutants on plates which do not contain egg-white lysozyme (Emrich, 1969). $\underline{psu}_b^-\Delta$ strains were derived from $\underline{psu}_b^+$ strains and characterized as deletions as described by Wilson & Abelson (1972). CR63 was used as the permissive host for $\underline{psu}_b^-\Delta$ strains, all of which carry the mutation amN133. S/6/5 was used as the host for growth of lysozyme deletion mutants.

(b) Media and buffers

Media and buffers are described in Wilson & Kells (1972) and Wilson & Abelson (1972).

(c) Transfer RNA from phage-infected cells

Methods for preparation of ^{32}P-labeled RNA, electrophoresis in 10% polyacrylamide gel slabs and autoradiography of the gel slabs are described by Wilson & Abelson (1972).

(d) DNA-RNA hybridization

Wild type T4B and the gene e deletion mutant carrying a spackle mutation, eG506:S12, were grown on E. coli B and purified by differential centrifugation and a step CsCl gradient (Thomas & Abelson, 1966). The purified phage were dialyzed at 4°C against 0.1M NaCl, 0.01 M Tris-HCl pH 7.4.

DNA was extracted as described by Thomas & Abelson (1966) and then sonicated for 2 min using a Branson sonifier. The sonicated DNA

was denatured by heating for 5 min in boiling water followed by quick chilling in ice.

The liquid hybridization technique of Nygaard & Hall (1964) was performed as described by Bolle, Epstein, Salser & Geiduschek (1968). Hybridization was for 6 hrs at 65°C.

^{32}P-labeled T4 RNA was prepared and separated by electrophoresis on a 10% polyacrylamide gel slab as described by Wilson & Abelson (1972). Bands were located by autoradiography and cut out of the gel slab. The RNA was removed from the gel by homogenizing the gel segment in 0.3 M NaCl. The gel slurry was centrifuged in glass centrifuge tubes and the supernatant containing most of the ^{32}P-labeled RNA was removed. The gel was reextracted twice with 0.3 M NaCl. This procedure removed more than 90% of the RNA from the gel. Small particles of gel were removed by filtration of the RNA solution through a Millipore filter. The RNA was concentrated by ethanol precipitation.

Two samples of T4 RNA which had been pulse labeled with ^{3}H-Uridine between 2 and 5 min and 17 and 20 min after phage infection at 37°C were given to us by Dr. G. Notani.

(e) Electron microscopy

Electron microscope techniques for examining heteroduplex DNA are described in Davis et al. (1971), and Westmoreland, Szybalski & Ris (1969). Heteroduplex DNA was prepared as follows. 1.3 µg of each of two phages were mixed with 0.25 ml. of 0.1 N NaOH, 0.02 M EDTA at room temperature for 10 min to lyse the phage and denature the DNA. To

neutralize the solution and renature the DNA 25 µl. of 1.8 M Tris-HCl, 0.2 M Tris-OH and 0.25 ml. formamide were added. Samples were dialyzed against 0.9 M NaCl, 0.1 M Tris pH 8.5, 0.01 M EDTA, 60% formamide for 1 hr and then exhaustively against 0.1 M NaCl, 0.01 M Tris pH 8.5, 0.001 M EDTA. 0.5 µg of the heteroduplex DNA was mixed with 50 µl. of hyperphase containing 0.1 M Tris pH 8.5, 0.05 mg/ml. cytochrome c, 50% formamide and spread onto 0.01 M Tris pH 8.5, 0.001 M EDTA, 10% formamide. Both single- and double-stranded ØX174 DNA (5200 bases long; N. Davidson, personal communication) were added as internal standards. Grids were stained with uranyl acetate and shadowed with platinum-palladium. Under these condition single-stranded DNA is extended into a measureable form and appears thinner and kinkier than does double-stranded DNA.

3. Results

(a) Production of Phage tRNAs in deletion-infected cells

The phage-coded nonsense suppressors which result from an alteration in the T4 ser-tRNA ($\underline{psu}_a^+$, $\underline{psu}_b^+$, and psu_1^+) are located between genes e and 57 about 8 map units from gene e (McClain, 1970; Wilson & Kells, 1972). To determine whether the genes for any of the other phage tRNAs are located in the same region of the genome, we compared the low molecular weight RNAs present in cells infected with wild type T4 and three strains carrying deletions of gene e. All three deletions were known to extend past the end of gene e toward gene 57 and two, eG192 and eG506, had been characterized genetically by the shrinkage

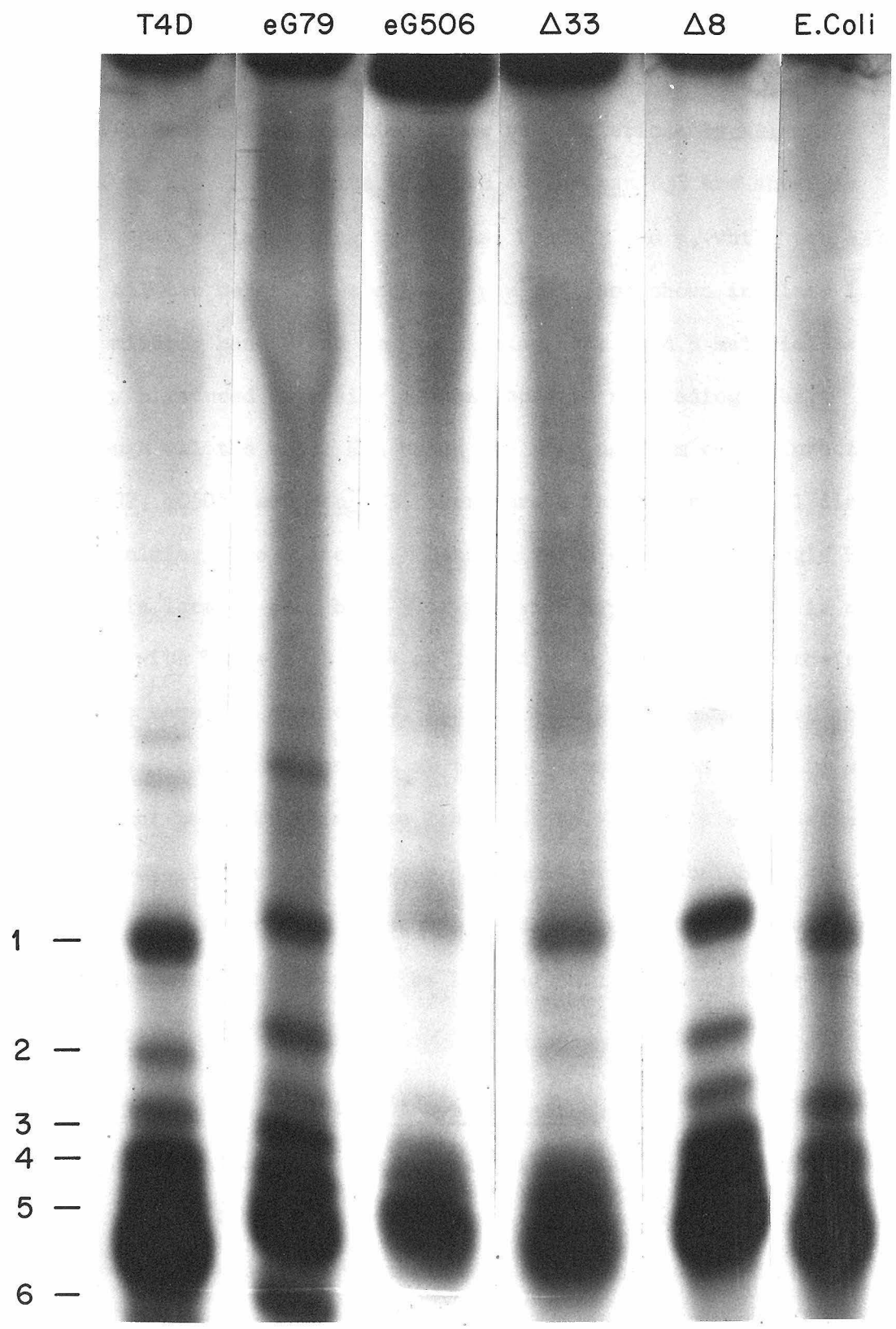
of outside markers to be quite large and were estimated to extend approximately half the way from gene e to gene 57 (J. Powers, personal communication).

Plate 1 shows the separation of low molecular weight RNA from infected cells obtained by electrophoresis in 10% polyacrylamide gels. The wild type T4 RNA separates into six distinct bands. Five of these bands (1, 2, 3, 4, and 6) contain single species of RNA as judged by their oligonucleotide maps and one, band 5, is a mixture of some 3 to 5 different species (J. Abelson, G. Paddock & T. Pinkerton, unpublished experiments; W. H. McClain & B. Barrell, personal communication). Bands 1 and 2 probably contain tRNA precursors (W. H. McClain & B. Barrell, personal communication; G. Paddock & J. Abelson, unpublished results). Band 3 contains a serine tRNA (W. H. McClain & B. Barrell, personal communication) and band 4 contains a leucine tRNA (Pinkerton, Paddock & Abelson, 1971). Bands 5 and 6 also contain tRNAs (W. H. McClain & B. Barrell, personal communication; J. Abelson, G. Paddock & T. Pinkerton, unpublished experiments).

As shown in Plate 1, eG79 and wild type are indistinguishable in their band patterns, while both eG192 and eG506 are missing most of the RNA bands prominent in wild type infected cells. These results suggest that the genes responsible for the missing bands are located in that portion of the genome which is deleted in both eG192 and eG506 but not deleted in eG79.

If the genes for these RNA species are clustered as indicated above, we should also see a decrease in the number of RNA bands in cells

Plate 1. Separation of ^{32}P-labeled RNAs from uninfected and T4-infected cells. ^{32}P-labeled RNA was extracted from cells, electrophoresed on 10% polyacrylamide gel slabs and antoradiographed as described in Material and Methods. eG506 carries S12. *psu*$_b^-$Δ8 and *psu*$_b^-$Δ33 carry *amN*133.


T4D
eG79
eG506
Δ33
Δ8
E.Coli
1
2
3
4
5
6

infected with deletions of $\underline{psu}_b^+$. To test this prediction we examined the low molecular weight RNA present in cells infected with three $psu_b^-\Delta$ strains (characterized genetically as $\underline{psu}_b^+$ deletions by Wilson & Abelson, 1972). The results with $psu_b^-\Delta 8$ and $\underline{psu}_b\Delta 33$ are shown in Plate 1. $\underline{psu}_b^-\Delta 8$ is missing two tRNAs, bands 3 and 6, while $\underline{psu}_b^-\Delta 33$ is lacking all the major RNA species. $\underline{psu}_b^-\Delta 53$, not shown in Plate 1, is clearly missing band 3 and may be missing some band 5 material as judged by a reduced intensity of that band in autoradiographs.

Though all the major RNA bands are missing from cells infected with eG192, eG506, and $\underline{psu}_b^-\Delta 33$, there are 2 fainter but still distinct bands remaining (see Plate 1). Band I, which contains a single RNA species, is located near band 3 of the wild type pattern but is not identical with bands 2, 3 or 4 as judged by a comparison of their oligonucleotide maps (J. Abelson, G. Paddock & T. Pinkerton, unpublished experiments). Band II is located at the position of band 5 but is much less intense than the wild type band. It is not yet clear whether these two bands are the result of phage synthesis or residual host cell synthesis. The existence of these two bands suggests the possibility that genes for some phage tRNAs may be located outside the cluster we have defined above.

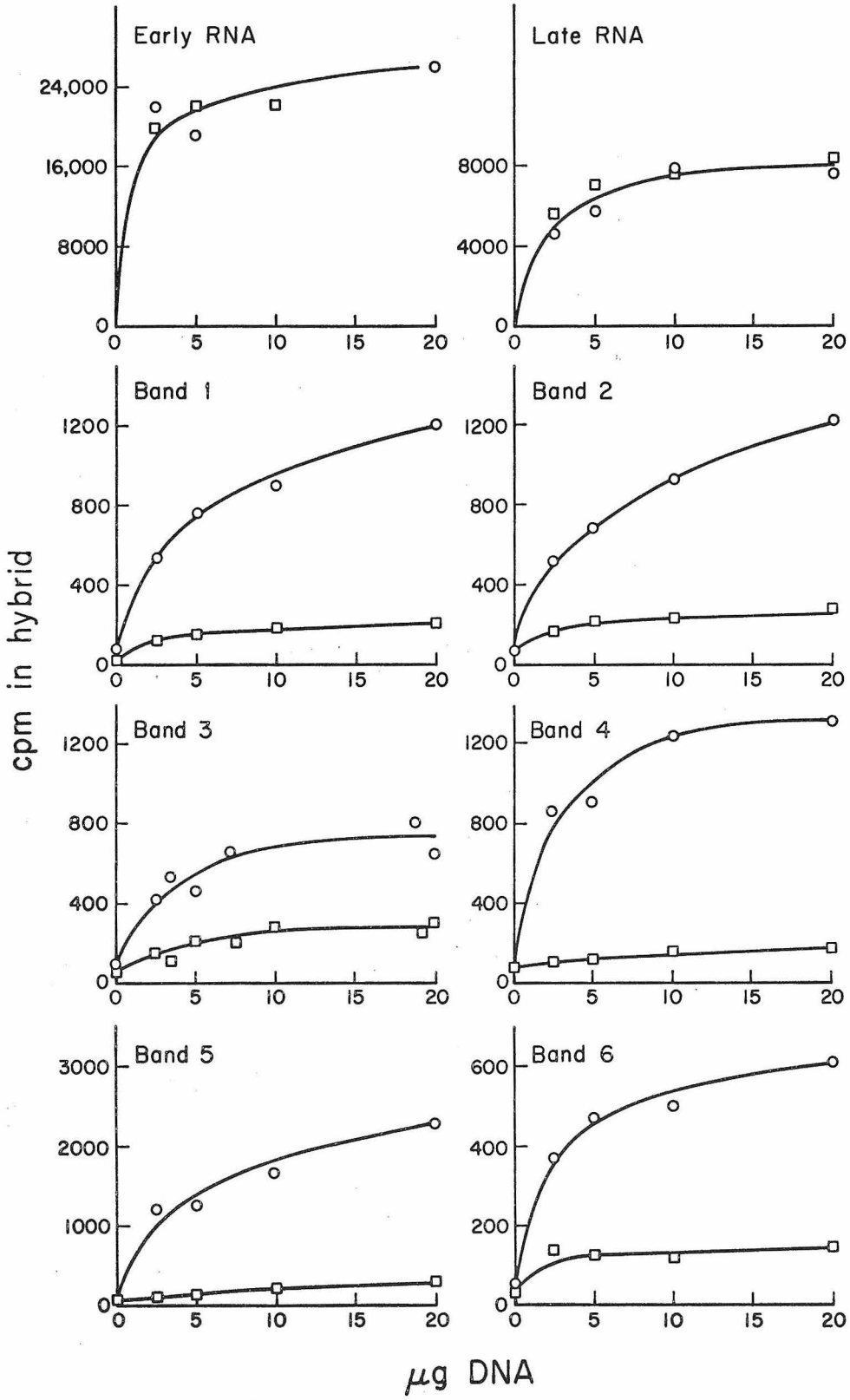
(b) DNA-RNA hybridization

To determine whether the absence of an RNA band means that the gene for that RNA has been deleted, we extracted the DNA from eG506 and wild type phage and compared their ability to hybridize RNA species

from wild type infected cells as shown in Figure 1. As a control to measure the relative quality of the two DNA preparations, we compared their hybridization of bulk RNA extracted from wild type infected cells which had been pulse labeled with ^{3}H-Uridine early or late after infection (these samples were kindly given to us by Dr. G. Notani). As shown in Figure 1 wild type and eG506 DNA hybridize both these RNA preparations equally well, indicating the DNA preparations are equivalent. The low hybridization values for bands 1, 2, 4 and 6 (each contains one unique RNA species) against eG506 DNA indicates that the DNA corresponding to these species is missing in eG506. Wild type band 5 RNA hybridizes only about 10% as much with eG506 DNA as with wild type DNA, suggesting that most, if not all, the genes for the 3 to 5 different RNA species present in band 5 are missing from eG506. If the material in the position of band 5 in eG506 infected cells, band II in Plate 1, is coded for by the phage, it represents a maximum of 10% of the total material present in wild type band 5.

The results with band 3 are anomalous. Band 3, which contains the phage ser-tRNA, seems to be composed of one unique species by examination of its oligonucleotide map (W. H. McClain & B. Barrell, personal communication) and as will be shown in sec. 3 (c) must be deleted in eG506. However, band 3 hybridizes nearly 50% as much with eG506 DNA as with wild type DNA. These results may indicate that band 3 actually contains a minor species of RNA in addition to the ser-tRNA, possibly corresponding to band I, which is present in eG506 infected cells (see Plate 1).

Fig. 1. DNA-RNA hybridization. Hybridization procedures are as described in Materials and Methods. Hybridization plateaus with wild type T4B DNA averaged 10% of the input radioactivity. Early and late RNA were pulse labeled with ^{3}H-Uridine between 2 and 5 min and 17 and 20 min after infection at 37°. O-O represents wild type T4B DNA. □-□ represents eG506 DNA.


Early RNA
Late RNA
Band 1
Band 2
Band 3
Band 4
Band 5
Band 6
cpm in hybrid
μg DNA

Taken together these results indicate that the absence of the RNA bands from deletion-infected cells means the corresponding genes have been deleted. Thus the genes for the majority of the T4 low molecular weight RNAs are located in a cluster in the region of the T4 genome which is missing in eG506 but present in eG79.

(c) Physical mapping of deletions of genes e and psu_b^+

In order to locate and define the size of the cluster of T4 tRNA genes we physically mapped several deletions of genes e and psu_b^+ by examination of heteroduplex DNA in the electron microscope. The general techniques we have used are those of Davis et al. (1971) and Westmoreland et al. (1969).

Heteroduplex DNA from a mixture of T2 and T4 phage (T2/T4 heteroduplex) shows a characteristic pattern of substitution and deletion loops when examined in the electron microscope (Kim & Davidson, in preparation). This pattern of loops has been oriented relative to the standard T4 genetic map by using deletions of genes e and rII as markers (Kim & Davidson, in preparation). Plate 2 shows an electron micrograph of the portion of a T2/T4 heteroduplex DNA molecule around gene e. Figure 2 is a schematic representation of the same picture. Except for loop A we do not know which strands of the loops correspond to T4 and which to T2. We have positioned the gene e deletions absolutely by mapping them relative to this loop pattern. The psu_b^+ deletions were subsequently mapped relative to defined gene e deletions.

The procedure for locating a particular gene e deletion relative

Plate 2. T2/T4 heteroduplex DNA. This electron micrograph shows the portion of a T2/T4 heteroduplex DNA molecule in the region of gene e. The letters relate the loop pattern in the micrograph to the schematic representation in Figure 2.

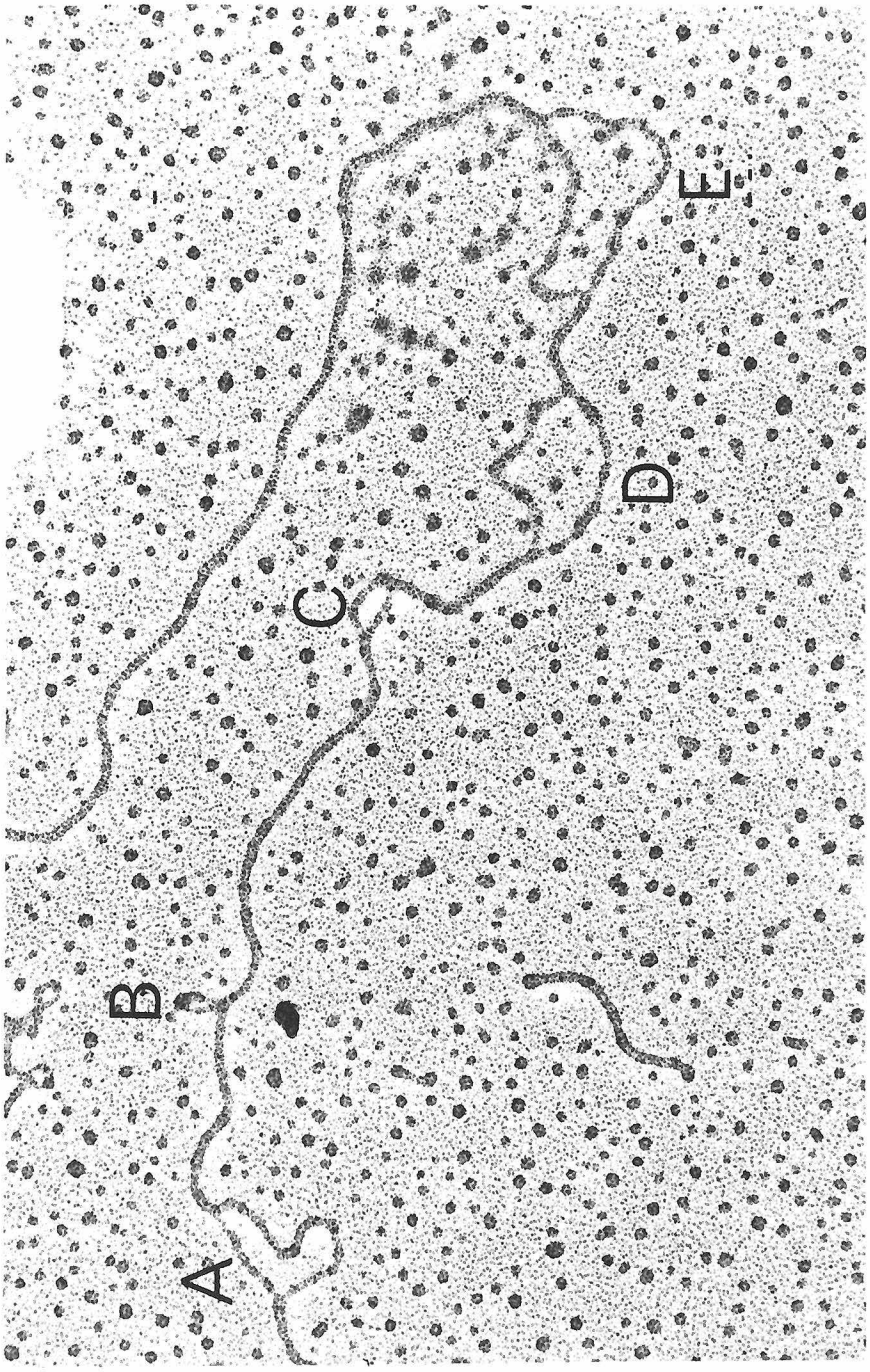
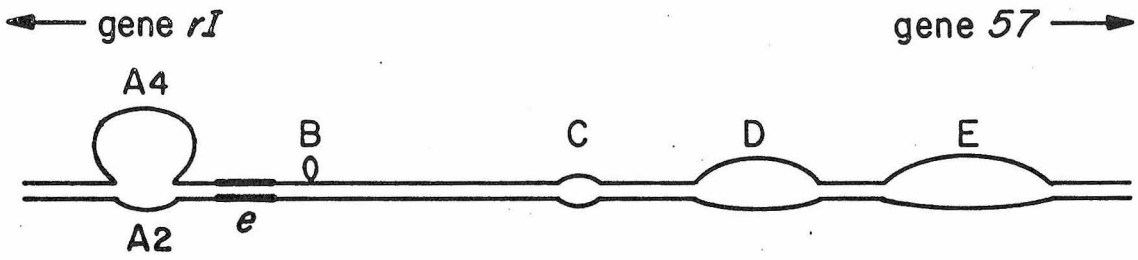

A
B
C
D
E'

Fig. 2. Schematic representation of T2/T4 heteroduplex DNA. The letters identify the loops in the electron micrograph in Plate 2. A2 and A4 indicate those strands of loop A which come from T2 and T4 respectively. The position of gene e is represented by the thickened segments on both strands.


gene rI
gene 57
A4
B
C
D
E
A2
e

to this loop pattern varied depending on the size of the deletion. We measured small deletions which did not extend past loop B or all the way through loop A in a single step by heteroduplexing with T2. Their size and location was determined from the decreases in length of A4 (the single-stranded portion of loop A which comes from T4) and of the double-stranded region between loops A and B. Deletions eG19, eG223, eG326, and eG342 were mapped in this way.

Those deletions which extended past B, or in the case of eG298 past the next marker loop in the direction of gene rI (not shown in Figure 2) were mapped in two or three steps. The first step was to define the size of the deletion by heteroduplexing it with wild type T4. The second step was to heteroduplex it with wild type T2. A typical eG79/T2 heteroduplex molecule is shown in Plate 3 and a schematic representation is shown in Figure 3. The loops E and D are intact while loops A, B and C have been replaced by a substitution loop. This pattern indicates that the right end of eG79 begins near loop D and that the left end starts in A4. The size of the shorter strand of the substitution loops gives the precise position within A4 at which the left end of eG79 begins and, thus, the position of eG79. Those deletions whose ends were both in double-stranded or single-stranded regions were mapped only approximately by this procedure. Such deletions were heteroduplexed to a defined gene e deletion which they partially overlapped to position one of their ends precisely. These heteroduplex molecules showed a single substitution loop. For such heteroduplexes one strand of the substitution loop will be equal to the length of the large deletion

Plate 3. eG79/T2 heteroduplex DNA. This electron micrograph shows the portion of an eG79/T2 heteroduplex DNA molecule in the region of gene e. The arrow, indicating the substitution loop formed by the eG79 deletion, and the letters relate the loop pattern in the micrograph to the schematic representation in Figure 3.

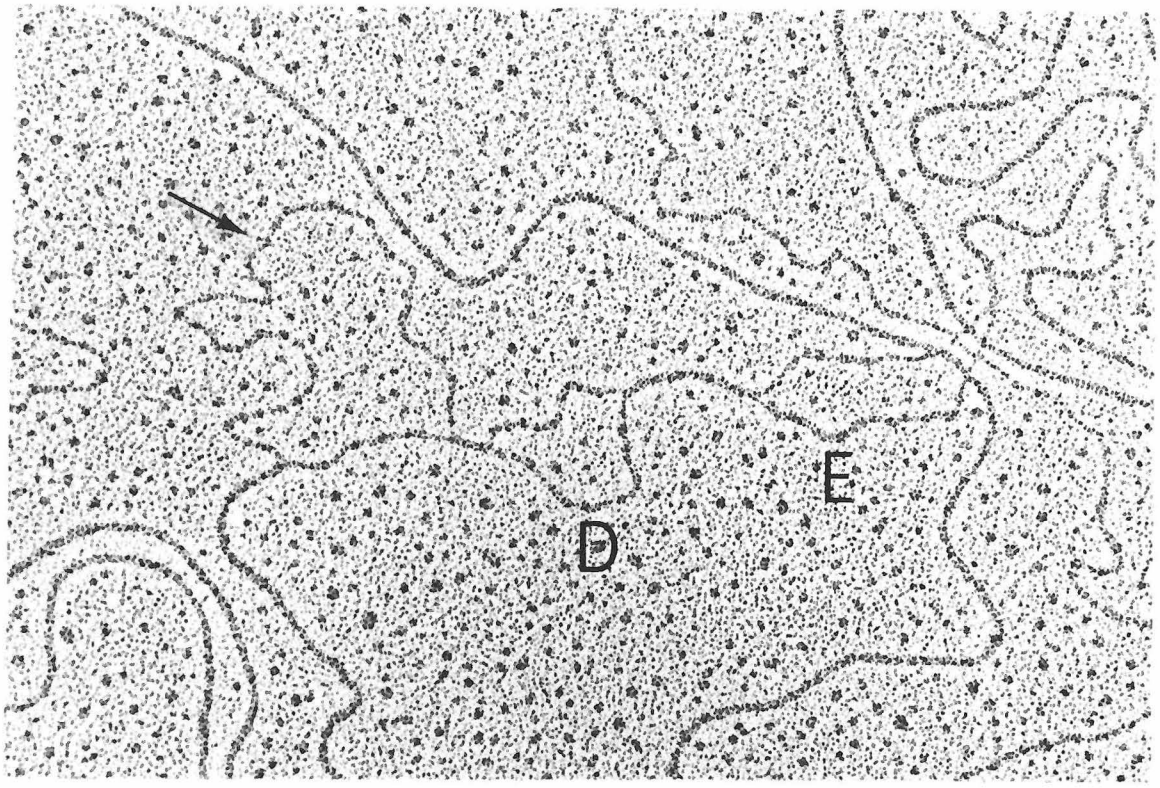
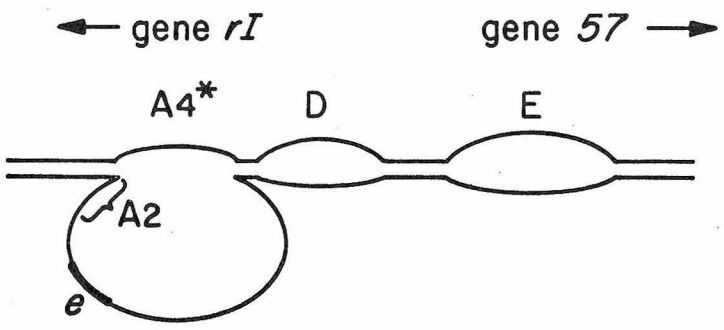

D
E

Fig. 3. Schematic representation of eG79/T2 heteroduplex DNA. The letters indentify the loops in the electron micrograph in Plate 3. $A4^{*}$ indicates that portion of A4 which is not deleted by eG79. The positions of A2 and T2 gene e are as indicated on the longer strand of the substitution loop.


gene rI
gene 57
A4*
D
E
A2
e

minus the length of the overlap and the other strand will be equal to the length of the small deletion minus the length of the overlap. This procedure defined the size of the overlap and thereby located precisely one end of the large deletion relative to the ends of the shorter deletion.

The three deletions of $\underline{psu}_b^+$ were mapped by heteroduplexing with a defined gene e deletion as shown for $\underline{psu}_b^-\Delta 8$ in Plate 4. Since this deletion and $\underline{psu}_b^-\Delta 53$ are so small, they could not be measured accurately. We estimate that $\underline{psu}_b^-\Delta 8$ is 200 bases long and $\underline{psu}_b^-\Delta 53$ is 150 bases long. The position of these two deletions was measured relative to both eG19 and eG79. The results indicate that these deletions are located to the right of gene e as expected from the genetic position of $\underline{psu}_b^+$ determined by Wilson & Kells (1972). The results for the physical mapping of all gene e and $\underline{psu}_b^+$ deletions are shown in Figure 4. The position of the overlap of $\underline{psu}_b^-\Delta 8$ and $\underline{psu}_b^-\Delta 53$ defines the site of $\underline{psu}_b^+$. From the positions of eG79, which contains all the RNA species, and $\underline{psu}_b^-\Delta 33$, which is missing all the RNA species, we conclude that the maximum size of this tRNA cluster is 2500 nucleotides.

4. Discussion

We have compared wild type T4 and strains carrying deletions of genes e and $\underline{psu}_b^+$ by gel electrophoresis of the RNA from infected cells, by DNA-RNA hybridization of wild type low molecular weight RNA and by electron microscope examination of heteroduplex DNA. A comparison of the capacity of DNA from wild type and the deletion, eG506, to hybridize

Plate 4. eG79/$\underline{psu}_b^-$Δ8 heteroduplex DNA. This electron micrograph shows the two deletion loops present in an eG79/$\underline{psu}_b^-$Δ8 heteroduplex DNA molecule. The larger deletion loop corresponds to eG79 and the smaller, indicated by the arrow, corresponds to $\underline{psu}_b^-$Δ8. The DNA circle at left is ØX174.

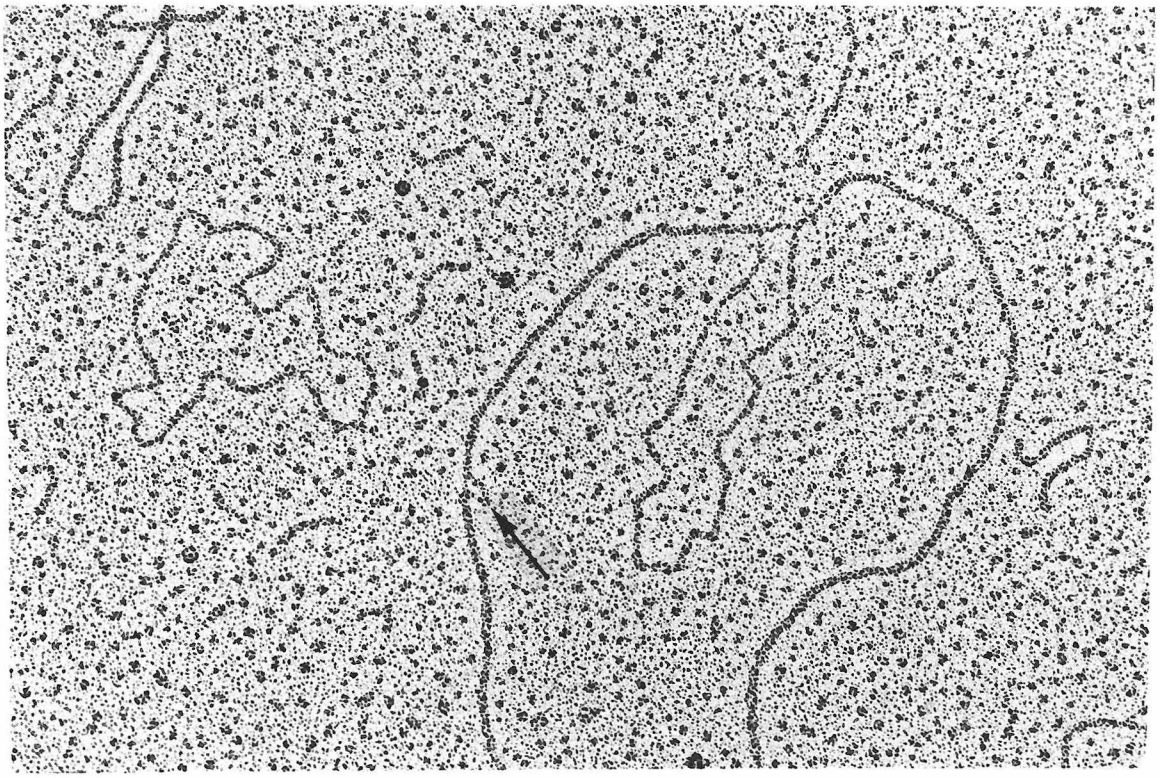
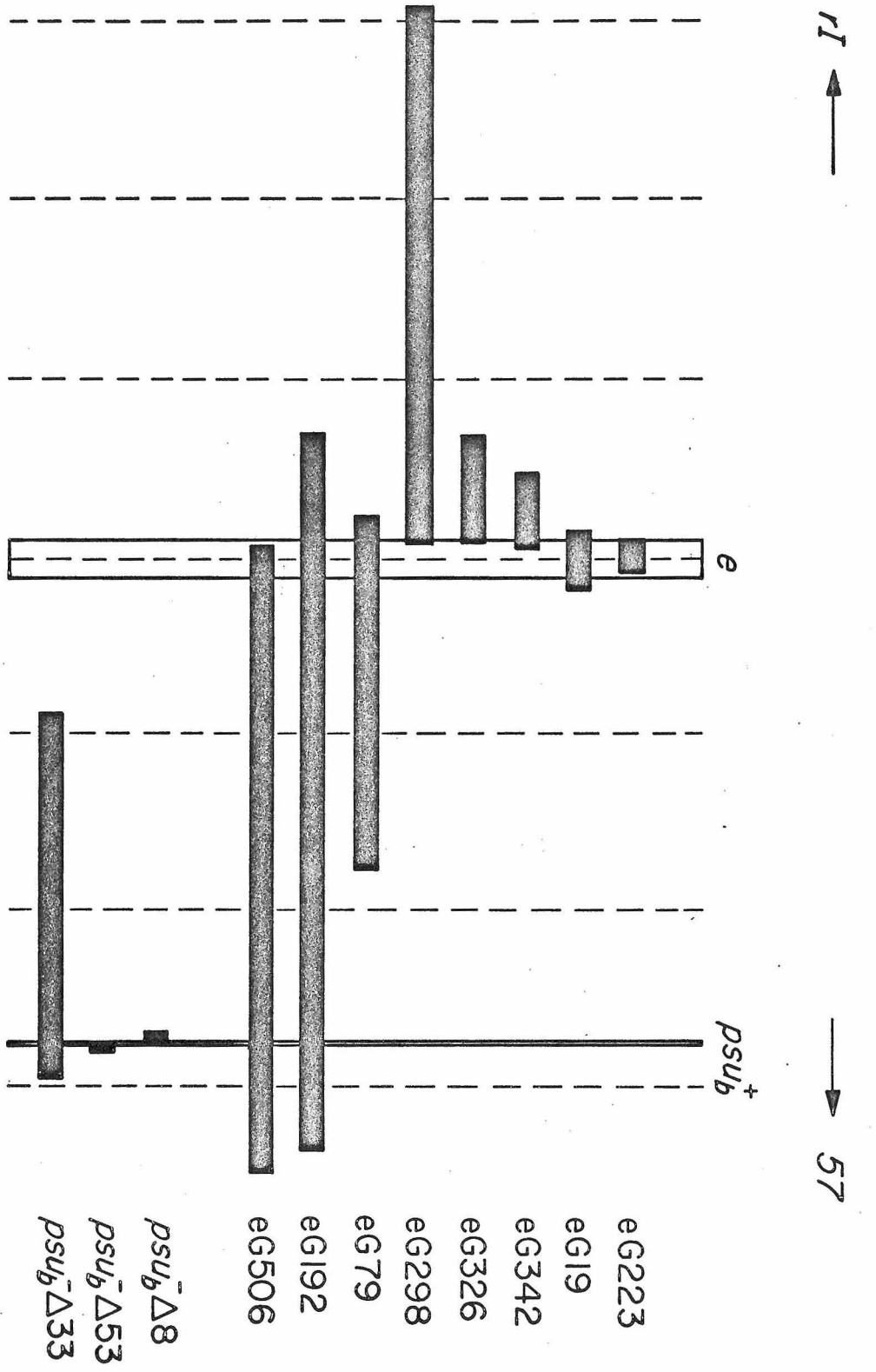


Fig. 4. Physical map of gene e and psu_b^+ deletions. The length of the black lines represents the length of the deletions. The distance between adjacent vertical dotted lines is 2000 nucleotides. The vertical rectangle labeled e represents the genetic position of gene e relative to the deletions (W. Salser, personal communication). The vertical rectangle designated psu_b^+ represents the minimum overlap of the psu_b^+ deletions. All gene e deletions except eG19, eG79 and eG298 carry the spackle mutation, S12. The $psu_b^-\Delta$ strains all carry amN133. The sizes and positions of the deletions represent an average of measurements on 5-10 different molecules.


rI
e
psu$_b^+$
57
eG223
eG19
eG342
eG326
eG298
eG79
eG192
eG506
psu$_b^-$Δ8
psu$_b^-$Δ53
psu$_b^-$Δ33

individual low molecular weight RNA bands from wild type infected cells suggests that the absence of an RNA species indicates the absence of the corresponding gene. We explore this relationship in more detail in a study in which we order several of the tRNA genes within the cluster (Wilson, Kim & Abelson, in preparation). By correlating the physical map position of the deletion with the presence or absence of RNA species from the deletion infected cells, we have determined that most of the T4 tRNA genes are clustered around the position of the ser-tRNA gene between genes e and 57. The existence of some ^{32}P-labeled low molecular weight RNA in cells infected with phage strains which delete this cluster leaves open the possibility that genes for some phage tRNAs are located outside the cluster as we have defined it.

Taken together these studies define the maximum size of this cluster as 2500 nucleotides. We can estimate a minimum size for the cluster by calculating the number of nucleotides in the RNA species present on the polyacrylamide gels. From the positions of the RNA bands on the gels and from nucleotide sequence data for some we estimate that the number of nucleotides in the RNA species in bands 1 through 6 are respectively 140, 100, 90, 90, 80, and 70 (Pinkerton et al., 1971; J. Abelson, G. Paddock & T. Pinkerton, unpublished experiments; W. H. McClain & B. Barrell, personal communication). If we assume band 5 contains 4 species, then we can estimate a minimum size for the cluster of about 800 nucleotides.

W. H. McClain and B. Barrell in a study of precursors of T4 tRNA precursors made an observation which independently suggested the clus-

tering of the tRNA genes and at the same time indicated that the individual tRNA genes may be quite close together. They have analyzed the nucleotide sequence of a precursor of the ser-tRNA and shown that it is composed of a ser-tRNA and a pro-tRNA which are separated by fewer than 10 nucleotides (W. H. McClain & B. Barrell, personal communication). That a precursor of this nature exists suggests the possibility that this entire cluster may be transcribed as a single unit and that the individual tRNAs may all be cleaved out of this one large RNA precursor.

The results of the physical mapping of gene e deletions suggest that the only absolutely essential T4 function in the 13,000 nucleotides defined by eG298 and eG506 is lysozyme, the product of gene e. In addition the fact that psu$_b^-$Δ33 grows as well as wild type phage indicates that all the T4 tRNAs from this cluster are dispensable under the conditions we have employed.

We are grateful to Dr. R. L. Russell, Dr. W. B. Wood, Dr. A. Sarabhai, and Dr. W. H. McClain for many helpful discussions. We are especially indebted to Dr. W. H. McClain and Dr. B. G. Barrell for generously offering us their unpublished results. This research was supported by grants (GM-06965, AI-09238, and CA-10984) from the U.S. Public Health Service. One of us (J.N.A.) is a Faculty Research Associate of the American Cancer Society.

REFERENCES

Bolle, A., Epstein, R. H., Salser, W., & Geiduschek, E. P. (1968). J. Mol. Biol. 31, 325.

Davis, R. W., Simon, M. & Davidson, N. (1971). Methods in Enzymology, vol. 21 (Grossman, L. & Moldave, K. eds.) Academic Press, p. 413.

Emrich, J. (1968). Virology, 35, 158.

McClain, W. H. (1970). FEBS Lett. 6, 99.

Nygaard, A. P. & Hall, B. D. (1964). J. Mol. Biol. 9, 125.

Pinkerton, T. C., Paddock, G., & Abelson, J. N. (1971) Fed. Proc. 30, 1218.

Thomas, C. A. & Abelson, J. N. (1966). Procedures in Nucleic Acid Research (Cantoni, G. L. & Davies, D. R., ed.) Harper and Row, p. 553.

Westmoreland, B. C., Szybalski, W., & Ris, H. (1969). Science, 163, 1343.

Wilson, J. H. & Kells, S. (1972). J. Mol. Biol.

Wilson, J. H. & Abelson, J. N. (1972). J. Mol. Biol.

General Discussion

Through investigations on phage strains which have been genetically altered in the nature or number of their tRNAs, we have shown:

1. There are only two phage genes which are essential for the production of functional ser-tRNA. One is the structural gene for the ser-tRNA and the second plays an undefined role which affects several tRNAs. The implication of only two phage genes in the maturation of the ser-tRNA suggests that there are very few phage genes other than the tRNA structural genes which are essential for phage tRNA production. Thus precursor cleavage, nucleotide modification and amino acid charging can be carried out by host enzymes, implying a great deal of similarity in the maturation and structure of host and phage-induced tRNAs.

2. The genes for 6 to 8 phage tRNAs and 2 probable tRNA precursors are clustered within a portion of the genome not more than 2500 nucleotides long.

3. The tRNAs within this cluster are dispensable in the laboratory host we have employed.

In continuing work which has not yet been completed we have expanded point 3 and have begun to answer the questions posed in the general introduction. These studies and results are listed below:

1. Investigations on differences in ^{14}C-labeled proteins as displayed by SDS polyacrylamide gel electrophoresis. We have pulse labeled wild type and eG506 infected cells at various times after infection and examined the resultant protein patterns. There are three differences

between eG506 and wild type.

a. An unidentified early protein of about 25,000 MW is absent in eG506 infected cells.

b. The three highest molecular weight late proteins (the products of genes 34, 7 and 37) are made in about 50% the wild type quantity in eG506 infected cells.

c. Cleavage rates of two proteins in eG506 infected cells seem to be altered. Gene 23 product seems to be cleaved at a much slower rate and the uncleaved internal protein IP3 is present in about twice the normal amount.

We have also examined $psu_b^-\Delta 8$, $psu_b^-\Delta 53$, $psu_b^-\Delta 33$, and mb5 infected cells for these differences. $psu_b^-\Delta 33$, which deletes all the tRNAs, shows the same effects as eG506. The others are all like wild type. It is not yet clear whether the differences we see are due to the absence of tRNAs or other products coded for in DNA which is deleted in both eG506 and $psu_b^-\Delta 33$. It should be emphasized that these differences are the only ones we see in the gel patterns. This means that host cell protein synthesis has been shut off in the absence of the phage tRNAs and that the turn-off and turn-on of all the proteins which are visible on the SDS gels appears normal.

2. Investigations on differences in growth of $psu_b^-\Delta 33$ on various strains of bacteria. The tRNAs are clearly not essential in the host we have employed and at most cause a very few changes in the pattern of protein synthesis. To determine whether the tRNAs might be essential in other hosts, we examined the growth of $psu_b^-\Delta 33$ on various strains of *E. coli* which had been isolated from patients in a hospital in Los Angeles.

633 of these strains were tested for their ability to support growth of several different phage by R. S. Edgar, J. Weigle and I. Lielausis (unpublished experiments). They demonstrated that wild type T4 grew on only 28 of these strains. In preliminary tests 4 of these 28 strains did not support the growth of $\underline{psu}_b^-\Delta 33$. 1 of these 4, CT439, also failed to support the growth of mb5, tentatively supporting the notion that the tRNAs may be required in other bacterial hosts.

3. Characterization of the other $\underline{psu}_b^-\Delta$ strains which don't grow on CT439. To determine whether the lack of growth of $\underline{psu}_b^-\Delta 33$ was due to the absence of the tRNAs, we screened all the rest of the $\underline{psu}_b^-\Delta$ strains for growth on CT439 in hopes of finding one whose ends are inside the limits of the cluster. 14 of the 51 deletions of $\underline{psu}_b^+$ do not grow on CT439. 11 of these are total deletions of the cluster as characterized by the absence of tRNA bands on 10% polyacrylamide gels of ^{32}P-labeled infected cells. 1 has not been characterized. 2 delete only a few of the bands. By physically mapping these 2 deletions in the electron microscope and correlating the map positions of these 2 and those described in paper III with the presence and absence of tRNA bands, we have been able to tentatively order within the cluster all the tRNA species except those in band 5. One of the newly characterized deletions has both ends flanked by tRNAs, suggesting that it is the absence of one or several tRNAs which causes the lack of growth on CT439.

From the detailed description of the genetic system within the body of this thesis and the partial physiological analysis of some of the mutants described above it is reasonable to expect that we shall be able

to determine the consequences of the lack of the phage tRNAs. Thus hopefully we shall be able to determine the functions of the phage tRNAs and possibly gain insight into the general problem of multiplicity of tRNA species and their relation, if any, to developmental events.