JOURNAL OF BACTERIOLOGY, Jan. 1979, p. 507-516
0021-9193/79/01-0507/10$02.00/0
Vol. 137, No. 1

Deletion Analysis of the Expression of rRNA Genes and Associated tRNA Genes Carried by a λ Transducing Bacteriophage†

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Received for publication 11 August 1978

Transducing phage λ*ilv*5 carries genes for rRNA's, spacer tRNA's ($tRNA_1^{Ile}$ and $tRNA_{1B}^{Ala}$), and two other tRNA's ($tRNA_1^{Asp}$ and $tRNA^{Trp}$). We have isolated a mutant of λ*ilv*5, λ*ilv*5*su7*, which carries an amber suppressor mutation in the $tRNA^{Trp}$ gene. A series of deletion mutants were isolated from the λ*ilv*5*su7* phage. Genetic and biochemical analyses of these deletion mutants have confirmed our previous conclusion (E. A. Morgan, T. Ikemura, L. Lindahl, A. M. Fallon, and M. Nomura, Cell **13**:335-344, 1978) that the genes for $tRNA_1^{Asp}$ and $tRNA^{Trp}$ located at the distal end of the rRNA operon (*rrnC*) are cotranscribed with other rRNA genes in that operon. In addition, these deletions were used to define roughly the physical location of the promoter(s) of the rRNA operon carried by the λ*ilv*5*su7* transducing phage.

It has previously been shown that there are tRNA genes located in association with each of the seven *Escherichia coli* rRNA operons (8, 11-13). These rRNA operons have been isolated on specialized transducing phages or on hybrid plasmids and have been analyzed previously (8, 10-13). Each of the seven rRNA operons has gene(s) for either $tRNA_2^{Glu}$ or both $tRNA_{1B}^{Ala}$ and $tRNA_1^{Ile}$, located between the genes for 16S and 23S rRNA (the "spacer tRNA genes"; 11, 13). Some operons also have tRNA genes at their distal ends (12). For example, the *rrnC* operon has genes for $tRNA^{Trp}$ and $tRNA_1^{Asp}$ at its distal end, and these genes are apparently cotranscribed with 16S, 23S, and 5S rRNA genes and the spacer tRNA genes. Transcription begins at promoter(s) located near the 5′ end of the 16S rRNA gene (12). The conclusion about cotranscription of "distal tRNA genes" and rRNA genes was based on the observations that, in strains carrying hybrid plasmids with $tRNA^{Trp}$ and $tRNA_1^{Asp}$ genes, the tRNA's are overproduced only if the promoter for the *rrnC* operon is present (12).

λ*ilv*5, a phage isolated by Jørgensen (9), carries a complete hybrid rRNA operon, which was probably formed by recombination between two rRNA operons during isolation of the phage (10). The rRNA promoter and the spacer region of this phage come from an as yet undetermined rRNA operon of *E. coli*. The distal end of this operon comes from *rrnC*. We have initiated deletion analysis of the promoter(s) for this rRNA operon. For this purpose, we first isolated a mutant (called λ*ilv*5*su7*) of λ*ilv*5. This mutant has an amber (Am) suppressor mutation in the gene for $tRNA^{Trp}$, *trpT*. We then isolated a series of deletion mutants and tested the expression of the suppressor tRNA and other tRNA genes as well as the rRNA genes. The results presented in this paper confirm our previous conclusion (12) that the genes for $tRNA_1^{Asp}$ and $tRNA^{Trp}$ are cotranscribed with other rRNA genes carried by the rRNA operon. In addition, deletions define roughly the region of the DNA necessary for the expression of this rRNA operon, that is, the physical location of the rRNA promoter(s).

MATERIALS AND METHODS

Bacterial strains and phages. The bacterial strains used in this study are listed in Table 1. The procedures used to construct some of the strains are described in the legend to this table. λ*ilv*5 has been previously described (9, 10). λ*ilv*5*su7*, a mutant of λ*ilv*5, carries the *su7* mutation in the $tRNA^{Trp}$ gene, *trpT* (see Results). λ*ilv*5*su7* was obtained by ethyl methane sulfonate mutagenesis of a λ*ilv*5 lysogen of NO869. Deletions were isolated from λ*ilv*5*su7* by citrate treatment as previously described (17, 20; see Results).

Synthesis of RNA in UV-irradiated cells. These techniques have been previously described (20). The strains used are S159 and NO1406, a λpapa lysogen of S159. Cells were grown to a density of 2×10^8/ml in synthetic AB minimal media (3) using maltose (0.4%) as a carbon source. Cells were concentrated to 10^9/ml by centrifugation, irradiated with UV light, and in-

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TABLE 1. *Bacterial strains used*[a]

E. coli strains used	Genotype	Source
KL16-99 (NO883)	Hfr *thi recA ser*	F. Engbaek
NO869	*ilv-1 his-29*(Am) *trpA9605*(Am) *trpR pro-2 ara tsx*	F. Engbaek
NO1966	NO869 (λ*ilv5*, λcI857*S7b515b519xis6*)	This work
NO1847	NO869 (λ*ilv5su7*, λcI857*S7b515b519xis6*)	This work
MB93 (NO1907)	*galK*(Am) *lac*(Am) *ara*(Am) *trp*(Am) *galE supF tsx*	J. Smith
NO1911	*galK*(Am) *lac*(Am) *ara*(Am) *trp*(Am) *galE tsx ilv recA*	This work
NO1912	NO1911 (λ*ilv5su7*, λcI857*S7b515b519xis6*)	This work
S159 (NO1348)	*uvrA gal str*	L. Siminovitch
S159(λ) (NO1406)	S159 (λpapa)	This laboratory

[a] NO1847 was constructed as follows. NO1966 was first spread on a glucose-AB minimal plate with only proline as a supplementary amino acid (for AB medium, see ref. 3). A disk soaked with ethyl methane sulfonate was then placed on the plate. After incubation at 30°C, Trp^+ His^+ colonies were isolated. One of them (NO1847) was found to have an amber suppressor mutation on λ*ilv5*. Strain NO1911 was constructed as follows. A suppressor-free derivative of MB93 was selected on a glycerol (0.2%)-AB minimal plate supplemented with lactose (0.2%), galactose (0.2%), and tryptophan (40 μg/ml). This strain (NO1908) was made Ilv^- by nitrosoguanidine mutagenesis followed by penicillin selection. The resulting strain (NO1909) was made Thy^- by trimethoprim selection (NO1910) and then Thy^+ $RecA^-$ by interrupted mating with KL16-99. The resulting strain is NO1911. All deletion phages subsequently produced from NO1847 or NO1912 were maintained as and produced from lysogens of NO869, using λcI857*S7* as a helper phage.

fected with purified phages at a multiplicity between 10 and 20. After a 10-min period of adsorption (at 37°C), the cultures were diluted fivefold with the prewarmed maltose media, and incubation with shaking was started at 37°C (time 0). Labeling of RNA was usually done between 10 and 25 min after the start of the incubation. The labeled RNA was extracted and analyzed either by RNA-DNA hybridization (6) or by two-dimensional gel electrophoresis (8).

Other procedures. Purifications of transducing phages (11) and plasmid DNA (10) were described previously. Fingerprinting analysis of RNA was done according to the method of Sanger (2, 16). The methods for preparation of heteroduplexes and for electron microscopy were according to Davis et al. (5). As in our previous work (e.g., refs. 10, 11, 20), single-strand DNA regions were measured using φX174 DNA (5.25 kilobase pairs [kb]) as a reference, and double-strand DNA regions were measured using PM2 DNA (9.5 kb) as a reference.

RESULTS

Isolation of the λ*ilv5su7* transducing phage. As described in the introduction, λ*ilv5*, isolated by Jørgensen (9), carries *ilv* genes and a hybrid rRNA operon (9, 10). The proximal part of the operon, including the rRNA promoter and the spacer tRNA genes (genes for $tRNA_1^{Ile}$ and $tRNA_{1B}^{Ala}$), comes from an operon different from *rrnC*. The distal end of the rRNA operon, which includes genes for $tRNA_1^{Asp}$ and $tRNA^{Trp}$, comes from *rrnC* (8, 10, 12, 13).

A mutant (λ*ilv5su7*) which carries an amber suppressor mutation has been isolated after ethyl methane sulfonate mutagenesis of λ*ilv5*. Restriction nuclease analysis and electron microscope heteroduplex analysis revealed no detectable insertions or deletions when λ*ilv5* and λ*ilv5su7* were compared (detailed data are not shown; see the legend to Fig. 1). The structure of λ*ilv5su7* is shown in Fig. 1 and Fig. 2a. The following experiments show that the suppressor mutation in λ*ilv5su7* is in the $tRNA^{Trp}$ gene (*trpT*) and is probably identical to the known *su7* suppressor mutation characterized previously.

First, λ*ilv5su7* lysogens of NO869 had Trp^+ His^+ phenotype and allowed growth of a subset of a number of independent T4 phage amber mutants (data not shown). φ80d3 lysogens of NO869 had identical properties in these respects. φ80d3, isolated by L. Soll, is a phage that carries *su7* (14, 18).

Second, UV-irradiated cells of E. coli strain S159(λ) were infected with λ*ilv5su7*, and the tRNA's synthesized were analyzed. Under the conditions used, synthesis of RNA is dependent on the introduction of bacterial genes on the infecting phage. The small RNAs produced were labeled with ^{32}P and analyzed by two-dimensional gel electrophoresis followed by autoradiography. The pattern of the tRNA's synthesized after infection with λ*ilv5su7* was very similar to that obtained after λ*ilv5* infection, except that there is a mobility difference in the $tRNA^{Trp}$ synthesized from λ*ilv5* compared to that from λ*ilv5su7* (Fig. 3). The $tRNA^{Trp}$ synthesized from each phage was isolated from the gel and fingerprinted after digestion with ribonuclease T1 (Fig. 4). Fingerprint patterns of the two RNAs

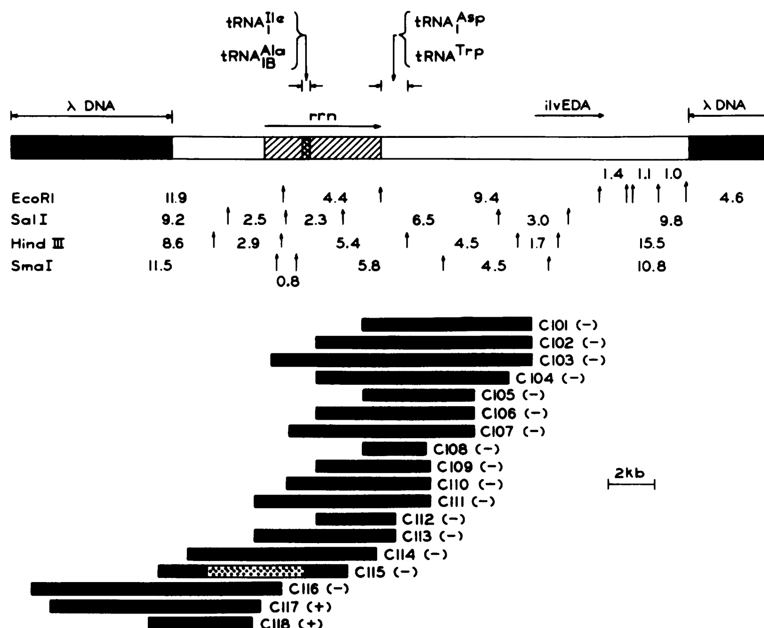

FIG. 1. *Restriction nuclease map of the bacterial DNA carried on λilv5su7 and locations of various deletion mutants. Only the left-hand portion of λilv5su7 is shown. EcoRI, SalI, SmaI, and HindIII cleavage sites are indicated by arrows. Sizes of the fragments are given in kilobase pairs (kb). The locations of some genes are also indicated. The horizontal bars below the λilv5su7 chromosome give the extent of some representative deletions. The termini of most of the deletions have been located only to the extent that it is known what restriction nuclease cleavage sites remain or are deleted. (−) or (+) after the names of deletions indicate the absence or the presence, respectively, of su7 suppressor ability as tested by a genetic method (see the text). The restriction map of λilv5 (and λilv5su7) was determined primarily by isolation and analysis of fragments. First, the restriction nuclease cleavage patterns for each enzyme were determined for λ, λilv5, pLC21-9, and pLC22-36 DNAs. The latter two are plasmids which carry a portion or all of rrnC and additional DNA homologous with that on λilv5 (10, 12). This allowed preliminary arrangement of restriction nuclease cleavage sites. The DNA of λilv5 was then digested with each restriction nuclease, and the fragments produced were isolated on a preparative scale. 16S and 23S rRNA were then hybridized to each fragment by standard filter hybridization techniques, enabling localization of rRNA genes. Each fragment was also digested individually with all other enzymes, and the mobility of the resulting products was compared with that of the starting fragment and with fragments produced from λilv5 by digestion with the same enzyme used for secondary digestion of the isolated fragment. In this way, all cleavage sites were accounted for and the distances between all cleavage sites were determined. The exact position of the cleavage sites in the rRNA operon was deduced from the secondary digestion data, from rRNA hybridization data, and by heteroduplex analysis (see refs. 10, 12). Deletion phages C103, C105, C106, C110, C111, C112, C114, C115, C116, C117, and C118 were obtained by citrate treatment of λilv5su7. Deletion phages C101 and C104 were obtained by screening phages produced from survivors of NO1912 after selection on media containing lactose plus galactose. Deletion phages C102, C107, C108, C109, and C113 were obtained by selecting survivors of NO1912 after growth in the presence of lactose and galactose, and subsequent citrate treatment of phages produced from these survivors, as described in the text.*

were identical except for oligonucleotides containing anticodons (see Fig. 4 and legend). The fingerprint of tRNA^{Trp} synthesized after λ*ilv5su7* infection is very similar to that previously reported for *su7* tRNA (7, 22). From these experiments, we conclude that the suppressor mutation carried by the λ*ilv5su7* phage is in the *trpT* gene and is identical to the known *su7*-(Am) suppressor mutation and involves a base substitution (C → U) in the anticodon of tRNA^{Trp}, as shown by Soll and his coworkers (18).

Isolation of mutants from λ*ilv5su7*. A number of point and deletion mutants were first isolated from λ*ilv5su7* by using a direct selection method. A λ*ilv5su7* lysogen of NO1911 was plated on minimal salts media containing lactose, galactose, glycerol, and tryptophan. In this lysogen, suppression of the *lac*(Am) and *gal*(Am) mutations by *su7* leads to death of the cells in the presence of lactose or galactose (see ref. 15). Surviving colonies were then picked and examined to determine whether they can produce transducing phages with reduced suppres-

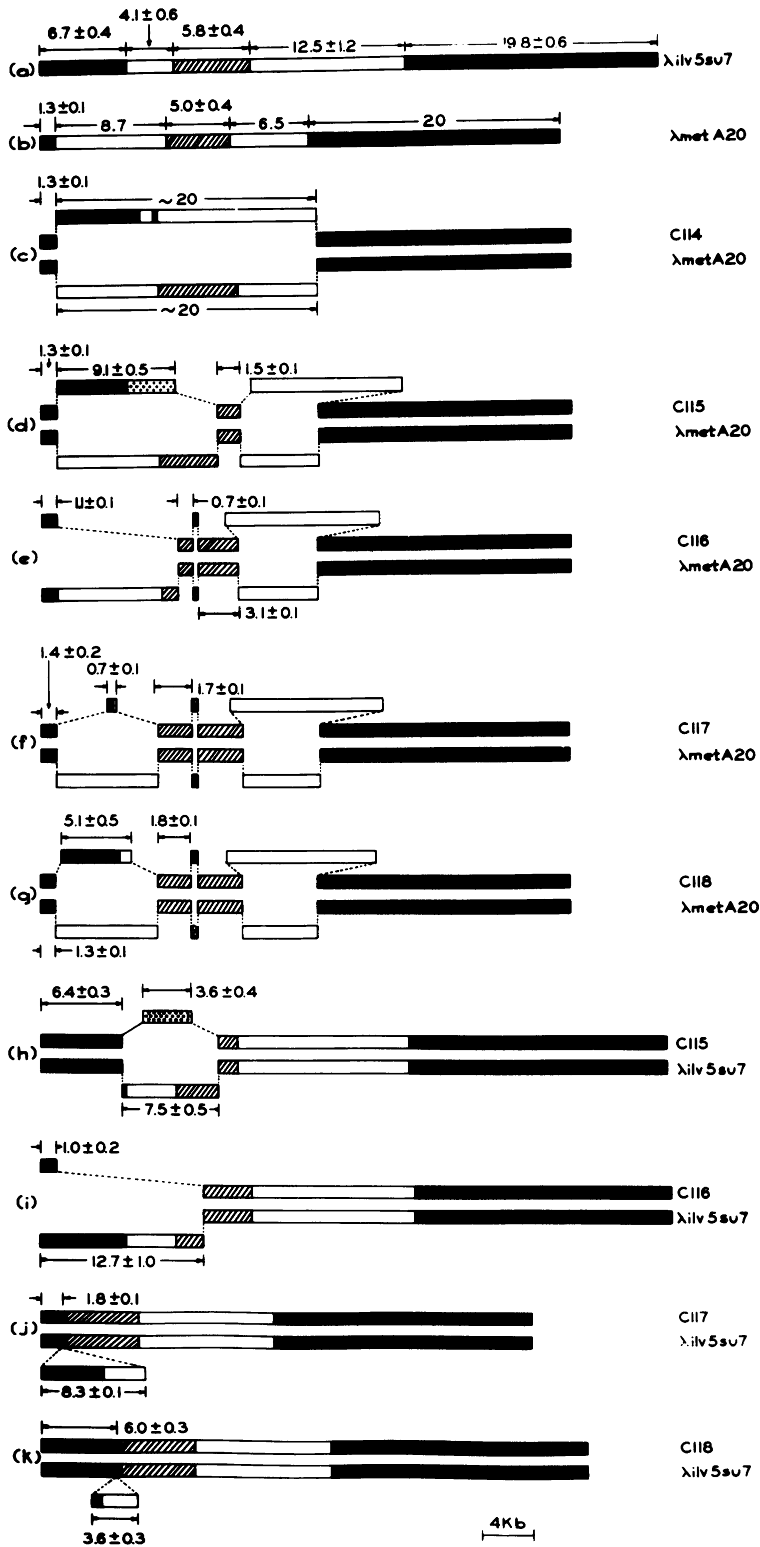

FIG. 2. *Summary of heteroduplex structures formed between λmetA20 or λilv5su7 and various deletion mutants of λilv5su7. As described in the text, λilv5su7 is a point mutant of λilv5. λmetA20 carries rrnE. The structures of λilv5 [i.e., λilv5su7 (a)] and λmetA20 (b) have been previously described (10, 21). The filled bars represent λ phage DNA; open bars represent non-ribosomal bacterial DNA; hatched bars represent rDNA containing rRNA genes; and stippled bars represent DNA of unknown origin found as an insertion in the mutant C115. Some pertinent distances are given in kb.*

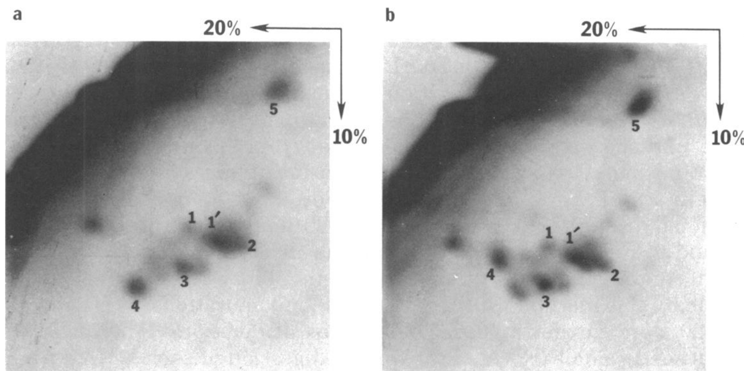

FIG. 3. *Two-dimensional polyacrylamide gel electrophoresis of ^{32}P-labeled small RNAs synthesized in UV-irradiated cells infected with λilv5 and λilv5su7. E. coli strain S159(λ) was irradiated with UV light and infected with λilv5 (a) or λilv5su7 (b), and the RNAs synthesized were labeled with [^{32}P]orthophosphate as described previously (11, 20). RNA was prepared and analyzed by two-dimensional polyacrylamide gel electrophoresis followed by autoradiography as described previously (8). The autoradiograms are shown. (1) $tRNA_1^{Ile}$ (an undermodified form); (1′) $tRNA_1^{Ile}$ (a mature form); (2) $tRNA_1^{Asp}$ (partially overlapped by $tRNA_1^{Ile}$); (3) $tRNA_{1B}^{Ala}$; (4) $tRNA^{Trp}$; (5) 5S RNA. Note a mobility difference between the two $tRNA^{Trp}$ species.*

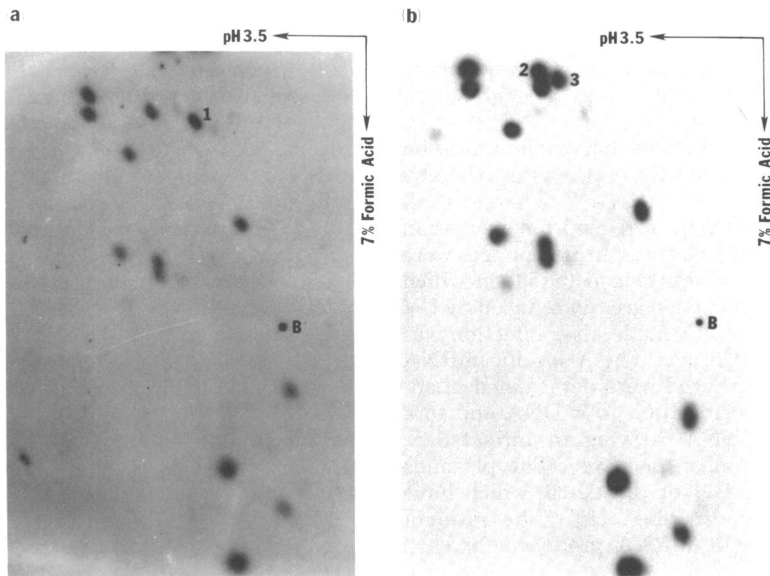

FIG. 4. *Autoradiograms of fingerprints of $tRNA^{Trp}$ synthesized in UV-irradiated E. coli after infection with λilv5 (a) and λilv5su7 (b). The tRNA's were isolated by two-dimensional polyacrylamide gel electrophoresis (see legend to Fig. 3), digested with ribonuclease T1, and fingerprinted. The oligonucleotides designated 1, 2, and 3 correspond to the anticodon oligonucleotides of the tRNA's. The sequence previously reported (22) for the anticodon oligonucleotides of $tRNA^{Trp}$ and $tRNA^{Trp}$ (su7) are $UC_mUCCA^{ms^2i^6}$ AAACCG and $UC_mUCUA^{ms^2i^6}$ AAACCG, respectively. Ribonuclease A digestion of oligonucleotides 1, 2, and 3 gives products with the mobility of U, C, AAAAC, and G when the products are analyzed at pH 3.5 (1). We believe that oligonucleotides 2 and 3 are related and that one of them is an undermodified form of the other.*

sor activity. The suppressor activity of the phages was analyzed by isolating Ilv^+ lysogens from NO869 and testing for suppression of *his*(Am) and *trp*(Am) in the lysogens. It was found that about 5% of the survivors produced *ilv* transducing phages with reduced suppressor activity. The mechanism that results in survival of the remaining 95% has not been investigated. The phages that lost suppressor activity were screened for insertions or deletions by digestion of isolated phage DNA with restriction nucleases, followed by comparison of the mobility

of the fragments produced by similar digestion of λ*ilv5su7* DNA. Each phage DNA was digested individually with *Hin*dIII, *Eco*RI, *Sal*I, or *Sma*I, or with *Eco*RI and *Sal*I together. The restriction nuclease map of λ*ilv5su7* is shown in Fig. 1. The *Eco*RI map of the parent (λ*ilv5*) of this phage was previously established (4; L. Lindahl, unpublished data). The *Sma*I, *Sal*I, and *Hin*dIII maps were determined during the course of this work (see the legend to Fig. 1). Eighty mutants were found which contained no insertions or deletions detectable by this method. Of these 80 "point" mutants, 20 were further analyzed for their ability to synthesize small tRNA's in UV-irradiated λ-lysogenic *E. coli* cells. As determined by polyacrylamide gel electrophoresis, normal 5S RNA, $tRNA_1^{Ile}$, $tRNA_1^{Asp}$, and $tRNA_{1B}^{Ala}$ were produced in each case. However, most of the mutants showed no identifiable $tRNA^{Trp}$ or produced a $tRNA^{Trp}$ that migrated to a slightly different position from that produced by λ*ilv5su7* (data not shown). Therefore, the majority of the mutations that arise in this selection system inactivate the *su7* suppressor tRNA by altering its primary structure or by causing incomplete maturation or degradation of the RNA transcript.

It was originally thought that a point mutation might inactivate the rRNA promoter, thereby preventing synthesis of $tRNA^{Trp}$. Therefore, all 80 point mutants were screened for expression of rRNA genes. To do this, purified phages were used to infect a λ nonlysogen (S159) in which RNA synthesis had been greatly reduced by UV irradiation. The RNA made after infection was labeled with [^{3}H]uracil. The λ-specific mRNA and rRNA synthesized were determined quantitatively by hybridization to λ DNA and to a hybrid plasmid DNA carrying an unrelated *E. coli* rRNA operon, respectively. The plasmids used were pLC22-11 or pLC23-30, which have been previously described (10). The ratio of rRNA to λ-specific mRNA produced for each mutant was indistinguishable from that produced after infection with λ*ilv5su7* (data not shown; see similar experiments in Table 3). Therefore, none of these point mutations inactivated the rRNA promoter(s).

Since the above selection method did not yield mutants useful for defining the rRNA promoter on the λ*ilv5su7* phage, we then used the citrate method, either alone or in combination with the above selection method, to isolate many deletion mutants. Random deletions were isolated by selecting Ilv^+, citrate-resistant phage survivors after three to five cycles of citrate treatment. Deletions specifically inactivating $tRNA^{Trp}$ were obtained in the following way. A λ*ilv5su7* lysogen of NO1911 was grown in the presence of lactose and galactose. Survivors containing a mixture of many independent suppressor-negative mutants were then subjected to thermal treatment to induce phages. Deletions were isolated from this phage population as Ilv^+, citrate-resistant phages after one cycle of citrate treatment.

Characterization of deletion mutants. The deletion mutants were first analyzed for their ability to suppress the *trp*(Am) and *his*(Am) mutations in NO869 and for the locations of deletions. The latter were determined by digestion of each DNA with *Eco*RI, *Hin*dIII, *Sal*I, and *Sma*I, and by agarose gel electrophoresis of the resulting fragments. The locations of some representative deletions and information about their suppressor activity are given in Fig. 1.

The $tRNA^{Trp}$ gene was previously localized to the right of the rRNA genes (in Fig. 1) and to the left of the *Sma*I-sensitive site (between the 5.8- and 4.5-kb fragments in Fig. 1) (12, 13). It was found that deletions C101 through C111 (see Fig. 1), which remove the DNA at the previously proposed location of the $tRNA^{Trp}$ gene, result in phages that do not confer suppressor activity in lysogens. Also, deletions C114, C115, and C116, which remove the 5′ end of the rRNA operon but do not delete the $tRNA^{Trp}$ gene (Fig. 1 and Table 2), resulted in phages which do not confer suppressor activity in lysogens. (Note that no selection against suppressor activity was used in obtaining deletions C114, C115, and C116; see

TABLE 2. *Hybridization of RNA species to DNA from λilv5su7 and deletion mutants*[a]

RNA species	Source of DNA			
	λ*ilv5su7*	C114	C115	C116
$tRNA^{Trp}$ (*su7*)	238	203	235	278
$tRNA_1^{Ile}$	551	7	0	396
$tRNA_{1B}^{Ala}$	452	23	51	484
16S rRNA	1,590	11	197	1,078
23S rRNA	3,222	546	2,505	3,448
5S rRNA	175	108	192	191

[a] A sample of 1.1 μg of DNA from each phage was fixed to filters and hybridized to various RNA species under conditions of DNA excess. Each RNA was hybridized to all phage DNAs in a single hybridization vial. The resulting hybrids were treated with ribonuclease A. Ribosomal RNA species were labeled with [^{3}H]uracil and prepared as described (10). tRNA species were labeled with [^{32}P]orthophosphate and prepared by two-dimensional gel electrophoresis of the small RNAs produced in UV-irradiated lysogenic *E. coli* [S159(λ)] infected with λ*ilv5su7*. Values are counts per minute; filters with no DNA gave 20 to 30 cpm, and this was subtracted from the values given.

legend to Fig. 1.) This latter observation confirms our previous conclusion (12) that the $tRNA^{Trp}$ gene is cotranscribed with the neighboring rRNA genes.

The deletion mutant C112 retains the promoter region of the rRNA operon, but does not express the *su7* suppressor gene. This is presumably due to a deletion of the $tRNA^{Trp}$ gene in this mutant. We have also found that radioactive $tRNA^{Trp}$ hybridizes to the 5.4-kb *Hin*dIII fragment, but not to the 4.5-kb *Hin*dIII fragment (unpublished experiments). Therefore, the $tRNA^{Trp}$ gene is probably located very close to the 5S RNA gene (see Fig. 1).

The structures of several deletion phages (C114, C115, C116, C117, and C118) were further analyzed by hybridization to rRNA and tRNA species (Table 2) and by electron microscope heteroduplex analysis (Fig. 2). These heteroduplex studies have established more detailed structures of these deletion phages (Fig. 5). Deletion phages C117 and C118 have deletions which do not cover the rRNA genes, whereas deletion phages C116, C115, and C114 have deletions of the rRNA promoter region which terminate in the middle of the 16S rRNA gene and the middle and end of the 23S rRNA gene, respectively.

Since deletion phages C117 and C118 have a functional amber suppressor and deletion phages C114, C115, and C116 do not, it would be predicted that phages C117 and C118 can express all the rRNA and tRNA genes on the phages, whereas deletions C114, C115, and C116 cannot. UV-irradiated λ-lysogenic cells were infected with these phages, and RNAs synthesized after infection were labeled with $[^{32}P]$orthophosphate. The small RNAs produced were analyzed by two-dimensional gel electrophoresis (Fig. 6). Deletions C114, C115, and C116 did not synthesize 5S RNA or any tRNA species whose genes are carried by λ*ilv5su7*. Deletions C117 and C118 produced normal amounts of 5S RNA and of these tRNA's.

To determine quantitatively the efficiency of expression of the rRNA operons in these deletion mutant phages, UV-irradiated, nonlysogenic *E. coli* cells were infected by these phages. The amounts of λ-specific mRNA and of rRNA produced in these cells were determined by filter hybridization to λ DNA and to DNA of hybrid plasmid pLC23-30, which carries an unrelated rRNA operon (10). The ratio of λ-specific mRNA to rRNA is a measure of the efficiency of expression of the rRNA operon. No expression or only a very weak expression of the rRNA operon was observed with deletions C114, C115, and C116 (Table 3), whereas deletions C117 and C118 did not affect the expression of the rRNA operon.

The right end of deletion C117 is close to the beginning of the rRNA operon as defined by the region of homology between the rRNA operons on λ*ilv5* and λ*metA20* in heteroduplex analysis. Using the data given in Fig. 2 (f and j), one can calculate that this distance is about 0.3 ± 0.2 kb. Thus, deletion C117 defines a maximum region necessary for rRNA operon expression.

DISCUSSION

We have isolated a mutant of λ*ilv5*, λ*ilv5su7*, which carries an amber suppressor mutation in the $tRNA^{Trp}$ gene. Since the expression of the *su7* gene is dependent on the promoter(s) of the rRNA operon (12; this paper), we thought that selection of mutants with inactivated suppressors would be able to detect point mutations in the promoter of the rRNA operon. However, our attempt to isolate such point promoter mutants was unsuccessful. The only mutants isolated that inactivated the rRNA promoter function were large deletion mutants. It is possible that point mutations inactivating rRNA synthesis will be difficult to obtain, as recent in vitro evidence indicates the presence of at least two promoters in each of two rRNA operons that have been examined (H. A. de Boer, S. F. Gil-

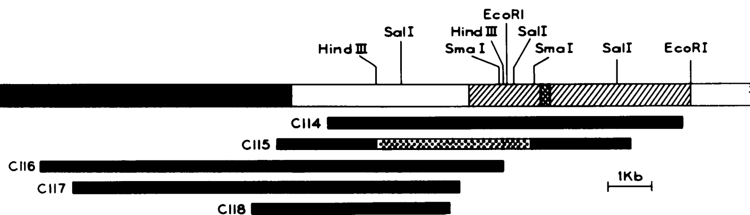

FIG. 5. *Detailed structure of the rRNA promoter region of λilv5su7. Under the restriction map (see legend to Fig. 1) of the promoter region, the extents of various deletions are indicated by the horizontal bars. The end points of the deletions are determined by restriction nuclease mapping (Fig. 1), RNA-DNA hybridization data (Table 2), and heteroduplex analysis (Fig. 2). "Deletion" phage C115 actually has a substitution of DNA of unknown origin, which is indicated by the stippled area on the deletion bar.*

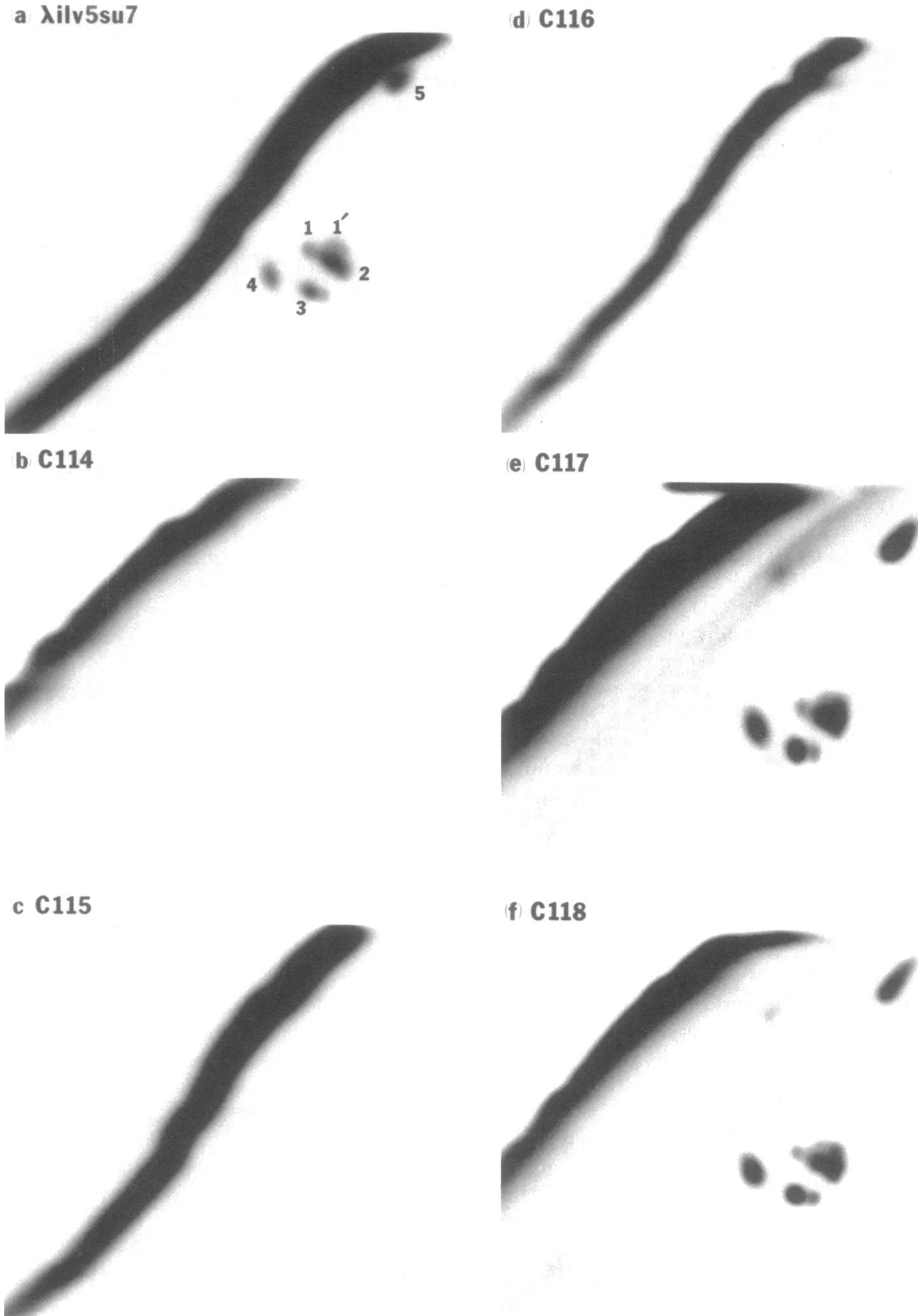

FIG. 6. *Autoradiograms of two-dimensional gels of small RNAs synthesized in UV-irradiated cells infected with λilv5su7 (a) and various deletion mutants of this phage (b through f). Experiments were done as described in the legend to Fig. 3. The key for tRNA's and 5S RNA is also given in the legend to Fig. 3. The autoradiogram of the control sample from uninfected cells was similar to those shown in (b), (c), and (d).*

bert, E. A. Morgan, and M. Nomura, unpublished data).

The analysis of deletion mutants of the λ*ilv*5*su7* phage has confirmed our previous conclusion that the genes for $tRNA_1^{Asp}$ and $tRNA^{Trp}$ located at the distal end of the *rrnC* operon are

TABLE 3. *Synthesis of rRNA and λ mRNA in cells infected with λilv5su7 and deletion mutants*[a]

Expt. no.	Infecting phage	RNA hybridized to (cpm)		rRNA/λ mRNA ratio
		λcI857S7 DNA	pLC23-30 DNA	
1	None	42	530	—
	λcI857S7	42,825	399	—
	λ*ilv5su7*	48,483	10,097	[1.0]
	C114	25,826	352	0
	C115	35,541	192	0
	C116	44,629	1,736	0.14
	C118	31,782	6,538	0.96
2	None	10	167	—
	λ*ilv5su7*	43,189	4,395	[1.0]
	C117	49,708	4,919	0.98
	C118	26,297	2,933	1.07

[a] UV-irradiated nonlysogenic cells (strain S159) were infected with the specialized transducing phages indicated in the table at a multiplicity of 20. [^{3}H]uracil (20 μCi) was added to each culture after infection. The RNA produced was extracted and hybridized to 3.4 μg of λ DNA or DNA from the plasmid pLC23-30. pLC23-30 carries an rRNA operon unrelated to that on λ*ilv5su7*. Filters with no DNA gave 30 to 70 cpm, and this was subtracted from all values. The ratio of rRNA hybridized to λ mRNA hybridized was normalized to the ratio resulting from infection by λ*ilv5su7*, which is given in a box. The values obtained from uninfected cells were subtracted from the values obtained from infected cells before the rRNA/λ mRNA ratio was calculated.

cotranscribed with other rRNA genes in that operon. This is inconsistent with the results reported by Wu and Davidson (19). These authors examined the rRNA and $tRNA^{Trp}$ genes carried by φ80d3 transducing phage by using an electron microscopic method involving the gene 32 protein method. Their results indicated that $tRNA^{Trp}$ and rRNA are transcribed from different strands on this phage. In addition, a large insertion (5.67 kb) of sequences derived from F was found to be between the proposed location of $tRNA^{Trp}$ and the end of the rRNA genes. Our previous studies indicated that the insertion of DNA derived from F has occurred in the immediate neighborhood of the end of the rRNA operon on φ80d3 (see heteroduplex structures formed between λ*ilv5* and φ80d3 described in Fig. 1 in ref. 10). It is possible that other chromosomal rearrangements have occurred during formation of φ80d3, and this may explain the discrepancy between the present results and the results reported by Wu and Davidson (19). We believe that the structure of λ*ilv5* in the region of $tRNA^{Trp}$ represents the structure of the *E. coli* chromosome in this region, because the two independently derived plasmids we analyzed are completely homologous to λ*ilv5* in this region (10). In addition, the cotranscription of rRNA genes and $tRNA^{Trp}$ and $tRNA_1^{Asp}$ is supported by studies with these plasmids as well as by rifampin run-out experiments using intact *E. coli* cells (12).

The deletion analysis described in this paper defines a maximum region necessary for the rRNA operon expression. Further work with these and similar mutant phages, in conjunction with DNA sequencing and RNA transcription experiments, should establish more precisely the extent of the regions on λ*ilv5su7* that are involved in initiation of rRNA synthesis.

ACKNOWLEDGMENTS

We thank G. D. Strycharz and M. Dietzman for their technical assistance.

This work was supported in part by the College of Agriculture and Life Sciences, University of Wisconsin, and by grants from the National Science Foundation (GB-31086 and PCM7818490) and the National Institutes of General Medical Sciences (Public Health Service grant GM-20427). E.A.M. is the recipient of Public Health Service postdoctoral fellowship GM-06033 from the National Institute of General Medical Sciences.

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