JOURNAL OF BACTERIOLOGY, Feb. 1983, p. 672–684
0021-9193/83/020672-13$02.00/0
Copyright © 1983, American Society for Microbiology
Vol. 153, No. 2

Efficient Read-Through of Tn*9* and IS*1* by RNA Polymerase Molecules That Initiate at rRNA Promoters

RICHARD J. SIEHNEL AND EDWARD A. MORGAN*

Department of Cell and Molecular Biology, State University of New York at Buffalo, Buffalo, New York 14260

Received 20 July 1982/Accepted 26 October 1982

Transcription and translation are coupled in most *Escherichia coli* operons. As a consequence, ribosomes must be present on an mRNA molecule while transcription of the mRNA is in progress or else premature termination of transcription may result. This requirement is most clearly manifested when premature nonsense codons result in polarity in multicistronic operons. Polarity can also result from insertions of transposons and insertion sequences. However, since rRNA operons are not translated, some property of these operons must allow transcription to be uncoupled from translation. In this paper we demonstrate that transposon Tn*9* and insertion sequence IS*1* are nonpolar or incompletely polar in rRNA operons during normal growth. We also show that essentially all expression of *rrn* sequences distal to IS*1* and Tn*9* results from transcripts that originate at rRNA promoters. These results suggest either that rRNA operons possess some mechanism which reduces or prevents termination within rRNA operons or that Tn*9* and IS*1* can be very inefficient at blocking normal transcription. Insertions of Tn*10* in rRNA operons are substantially but incompletely polar. We could not determine whether the residual downstream transcription observed results from promoters within Tn*10* or from read-through of Tn*10*. We discuss the meaning of read-through of Tn*9* and IS*1* and the residual expression of genes downstream from Tn*10* with regard to rRNA operon structure and previous experiments in which polarity of transposons or insertion sequences was observed in protein-encoding operons.

Transcription is coupled to translation in bacterial operons that code for proteins. Ribosomes must be on mRNA molecules while transcription of the mRNA is in progress, or transcription can terminate prematurely (1). Premature termination of transcription occurs when RNA polymerase can interact with termination signals normally masked by ribosomes (1, 5). If premature termination of translation occurs in promoter-proximal genes in a polycistronic operon, downstream genes are not transcribed, resulting in a phenomenon known as polarity (1). Polarity can also be caused by transcription termination resulting from insertion sequences or transposons (21).

Despite the fact that transcription is not coupled to translation in rRNA operons, rRNA operons (*rrn* operons) are transcribed without significant polarity (16, 25, 27). Therefore, *rrn* operons must possess some mechanism or property which circumvents the requirement for coupling of transcription and translation that exists in other operons. The requirement for coupling of transcription and translation can also be relieved by the antitermination mechanism mediated by the phage lambda gene *N* product, which allows read-through of normal transcription termination signals and insertion sequences and relieves polarity caused by nonsense mutations (1). The absence of transcriptional-translational coupling in *rrn* operons suggested that these operons could have a similar mechanism that controls termination within *rrn* operons. Therefore, we previously introduced Tn*10*, Tn*9*, and IS*1* into *rrnX* and examined the polarity caused by these elements. Genetic results proved that expression of the tRNA^{Trp}-*Su7* gene of *rrnX* (located on lambda specialized transducing phages) occurred at a detectable level in growing lysogens that contained *rrnX* transcription units with Tn*10*, Tn*9*, or IS*1* insertions located upstream from the tRNA^{Trp}-*Su7* gene (4, 25). However, previous quantitative measurements of the level of downstream transcription depended on the use of UV-irradiated cells infected by these phages. When this method was used, Tn*9* and IS*1* were found to reduce transcription of downstream regions by at most 20% (4), and three different insertions of Tn*10* could reduce transcription of downstream regions to variable lev-

els, which depended on the location of Tn*10* in *rrnX* (25). However, evidence that prior UV irradiation could reduce polarity (12, 33) and the possibility of involvement of lambda antitermination mechanisms in these experiments raised questions about the results obtained by these methods. It was also possible that the transcription of downstream regions originated within Tn*10*, Tn*9*, or IS*1*.

In this paper we show that (i) Tn*9* and IS*1* insertions in *rrn* operons are essentially nonpolar in growing cells under conditions where lambda antitermination mechanisms cannot contribute to the observed lack of polarity of these insertions, (ii) the lack of polarity of Tn*9* and IS*1* in *rrn* operons requires transcripts which initiate at *rrn* promoters, and (iii) Tn*10* is substantially but incompletely polar in *rrn* operons. We also describe an assay that is capable of measuring transcription of a single *rrn* operon in growing cells.

MATERIALS AND METHODS

Strains and phages. The starting bacterial strains, plasmids, and phages used for the experiments described in Fig. 1, 3, and 7 have been described previously in detail (4, 25). A hybridization and elution analysis was done by using RNA prepared from *Escherichia coli* strain EM4 lysogenized with λ *c*I857 *S7* and the desired specialized transducing phage. Lysogens were constructed under conditions where lysogenization occurred after infection of cells by an average of much less than one specialized transducing phage per cell. The characteristics of bacterial strains, phages, and plasmids that are relevant to this study are as follows: EM22 *recA ilv trp*(Am); EM2 RecA$^+$ *ilv his*(Am) *trp*(Am); EM4, a *recA* derivative of EM2; pLC22-36 carries *ilv* genes which complement the *ilv* mutations in EM2, EM4, and EM22 and also carries colicin immunity. All of the phages described in this paper also carry *ilv* genes. T4(amber) phages were obtained from W. McClain and M. Sussman.

Construction of plasmids with insertions and deletions. EM2 was first lysogenized with one of the defective specialized transducing phages carrying transposons, but without helper phage. Each strain was then transformed with pLC22-36, selecting for colicin immunity. Cultures derived from these cells were then mated with EM22 (λ^r Str^r), followed by selection for Ilv$^+$ Str^r λvir^r cells that were also resistant to an antibiotic due to the desired transposon. Deletion derivatives of these plasmids were then isolated by restriction nuclease digestion of the plasmids with *Sma*I or *Xma*I, followed by ligation of the mixture, transformation, and selection for antibiotic-resistant, colicin-immune cells. IS*1* insertions were obtained from Tn*9* insertions by first transforming plasmids with Tn*9* into strain EM2. The resulting cultures were then enriched by chloramphenicol-ampicillin treatment (4) for Tn*9* insertions which had undergone recombination between the direct repeats of IS*1* found at the ends of Tn*9*, followed by recovery of chloramphenicol-sensitive, colicin-immune cells after mating with EM22 (λ^r Str^r). The desired plasmids were identified by further screening of purified plasmids by digestion with restriction nucleases.

Hybridization and elution analysis. Cultures (15 ml) were grown at 30°C in MOPS medium (31) containing 0.5 mM phosphate, 0.4% glucose, 18 amino acids (no isoleucine or valine) (50 μg/ml each), 20 μg of each of the five nucleic acid bases per ml, and 1 μg of thiamine per ml. At 30°C the doubling times of the cultures in this medium were about 70 min; 1 mCi of $^{32}P_i$ was added at an optical density at 550 nm of 0.5. After 1 h the cells were chilled on ice, lysed, and extracted by the hot phenol-sodium dodecyl sulfate method (16). The isolated RNA was then ethanol precipitated and suspended in 50 μl of a solution containing 50% deionized, charcoal-treated formamide, 5× SSC (1× SSC is 0.15 M NaCl plus 0.015 M sodium citrate), 0.02% bovine serum albumin, 0.02% Ficoll, 0.02% polyvinylpyrollidone, 20 mM sodium phosphate (pH 6.5), and 250 μg of denatured calf thymus DNA per ml. This solution was then sealed in a plastic bag (150 by 150 mm) containing a nitrocellulose filter (diameter, 150 mm) to which 20 μg of *Sma*I-cut pLC21-9 DNA was bound. Just before hybridization, the filter was prewashed in the same hybridization solution and drained. For experiments requiring fingerprint analysis of eluted tRNAs, each filter contained 50 to 500 μg of *Sma*I-cut pLC21-9 DNA, and the cells were labeled with 3 to 20 mCi of $^{32}P_i$. After hybridization for 18 h at 47°C, the hybridization mixtures were chilled on ice, rinsed in cold 6× SSC, and then washed at 30°C in 6× SSC; this was followed by additional washes in 2× SSC for 4 to 12 h with vigorous agitation. The amount of $tRNA_f^{Met}$ recovered (but not the amounts of other tRNAs) depended on the length of washing. The tRNAs were then eluted from the filter for 5 min at 67°C in 3 ml of a solution containing 90% formamide, 20 mM PIPES [piperazine-*N*,*N'*-bis(2-ethanesulfonic acid)] (pH 6.4), 0.5% sodium dodecyl sulfate, 1 mM EDTA, and 200 μg of yeast tRNA; 5 ml of water and 0.7 ml of 20% sodium acetate (pH 5) were then added, and the RNA was ethanol precipitated. Two-dimensional gel electrophoresis and fingerprint analysis were performed as described previously (16), except that the gels were frequently frozen before autoradiography at −70°C. Fingerprint analysis and further sequence analysis required the use of intensifying screens.

Other procedures. Isolation of deletions in lambda (30) and conversions of Tn*9* to IS*1* (4) were done as described previously. Heteroduplex analysis, gel electrophoresis, and fingerprint analysis were done as previously described (25). Plasmid copy number was calculated by determining how much [^{3}H]uracil-labeled 23S rRNA hybridized to 4 μg of total cellular DNA (27).

RESULTS

Polarity in growing cells: expression from lysogenic phages. We developed an assay that is capable of measuring expression of *rrnX* relative to expression of *rrnC* in growing cells lysogenic for lambda specialized transducing phages which carry *rrnX*. This assay makes use of the fact that *rrnX* is a hybrid transcription unit that contains the promoter region of *rrnH* and the

distal portion of *rrnC* (26). Therefore, the most distal gene of both *rrnX* and *rrnC* is the $tRNA^{Trp}$ gene (Fig. 1). Preliminary experiments demonstrated that $tRNA^{Trp}$ and $tRNA^{Trp}$-*Su7* are separable by two-dimensional gel electrophoresis when these tRNAs are produced in UV-irradiated cells after infection with lambda specialized transducing phages (Fig. 2 and 3). RNase T_1 digestion of these tRNAs results in an oligonucleotide from the anticodon region of $tRNA^{Trp}$ that differs in sequence from the anticodon oligonucleotide of $tRNA^{Trp}$-*Su7*. This allows $tRNA^{Trp}$ and $tRNA^{Trp}$-*Su7* to be distinguished by fingerprinting (Fig. 4). The ability to separate these tRNAs and confirm their identity made it possible to assay for expression of *rrnX* relative to expression of *rrnC* in growing cells when the *rrnX* operon contained a $tRNA^{Trp}$-*Su7* gene. Therefore, we constructed lysogens in which a single copy of a $tRNA^{Trp}$ gene (in *rrnC*) and a single copy of a $tRNA^{Trp}$-*Su7* gene (in *rrnX* on a lambda specialized transducing phage) were present per chromosome. Since it was not possible to locate reproducibly and to measure $tRNA^{Trp}$ and $tRNA^{Trp}$-*Su7* after two-dimensional gel electrophoresis of total cellular RNA prepared from these lysogens, $tRNA^{Trp}$ and $tRNA^{Trp}$-*Su7* were first greatly enriched from total RNA by filter hybridization to DNA from pLC21-9, a recombinant plasmid which carries the distal half of *rrnC* (28). The tRNAs were then eluted from the filter and separated by two-dimensional acrylamide gel electrophoresis (Fig. 2 [some portions of the gel are not shown]). In addition to equimolar levels of $tRNA_2^{Glu}$, $tRNA_1^{Asp}$, $tRNA^{Trp}$, and 5S rRNA, which hybridize to genes on pLC21-9 (28), variable levels of $tRNA_f^{Met}$ were also observed (Fig. 2 and 4). $tRNA_f^{Met}$ hybridizes to 23S rRNA sequences (8, 28). In contrast to the single form of $tRNA^{Trp}$ produced in UV-irradiated cells, two forms of $tRNA^{Trp}$ were observed after two-dimensional gel electrophoresis of eluted tRNAs (Fig. 2). These two forms gave rise to identical RNase T_1 fingerprints (Fig. 4) and pancreatic RNase digestion products of RNase T_1 oligonucleotides (data not shown). The two forms of $tRNA^{Trp}$ may be identical to the previously characterized native and denatured forms of $tRNA^{Trp}$ (14). An additional tRNA was present on two-dimensional gels when tRNAs were produced from a lysogen that also expressed $tRNA^{Trp}$-*Su7* from *rrnX* (Fig. 2). This tRNA was firmly identified as $tRNA^{Trp}$-*Su7* by fingerprinting (Fig. 4). Measurements of the radioactivity in each tRNA indicated that $tRNA^{Trp}$-*Su7* was present at a level which was 10 to 20% of the yield of $tRNA^{Trp}$ (data not shown). Unfortunately, many experiments have revealed that accurate measurement of $tRNA^{Trp}$-*Su7* relative to $tRNA^{Trp}$ is not possible due to the diffuse background often present in the gels and due to the proximity of $tRNA^{Trp}$-*Su7* to more abundant tRNAs. The relative yields of $tRNA^{Trp}$ and $tRNA^{Trp}$-*Su7* were not greatly influenced by hybridizing to DNA which contained a $tRNA^{Trp}$-*Su7* gene (data not shown). This difference in yield between $tRNA^{Trp}$ and $tRNA^{Trp}$-*Su7* must have been in part due to a gene dosage difference in replicating chromosomes, because *rrnC* is located near the origin of DNA replication and *rrnX* is located at the lambda attachment site close to the replication terminus. This gene dosage difference was only 1.7-fold, as calculated by the methods of Chandler and Pritchard (6) and Cooper and Helmstetter (7). Therefore, the observed

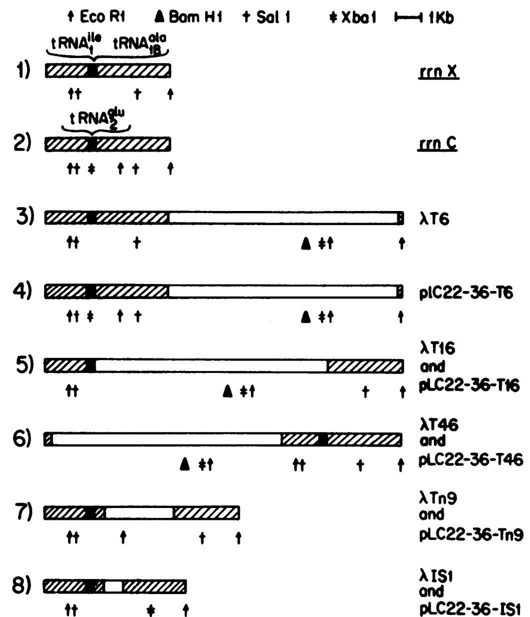

FIG. 1. Structures of the *rrn* operons on the plasmids and phages used in this study. The restriction nuclease sites used to characterize the *rrn* operons containing Tn*9*, IS*1*, and three insertions of Tn*10* (designated T6, T16, T46) are shown. All lambda phages are derivatives of λC117 or λC118 and have been described previously (4, 25), and all plasmids are derivatives of pLC22-36, which carries *rrnC* (27). Only the *rrn* portions are shown; all DNA outside the *rrn* sequences retained the structure of λC117, λC118, or pLC22-36. The *rrn* sequences are indicated by cross-hatched bars, whereas the insertions are indicated by open bars. The heterogeneity in the restriction nuclease sites of *rrnX* and *rrnC* is due to a minor difference in the sequences of the 23S rRNA genes and to the presence of an *Xba*I site in the gene for $tRNA_2^{Glu}$ (29). Both *rrnX* and *rrnC* have $tRNA_1^{Asp}$ and $tRNA^{Trp}$ genes just before the distal end of the *rrn* operon (27, 30). Kb, Kilobase pair.

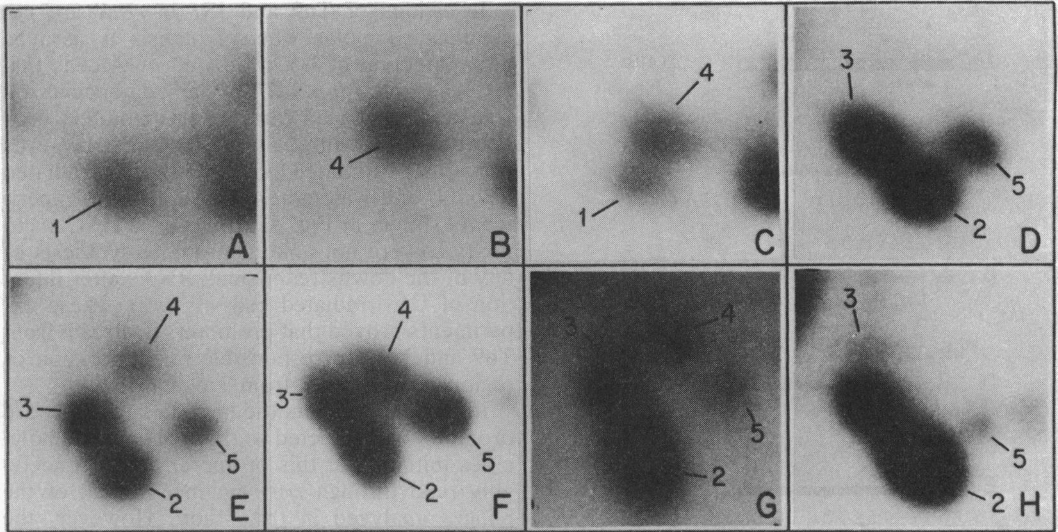

FIG. 2. Separation of $tRNA^{Trp}$ and $tRNA^{Trp}$-*Su7* on two-dimensional gels. Only small portions of the gels are shown. (A) tRNAs produced from λ *ilv5* in UV-irradiated cells. (B) tRNAs produced from λ *ilv5 Su7* in UV-irradiated cells. (C) Mixture of tRNAs produced from λ *ilv5* and λ *ilv5 Su7* in UV-irradiated cells, showing that $tRNA^{Trp}$ and $tRNA^{Trp}$-*Su7* did not comigrate. (D) RNAs synthesized in growing λ *ilv5* lysogen, selected by hybridization to pLC21-9 DNA, eluted, and separated by gel electrophoresis. (E) $tRNA^{Trp}$ species selected by hybridization after synthesis in a growing λ *ilv5 Su7* lysogen. (F) $tRNA^{Trp}$ species synthesized in a growing λ Tn*9* lysogen. (G) $tRNA^{Trp}$ species synthesized in a growing λIS*1* lysogen. (H) $tRNA^{Trp}$ species synthesized in a growing λT46 lysogen. RNA species were identified by fingerprints, some of which are shown in Fig. 4. 1, $tRNA^{Trp}$; 2, $tRNA_A^{Trp}$; 3, $tRNA_B^{Trp}$; 4, $tRNA^{Trp}$-*Su7*; 5, $tRNA_f^{Met}$.

difference in yield must also have been due in part to a difference in strength of the *rrnX* and *rrnC* promoters, due to a difference in the ability of these tRNAs to hybridize to DNA, or possibly due to a more rapid degradation rate of $tRNA^{Trp}$-*Su7* compared with $tRNA^{Trp}$. Although $tRNA^{Trp}$-*Su7* was present in low molar yields relative to other tRNAs, the presence of $tRNA^{Trp}$-*Su7* on autoradiograms was invariably observed at nearly a constant intensity above background when strains expressing $tRNA^{Trp}$-*Su7* were used, but was never observed in strains expressing only $tRNA^{Trp}$. Despite the shortcomings of this assay in its present form, it is valuable because a twofold reduction in expression of $tRNA^{Trp}$-*Su7* in growing cells is visually apparent and the identity of $tRNA^{Trp}$-*Su7* can be confirmed by fingerprinting.

Two-dimensional gel electrophoresis and fingerprinting of tRNAs isolated by hybridization confirmed that phages λC117, λC118, and λ *ilv5 Su7* made $tRNA^{Trp}$-*Su7* in comparable amounts in lysogens (data not shown). λC117 and λC118 are deletion derivatives of λ *ilv5 Su7* which have lost different amounts of DNA upstream from the *rrnX* promoter region (Fig. 1 and 3) (30). These two different deletion phages were used to accomodate the different transposable elements used in this study. The deletions did not affect *rrn* DNA or lambda DNA in a way that should have influenced the results of this study. λTn*9* and λIS*1* are derivatives of λC118 which contain insertions of Tn*9* and IS*1*, respectively, in *rrnX* upstream from the $tRNA^{Trp}$-*Su7* gene (Fig. 1). λTn*9* and λIS*1* produced levels of $tRNA^{Trp}$-*Su7* comparable to the levels of $tRNA^{Trp}$-*Su7* produced from *rrnX* transcription units without insertions (Fig. 2). Therefore, Tn*9* and IS*1* did not strongly affect expression of the $tRNA^{Trp}$-*Su7* gene located downstream from the insertions. λT6, λT16, and λT46 are derivatives of λC117 that contain Tn*10* insertions upstream from $tRNA^{Trp}$-*Su7* (Fig. 1). The Tn*10* insertions in these three phages were previously shown to be incompletely polar in UV-irradiated cells, and genetic methods demonstrated some expression of $tRNA^{Trp}$-*Su7* in growing cells (25). No $tRNA^{Trp}$-*Su7* was detected by two-dimensional gel electrophoresis of tRNAs produced in growing lysogens which contained these phages, regardless of whether tetracycline (12.5 μg/ml) was present in the growth medium (Fig. 2; data not shown). These results indicate that $tRNA^{Trp}$-*Su7* synthesis from genes downstream from Tn*10* in λT6, λT16, and λT46 must be reduced to one-half or less as a result of the insertion (an estimate based on our inability to detect $tRNA^{Trp}$-*Su7* visually).

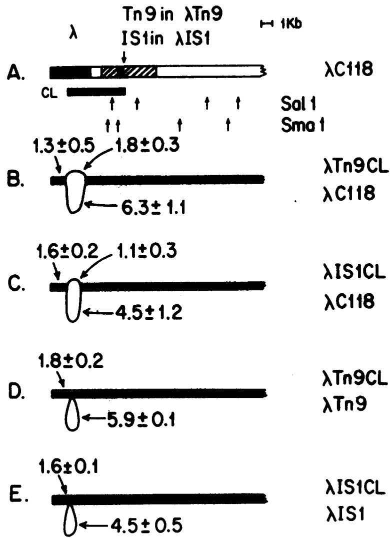

FIG. 3. Structures of λTn*9*CL and λIS*1*CL. (A) Structure of λC118, as previously determined (30), and locations of insertions which gave rise to λTn*9* and λIS*1*. Lambda DNA is indicated by the solid bars, *rrnX* DNA is indicated by the cross-hatched bars, and other bacterial DNA is indicated by the open bars. The location of the deletion (CL) in λTn*9*CL and λIS*1*CL was determined by restriction nuclease digestion and heteroduplex analysis (B through E). λTn*9*CL retained the *Eco*RI site within the coding region of Tn*9* (data not shown). The distances are given in kilobase pairs (Kb). Only the leftmost portion of the phage DNA is shown.

Expression of tRNA genes downstream from insertions requires *rrn* promoters. Deletions in λTn*9*, λT6, λT16, and λT46 were selected by citrate treatment of purified specialized transducing phages. Only 1 *rrn* promoter deletion was found among 50 λTn*9* deletions, and no *rrn* promoter deletions were found among 60 deletions in phages which carried Tn*10* insertions. In the single phage with an *rrn* promoter deletion, an IS*1* insertion was generated at the site of Tn*9*, as previously described (4). These two deletion phages (λTn*9*CL and λIS*1*CL) were further characterized (Fig. 3) and appear to have resulted from simple deletions extending from the terminus of the insertion. These deletions fuse the lambda late rightward promoter to *rrn* sequences. λTn*9* and λIS*1* both produce $tRNA^{Trp}$-*Su7*, but λTn*9*CL and λIS*1*CL do not, as judged by suppression of the *trp*(Am) *his*(Am) mutations in bacterial strain EM4 made lysogenic for these phages (data not shown) and by the inability of T4 phages with amber mutations to plaque on these lysogens (Table 1). It has been shown previously that deletion of the *rrn* promoters abolishes $tRNA^{Trp}$-*Su7* expression in operons without insertions (30).

Insertions of Tn*9* and IS*1* in *rrnX* are not significantly polar when synthesis is assayed after infection of UV-irradiated *uvrA* cells that are lysogenic for λ cI^{ind} by lambda specialized transducing phages carrying the modified *rrnX* operons. Read-through of Tn*9* and IS*1* was previously estimated to be 80% in UV-irradiated cells (4) and was more than 90% in the experiments shown in Fig. 5. However, λTn*9*CL and λIS*1*CL were not able to promote synthesis of any of the downstream small RNAs after infection of UV-irradiated cells (Fig. 5). These experiments proved that promoters upstream from Tn*9* and IS*1* are responsible for expression of regions downstream from Tn*9* and IS*1*.

In our experiments the lambda late rightward promoter was directed so that polymerase molecules initiating at this promoter could conceivably read through *rrn* sequences of all of the phages analyzed in this study. However, the deletion phages analyzed in our previous work (30) and in the present study have the lambda rightward transcription unit directly fused to *rrn* sequences (Fig. 1 and 3), and yet no *rrn* tRNAs were made. This proves that the provision of a lambda repressor in these experiments effectively silenced this lambda promoter and that read-through of *rrn* sequences due to lambda antitermination mechanisms could not be responsible for the results presented here.

Polarity in growing cells: expression from plasmids. The Tn*9* and Tn*10* insertions were introduced into *rrnC* located on a hybrid plasmid (pLC22-36) by means of *recA*-dependent in vivo recombination with the phages that carried the insertions in *rrnX*. As a result, all three Tn*10* insertions and the Tn*9* insertion were introduced into an *rrn* operon which in each case contained the distal region of *rrnC* and probably the *rrnC* promoter, but which in some (and perhaps all) resulting recombinant operons contained some internal sequences derived from *rrnH* (Fig. 1). Plasmids containing an IS*1* insertion were isolated by recombination between the two IS*1* elements of Tn*9*, but these were not sufficiently stable to use in further experiments. Digestion of the resulting plasmids with restriction nucleases showed that the plasmids had insertions at the same location in the *rrn* operons as the original phages did. Several of the plasmids chosen for further study acquired the $tRNA_1^{Ile}$ and $tRNA_{1B}^{Ala}$ genes from *rrnH* and lost an *Eco*RI recognition site in the 23S rRNA gene present in *rrnC* (Fig. 1; see below). All plasmids chosen for study also acquired the $tRNA^{Trp}$-*Su7* gene derived from *rrnX* on the phages. The $tRNA^{Trp}$-*Su7* gene was an unselected marker identified by suppression of a *trp*(Am) mutation in strain EM22 (data not shown) and by the ability of the strains which carried the plasmids to plaque T4

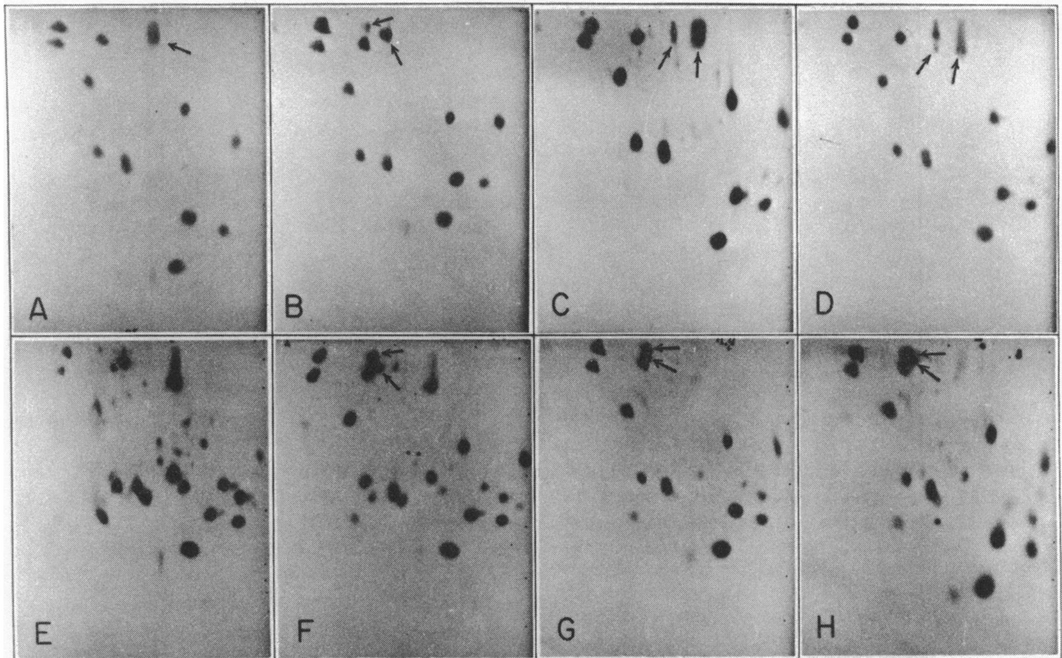

FIG. 4. Identities of tRNA species established by fingerprinting RNase T_1 digests of tRNAs eluted from the acrylamide gels shown in Fig. 2. (A) $tRNA^{Trp}$ synthesized from λ *ilv5* in UV-irradiated cells. (B) $tRNA^{Trp}$-*Su7* synthesized from λ *ilv5 Su7* in UV-irradiated cells. (C) $tRNA_A^{Trp}$ synthesized in growing λ *ilv5* lysogens and selected by hybridization. (D) $tRNA_B^{Trp}$ synthesized in growing λ *ilv5* lysogens and selected by hybridization. (E) $tRNA_f^{Met}$ from growing λ *ilv5* lysogens, selected by hybridization. (F) $tRNA^{Trp}$-*Su7* synthesized in growing λ *ilv5 Su7* lysogens and selected by hybridization. (G) $tRNA^{Trp}$-*Su7* synthesized in growing λTn*9* lysogens and selected by hybridization. (H) $tRNA^{Trp}$-*Su7* synthesized in growing λIS*1* lysogens and selected by hybridization. All oligonucleotides longer than 2 nucleotides were further analyzed by pancreatic RNase digestion. No difference between $tRNA_A^{Trp}$ and $tRNA_B^{Trp}$ was distinguished by these techniques. The oligonucleotides indicated by the arrows all gave rise to pancreatic RNase redigestion products of Up, Cp, Gp, and "AAAACp" and "CmUp", which is consistent with the assignment of these oligonucleotides to the anticodon loop of $tRNA^{Trp}$ (UCmUCCAms^{2}i^{6}AAACGp) and $tRNA^{Trp}$-*Su7* (UCmUCUAms^{2}i^{6}AAACGp). Thin-layer chromatography of complete RNase T_2 digests of both of the anticodon-containing spots of $tRNA^{Trp}$-*Su7* by the method of Nishimura (32) resulted in identification of Up, Cp, Ap, Gp, CmUp, and a product with a mobility shared by i^{6}Ap and ms^{2}i^{6}Ap. Therefore, the difference between the last two RNase T_1 oligonucleotides is not certain, but may be due to differences in the completeness of modification of ms^{2}i^{6}A. Identification of fingerprints was assisted by comparison with previously published fingerprints (8, 30).

phages which carried amber mutations in essential genes (Table 1). Expression of suppressor tRNA was independent of the presence of antibiotics in the cases where the plasmids carried Tn*9* or Tn*10*.

Synthesis of tRNAs from genes on plasmids can be measured because these tRNAs are overproduced, and the polarity of insertions can be determined by measuring overproduction of tRNAs from genes located upstream from the insertion compared with tRNA overproduction from genes located downstream from the insertion. Overproduction of various RNAs is presented relative to synthesis of the same RNAs in cells without a plasmid, and therefore the apparent overproduction of a given RNA (Table 2) is dependent on the rate of chromosomal synthesis of that RNA. RNA was labeled with $^{32}P_i$ in growing cells or in cells that had been previously exposed for 4 h to an inhibitor of protein synthesis to cause plasmid amplification. Inhibitors were used to increase plasmid copy number to obtain better data for RNAs showing low relative amplification or when the plasmid copy number was too low to obtain the desired RNA overproduction. Although inhibitors could conceivably have altered the polarity which we observed, we note that in cases where good RNA overproduction occurred from unamplified plasmids, the polarity observed was similar to the polarity observed after plasmid amplification. After labeling of cells, the RNA was then subjected to two-dimensional gel electrophoresis (Fig. 6). Individual RNAs were excised from

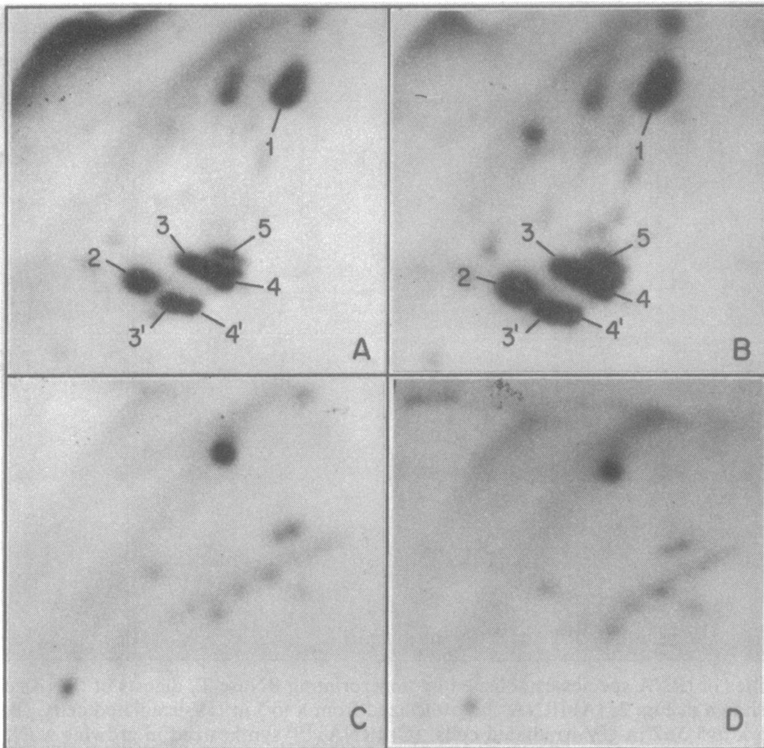

FIG. 5. Small RNAs produced in UV-irradiated cells infected with λTn*9* (A), λIS*1* (B), λTn*9*CL (C), and λIS*1*CL (D) and separated by two-dimensional gel electrophoresis. The results from uninfected cells were similar to the results for λTn9CL and λIS1CL. 1, 5S rRNA; 2, $tRNA^{Trp}$-*Su7*; 3 and 3′, mature and immature forms of $tRNA_1^{Ile}$; 4 and 4′, mature and immature forms of $tRNA_{1B}^{Ala}$; 5, $tRNA_1^{Asp}$. The dark spot in (C) and (D) does not have a fingerprint related to any of the small RNAs encoded by *rrnX* (data not shown).

the gel, and the radioactivity in each RNA species was determined. tRNAs not encoded in *rrn* operons were used to compare tRNA synthesis in cells with plasmids to tRNA synthesis in cells without plasmids, thereby allowing the extent of overproduction of plasmid-encoded RNAs to be determined (Table 2). Unlabeled portions of each culture were used as a source of

TABLE 1. Use of a battery of T4(amber) phages to detect and estimate the levels of $tRNA^{Trp}$-*Su7* in growing cells that are lysogenic for lambda specialized transducing phages or contain plasmids[a]

Phage or plasmid	Plaque formation by T4(amber) phages				
	EL3/a (endolysin)	T4amB22 (gene 43)	T4amB278 (gene 23)	T4amH11 (gene 23)	T4amN122 (gene 42)
Phages					
λ *ilv5*	−	−	−	−	−
λ *ilv5 Su7*	+	+	+	+	+
λTn*9*	+	+	+	+	+
λTn*9*CL	−	−	−	−	−
λIS*1*	+	+	+	+	+
λIS*1*CL	−	−	−	−	−
Plasmids					
pLC22-36	−	−	−	−	−
pLC22-36-Tn*9*	+	+	+	+	+
pLC22-36-Tn*9*-D	+	+	−	−	−
pLC22-36-IS*1*-D	+	+	−	−	−

[a] In the case of phages or plasmids containing Tn*9* and Tn*10*, inclusion of chloramphenicol and tetracycline, respectively, in the medium did not alter the results.

TABLE 2. Accumulation of tRNAs and 5S rRNA from genes in *rrn* operons[a]

Plasmid	Amplification	Plasmid copy no.	Relative accumulation				
			$tRNA_2^{Glu}$	$tRNA_1^{Ile}$ + $tRNA_{1B}^{Ala}$	$tRNA_1^{Asp}$	$tRNA^{Trp}$[b]	5S rRNA
None	None	0	1	1	1	1	1
None	Chloramphenicol	0	1.4	1.3	0.59	2.4	2.0
None	Tetracycline	0	1.5	1.5	0.99	0.74	1.6
pLC22-36	None	11	2.4[c]	0.99	1.2	4.7[c]	1.9
pLC22-36	Chloramphenicol	28	2.8[c]	1.2	10[c]	110[c]	16[c]
pLC22-36-Tn*9*	None	14	1.1	2.3[c]	1.0	1.1	1.3
pLC22-36-Tn*9*	Tetracycline	19	2.0	9.6[c]	3.5[c]	2.7	4.9[c]
pLC22-36-Tn*9*-D	Tetracycline	14	2.1	1.6	0.59	3.4	2.5
pLC22-36-IS*1*-D	Chloramphenicol	32	2.1	1.4	1.2	1.2	3.4
pLC22-36-T6	Chloramphenicol	8	6.5[c]	1.1	0.70	2.0	2.8
pLC22-36-T6-D	Chloramphenicol	13	1.7	1.4	0.86	2.4	2.3
pLC22-36-T16	Chloramphenicol	8	2.2	6.8[c]	1.5	2.8	2.1
pLC22-36-T16-D	Chloramphenicol	9	2.0	1.3	0.75	3.4	2.1
pLC22-36-T46	Chloramphenicol	12	0.81	0.96	0.80	0.91	0.97

[a] tRNA levels were determined by measuring radioactivity in RNA species that were separated by two-dimensional gel electrophoresis (Fig. 6) and are expressed relative to the RNA levels in a growing bacterial strain (strain EM22) that contained no plasmid, which were normalized to 1.0. RNA was labeled for 1 h in growing cells or after 4 h of exposure to tetracycline or chloramphenicol to cause plasmid amplification.

[b] The accumulation of $tRNA^{Trp}$-*Su7* is not included in the values for $tRNA^{Trp}$ accumulation.

[c] Values believed to represent significant amplification.

DNA to determine plasmid copy number by hybridization to purified 23S rRNA, as previously described (27). Without plasmid amplification, only pLC22-36-Tn*9* had a copy number sufficient to result in detectable amplification of plasmid-encoded small RNAs (Fig. 6 and Table 2; data not shown). After plasmid amplification, the copy numbers of pLC22-36-T6, pLC22-36-T16, and pLC22-36-T46 also increased to levels sufficient to enable potential observable overproduction of small RNAs (Fig. 6 and Table 2). With or without plasmid amplification, the Tn*9* insertion in pLC22-36-Tn*9* reduced transcription of downstream regions by 50%, whereas the Tn*10* insertions in pLC22-36-T6, pLC22-36-T16, and pLC22-36-T46 reduced downstream transcription to levels below the level of detection (Fig. 6 and Tables 2 and 3). We estimated that transcription of regions downstream from the Tn*10* insertions was 30% or less of the transcription of regions upstream from the insertions, as we could not detect overproduction of RNAs from genes downstream from these insertions. The read-through of Tn*9* and IS*1* insertions on plasmids was less than the read-through observed in the UV experiments described above. It is possible that the increase in copies of *rrn* operons resulted in depletion of a factor necessary for read-through of these insertions or that UV irradiation of cells increased the read-through of Tn*9* and IS*1*.

The identities of the $tRNA_1^{Asp}$ and $tRNA^{Trp}$-*Su7* molecules that were overproduced from pLC22-36-Tn*9* with and without plasmid amplifi-

TABLE 3. Relative molar yields of individual distal RNAs[a]

Plasmid	Amplification	Molar yield of individual distal RNA/molar yield of individual spacer tRNA		
		5S rRNA	$tRNA_1^{Asp}$	$tRNA^{Trp}$ or $tRNA^{Trp}$-*Su7*
pLC22-36	None	1.6	1.7	0.56
pLC22-36	Chloramphenicol	1.1	1.2	1.1
pLC22-36-Tn*9*	None	0.36	0.48	0.37
pLC22-36-Tn*9*	Tetracycline	0.33	0.40	0.45

[a] We calculated the relative molar yields of individual distal RNAs (5S rRNA, $tRNA_1^{Asp}$, and $tRNA^{Trp}$ or $tRNA^{Trp}$-*Su7*) relative to the molar yields of individual spacer region tRNAs ($tRNA_2^{Glu}$ in the case of pLC22-36 or an average yield of $tRNA_1^{Ile}$ and $tRNA_{1B}^{Ala}$ in the case of pLC22-36-Tn9) in cells containing plasmids (Fig. 6 and Table 1). This table includes data only from experiments in which the increase in all plasmid-encoded tRNAs was large enough for this value to be a useful indication of polarity across the interval between the two clusters of genes for small RNAs.

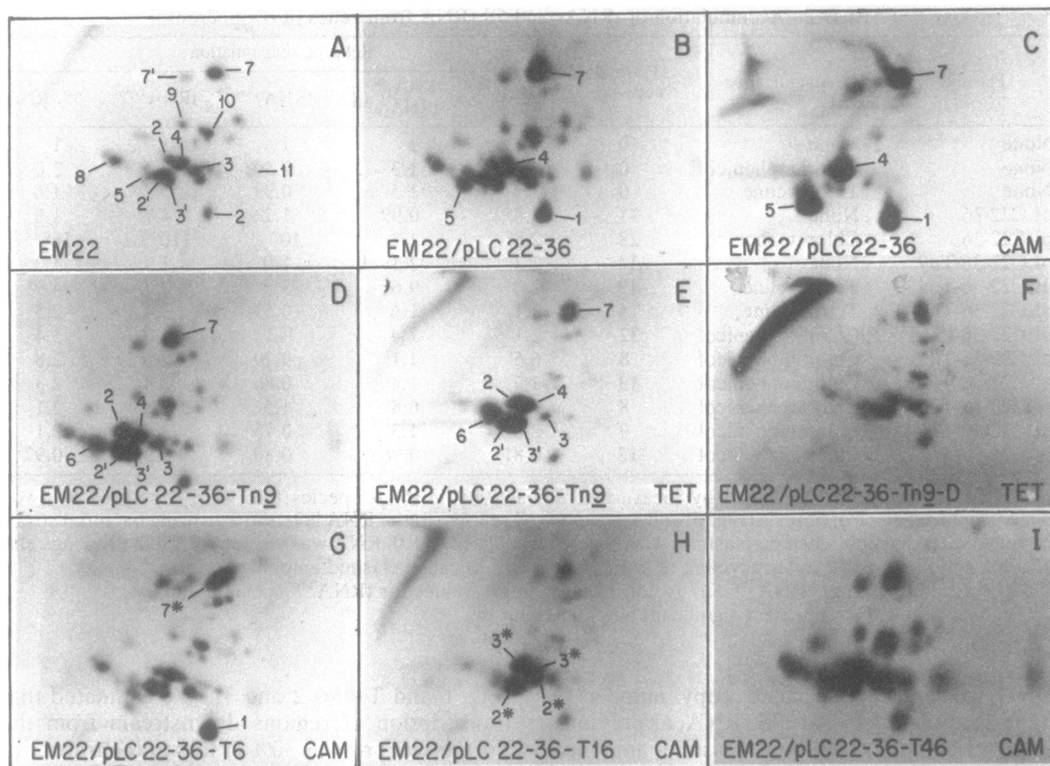

FIG. 6. Autoradiograms of small RNAs separated by two-dimensional gel electrophoresis. tRNAs were produced in cells containing the plasmids indicated. Each plasmid was amplified by treatment for 4 h with chloramphenicol (C and G through I) or tetracycline (E and F) before labeling of RNAs. The tRNAs used to standardize each gel (Table 2) were tRNAs 8 through 11 in the autoradiogram of the tRNAs produced from EM22 (A); some of these have been identified previously (16). 1, $tRNA_2^{Glu}$; 2 and 2′, $tRNA_1^{Ile}$; 3 and 3′, $tRNA_{1B}^{Ala}$; 4, $tRNA_1^{Asp}$; 5, $tRNA^{Trp}$; 6, $tRNA^{Trp}$-*Su7*; 7 and 7′, 5S rRNA. In (B) through (I) only amplified RNAs synthesized from plasmids are indicated. In (G) and (H) RNAs 2*, 3*, and 7* are unusual RNA species which have been observed previously (25) and are produced as a result of particular Tn*10* insertions. CAM, Chloramphenicol; TET, tetracycline.

cation were confirmed by fingerprinting (data not shown). Since a $tRNA^{Trp}$-*Su7* gene was present only on the plasmid, the $tRNA^{Trp}$-*Su7* identified by these fingerprints must have resulted from transcription of the $tRNA^{Trp}$-*Su7* gene downstream from the Tn*9* insertion and could not have come from any other gene.

Deletion of promoters on plasmids. Deletions of the *rrn* promoters on pLC22-36-Tn*9*, pLC22-36-T6, and pLC22-36-T16 were obtained by removing DNA fragments generated by digestion with *Sma*I or *Xma*I (Fig. 7). The deletion plasmids were designated pLC22-36-Tn*9*-D, pLC22-36-T6-D, and pLC22-36-T16-D. An IS*1* insertion was generated at the site of Tn*9* as previously described (4) to give rise to pLC22-36-IS*1*-D. In these plasmids the colicin E1 promoter (38) on the ColE1 vector was fused to the 16S rRNA gene upstream from the Tn*9* or Tn*10* insertions. The colicin E1 promoter is inactive or weakly active except after treatments that cause DNA damage (38).

Small RNAs from plasmids with promoter deletions were not overproduced, as determined by two-dimensional gel electrophoresis of RNAs extracted from cells that contained deletion plasmids (Fig. 6 and Table 2). Therefore, *rrn* promoters were required for most of the expression of $tRNA^{Trp}$-*Su7* from genes downstream from Tn*9* and IS*1*.

The deletion plasmids were tested for suppressor activity by using T4(amber) phages sensitive to the level of suppressor tRNA (Table 1). Our results indicated that pLC22-36-Tn*9*-D and pLC22-36-IS*1*-D had a reduced level of expression of $tRNA^{Trp}$-*Su7* compared with pLC22-36-Tn*9*, λTn*9*, or λIS*1*. The difference in the levels of $tRNA^{Trp}$-*Su7* was not due to a difference in the copy number of the plasmids since pLC22-36-Tn*9*, pLC22-36-Tn*9*-D, and pLC22-36-IS*1*-D

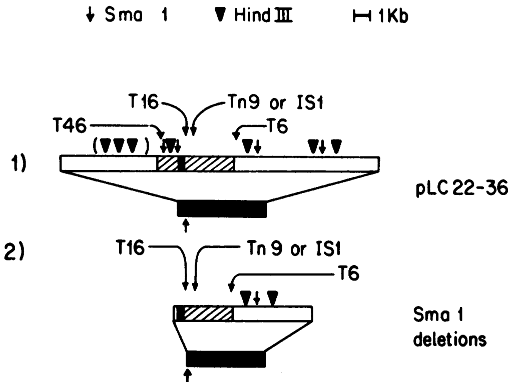

FIG. 7. Structure of pLC22-36, the insertion and deletion plasmids derived from it, and some of the restriction nuclease sites used to generate the deletions and characterize the structures of the deletion plasmids. The sites of Tn*9* or IS*1* insertions and the sites of three Tn*10* insertions (designated T6, T16, T46) in *rrn* operons are indicated (see Fig. 1). Complete digestion of pLC22-36 derivatives by *Sma*I or its isoschizomer, *Xma*I, followed by ligation, resulted in isolation of plasmids with deletions of the *rrn* promoters. Tn*10*, Tn*9*, and IS*1* contain no *Sma*I sites. The three *Hin*dIII sites in parentheses have not been fully mapped. The deletion plasmids were also characterized by digestion with the restriction nucleases shown in Fig. 1 and combinations of these restriction nucleases to verify the presence and identities of the insertions. Kb, Kilobase pair.

have similar copy numbers (14, 5, and 5, respectively), as determined by hybridization of total cellular DNA to 23S rRNA. In the absence of *rrn* promoters, accumulation of tRNA^{Trp}-*Su7* from genes downstream from Tn*9* or IS*1* insertions in *rrn* operons on plasmids appeared to be greater than accumulation of tRNA^{Trp}-*Su7* from similar operons on λTn*9*CL or λIS*1*CL. This may have been due to the higher copy number of plasmids compared with lysogenic lambda phages, resulting in a greater accumulation of tRNA^{Trp}-*Su7* from weak or nonspecific promoters. Unfortunately, when T4(amber) phages were used, the level of expression of tRNA^{Trp}-*Su7* in the absence of *rrn* promoters could not be distinguished from the low level of expression of tRNA^{Trp}-*Su7* from complete *rrn* operons with Tn*10* insertions or from the low level of expression of tRNA^{Trp}-*Su7* from *rrn* operons with both Tn*10* insertions and deletions of *rrn* promoters (data not shown). This fact, coupled with the low sensitivity of our other assays for expression of *rrn* operons on plasmids and our inability to generate the required *rrn* promoter deletion on a lambda specialized transducing phage (due to the proximity of the lambda *cos* site to the *rrn* promoters), made it impossible to determine whether RNA polymerase molecules that initiate at *rrn* promoters are responsible for the low level of transcription which we observed downstream from Tn*10*.

DISCUSSION

rrn operons containing insertions of Tn*9*, Tn*10*, and IS*1* were examined for transcription of *rrn* sequences downstream from the insertions. Strong transcription of portions of *rrn* operons downstream from Tn*9* and IS*1* was demonstrated in growing cells and also in cells that were treated with inhibitors of protein synthesis to cause amplification of *rrn* operons on plasmids. The level of transcription of *rrn* operons downstream from Tn*9* and IS*1* was 40 to 95% of the level of transcription upstream from the insertions. The level of transcription of *rrn* sequences downstream from Tn*10* insertions was less than 30% of the level of upstream transcription, but was not zero. Efficient transcription of portions of *rrn* operons downstream from Tn*9* and IS*1* insertions required *rrn* promoters located upstream from the insertions. Some of these results were obtained by using an assay for *rrn* operon expression that allows comparative measurements of tRNA^{Trp} synthesis and tRNA^{Trp}-*Su7* synthesis when each tRNA is synthesized from a different *rrn* operon during steady-state growth.

The experiments described here could not rigorously exclude the possibility that RNA polymerase molecules that initiate at *rrn* promoters might cause RNA polymerase molecules that initiate within Tn*9* or IS*1* to read out from the insertions into *rrn* sequences. However, it is not clear why this would occur. The possibility that simple read-through of the insertions occurs is supported by the observation that proteins encoded by Tn*10* and Tn*9* in *rrn* operons are overproduced relative to proteins produced from insertions at other locations (data not shown). This suggests that extensive *rrn* promoter-dependent transcription occurs throughout these insertions in *rrn* operons. At present, we favor the possibility of simple read-through of Tn*9*, IS*1*, and, possibly, Tn*10* by RNA polymerase molecules that initiate at *rrn* promoters. The results of the experiments described above cannot exclude the possibility that transcription downstream from Tn*10* results from outwardly directed promoters in Tn*10*.

The mechanisms by which Tn*10*, Tn*9*, and IS*1* cause polarity are not well understood (21). Strongly polar insertions in both orientations have been documented in protein-encoding operons for Tn*10* (22) and IS*1* (13, 15, 19, 20, 24, 35). The polarity of Tn*10* and IS*1* is much greater (3, 19, 22, 23, 36) than the polarity due to nonsense mutations (18, 36). In the *gal* operon, genes downstream from IS*1* are expressed at a

level which is 0.5% or less of the level of expression from uninterrupted operons (3, 36). Polarity due to IS*1* is partially relieved by mutations in the gene for transcription termination factor *rho* (3, 9). de Crombrugghe et al. (10) were not able to identify a *rho*-dependent transcription termination signal in IS*1* in vitro and proposed that these elements might cause polarity by introduction of nonsense mutations in all three reading frames, with transcription termination then resulting because of the requirement for transcriptional-translational coupling. If this is the case, the previously published data examining the polarity of IS*1* suggest that termination of most transcripts must occur within IS*1* itself, or else the probability of premature termination by RNA polymerase molecules that read through would likely have been similar to the probability of termination caused by a premature nonsense codon at the same position. However, as noted above, the polarity caused by premature nonsense codons is much less than the polarity due to IS*1*, and some nonsense codons are essentially nonpolar (18, 39), whereas no nonpolar insertions of Tn*9*, Tn*10*, or IS*1* have been reported except when preparations were examined under the influence of a phage antitermination mechanism or after UV irradiation of cells (17) (see below). Therefore, based on these previous data, it seemed likely that the polarity caused by Tn*9*, Tn*10*, and IS*1* is largely independent of the sequences in which the DNA elements is inserted.

Although Tn*10* insertions in protein-encoding operons have been polar in previously described instances, Kleckner (21) has demonstrated in vitro that Tn*10* contains promoters that read out of Tn*10* into surrounding sequences on both sides of the Tn*10* insertions. We have shown that little or no transcription of regions of *rrn* operons downstream from Tn*9* or IS*1* occurs when the *rrn* promoters are removed. However, the chloramphenicol acetyl transferase gene of Tn*9* and a presumptive protein within IS*1* are transcribed in the same direction as the rRNA in the insertions which we used in this study (data not shown). Therefore, the promoters for these genes must be very weak relative to *rrn* promoters or else must be followed by termination signals within the insertion or within flanking *rrn* sequences. We have demonstrated efficient read-through of Tn*9* and IS*1* insertions in *rrn* operons, and our results are consistent with modification of RNA polymerase at or near *rrn* promoters to make a polymerase resistant to termination or consistent with an absence of termination signals within Tn*9* and IS*1*.

Our results suggest that the properties of transcription within *rrn* operons must be unique or that Tn*10*, Tn*9*, and IS*1* contain outwardly directed promoters or do not contain transcription termination signals or both. If the properties of transcription in *rrn* operons are not unusual, then the Tn*10*, Tn*9*, and IS*1* insertions previously characterized in protein-encoding operons must be polar only because the insertions disrupt protein-encoding sequences, thereby preventing the coupling of transcription to translation and causing polarity by allowing premature termination of transcription at *rho*-dependent transcription termination sites within the operon that are normally masked by ribosomes. This scenario predicts that some nonpolar insertions of Tn*10*, Tn*9*, or IS*1* should result when the insertion is located such that no *rho*-dependent termination sequences intervene between the insertion and the next gene in the operon.

IS*1* is nonpolar, and transcription is also not coupled to translation when RNA polymerase is under the influence of an antitermination system mediated by lambda *N* protein (2, 34). Since IS*1* is nonpolar and transcription is not coupled to translation in *rrn* operons, it is possible that *rrn* operons have a similar antitermination mechanism. The fact that Tn*10* is substantially polar does not rule out the possibility that *rrn* operons have an antitermination mechanism. It has been observed that Tn*1* blocks transcription from phage promoters in one orientation but does not block transcription from phage promoters in the other orientation when it is inserted under the influence of a probable antitermination system of *Salmonella* phage P22 (37). Tn*10* is not polar in the same phage operon (D. Botstein, personal communication). These results are not unexpected, as an RNA polymerase molecule modified by an antitermination protein could be immune to only certain types of termination signals. Morgan has observed unique features of *rrn* termination sequences that could confer special termination properties capable of terminating an RNA polymerase molecule modified to allow read-through of some termination signals (26).

In summary, it is possible that our data are the result of an alteration of RNA polymerase molecules at or near *rrn* promoters that makes the RNA polymerase resistant to termination. An equally likely possibility is that Tn*10*, Tn*9*, and IS*1* allow transcription of downstream regions as a result of internal promoters or as a result of read-through of these elements by "normal" RNA polymerase molecules. Perhaps polarity was observed in previous experiments but not our present experiments because rRNA and *rrn* DNA are somehow unusual in their ability to prevent premature termination in *rrn* sequences due to a loss of all sequences that resemble termination sequences or a loss of all sequences that permit *rho* to act. These structural features

might be the evolutionary consequence of the untranslated nature of rRNA. Further experiments will be necessary to distinguish between these alternatives. Clearly, complete interpretation of experiments in which transposons or insertion sequences are used as transcriptional barriers depends on full characterization of promoters within the DNA element and knowledge of the transcription termination signals within and downstream from the inserted DNA. The required information may not be available yet for any transposon or insertion element.

ACKNOWLEDGMENTS

We thank Marilyn Melithoniotes for technical assistance.

This work was supported by a Public Health Service grant from the National Institutes of Health and by a Junior Faculty Research Career Development Award from the American Cancer Society.

LITERATURE CITED

1. **Adhya, S., and M. Gottesman.** 1978. Control of transcription termination. Annu. Rev. Biochem. 47:967–996.
2. **Adhya, S., M. Gottesman, and B. de Crombrugghe.** 1974. Release of polarity in *Escherichia coli* by gene *N* of phage λ: termination and antitermination of transcription. Proc. Natl. Acad. Sci. U.S.A. 71:2534–2538.
3. **Besemer, J., and M. Herpers.** 1977. Suppression of polarity of insertion mutations within the *gal* operon of *E. coli*. Mol. Gen. Genet. 151:295–304.
4. **Brewster, J. M., and E. A. Morgan.** 1981. Tn*9* and IS*1* inserts in a ribosomal RNA operon of *Escherichia coli* are incompletely polar. J. Bacteriol. 148:897–903.
5. **Calva, E., and R. R. Burgess.** 1980. Characterization of a *rho*-dependent termination site within the *cro* gene of bacteriophage lambda. J. Biol. Chem. 225:11017–11022.
6. **Chandler, M. G., and R. H. Pritchard.** 1975. The effect of gene concentration and relative gene dosage on gene output in *Escherichia coli*. Mol. Gen. Genet. 138:127–141.
7. **Cooper, S., and C. E. Helmstetter.** 1968. Chromosome replication and the division cycle of *Escherichia coli* B/r. J. Mol. Biol. 31:519–540.
8. **Dahlberg, J. E., C. Kintner, and E. Lund.** 1978. Specific binding of $tRNA_f^{met}$ to 23S rRNA of *Escherichia coli*. Proc. Natl. Acad. Sci. U.S.A. 75:1071–1075.
9. **Das, A., D. Court, M. Gottesman, and S. Adhya.** 1977. Polarity of insertion mutations is caused by *rho*-mediated termination of transcription, p. 93–98. *In* A. I. Buckhari, J. A. Shapiro, and S. L. Adhya (ed.), DNA insertion elements, plasmids, and episomes. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
10. **de Crombrugghe, B., S. Adhya, M. Gottesman, and I. Pastan.** 1973. Effect of *rho* on transcription of bacterial operons. Nature (London) New Biol. 241:260–264.
11. **Farnham, P. J., and T. Platt.** 1981. *rho*-dependent termination: dyad symmetry in DNA causes RNA polymerase to pause during transcription *in vitro*. Nucleic Acids Res. 9:563–577.
12. **Fassler, J. S., and I. Tessman.** 1980. Suppression of polarity in the *gal* operon by ultraviolet irradiation. J. Bacteriol. 144:1205–1207.
13. **Fiandt, M. W., W. Szybalski, and M. H. Malamy.** 1972. Polar mutations in *lac*, *gal*, and phage λ consist of a few IS DNA sequences inserted in either orientation. Mol. Gen. Genet. 119:223–231.
14. **Gartland, W., and N. Sueoka.** 1966. Two interconvertable forms of tryptophanyl sRNA in *E. coli*. Proc. Natl. Acad. Sci. U.S.A. 55:948–956.
15. **Hirsch, H. J., P. Starlinger, and P. Bracket.** 1972. Two kinds of insertions in bacterial genes. Mol. Gen. Genet. 119:191–206.
16. **Ikemura, T., and M. Nomura.** 1977. Expression of spacer tRNA genes in ribosomal RNA transcription units carried by hybrid ColE1 plasmids in *E. coli*. Cell 11:779–793.
17. **Jaskunas, S. R., and M. Nomura.** 1977. Mapping ribosome protein genes in *E. coli* by means of insertion mutations, p. 487–496. *In* A. I. Bukhari, J. A. Shapiro, and S. L. Adhya (ed.), DNA insertion elements, plasmids, and episomes. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
18. **Jordan, E., and H. Saedler.** 1967. Polarity of amber mutations and suppressed amber mutations in the galactose operon of *E. coli*. Mol. Gen. Genet. 100:283–295.
19. **Jordan, E., H. Saedler, and P. Starlinger.** 1967. Strong polar mutations in the transferase gene of the *gal* operon of *E. coli*. Mol. Gen. Genet. 100:296–306.
20. **Jordan, E., H. Saedler, and P. Starlinger.** 1968. 0° and strong polar mutations in the *gal* operon are insertions. Mol. Gen. Genet. 102:353–363.
21. **Kleckner, N.** 1981. Transposable elements in prokaryotes. Annu. Rev. Genet. 15:341–404.
22. **Kleckner, N., R. K. Chan, B. K. Tye, and D. Botstein.** 1975. Mutagenesis by insertion of a drug-resistance element carrying an inverted repetition. J. Mol. Biol. 97:561–575.
23. **Malamy, M. H.** 1970. Some properties of insertion mutations in the *lac* operon, p. 359–374. *In* J. R. Beckwith and D. Zipser (ed.), The lactose operon. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
24. **Malamy, M. H., M. Fiandt, and W. Szybalski.** 1972. Electron microscopy of polar insertions in the *lac* operon of *Escherichia coli*. Mol. Gen. Genet. 119:207–222.
25. **Morgan, E. A.** 1980. Insertions of Tn*10* into an *E. coli* ribosomal RNA operon are incompletely polar. Cell 21:257–265.
26. **Morgan, E. A.** 1982. Ribosomal RNA genes in *Escherichia coli*. Cell Nucleus 10:1–29.
27. **Morgan, E. A., T. Ikemura, L. Lindahl, A. M. Fallon, and M. Nomura.** 1978. Some rRNA operons in *E. coli* have tRNA genes at their distal ends. Cell 13:335–344.
28. **Morgan, E. A., T. Ikemura, and M. Nomura.** 1977. Identification of spacer tRNA genes in individual ribosomal RNA transcription units of *Escherichia coli*. Proc. Natl. Acad. Sci. U.S.A. 74:2710–2714.
29. **Morgan, E. A., T. Ikemura, L. E. Post, and M. Nomura.** 1980. tRNA genes in ribosomal RNA operons of *Escherichia coli*, p. 259–266. *In* J. Abelson, P. Schimmel, and D. Soll (ed.), tRNA: biological aspects. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
30. **Morgan, E. A. and M. Nomura.** 1979. Deletion analysis of the expression of rRNA genes and associated tRNA genes carried by a λ specialized transducing bacteriophage. J. Bacteriol. 139:507–516.
31. **Neidhardt, F., P. Bloch, and P. Smith.** 1974. Culture medium for enterobacteria. J. Bacteriol. 119:736–747.
32. **Nishimura, S.** 1979. Chromatographic mobilities of modified nucleotides, p. 487–496. *In* P. R. Schimmel, D. Soll, and J. N. Abelson (ed.), Transfer RNA: structure, properties, and recognition. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
33. **Pollack, T. J., E. S. Tessman, and I. Tessman.** 1979. Suppression of polar effects of nonsense mutations by ultraviolet irradiation. J. Bacteriol. 138:122–154.
34. **Reyes, O., M. Gottesman, and S. Adhya.** 1976. Suppression of polarity of insertion mutations in the *gal* operon and *N* mutation in bacteriophage lambda. J. Bacteriol. 126:1108–1112.
35. **Shapiro, J. A.** 1969. Mutations caused by the insertion of genetic material into the *gal* operon of *E. coli*. J. Mol. Biol. 40:93–105.
36. **Starlinger, P., H. Saedler, B. Rak, E. Tillman, P. Venkov, and L. Waltshewa.** 1973. mRNA distal to polar nonsense mutations in the *gal* operon of *E. coli*. Mol. Gen. Genet.

122:279–286.

37. **Weinstock, G. M., P. D. Riggs, and D. Bostein.** 1980. Genetics of bacteriophage P22. III. The late operon. Virology **106**:82–91.
38. **Yamada, M., Y. Ebina, T. Miyata, T. Nakazawa, and A. Nakazawa.** 1982. Nucleotide sequence of the structural gene for colicin E1 and predicted structure of the protein. Proc. Natl. Acad. Sci. U.S.A. **79**:2827–2831.
39. **Zipser, D., S. Zabell, J. Rothman, T. Gradzicker, M. Wenk, and M. Novilski.** 1980. Fine structure of the gradient of polarity of the *Z* gene of the *lac* operon of *Escherichia coli*. J. Mol. Biol. **49**:251–254.