JOURNAL OF BACTERIOLOGY, Dec. 1981, p. 897-903
0021-9193/81/120897-07$02.00/0

Vol. 148, No. 3

Tn*9* and IS*1* Inserts in a Ribosomal Ribonucleic Acid Operon of *Escherichia coli* Are Incompletely Polar

JAMES M. BREWSTER AND EDWARD A. MORGAN*

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

Received 20 May 1981/Accepted 11 August 1981

Transcription is known to be coupled to translation in many or all bacterial operons which code for proteins. In these operons, nonsense codons which prevent normal translation often result in premature termination of transcription (polarity). However, efficient transcription of ribosomal ribonucleic acid operons (*rrn* operons) occurs, although *rrn* transcripts are not translated. It therefore seemed possible that insertion sequences and transposable elements which are polar in protein-coding operons might not be polar in *rrn* operons. Previously, it has been shown (E. A. Morgan, Cell **21**:257-265, 1980) that Tn*10* is incompletely polar in the *rrnX* operon. Here we show that the transposon Tn*9* and the insertion sequence IS*1* are also incompletely polar in *rrnX*. In normal cells expression of sequences distal to the insertions can be detected by genetic methods. In ultraviolet-irradiated cells expression of distal sequences is about 80% of that observed in uninterrupted *rrnX* operons. These observations provide evidence that ribonucleic acid polymerase molecules beginning at *rrnX* promoters can read through Tn*9* and IS*1* and that, at least in ultraviolet-irradiated cells, read-through is very efficient.

In *Escherichia coli*, mRNA begins to be translated by ribosomes while mRNA elongation is still occurring. Simultaneous translation of the mRNA seems necessary for transcription to be completed. When translation is interrupted by introduction of premature translation termination codons, transcription also frequently ceases prematurely, resulting in a phenomenon known as polarity (1, 5, 9, 11). This premature termination of transcription within protein coding regions occurs at sites which by chance resemble *Rho*-dependent transcription termination signals and which require *Rho*. These premature termination signals are apparently unmasked in the absence of ribosomes (1, 4). The coupling of transcription and translation may therefore have evolved to reduce disadvantageous premature termination of transcription while allowing correct termination of transcription at termination sites at the end of mRNA coding regions. Transcriptional-translational coupling may also have evolved to allow attenuation mechanisms to function in regulation of bacterial operons (22).

Despite the fact that transcription is coupled to translation in many operons, *rrn* operons are transcribed without detectable polarity even though they are not translated (10, 16). It is possible that some specific mechanism or property of *rrn* operons allows transcription to proceed uncoupled from translation. To test this hypothesis, inserts of the transposable element Tn*10* were isolated in the *rrnX* operon and tested for polarity (16). Tn*10* is extremely polar when inserted in bacterial operons which code for proteins (14, 15), but is incompletely polar in two sites in *rrnX* and almost completely nonpolar at one site (16). To establish the generality of this phenomenon and to show that transcripts of downstream portions are unlikely to arise from promotors within the inserts, we here examine the effects of a Tn*9* insert and an IS*1* insert in *rrnX*. The results of genetic experiments indicate these insertions are incompletely polar. Direct determination of the extent of polarity in UV-irradiated cells shows that these inserts reduce transcription of downstream portions of *rrnX* by only 20%. These findings provide a groundwork for the use of transposons and insertion sequences for further study of unique properties or mechanisms governing transcription of *rrn* operons.

MATERIALS AND METHODS

Bacterial strains and phages. P1 *cam* (obtained from M. M. Gottesman), λ *c*118, and λ *c*I857 *S*7 1*xis*-6 *b*515 *b*519 were lysogenized in EM4 [*ilv-1, his-29* (Am), *pro-2, tsx-2, trpA9605*(Am), *recA, trpR, araE*]. Phages were temperature induced in this strain, suspended in 1/10 volume of 10 mM Tris (pH 7.4)-10 mM $MgSO_4$-100 mM NaCl, and released from the cells by the addition of $CHCl_3$. The phages were then read-

sorbed to EM2 (λ *c*-I857 *S7*). EM2 is the *recA*$^+$ parent of EM4. For these mass adsorption experiments cells were grown in Luria broth plus 0.2% maltose, resuspended in 0.25 volume of AB minimal salts plus 10 mM $MgSO_4$, and shaken at 30°C for 30 min before the addition of phage. After adsorption of phage for 30 min, the cells were diluted to their original volume in Luria broth. After 60 min of growth, chloramphenicol was added to 20 μg/ml. The cells were grown into stationary phase, collected by centrifugation, and diluted into 20 volumes of AB minimal salts medium containing glucose, histidine, proline, tryptophan, and chloramphenicol, and then grown to stationary phase. After another dilution, phages were temperature induced, and the cycle of adsorption and selection was repeated. Ilv$^+$ chloramphenicol-resistant single colonies were then isolated and screened for temperature inducible phage (transducing *ilv*$^+$ genes and chloramphenicol resistance) by performing spot tests of phage on a lawn of EM2(λ *c*-I857 *S7*) spread on glucose minimal medium plates containing proline, tryptophan, and histidine. To test for chloramphenicol resistance, growing lysogens were patch tested on similar plates with chloramphenicol. Independently isolated phages were purified from these lysogens by use of CsCl gradients and screened for inserts by restriction nuclease digestion of phage DNA using *Sal*I and *Eco*RI. To obtain a single IS1 insert at the site of previous Tn*9* insertion, EM2(λ *c*I857 *S7*) lysogens containing Tn*9* phages were grown to mid-log phase in glucose minimal media with proline, tryptophan, and histidine, followed by the addition of 20 μg of chloramphenicol per ml and 15 min later by the addition of 200 μg of ampicillin per ml. After 4 h the antibiotics were removed, and the survivors were grown to stationary phase in fresh medium. The cycle of chloramphenicol-ampicillin selection was repeated twice more, and survivors were screened for temperature-inducible phages which transduced *ilv*$^+$ genes but not chloramphenicol resistance. All genetic and physical properties of the phages described in this paper were determined by spot tests on EM4(λ *c*I857 *S7*) or with lysogens constructed in this strain at minimal multiplicity of infection or by using phages purified from lysogens constructed in this strain.

Other procedures. The filter hybridization described in Table 2 was done for 16 h at 42°C in 2 ml of 50% deionized formamide–0.15 M NaCl–0.015 M sodium citrate–0.02% each bovine serum albumin, Ficoll type 400, and polyvinyl pyrrolidone (360,000)–20 mM sodium phosphate (pH 6.5–125 μg of denatured calf thymus DNA per ml–10% dextran sulfate (500,000). RNA was labeled with 20 μCi of [^{3}H]uracil per ml for 45 min starting 15 min after phage infection, as previously described (16). After phenol extraction, nucleic acid corresponding to 0.1 ml of UV-irradiated cells or 0.04 ml of unirradiated cells labeled under the same conditions (9,000 to 25,000 cpm in all cases, as phage infection of UV-irradiated cells increases incorporation of [^{3}H]uracil into trichloroacetic acid-precipitable material by threefold or less) was hybridized under stringent conditions to denatured DNA immobilized on nitrocellulose filters. Each hybridization reaction contained RNA from each cell type, duplicate blank filters, and duplicate filters with all types of DNA and was done twice. The average counts per minute per filter is presented. Backgrounds of 15 to 25 cpm for filters without DNA are subtracted. The amount of DNA per filter was λ*S7*, 6 μg; pLC23-30, 8 μg; fragment A, 0.35 μg; fragment B, 0.42 μg; and fragment C, 1.3 μg. Reduction of RNA input by half reduced the counts hybridized by half (data not shown). These data indicate that this procedure measures levels of RNA accumulation without strong bias for precursor-specific sequences, despite the large pool of unlabeled ribosomal RNA in UV-irradiated cells. All other procedures are similar to those reported previously (16).

RESULTS

rrnX is a hybrid *rrn* operon isolated on λ d*ilv*$_5$ (18). This operon contains genes for all three rRNA species and four tRNA species, in the order promotor-promotor-16S-tRNA$_1^{Ile}$-RNA$_{1B}^{Ala}$-23S-5S-tRNA$_1^{Asp}$-tRNA^{Trp}-terminator (reviewed in reference 16). The tandem promotors for this operon and the tRNA$_1^{Ile}$ and tRNA$_{1B}^{Ala}$ genes probably come from the "group I" *rrn* operon of Nomura and co-workers (18), and the distal tRNA genes probably come from *rrnC*. *rrnX* was probably formed by a reciprocal recombination event within the 23S rRNA genes of these two *rrn* operons during the isolation of λ d*ilv*$_5$ (18). Previous experiments (16, 17) indicate that *rrnX* is functionally similar to *rrn* operons which occur naturally in *E. coli*. The most distal gene of *rrnX* codes for tRNA^{Trp}. This gene has been altered to give rise to an amber suppressor tRNA gene on the phage λ *ilv*$_5$ Su7, which therefore provides a genetic system for study of *rrnX* expression (17). All phages used in this study which contain *rrnX* are derivatives of λ *ilv*$_5$ Su7.

A Tn*9* insertion into *rrnX* was isolated. To accomplish this, a lysogen was constructed which carried λ*c*118, a derivative of λ *ilv*$_5$ Su7 which has a deletion of non-*rrnX* DNA (17). This strain was then lysogenized with Pl *cam*, which carries Tn*9* (21). In the resulting strain Tn*9* transpositions occurred, some of which resulted in Tn*9* inserts in λ *c*118. λ *c*118 was then temperature induced from this strain and Ilv$^+$, chloramphenicol-resistant lysogens selected after adsorption to Ilv$^-$, chloramphenicol-sensitive bacteria. These lysogens were then screened genetically to identify those capable of producing temperature-inducible high-frequency specialized transducing phage which carried *ilv*$^+$ genes and a chloramphenicol resistance determinant. Phages were purified from Ilv$^+$, chloramphenicol-resistant lysogens, and phage DNA was screened for inserts in *rrnX* by use of the restriction nucleases *Eco*RI and *Sal*I. During analysis of about 20 phages, one phage (λ *rrnX*-1::Tn*9*) was found to contain an insert in *rrnX*. This phage is referred to in this paper as λ Tn*9*.

This insert was further characterized (Fig. 1 and 2) by heteroduplex analysis and restriction nuclease mapping.

An IS1 insert in *rrnX* was then obtained using λ Tn9 as starting material. Since Tn9 has direct repeats of IS1 at its ends (21), reciprocal recombination between the IS1 elements would leave an IS1 insert in *rrnX* with loss of one IS1 sequence and the chloramphenicol resistance determinant, as previously shown to occur in P1 *cam* (21). This procedure was used to obtain an IS1 insertion because there is no direct selection for the presence of insertion sequences in *rrnX*, and because IS1 inserts obtained in this manner should exactly mimic IS1 inserts obtained by transposition of single IS1 elements. To isolate the desired recombinant, λ Tn9 was lysogenized in an Ilv⁻, recombination proficient strain, and chloramphenicol sensitive, Ilv⁺ bacteria isolated after ampicillin enrichment of cells incapable of growth in the presence of chloramphenicol. The lysogens were then screened for those capable of production of temperature-inducible transducing phage carrying *ilv*⁺ genes but not chloramphenicol resistance. One of these phages (λ *rrnX*-1::IS1) was further analyzed by heteroduplex analysis and restriction nuclease mapping (Fig. 1 and 2) and will be referred to here as λ IS1.

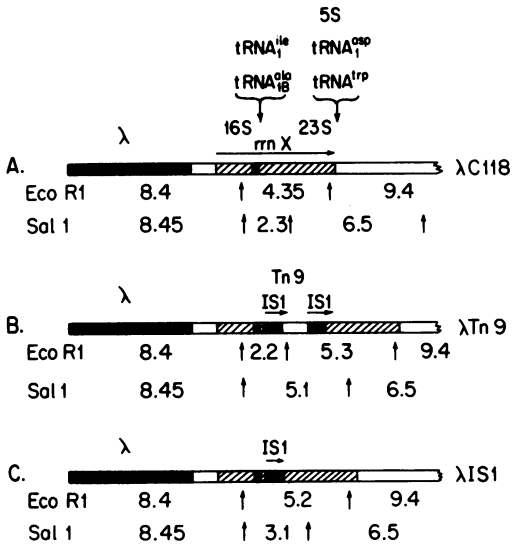

FIG. 1. *Restriction nuclease maps of phages used in this study. Only the leftmost portions of the phages are shown. The structure of λ c118 has been previously determined (17). The structures of λ Tn9 and λ IS1 were determined by heteroduplex analysis (Fig. 2) and by agarose gel electrophoresis of phage DNA digested with SalI and EcoRI alone and in combination. Lambda DNA is indicated in black, rrnX DNA is cross-hatched, and non-rrnX bacterial DNA is white. The IS1 portion of inserts is indicated in black, and the arrow over IS1 indicates an arbitrary orientation. The direction of transcription within Tn9 is believed to be from left to right (unpublished data, see text). Distances are given in kilobase pairs of DNA.*

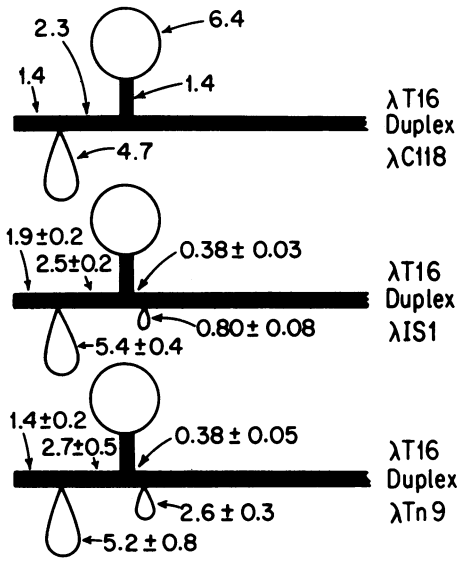

FIG. 2. *Summary of heteroduplex results which determine the structure of phages used in this study. Measurements of the indicated segments of heteroduplex molecules are given in kilobase pairs of DNA. Only the leftmost portions of the heteroduplex structures are presented. A summary of the structure of the phages used in this analysis is given in Fig. 1 and in references 16 and 17. λ T16 is a derivative of λ ilv5 Su7 and carries a Tn10 insertion between the 16S and 23S rRNA genes, probably just downstream of the $tRNA_1^{Ile}$ and $tRNA_{1B}^{Ala}$ genes (16).*

Restriction enzyme mapping (Fig. 1) of λ Tn9 and λ IS1 confirmed inserts of about 2,800 and 850 base pairs, respectively, in *rrnX*. λ Tn9 also contained an *Eco*RI recognition site within the insert, as expected (2). These data and heteroduplex analysis of λ Tn9 and λ IS1 (Fig. 2) indicate that Tn9 in λ Tn9 and IS1 in λ IS1 were of the expected sizes and are simple insertions of these DNA elements at the same site in *rrnX*, as expected from the method of isolation of the insertion phages. Both Tn9 and IS1 in *rrnX* probably have a nine-base-pair duplication of *rrnX* sequences at the site of insertion (13, 19). Both inserts are located 380 bases downstream from the Tn10 insert in *rrnX* isolated previously on λ T16 (see legend to Fig. 2). The inserts are therefore located about 200 to 350 base pairs downstream from the promotor proximal end of the 23S rRNA gene. (Variation observed in the 16 to 23S intergenic region in heteroduplexes prevented more precise determination of the

location of Tn*10* in λ T16 [16].) This location is also supported by preliminary experiments (not shown) which demonstrate that these inserts prevent accumulation of 23S molecules after λ Tn*9* or λ IS*1* infect UV-irradiated cells, although transcription of the 23S rRNA gene distal to the inserts is not greatly reduced (see below).

During isolation of these phages no selective pressure had been applied for expression of Su7-tRNA^{Trp}. Since expression of the Su7-tRNA^{Trp} gene requires *rrnX* promotors (17), λ IS*1* or λ Tn*9* would not produce Su7-tRNA^{Trp} if Tn*9* or IS*1* were completely polar in *rrnX*. To test the polarity of Tn*9* or IS*1* in *rrnX*, *recA* mutant bacteria which are *his*(Am) *trp*(Am) were lysogenized with λ *c*118, λ Tn*9*, and λ IS*1*. Lysogens which contain any one of these three phages grow equally well in the absence of histidine and tryptophan, whereas bacteria lysogenic with λ d*ilv*$_5$ (lacking Su7-tRNA^{Trp}) do not grow at all in the same medium. When lysogens were made at a minimal multiplicity of infection using an endpoint dilution procedure, suppressor activity always cotransduced as an unselected marker with *ilv*$^+$ genes. In the case of λ Tn*9*, *ilv*$^+$ or Su7 always cotransduced as unselected markers with chloramphenicol resistance. These results prove that expression of Su7-tRNA^{Trp} comes from *rrnX* operons which contain IS*1* or Tn*9* inserts, and not from unaltered *rrnX* operons present on other phages lysogenized in the same cell. Another phage isolated during the course of this study (λ Tn*9*-4.55) contains a Tn*9* insert in the 4.55-kilobase *Eco*RI fragment of λ *c*118 as determined by restriction nuclease mapping and heteroduplex analysis (data not shown). The 4.55-kilobase *Eco*RI fragment contains no *rrnX* sequences (17). Without histidine and tryptophan in the medium, *his*(Am) *trp*(Am) bacteria lysogenic for (λ Tn*9*-4.55) grow as well as λ Tn*9* lysogens in either the presence or absence of 20 μg of chloramphenicol per ml. These experiments show that synthesis of Su7-tRNA^{Trp} from λ Tn*9* does not strongly depend on possible chloramphenicol-induced changes in expression of Tn*9* genes.

To quantitatively measure read-through of the inserts in *rrnX*, UV-irradiated λ *papa* lysogens were infected with λ *c*118, λ Tn*9*, and λ IS*1*, followed by the addition of $^{32}P_i$. Under these conditions, the only labeled RNA is synthesized from promotors on the phage which are not repressed by lambda repressor (17) (see Table 2). The resulting tRNA's were separated by two-dimensional gel electrophoresis (Fig. 3), and the tRNA's were excised from the gel, and the radioactivity in each tRNA species was determined (Table 1). The results indicate that IS*1* or Tn*9* inserts in *rrnX* decrease transcription of

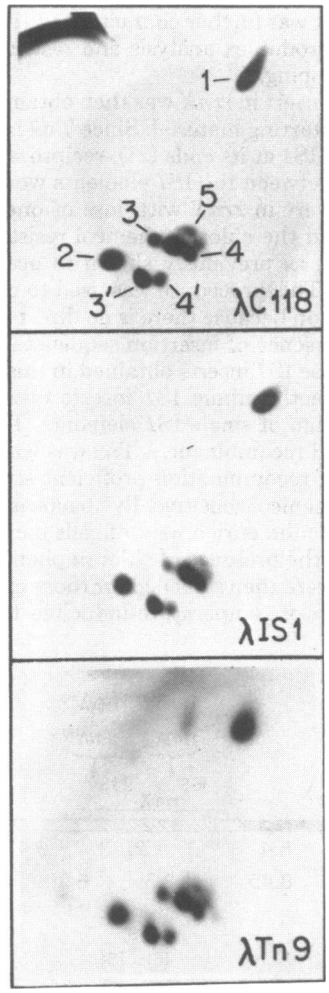

FIG. 3. *Two-dimensional gel electrophoresis of small RNAs synthesized in UV-irradiated E. coli infected with the indicated purified specialized transducing phages. Spots: 1, 5S rRNA; 2, tRNA^{Trp}, 3, tRNA$_1^{Ile}$ (m); 3, tRNA$_1^{Ile}$ (imm); 4, tRNA$_{1B}^{Ala}$ (m); 4, tRNA$_{1B}^{Ala}$ (imm); 5, tRNA$_1^{Asp}$. The relative molar yields of these RNAs are presented in Table 1. RNAs were identified by position and comparison to previous results (16, 17). m and imm refer to mature and immature forms of tRNA which differ in their content of modified bases (10, unpublished data).*

5S rRNA and downstream tRNA's by only 20% when compared with transcription of upstream tRNA's. To quantitatively measure other portions of *rrnX* transcripts, total RNA was extracted from UV-irradiated cells labeled with [^{3}H]uracil after phage infection. The RNA was then hybridized to denatured restriction nuclease fragments which contain various portions of an *rrn* operon (Fig. 4, Table 2) under conditions where RNA did not saturate the DNA.

TABLE 1. *Relative yield of counts per minute in small RNA species synthesized from lambda specialized transducing phages and then separated as described in the legend to Fig. 3*

Phage	cpm of RNA species[a]				
	$tRNA_1^{Ile}$ (m + imm)[b]	$tRNA_{1B}^{Ala}$ (m + imm)[b]	$tRNA_1^{Asp}$	$tRNA^{Trp}$	5S rRNA
λ c118	1	0.75	0.67	0.94	1.81
λ IS1	1	0.91	0.64	0.78	1.25
λ Tn9	1	0.87	0.63	0.80	1.39

[a] For each phage the counts per minute present in $tRNA_1^{Ile}$ are normalized to 1, and the counts per minute consist of other RNA species are presented relative to this value. Each tRNA and 5S rRNA species contained over 10,000 cpm. No correction has been made for differences in size of RNA species.

[b] Mature and immature forms of tRNA.

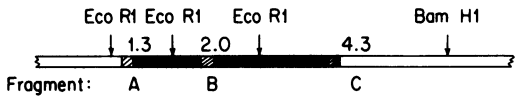

FIG. 4. *Source of restriction nuclease fragments used for filter hybridization in Table 2. The fragments are derived from λ metA20 (6), which carries rrnE. rRNA coding regions are drawn in black, and precursor specific sequences are lined. Surrounding bacterial DNA is white and is nonhomologous to λ c118. Only a portion of λ metA20 DNA is shown. The sizes of the fragments are given in kilobase pairs of DNA.*

TABLE 2. *Measurement of Tn9- or IS1-induced polarity in rrnX by hybridization of RNA produced in UV-irradiated cells after phage infection at multiplicities of infection of 5 to 10[a]*

Source of RNA	cpm of RNA hybridized to DNA fragments:				
	λ S7	pLC23-30	A	B	C
UV, no phage	4	1	15	15	19
UV, λ S7	14	0	15	15	22
UV, λ c118	18	295	86 (1.0)	161 (1.0)	181 (1.0)
UV, λ IS1	25	183	72 (1.3)	162 (1.6)	96 (0.9)
UV, λ Tn9	11	130	63 (1.7)	70 (1.0)	56 (0.7)
No UV, no phage	0	194	65 (1.2)	127 (1.2)	129 (1.1)

[a] pLC23-30 is a recombinant DNA plasmid which contains a complete ribosomal RNA operon but no other DNA sequences homologous to λ c118 (16). Fragments A, B, and C contain portions of a ribosomal RNA operon and were produced by preparative restriction nuclease digestion and agarose gel electrophoresis of DNA from λ metA20 (Fig. 4). Hybridization of RNA to these fragments allows measurement of transcription of different portions of *rrnX*. For RNA synthesized in cells infected by λ c118, the (counts per minute of λ c118 RNA hybridized per fragment/counts per minute of λ c118 RNA hybridized to pLC23-30) is normalized to 1.0. For all RNA produced in other cells, the (counts per minute hybridized per fragment/counts per minute hybridized to pLC23-30)/(counts per minute of λ c118 RNA hybridized per fragment/counts per minute of λ c118 RNA hybridized to pLC23-30) is presented in parenthesis. Therefore, if Tn9 or IS1 inserts completely prevented accumulation of downstream *rrnX* transcript the values in parenthesis would be about 2.5 for fragment A, and 1 for fragment B, and zero for fragment C.

The results (Table 2) indicate that accumulation of RNA synthesized from portions of *rrnX* downstream of the insertions is reduced only about 20% as compared to transcription of upstream portions. In these experiments it is likely that the observed transcription originates at *rrnX* promotors, as no *rrnX* transcripts are detectable by similar methods when most of *rrnX* is present but the *rrnX* promotors are absent (17).

DISCUSSION

Transcription is coupled to translation in bacterial operons which code for proteins. In these operons nonsense mutations which cause premature termination of translation also often cause premature termination of transcription (manifested by polarity). Premature nonsense mutations cause polarity by unmasking sites which resemble *rho*-dependent transcription termination sites that are normally masked by ribosomes (1, 4). However, because *rrn* transcripts are not translated the coupling of transcription to translation must somehow be circumvented in these operons. One possibility is that *rrn* operons have an antitermination mechanism similar to that mediated in lambda by interaction of *N* protein and *nut* sites (1). Under the influence of this antitermination system, transcription is apparently uncoupled from translation, and RNA polymerase molecules initiating at the lambda promotors p_R or p_L can read through transcription termination signals and insertion sequences (1).

Nonsense mutations cause polarity only when followed by a premature transcription termination signal normally masked by ribosomes, and therefore not all premature nonsense mutations cause polarity (1). However, no nonpolar inserts of Tn*10*, Tn*9*, or IS*1* have been reported. Therefore, it is likely that the transcriptional barriers provided by these insertions rely only on sequences internal to these DNA elements. Therefore, we have inserted these elements in *rrnX* to determine whether RNA polymerase molecules transcribing *rrnX* can read through DNA sequences which are transcriptional barriers in other operons. Read-through would provide preliminary evidence for an antitermination mechanism or property in *rrnX*. However, any mechanism allowing read-through in *rrnX* need not necessarily resemble the antitermination mechanism used by lambda.

Three inserts of Tn*10* into *rrnX* have been

previously shown to be incompletely polar (16). The degree of polarity was dependent on the site of insertion. A Tn*10* insert in the 16S rRNA gene did not detectably reduce synthesis of tRNA's encoded in *rrnX* downstream of the insert when analyzed in UV-irradiated cells whereas two inserts of Tn*10* in or near sequences involved in *rrn* transcript processing reduced accumulation of mature tRNA's encoded downstream in *rrnX* by 90% in the case of one insert and greater than 90% in the case of the other insert (16). Measurements by means of filter hybridization of total *rrnX* transcript for all three Tn*10* insertions into *rrnX* showed that all three Tn*10* insertions did not substantially reduce accumulation of downstream *rrnX* transcript. Because these experiments rely on a single transposon it is possible that expression of *rrnX* downstream of Tn*10* could result from promotors within Tn*10* or be the result of some unique property of Tn*10*. It is, therefore, necessary to show that another transposon is also not polar in *rrnX* and to show that an insertion element which contains no known internal promotors is also not polar in *rrnX*. In this paper we show that Tn*9* and IS*1* inserts are also incompletely polar in growing cells. Quantitative measurements of polarity by using UV-irradiated cells show that Tn*9* and IS*1* reduce transcription of downstream portions of *rrnX* by only 20%. These observations, therefore, demonstrate that inserts of Tn*9*, Tn*10*, and IS*1*, which are all highly polar in operons which code for proteins (8, 13–15), are not very polar when inserted into *rrnX* structural genes. Because both Tn*9* and Tn*10* can be inserted into *rrnX* with limited polarity, and because IS*1* is also nonpolar in *rrnX* although it contains no identifiable promotors (19), it is very unlikely that transcripts of downstream genes originate from promotors within these inserts or result from a rare event creating a promotor during insertion. In preliminary experiments (data not shown) we have also demonstrated that three different Tn*10* insertions in *rrnX* (16) and the Tn*9* insertion in *rrnX* isolated here, produce greatly elevated levels of transposon-encoded proteins in UV-irradiated cells when compared with transposons inserted in other locations in lambda DNA. Therefore, at least in UV-irradiated cells, it seems certain that RNA polymerase molecules which initiate at *rrnX* promotors are capable of efficiently reading into and through Tn*10*, Tn*9*, and IS*1*.

From the genetic experiments described above it is clear that in growing cells some transcription of downstream portions of *rrnX* occurs in *rrnX* operons interrupted by Tn*9*, Tn*10*, and IS*1*. It is also clear that these elements can be substantially nonpolar in UV-irradiated cells. However, it is not clear whether our quantitative measurements of polarity in UV-irradiated cells accurately reflect polarity in growing cells. Using very similar experimental protocols, workers in other laboratories have used UV-irradiated cells to measure polar effects on protein synthesis caused by insertion sequences (12) and nonsense mutations (3). However, it has been shown, by use of different procedures, that UV irradiation can partially suppress polarity caused by some amber mutations (7, 20). These latter studies used a UV-sensitive (Hcr$^-$) derivative of *E. coli* C which was not further described (20) and an *E. coli* K-12 strain which apparently has normal UV sensitivity (7). Our studies (and those mentioned above which use protocols similar to ours) use SA159(λ), an *E. coli* K-12 *uvrA* strain which is very UV sensitive due to a deficiency in excision of photoadducts. The environment in the cell may be substantially different when these different strains are UV irradiated to reduce transcription before phage infection. We also note that transposons and insertion sequences may pose a much stronger barrier to transcription than simple nonsense mutations. However, quantitative measurements of polarity in *rrnX* in growing cells will be necessary to completely resolve the effects of prior UV irradiation of cells on subsequent expression of *rrnX* transcripts from infecting phage. This should be possible with assays for *rrnX* expression presently being developed in this laboratory.

The experiments described in this paper and our previous experiments (16) show that Tn*9*, Tn*10*, and IS*1* are at least incompletely polar in normal cells and can be virtually nonpolar in UV-irradiated cells. This property of *rrnX* may distinguish *rrnX* from operons which code for proteins. The mechanisms or properties of *rrnX* operons which are responsible for lack of Tn*9*-, Tn*10*-, or IS*1*-induced polarity are not known, but there are several conceivable strategies of gene organization which could account for this property. An antitermination mechanism or property could have important implications for *rrnX* expression (discussed in reference 16). Future experiments made possible by the insertions isolated in this work may be capable of further defining this mechanism or property.

ACKNOWLEDGMENTS

We thank Marilyn Melithoniotes for heteroduplex analysis and superb technical assistance and M. M. Gottesman for P1 *cam*.

Work in this laboratory is supported by Public Health Service grants from the National Institutes of Health and a Junior Faculty Research Career Development Award to E. M. 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. **Alton, N. K., and D. Vapnek.** 1979. Nucleotide sequence analysis of the chloramphenicol resistance transposon *Tn9*. Nature (London) **282**:864-869.
3. **Cabezon, T., G. Decluve, M. Faelen, L. Desmet, and A. Bollen.** 1980. Polarity of amber mutations in ribosomal protein genes of *Escherichia coli*. J. Bacteriol. **141**:41-51.
4. **Calva, E., and R. R. Burgess.** 1980. Characterization of a rho-dependent termination site within the *cro* gene of bacteriophage lambda. J. Biol. Chem. **255**:11017-11022.
5. **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. Bukhari, J. A. Shapiro, and S. L. Adhya (ed.), DNA insertion elements, plasmids, and episomes. Cold Spring Harbor Laboratory, Cold Spring Harbor, N. Y.
6. **de Boer, H. A., S. F. Gilbert, and M. Nomura.** 1979. DNA sequences of promotor regions for rRNA operons *rrnE* and *rrnA* in *E. coli*. Cell **17**:201-209.
7. **Fassler, J. S., and I. Tessman.** 1980. Suppression of polarity in the *gal* operon by ultraviolet irradiation. J. Bacteriol. **144**:1205-1207.
8. **Fiandt, M., W. Szybalski, and M. H. Malamy.** 1972. Polar mutations in *lac, gal,* and phage λ consist of a few IS-DNA sequences inserted with either orientation. Mol. Gen. Genet. **119**:223-231.
9. **Franklin, N. C., and S. E. Luria.** 1961. Transduction by bacteriophage P1 and the properties of the *lac* genetic region in *E. coli* and *S. dysenteriae*. Virology **15**:299-311.
10. **Ikemura, T., and M. Nomura.** 1977. Expression of spacer tRNA genes in ribosomal RNA transcription units carried by hybrid Col E1 plasmids in *E. coli*. Cell **11**:779-793.
11. **Jacob, R., and J. Monod.** 1962. On the regulation of gene activity. Cold Spring Harbor Symp. Quant. Biol. **26**:193-209.
12. **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.
13. **Johnsrud, L., M. P. Calos, and J. H. Miller.** 1978. The transposon *Tn9* generates a 9 bp repeated sequence during integration. Cell. **15**:1209-1219.
14. **Johnston, H. M., W. M. Barnes, F. G. Chumley, L. Bossi, and X. Roth, Jr.** 1980. Model for the regulation of the histidine operon of Salmonella. Proc. Natl. Acad. Sci. U.S.A. **77**:508-512.
15. **Kleckner, N., D. F. Barker, D. G. Ross, and D. Botstein.** 1978. Properties of the translocatable tetracycline-resistance element *Tn10* in *Escherichia coli* and bacteriophage λ. Genetics **90**:427-461.
16. **Morgan, E. A.** 1980. Insertions of *Tn10* into an *E. coli* ribosomal RNA operon are incompletely polar. Cell **21**: 257-265.
17. **Morgan, E. A., and M. Nomura.** 1979. Deletion analysis of the expression of rRNA genes and associated tRNA genes carried by a λ transducing bacteriophage. J. Bacteriol. **137**:507-516.
18. **Nomura, M., E. A. Morgan, and S. R. Jaskunas.** 1977. Genetics of bacterial ribosomes. Annu. Rev. Genet. **11**: 297-347.
19. **Ohtsubo, H., and E. Ohtsubo.** 1978. Nucleotide sequence of an insertion element, *IS1*. Proc. Natl. Acad. Sci. U.S.A. **75**:615-619.
20. **Pollock, T. J., E. S. Tessman, and I. Tessman.** 1979. Suppression of polar effects of nonsense mutations by ultraviolet irradiation. J. Bacteriol. **138**:122-154.
21. **Rosner, J. L., and M. M. Gottesman.** 1977. Transposition and deletion of *Tn9*: a transposable element carrying the gene for chloramphenicol resistance, p. 213-218. *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.
22. **Yanofsky, C.** 1981. Attenuation in the control of expression of bacterial operons. Nature (London) **289**:751-757.