JOURNAL OF BACTERIOLOGY, July 1982, p. 251–261
0021-9193/82/070251-11$02.00/0

Vol. 151, No. 1

lac Transcription in *Escherichia coli* Cells Treated with Chloramphenicol

MADGE Y. GRAHAM, MOSHE TAL†, AND DAVID SCHLESSINGER*

Department of Microbiology and Immunology, Division of Biology and Biomedical Sciences, Washington University School of Medicine, St. Louis, Missouri 63110

Received 4 November 1981/Accepted 17 March 1982

When protein synthesis was blocked by chloramphenicol in vivo, transcription initiation of *lac* mRNA was severely inhibited. In a promoter mutant (L8-UV5) or in wild-type cells supplemented with adenosine 3',5'-phosphate (≥5 mM), nearly normal initiation could be achieved, and when the mRNA chains formed were extracted, they coded for the 5'-terminal α-peptide of the *lacZ* gene in vitro. However, even under such conditions, only a fraction of RNA polymerases proceeded to the end of the *Z* gene in the presence of chloramphenicol; as a consequence, a wide range of sizes of mRNA was produced, and very few transcripts were formed all the way to the natural termination site of the operon. In other words, premature transcription termination occurred in chloramphenicol-treated cells, as current models predict, but terminations occurred to variable extents at several intragenic sites and apparently at least one intergenic site. Termination at intragenic sites occurred far less in cells bearing a mutation in the transcription termination factor rho.

When protein synthesis is blocked, bacterial mRNA metabolism is greatly affected. Preexisting mRNA, for example, is stabilized in the presence of chloramphenicol (8, 40). More unexpectedly, the formation of some mRNA species is inhibited (12, 19, 28, 30, 45). In those cases, transcription of mRNA is tightly coupled to its translation. Transcription of *trp* and *lac* mRNA, for example, is reduced more than 20-fold when translation is stopped by any of a number of means, including protein synthesis inhibitors, amino acid starvation, or temperature inactivation of thermolabile components of the translation machinery (6, 19, 22, 30, 45). A phenomenon similar to this coupling is polarity: termination of translation at a nonsense codon in a gene often severely inhibits the formation of distal mRNA (2, 13).

Recent studies have suggested a mechanism for both coupling and polarity that involves mainly the premature termination of nascent mRNA beyond blocked ribosomes, mediated by the rho termination factor (reviewed in 2, 13). For example, when chloramphenicol is added to cells in which the rho factor is altered by mutation, or when such a mutation is introduced into cells with a nonsense lesion in *lac*, *trp*, or *gal* genes, the levels of operon-specific mRNA are not reduced as much as in cells with wild-type rho factor (2, 9, 23, 30, 45). On the other hand, it was recently found that certain promoter mutations could alleviate part of the inhibition of RNA synthesis by chloramphenicol (18). Thus, at least part of the nearly total inhibition of *lac* transcription by chloramphenicol seemed to occur at the initiation rather than solely at the elongation of RNA chains.

Even in cases in which the block at mRNA initiation can be bypassed, it has not been clear how far RNA transcription proceeds. For example, in vitro studies with RNA polymerase and rho factor suggested that *lac* transcription in the absence of translation (10) proceeds about 1,000 nucleotides to a major termination point in the initial *lacZ* gene (2, 10). In contrast, in vivo studies of polarity gradients implied the possibility of a number of termination sites (48). Similar uncertainties about the extent of transcription have arisen in studies of other operons (2; see Discussion).

We wanted to refine the analysis of the extent of *lac* transcription. The *lacZ* gene provides especially favorable material, because (i) the block at transcription initiation in chloramphenicol-treated cells can be specifically bypassed in vivo in the L8-UV5 strain (or in normal strains; see below); (ii) the *lacZ* gene is very long, and as a result, by using composite gel electrophoresis (1), one can easily resolve major truncated mRNA species that might be formed within the gene; (iii) DNA fragments corresponding to various segments of the operon are available as

† Permanent address: Biology Department, Technion, Haifa, Israel.

hybridization probes to verify the sequence content of mRNA species; and (iv) the coding activity of mRNA from the initial 300 nucleotides of the mRNA can be assayed directly in vitro (24–26).

The functional assay and the available probes have enabled us to confirm that for *lac* mRNA, the major block of transcription when translation stops is at initiation. However, when this coupling block is bypassed, termination at a number of sites further in the *lacZ* gene is observed. Much of the chain termination does not occur, as expected, in cells containing a defective rho factor.

MATERIALS AND METHODS

Strains, media, and growth condition. Table 1 lists the strains used. The growth medium contained minimal salts (29) supplemented with 0.8% Casamino Acids, 0.4% glycerol, and 1 μg of thiamine per ml. For experiments, cultures were freshly grown to mid-log phase at 30°C with shaking.

Preparation of RNA. Five milliliters of culture was mixed with an equal volume of ice-cold or frozen killing buffer (0.1 M sodium acetate, pH 5.5, 0.02 M NaN_3, and 0.01 M NaF). Cells were pelleted at 12,000 × *g* for 10 min and suspended in a mixture of 2.5 ml of 10-fold-diluted killing buffer, 50 μl of 20% sodium dodecyl sulfate, and 1 ml of freshly distilled phenol. Suspensions were heated at 60°C with occasional agitation for 5 to 10 min. They were then centrifuged at 12,000 × *g* for 15 min, and the upper aqueous phase was carefully removed and extracted with 0.5 ml of phenol twice more as above. To the final aqueous phase, 0.2 ml of sodium acetate (1 M, pH 5.5) and 2.5 volumes of ethanol were added. The suspension was chilled overnight at −18°C (or 1 h at −70°C), and the precipitate was collected at 12,000 × *g* for 30 min.

When the RNA was to be used for translation of the α-peptide of β-galactosidase (24), 5 ml of 95% ethanol was added to wash the RNA precipitate. After 1 h at room temperature, the ethanol phase was discarded and the process was repeated with 66% ethanol. The final precipitate was dissolved in 1 ml of water, lyophilized, redissolved in 120 μl of water, and used immediately.

When the RNA was to be used for DNA:RNA hybridization, the pellet from the first ethanol precipitation was dissolved in 1 ml of water. Then 0.2 ml of 1 M sodium acetate was added, and the RNA was ethanol precipitated once again. The final precipitate was dissolved in 1.2 ml of hybridization buffer containing 50% formamide, 3× SSC (SSC is 0.15 M NaCl plus 0.015 M sodium citrate), and 0.1% sodium dodecyl sulfate.

For hybridization assays with RNA from gel slices, a milder and faster RNA extraction procedure, which involves high-salt precipitation in an extract of cells lysed with EDTA-lysozyme, was used to minimize the breakdown of RNA (1, 18).

Functional assay of 5′-*lacZ* mRNA. The assays were modified from an earlier procedure (20, 21). Each 100 μl of reaction mixture contained 30 μl of S30 extract from strain 514K2, 30 μl of RNA (about 20 to 80 μg) prepared as above, and 2.3 μmol of phospho(enol)pyruvate (Sigma Chemical Co.); 0.02 μmol of each of 20 amino acids (Sigma); 10 μg of tRNA (GIBCO Laboratories); 4.4 μmol of Tris-acetate (pH 8.2); 7 μmol of potassium acetate; 3 μg of folinic acid; 0.22 μmol of ATP and 0.05 μmol of GTP (Sigma); 2 mg of polyethylene glycol 6000; 1.2 μmol of magnesium acetate; 0.14 μmol of dithiothreitol; and DNase as previously described (20).

The reaction mixture was incubated at 37°C for 10 min. Then 25 μl of DZ291 (26) extract (α-acceptor) was added, followed by 1 ml of 0.25 M phosphate buffer, pH 7, 0.1 M β-mercapthoethanol, 400 μg of *o*-nitrophenyl-β,D-galactoside (Sigma), and 50 μg of chloramphenicol (Sigma). This mixture was incubated at 30°C until sufficient yellow color was developed. The reaction was stopped by adding 1 drop of glacial acetic acid. The tubes were centrifuged at 2,000 × *g* for 10 min. To the clear supernatant, 0.6 ml of 1 M Na_2CO_3 containing 0.005 M EDTA was added, and the absorbance at 420 nm was measured. Reaction mixtures lacking RNA were used as blanks, and their spectral reading, usually less than 2% of the total absorbance, was subtracted.

DNA:RNA hybridization. Phage and plasmid DNAs were prepared as previously described (29, 42); DNA filters were prepared by an established method (29). For a 27-mm nitrocellulose filter, 50 μg of phage DNA or 5 μg of plasmid DNA was used. For each hybridization, 200 μl of RNA solution prepared as described above was incubated with a 1/16 sector of a filter at 53°C for 40 to 48 h. Both probe and corresponding background DNA filters were included in each hybridization mixture. DNAs of phages λ *plac*5, plasmid pMC3, and plasmid pTE21 were used as probes for *lacZ*; for the 5′-terminal portion of the *lacZ* gene; and for *lacY* and *lacA* mRNA, respectively; DNAs of phage λ *c*I857 *S*7 and plasmid vehicles pMB9 (for pMC3) and pBR322 (for pTE21) were used to determine corresponding background levels of hybridiza-

TABLE 1. Bacterial strains

Name	Genotype	Provenance	Reference
E8027 (L8-UV5)	F′ *lac* L8-UV5 *pro*/Δ(*lac-pro*) *thi*	W. Reznikoff	3
E5014 (Wild type)	F′ *lac pro*/Δ(*lac-pro*) *thi*	W. Reznikoff	3
U118	*lacZ*U118 *trpR trpE trpA*	C. Yanofsky	23
CSH1	*lacZ*(Am) *trp rpsL thi*	Cold Spring Harbor Collection	29
CSH2	*lacZ*(Am) *trp rpsL thi*	Cold Spring Harbor Collection	29
514K2	*rpsL* Δ*lac* Ksg^r	K. Jacobs	20
DZ291	F′ *lacI2* ΔM15/*lacI2* ΔM15	I. Zabin	26
AD1600	F$^-$ *galE3*::IS2 *his rpsL rho-15*	S. Adhya	9
SA1030	F$^-$ *galE3*::IS2 *his rpsL*	S. Adhya	9

tion. Each hybridization was done at least in duplicate. (Plasmid pMC3 has an insert extending from the end of the *lacI* gene through the initial one-sixth of *lacZ*. The *Hin*II insert in pMB9 totals 789 base pairs and covers the region that codes for the N-terminal α-peptide of β-galactosidase [24]. Plasmid pTE21 has a 1.76-megadalton *Eco*RI insert, in pBR322, including *lac* sequences with *lacZ* almost completely deleted [R. Teather, personal communication]; therefore, the *lac* portion of this plasmid represents mainly the *lacYA* region of the operon and serves as a good probe for promoter distal *lacY* and *lacA* mRNA.)

Gel electrophoresis and hybridization with RNA from gel slices were modified from references 1 and 18. RNA labeled as indicated in the figures was electrophoresed in a 2.2% polyacrylamide–0.5% agarose slab gel at 5 V/cm for 5 h. Each slot was sliced into 1-mm slices; RNA was then eluted from each slice and hybridized in 300 μl of hybridization mix with DNA filters as described above (1, 18).

After the RNA:DNA hybridization, filters were treated with 20μg of RNase per ml at 37°C for 1 h and washed four times with 2× SSC. Dry filters were treated with 0.3 N NaOH for 40 min to render RNA soluble, and the liquid was mixed and counted in Ready-Solv (Beckman Instruments, Inc.).

RESULTS

In wild-type *Escherichia coli*, less than 5% as much *lac* mRNA is made in cells treated with chloramphenicol (12, 45). Here we characterize further both the block at initiation (18) and the mRNA formed when the block at initiation is bypassed.

Initiation of functional *lac* mRNA in chloramphenicol-treated cells. Hirschel et al. (18) found that in contrast to wild-type cells, strains bearing a class III-type *lac* promoter (38) formed some *lac* mRNA even in the presence of chloramphenicol. When a DNA probe for the entire *lacZ* gene was used, 30 to 40% of the mRNA formed in the absence of the drug was induced in its presence.

Because it is specifically the promoter that is altered in class III mutants, at least part of the block of transcription in wild-type cells should be at or near the initiation step. Transcription from distal portions of the very long *lacZ* gene in the class III strains might still be prematurely arrested (2), or the mRNA might be hyperlabile (13, 17). The relative relief of an initiation block could therefore probably be better assessed by hybridization to a DNA probe for the initial segment of the *lacZ* gene. We used a probe for "*5'-lacZ*" mRNA that contained the first 479 nucleotides of the *lac* operon mRNA sequence (24). The amount of *5'-lacZ* mRNA formed in the presence of chloramphenicol was as much as 80% of that in control cells (Table 2, row 1). Presumably, even more relief of the apparent inhibition by chloramphenicol would be detected with an even shorter promoter-proximal DNA probe; and extrapolating to the start of transcription, there would be scarcely any inhibition of chain initiation in chloramphenicol-treated L8-UV5 cells.

The class III promoter mutation L8-UV5 was originally selected as a strain insensitive to catabolite repression of the *lac* operon (41). Restoration of *lac* mRNA formation in the presence of chloramphenicol could be related to this lack of a requirement for cAMP. Earlier studies, including our own, showed no significant restoration of *lacZ* message formation (or only a small effect) when cAMP was added to chloramphenicol-treated cells (12, 18, 45). However, we found that the estimate of mRNA formation in the presence of chloramphenicol depended on (i)

TABLE 2. 5'-Proximal *lacZ*-, *lacZ*-, and *lacYA*-specific RNA formed in the presence and absence of chloramphenicol[a]

Expt	Addition		% Hybridization with given probe		
	Cm	IPTG	*5'-lacZ*	*lacZ*	*lacYA*
A	–	–	0.014	0.004	0.018
B	–	–			
C	–	–			
A	–	+	0.065	0.194	0.088
B	–	+	0.061	0.209	0.069
C	–	+	0.084	0.444	0.144
A	+	–	0.007	−0.003	0.026
B	+	–	0.004	−0.002	0.008
C	+	–	0.003	0.006	0.019
A	+	+	0.045	0.068	0.029
B	+	+	0.050	0.087	0.008
C	+	+	0.009	0.010	0.027

[a] Where indicated, 100 μg of chloramphenicol (Cm) per ml was added to an exponentially growing culture of a wild-type strain or one bearing the L8-UV5 mutation. Five minutes later, the β-galactosidase inducer isopropyl-β-D-thiogalactopyranoside (IPTG) was added to 1 mM. After another 5 min, [^{3}H]uridine (New England Nuclear Corp.; specific activity, 29 Ci/mmol) was added to 50 μCi/ml to label the RNA for 1 min for A (L8-UV5; average of four to eight independent experiments) and for C (wild type; average of two independent experiments). For B, the L8-UV5 strain was labeled as in the legend to Fig. 4 (with rifampin and [^{3}H]uridine added for 3 min before the cells were killed). After labeling, RNA was extracted from the pulse-labeled cells. Hybridizations to various DNA probes were then carried out (see Materials and Methods) to estimate the levels of *5'-lacZ*, *lacZ*, and *lacYA* RNA. The values are expressed as the percentage of input counts over background which were hybridizable. (The input for each hybridization reaction was usually 1×10^6 to 2×10^6 cpm. The backgrounds, which were subtracted before the calculation of percentage of hybridization, were 0.010 to 0.013% of input counts for λ *S7* and <0.001% for pMB9 and pBR322).

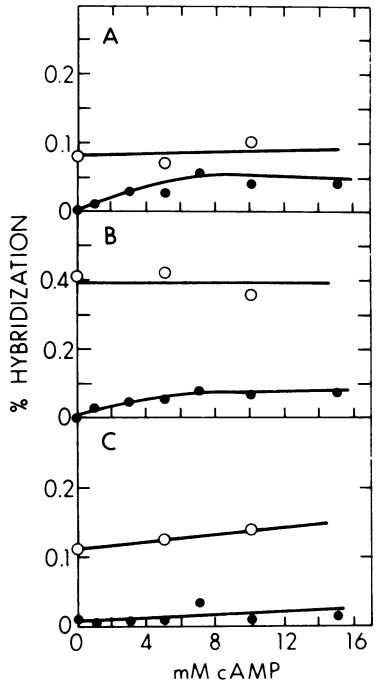

FIG. 1. Restoration of mRNA synthesis in the presence of chloramphenicol by cAMP. A culture of E5014 (wild-type *lac* promoter) was induced and labeled, and the hybridizations were expressed as in Table 2, except that cAMP at the indicated concentrations was added along with IPTG. Uninduced backgrounds were substracted. Symbols: (○, without chloramphenicol; ●, with chloramphenicol. (A) 5′-*lac* probe; (B) *lacZ* probe; C: *lacY A* probe.

cAMP concentration and (ii) the specific region of mRNA assayed (Fig. 1). If the 5′-*lacZ* mRNA was assayed by hybridization and the concentrations of cAMP were high enough (≥5 mM), restoration of 5′-*lacZ* message up to 70% of the level of mRNA formed in the absence of chloramphenicol was achieved in a wild-type strain. This level is comparable to that in the strain bearing the L8-UV5 mutations.

It is possible to test whether the 5′-*lac* mRNA formed is functionally intact. Activity can be determined by translating the mRNA into the 5′-"α-peptide" of β-galactosidase (no more than 81 amino acids long), which can be assayed by α-complementation (44). In the experiments depicted in Fig. 2, cells were induced for β-galactosidase for brief times at intervals after the addition of chloramphenicol. The mRNA was then extracted and tested for its capacity to code for the α-peptide in vitro. The level of inducibility of α-coding mRNA changed very slowly in the L8-UV5 promoter mutant. Thus, the 5′-proximal segment of the messages formed in the cells was functionally intact. As expected, the inducibility of active mRNA dropped very rapidly in the wild-type strain, to a negligible level within 3 min.

Chloramphenicol affects protein synthesis by blocking ribosome movement along the mRNA molecule (8). Other protein synthesis inhibitors which act differently are also known to inhibit transcription (12, 19), and it was of interest to see their effect on the formation of functional 5′-*lacZ* mRNA in the E8027 strain. Puromycin, which causes release of any ribosomes bound to mRNA (32), and kasugamycin, which prevents the addition of any ribosomes to the mRNA (33), were chosen for this purpose. They were as effective as chloramphenicol in inhibiting mRNA synthesis in the wild-type cells (data not shown). Again, when they were added to L8-UV5 cells, the synthesis of functional α-message did not seem to be affected (Fig. 3).

These results indicate that when protein synthesis is inhibited by any of a variety of means, *lac* mRNA becomes uninducible; but in the presence of sufficient exogenous cAMP, or with a promoter that needs no cAMP, a similar number of mRNA chains are initiated in the presence and in the absence of translation.

Transcription termination sites in the *lac* operon in chloramphenicol-treated cells. Even when 5′-*lacZ* mRNA was formed at high levels in the

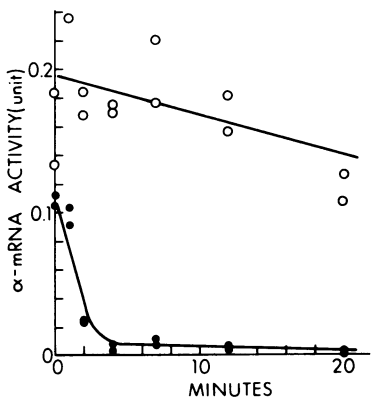

FIG. 2. *lac* transcription capability of cells after addition of chloramphenicol. Sixty milliliters of exponentially growing bacteria was supplemented at zero time with 3 ml (2 mg/ml) of chloramphenicol. At the times indicated, 6-ml portions were transferred to vials containing 60 μl of IPTG (0.1 M). After a 2-min period to permit enzyme induction, the bacteria were killed and RNA was prepared as described in Materials and Methods. A modified Zubay-type system (see Materials and Methods) supplemented with DNase was used for the translation of the *lac* mRNA synthesized in vivo. The activity is expressed as absorbancy at 420 nm × 1,000 per 10 μg of RNA input per 10 min of incubation at 30°. Symbols: ○, E8027 (L8-UV5 *lac* promoter); ●, E5014 (wild-type *lac* promoter).

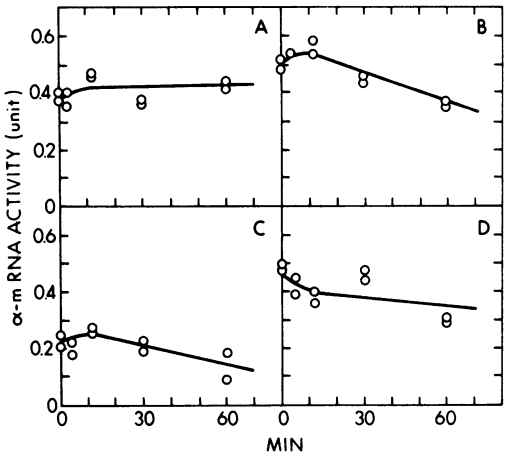

FIG. 3. *lac* transcription capability of cells after addition of various antibiotics. One hundred twenty milliliters of exponentially-growing L8-UV5 cells were washed twice in 0.12 M Tris, pH 8, and treated with EDTA according to reference 1. For the experiment, the bacteria were suspended in Casamino Acids medium (supplemented with 0.4% glycerol) at concentrations comparable to those in the starting culture. cAMP was then added to a final concentration of 1 mM. After 5 min of preincubation, puromycin, kasugamycin, or chloramphenicol was added to give a final concentration of 600 μg/ml, 82 mg/ml, or 300 μg/ml, respectively, at the times indicated. Portions of 4 ml were then transferred to vials containing IPTG at a final concentration of 2 mM. After a 2-min induction period, the bacteria were killed and RNA was prepared as described under Materials and Methods. The protocol for the Zubay system and the expression of the activity were as described in the legend to Fig. 2. (A) Control without antibiotics; (B) chloramphenicol-treated cells; (C) kasugamycin-treated cells; (D) puromycin-treated cells.

presence of chloramphenicol, total *lacZ* mRNA was found at lower levels (Table 1). When we used a DNA hybridization probe that corresponded to the distal genes of the operon (*Y* and *A* mRNA), the deficit was even more marked. As in wild-type cells, scarcely any *Y* and *A* mRNA above uninduced levels was observed in chloramphenicol-treated L8-UV5 cells (Table 2, row 3).

To assess the size and sequence content of *lac* mRNA formed, we fractionated the messages made in the presence of chloramphenicol by gel electrophoresis and then tested them for hybridization to probes for different parts of the operon: *5'-lacZ*, *lacZ*, and *lacYA* (Fig. 4). Labeling was done in the presence of rifampin to prevent further transcription initiation while RNA synthesis was completed. Thus, RNA chains would be accumulated as finished products. In the control cells in the absence of chloramphenicol, the labeled RNA hybridizable to all three probes was almost entirely associated with large species of messages (23S to 30S, corresponding to the chains expected for *lacZ*-size to full *lacZYA*-size RNA (27, 40)). When chloramphenicol was present, both *5'-lacZ* and total *Z* mRNA sequences were found in RNA chains over a range of smaller sizes (6S to 16S). Although some full-size *lacZ*-length message was observed (22S to 25S), there was very little RNA larger than that size. That these were the largest transcripts formed was consistent with the lack of significant hybridization to the *lacYA* DNA probe across the gel; i.e., few if any transcripts extended into the *Y* and *A* genes. (These results are somewhat reminiscent of earlier findings in which cells recovering from chloramphenicol treatment showed some "polarity" effects even after chloramphenicol had been removed [35].)

Even though mRNA is generally more stable in chloramphenicol-treated cells (8), and endonuclease cleavage of preformed *lac* mRNA is inhibited when the drug is added (40), it still seemed possible that the shorter messages observed were degradation products of longer transcripts, especially because the first ribosome can bind to mRNA in chloramphenicol-treated cells but is immediately prevented from any but slow movement (8); hence, the mRNA is mostly "naked." However, Fig. 5 confirms that even for *5'-lacZ* and total *lacZ* mRNA formed after the addition of chloramphenicol, half-lives were about threefold longer than in untreated cells (10 min compared with 3.5 min). (The low level of *Y* and *A* mRNA formed in the presence of chloramphenicol made meaningful determination of its half-life difficult.) Consistently, the functional half-life of *5'-lacZ* message formed in the presence of chloramphenicol was also 10 min in both L8-UV5 and wild-type strains (data not shown). Thus, the short transcripts seen in chloramphen-

TABLE 3. Partial relief of the blockage of *lac* transcription by chloramphenicol (Cm) in a mutant defective in rho factor[a]

Probe	% Hybridization					
	SA1030 (wild type)			AD1600 (*rho-15*)		
	−Cm	+Cm	Ratio, +Cm/−Cm	−Cm	+Cm	Ratio, +Cm/−Cm
5'-lacZ	0.181	0.012	0.11	0.107	0.059	0.55
lacZ	0.337	0.020	0.04	0.222	0.111	0.50
lacYA	0.167	<0		0.178	0.019	0.11

[a] Protocols as in Table 2, except that 7 mM cAMP was included. The tabulated values are the percentage of hybridization from at least two independent experiments and were corrected for the background observed in corresponding cultures to which no inducer was added.

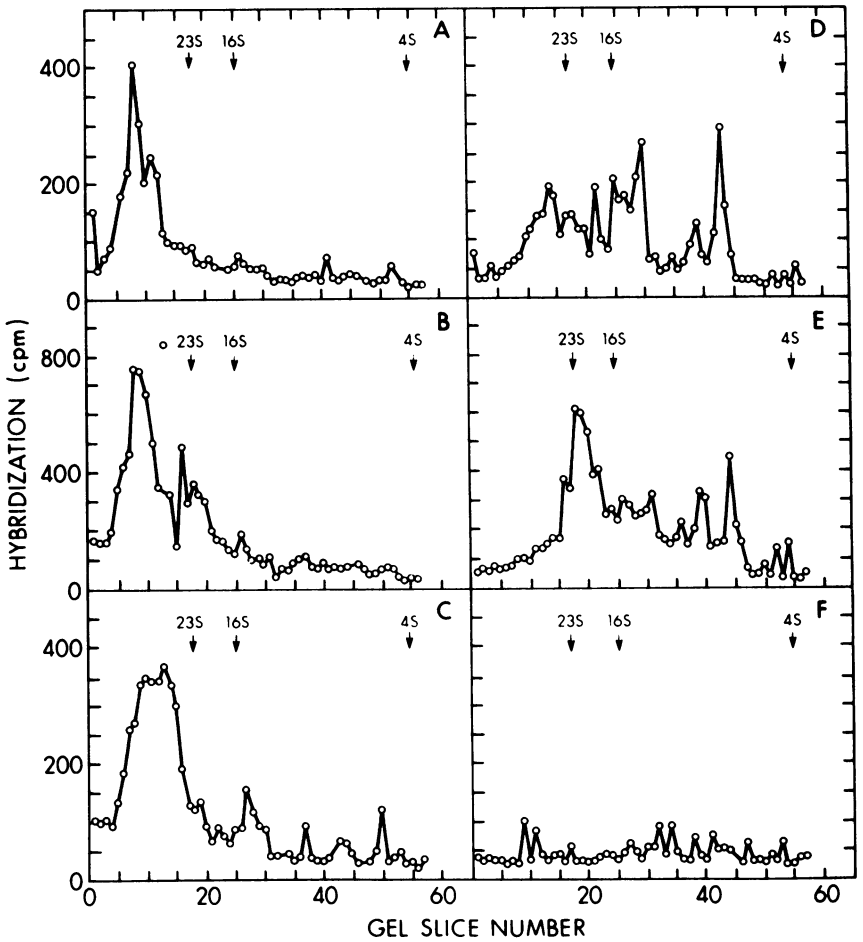

FIG. 4. Size distributions of mRNA synthesized in the L8-UV5 strain in the absence and presence of chloramphenicol. L8-UV5 cells were prelabeled with 0.05 μCi of [^{14}C]uridine per ml for two to three generations before the experiments to provide internal markers. Chloramphenicol and IPTG were then added to exponentially growing cultures, at time zero and 5 min, respectively, as in Table 2. [^{3}H]uridine at 100 μCi/ml and rifampin at 200 μg/ml were added at the 10th min. The cells were killed 3 min later, and RNA was extracted. Gel electrophoresis and hybridization were as described in Materials and Methods. Background levels are not subtracted; they were about 30 cpm for each slice. (A) *5'-lac*; (B) *lacZ*; (C) *lacYA*; message synthesized in the absence of chloramphenicol. (D) *5'-lac*; (E) *lacZ*; (F) *lacYA*; message synthesized in the presence of chloramphenicol (100 μg/ml).

icol-treated cells were unlikely to result from rapid degradation of longer RNA chains.

Experiments with a strain defective in the termination factor rho support the notion that the short transcripts result from premature transcription termination. A mutation in the chain termination factor rho (*suA*; 39, 45) is known to relieve partially the effects of chloramphenicol on transcription. We used such a strain to test for a reversal of putative premature termination events. In strain *rho-15*, rho factor function is diminished (9). The levels of inducible *5'-lacZ* and total *lacZ* mRNA made in the *rho-15* strain treated with chloramphenicol were as much as 50% those in untreated cells (Table 3). The increased levels of mRNA were found in larger, *lacZ*-sized chains (Fig. 6). We noted, however, that even in the *rho-15* mutant, little full-size *lac* mRNA (*lacZ* plus *lacY* plus *lacA*) was formed (Fig. 6 and Table 3).

mRNA in polar mutants. We also determined the message levels in three polar mutants: U118, CSH1, and CSH2. These provide another case in which no block of chain initiation occurs, but one can expect the termination of transcription beyond the ribosome at the termination codon. The nonsense codons are all located within the initial 10% of the *lacZ* gene, but at progressively

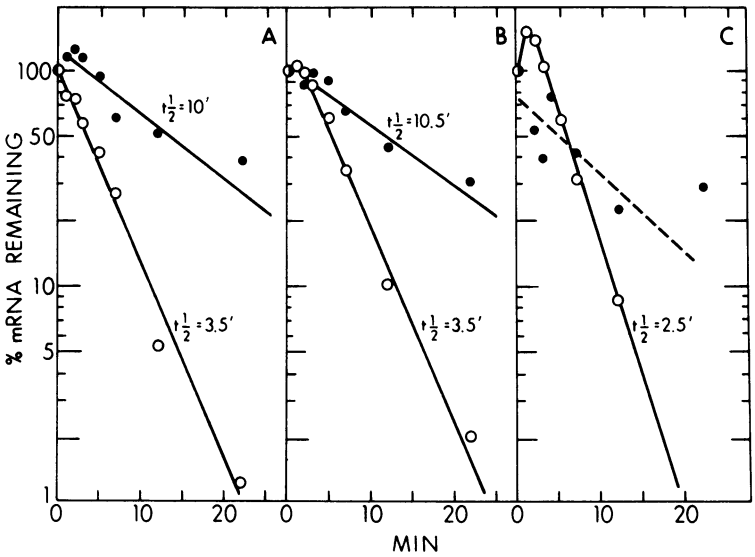

FIG. 5. Decay of *lac* mRNA made in the presence or in the absence of chloramphenicol. L8-UV5 cells were induced either in the absence of chloramphenicol or after 5 min in its presence. Five minutes later, [^{3}H]uridine (50 μCi/ml) was added to the cells. Rifampin (200 μg/ml) was added after another 1 min. The first portion of culture (5 ml) was sampled 1 more min later (zero time), and the rest was sampled at the indicated times. The cells were killed immediately and processed as in Materials and Methods. Symbols: ○, without chloramphenicol; ●, with chloramphenicol. (A) 5'-*lac* probe; (B) *lacZ* probe; (C) *lacYA* probe.

more distal positions (6, 7, 26, 46). The measurements shown in Table 4 were made on RNA from cells induced for 5 min in the absence of chloramphenicol. The levels of mRNA in strains CSH1 and CSH2 were considerable and rather comparable to that in chloramphenicol-treated L8-UV5 cells. U118 is the earliest known nonsense mutant, with a lesion at the 17th amino acid of β-galactosidase (46). Its message levels were higher than those of wild-type cells treated with chloramphenicol, although lower than those of the L8-UV5 mutant. As one might expect, the level was not much stimulated by cAMP and could be further lowered to the value observed in uninduced cells when chloramphenicol was added to the cells (data not shown).

DISCUSSION

The effects of chloramphenicol on transcription in *E. coli* cells vary for different transcripts. For rRNA and ribosomal protein gene mRNAs (11), and for lambda phage early mRNA (13), blockage of translation has no effect on RNA formation. Also for *trp* mRNA, chain initiation seems to occur at a very similar rate in growing and chloramphenicol-treated cells; however, only very short transcripts containing leader sequences are formed in the presence of chloramphenicol (22). Thus, the inhibition of *trp* mRNA formation occurs at RNA chain elongation. For *lac* mRNA, the effects of chloramphenicol are more complex and result in an inhibition of initiation as well as elongation.

Effect of chloramphenicol on transcript initiation. In a wild-type strain treated with chloramphenicol, very little mRNA from any part of the *lac* operon was detected. In contrast, in an L8-UV5 promoter mutant treated with chloramphenicol, hybridizable and functional 5'-*lacZ* mRNA was induced at at least 80% the level of untreated cells. The L8-UV5 mutant does not require cAMP for *lac* induction, and relief of a cAMP requirement in chloramphenicol-treated cells explains the difference in the behavior of

TABLE 4. *lac* mRNA made in polar mutants[a]

Strain	% Hybridization with given probe		
	5'-*lac*	*lacZ*	*lacYA*
U118[b]	0.020	0.018	−0.013
CSH1	0.059	0.056	0.002
CSH2	0.159	0.123	0.023

[a] The experimental protocols were as in Table 2. Cells were again induced for 5 min and then pulse-labeled for 1 min in the absence of chloramphenicol. Strains U118, CSH1, and CSH2 are nonsense mutants (see text). The numbers shown are the percentage of hybridization with respect to total input after the uninduced level of mRNA has been substracted.

[b] Average from three independent experiments.

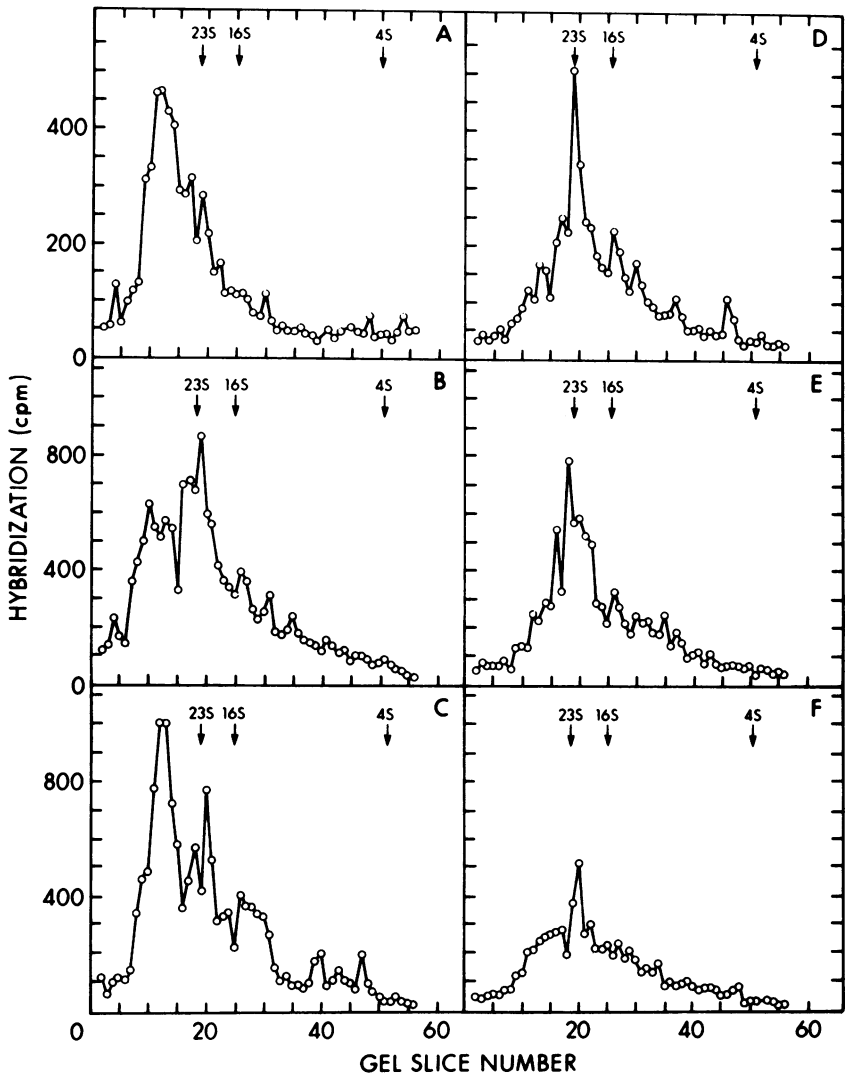

FIG. 6. Size distributions of mRNA synthesized in the *rho-15* strain in the absence and presence of chloramphenicol. The experiments were done as in Fig. 4, except that the *rho-15* strain was used and 7 mM cAMP was included. (A) 5'-*lac*; (B) *lacZ*; (C) *lacYA*; mRNA synthesized in the absence of chloramphenicol. (D) 5'-*lac*; (E) *lacZ*; (F) *lacYA*; mRNA synthesized in the presence of chloramphenicol 100 μg/ml.

the mutant and wild-type strains: added cAMP permitted wild-type cells to form *lac* mRNA as well in the presence of chloramphenicol. Either the level of cAMP falls in chloramphenicol-treated cells, or the cAMP requirement for induction increases (for example, cAMP receptor protein might otherwise become unstable).

Earlier efforts to assess the contribution of "catabolite repression" and altered cAMP levels to the inhibition of transcription in wild-type cells by chloramphenicol concluded that there is only a small effect (12, 45). However, the relief of transcription initiation in those studies was largely masked by premature termination of initiated transcripts, since entire *lac* or *lacZ* message content was measured (see below). When a 5'-*lacZ* probe was used and a higher concentration of cAMP was added, relief of up to 70% could be achieved (Fig. 3). Moreover, messages made in wild-type cells in the presence of cAMP showed the same sequence content as those made in L8-UV5 when both were treated with chloramphenicol (i.e., more 5'-*lacZ* than *Z*; little *Y* and *A* mRNA). cAMP seems to act at chain initiation in this case, with no action at chain elongation (43), nor is there any apparent need for ppGpp (36, 37, 47), which is destroyed rapidly in chloramphenicol-treated cells (15).

Effect of chloramphenicol on *lac* RNA chain elongation. Once mRNA chains are started, translation blockage leads to the well-known polar effects. In operons like *lac*, *trp*, and *gal*, RNA polymerase tends to stop some distance beyond the point at which ribosomes are stopped, whether ribosome progression is blocked by chloramphenicol or by a nonsense codon (cf. Tables 2 and 4).

Many suggestions have been made about how many transcription termination points there are and how efficient they are. From experiments with *trp* operon nonsense mutants, for example, it seemed possible from one study that the polymerase may continue to the end of the gene containing the nonsense allele (17), whereas other studies have suggested a number of weak termination sites within a gene (22, 31).

For *lac* mRNA, the three hybridization probes we used (Tables 2 to 4) and the length of the *lacZ* gene facilitated the analysis. The results are in agreement for this case with the notion of a number of weak intragenic termination sites. Several peaks ranging from 6S to 16S were seen, and even a peak corresponding to the full-size *lacZ* transcript was formed in chloramphenicol-treated cells (Fig. 4). In other words, a fraction of transcripts would terminate at each of several sites, but some nascent transcripts still reached the end of the *Z* gene. In the *rho-15* strain, in which rho-dependent termination occurs less efficiently, the shorter transcripts were largely not seen, and most of the transcripts were correspondingly longer (Fig. 6). In no case did we observe a single peak of 12S (about 1,000 nucleotides long) to correspond to the major *lac* mRNA transcript made in the presence of rho factor in vitro (2, 10).

In mutants bearing early *Z* nonsense alleles (U118, CSH1, and CSH2), the amounts of both 5'-*lacZ* and total *lacZ* mRNA increased roughly in proportion to the positions of the mutations. These data are in general agreement with earlier results that the closer the nonsense mutation to the end of the *Z* gene, the larger the mRNAs that were formed (6, 7, 48). Multiple termination sites are thus consistent with the observations on polar mutants as well.

In contrast to the relief of rho-dependent termination within the *lacZ* gene, little *lacY* and *lacA* mRNA was observed even in the *rho-15* strain. This suggests that a stronger barrier to transcription may be present just before the *lacY*-coding sequence (e.g., in the intercistronic region). From the DNA sequence in the *lacZ-lacY* junction region (4), some potential hairpin loops can be hypothesized, but whether these potential structures are related to strong transcription termination is not clear at present.

Basis of coupling. Why does RNA polymerase stop distal to blocked ribosomes? And why does it stop on only some transcripts? Two major factors seem to affect the extent of chain termination. One is the sequence of the RNA; presumably a number of sites exist in the *lacZ* gene at which the conformation of the mRNA or the neighboring sequence in DNA, or both, can promote chain termination in the absence of ribosomes (Fig. 4). Many of these termination events require rho factor (Fig. 6).

The other important factor is the nature of the transcribing RNA polymerase: it can function in such situations when bound to lambda N protein (13) or "L factor" ("*nusA* protein"; 16, 25), or both, which promote continued transcription on lambda DNA. L factor was discovered by its capacity to promote the elongation of *lac* transcripts in vitro (25).

Thus, RNA polymerase might not stop on some transcripts because it is associated with factors that inhibit termination or because the transcripts contain no sequences at which transcription arrest is likely. Viewed in this way, the requirement of translation for continued *lac* RNA transcription in vivo and in vitro (20, 21) can be understood in at least two ways. In one, ribosomes interact actively with RNA polymerase, either as a modulating factor (2) or by the formation of a regulatory molecule like ppGpp (15). Such an interaction has been suggested by the observations that mutations in ribosomal proteins can affect RNA polymerase action (5, 14). Alternatively, ribosomes may affect the structure of mRNA, in analogy to their proposed role in attenuation (34). They could, for example, prevent the formation of a loop or RNA hairpin that would otherwise promote rho factor-mediated termination.

These mechanisms are not mutually exclusive, and both suggest the teleological function of coupling. When mRNA cannot be translated, the effective reduction of intracellular cAMP blocks even the initiation of more *lac* mRNA transcripts, and very likely of all catabolite-repressible mRNAs. The blocks in mRNA chain elongation then also help to reduce further the production of untranslatable mRNA.

ACKNOWLEDGMENTS

We especially thank R. Teather, who made available to us the plasmid PTE21-containing strain. We thank V. Shen for helpful discussions in part of the work and T. King for reading the manuscript.

These studies were supported by grant CD-69C from the American Cancer Society.

LITERATURE CITED

1. **Achord, D., and D. E. Kennell.** 1974. Metabolism of messenger RNA from the gal operon of *Escherichia coli*. J. Mol. Biol. **90**:581–599.

2. **Adhya, S., and M. Gottesman.** 1978. Control of transcription termination. Annu. Rev. Biochem. **47**:967–996.
3. **Arditti, R. R., R. T. Grodzicker, and J. Beckwith.** 1973. Cyclic adenosine monophosphate-independent mutants of the lactose operon of *Escherichia coli*. J. Bacteriol. **114**:652–655.
4. **Büchel, D. E., J. S. Gronenborn, and B. Müller-Hill.** 1980. Sequence of the lactose permease gene. Nature (London) **283**:541–545.
5. **Chakrabarti, S. L., and L. Gorini.** 1979. Interaction between mutations of ribosomes and RNA polymerase: a pair of *str*A and *rif* mutants individually temperature-sensitive but temperature-sensitive in combination. Proc. Natl. Acad. Sci. U.S.A. **74**:1157–1161.
6. **Cohen, T., A. Silberstein, J. Kuhn, and M. Tal.** 1979. Relief of polarity in *E. coli* depleted of 30S ribosomal subunits. Mol. Gen. Genet. **173**:127–134.
7. **Contesse, G., H. Crepin, and F. Gros.** 1970. Transcription of the lactose operon in *E. coli*, p. 111–141. *In* D. Zipser (ed.), The lactose operon. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
8. **Cremer, K. J., L. Silengo, and D. Schlessinger.** 1974. Polypeptide formation and polyribosomes in *Escherichia coli* treated with chloramphenicol. J. Bacteriol. **118**:582–589.
9. **Das, A., D. Court, and S. Adhya.** 1976. Isolation and characterization of conditional lethal mutants of *Escherichia coli* defective in termination factor rho. Proc. Natl. Acad. Sci. U.S.A. **73**:1959–1963.
10. **DeCrombrugghe, B., A. Adhya, M. Gottesman, and I. Pastan.** 1973. Effects of rho on transcription of bacterial operons. Nature (London) New Biol. **241**:260–264.
11. **Dennis, P. P.** 1976. Effects of chloramphenicol on the transcriptional activities of ribosomal RNA and ribosomal protein genes in *Escherichia coli*. J. Mol. Biol. **108**:535–546.
12. **Dütting, D., and L. Hübner.** 1972. The effect of antibiotics on the *in vivo* synthesis of messenger ribonucleic acid from the lactose operon of *Escherichia coli*. Mol. Gen. Genet. **116**:277–290.
13. **Franklin, N. C., and C. Yanofsky.** 1976. The N protein of λ: evidence bearing on transcription termination, polarity and the alteration of *E. coli*, p. 693–706. *In* R. Losick and H. Chamberlin (ed.), RNA polymerase. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
14. **Friedman, D. I., A. T. Schauer, M. R. Baumann, L. S. Baron, and S. L. Adhya.** 1981. Evidence that ribosomal protein S10 participates in control of transcription termination. Proc. Natl. Acad. Sci. U.S.A. **78**:1115–1118.
15. **Gallant, J., G. Margason, and B. Finch.** 1972. On the turnover of ppGpp in *Escherichia coli*. J. Biol. Chem. **247**:6055–6058.
16. **Greenblatt, J., J. Li, S. Adhya, D. I. Friedman, L. S. Baron, B. Redfield, H. F. Kung, and H. Weissbach.** 1980. L factor that is required for β-galactosidase synthesis is the *nusA* gene product involved in transcription termination. Proc. Natl. Acad. Sci. U.S.A. **77**:1991–1994.
17. **Hiraga, S., and C. Yanofsky.** 1972. Hyperlabile messenger RNA in polar mutants of the *trp* operon of *Escherichia coli*. J. Mol. Biol. **72**:103–110.
18. **Hirschel, B. J., V. Shen, and D. Schlessinger.** 1980. Lactose operon transcription from wild-type and L8-UV5 *lac* promoters in *Escherichia coli* treated with chloramphenicol. J. Bacteriol. **143**:1534–1537.
19. **Imamoto, F.** 1973. Diversity of regulation of genetic transcription. I. Effect of antibiotics which inhibit the process of translation on RNA metabolism in *Escherichia coli*. J. Mol. Biol. **74**:113–136.
20. **Jacobs, K. A., and D. Schlessinger.** 1977. *Escherichia coli* DNA-directed β-galactosidase synthesis in presence and absence of Ca^{2+}. Biochemistry **16**:914–920.
21. **Jacobs, K. A., V. Shen, and D. Schlessinger.** 1978. Coupling of *lac* mRNA transcription to translation in *Escherichia coli* cell extracts. Proc. Natl. Acad. Sci. U.S.A. **75**:158–161.
22. **Kano, Y., M. Kuwano, and F. Imamoto.** 1976. Initial *trp* operon sequence in *Escherichia coli* transcribed without coupling to translation. Mol. Gen. Genet. **46**:179–188.
23. **Korn, L. J., and C. Yanofsky.** 1976. Polarity suppressors increase expression of the wild-type tryptophan operon of *Escherichia coli*. J. Mol. Biol. **103**:395–409.
24. **Kung, H. F., M. Tainsky, and H. Weissbach.** 1978. Regulation of the *in vitro* synthesis of the α-peptide of β-galactosidase directed by a restriction fragment of the lactose operon. Biochem. Biophys. Res. Commun. **81**:1000–1010.
25. **Kung, H. F., and H. Weissbach.** 1980. Further characterization of L-factor, a protein required for β-galactosidase synthesis. Arch. Biochem. Biophys. **201**:544–580.
26. **Langley, K. E., M. R. Villarejo, A. V. Fowler, P. J. Zamenhof, and I. Zabin.** 1975. Molecular basis of β-galactosidase α-complementation. Proc. Natl. Acad. Sci. U.S.A. **72**:1254–1257.
27. **Lim, L. W., and D. Kennell.** 1979. Models for decay of *Escherichia coli lac* messenger RNA and evidence for inactivating cleavages between its messages. J. Mol. Biol. **135**:369–390.
28. **Mackie, G., and D. B. Wilson.** 1972. Polarity and transcription in the galactose operon of *E. coli*. Biochem. Biophys. Res. Commun. **48**:226–234.
29. **Miller, J. H.** 1972. Experiments in molecular genetics. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
30. **Morse, D. E.** 1971. Polarity induced by chloramphenicol and relief by *suA*. J. Mol. Biol. **55**:113–118.
31. **Nakamura, H., Y. Kano, D. Schlessinger, F. Imamoto, A. McPartland, and R. C. Somerville.** 1979. Translation-uncoupled transcription of promoter-proximal DNA sequences in *E. coli* strains harboring mutationally-generated constitutive promoters within genes of the *trp* operon. Mol. Gen. Genet. **172**:127–136.
32. **Nathans, D.** 1964. Puromycin inhibition of protein synthesis: incorporation of puromycin into peptide chains, Proc. Natl. Acad. Sci. U.S.A. **51**:585–592.
33. **Okuyama, A., N. Machiyama, T. Kinoshita, and N. Tanaka.** 1971. Inhibition by kasugamycin of initiation complex formation on 30S ribosomes. Biochem. Biophys. Res. Commun. **43**:196–199.
34. **Oxender, D. L., G. Zurawski, and C. Yanofsky.** 1979. Attenuation in the *Escherichia coli* tryptophan operon: role of RNA secondary structure involving the tryptophan codon region. Proc. Natl. Acad. Sci. U.S.A. **76**:5524–5528.
35. **Pastushok, C., and D. Kennell.** 1974. Residual polarity and transcription/translation coupling during recovery from chloramphenicol or fusidic acid. J. Bacteriol. **117**:631–640.
36. **Primakoff, P.** 1981. In vivo role of the *relA*$^+$ gene in regulation of the *lac* operon. J. Bacteriol. **145**:410–416.
37. **Primakoff, P., and S. W. Artz.** 1979. Positive control of *lac* operon expression *in vitro* by guanosine 5′-diphosphate 3′-diphosphate. Proc. Natl. Acad. Sci. U.S.A. **76**:1726–1730.
38. **Reznikoff, W. S.** 1976. Formation of the RNA polymerase-lac promoter open complex, p. 441–445. *In* R. Losick and M. Chamberlin (ed.), RNA polymerase. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
39. **Richardson, J. P., C. Grimley, and C. Lowery.** 1975. Transcription termination factor rho activity is altered in *Escherichia coli* with *suA* gene mutations. Proc. Natl. Acad. Sci. U.S.A. **72**:1725–1728.
40. **Schneider, E., M. Blundell, and D. Kennell.** 1978. Translation and mRNA decay. Mol. Gen. Genet. **160**:121–129.
41. **Silverstone, A. E., R. R. Arditti, and B. Magasanik.** 1970. Catabolite-insensitive revertants of *lac* promoter mutants. Proc. Natl. Acad. Sci. U.S.A. **66**:773–779.
42. **Tanaka, T., and B. Weisblum.** 1975. Construction of a colicin E1-R factor composite plasmid in vitro: means for amplification of deoxyribonucleic acid. J. Bacteriol. **121**:354–362.

43. **Ullmann, A., E. Joseph, and A. Danchin.** 1979. Cyclic AMP as a modulator of polarity in polycistronic transcriptional units. Proc. Natl. Acad. Sci. U.S.A. **76**:3194–3197.
44. **Ullmann, A., and D. Perrin.** 1970. Complementation in β-galactosidase, p. 143–172. *In* D. Zipser (ed.), The lactose operon. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
45. **Varmus, H. E., R. L. Perlman, and I. Pastan.** 1971. Regulation of *lac* transcription in antibiotic-treated *E. coli*. Nature (London) New Biol. **230**:41–44.
46. **Welply, J. K., A. V. Fowler, J. R. Beckwith, and I. Zabin.** 1980. Position of early nonsense and deletion mutations in *lacZ*. J. Bacteriol. **142**:732–734.
47. **Yang, H. L., G. Zubay, E. Urm, G. Reiness, and M. Cashel.** 1974. Effects of guanosine tetraphosphate, guanosine pentaphosphate, and β-γ methylenyl-guanosine pentaphosphate on gene expression of *Escherichia coli in vitro*. Proc. Natl. Acad. Sci. U.S.A. **71**:63–67.
48. **Zipser, D., S. Zabell, J. Rothman, T. Grodzicker, H. Wenk, and M. Novitski.** 1970. Fine structure of the gradient of polarity in the *Z* gene of the *lac* operon of *Escherichia coli*. J. Mol. Biol. **49**:251–254.