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lac repressor blocks *in vivo* transcription of *lac* control region DNA

(*lac* operon regulation/transcriptional elongation/termination)

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ABSTRACT **Transcription of the *Escherichia coli lac* repressor gene (*lacI*) *in vivo* produces monocistronic mRNAs with discrete 3′ ends in the *lac* control region, although the DNA sequence of this region does not specify a strong termination signal of the traditional form. Direct analysis of *lac* transcripts was used to show that the DNA sequence alone does not provide the signal to end the repressor mRNA and to establish that of the proteins with specific binding sites on control region DNA only the *lac* repressor has a striking effect on the continuity of *lacI* gene transcription. RNAs with 3′ ends in the control region sequence are major mRNA species produced from a repressor-bound template, reflecting as much as a 50-fold increase over their levels in the repressor's absence. Repressor binding to the operator thus has a dual function. In addition to blocking initiation of transcription from the *lacZ* promoter, repressor serves as a termination factor by setting the length of its own transcript and separating *lacI* and *lacZYA* into two distinct transcription units.**

The gene encoding the *lac* repressor (*lacI*) in *Escherichia coli* is just upstream from and is transcribed in the same direction as the three *lac* operon structural genes *lacZ*, *-Y*, and *-A*. A control region of 84 nucleotides between the repressor coding region and the *lacZ* transcription start (see Fig. 1) contains binding sites for RNA polymerase and two proteins that regulate initiation of *lacZ* transcription, the *lac* repressor and cAMP receptor protein (CRP). When RNA polymerase engaged in *lacI* gene transcription enters the control region, it could either continue into downstream structural gene sequences, respond to a termination signal in the DNA template, or cease elongation as it encounters DNA-bound protein. What response elongating polymerase makes is a question of general interest as well as a specific issue in *lac* operon regulation. Under conditions of repression, the structural gene products β-galactosidase and *lac* permease must be present in some small amount for specific uptake of lactose and its subsequent conversion to allolactose, the physiological inducer of gene expression. If *lacI* gene transcription continues into structural gene sequences, read-through transcription would provide one explanation for the basal levels of these enzymes observed in the uninduced cell (1).

The *in vivo* repressor mRNA, as isolated from cells containing the *lacI* gene on a plasmid, is a monocistronic message with discrete 3′ ends mapping to positions within the *lac* control region (2). One 3′ end point (III) is in sequences corresponding to the −10 region of the *lacZ* promoter, and a second (II) is ≈25 nucleotides upstream (see Fig. 1). Although analysis of RNA 3′ ends does not reveal how they are produced, two observations initially suggested that these 3′ ends reflect responses to termination signals in the *lac* control region DNA. An RNA with a 3′ end similar to II is obtained as a product of *in vitro* transcription (3, 4). In addition, DNA fragments from the *lacI* gene end, when cloned in a plasmid designed to measure transcription termination efficiency, lead to an 80% reduction in downstream gene expression. This effect is observed even with *lac* DNA fragments whose sequences preclude binding of *lac* regulatory proteins (2).

In this paper, we present evidence from direct examination of *in vivo* transcription patterns that *lac* control region DNA does not itself specify a strong termination signal. However, if the *lac* repressor is present on the DNA template, a greatly increased fraction of the transcripts produced have 3′ ends in *lac* control region sequences. A preliminary report of these results (5) and data of Deuschle *et al.* (6) showing the same effect of *lac* repressor at an operator sequence cloned elsewhere have been published.

MATERIALS AND METHODS

Bacterial Strains and Plasmids. Strain sources were as follows: BD1528, F⁻ *thi met nadB7 ung-1 galK rpsL supE supF hsdR⁻ hsdM⁺*, B. Duncan; LS854, *trpA9605*(Am) *his-85*(Am) *his-85*(Am) *rpoL-136* Δ(*crp-3*) *metE-70 trpR55* IN(*rrnD-rrnE*)*1* (λ⁻), B. Bachmann; and CAN20/12, *lacI⁺Z-125*(Am) *supF can rnb rnd* (λ⁺), M. Deutscher (7). Plasmid pMST190 is a pMC1403 (8) derivative containing the abbreviated $lacI^Q$ transcription unit described in *Results*; in pMST190rep, the $lacI^Q$ gene is also present elsewhere in the plasmid. The $lacI^Q$ gene was obtained from a pACYC184 derivative (pKC1700) containing the $lacI^Q$ gene from pMC7 (9) on *Eco*RI linkers. The *lac* deletion (DS70) and *galK⁻* (N100) strains as well as plasmids pMS106 and pMS190 have been described (2). Sodium bisulfite mutagenesis (10) was carried out *in vitro* on heteroduplexes formed between linearized plasmid DNAs in buffered 70% formamide (11) essentially as described (12). Treated DNA was introduced by transformation into the *ung⁻* strain BD1528. Plasmids were transferred to strain N100 for characterization, sequencing by Maxam–Gilbert methods (13), and measurements of galactokinase activity (14).

Cell Growth and RNA Preparation. Plasmid-bearing strains grown overnight at 37°C in LB broth containing ampicillin (50 μg/ml) were subcultured into LB broth or M9 medium supplemented with 0.4% Casamino acids. The carbon source was 0.2% glucose or, for *lac* operon induction, 0.2% glycerol. For the latter, mid-logarithmic cultures were diluted 1:4 and adjusted to 5 mM isopropyl β-D-thiogalactoside. At a density of 5×10^8 cells per ml, cells were collected by centrifugation, resuspended at 0°C in 5 ml of 1× lysis buffer (10 mM Tris·HCl, pH 7.5/10 mM EDTA/0.5% $NaDodSO_4$), and incubated 1 min at 80°C. For time course experiments, 5-ml samples of LB broth cultures were removed at times after addition of rifampicin to 200 μg/ml and were added to 2.5 ml of 3× lysis buffer at 65°C for immediate cell lysis. The nucleic acids were isolated by phenol extraction and ethanol pre-

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Abbreviations: CRP, cAMP receptor protein; CAP site, DNA binding site for CRP.

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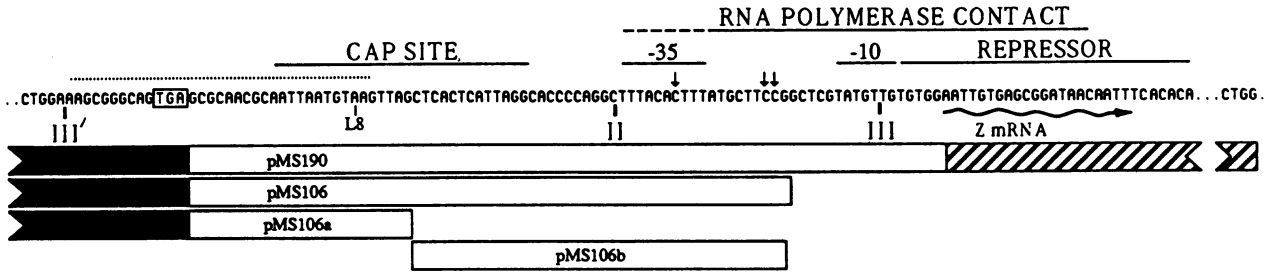

FIG. 1. *lac* control region sequences and DNA templates. Extending from the 3′ end of the *lac* repressor coding sequence through the promoter-operator, this segment of DNA contains binding sites (shown overlined) for CRP (CAP site), *lac* repressor, and RNA polymerase (16). Data shown throughout are for DNA templates containing the CAP site mutation L8, which reduces CRP stimulation by a factor of 15 (17). A secondary repressor binding site at the *lacI* gene end (18) is indicated (dotted overline). *In vivo lacI* mRNA end points discussed in the text are designated III′, II, and III. Horizontal bars represent the *lac* inserts present in the plasmid pKG1800 (14) derivatives pMS106, pMS190 (2), and two clones containing smaller segments of the 106-base-pair (bp) insert: *lacI* (solid bar), *lac* control region (open bar), *lacZ* (hatched bar). The *lacZ* promoter in the 190-bp insert has a wild-type −10 region. Arrows point to C residues that, when substituted by T residues after sodium bisulfite mutagenesis, restore full galactokinase activity. Relative to pKG1800 (100%), galactokinase levels were as follows: pMS190, 13%; pMS106, 15%; pMS106a, 100%; and pMS106b, 14%.

cipitation. The pMST190- and pMST190rep-containing strains gave equivalent yields. The *in vitro* transcript was prepared as described (15).

RESULTS

A Partial *lac* Control Region Sequence Lacks Sufficient Information to Signal *lacI* Gene Transcription Stops. To characterize putative transcription termination signals at the *lacI* gene end, plasmids pMS106 and pMS190 were constructed earlier (2) by inserting the *lac* DNA fragments diagrammed in Fig. 1 between the *E. coli gal* operon promoter and the galactokinase (*galK*) gene present in plasmid pKG1800 (14). pKG1800 has been used widely to assay the termination activity of inserted DNA fragments indirectly by their effects on downstream galactokinase activity. The 106-bp *lac* fragment lacks the repressor binding site and the −10 region of the *lacZ* promoter, while the 190-bp fragment extends 63 bp into *lacZ*. The cloned *lac* sequences are translated in the *lacI* gene reading frame up to the repressor stop codon and leave the transcription complex on the recombinant DNA template with the same potential for termination as normally exists in the *lac* operon. Both *lac* inserts lead to at least an 80% reduction in downstream *galK* expression, a result implying that termination occurs at 80% efficiency in a region between the repressor stop codon and −10 region of the *lacZ* promoter. As indicated by the results of cloning smaller DNA segments into pKG1800 (Fig. 1), a region of ≈40 nucleotides between the CAP site and the −10 region (pMS106b) suffices to give the same reduction in galactokinase activity. If these sequences are not included (pMS106a), *galK* expression returns to the level characteristic of the parent plasmid pKG1800. Point mutations in the 106-bp *lac* insert that fully restore *galK* expression cluster at the 3′ end of the same region.

If the RNAs synthesized from the pMS106 template *in vivo* are examined directly, a different picture of transcription in the *lac* control region is obtained. Specific fragments of RNA pulse-labeled with $^{32}PO_4$ were prepared by T1 RNase trimming of the RNA·DNA hybrids formed with a restriction

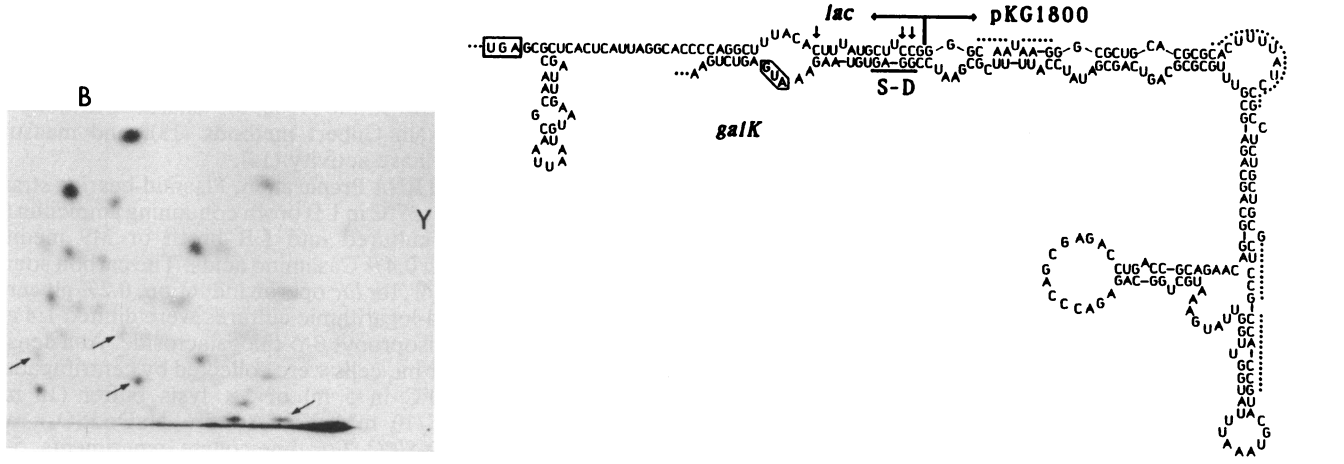

FIG. 2. T1 RNase fingerprint and predicted structure of the *lac/gal* RNA transcripts encoded by pMS106. The region containing the 3′ ends of RNAs synthesized *in vivo* from the pMS106 template was obtained by hybridizing RNAs labeled for 5 min with 5 mCi of $^{32}PO_4$ (1 Ci = 37 GBq) (2) at DNA excess to filters (GeneScreen, New England Nuclear) bearing the separated strands of the 183-bp fragment from pMS106, which spans the junction between *lac* and *gal* sequences. Hybridization was at 42°C for 16–20 hr in 5 ml of 1 M NaCl/50% formamide; subsequent washing of the filters, trimming of hybrids with RNase T1, and elution were as described (2). Horizontal dimension, electrophoresis on cellulose acetate strips at pH 3.5. Vertical dimension, chromatography on polyethyleneimine cellulose plates in homomix C. Tracking dyes: B, xylene cyanole FF; Y, orange G. The horizontal smear is ^{32}P-labeled DNA in the preparation. In the predicted structure, the *lacI* stop codon and *galK* initiator AUG are boxed, and the Shine–Dalgarno sequence is denoted S–D. Vertical arrows mark the position of C → T mutations shown in Fig. 1. Unique *gal*-specific oligonucleotides (14, 19) are indicated on the structure (dotted line) and in the fingerprint (arrows). The RNA-folding program of Zuker and Stiegler (20) was used for structure predictions.

fragment from pMS106 that includes the *lac* insert and ≈60 bp of downstream *gal* sequence. Analysis of the fingerprint (Fig. 2) reveals diagnostic oligonucleotides representing downstream *gal* sequences. As assessed by the molar yields of *lac*- and *gal*-specific oligonucleotides, 80–90% of the transcripts extend beyond *lac* sequences.

An explanation for this conflicting evidence is suggested by the extensive structure predicted to form within pMS106 sequences between the repressor stop codon and the *galK* initiator AUG (Fig. 2). The structure's core is comprised of *gal* sequences in the parent plasmid pKG1800. In the RNAs transcribed from most pKG1800 derivatives, this same core would form and perhaps provide the constant environment around the *galK* translation initiator region which generally ensures that galactokinase synthesis is proportional to read-through transcription levels (14). For the pMS106 sequence, however, as well as for pMS190 (data not shown), the structure is extended by base-pairing of nucleotides in the *galK* translational initiation site with those *lac* sequences implicated in apparent terminator function by the genetic analysis described above. The expected effect of sequestering the *galK* initiator in secondary structure would be an inhibition of translation, the consequence of which would be a reduction in *galK* expression indistinguishable from that due to upstream transcription termination.

***In Vivo* Transcription Patterns and *lac* Control Region Proteins.** *In vivo* transcription was monitored by S1 nuclease mapping to find conditions under which the previously observed *lacI* mRNA 3′ ends within the control region are formed. Since, presumably, transcription on the 106-bp *lac* template is continuous because of the absence of a required DNA sequence or additional protein(s) necessary for efficient generation of RNA 3′ ends, the plasmid DNA templates had 190-bp *lac* inserts that contained the complete *lac* control region in one of three forms: wild-type; L8, a CAP site mutation; or L8UV5, both the CAP site mutation and a mutation in the −10 region making *lacZ* transcription independent of CRP and cAMP. Growth conditions and templates were chosen to achieve the following conditions: (*i*) an unoccupied *lac* control region; (*ii*) DNA occupied by CRP and RNA polymerase, the natural state of an induced *lac* operon; (*iii*) an RNA polymerase-bound template; and (*iv*) a repressor-bound template. For the latter condition, the $lacI^Q$ gene was cloned in the same plasmid to ensure sufficient production of *lac* repressor to saturate multiple copies of the operator. The results revealed the two transcription patterns described below: one shared by the first three conditions in which repressor is not bound to the operator, and one in which transcription is on the repressor-bound template.

The data in Fig. 3*D* illustrate the pattern of RNA 3′ end points generated during transcription in the absence and presence of *lac* repressor. The 190-bp *lac* insert here has been cloned in a compact transcription unit (pMST190; Fig. 3*A*) bounded by the $lacI^Q$ promoter and the strong rho-independent termination site from the phage f1 genome (21). This has been done to reduce transcript length and precisely define the read-through RNA species. In the absence of repressor (lanes −REP), the RNA population includes the previously seen species with 3′ ends in the *lac* control region, II and III, but at trace levels. The predominant products are read-through RNAs that have stopped at the f1 terminator. End point IV, which maps a few nucleotides 5′ to the *lacZ* initiator AUG, is probably due to processing of the larger RNAs, since it is not present in RNA prepared from a strain (CAN20/12) deficient in exoribonucleases II, D, and BN. In contrast to this result, the transcripts synthesized on a *lac* repressor-bound template (lanes +REP) show at least a 50-fold increase in species with 3′ ends in the control region, as assessed by densitometric tracing. Species III, which ends at a position just upstream from the operator, is particularly enhanced.

Visualization of *lac*-Specific Transcription *in Vivo*. Further evidence that operator-bound *lac* repressor interferes with transcription through the control region was obtained using RNA blot hybridization. *In vivo* RNAs produced in the

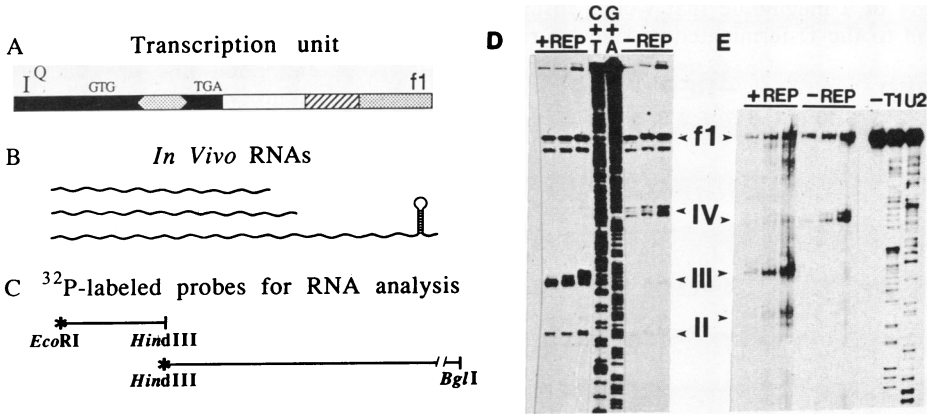

FIG. 3. Nuclease S1 and blot hybridization analysis of *in vivo* RNAs synthesized in the presence and absence of *lac* repressor. (*A*) Abbreviated *lacI* transcription/translation unit in plasmid pMST190. Origins of the major elements, designated as in Fig. 1, are as follows: promoter and translation start signal, $lacI^Q$ gene in pMC7 (9); *lacI* gene end and *lac* control region, pMS190; and backstop terminator (f1), phage f1 sequence following gene VIII (21). pKG1800 and f1 sequences are denoted by stippled bars. Translation occurs in the *lacI* gene reading frame up to the repressor UGA stop codon. (*B*) *In vivo* RNAs. f1-terminated transcript, 386 nucleotides. (*C*) ^{32}P-labeled probes for RNA analysis. DNA fragments were labeled by repair synthesis with [α-^{32}P]TTP using the Klenow fragment of DNA polymerase I. (*D*) Nuclease S1 mapping of RNA 3′ ends. RNA samples were from DS70 derivatives bearing pMST190 (lanes −REP) or pMST190rep (lanes +REP) cultured under inducing and noninducing conditions, respectively. Hybridizations with the end-labeled *Hin*dIII/*Bgl* I fragment were in buffered 70% formamide (11) for 16 hr at 50°C. Hybrids were treated with a range of S1 nuclease concentrations (2) and were fractionated on 8% sequencing gels in parallel with the Maxam–Gilbert cleavage products of the same DNA fragment (13). S1 nuclease concentrations in each set of lanes (in units per μg of RNA) were, from left to right, 0.5, 1.8, and 3.6. II, III, IV, and f1 indicate the protected fragments identifying RNA 3′ end points discussed in the text. The upper of two bands at the position of the f1 terminator identifies the actual 3′ end of the RNA, and the lower band is due to S1 nuclease cleavage in the DNA probe opposite the terminator stem–loop structure formed in the RNA (21). (*E*) RNA blot hybridization of *in vivo* RNAs. RNA samples as described above were fractionated on 8% sequencing gels, transferred to GeneScreen*Plus* (New England Nuclear) and hybridized with the end-labeled *Eco*RI/*Hin*dIII as recommended by the manufacturer. Each set of lanes contained, from left to right, 5, 10, and 25 μg of *in vivo* RNA. An *in vitro* transcript from the same DNA template and partial digestion products (22), labeled −, T1, and U2, respectively, are the size standards.

absence and presence of repressor in strains bearing the pMST190 transcription unit (Fig. 3*A*) were visualized by hybridization to an end-labeled DNA fragment specific for the 5′ proximal portion of the transcription unit. As shown by the autoradiogram in Fig. 3*E*, the bands visible in the blot clearly resemble the pattern of protected fragments observed by S1 nuclease mapping analysis (Fig. 3*D*). When repressor is not bound to the template, most of the RNAs are products of read-through transcription. By contrast, the RNAs with 3′ ends in the *lac* control region, II and III, comprise as much as 40% of the steady-state RNAs transcribed from a repressor-bound template.

In RNA blots, the distribution of the various *lac*-specific RNA species over time after further chain initiation has been inhibited with rifampicin suggests that repressor does not simply induce a transient pause during *in vivo* transcription. Were this the case, one should observe conversion of a paused species to the larger f1-terminated read-through product, provided that the f1-terminated RNA survives long enough to be detected. It is apparent in RNA profiles such as that shown in Fig. 4 (lanes −REP) that the f1-terminated RNA is a relatively long-lived species as well as the major product of transcription in the absence of repressor. Moreover, the number of transcription complexes present at the time of rifampicin addition appears small relative to the number of completed chains. No increase in the amount of the f1-terminated species is visible 0.3 min after rifampicin addition, even though all elongating RNA chains on the short pMST190 transcription unit should be completed within this time. When the same template is transcribed in the presence of repressor, shorter RNAs predominate over the f1-terminated product. Species III is the major RNA, but species II is visible as well. Also appearing under these conditions is another RNA (III′), which, intriguingly, has a 3′ end just upstream of the weak repressor binding site at the 3′ border of the repressor coding region (18). The abundance of III and III′ is greater than the small number of growing chains expected and certainly of a magnitude that would ensure observable conversion to the f1-terminated product, if repressor-induced pauses were eventually overcome. Significant conversion is not observed; instead, III and III′ seem to decay with increasing time. The results favor the interpretation that in the cell, the presence of bound repressor leads to transcription termination, with net production of discrete RNA species ending in the *lac* control region.

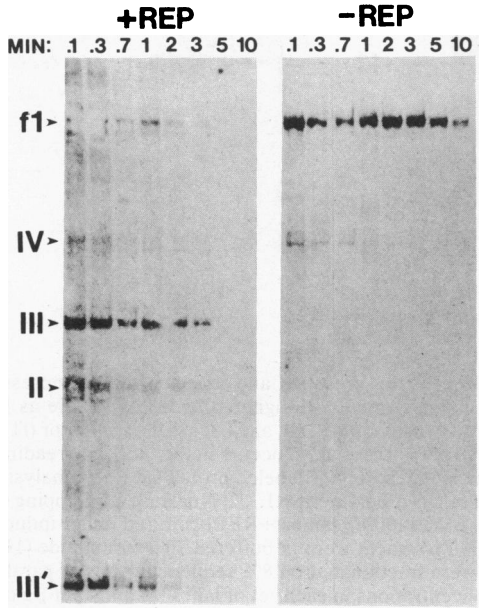

FIG. 4. RNA blots visualizing the distribution of *in vivo* RNAs over time in rifampicin-treated cultures. Each lane of the 8% sequencing gel contained RNA (9 μg) isolated at the indicated time points (0.1–10 min) after rifampicin addition (0 time) from DS70 derivatives bearing pMST190rep (+REP) or pMST190 (−REP). The two sample sets were taken in parallel through RNA blot analysis as in Fig. 3*E*.

DISCUSSION

The results provide direct evidence that *lac* repressor bound to an operator sequence interrupts transcription through the *lac* control region *in vivo* and thereby plays a key role in generating major species of *lacI* mRNA found in the cell. Although such an effect on transcription by repressor has previously been suggested from genetic studies of *trp–lac* fusions (23, 24) and more recent operator-containing constructs (25, 26), the results of RNA fingerprinting, S1 nuclease mapping, and RNA blot analysis directly visualize the relevant *in vivo* RNA species. The importance of direct methods to assay transcription products is underscored by the contradictory results obtained when transcription was monitored by downstream gene expression (2).

It is perhaps not surprising that we observe little termination on a naked *lac* control region DNA template or that a standard termination signal has not evolved here. The short region between the *lacI* gene end and the *lacZ* transcription start is densely packed with the binding sites for *lac* regulatory proteins and, depending on the carbohydrate substrate available to the cell, is normally occupied by one or more of these proteins. Any or all might be expected to impinge on the movement of RNA polymerase along this segment of DNA. The actively transcribing RNA polymerase appears under conditions of induction not to be interrupted by either CRP or other RNA polymerase molecules present on the DNA template. However, binding of the *lac* repressor to the operator is observed by both S1 nuclease mapping and RNA blot analysis to result in a markedly increased fraction of *lacI* transcripts with the previously identified end points in *lac* control region sequences (2–4) at positions immediately 5′ to the natural *lac* operator (III) and ≈25 nucleotides upstream (II). A 3′ end point just upstream from the secondary repressor binding site at the *lacI* gene end (III′) is also observed, although thus far only in experiments in which rifampicin has been added and RNA has been extracted very quickly from aliquots of the culture. The data suggest that the discrete RNA species enhanced by repressor binding are not simply reflections of polymerase colliding with repressor. Whereas two end points (III and III′) are located such that they could represent RNA termini generated at the upstream edge of bound repressor molecules, end point II cannot as easily be attributed to polymerase encountering a bound repressor. One possible explanation for end point II is that bound repressor enhances termination at this otherwise weak site by in some way altering either the DNA topology or the stability of the ternary complex. An alternative possibility, that end point II is due to the upstream polymerase encountering a polymerase bound to the P2 *lac* promoter, cannot be excluded. RNA polymerase binds to P2 in the presence of repressor but does not elongate (27). If this were the case, we might have expected to see end point II accentuated in the rifampicin experiments, even in the absence of repressor, since any polymerase bound to P2 might be trapped as an open promoter complex, which is known to block elongating polymerases *in vitro* (28).

Whereas *in vitro* studies demonstrate a repressor-induced pause in the *lac* control region that is overcome (3, 4), we observe that *in vivo* transcription in the presence of rifampicin produces long-lived RNAs that are not extended to the read-through product. The differences among the three *in vivo* transcription cases directly examined suggest a number

of factors that may determine the efficiency with which *lac* repressor acts as a transcriptional roadblock and the spectrum of RNA 3′ ends produced. These are the angle by which repressor unwinds DNA, the affinity of repressor for binding sites in a given context, whether the region containing the binding site is translated, and the effective repressor concentration. The latter would reflect the repressor/operator ratios and take into account any effects of nearby secondary repressor binding sites. Whether or not the repressor itself is translated from sequences immediately upstream from its binding sites could also influence local repressor concentration. We previously observed at least 90% termination within a *lac* control region in its natural context downstream from the $lacI^Q$ gene (2). Both pseudooperators were present, and repressor was being translated just upstream. As reported here, as little as 50% termination may occur on an abbreviated I^Q template that has the upstream pseudooperator site but is separated in the plasmid construct from the $lacI^Q$ gene producing repressor. Deuschle *et al.* (6) report 90% termination efficiency in a transcription unit that contains an isolated copy of the natural operator inserted in untranslated regions either upstream or downstream from the dihydrofolate reductase coding sequence. A $lacI^Q$ gene on a compatible plasmid provided repressor. These authors also observed two repressor-induced sites of termination, one immediately bordering the operator and a second ≈10 nucleotides upstream. Present indications thus are that while repressor interrupts transcription in several sequence contexts, there is variation in the number and spacing of the RNA 3′ ends produced. Further conclusions about the means by which repressor induces termination or how any of these factors contribute to efficiency are probably not yet warranted. The efficiency with which repressor blocks transcription in a wild-type *lac* operon in the bacterial chromosome can likewise only be predicted with caution from data for plasmid DNA templates under conditions of repressor overproduction. The *lac* operon contains the operator and two pseudooperators and produces, from just upstream, ≈10 repressor molecules (29). Interestingly, previous estimates of repressor blockage inferred from reductions in downstream *lacZ* expression (23) are compatible with the termination levels observed here.

Whatever the means by which the 3′ ends in the *lac* control region are produced, the ability of the repressor–operator complex to halt transcriptional elongation establishes a second biological function for repressor in *lac* operon regulation. Although repressor's role in blocking initiation from the *lacZ* promoter is long-known, it is now apparent that repressor also serves in a sense as a termination factor dividing the *lac* region into two distinct transcription units. Under conditions of repression, monocistronic *lac* repressor mRNA species are generated, and only to the extent that transcription proceeds past the repressor block are read-through RNAs produced. This strongly suggests that basal levels of downstream *lac* operon gene products in the uninduced cell are controlled, at least in part, by the amount of transcriptional read-through from the *lacI* gene. In light of observations that tight-binding mutants of repressor cause decreases in the basal levels of *lac* enzymes (30), it would be interesting to quantitate the effect of such mutants on terminated versus read-through transcription.

The data presented here bear on the ability of one *lac* operon regulatory protein to influence transcription at the *lacI* gene end, but also have implications on the possible function of other sequence-specific DNA binding proteins as transcriptional roadblocks. Some repressors appear to be displaced from the template by a ternary complex containing elongating polymerase, but at least one other protein has been shown to interfere with a transcribing polymerase *in vitro* (31), although termination and release of the upstream transcript may not occur. When the LexA protein is bound to its site overlapping the *uvrB* P2 promoter, RNA polymerases apparently stall and accumulate at positions immediately upstream. Only in single-round transcription experiments is a discrete RNA with a 3′ end abutting the LexA binding site visualized. In view of the number of DNA binding proteins with specific target sequences, it would not be surprising if protein blockage of transcriptional elongation proved in other instances to be a mechanism for regulating gene expression.

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