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Lactose Operon Transcription from Wild-Type and L8-UV5 *lac* Promoters in *Escherichia coli* Treated with Chloramphenicol

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In cells treated with chloramphenicol and the inducer isopropyl-β-D-thiogalacto-pyranoside, messenger ribonucleic acid transcription from the wild-type *lac* promoter was not detected. Transcription occurred from the mutant UV5-L8 promoter. The transcripts were of variable length; some included the whole *Z* gene. No major site of transcription arrest within the *Z* gene was apparent.

Chloramphenicol rapidly stops translation of RNA in susceptible *Escherichia coli*; protein synthesis ceases within a few minutes after its addition (18). Transcription continues. Some mRNA's and all rRNA's accumulate at normal or even increased rates without translation; such RNA synthesis is called "uncoupled." Not all transcription, however, is uncoupled. In certain operons, e.g., the lactose (*lac*) and the tryptophan (*trp*) operons, transcription is tightly coupled to translation, and these mRNA's are usually undetectable in cells treated with chloramphenicol (10) or other inhibitors of translation (5, 7, 9, 20). Most of the recent research on coupling (and on the analogous phenomenon of "polarity" [11, 22, 25]) has concentrated on the *trp* operon, with its five relatively short genes (19). Using the *lac* operon instead, with its very long initial *Z* gene, and measuring the size of mRNA produced in the presence of chloramphenicol, we hoped to determine whether transcription arrest occurred at discrete intragenic sites. In cells treated with chlorampenicol, we detected no transcription at all from the wild-type *lac* promoter. Transcription occurred only when the mutant *lac* L8-UV5 promoter was used. Even then we did not find discrete transcription termination sites within *Z*, although transcripts long enough to include the entire *Z* gene message were observed. Thus, the L8-UV5 promoter mutation effectively alleviated coupling.

Strains C3 and ColE1-*lac* (for a description of the strains, see Table 1) have a wild-type *lac* promoter. As expected, chloramphenicol severely inhibited *lac Z* transcription in all these strains. The amount of mRNA made after additon of chloramphenicol and isopropyl-β-D-thiogalactopyranoside did not exceed the amount made by an uninduced culture with or without chloramphenicol (Table 1). In contrast, the strains 5'-plasmid and F'UV5 contain the L8-UV5 promoter. This mutant promoter has three base pair changes: GC to AT at position −66, GC to AT at −9, and TA to AT at −8 (reference 21). In the presence of chloramphenicol and inducer, their *lac* mRNA production clearly exceeded that of the uninduced control, attaining 30 to 55% of the amount produced in the absence of chloramphenicol. This was true even in the 5'-plasmid strain, which is partially constitutive for *lac* (probably because there is only one copy of the repressor gene, but multiple copies of the operator on the plasmid). Lambda p^s, which has another "superpromoter," TA to AT at −8, behaves like L8-UV5 (see Table 1).

Figure 1 shows the result of gel electrophoresis of (^{3}H)-labeled *lac Z* mRNA isolated from strain F'UV5 *lac*. In the *absence* of chloramphenicol, nascent RNA species were observed at 30S, the position expected for *lacZYA* mRNA; at 23S, corresponding to *lacZ* mRNA (1); and at a range of smaller sizes. In the presence of chloramphenicol, a wide range of transcript sizes again was seen. No major transcription termination site (6) was apparent early in the *lacZ* gene.

These results suggest a role for the promoter in the determination of coupling because a promoter mutation allevates it. Imamoto et al. (8, 11, 22) have proposed that the RNA polymerase starting from a lambda phage promoter can be "programmed" to resist transcriptional arrest by chloramphenicol. Similar observations were made by Dütting and Hübner (7).

Some possible mechanisms are suggested by the observations that the *lac* UV5-L8 promoter is cyclic AMP (23) and guanosine-5'-diphosphate-3'-diphosphate (ppGpp) independent (20). Could a reduced cyclic AMP, caused by adding chloramphenicol, explain the difference in transcription between the two promoters? This is unlikely, because adding cyclic AMP with chlor-

TABLE 1. *lac mRNA produced in strains containing wild-type or mutant lac promotor in the presence and absence of chloramphenicol*[a]

RNA from strain	Promotor	IPTG	Chloramphenicol	Total input (10^6 cpm)	% λ p*lac* 5 hybrid	% λ hybrid	% *lac* hybrid	Normalized to +IPTG −chloramphenicol in each strain
C3	WT[b]	+	−	0.21	0.48	0.01	0.47	100
		−	−	0.13	0.07	0.03	0.04	8
		−	+	0.19	0.05	0.02	0.03	6
		+	+	0.25	0.04	0.01	0.03	6
S232	WT	+	−	0.80	0.11	0.02	0.09	100
		−	−	0.68	0.03	0.02	0.01	10
		−	+	0.64	0.02	0.02	0.00	0
		+	+	0.56	0.02	0.02	0.00	0
5′-plasmid	UV5	+	−	0.50	1.01	0.02	0.99	100[d]
		−	−	0.52	0.14	0.01	0.13	13
		−	+	0.60	0.15	0.04	0.11	11
		+	+	0.62	0.57	0.02	0.55	55
F′-UV5	UV	+	−	1.3	0.28	0.05	0.23	100
		−	−	1.2	0.02	0.04	(0.02)[c]	0
		−	+	1.2	0.02	0.04	(0.02)	0
		+	+	0.9	0.15	0.03	0.12	52
Lambda p$^+$	p$^+$	+	−	1.04			0.183[e]	100
		−	+	1.2			0.023	12
		+	+	1.2			0.074	40

[a] C3 is described in Achord (1). Strain S232, containing a ColE1 *lac* plasmid, comes from J. Carbon. The 5′-plasmid is PMB9 with a 789-base-pair fragment containing the *lac* L8-UV5 promoter and the beginning of the *z* gene (12). F′-UV5: F′ p*lacz*(L8-UV5) *lacz*$^+$*y*$^+$*A*$^+$ *proA*$^+$*B*$^+$/Δ(*lac-proAB*) B1 SuII$^+$ was from Reznikoff (21). λ p$^+$ (λ *h80* d*lac* p$^+$ *c*I857 *t68*) was from Kung (12). λ p*lac*5 [λ *c*I857 *S7* p*lac*(*I*$^-$*Z*$^+$*Y*$^-$*A*$^-$) in strain M7173] was from Miller (16). Fresh overnight cultures were diluted 1:50 into 10 ml of Casamino Acids (0.8%) with minimal salts and glycerol (0.4%), grown for three generations at 30°C to a density of 5×10^8 bacteria per ml, and prelabeled with 0.01 μCi of [^{14}C]guanosine per ml to provide labeled internal markers for gel analyses. Whenever cells were incubated in chloramphenicol, the antibiotic was added 5 min before induction to a final concentration of 100 μg/ml. Induction was by addition of 1 mM isopropyl-β-D-thiogalactopyranoside (IPTG). Five minutes later, [^{3}H]uridine, 30 Ci/mmol, was added to a final level of 50 μCi/ml. Labeling was stopped 1 min later by pouring the cultures over frozen 100 mM Tris-hydrochloride (pH 8.0) containing 200 μg of chloramphenicol per ml and 20 mM sodium azide. After centrifugation, the cells were resuspended in 0.5 ml of Tris-hydrochloride (pH 8.0), 20% sucrose, and 10 mM EDTA. Lysozyme (50 μg/ml) was added, and 5 min later 2 ml of $MgCl_2$ (25 mM), 1.25% sodium dodecyl sulfate, and 0.1 ml of diethylpyrocarbonate were added. The suspension was incubated for 5 min at 37°C to inactivate nucleases. A 2.5-ml amount of cold (−20°C) 5 M NaCl was added. After 45 min at 10,000 rpm the supernatant was carefully aspirated. Water (2.5 ml) and ethanol (15 ml) were added. The RNA was precipitated after immersion in an acetone-dry ice bath for 15 min and was collected by centrifugation; dissolved in water (1 ml), 1 M sodium acetate (pH 5.2, 0.1 ml), and 1 M NaCl (0.4 ml); and reprecipitated by 3 ml of ethanol. The RNA pellet after the second ethanol precipitation was dissolved in a small volume of water for hybridization or gel electrophoresis. For DNA-RNA hybridization, filters with DNA probes were prepared from λ p*lac* 5 carrying the *Z* gene and from λ *S7* (no *lac* sequences), as described previously (14, 16, 24). Hybridization was done in 50% formamide, 0.1% sodium dodecyl sulfate, 2× SSC (1× SSC = 0.15 M NaCl–0.15 M sodium citrate) at a temperature of 53°C, for 40 to 48 h. When investigating RNA from λ p$^+$, we first absorbed λ mRNA from the solution by preincubation with a λ *S7* filter for 2 days; only then did we add the probe for *lac* sequences. *lac*-specific counts were those remaining after subtraction of λ background (obtained by hybridization to filters containing only λ DNA). The counts given are the average of triplicate samples.

[b] WT, Wild type.

[c] Figures in parentheses indicate negative values.

[d] 5′-plasmid strain is partially constitutive for *lac*.

[e] After prehybridication to *S7* λ DNA.

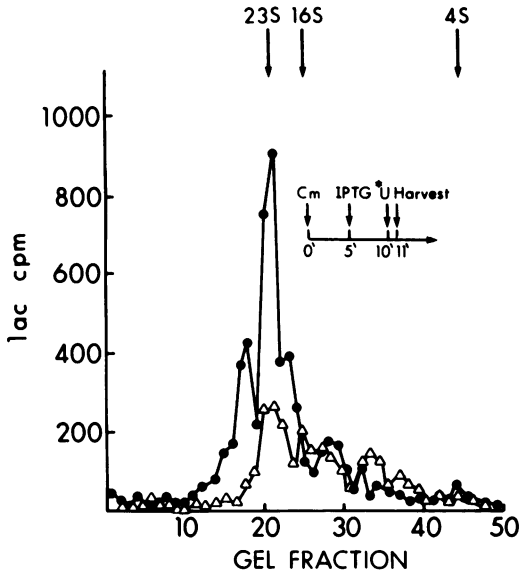

FIG. 1. *Size of mRNA produced in the presence and absence of chloramphenicol. Symbols: ●, without chloramphenicol; △, with chloramphenicol. The RNA was prepared from an F' lac L8-UV5 strain as described in the legend to Table 1, and electrophoresed on 2.2% acrylamide–0.5% agarose gels (1). After about 3 h of electrophoresis at 8 V/cm, the gels were frozen and sliced, and lac-specific sequences were detected by incubation of individual slices with DNA probes as described in the legend to Table 1. IPTG, isopropyl-β-D-thiogalactopyranoside. *U, [^{3}H]uridine. Background, i.e., hybridization to filters containing only λ DNA, was less than 30 cpm in all slices and was not subtracted.*

amphenicol does not alleviate the block of transcription from the wild-type promotor (7, 25; our own results, not shown), and because cyclic AMP formation in chloramphenicol-treated cells remains substantial (H. Rickenberg, personal communication). On the other hand, chloramphenicol rapidly depletes intracellular ppGpp (13). Primakoff and Artz (20), using a *Salmonella*-based in vitro system, have shown that ppGpp increased *lac* transcription from the wild-type promoter 20-fold, but had no effect on the already high levels of transcription from the UV5-L8 promoter. This could explain why transcription only occurs from the ppGpp-independent UV5-L8 promoter. Alternatively, there may be a direct interaction of RNA polymerase and ribosomes required for *lac* transcription in vivo (3, 17).

One can ask at what sites transcription termination occurs distal to blocked ribosomes once transcription has started. In current models for the analogous phenomenon of polarity, mRNA denuded of ribosomes can interact with the termination factor rho, which then stops RNA polymerase at a specific rho-dependent site (2). One possible termination site was observed early in the *Z* gene in transcription experiments in vitro (6). However, we did not observe such a site in vivo (see Fig. 1). Instead, a large range of sizes was observed. Most of these are very likely nascent RNA molecules, and not degraded RNA chains, since (i) all the *lac* mRNA is stabilized in the presence of chloramphenicol (9); (ii) the size distribution was similar when RNA was analyzed after different brief pulse times; and (iii) some polar *lacZ* mutants produce a similarly large range of sizes of *lac* transcripts.

Further work is required to determine how many transcripts formed in chloramphenicol-treated cells include distal *Z*, *Y*, and *A* sequences; i.e., whether the alleviation of the block at the promoter cancels all the effects of chloramphenicol on *lac* mRNA transcription. In this study, little labeled *lac* mRNA of the full "*ZYA*" size was seen; but only probes for *Z* mRNA were used, and active cleavage of the mRNA between *Z* and *Y* genes (15) could eliminate full-sized transcripts.

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