Proc. Nat. Acad. Sci. USA
Vol. 71, No. 4, pp. 1088–1092, April 1974

Translational Control Induced by Bacteriophage T7

(enzyme synthesis/uncoupled system/initiation factors/RNA shut-off/gene UV sensitivities)

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Communicated by F. Lynen, November 20, 1973

ABSTRACT **Phage T7 discontinues host gene expression by translational and transcriptional control mechanisms. Translational control is exerted by the T7 translational-repressor. This protein inhibits the synthesis of β-galactosidase (EC 3.2.1.23) *in vivo* and *in vitro* and the synthesis of the T3 enzyme S-adenosylmethioninehydrolase (EC 3.3.1.-). The translational-repressor does not interfere with T7-specific enzyme synthesis. The T7 translational-repressor purifies with the initiation factors. The repressor interacts with the initiation of translation of host enzymes. The translational-repressor gene is close to the promotor for RNA polymerase of *Escherichia coli*.**

The mechanisms involved in the control of gene expression are at the center of interest in modern molecular biology. Valuable model systems for attempts to elucidate these processes are presented by the reorientation of macromolecular synthesis, which is caused by certain virus infections. For example, after infection of *Escherichia coli* by bacteriophage T7, complex changes occur at the levels of both protein and RNA synthesis. Soon after infection the expression of the bacterial genome is inhibited (1–5) and early T7 proteins are synthesized in a defined sequence (6). In addition, later in infection the transcription of late bacteriophage genes is directed by a newly formed T7-specific polymerase (7) and the expression of early genes is prevented (1, 3).

We have previously reported that host gene expression is blocked by a T7-specific protein that prevents the induction of host enzymes (1) and that this protein is specified by an early bacteriophage gene that maps in the region between the early promotor and gene 1. Here we report an analysis of the specificity and mechanism of this control and conclude that the expression of the genetic information of either the host or of other bacteriophages is specifically inhibited after T7 infection as a result of action of a translational-repressor* at the level of initiation of translation.

METHODS

DNA-Dependent Synthesis of Enzymes in Vitro. The incubation mixtures for cell-free synthesis of phage enzymes have been described (1, 8, 9) (Fig. 1). The synthesis of β-galactosidase *in vitro* was performed essentially by the method of G. Zubay (10). However, *p*-aminobenzoic acid, pyridoxine, triphosphopyridine nucleotide, and flavine adenine dinucleotide were omitted from the incubation mixture and $MgCl_2$ and Tris·HCl were used instead of Mg-acetate and Tris-acetate.

Assay of Enzyme Activity. The tests for *S*-adenosylmethioninehydrolase (EC 3.3.1.-), lysozyme (EC 3.2.1.17), T7 RNA polymerase (EC 2.7.7.6), and DNA ligase (EC 6.5.1.1) have been described (9). The assay for β-galactosidase (EC 3.2.1.23) activity was performed as described by Zubay (10).

Infection of E. coli. E. coli strains specified in the individual experiments were grown at 30° in rich medium or M9 minimal medium to an optical density at 600 nm of 0.4. CsCl-purified T7 phage (11) were added at a multiplicity of infection of 10. One percent of the cells survived at 2 min after infection. At various times after infection, aliquots of 2–10 ml were harvested on ice, or treated otherwise as shown in figures.

Partial Purification of the Translational-Repressor. E. coli XA7007 *su*A (12) were grown in rich medium (30°) to an optical density of 0.4. The culture was divided. One-half was harvested; the other half was infected at a multiplicity of infection of 10 and harvested at 8 min after infection. From each half, 2.5 g of wet-cell paste was collected. The cells were disrupted (separately) by glass beads (9) to make a total of 26 ml of crude extract [TMA buffer: 10 mM Tris·HCl (pH 7.5), 10 mM $MgCl_2$, 22 mM NH_4Cl, 1 mM dithiothreitol, 5% (v/v) glycerol]. The crude extracts were centrifuged at 100,000 × *g* for 20 min to yield "S30" supernatant. The ribosomes were pelleted at 165,000 × *g* for 5 hr. The resulting "S100" supernatant was chromatographed on DEAE–cellulose (yielding DEAE protein) (9). The crude ribosomes were dissolved in 1 ml of TMA buffer at 750 A_{260}/ml. Ribosomes washing buffer (9 ml) was added [100 mM Tris·HCl (pH 7.5), 50 mM KCl, 10 mM $MgCl_2$, 1.1 M NH_4Cl, 5% glycerol, 1 mM dithiothreitol]. After 3 hr of stirring at 0°, the ribosomes were again pelleted at 165,000 × *g* for 9 hr. The procedure was repeated and both washing fluids were combined. The washing fluid was diluted 1:3.9 (μ = 0.3) and passed through 1 g of DEAE–cellulose that had been equilibrated with ribosome washing buffer that had been diluted 1:3.9. The nonadsorbed material was treated with 16.7 g of $(NH_4)_2SO_4$/100 ml. The precipitate was discarded; the initiation factor fraction (IF fraction) was precipitated by an additional 26 g of $(NH_4)_2SO_4$/-100 ml, redissolved in 1 ml of TMA buffer, and dialyzed against TMA buffer. This fraction contained the translational-repressor activity. The ribosomes were dissolved in TMA buffer at 750 A_{260}/ml.

The various fractions containing translational-repressor

Abbreviation: IF, initiation factor.

* We are aware that repressors are generally understood to act at the level of transcription, although the original definition does not restrict repressor action to transcription [Jacob, F. and Monod, J., (1961) *J. Mol. Biol.* **3**, 318]. We describe here a specific translational repression, not a general inhibition.

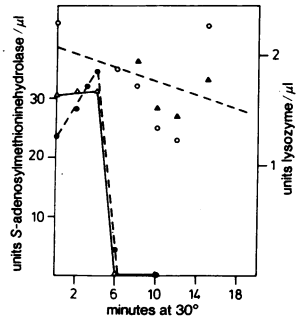

FIG. 1. Enzyme synthesis in extracts from cells at various times after T7 infection. *E. coli* XA7007 (12) were grown in rich medium to O.D.$_{600}$ = 0.4 and infected with T7 carrying an amber mutation in the lysozyme gene (6). At various times after infection, aliquots of 10 ml were harvested on ice. Extracts were prepared as described (1, 8); lysozyme, however, was omitted and the cells were disrupted by brief sonication. The incubation mixtures for protein synthesis contained in 0.05 ml: 20 μl of extract, 3.3% sucrose, 1.43 mM EDTA, 0.2% Brij 58, 59.1 mM Tris·HCl (pH 8.0), 50 mM K-acetate, 0.2 mM each of 20 amino acids, 2 mM ATP, 0.5 mM each of UTP, CTP, and GTP, 20 mM phosphoenolypyruvate, 2.5 mM dithiothreitol, 500 μg/ml of tRNA, 30 mM NH_4Cl, 50 μg/ml of DNA or 100 μg/ml of RNA, and 17 mM $MgCl_2$ (cell-sap contents were neglected). (Δ——Δ) T3 DNA-dependent *S*-adenosylmethioninehydrolase synthesis. The enzyme activity relates to units/0.2 μl of the protein-synthesis mixture. (●- - -●) *S*-adenosylmethioninehydrolase synthesis under the direction of RNA that had been isolated from *E. coli* B, 6 min after T3 infection (9). Units of *S*-adenosylmethioninehydrolase per μl. (▲- - -▲) T7 DNA-directed lysozyme synthesis. (○- - -○) T7 RNA-directed lysozyme synthesis. The RNA was isolated from *E. coli* B, 8 min after T7 infection. Similar results were obtained for synthesis of *S*-adenosylmethioninehydrolase and DNA ligase *in vitro* with Brij-extracts from cells infected with T7 carrying a missense mutation in the ligase gene. Note that the inhibition of DNA-directed synthesis of T3 *S*-adenosylmethioninehydrolase is stronger than the inhibition of T3 RNA-directed synthesis (also see Tables 1 and 2). This is due to an additional transcriptional control. A transcriptional inhibitor was separated from the translational-repressor (16).

and the corresponding control fractions from uninfected cells contained equal amounts of ribonuclease. The same can be concluded from the yields of protein synthesis in cell-free systems from uninfected and infected cells.

RESULTS

After infection with bacteriophage T7, cells lose their ability to synthesize host enzymes (1). Cell-free systems prepared from these cells are unable to support the T3 DNA-directed synthesis of *S*-adenosylmethioninehydrolase, a representative non-T7 enzyme (Fig. 1). However, they remain active in T7-specific enzyme synthesis. In the original experiments, which indicated that *S*-adenosylmethioninehydrolase formation was inhibited whereas T7 enzyme synthesis was not affected, cell-free systems prepared from infected cells were used. The results shown in Fig. 2 and Table 3 also indicate that the T7 translational-repressor affects the synthesis of enzymes when added to cell-free systems from uninfected cells. In these experiments the synthesis of either β-galactosidase, a host enzyme, or T3 DNA-directed *S*-adenosylmethioninehydrolase was specifically inhibited upon addition of T7 translational-

TABLE 1. *Partial purification of the translational-repressor of bacteriophage T7*

	Components from uninfected cells	Components from infected cells	*S*-adenosylmethionine-hydrolase (units/μl)
1	Crude extract		28
2		Crude extract	1.5
3	S30		26
4		S30	0.8
5	S100 + ribosomes		44
6		S100 + ribosomes	0.3
7	S100	Ribosomes	0.2
8	Ribosomes	S100	23.2
9	DEAE protein + crude ribosomes		45.4
10	DEAE protein	Crude ribosomes	<0.1
11	DEAE protein + 1× washed ribosomes		0.8
12	DEAE protein + 2× washed ribosomes		0.1
13	DEAE protein + 2× washed ribosomes + IF fraction		80.3
14	DEAE protein	1× washed ribosomes	0.2
15	DEAE protein	2× washed ribosomes	0.2
16	DEAE protein	2× washed ribosomes + IF fraction	0.2
17	DEAE protein + IF fraction	2× washed ribosomes	35.4
18	DEAE protein + 2× washed ribosomes	IF fraction	0.4
19	DEAE protein + IF fraction		0.2
20	DEAE protein	IF fraction	0.1
21	IF fraction + 2× washed ribosomes	DEAE protein	75.0

S-adenosylmethioninehydrolase was synthesized *in vitro* under the direction of T3 DNA in crude extracts, S30 supernatant, or further purified systems. The cellular components for synthesis of *S*-adenosylmethioninehydrolase *in vitro* were either from infected (8 min after infection) or uninfected cells, as indicated in the table. For experiments 9 to 20, a complete DEAE system (8, 9) from uninfected cells was used; however, the ribosomal fraction was omitted. Ribosomes or ribosomal subfractions were added in equivalent amounts (always corresponding to 10 mg/ml of crude ribosomes). In experiment 21, the DEAE protein was derived from T7-infected cells; all other cellular components were from uninfected cells.

TABLE 2. *Action of translational-repressor on RNA-dependent S-adenosylmethioninehydrolase synthesis in vitro*

Components from uninfected cells	Components from infected cells	*S*-adenosylmethionine units/μl
S100 + ribosomes		92
	S100 + ribosomes	11.0
S100	Ribosomes	11.6
Ribosomes	S100	80.2

Experiment was identical to the one described in Table 1 except that RNA from cells 8 min after T3 infection was used as template.

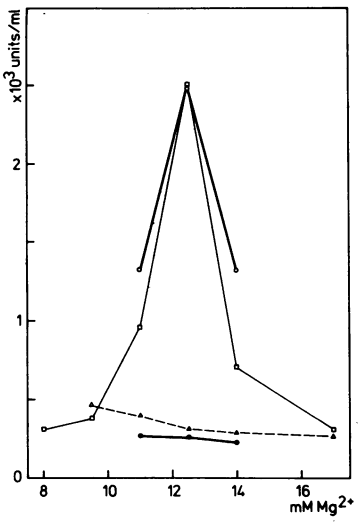

FIG. 2. Inhibition of β-galactosidase synthesis *in vitro* by the T7 translational-repressor. In a preincubated S30 system from strain 514, β-galactosidase was synthesized under the direction of lambda *plac* DNA (λ*plac* $z^+y^-i^-$ obtained from R. Ehring). Enzyme synthesis was allowed to proceed under various concentrations of $MgCl_2$. β-Galactosidase activity was determined and the units were defined as described (10). (O——O) Synthesis in the presence of 6×10^{-4} M cyclic AMP; (●——●) in the absence of cyclic AMP; (□——□) in the presence of 6×10^{-4} M cyclic AMP plus 50 μg/ml of ribosomal wash protein from uninfected *E. coli* 514; (Δ- - -Δ) in the presence of 6×10^{-4} M cyclic AMP plus 50 μg/ml of ribosomal wash protein from $T7^+$-infected *E. coli* 514.

TABLE 3. *Interference of T7 translational-repressor with enzyme synthesis in vitro*

Enzyme synthesized	IF (uninfected)	IF (infected)
β-Galactosidase (units/ml)	2×10^{-3}	0.06×10^{-3}
T3 *S*-adenosylmethioninehydrolase (units/μl)	178.5	42.5
	201*	3.5*
		2.8* (50°)
		6.8* (60°)
		63.8* (70°)
		132* (100°)
T3 lysozyme (units/μl)	33.4	21.2
	7.4*	3.4*
T7 DNA ligase (units/μl)	9015	8090
T7 lysozyme (units/μl)	40.9	35.1
	19*	9.6*

Partially purified repressor (IF fraction) was added to cell-free enzyme-synthesizing systems from uninfected cells: a DEAE system under the direction of T3 or T7 DNA was used for the synthesis of all enzymes (except for the synthesis of β-galactosidase, which was performed in an S30 system under the direction of λ *plac* DNA). 5 μg or 25 μg (*) of IF-fraction protein was added per 0.05 ml of incubation mixture. In part of the experiment, the IF fraction was heated at various temperatures (numbers in *parentheses*) for 3 min before addition to the system. Test backgrounds have been subtracted.

repressor to the uninfected cell extracts, whereas the synthesis of other enzymes, T7 DNA ligase, T7 lysozyme, and T3 lysozyme (Table 3), was inhibited much less. It is not too surprising that not all T3 enzymes are affected by the translational-repressor because the DNAs of T3 and T7 have long regions of homology (13).

In subsequent experiments, the specific inhibition of *S*-adenosylmethioninehydrolase synthesis in cell-free systems by translational-repressor was used to assess possible procedures for the purification and identification of the translational-repressor molecule. The results shown in Tables 1 and 2 indicate that the translational-repressor was sedimented with the crude ribosomes and could be liberated together with the translation initiation factors by treatment with molar ammonium chloride. The washed ribosomes had little or no translational-repressor activity. Similarly to the initiation factors, the translational-repressor was precipitated at concentrations of ammonium sulfate between 40 and 60%. The salt-precipitation and the heat sensitivity of translational-repressor activity in either crude extracts or partially purified extracts indicate that the factor is a protein (Table 3).

The translational-repressor acts at the level of translation. This was found by determining the extent of *S*-adenosylmethioninehydrolase synthesis directed by either T3 DNA or T3-specific messenger RNA in extracts from infected cells. As can be seen from the results shown in Fig. 1, enzyme syn-

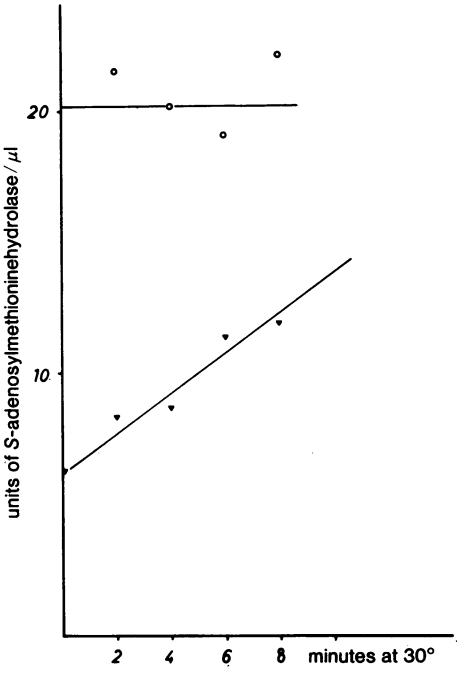

FIG. 3. Influence of translational-repressor on uncoupled enzyme synthesis. RNA synthesis on T3 DNA was allowed to proceed in a purified transcription system with *E. coli* RNA polymerase (0.025 ml of incubation mixture, 2.5 μg of DNA, 0.5 units of enzyme) (9, 19). The reaction was stopped by the addition of actinomycin after 15 min at 37°. The protein-synthesizing system was completed, and translation was permitted in the absence of transcription. At various times after the start of incubation for translation, either the IF fraction from uninfected cells (O) or the repressor-containing IF fraction from T7-infected cells (▽) was added to aliquots of the mixture and incubation was continued for 15 min.

thesis directed by either template was affected by the translational-repressor and, therefore, a direct inhibition of mRNA translation was indicated. This conclusion is also supported by the results shown in Table 2, where the translational-repressor is shown to inhibit RNA-dependent synthesis of *S*-adenosylmethioninehydrolase directly, and by those in Fig. 3. In these latter experiments partially purified translational-repressor effectively inhibited *S*-adenosylmethioninehydrolase synthesis when added after the initial DNA transcription phase of the uncoupled cell-free system. In addition, by varying the time of addition of translational-repressor to such transcription–translation uncoupled systems, it was observed that complete inhibition of *S*-adenosylmethioninehydrolase synthesis was obtained only when translational-repressor was added either before or at the start of translation (Fig. 3). It is therefore concluded that the repressor specifically acts at the level of the initiation of translation.

To obtain further information on the nature of the translational-repressor molecule and its significance in T7-infected cells, we analyzed the sensitivity of translational-repressor synthesis to UV irradiation of the infecting T7 genome. The results shown in Fig. 4 clearly indicate that the translational-repressor is a T7-specific gene product. Furthermore, determination of the kinetics of inactivation also reveals that the translational-repressor gene is located close to the promotor used by *E. coli* RNA polymerase at the left end of the T7 genome and that it is distinct from the gene that possibly controls the bacteriophage-specific inhibition of host RNA synthesis (2, 14) (for the principle of this determination, see the legend to Fig. 4) and distinct from the genes for protein-phosphokinase (15) and for the T7 transcriptional inhibitor (16).

DISCUSSION

Soon after T7 phage DNA becomes available for the direction of protein synthesis in the infected cell, various control functions are expressed: e.g. (*1*) a translational control, which specifically stops host translation, (*2*) a transcriptional interference that blocks host RNA synthesis, and (*3*) a phage-specific RNA polymerase that transcribes the phage genome.

The translational control protein, the translational-repressor, is expressed as the first detectable phage protein.

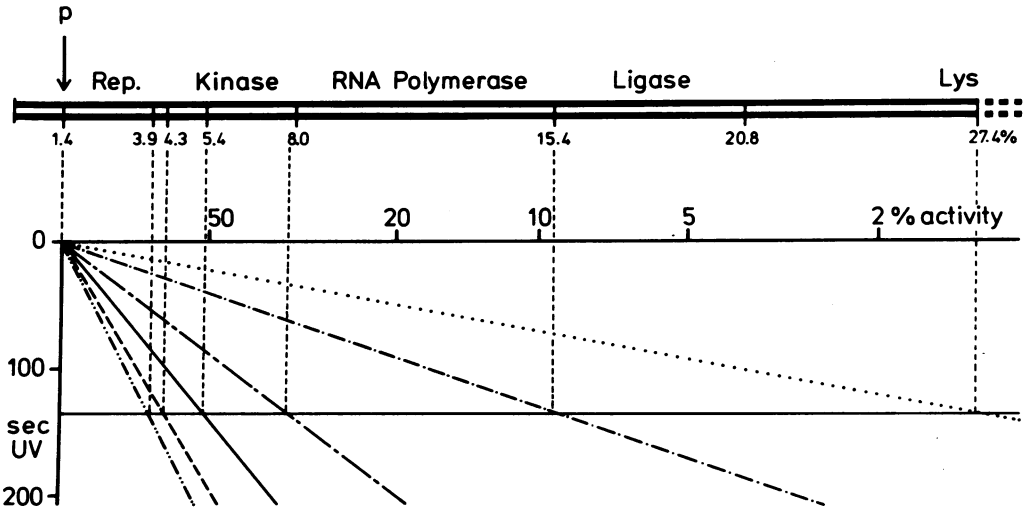

FIG. 4. The control region of phage T7. The various genes in the control region of phage T7 were mapped by measurement of the UV sensitivity of their transcription. The method follows a rationale that had been developed and used in a similar context earlier (6). The UV sensitivity of expression of a gene is correlated to the distance between the promotor and the promotor-distal end of the gene. UV causes transcriptional blocks in the DNA (20). The UV sensitivity of the expression of various functions was tested after infection of *E. coli* B_{s-1} with irradiated phage (T3 or T7) in dim yellow light. The sensitivity of *S*-adenosylmethioninehydrolase synthesis *in vivo* is the only marker of phage T3 used here. The ability to repress the induction of β-galactosidase *in vivo* and to repress the synthesis of *S*-adenosylmethioninehydrolase *in vitro* was tested as in ref. 1 and Fig. 1. The decrease in the rate of total RNA synthesis, which could represent a shut-off of RNA synthesis, was tested in *E. coli* B_{s-1} infected with gene-1$^-$ mutants: The cells were grown in M9 1% glucose at 30°. At various times after infection, 0.1 ml of culture was mixed with 0.02 ml of a solution containing 0.2 μg/ml of nalidixic acid and 1 mM uridine (labeled with ^{14}C, 0.5 μCi/μmol) and incubated further at 30° for 2 min; 0.1 ml of each sample was applied to a Whatman no. 3 filter-paper disc (23 mm in diameter). The disc was submerged into cold 10% trichloroacetic acid and the acid-precipitable cpm were determined. T7 protein phosphokinase activity in cell extracts was determined as described (15). Kinase activity was also determined by labeling proteins with phosphate *in vivo:* cells were grown in TG medium (21) that had been supplemented with amino acids (2 mM each), 50 μM phosphate, and 50 μM $MgSO_4$. Protein was pulse-labeled with [^{32}P]orthophosphate (25 μCi/ml) after infection of the cells with T7. Pulse duration was usually 5 min. The cells were chilled and washed and the pellet was disrupted by 0.1% sodium dodecyl sulfate. Aliquots were precipitated by 10% trichloroacetic acid and heated to 100° for 15 min. Radioactivity in the precipitates was measured. The average of several kinase determinations was plotted. The UV dose received by the infecting phage was plotted against remaining enzyme activity or gene-product activity that is induced by the phage. The UV data (compiled from 15 independent experiments) yield a physical map of T7. The physical map was calibrated with the genetic map by identifying the promotor-distal end of the RNA polymerase gene with 15.4% (3). The UV irradiation dose of 20 sec produces an average of 5 hits per genome. *p* = promotor used by *E. coli* RNA polymerase; *Lys* = lysozyme. (····) lysozyme activity after infection with gene-1$^-$ mutant; (—·—) rifampicin-resistant RNA polymerase; (— — —) protein phosphokinase; (——) shut-off of RNA synthesis after infection with gene-1$^-$ mutant as determined by the decrease in synthesis rate of total RNA; (– – –) repression of β-galactosidase induction *in vivo*; (–··–) repressor activity in T3 DNA-directed synthesis of *S*-adenosylmethioninehydrolase *in vitro*, and (identical curve) *S*-adenosylmethioninehydrolase synthesis *in vivo* after T3 infection.

This is a consequence of the location of the repressor gene, which is close to the promotor for *E. coli* RNA polymerase and ends at a position equivalent to approximately 4% of the T7 genome. As expected from this map position, the translational-repressor is also detected in nonpermissive cells infected with gene-1 mutants, which do not synthesize phage-specific RNA polymerase.

The results presented here indicate that the T7 translational-repressor specifically regulates translation by preventing the initiation of synthesis of host proteins and allowing T7 protein synthesis to continue. However, the mechanism of this action is not yet clear. It is possible that the translational-repressor is an initiation factor of the IF-3 type which after T7 infection may replace or modify the host initiation factor. It should be noted in this context that in the special case of infection of *E. coli* F^+ IF-3 antigenicity is reduced (17); this drastic change was not observed after productive infection of an F^- *E. coli* strain.

In addition to this translational control, host gene expression after T7 infection is possibly also inhibited at the level of transcription. This is suggested by the decrease of overall RNA synthesis after infection with a T7 gene-1 mutant. The cooperation of these various controls has complicated their analysis; possibly, as a consequence, extensive screening in our laboratory for T7 mutants defective in blocking host-enzyme induction has been unsuccessful.

The results presented here concerning the location of the RNA shut-off gene also reflect the complementary action of these control processes. Thus, by the technique of UV inactivation, the RNA shut-off gene maps at a position promotor-distal to the translational-repressor gene. Other reports (2, 4) showed that a deletion mutant between 6 and 8% displayed a defective host RNA shut-off with normal inhibition of β-galactosidase synthesis. The relatively low UV sensitivity of RNA shut-off may be explained by RNA breakdown caused by the translational-repressor mediated translational block. It is of note that similar relationships between RNA degradation and inhibition of protein synthesis have been observed previously and have been suggested as the basis for polarity in the tryptophan operon (18).

John Skehel spent much of his time helping us to write this manuscript. We cordially thank him for his effort and for stimulating discussions. We are grateful for ligase assays performed by Helmut Ponta. We acknowledge the expert assistance of Jutta Kodrzynski and the language advice of Robert Crichton.

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