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LETTER TO THE EDITOR

Fusions of the *lac* and *trp* Regions of *Escherichia coli*: Covalently Fused Messenger RNA

A bacterial strain is described in which the structural genes of the *lac* operon have been fused by deletion to the *trp* operon. It is shown that in this strain *lac* messenger RNA synthesis is controlled by the *trp* regulatory gene. DNA–RNA hybridization studies show that a fraction of the *lac* messenger RNA is covalently linked to the *trp* messenger RNA. These results demonstrate that at least a fraction of the RNA polymerase molecules which initiate in the *trp* operon continue transcribing into the *lac* genes.

Two operons are said to be fused if the genes of one operon are regulated by the controlling elements of a second adjacent operon. Operon fusion was first described in a strain with a deletion between the purine operon and the *lac z* gene, where *lac* expression is under purine operon control (Jacob, Ullman & Monod, 1965). With the transposition of the *lac* region to a site near the *trp* operon (Beckwith, Signer & Epstein, 1966), fusions between *lac* and *trp* were obtained whereby *lac* expression is determined by *trp* controlling elements. In these strains, when *trp* repressor is present (the *trp* R^+ state), the *lac* enzymes are repressed, and when *trp* repressor is inactive or absent (the *trp* R^- state), the *lac* enzymes are synthesized constitutively (Reznikoff, Miller, Scaife & Beckwith, 1969).

Control of *lac* operon expression in these fusion strains may occur in either one (or a combination) of two ways. (1) An RNA polymerase molecule could initiate transcription at the *trp* promoter and read-through into the *lac* genes, due to the deletion of the transcription stop signal at the end of the *trp* operon. (2) Transcription of *lac* could result from independent transcription initiations at the *trp–lac* junction stimulated by the approach of a *trp*-initiated RNA polymerase that does not itself proceed into the *lac* operon.

To ascertain whether read-through occurs *in vivo*, experiments were directed toward the detection of a messenger RNA species, composed partly of *trp* and partly of *lac* genetic information. These experiments were done with strain W211, in which the *lac* operon is fused to the *trp* operon in such a way that all three structural genes of the *lac* operon, *z*, *y* and *a*, and all but the *trp A* gene in the *trp* operon, are intact (Fig. 1). For hybridization studies we can use the ϕ80p*trp* phages (Morse & Yanofsky, 1969) and ϕ80p*lac* (Shapiro *et al.*, 1969) already described. Using this system, we have shown that at least a small fraction of *lac* message made in strain W211 is fused to messenger RNA from the *trp* genes, and *vice versa*.

The messenger RNA made from the *trp* operon hybridizes to the ϕ80ptrp_L strand, while the *lac* messenger RNA hybridizes to the ϕ80plac_H strand (Table 1). Although normally the *lac* and *trp* operons are oriented in the same direction on the chromosome, the ϕ80p*lac* was derived from a strain which carries *lac* in the inverted orientation (Beckwith *et al.*, 1966).

In Table 2 it can be seen that in a strain (W211) in which the *lac* and *trp* operons are fused (Fig. 1), the amount of *lac*-specific messenger RNA varies co-ordinately

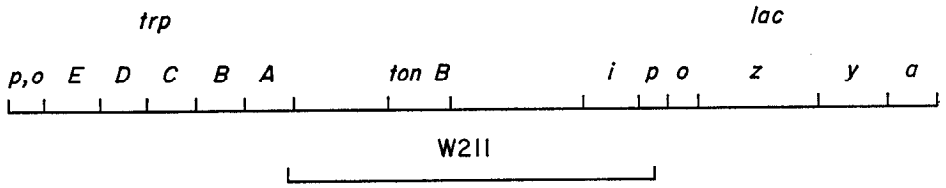

FIG. 1. Structure of the *lac-trp* fusion deletion, W211.

Deletion W211 is presumed to have the structure indicated (Reznikoff *et al.*, 1969). The strain is trp^+, but has no detectable *A* protein activity in cell extracts. Presumably, the deletion has removed only a small terminal portion of the *A* gene. By physiological tests, W211 still has the *lac* operator. With the F*lac* i^+z^- episome present, β-galactosidase levels (expressed as % fully induced wild-type levels) are in $trpR^-$ cells 100% with IPTG, 31% without IPTG and in $trpR^+$ cells 11% with IPTG, 2·3% without IPTG. To date, we have not been able to obtain recombinants of W211 with *lac* promoter mutants. The isolation of W211 will be described elsewhere (Reznikoff, Mitchell & Beckwith, unpublished results). This strain carries a deletion (XIII) of the entire *lac*, *proA*, and *proB* regions (Cuzin & Jacob, 1964).

Genes *i*, *z*, *y*, and *a* code for *lac* repressor, β-galactosidase, galactoside permease, and galactoside transacetylase, respectively. Genes *p* and *o* are promoter and operator loci for their respective operons. *tonB* codes for the receptor site for phage T1. Genes *E*, *D*, *C*, *B*, and *A* code for anthranilate synthetase, anthranilate–phosphoribosyl transferase, indoleglycerol–phosphate synthetase, tryptophan synthetase β2, and tryptophan synthetase α, respectively. Gene *R* codes for the *trp* repressor.

TABLE 1

Hybridization of lac *and* trp *messenger RNA from an unfused strain to separated strands of transducing phage DNA*

Source of RNA	DNA strand†	Cts/min hybridized	% of input
D-7011 + IPTG	ϕ80p*lac*$_H$	3454	1
D-7011 + IPTG	ϕ80p*lac*$_L$	57	0·02
D-7011 − IPTG	ϕ80p*lac*$_H$	112	0·03
D-7011 − IPTG	ϕ80p*lac*$_L$	21	0·01
D-7011 ($trpR^-$)	ϕ80p*trpE-A*$_L$	3421	1
D-7011 ($trpR^-$)	ϕ80p*trpE-A*$_H$	105	0·04
trp deletion	ϕ80p*trpE-A*$_L$	33	0·01
trp deletion	ϕ80p*trpE-A*$_H$	28	0·01
D-7011 ($trpR^-$)	ϕ80p*trpB-A*$_L$	1784	0·5
D-7011 ($trpR^-$)	ϕ80p*trpB-A*$_H$	188	0·06
trp deletion	ϕ80p*trpB-A*$_L$	120	0·04
trp deletion	ϕ80p*trpB-A*$_H$	98	0·03

† Subscript H or L following phage indicates whether it is the heavy or light strand in the CsCl centrifugation technique for separating DNA strands.

RNA input was 350,000 cts/min per tube.

D-7011 is an *E. coli* Hfr Cavalli derivative which carries wild-type *lac* and *trp* operons in their normal positions. It also carries a constitutive allele ($trpR^-$) of the *trp* regulatory gene (Yanofsky & Ito, 1966). The *trp*-deletion strain carries a *tonB* deletion which removes the entire *trp* operon (Yanofsky & Ito, 1966). The ϕ80p*lac* plaque-forming phage carries intact *lac i* and *z* genes and a portion of the *y* gene (Shapiro *et al.*, 1969). The ϕ80p*trpE-A* (carrying the entire *trp* operon) and ϕ80p*trpB-A* (carrying the *trpB* and *trpA* genes) were provided by C. Yanofsky and have been described elsewhere (Morse & Yanofsky, 1969). Plate lysates of phage, the phage purification, and the DNA extraction and strand separation are described elsewhere (Arditti *et al.*, 1970; Eron, Arditti, Zubay, Connaway & Beckwith, 1971).

Labeling of mRNA and extraction of RNA. Cells, grown to saturation in Tryptone–yeast extract at 37°C, were inoculated into a medium containing 0·4% glycerol, 0·2% Casamino acids, 0·005% thiamine, 0·05% tryptophan, and 0·05% proline in a phosphate buffer, and grown overnight at 37°C either in the presence or absence of 10^{-3} M-IPTG. The cultures were then re-inoculated into the same medium, grown, and at a cell density of 4×10^8/ml., [^{3}H]uridine (20 Ci/mole, Schwarz

TABLE 2

Trp *and* lac *message in fused and unfused strains*

Bacterial strain	*trpR*	IPTG Present	Cts/min hybridized to DNA of $\phi 80 plac_H$	$\phi 80 ptrpE\text{-}A_L$	$\phi 80 ptrpB\text{-}A_L$
W211	−	−	1220	3014	1460
W211	+	−	181	354	180
D-7011	−	−	112	3655	1821
D-7011	−	+	3454	3421	1786

Total input of counts was 350,000.

with the amount of *trp* mRNA. The inducer, isopropyl-β-D-thiogalactoside, is not necessary for high levels of *lac* transcription. In contrast, in a control strain (D-7011), in which the *lac* and *trp* operons are unfused and at their normal locations, there is essentially no *lac* mRNA synthesis when the *trp* genes are derepressed (*trp* R^- state). Synthesis of *lac* mRNA is controlled, as expected, by IPTG†.

To determine whether any of the *lac* mRNA in the fusion strain W211 is part of a

† Abbreviation used: IPTG, isopropyl-β-D-thiogalactoside.

Bioresearch) was added to a final concentration of 10 mCi/ml. Cultures were further incubated for 75 sec, during which time the rate of [^{3}H]uridine uptake into trichloroacetic acid-precipitable material was maximal, and then poured over 2 vol. of crushed, frozen buffer containing 0·01 M-Tris (pH 7·8), 0·005 M-$MgCl_2$, 0·01 M-sodium azide. After warming to 4°C, the cells were centrifuged and then resuspended in either 1 ml. of 0·01 M-potassium acetate (pH 5·2) or 1 ml. of 0·01 M-Tris (pH 7·9) plus 0·01 M-EDTA. To each was added lysozyme (Sigma, grade I) to a final concentration of 400 μg/ml. After 5 min at 4°C, sodium dodecyl sulfate (Fischer, twice recrystallized) was added to a final concentration of 2%, and the mixtures were shaken gently at 4°C for 15 min, at which time 2 ml. of 1 M-potassium acetate (pH 5·2) was added. The first solution without EDTA was extracted 3 times with phenol (freshly distilled) at 60°C. The second solution with EDTA was extracted 3 times with phenol at 20°C. This latter "cool phenol extraction" was attempted since hot phenol apparently degrades RNA (Tocchini-Valentini, personal communication; Morse, unpublished results). The aqueous phase of both preparations was then dialyzed overnight at 4°C against 2 changes of 100 vol. of 2 × SSC (SSC is 0·15 M-NaCl and 0·015 M-sodium citrate). The hot-phenol extraction yields twofold more RNA than the cool-phenol extraction. The hybridization background (i.e. % of RNA retained on filters following hybridization reaction in the absence of DNA) of the hot phenol-extracted RNA is 1·5% of the total input in the absence of RNase treatment and 0·01% in the presence of RNase treatment. The background of the cool phenol-extracted RNA is 1·5% in the absence of RNase and 0·1% in the presence of RNase. Trichloroacetic acid-precipitable label extracted by these procedures was more than 99% sensitive to heat-treated RNase (pancreatic ribonuclease, Worthington), but not at all to DNase (deoxyribonuclease I, Worthington).

DNA–RNA hybridization. Hybridization was carried out in liquid with 5 μg of DNA separated strands per ml. of 2 × SSC for 4 hr at 65°C, by which time it was 85% complete. Under these conditions (Nygaard & Hall, 1963), the rate of hybridization was maximal at DNA concentrations exceeding 2 μg/ml. and was linearly dependent on the total RNA input. The hybridization solutions were chilled to 4°C following the 4-hr incubation, and DNA–RNA hybrids were collected on nitrocellulose filters (Bac-T-flex B6, Schleicher & Schuell Co., 25 mm diameter). When the hybrids on the filters were to be directly assayed and not eluted (see also Tables 3 and 4), the filters were washed, treated with RNase, and rewashed as explained elsewhere (Gillespie & Spiegelman, 1965) except that a 15-ml. wash was used instead of 100 ml. The filters were heat-dried and counted in toluene liquifluor. In Tables 1 to 4, "cts/min hybridized" represents total cts/min hybridized minus the hybridization background (0·01% of total input, except for cool phenol-extracted RNA in Tables 3 and 4, where it is 0·1% of the total input of counts).

TABLE 3

Amount of trp *mRNA fused to* lac *mRNA*

RNA extracted from	Extraction procedure	First hybridization: RNA hybridized to	First hybridization: Cts/min hybridized	Second hybridization: RNA eluted from $\phi 80 p lac_H$ DNA and hybridized to	Second hybridization: Cts/min hybridized	% *trp* mRNA fused to *lac* mRNA
W211	cool-phenol	$\phi 80 p lac_H$	6098	$\phi 80 p lac_H$	5033	—
(*trpR*$^-$)		$\phi 80 p trpE\text{-}A_L$	15,066	$\phi 80 p trpE\text{-}A_L$	620	4
fused *lac-trp*		$\phi 80 p trpB\text{-}A_L$	6984	$\phi 80 p trpB\text{-}A_L$	570	8
,,	hot-phenol	$\phi 80 p lac_H$	6075	$\phi 80 p lac_H$	5312	—
		$\phi 80 p trpE\text{-}A_L$	12,031	$\phi 80 p trpE\text{-}A_L$	506	4
		$\phi 80 p trpB\text{-}A_L$	5637	$\phi 80 p trpB\text{-}A_L$	406	7
D-7011	hot-phenol	$\phi 80 p lac_H$	7457	$\phi 80 p lac_H$	6070	—
(*trpR*$^-$)		$\phi 80 p trpE\text{-}A_L$	7088	$\phi 80 p trpE\text{-}A_L$	63	1
unfused +IPTG		$\phi 80 p trpB\text{-}A_L$	3568	$\phi 80 p trpB\text{-}A_L$	54	1·4

The % fused RNA is already corrected for the carry-over and for the hybridization efficiency.

In the "first hybridization," the amounts of *lac* and *trp* mRNA in the phenol-extracted solutions were measured by hybridization followed by RNase treatment as described in Table 1. In addition, a portion (three times the volume of that assayed directly by filtration with RNase treatment) of the $\phi 80 p lac_H$ DNA–RNA hybrid solution was filtered separately without RNase treatment, washed with 100 ml. of 2 × SSC, and the RNA eluted. Elution was accomplished by immersing the filters in 1 ml. of 0·01 × SSC in a 1-ml. glass-stoppered vial and heating for 15 min at 95°C. The vial was then quickly cooled and the filter removed. A 0·1 vol. of 0·5 M-Tris (pH 7·4) and 0·04 M-$MgCl_2$ was added, as well as DNase I (Worthington, electrophoretically purified from RNase) to a final concentration of 10 μg/ml. The mixture was incubated for 20 min at 37°C with no loss of trichloroacetic acid-precipitable label. The reaction was terminated by adjusting the solution to 0·01 M-EDTA, 2 × SSC, and 5% phenol, and heating 10 min at 95°C to denature the DNase. Following elution of this fraction, the eluted RNA sample was divided into 3 equal portions and rehybridized to separated strands of DNA (5 μg/ml.) from $\phi 80 p lac_H$, $\phi 80 trpE\text{-}A_L$, and $\phi 80 p trpB\text{-}A_L$, as shown in the column under "second hybridization". After 4 hr incubation at 65°C, hybrids were collected on filters, washed, treated with RNase, and rewashed as in Table 1. Total radioactivity of extracted RNA used in the first hybridization was 2,000,000 cts/min for experiments with W211 RNA and 700,000 cts/min for D-7011 RNA.

TABLE 4

Amount of lac *mRNA fused to* trp *mRNA*

RNA extracted from	Extraction procedure	First hybridization RNA hybridized to	First hybridization Cts/min hybridized	Second hybridization RNA eluted from ϕ80p*trpE-A*$_L$ DNA and hybridized to	Second hybridization Cts/min hybridized	%*lac* mRNA fused to *trp* mRNA
W211 (*trpR*$^-$) fused *lac-trp*	cool-phenol	ϕ80p*trpE-A*$_L$	10,048	ϕ80p*trpE-A*$_L$	6897	—
		ϕ80p*lac*$_H$	4922	ϕ80p*lac*$_H$	516	11
	hot-phenol	ϕ80p*trpE-A*$_L$	20,140	ϕ80p*trpE-A*$_L$	14,332	—
		ϕ80p*lac*$_H$	9800	ϕ80p*lac*$_H$	1050	11
D-7011 (*trpR*$^-$) unfused +IPTG	hot-phenol	ϕ80p*trpE-A*$_L$	22,307	ϕ80p*trpE-A*$_L$	20,004	—
		ϕ80p*lac*$_H$	24,057	ϕ80p*lac*$_H$	179	0·7

The experiments follow the same principles as in Table 3. Total radioactivity of extracted RNA used in the first hybridization was 1,000,000 cts/min for cool-phenol extraction and 2,000,000 cts/min for hot-phenol extraction.

trp mRNA molecule, and *vice versa*, we have carried out the following procedure. A pulse of [^{3}H]uridine is incorporated into RNA and the RNA extracted from the cell. This RNA is hybridized to the ϕ80p*lac*$_H$ DNA. If there is any fused mRNA then a portion of *trp* mRNA should be carried along with the *lac* mRNA which hydridizes to ϕ80p*lac*$_H$. There would then be a DNA–RNA hybrid for the *lac* region with an unhybridized single-stranded RNA tail representing a portion of the *trp* region. We have eliminated the RNase treatment of the DNA–RNA hybrid since this would also remove the single-stranded unhybridized *trp* mRNA tail.

The RNA from the non-RNase-treated complex is then eluted and hybridized to ϕ80p*trp* strands to determine whether any *trp* mRNA was covalently bound to the *lac* RNA–DNA hybrid. To ensure that the *trp* mRNA counts seen were not due to non-specific sticking of the *trp* mRNA to the filter or to the ϕ80p*lac* DNA, the same experiment was done with a control strain, in which the *lac* and *trp* operons were not fused and were at their normal positions on the *E. coli* chromosome. The same principle was used to determine whether any *lac* mRNA is carried along with *trp* mRNA.

The results in Tables 3 and 4 show that there is a significant percentage of *trp* mRNA attached to *lac* mRNA and *vice versa*. There is also an apparently higher percentage of the operator-distal *trpB-A* mRNA attached to *lac* mRNA than of the total *trpE-A* mRNA. The non-specific counts carried over when the control strain (D-7011) is used are always considerably lower (5- to 12-fold) than those seen in the fused strain.

These results demonstrate the existence of a single mRNA species which contains genetic information from both the *lac* and *trp* operons. However, the percentage of messages which contain substantial amounts of both *lac* and *trp* genetic information is small, 4 to 11% (Tables 3 and 4). We can consider several explanations for this low figure. (1) Only a fraction of the *lac* messages made are due to read-through transcription from the *trp* operon. The majority of the *lac* messages may result from independent transcription initiations stimulated by the read-through. (2) The *trp* and *lac* mRNA is synthesized in pieces of monocistronic messenger. In this case the fused mRNA that we detect is messenger initiated on the *trp A* gene and extending to the end of the *z* gene, because the *A* gene transcription stop signal is deleted in the fusion strain. However, Morse (unpublished results) has found that between 10 and 20% of the mRNA of one gene in the *trp* operon is covalently linked to the mRNA of adjacent genes in the same operon. Thus *trp* mRNA is at least in part polycistronic. (3) Extraction or hybridization procedures may cause substantial breakage of mRNA molecules, so that we would only recover a fraction of the original polycistronic mRNA molecules which existed inside the bacterial cells. However, different extraction procedures using hot phenol or cool phenol (Tables 3 and 4) and hybridization at lower temperatures (Bonner, 1967) yield RNA with essentially identical linkage of *trp* and *lac* mRNA. (4) The normal *in vivo* degradation of nascent *trp* mRNA proceeds so rapidly from the 5′-end that most mRNA molecules may exist only as relatively short growing chains (Morse, Mosteller, Baker & Yanofsky, 1969).

Consistent with either of the latter three explanations is the finding that the fusion of *trp* to *lac* mRNA is greater for the operator-distal *B-A* segment of *trp* (closest to *lac*) than for the entire *E-A* transcript (Table 3). If *trp* mRNA is assumed to be polycistronic *in vivo*, the low linkage (10 to 20%) of adjacent cistrons on the *trp* transcript (Morse, unpublished results) may be due to breakage of RNA during extraction and hybridization or by rapid degradation of RNA *in vivo*. Then, the *trp-lac* mRNA may

for the same reasons also be polycistronic *in vivo* despite the low figure (4 to 11%) for linkage of *trp* to *lac* mRNA in the fusion strain. Because of the low fraction of counts in the fused messengers, these experiments do not indicate whether the fused message is monocistronic or polycistronic. Direct demonstration of a polycistronic messenger RNA molecule may then be very difficult, if the results are explained by breakage or degradation of RNA.

The experiments described in this paper have shown that in a *trp-lac* fusion strain (W211), mRNA can be isolated in which *trp* mRNA is covalently linked to *lac* mRNA, thereby directly demonstrating that an RNA polymerase molecule that initiates in the *trp* operon can continue transcribing into the *lac* genes.

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REFERENCES

Arditti, R., Eron, L., Zubay, G., Tocchini-Valentini, G., Connaway, S. & Beckwith, J. (1970). *Cold Spr. Harb. Symp. Quant. Biol.* **35**, 437.
Beckwith, J., Signer, E. & Epstein, W. (1966). *Cold Spr. Harb. Symp. Quant. Biol.* **31**, 393.
Bonner, J. (1967). *Biochemistry*, **6**, 3650.
Cuzin, F. & Jacob, F. (1964). *C. R. Acad. Sci. Paris*, **258**, 1350.
Eron, L., Arditti, R., Zubay, G., Connaway, S. & Beckwith, J. (1971). *Proc. Nat. Acad. Sci., Wash.* **68**, 215.
Gillespie, D. & Spiegelman, S. (1965). *J. Mol. Biol.* **12**, 829.
Jacob, F., Ullman, A. & Monod, J. (1965). *J. Mol. Biol.* **13**, 704.
Morse, D., Mosteller, R., Baker, R. & Yanofsky, C. (1969). *Nature*, **223**, 40.
Morse, D. & Yanofsky, C. (1969). *J. Mol. Biol.* **41**, 317.
Nygaard, A. & Hall, B. (1963). *Biochem. Biophys. Res. Comm.* **12**, 98.
Reznikoff, W., Miller, J., Scaife, J. & Beckwith, J. (1969). *J. Mol. Biol.* **43**, 210.
Shapiro, J., MacHattie, L., Eron, L., Ihler, G., Ippen, K. & Beckwith, J. (1969). *Nature*, **224**, 768.
Yanofsky, C. & Ito, J. (1966). *J. Mol. Biol.* **21**, 313.

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