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IN VIVO EVIDENCE FOR *nusA* AND *nusB* GENE FUNCTION IN GENERAL TRANSCRIPTION OF THE *ESCHERICHIA COLI* GENOME

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SUMMARY The requirements for *nusA* and *nusB* gene products for the effective transcription of *E. coli* genes, including the *trp* operon, were demonstrated by analysis of RNA transcripts produced in *nusA*, *nusB* and *nusAnusB* mutants. In the *nusA1* mutant, the levels of overall synthesis of both bulk RNA and *trp* mRNA were reduced more at 37 C than at 30 C, consistent with the idea of temperature-sensitive *nus* protein function (Friedman et al., 1973). In the *nusB27* and *nusA1nusB27* mutants (Friedman et al., 1976), decrease in the overall rates of these RNA synthesis was notable even at 30 C. In *nus* mutants harboring *trpΔLD1412*, in which the *trp* operon attenuator site is deleted, the reduction in the *trp* mRNA level was less severe. Thus, the effect of *nus* mutations seems to result in part from more frequent transcription termination at the attenuator site. Also in *nusA1nusB27* mutants, in which the distal portion of the *trp* operon is deleted, the *trp* mRNA level was not reduced much. Thus, transcription in the *nus*⁻ mutants seems to be frequently and prematurely arrested at sites along the *trp* operon.

INTRODUCTION

Transcription of the bacteriophage λ genome beyond certain stop signals is thought to require a multi-protein complex containing at least RNA polymerase, the phage gene *N* product, and the host bacteria-specified *nusA* protein (Friedman et al., 1973; Greenblatt et al., 1980; Ishii et al., 1980; Ishii, S., Maekawa, T., Itamura, S., Kuroki, K., Kano, Y., and Imamoto, F., unpublished data). However, effective anti-termination also seems to require other host functions. For example, when bacterial mutants are isolated with mutations that interfere with antitermination, these mutations are mapped not only in the *nusA* gene and in the RNA polymerase β subunit at 89 min (Georgopoulos, 1971; Ghysen

and Pironio, 1972; Baumann and Friedman, 1976; Sternberg, 1976), but also in the *nusB* gene at 11 min (Friedman et al., 1976), in the *groNB* gene at 9 min (Georgopoulos, et al., 1980), in the *nusC* gene at 88 min (cited in Friedman et al., 1981), in the structural gene for ribosomal protein S10 (*nusE*) at 72 min (Friedman et al., 1981), and in the σ subunit of *E. coli* RNA polymerase at 66 min (Nakamura et al., 1979). He is unknown how many of these gene products, including ribosomal proteins, are directly involved in transcription, and how they act.

In attempts to study the transcription process in more detail, some groups have studied the effects on bacterial RNA formation of gene products that affect λ transcription termination. In a hybridization system, with the *trp* operon under λ phage promoter control, P_L-promoted transcription of distal *trp* mRNA in the absence of *N* function was frequently terminated at least two sites in the *trpD* and *trpC* genes (Nakamura et al., 1978). In contrast, transcription from the authentic *trp* promoter (P_{trp}) of the *trp* operon translocated into the λ_{trp} phage (as well as in uninfected cells) was apparently not arrested at these sites (Segawa and Imamoto, 1974; Ishii, S. et al., unpublished data). These observations suggested that RNA polymerase might be differentially programmed at P_L and P_{trp} promoters, making it less responsive to the arrest of transcription when it starts from the P_{trp} promoter. For example, the transcribing complex may vary according to the protein factor bound.

One factor certainly involved in promoting some bacterial RNA synthesis is the *nusA* or L factor. It was shown to stimulate the synthesis of β-galactosidase in a DNA-directed in vitro system by preventing the premature termination of transcription (Kung et al., 1975; Kung and Weisbach, 1980). L factor also stimulated the *in vitro* synthesis of the β and β' subunits of RNA polymerase, directed by λrif^d 18 DNA template, although the synthesis of proteins from several other genes was not affected (Zarucki-Schulz et al., 1979). Consistent with this observation, there is evidence for an attenuator site that regulates the expression of the β and β' genes (Barry et al., 1979; Kajitani et al., 1980; Barry et al., 1980). A comparison of physical, immunological and biochemical properties has shown that L factor is the product of the *nusA* gene (Greenblatt et al., 1980).

In this report, we show that effective transcription of the bacterial *trp* operon requires functional products of both the *nusA* and *nusB* gene.

MATERIALS AND METHODS

1. *Bacterial and phage strains*

Strains bearing the *nus*$^+$ (K37) and *nus*$^-$ (*nusA*1, *nusB*27 and *nusA*1*nusB*27) alleles (Friedman et al., 1978) were kindly donated by Dr. D. I. Friedman. The *nus*$^+$ and *nus*$^-$ strains, which bear a missense mutation in the *trpC* gene, *trpC*9941 (Yanofsky et al., 1971), or deletions in the *trp* operon (*trpΔLD*-1412, *trpΔED*24, *trpAD*5, *trpAD*28, *trpOAE*2 or *trpOAE*14) were constructed by PlKc transduction of these *trp* mutations into isogenic *nus*$^+$ and *nus*$^-$ strains. The *trp* operon segments carried by the deletion mutants are shown in Fig. 1. These strains were the sources of RNA for analysis of transcription.

The bacteriophage sources of DNA probes for *trp* mRNA assay by DNA-RNA hybridization were ϕ80, the nontransducing parental phage, and nondefective transducing phages ϕ80*trpED*, ϕ80*trpCBA* and ϕ80*hΔtonBtrpA*905*ΔtrpLD*142. The *trp* operon segments carried by the ϕ80*trp* phage are shown in Fig. 1.

2. *Preparation of DNA*

Phage DNA was prepared as described previously (Kano et al., 1975).

3. *Preparation of pulse-labeled RNA*

Bacteria were grown exponentially at 30 C in L-broth (Lennox et al., 1955) supplemented with L-tryptophan (50 μg/ml). The cells were precipitated and washed twice with cold minimal medium (Vogel and Bonner, 1956), supplemented with glucose (1%), and then resuspended in the same me-

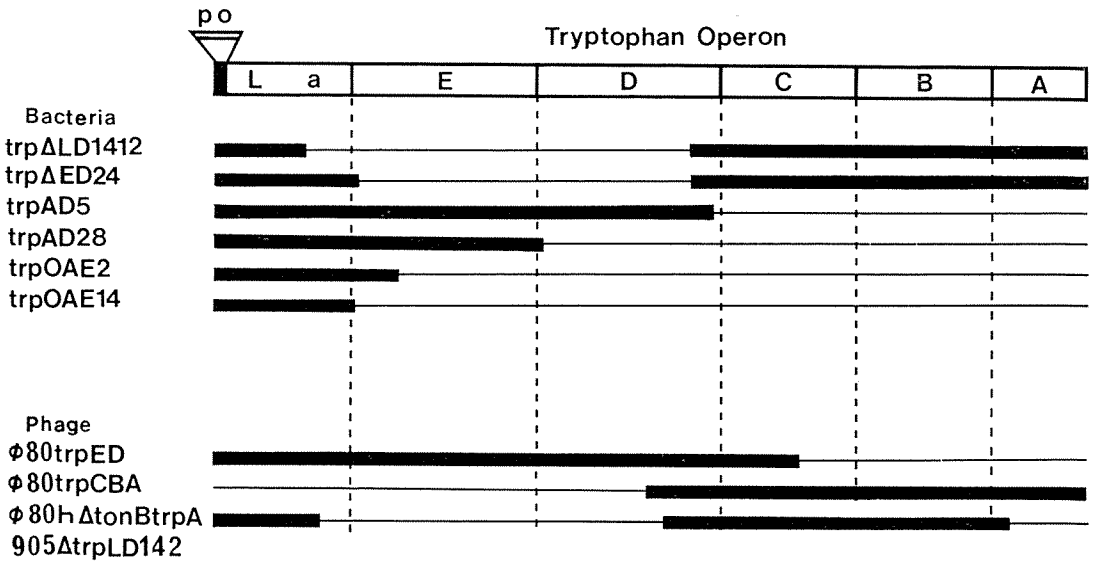

FIGURE 1. Tryptophan operon segments in deletion mutants and phages.

Regions indicated by solid lines are *trp* segments carried by deletion mutants and transducing phages. The relative sizes of *trp* structural genes are only approximate; *trpL*, the leader region, is expanded by 7.5-fold. The end-points of deletion termini of *trpΔED*24 and *trpΔLD*1412 are based on the studies reported by Bertrand et al., (1976). The deletion tremini of the *trp* operon in the bacterial strains *trpAD*5, *trpAD*28, *trpOAE*2, and *trpOAE*14, and bacteriophages ϕ80*trpCBA* and ϕ80*trpED* were based on previous studies (see Imamoto et al., 1970). The map of ϕ80*hΔtonBtrpA*905*ΔtrpLD*142 is based on the data by Bertrand et al. (1976). The regulatory elements, promoter, operator and attenuator, of the *trp* operon are abbreviated to p, o and a, respectively.

dium to a density of 3×10^{10} cells/ml. A portion of the cell suspension (containing 6×10^{9} cells) was diluted 15-fold with prewarmed minimal medium supplemented with 19 amino acids (each at 0.5 m*M*), but lacking tryptophan. The culture was shaken vigorously at 30 C or 37 C. After incubation for 8 min at 37 C or 10 min at 30 C. the cells were pulse-labeled with 100 μCi of [^{3}H]uridine (17 Ci/mmole)/ml for 1 min at 37 C or 2 min at 30 C. After pulse-labeling, the cell suspension was poured onto 27 ml of crushed frozen medium containing 0.01 *M* Tris-HCl buffer (pH 7.3), 0.005 *M* $MgCl_2$, 0.01 *M* NaN_3, and 150 μg/ml of chloramphenicol. RNA was extracted with phenol as described previously (Imamoto, 1969). The RNA obtained was filtered through a Millipore filter (type HA, 0.45 μm pore size, 25 mm diameter), precipitated with ethanol, and redissolved in H_2O.

4. *DNA-RNA hybridization*

The hybridization procedure was as follows: Phage DNA was diluted to a concentration of 100 μg/ml with 1×SSC, heated in boiling water for 11 min and then rapidly cooled in ice water. The DNA was then further diluted to 8 μg/ml with 6× SSC, and 5 ml was filtered through a Millipore filter. The filter was washed with 10 ml of 6×SSC, cut into 8 sectors, and dried at 80 C for 2 h. DNA-RNA hybridization was carried out by immersing the sectors in 100 μl of [^{3}H]RNA solution in 10^{-2} *M* Tris-HCl buffer (pH 7.3), containing 0.5 *M* KCl and 10^{-3} *M* Na_2EDTA, and incubating them at 66 C for 18 h. Then the filters were treated with RNase (5 μg/ml) in 1×SSC at 37 C for 30 min, washed with 1×SSC, dried and counted in toluene-based scintillation fluid. The total redioactivity of [^{3}H] uridine incorporated into RNA (total [^{3}H]RNA) was measured in material precipitated with cold trichloroacetic acid.

5. *Reagents*

[^{3}H]uridine was purchased from the Daiichi

TABLE 1. *Comparison of the rates of RNA synthesis in nus mutans of strain trpC9941.*

Temperature of assay (C)	Strain	Radioactivity incorporated (cpm)			Relative ratio of *trp* mRNA to bulk RNA (%)	
		bulk RNA	*trpED* mRNA	*trpCBA* mRNA	*trpED* mRNA	*trpCBA* mRNA
		($\times 10^{-2}$)				
30	*nus*$^+$	2,995 (100)	5,463 (100)	2,275 (100)	1.82 (100)	0.76 (100)
	*nusA*1	2,526 (84)	4,465 (82)	1,576 (69)	1.77 (97)	0.62 (82)
	*nusB*27	1,475 (49)	2,003 (37)	1,196 (53)	1.36 (75)	0.81 (107)
	*nusA*1*nusB*27	1,152 (38)	1,627 (30)	1,086 (48)	1.41 (77)	0.94 (124)
37	*nus*$^+$	3,609 (100)	6,770 (100)	2,829 (100)	1.88 (100)	0.78 (100)
	*nusA*1	2,376 (66)	3,753 (55)	1,379 (49)	1.58 (84)	0.58 (74)
	*nusB*27	1,518 (42)	2,192 (32)	1,149 (41)	1.44 (77)	0.76 (97)
	*nusA*1*nusB*27	1,210 (34)	1,622 (24)	1,245 (44)	1.34 (71)	1.03 (132)

Under standard conditions, samples of 3.7–5.1 μg of [^{3}H]RNA were hybridized with 5 μg of heart-denatured DNA from ϕ80, ϕ80*trpED* or ϕ80*trpCBA*. The differences between hybrid values with ϕ80*trpED* DNA and ϕ80 DNA and with ϕ80*trpCBA* DNA and ϕ80 DNA are represented as *trpED* mRNA and *trpCBA* mRNA, respectively. RNA values are expressed as radioactivities of [^{3}H]RNA/3×10^8 cells (bulk RNA) or radioactivities of [^{3}H]RNA hybridized with ϕ80*trp* DNA/3×10^8 cells ((*trp* mRNA) and normalized to 100% for the value of the *nus*$^+$ control (numbers in parenthesis). The *trp* mRNA values relative to bulk RNA are also listed as percentages of total radioactivity and the values normalized to 100% for the *nus*$^+$ control value are shown in parenthesis. Values are average for duplicate determinations. Other conditions are described in the Materials and Methods.

Chemical Co., Tokyo, and was used without addition of carrier. Millipore filters were purchased from the Millipore Filter Co., Bedford, Mass. RNase was used after heating at 80 C for 20 min in 0.15 *M* NaCl to inactive contaminating DNase.

RESULTS

1. *Reduction in the overall rate of RNA synthesis in the nus$^-$ strains*

In this section, we show that in vivo transcription of the bacterial genome is generally affected by mutation of the *nusA* and *nusB* genes.

The characteristics of RNA synthesis in the *trpC*9941*nus*$^-$ mutants, that bear the *nusA*1 or *nusB*27 mutation or both were investigated. The missense mutant of the *trpC* gene was employed to avoid the possible complication of repression of *trp* mRNA synthesis by accumulation of tryptophan in the cell. The rate of *trp* mRNA and bulk RNA synthesis measured by pulse-labeling with [^{3}H] uridine in steady-state transcription in tryptophan-derepressed cells is shown in Table 1. The most pronounced effect of the *nus*$^-$ mutations on RNA synthesis was that a deficiency of *nusA* or *nusB* function caused substantial decreases in synthesis of both *trp* mRNA and bulk RNA. In the *nusA*1 mutant, the overall synthesis of RNA was more severely affected at 37 C. This may be due to temperature-sensitivity of the mutated *nusA* gene product (Friedman et al., 1973; Friedman et al., 1976). In fact, growth of bacteriophage λ*cI*90 in the *trpC*9941*nusA*1 host was severely restricted at a higher temperature of 42 C, but only moderately at a lower temperature of 30 C, although the phage λN^-*nin*5 gave a normal burst size in the *nusA*1 host at the high temperature (data not shown). The effect of the combination of *nusA*1 and *nusB*27 mutations

TABLE 2. *Comparison of the rates of RNA synthesis in strain trapΔLD1412 and trpΔED24.*

Temperature of assay (C)	Strain	Radioactivity incorporated (cpm)			Relative ratio of *trp* mRNA to bulk RNA (%)	
		bulk RNA	*trpED* mRNA	*trpCB* mRNA	*trpED* mRNA	*trpCB* mRNA
		($\times 10^{-2}$)				
30	*trpΔLD*1412*nus*$^+$	3,820 (100)	1,217 (100)	4,207 (100)	0.32 (100)	1.10 (100)
	*trpΔLD*1412*nusA*1*nusB*27	1,347 (35)	694 (57)	2,520 (60)	0.52 (163)	1.87 (170)
	*trpΔED*24*nus*$^+$	2,915 (100)	1,240 (100)	3,060 (100)	0.43 (100)	1.05 (100)
	*trpΔED*24*nusA*1*nusB*27	1,704 (58)	678 (55)	2,148 (75)	0.40 (93)	1.26 (120)
37	*trpΔLD*1412*nus*$^+$	3,498 (100)	803 (100)	2,797 (100)	0.23 (100)	0.80 (100)
	*trpΔLD*1412*nusA*1*nusB*27	1,496 (43)	714 (80)	2,660 (95)	0.48 (209)	1.78 (223)
	*trpΔED*24*nus*$^+$	3,045 (100)	1,240 (100)	2,823 (100)	0.41 (100)	0.93 (100)
	*trpΔED*24*nusA*1*nusB*27	1,742 (57)	609 (49)	1,960 (69)	0.35 (85)	1.13 (122)

Under standard conditions, samples of 4.6–5.3 μg of [^{3}H]RNA were hybridized with 5 μg of heat-denatured DNA from φ80, φ80*trpED* and φ80*hΔtonBtrpA*905Δ*trpLD*142. The other conditions and representations are as described in the Materials and Methods.

on RNA synthesis very slightly, but distinctly, more severe than that of only *nusB*27. Decrease in RNA synthesis in the *nusB*27 mutant was detectable at 30 C and at a higher temperature the inhibition of RNA was only slightly greater, although some temperature-sensitive λ growth of the *nusB* gene product was observed in the *trpC*9941*nusB*27 mutant employed (data not shown; cf. Friedman et al., 1976). A similar effect of *nus*$^-$ mutations on RNA synthesis was observed in the prototrophic strains for tryptophan [K37(*nus*$^+$), K95 (*nusA*1), K37*nusB*27 and K37*nusA*1*nusB*27, originally isolated by Friedman et al., (1973; 1976)].

Changes in the rate of rRNA synthesis in the *nus*$^-$ mutants were investigated using DNA from a transducing phage φ80*rrn*, which carries a ribsomal RNA gene, as a DNA probe in DNA-RNA hybridization (cf. Kano et al., 1976). The results indicated that the apparent rate of rRNA synthesis at 37 C was reduced to 65% in the *nusA*1 mutant, 37% in the *nusB* 27 mutant and 23% in the double *nusA*1*nusB* 27 mutant of the level in the *nus*$^+$ parent strain *trpC*9941, under conditions comparable to those for the results in Table 1. Thus, rRNA synthesis was also affected by deficiency of functional *nus* gene products.

It is known that transcription is frequently arrested in the leader region of the *trp* operon at the " attenuator ", where the level of transcription is believed to be limited (Bertrand et al., 1975). Thus a deficiency of *nus* gene function could increase the frequency of early transcription termination at the attenuator site. To examine this possibility, we isolated strains with internal deletions of the *trp* operon bearing double *nus*$^-$ mutations or the *nus*$^+$ allele. In strain *trpΔLD*1412, one deletion terminus is located in the leader region immediately preceding the attenuator site and the other terminus is in the *trpD* gene (Bertrand et al., 1976). Another strain *trpΔED*24 has one deletion terminus in the operator-proximal extremity of the *trpE*, retaining the attenuator site; the other terminus is in the *trpD* gene (Bertrand et al., 1976). Typical data on *trp* mRNA and bulk RNA assay are given in Table 2. In these experiments, *trp* mRNA corresponding to the operator-distal *trp* genes was assayed with DNA from φ80*trpCB* phage which deletes the *tonB* gene and part of the *trpA* gene (Bertrand and Yanofsky, 1976), to

avoid possible complications in hybridization assay caused by RNA from the *tonB* region. The level of *trp* mRNA synthesized in these deletion mutants correlated with the location of the deletion endopoints; the overall rate of production of mRNA from the *trpC-trpB* region was about 2.5 and 3.5 times higher in the *trpΔED*24*nus*$^+$ mutant and *trpΔLD*1412*nus*$^+$ mutant, respectively, than that of mRNA from the operator-proximal short segment preceding the *trpC* gene. The amount of *trp* mRNA that was detected at 30 C in the *trpΔLD*1412 *nus*$^-$ mutant was 57% for *trpED* mRNA and 60% for *trpCB* mRNA of the level in the control *trpΔLD*1412*nus*$^+$ strain. There was some partial alleviation at 37 C of the diminution in level of *trp* mRNA synthesis in the *trpΔLD* 1412*nus*$^-$ mutant, although the reduced level of bulk RNA synthesis in this mutant was not nearly so greatly affected by the temperature shift-up. It was repeatedly observed that the relative level of *trp* mRNA produced in the *trpΔLD*1412*nus*$^-$ mutant was slightly, but distinctly, higher even at 30 C than that of the mRNA production in the *trpΔED*24*nus*$^-$ mutant. Consistent with this finding, is the significantly higher ratio of *trp* mRNA production relative to the production of bulk RNA in the *trpΔLD*1412*nus*$^-$ mutant than that in the *trpΔED*24*nus*$^-$ mutant. Thus the decreased *trp* mRNA level in the *nus*$^-$ mutants possible results in part, but not all, from increase in the frequency of transcription termination at the attenuator site.

2. *Alleviation of limitation of trp mRNA synthesis in the nus$^-$ mutants by deletions of the distal trp operon region*

The reduction of the overall rate of *trp* mRNA synthesis upon deficiency of *nus* function could result either from a decrease in the frequency of initiation of RNA synthesis or premature arrest of RNA chain elongation. To distinguish between these alternatives, we examined how the reduction in the overall rate of *trp* mRNA synthesis is affected by deletion of the promoter-distal portion of the operon. The expectation was that, if transcription is frequently arrested at sites located in the *trp* operon upon a deficiency of *nus* function while the frequency of transcriptional initiation is normal, then the deletion of the operon region containing the transcriptional termination sites would selectively alleviate diminution in the overall rate of *trp* mRNA synthesis in the *nus*$^-$ mutant. In these experiments, we employed four deletion mutants of the *trp* operon, each of which was transduced into *nusA*1*nusB* 27 and *nus*$^+$ strains. Deletions *trpAD*5 and *trpAD*28 delete the promoter-distal *trp* genes but retain the whole region for the " leader " and the *trpE* and the promoter-proximal portion of *trpED* (Fig. 1). Other deletion mutants, such as *trpOAE*2 *trpOAE*14, retain the whole " leader " region and the promoter-proximal portion of the *trpE* (Fig. 1).

The results in Table 3 show that in these deletion mutants a significant reduction in the overall rate of *trp* mRNA production at 30 C was observed on deficiency of *nus* function, but the reduction in *trp* mRNA production in the *nus*$^-$ mutants was significantly less as the operon segment retained in the mutant became shorter. Since the production of bulk RNA did not vary so much in these four double mutants, the ratio of *trp* mRNA production to bulk RNA production in the longer deletion mutant was distinctly higher than that of mRNA production in the *nus*$^-$ mutant which retains the whole *trp* operon (Table 1) or the longer operon segment, trpΔED24 (Table 2). This was seen more clearly when the levels of *trp* mRNA production at 37 C of mutants harboring the promoter-distal deletions and the intact operon were compared. As seen with *trp* mRNA production in the *trpΔLD*1412*nus*$^-$ mutant (Table 2), diminution in the *trp* mRNA level in the *nus*$^-$ mutants bearing a short *trp* operon segment was also alleviated by the temperature shift-up.

DISCUSSION

The experiments presented in this paper show

TABLE 3. *Comparison of the rates of RNA synthesis in various deletion mutants of the trp operon harboring nus mutations.*

Temperature of assay (C)	Strain	Radioactivity incorporated (cpm)		Relative ratio of *trp* mRNA to bulk RNA (%)
		bulk RNA	*trp* mRNA	
		($\times 10^{-2}$)		
30	*trpAD5nus*$^+$	1,603 (100)	1,085 (100)	0.68 (100)
	trpAD5nusA1nusB27	424 (26)	425 (39)	1.00 (147)
	trpAD28nus$^+$	1,407 (100)	738 (100)	0.53 (100)
	trpAD28nusA1nusB27	436 (31)	340 (46)	0.78 (147)
	trpOAE2nus$^+$	1,846 (100)	546 (100)	0.30 (100)
	trpOAE2nusA1nusB27	660 (36)	313 (57)	0.47 (157)
	trpOAE14nus$^+$	1,674 (100)	303 (100)	0.18 (100)
	trpOAE14nusA1nusB27	615 (37)	194 (64)	0.32 (178)
37	*trpAD5nus*$^+$	1,881 (100)	1,443 (100)	0.77 (100)
	trpAD5nusA1nusB27	867 (46)	1,096 (76)	1.27 (165)
	trpAD28nus$^+$	1,814 (100)	904 (100)	0.50 (100)
	trpAD28nusA1nusB27	980 (54)	789 (87)	0.81 (162)
	trpOAE2nus$^+$	1,695 (100)	348 (100)	0.21 (100)
	trpOAE2nusA1nusB27	1,046 (62)	438 (126)	0.42 (200)
	trpOAE14nus$^+$	2,242 (100)	305 (100)	0.14 (100)
	trpOAE14nusA1nusB27	1,214 (54)	296 (97)	0.24 (171)

Under standard conditions, samples 4.7–17.0 μg of [^{3}H]RNA were hybridized with 5 μg of heart-denatured DNA from ϕ80*trpED*. The RNA hybridized specifically with ϕ80*trpED* DNA is represented as *trp* mRNA. The other conditions and representations are as described in the Materials and Methods and Table 1.

that a mutation in either the *nusA* or *nusB* gene prevents fruitful progress of transcription of the genes. The lower overall rate of *trp* mRNA synthesis in the *nus*$^-$ mutants could result from frequent premature termination of transcription. One site of termination appears to be the " attenuator " in the leader region. Other sites could include the barriers at which transcription initiated at the P_L promoter of the fused *N-trp* operon is arrested in the absence of λN protein (Nakamura et al., 1978; Ishii et al., 1980). Intragenic transcriptional barriers associated with premature termination have also been reported in the lactose operon (deCrombrugghe et al., 1971; Kung et al., 1975), the galactose operon (Adhya and Miller, 1979), the *cro* gene of bacteriophage λ (Calva and Burgess, 1980), and the early (Darlix and Horaist, 1975) and late (Colomb and Chamberlin, 1974) regions of the T7 phage genome. Similar transcriptional barriers could exist in general in bacterial and phage DNA. Termination of RNA transcripts at intragenic transcriptional barriers has been demonstrated in most cases for in vitro transcription by *E. coli* RNA polymerase. Under normal in vivo conditions of transcription and translation, premature arrest is presumably prevented by anti-termination factors to allow efficient expression.

Intragenic termination signals are generally weak terminators. For example, in the case of the fused *N-trp* operon, termination of *trp* mRNA chain propagation in vivo at intragenic sites in the absence of *N* gene function is leaky, producing a significant amount of *trp* mRNA from the promoter-distal *trp* genes (Nakamura et al., 1978). Preferential relief

of the reduced level in *trp* mRNA synthesis, for example at high temperature in the double *nus*⁻ mutants of strain *trpΔLD*1412 (Table 2), requires further comment. Possibly RNA stem-loop structures that play a role in transcription termination (see Adhya and Gottesman, 1978) are weaker in such cases.

If transcription in the *nus*⁻ mutants is indeed frequently but inefficiently arrested at sites distributed through out the *trp* operon, one would expect to find a gradient of reduction in the *trp* mRNA level, with the orientation running in the same direction as transcription; i.e., the level of *trp* mRNA produced from the promoter-distal end of the operon would be extremely reduced. This expectation has been seen in vitro, in assays for *nus* function based on the *nus*-dependent stimulation of *trp* mRNA synthesis from *trp* operon DNA (Kuroki et al., 1982). In the S-100 extracts from *nusA*1, *nusB*27 and *nusA*1*nusB*27 mutants with deletion of the whole *trp* operon, the levels of *trpCBA* mRNA synthesized relative to *trpED* mRNA were 71%, 24% and 33%, respectively, of the level of that in the control *nus*⁺ extract. In contrast, the reduced level of *trp* mRNA in vivo in the *nus*⁻ mutants is uniform throughout the *trp* operon (Table 1, 2). Perhaps only a few early blocks (like the attenuator) are usually expressed in vivo.

While antitermination of transcription by the *N* gene product of *λ* is essentially complete for most transcriptional termination sites (Hayes and Szybalsky, 1973; Franklin, 1974; Adhya et al., 1974; Segawa and Imamoto, 1976; Gottesman et al., 1980), the function of *nus* gene products alone seems to counteract only weak termination signals. Nevertheless, the present findings emphasize that this secondary effect makes these products required in general for expression of many of the genes of *E. coli*.

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