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Effects of Rifampicin Resistant *rpoB* Mutations on Antitermination and Interaction with *nusA* in *Escherichia coli*

Ding Jun Jin[1], Michael Cashel[2], David I. Friedman[3], Yoshikazu Nakamura[4] William A. Walter[1] and Carol A. Gross[1]

[1]*Department of Bacteriology University of Wisconsin Madison, WI 53706, U.S.A.*

[2]*Laboratory of Molecular Genetics National Institute of Child Health and Human Development, Bethesda MD 20892, U.S.A.*

[3]*Department of Microbiology and Immunology The University of Michigan Medical School Ann Arbor, MI 48109, U.S.A.*

[4]*The Institute of Medical Science The University of Tokyo, P.O. Takanawa Tokyo 108, Japan*

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Rifampicin resistant (Rif^r mutations map in the *rpoB* gene encoding the β subunit of *Escherichia coli* RNA polymerase. We have used our collection of 17 sequenced Rif^r mutations to investigate the involvement of *E. coli* RNA polymerase in the antitermination systems enhancing expression of delayed early λ genes or stable RNA. We have found that Rif^r mutations affect both λ N-mediated antitermination and the cellular antitermination system involved in synthesis of stable RNA. Because NusA is involved in antitermination and termination, we also investigated the interaction of NusA and RNA polymerase by determining whether Rif^r mutations alter NusA-dependent termination or antitermination in cells with defective *nusA* alleles. We have shown that Rif^r mutations can either enhance or suppress the phenotypes of defective *nusA* alleles. Most Rif^r mutations alter the temperature range over which the *nusA1* allele supports λ N-mediated antitermination. In addition, a number of Rif^r alleles restore termination to the *nusA10*(Cs) and the *nusA11*(Ts) mutants defective in this process. Our results indicate that the region of the *rpoB* gene defined by the Rif^r mutations is involved in the antitermination process and affects the activity of the NusA protein directly or indirectly.

1. Introduction

Antitermination of transcription was first identified as an important genetic regulatory mechanism from studies on bacteriophage λ (for a review, see Friedman & Gottesman, 1983; Friedman *et al.*, 1984). Progression through the λ life cycle is controlled by two successive antitermination events, each of which enhances the transcription of genes downstream from terminators. The first antitermination event, mediated by λN protein, results in increased expression of the λ DNA replication genes and the *Q* gene while the second, mediated by the Q protein, allows expression of the genes coding for host lysis and structural components of the bacteriophage.

The involvement of *Escherichia coli* proteins in N-mediated antitermination has been investigated both genetically and biochemically. The Nus mutants identify a group of genetic loci that encode gene products involved in N-mediated antitermination (for a review, see Friedman & Gottesman, 1983). Nus mutants do not allow wild-type λ to grow but permit the growth of λ N-independent

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derivatives such as *λnin5*. The *nin5* deletion removes the strong terminators interposed between the λP_R promoter and the *Q* structural gene thus eliminating or reducing the requirement for N-mediated antitermination. To date, five *nus* loci have been identified. The *nusA* locus encodes an acidic 54,000 M_r protein that binds to core RNA polymerase with high efficiency (Friedman, 1971; Kung *et al.*, 1975; Greenblatt & Li, 1981*a*,*b*; Ishii *et al.*, 1984*b*). Functionally, NusA modulates elongation and pausing (Kingston & Chamberlin, 1981; Greenblatt *et al.*, 1981; Schmidt & Chamberlin, 1984; Greenblatt, 1984; Lau *et al.*, 1983; Farnham *et al.*, 1982; Fisher & Yanofsky, 1983; Landick & Yanofsky, 1984) and participates in both termination and antitermination (Greenblatt *et al.*, 1981; Ward & Gottesman, 1981; Nakamura *et al.*, 1986*a*,*b*; Schmidt & Chamberlin, 1987; Chamberlin *et al.*, 1987). The *nusB* locus encodes a very basic 15,000 M_r protein (Swindle *et al.*, 1981; Georgopoulos *et al.*, 1980; Strauch & Friedman, 1981; Ishii *et al.*, 1984*a*). The other three *nus* alleles are in previously identified genes: *nusC* mutation map in *rpoB* (Friedman *et al.*, 1984), encoding the β subunit of RNA polymerase; *nusD* mutations map in *rho* (Simon *et al.*, 1979) encoding the transcription termination factor Rho and the *nusE* mutation (Friedman *et al.*, 1981) alters *rpsJ*, encoding ribosomal protein S10. Recently, a 23,000 M_r *E. coli* protein called NusG was identified biochemically (Horwitz *et al.*, 1987). The NusA, NusB, S10 and NusG proteins, in concert with N protein, modify host RNA polymerase at *nut* sites (Friedman *et al.*, 1973; Adhya *et al.*, 1974; Franklin, 1974; Salstrom & Szybalski, 1978; Rosenberg *et al.*, 1978; de Crombrugghe *et al.*, 1979; Das & Wolska, 1984; Goda & Greenblatt, 1985; Greenblatt *et al.*, 1986; Barik *et al.*, 1987; Horwitz *et al.*, 1987) rendering transcription resistant to termination at some, but not all, downstream terminators. The *nut* sites are likely to be recognized in the transcript and contain a stem and loop structure called *boxB* required for N-entry, as well as the octamer sequence CGCTCTTA called *boxA* (Salstrom & Szybalski, 1978; Friedman & Olson, 1983; Warren & Das, 1984; Olson *et al.*, 1984; Peltz *et al.*, 1985).

The extensive involvement of host proteins in N-mediated antitermination suggests that antitermination might also be involved in host gene expression. In fact, expression of stable RNA has been found to be dependent upon an antitermination system, presumably to prevent termination at Rho-dependent terminators and other terminators that exist in the nascent non-translated stable RNA transcripts (Aksoy *et al.*, 1984; Li *et al.*, 1984; Holben & Morgan, 1984; also reviewed by Morgan, 1986). This antitermination system shares some components with N-mediated antitermination. *E. coli* carrying *nusB5* mutations are defective in both antitermination systems (Sharrock *et al.*, 1985). In addition, both types of antitermination seem to involve the *boxA* sequence (Li *et al.*, 1984). It is likely, although it has not been demonstrated conclusively, that NusA is involved in cellular antitermination (Sharrock *et al.*, 1985).

Little is known about the intrinsic terminating capacity of RNA polymerase or the nature of the interaction of RNA polymerase with the antitermination apparatus or with NusA. One way to probe these interactions is to examine the effect of RNA polymerase mutations on these processes. Mutations leading to rifampicin resistance map in *rpoB* and some are known to affect the λ antitermination process and other events involving the NusA protein (Georgopoulos, 1971; Ghysen & Pironio, 1972; Sternberg, 1973). The Rif^r mutant, *rif501*, confers partial N-independence and also affects the ability of RNA polymerase to terminate at NusA-dependent terminators (Lecocq & Dambly, 1976; Greenblatt *et al.*, 1981). Other rifampicin-resistant (Rif^r) mutations are reported to enhance the antitermination defect of cells containing the *nusA1* mutation (Sternberg, 1976; Baumann & Friedman, 1976). However, there has been no systematic study of the effects of Rif^r mutations on antitermination of *nusA* mutants.

We have identified 17 rifampicin-resistant (Rif^r) mutations, affecting 14 different amino acids in the middle of the β subunit, many of which alter the ability of RNA polymerase to terminate *in vivo* at Rho-dependent or Rho-independent terminators (Jin & Gross, 1988; Jin *et al.*, 1988). Based upon the number of identical isolates at each position, we have argued that this set of mutations is likely to include most of the Rif^r mutations viable in haploid cells (Jin & Gross, 1988). We report the effects of each of these Rif^r mutations on λ N-mediated and cellular antitermination. Because of the involvement of NusA in these antitermination processes as well as in termination, we also specifically ask about the interaction of RNA polymerase with NusA. We determine whether any of the Rif^r mutations alter NusA-dependent antitermination and termination in cells with defective *nusA* alleles. Our results indicate that the Rif^r mutations affect antitermination and they either enhance or suppress the phenotypes of defective *nusA* mutants. Some of the effects on antitermination may be due to effects on termination capabilities, whereas other effects on antitermination may be due to altered interactions with NusA. These results suggest that the region of the β subunit defined by the Rif^r mutations is involved in the antitermination process and affects the activity of NusA protein either directly or indirectly.

2. Materials and Methods

(a) *Bacterial strains, bacteriophage and plasmids*

A description of the Rif^r mutations used in this study and the strains employed for measuring their phenotypes are presented in Table 1. In every case, the Rif^r alleles were introduced into the strains by cotransduction with a linked Tn*10* marker as described (Jin & Gross, 1988). The

Table 1
Strains used in this study

Strain	Relevant genotype	Source/reference
MG1655	*E. coli* K12 wild-type su°	CGSC
CAG3307	*nusA1* derivative of MG1655	This work
N5261	SA500*his ilv galE490* (*chlD-blu*)$^{\Delta 8}$(λΔBAM *N*$^+$*cI14* ΔH)	Ward *et al.* (1983)
N5283	Same as N5261 except λΔBAM carries *Nam* mutations	M. Gottesman
CAG8333	*nusA1* derivative of N5261	M. Singer
K37	*galK2 rpsL200*	CGSC
K1914	*nusA10*(Cs) derivative of K37	Schauer *et al.* (1987)
CAG8102	*rpsL*$^+$ derivative of K37	M. Singer
CAG3844	*nusA10*(Cs) derivative of CAG8102	This work
CAG3846	*nusA11*(Ts) derivative of CAG8102	This work
YN2458	R594 *nusA11*(Ts)	Nakamura *et al.* (1986*a*)

Rif^r (*rpoB*) allele	Amino acid residue affected	Amino acid change
3445	Δ(507–511)	Δ Gly, Ser, Ser, Gln, Leu and inserts Val
101	513	Gln to Leu
8	513	Gln to Pro
113	516	Asp to Asn
148	516	Asp to Val
3051	517	Insert Gln and Asp
3595	522	Ser to Phe
2	526	His to Tyr
3401	529	Arg to Cys
3402	529	Arg to Ser
114	531	Ser to Phe
3449	Δ532	ΔAla
3443	533	Leu to Pro
3370	563	Thr to Pro
111	564	Pro to Leu
7	572	Ilv to Phe
3406	687	Arg to His

efficiency of λ plating (e.o.p.†: see below) was determined in Rif^r derivatives of MG1655 and CAG3307. N-mediated antitermination was assayed in Rif^r derivatives of N5261 and CAG8333. Cellular antitermination was assayed in Rif^r derivatives of CAG8102 carrying plasmid pES3 (described below). The effect of the Rif^r mutations on the termination efficiency of *nusA10*(Cs) and *nusA11*(Ts) was determined in Rif^r derivatives of K1914 and CAG3846 carrying pES4 (described below), respectively.

Bacteriophage λ$^+$, λ*Nam53* and λ*nin5* were from W. Dove.

Plasmid pKG1800 contains promoter P_{gal} inserted upstream from the *galK* structural gene, while pKG1810 contains the terminator T_{IS2} interposed between P_{gal} and *galK*. Both plasmids were obtained from K. McKenney and derived from plasmid pK01 (McKenney *et al.*, 1981).

(b) *Construction of pES3 and pES4*

Plasmids pES3 (see Fig. 2) and pES4 (see Fig. 3) were constructed to allow measurement of *rrnA* transcription antitermination activity by comparative measurements of *galK* gene expression. Both plasmids contain the strong *rrnA* P1 promoter transcribing the *galK* gene with 2 tandem transcriptional terminators (*rrnB* T1 and T_{IS2}) interposed to dampen *gal*K expression to the point where colony color on galactose MacConkey plates is sensitive to the presence of a modified *boxA* region in pES3. Both plasmids were derived from a pKG1800 plasmid containing an IS*2* terminator in the *Sma*I site 180 bp upstream from the ATG of the *galK* gene (McKenney *et al.*, 1981). The pES3 and pES4 plasmids were constructed by replacing the *Eco*RI–*Hin*dIII *gal* promoter fragment of pKG1800-IS2 with various portions of a pPS1 plasmid (or its subclones) that contains a fusion between the *rrnA* promoter region and the *rrnB* terminator region (Sarmientos *et al.*, 1983). The *rrnA* P1 promoter is on an *Eco*RI–*Hin*dIII fragment containing sequences from −262 to +31 (relative to the RNA start site). The *rrnB* T1 terminator was inserted as a *Hin*dIII fragment (containing positions 2418 to 2607 of Brosius *et al.*, 1981). In pES3, but not pES4, a modified *boxA* region fragment spanning positions 1347 to 1426 of Brosius *et al.* (1981) was obtained from pPS1 subclones that contained a T to G change at position 1379 and a deletion of 3 bases (ATC) at positions 1390 to 1392. These spontaneous changes result in a *boxA* region sequence of ...TGCTCGTTAACAATTT---AGA..., which permits less readthrough than the wild-type *boxA* sequence (M. Cashel, unpublished). Nonetheless, the presence of this modified *boxA* fragment in pES3 enables *galK* expression despite the presence of 2 otherwise highly efficient terminators in wild-type hosts (see Results).

(c) *General bacterial and bacteriophage techniques and media*

Cells were grown in M9-glucose complete medium (M9-glucose supplemented with amino acids, nucleosides and

† Abbreviations used: e.o.p., efficiency of plating; Cs, cold-sensitive; Ts, temperature-sensitive; bp, base-pair(s).

vitamins) (Miller, 1972), NZY medium + maltose (Maniatis *et al.*, 1982) or LB (Miller, 1972). LB, NZY and MacConkey-galactose plates were made as described by Miller (1972) or Maniatis *et al.* (1982). Tetracycline (10 μg/ml), ampicillin (50 μg/ml) and rifampicin (50 μg/ml) were added when indicated.

Competent cells were prepared by the Ca^{2+} shock method following growth in LB (Mandel & Higa, 1970) and were stored at −70 °C. Transformations were performed as described by Morrison (1979) and transformants were plated on selective plates after outgrowth for 2 h.

Bacterial growth was followed by measuring a change in optical density at 450 nm (minimal medium) or 600 nm (broth).

P1 transductions were performed as described by Miller (1972).

(d) *Efficiency of λ plating (e.o.p.)*

Cells (0·1 ml) of a fresh overnight culture grown on NZY + maltose were infected with about 5×10^3 λ phage. Following adsorption at 37 °C for 15 min, cells were plated with 3 ml of NZY top soft agar on prewarmed fresh NZY plates. Plates were incubated overnight at the indicated temperature and λ plaques were counted. When the e.o.p. was within a factor of 2 of that exhibited by wild-type cells, the strain was considered permissive for λ growth. Strains unable to grow λ exhibited an e.o.p. of 2×10^{-4} or lower.

(e) *Enzyme assays*

Galactokinase (GalK) activity was measured from cells grown in M9-glucose complete medium using the assay described by McKenney *et al.* (1981) except that sodium deoxycholate (0·3%, w/v) was present in the lysis buffer. GalK activity is expressed as a differential rate of synthesis and is calculated from the slope of the line generated when enzyme activity is plotted *versus* cell growth. The slopes reported were based on samples taken at 3 to 4 times during log phase growth and are calculated as cts/min × 10^3 Gal-PO_4/A_{450} normalized to a reaction mixture containing 8 μCi/μmol [^{14}C]galactose. A slope of 1000 cts/min Gal-PO_4/A_{450} is defined as 1 U. Every differential rate was determined at least twice. Duplicate determinations deviated less than 25% from the average value. When the *galK* gene was carried on a plasmid, the differential rate of synthesis was divided by the β-lactamase activity determined as described by Tomizawa (1985) to correct for copy number differences between strains. The β-lactamase activity was determined in duplicate on one of the lysates used for measuring the differential rate of galactokinase synthesis. We verified that the β-lactamase activity measurements were an accurate reflection of copy number by quantifying copy number by hybridization with ^{32}P-labeled pES4 as described by Adams & Hatfield (1984). The relative copy number measurements obtained by dot blots analysis agreed with that obtained from β-lactamase assay (D. J. Jin, unpublished results).

(f) *S_1 mapping*

We used the S_1 nuclease method of Berk & Sharp (1978) to compare the fraction of transcripts that read through terminators in pES3 or pES4 following initiation at promoter *rrnA* P1. Total *E. coli* RNA was prepared from mid-log cultures (A_{450nm} = 0·5) growth at 37 °C in M9-glucose complete medium + ampicillin by the hot phenol extraction method of Salser *et al.* (1967). RNA/DNA hybridization conditions were those used by Berk & Sharp (1978). All hybridizations were done with excess DNA. RNA samples (adjusted to 100 μg of RNA per sample with tRNA) were hybridized to labeled DNA probe for overnight at 37 °C, digested with S_1 nuclease (170 units/reaction; Boehringer-Mannheim) for 1 h at 37 °C. The S_1-resistant DNA fragments were resolved on a 12% or 5% (w/v) polyacrylamide gel containing 50% (w/v) urea (Maxam & Gilbert, 1980). The experiments were quantified by measuring the radioactivity in DNA fragments cut from gels. Data for each transcript were based on duplicate determinations at several RNA concentrations.

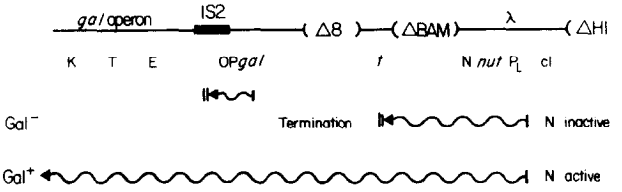


Figure 1. P_L–*gal* fusion. The lambda P_L promoter is contained on a cryptic prophage located close to the *E. coli gal* operon. Deletions ΔBAM and ΔHI remove all λ genes with the exception of *N*, *rex*, and *cI*. The *cI* repressor gene is inactivated by the *cI14* mutation, thereby allowing constitutive leftward transcription from P_L towards the nearby *E. coli gal* operon. The only λ gene expressed is N. Expression of *gal* from its cognate promoter is prevented by the *galE490* mutation, an IS2 insertion in the leader region that causes termination of transcription. When the λN function is active, transcription from P_L is insensitive to the termination signals of *t* and IS2 enabling expression of the *gal* operon. To maximize expression of *gal*, the *gal* operon was brought closer to the P_L promoter by the large chromosomal deletion *chlD-blu*$^{\Delta 8}$ (adapted from Ward *et al.*, 1983, with permission).

3. Results

(a) *Most Rif^r mutations affect N-mediated antitermination*

We used two different types of assays to determine whether any of the Rif^r mutations affected N-mediated antitermination. First, we examined the efficiency of plating (e.o.p.) of λ and several of its derivatives on the mutant strains (see Materials and Methods). We determined whether any of the Rif^r mutations affected the e.o.p. of λ$^+$, but not *λnin5* on lawns of either *nusA*$^+$ or *nusA1* bacteria. Because λ$^+$ requires N-mediated antitermination for its lytic growth while *λnin5* does not, bacterial strains able to grow *λnin5* but not λ$^+$ are likely to be defective in N-mediated antitermination. In addition, we determined whether any Rif^r mutations render λ growth independent of N by asking if any of the Rif^r mutations can support the growth of the *λNam53* phage, which cannot express functional N protein in hosts lacking a suppressor tRNA.

Table 2
rpoB3595 *bypasses the requirement for N from* λP_R *but not from* λP_L

rpoB allele	*λNam53* plaque formation in MG1655[a]		Expression of *galK* from λP_L in N5283 (N5261N^-)[b]	
	32°C	40°C	32°C	40°C
rpoB$^+$	−	−	≤0·5[c]	≤0·5
3595	−	+	≤0·5	≤0·5
Others[d]	−	−	≤0·5	≤0·5

[a] +, permissive for phage growth; −, e.o.p. $<2{\cdot}0\times10^{-4}$ relative to that in nonsense-suppressing bacteria.
[b] Values are expressed in GalK units as defined in Materials and Methods.
[c] A GalK value ≤0·5 is not distinguishable from the background value.
[d] See Table 1.

To determine the magnitude of these effects, we used a second assay in which the rate of *galK* synthesis reflected the extent of N-mediated antitermination. In strain N5261, expression of the *gal* operon is dependent on antiterminated transcripts originating from the λP_L promoter (Fig. 1). We transduced each of the Rif^r alleles into N5261 and CAG8333 (N5261 *nusA1*) and then measured *galK* expression to quantify the effect of each Rif^r mutation on N-mediated antitermination.

(i) *Bypass of the need for N-mediated antitermination*

One Rif^r mutation, *rpoB3595*, allowed *λNam53* to grow at 40°C but not 32°C indicating that it permitted *λ* growth independent of N at high temperature (Table 2). The wild-type strain and other Rif^r mutants did not permit *λNam53* to form plaques (Table 2). Interestingly, although transcription from λP_R (as assayed by *λ* growth) is N-independent, transcription from λP_L as assayed by *galK* expression from λP_L was not increased above the background in the *rpoB3595* derivative of N5261N$^-$ (Table 2). The fact that expression from P_L is not increased rules out the possibility that the *λ* N-independent growth arises from partial suppression of the *Nam* defect in *rpoB3595* strains. The *rif501* mutation has the same phenotype as *rpoB3595* (Lecocq & Dambly, 1976). As discussed below, the sequence change leading to Rif^r, is shared between the two strains (Jin & Gross, 1988).

(ii) *Defects in N-mediated antitermination in* nusA$^+$ *strains*

λ$^+$ forms plaques on a strain containing the *rpoB111* allele only at high temperature (Table 3). The inability of a strain with the *rpoB111* allele to allow *λ*$^+$ plaque formation at 32°C results from a defect in N-mediated antitermination since *rpoB111* allows *λnin5* plaque formation at 32°C (Table 3). K. Hammer and M. Gottesman, who originally isolated the *rpoB111* allele (which they called sck-2), have also found that it restricts *λ* growth at low temperature (Hammer *et al.*, 1987). Consistent with the *λ* plaque formation phenotype, expression of *galK* in N5261 *rpoB111* was reduced tenfold at 32°C (Table 3). Measurement of GalK indicated that the *rpoB111* strain is also defective in N-mediated antitermination at 42°C, although the defect is apparently not severe enough to inhibit *λ* growth (Table 3). *rpoB111* was the only Rif^r allele found to affect N-mediated antitermination in a *nusA*$^+$ strain (Table 3).

(iii) *Alterations in N-mediated antitermination in* nusA1 *strains*

In *nusA1* strains, N-mediated antitermination is defective at high temperature (Friedman, 1971;

Table 3
rpoB111 *inhibits λ N-mediated antitermination in a* nusA$^+$ *strain*

rpoB allele	*λ* Plaque formation in MG1655[a] *λ*$^+$		*λnin5*		Expression of *galK* from *λ* P_L in N5261[b]	
	32°C	42°C	32°C	42°C	32°C	42°C
rpoB$^+$	+	+	+	+	1·0	1·0
111	−	+	+	+	0·1	0·2
Others[c]	+	+	+	+	[d]	[d]

[a] +, permissive for phage growth; −, e.o.p. $<2{\cdot}0\times10^{-4}$.
[b] The GalK units (measured as described in Materials and Methods) in each of the mutant strains are expressed relative to the units in the *rpoB*$^+$ strain. The GalK units for the *rpoB*$^+$ strain at 32°C and 42°C are 30·0 and 90·0 units, respectively.
[c] See Table 1.
[d] GalK values for each of the other Rif^r mutants differed less than 2-fold from that of the wild-type strain.

Table 4

Some Rif^r mutations interfered with λ N-mediated antitermination in nusA1 *strains*

rpoB allele	λ Plaque formation in CAG3307 (MG1655 *nusA1*)[a]		Expression of *galK* from λP_L in CAG8333 (N5261 *nusA1*)[b]	
	32°C	36°C	32°C	36°C
rpoB$^+$	+	+	1·0	1·0
3445	−	−	0·1	0·1
8	+	−	0·3	0·3
113	+	−	0·3	0·2
148	+	−	0·6	0·3
111	−	−	<0·1	<0·1

[a] +, permissive for phage growth; −, e.o.p. $<2{\cdot}0\times10^{-4}$.

[b] The GalK units (measured as described in Materials and Methods) in each of the mutant strains are expressed relative to the units in the *rpoB*$^+$ strain. The GalK units for the *rpoB*$^+$ strain at 32°C and 36°C are 20·0 and 12·0 units, respectively.

Friedman & Baron, 1974). Strains carrying the *nusA1* allele allow λ plaque formation normally at low temperature but are unable to allow λ plaque formation above 37°C (Tables 4 and 5). We asked if any of the Rif^r alleles altered N-mediated antitermination in *nusA1* strains. All but one Rif^r mutations altered the temperature range over which the N-mediated antitermination system was functional in *nusA1* strains (Tables 4 and 5), suggesting that the Rif region of the β subunit plays an important role in modulating N-mediated antitermination.

Five of the Rif^r mutations inhibited antitermination in *nusA1* strains. *nusA1* strains containing these Rif^r mutations were unable to support λ plaque formation at 36°C (Table 4). Of these Rif^r mutants, two were unable to allow λ plaque formation at 32°C. These five Rif^r *nusA1* mutants all allowed *λnin5* plaque formation at 32°C (D. J. Jin, data not shown) suggesting that the defect in λ plaque formation reflected inability to carry out N-mediated antitermination. Measurement of GalK in N5261 *nusA1* confirmed that these five Rif^r mutations decreased N-mediated antitermination (Table 4). These strains have reduced expression of *galK* at both 32°C and 36°C. Strains containing *rpoB111* and *rpoB3445* that were most restrictive for λ growth also showed the greatest reduction in *galK* expression. Comparison of the extent of N-mediated antitermination with the ability to allow λ plaque formation indicates that λ plaque formation is prevented (e.o.p. $<2\times10^{-4}$) when the amount of antitermination is reduced about tenfold from that in wild-type cells at 32°C.

A total of 11 of the Rif^r mutations partially suppressed the *nusA1* phenotype and permitted λ

Table 5

Some Rif^r mutations suppress the defect of nusA1 *mutant in λ N-mediated antitermination*

rpoB allele	λ Plaque formation in CAG3307 (MG1655 *nusA1*)[a]		Expression of *galK* from λP_L in CAG8333 (N5261 *nusA1*)[b]		
	32°C	38°C	32°C	38°C	42°C
rpoB$^+$	+	−	1·0	1·0	1·0
101	+	+	1·2	5·1	12·0
3051	+	+	0·9	2·7	2·0
3595	+	+	0·7	2·3	4·0
501	+	+	0·6	5·1	30·0
2	+	+	1·6	3·3	5·4
3401	+	+	1·8	5·8	[c]
3402	+	+	0·8	2·2	3·4
3449	+	+	1·4	2·2	4·0
3443	+	+	1·2	1·8	2·4
3370	+	+	2·4	7·3	23·0
7	+	+	0·8	1·7	2·4
3406	+	+	2·3	5·1	8·0

[a] +, permissive for phage growth; −, e.o.p. $<2{\cdot}0\times10^{-4}$.

[b] The GalK units (measured as described in Materials and Methods) in each of the mutant strains are expressed relative to the units in the *rpoB*$^+$ strain. The GalK units for the *rpoB*$^+$ strain at 32°C, 38°C and 42°C are 20·0, 4·8 and ≤0·5 units, respectively. A GalK value ≤0·5 is not distinguishable from the background value.

[c] The strain containing *rpoB3401* was unable to grow at 42°C.

plaque formation at 38°C (Table 5). These same mutations also caused increased *galK* expression at 38°C in N5261 *nusA1* (Table 5). This effect was dependent upon a functional N; in the absence of N, *galK* expression was undetectable (D. J. Jin, data not shown). Since these Rif^r alleles have little (<2-fold) effect on *galK* expression in the N5261 *nusA*$^+$ strain, they are unlikely to affect initiation from the P_L promoter (D. J. Jin, data not shown). Therefore, we conclude that the increased *galK* expression of these 11 Rif^r mutations results from increased antitermination. Among these 11 strains, some showed significant enhancement of *galK* expression even at 42°C. Two alleles, *rpoB101* and *rpoB3370*, had the greatest effects and increased N-mediated antitermination 10 to 20-fold in *nusA1* strains at this temperature (Table 5).

We present the *nusA1* suppression data for *rif501* in Table 5 because these data suggested to us that *rif501* has more than one mutation. The *rif501* mutation is identical with that in *rpoB3595* within the 200 bp region sequenced to ascertain the mutational change conferring Rif^r (Jin & Gross, 1988). The phenotypes of these strains were also identical (D. J. Jin, unpublished data) except when we examined the *nusA* suppression phenotype. The expression of *galK* is about sevenfold higher at 42°C in CAG8333 *rif501* than in the CAG8333 *rpoB3595* isogenic strain (Table 5). The mutation *rpoB3595* arose spontaneously (J. Gardner, personal communication) while *rif501* was obtained after nitrosoguanidine mutagenesis (Lecocq & Dambly, 1976). The enhanced suppression of the *nusA1* defect by *rif501* could be explained if this strain carries additional mutations outside of the Rif region of *rpoB*.

(b) *The effects of Rif^r mutations on the cellular antitermination system*

We used the pES3 vector described in Materials and Methods to determine whether any of the Rif^r mutations affected the cellular antitermination system involved in transcription of stable RNA. In pES3, sequences derived from the *rrnA* operon, including a *boxA* site, allow transcription through the strong *rrn*T1 and IS2 terminators resulting in expression of *galK*. When the pES3 plasmid is carried in a *galK*$^-$ strain, alterations in the amount of antitermination were detected as altered expression of *galK*.

Rif^r derivatives of CAG8102, a *galK*$^-$ strain, were transformed with pES3 and screened on MacConkey-galactose-ampicillin plates (MGA plates) to determine whether they decreased *galK* expression from pES3. Most of the Rif^r mutants gave the expected Gal$^+$ phenotype and had GalK activity similar to that in the *rpoB*$^+$ strain (data not shown). However, three Rif^r mutants, *rpoB114*, *rpoB3449* and *rpoB3443* were Gal$^-$ when transformed with pES3. The rate of *galK* expression in these strains was about tenfold lower than in the isogenic *rpoB*$^+$ parental strain (Table 6).

Table 6

Some Rif^r mutants are defective in cellular antitermination

	GalK phenotype of pES3 in CAG8102	
rpoB allele	Colony color on MGA plates	*galK* expression[a]
rpoB$^+$	Red	1·0 (75·0)
114	White/pink	0·1
3449	White/pink	0·1
3443	White/pink	0·1
Others[b]	Red	[c]

[a] The GalK units (measured as described in Materials and Methods) in each of the mutant strains are expressed relative to the units in the *rpoB*$^+$ strain. The value in parentheses indicates the value of GalK units in the *rpoB*$^+$ strain.

[b] See Table 1.

[c] GalK values for each of the other mutants differed less than 2-fold from that of the *rpoB*$^+$ strain.

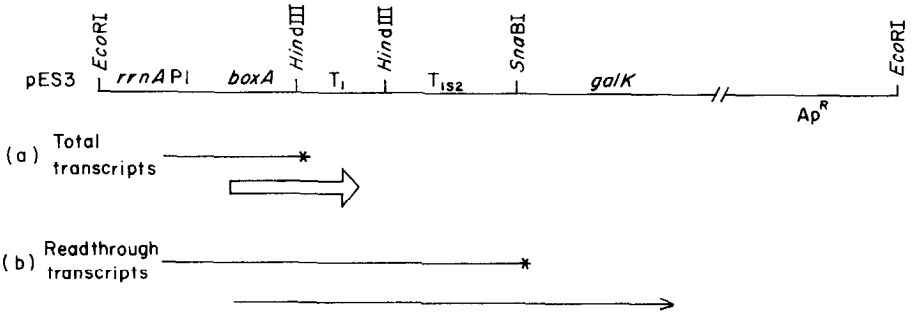
Interestingly, these were the only three Rif^r alleles that do not affect any of the Rho-dependent or Rho-independent terminators we have assayed (Jin *et al.*, 1988).

The decreased expression of *galK* in strains containing these three Rif^r mutants could result either from decreased initiation at *rrnA* P1 or from increased termination at one or both of the terminators located upstream of *galK* in pES3. To distinguish these possibilities, we "S_1 mapped" *in vivo* RNA originating from *rrnA P1*, using a probe that was 5′-end-labeled at the *Hin*dIII site (see schematic in Fig. 2). We found that the initiated transcript represents a similar fraction of total RNA in the *rpoB*$^+$ and the three Rif^r mutant strains indicating that initiation at promoter P1 is not affected by the Rif^r mutations (Fig. 2(a)). A further S_1 mapping experiment using a probe 5′-end-labeled at the *Sna*B1 site downstream from the terminators (see schematic in Fig. 2) indicated that these three Rif^r mutants decreased read-through of the pES3 terminators at least tenfold (Fig. 2(b)). These results indicate that the dramatic decrease in *galK* expression results from the fact that these three Rif^r mutations decrease the effectiveness of the cellular antitermination system involved in the synthesis of stable RNA.

(c) *The* nusA10*(Cs) mutation affects termination in pES4*

Plasmid pES4 differs from pES3 in that it lacks the *boxA* sequence necessary for antitermination. Expression of *galK* in cells carrying pES4 is very low because transcription stops at the *rrn*T1 and T_{IS2} terminators upstream from the *galK* structural gene. None of the Rif^r mutations increased expression of *galK* from pES4 (data not shown).

However, the *nusA10*(Cs) mutation, which prevents cell growth at 30°C and decreases the efficiency of the N-mediated antitermination system (Schauer *et al.*, 1987), does affect *galK* expression



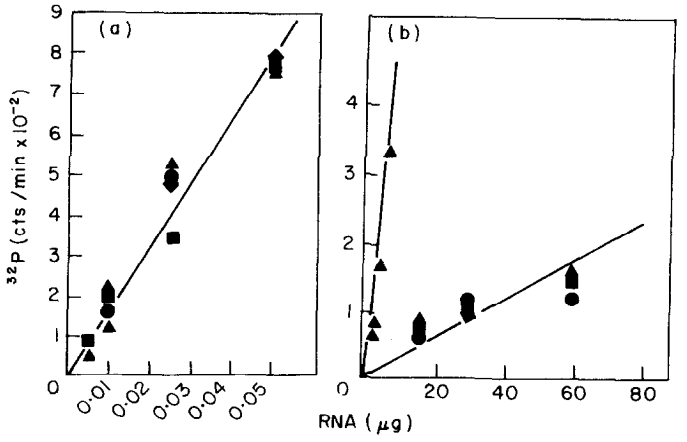


Figure 2. S_1 nuclease mapping of the transcripts from *rrnA* P1 in pES3 to analyze cellular antitermination in wild-type and 3 Rif^r mutant strains. The schematic (not drawn to scale) outlines the strategy for mapping the transcripts initiating from *rrnA* P1 and the transcripts that readthrough both the T1 and T_{IS2} terminators. The mRNA transcripts are indicated with an arrowhead. The 5′-end-labeled [^{32}P]DNA fragment (^{32}P indicated by *) used for hybridization is shown above the respective mRNA transcript. (a). Total transcripts initiating from *rrnA* P1. A 410 bp *Hin*dIII–*Eco*RI fragment 5′ end-labeled at the *Hin*dIII site was hybridized with increasing amounts of RNA extracted from *rpoB*$^+$(▲), *rpoB114* (■), *rpoB3449* (◆), or *rpoB3443* (●), digested with S_1 nuclease and electrophoresed on a 5% (w/v) polyacrylamide gel containing 50% (w/v) urea. The radioactivity in the protected fragment (110 bp) was quantified by counting the excised fragment in 5 ml of scintillation fluid (EcoLite). The amount of transcript hybridized is plotted *versus* increasing amount of total cellular RNA. (b). Transcripts extending beyond terminators *rrnB* T1 and T_{IS2}. A 1100 bp *Sna*BI–*Eco*RI fragment 5′-end-labeled at the *Sna*BI site was used for hybridization with RNA from the *rpoB*$^+$(▲), *rpoB114* (■), *rpoB3449* (◆), or *rpoB3443* (●). After S_1 nuclease digestion and electrophoresis on a 5% (w/v) polyacrylamide gel containing 50% (w/v) urea, the protected fragment was quantified by measuring the radioactivity in the fragment. The amount of transcript hybridized is plotted *versus* increasing amounts of total cellular RNA.

from plasmid pES4. When pES4 is carried in strains containing *nusA10*(Cs), the cells are red on MGA plates and expression of *galK* is at least tenfold higher than in isogenic *nusA*$^+$ strains at 37 °C (Table 7). In fact, the level of *galK* expression from pES4 in *nusA10*(Cs) cells is comparable to that of pES3 in *nusA*$^+$ cells (compare Tables 6 and 7).

The increased expression of *galK* from pES4 in the *nusA10*(Cs) strain resulted either from increased initiation at *rrnAP1* or from increased readthrough of one or both of the terminators located upstream from *galK* in pES4. To distinguish these possibilities, we used S_1 mapping of *in vivo* RNA. We measured total RNA initiated from promoter P1 of *rrnA* by S_1 mapping with a probe that was 5′-end-labeled at the *Hin*dIII site and readthrough RNA using a probe that was 5′-end-labeled at the *Sna*BI site located downstream of both terminators (Fig. 3). We found that the initiated transcript represents a similar fraction of total RNA in the *nusA10*(Cs) and *nusA*$^+$ strains indicating that initiation at promoter P1 is not significantly affected by the *nusA10*(Cs) mutation (Fig. 3(a)). In contrast, readthrough RNA is about 15 times more abundant in the *nusA10*(Cs) strain than in the *nusA*$^+$ strain (Fig. 3(b)). This result indicated that the *nusA10*(Cs) allele is altered in terminator readthrough rather than initiation.

The pES4 plasmid might contain cryptic *boxA* sites. In this case, the increased terminator readthrough by the *nusA10*(Cs) allele could result from increased antitermination at cryptic *boxA* sites

Table 7

Some Rif^r *mutations suppress the termination defects of* nusA *mutants, as measured by* galK *expression from pES4*

	nusA10(Cs)[a]		*nusA11*(Ts)[b]	
rpoB allele	Colony color on MGA plates	*galK* expression[c]	Colony color on MGA plates	*galK* expression[c]
rpoB$^+$	Red	1·0 (70·0)	Red	1·0 (75·0)
114	White	<0·1	White	<0·1
3449	White	<0·1	White	<0·1
3443	White	<0·1	White	<0·1
3370	Red	0·2	Red	0·2
111	Red	0·2	Red	1·0
7	Pink/red	0·2	Red	0·2
3406	Pink/red	0·2	[d]	[d]
Others[e]	Red	[f]	Red	[f]

In the *rpoB*$^+$ *nusA*$^+$ strains containing pES4, GalK is 5·0 units (measured as described in Materials and Methods) and cells are white on MGA plates.

[a] Assayed at 37 °C.

[b] Assayed at 30 °C.

[c] The GalK units (measured as described in Materials and Methods) in each of the mutant strains are expressed relative to the units in the *rpoB*$^+$ strain. The value in parentheses indicates the value of GalK units in the *rpoB*$^+$ strain.

[d] This double mutant grew too poorly to be assayed.

[e] See Table 1.

[f] GalK values for the other Rif^r mutants differed less than 2-fold from that of the *rpoB*$^+$ strain.

rather than from decreased termination at one or both of the terminators. To distinguish between these possibilities we asked if the *nusA10*(Cs) allele can decrease termination of transcripts initiated from P_{gal} that do not contain a *boxA* sequence. Plasmid pKG1810 contains T_{IS2} interposed between P_{gal} and *galK*. GalK expression is significantly higher in the *nusA10*(Cs) cells containing pKG1810 than in the isogenic wild-type cells (Fig. 4(a)). The effect of the *nusA10*(Cs) allele on readthrough of T_{IS2} from P_{gal} is at least fivefold but could be much greater. We cannot calculate the magnitude of the effect since expression of *galK* in the *nusA*$^+$ strain containing pKG1810 is not significantly different from the background value for the assay (see legend to Fig. 4), and we cannot reliably measure terminator readthrough in the *nusA*$^+$ control. Increased *galK* expression in the *nusA10*(Cs) strain does not result from increased initiation at P_{gal}. *galK* expression is virtually identical in *nusA*$^+$ and *nusA10*(Cs) strains containing the control plasmid pKG1800 lacking the terminator (Fig. 4(b)).

Taken together, these experiments establish that the *nusA10*(Cs) strain has a defect in termination at some terminators. Our conclusion that *nusA10*(Cs) is defective in termination is consistent with recent work of Schmidt & Chamberlin (1987) indicating that termination at *rrn*T1, one of the two terminators present in pES4, is *nusA*-dependent *in vitro*.

(d) *Some Rif*^{r} *mutations affect termination in* nusA *mutant strains*

We determined whether any of the Rif^r mutations suppressed the termination defect of the *nusA10*(Cs) strain at the terminators present in pES4. A total of seven of the Rif^r mutations suppressed this defect to some extent (Table 7). Among these, the three Rif^r mutations depressing cellular antitermination showed virtually complete suppression. These *nusA10*(Cs) Rif^r mutants are white on MacConkey-galactose plates and have the same low level of *galK* expression from pES4 as does *nusA*$^+$ (Table 7). These data indicate that a number of the Rif^r alleles significantly restore the ability of this mutant NusA protein to carry out termination.

The *nusA11*(Ts) allele prevents cell growth at high temperature, is altered in N-mediated antitermination and has been shown to be defective in termination at several terminators *in vivo* (Nakamura *et al.*, 1986*a*,*b*). The level of *galK* expression in *nusA11*(Ts) cells containing pES4 is similar to that in *nusA10*(Cs) cells, indicating that *nusA11*(Ts) is also defective in termination at pES4 terminators (Table 7). We tested the Rif^r mutations to determine if any suppressed the *nusA11*(Ts) termination defect. Of the seven Rif^r alleles that decreased *galK* expression in the *nusA10*(Cs) strain, five also decreased *galK* expression in the *nusA11*(Ts) strain. There were no alleles that suppressed *nusA11*(Ts) that did not also suppress *nusA10*(Cs).

Two of the Rif^r alleles, *rpoB3595* and *rpoB2*, are incompatible with *nusA10*(Cs) and *nusA11*(Ts) at 30 °C, 37 °C and 40 °C while bacteria carrying *rpoB3401* and either *nusA* mutation grow extremely poorly (D. J. Jin, data not shown). These three Rif^r mutations are located near each other. Each of these Rif^r mutations permitted a great amount of readthrough at one or more of the Rho-

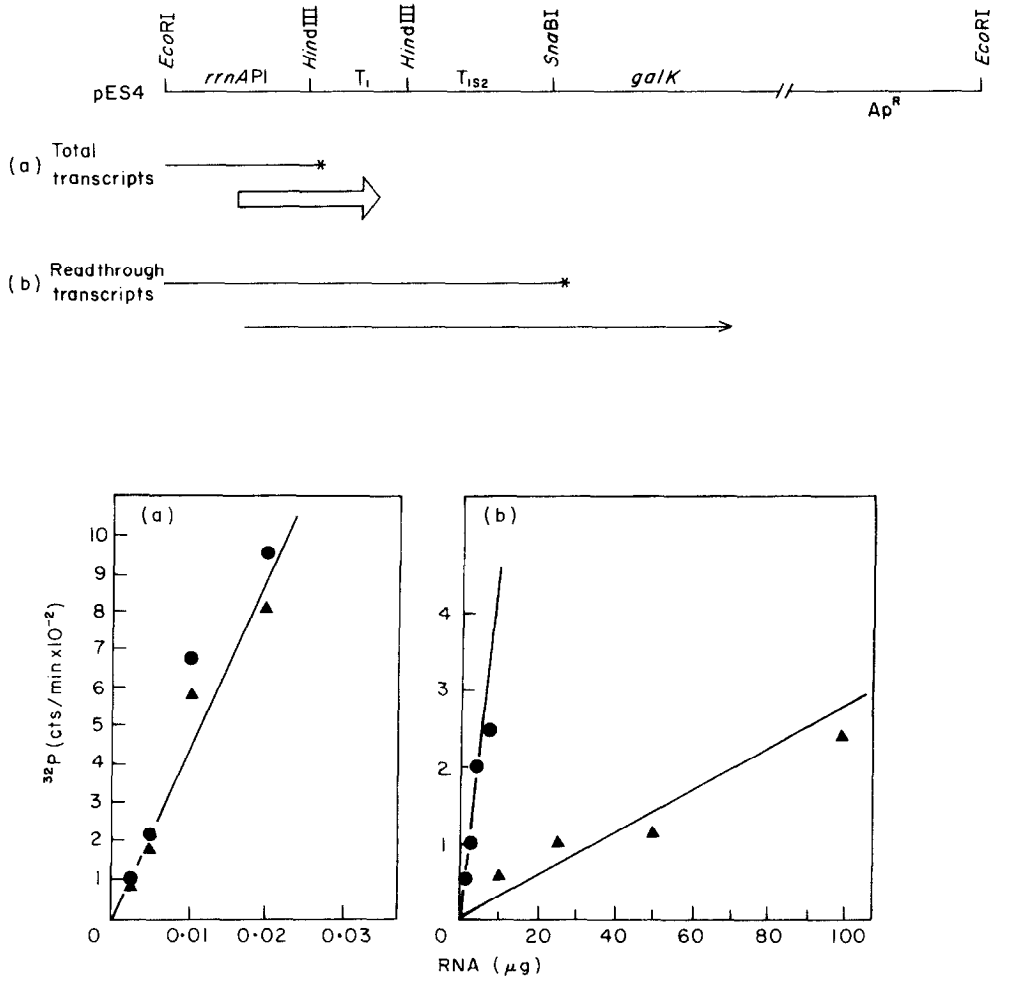


Figure 3. S_1 nuclease mapping of the transcripts from pES4 to compare termination in *nusA10*(Cs) and *nusA*$^+$ strains. The schematic (not drawn to scale) outlines the strategy for mapping the transcripts initiating from *rrnA* P1 and the transcripts that readthrough both the T1 and T_{IS2} terminators. The mRNA transcripts are indicated with an arrowhead. The 5′-end-labeled [^{32}P]DNA fragment (^{32}P indicated by *) used for hybridization is shown above the respective mRNA transcript. (a) Total transcripts initiating from *rrnA* P1. A 330 bp *Hin*dIII–*Eco*RI fragment 5′-end-labeled at the *Hin*dIII site was hybridized with increasing amounts of RNA extracted from either the *nusA*$^+$ strain (▲) or the *nusA10*(Cs) strain (●), digested with S_1 nuclease and electrophoresed on a 12% (w/v) polyacrylamide gel containing 50% (w/v) urea. The radioactivity in the protected fragment (30 bp) was quantified by counting the excised fragments in 5 ml of scintillation fluid (EcoLite). The amount of transcript hybridized is plotted *versus* increasing amounts of total cellular RNA. (b). Transcripts extending beyond terminators *rrnB* T1 and T_{IS2}. A 1020 bp *Sna*BI *Eco*RI fragment 5′-end-labeled at the *Sna*BI site was used for hybridization with RNA from either the *nusA*$^+$ strain (▲) or *nusA10*(Cs) strain (●). After S_1 nuclease digestion and electrophoresis on a 5% (w/v) polyacrylamide gel containing 50% (w/v) urea, the protected fragment was quantified by measuring the radioactivity in the fragment. The amount of transcript hybridized is plotted *versus* increasing amounts of total cellular RNA.

dependent or Rho-independent terminators on which their effects were determined (Jin *et al.*, 1988). Enhanced terminator readthrough may account for the incompatibility of these mutations with the *nusA* mutants defective in termination.

4. Discussion

We have investigated the effects of 17 Rif^r mutations on processes involving the NusA protein. We determined whether any of the Rif^r mutations alter the ability of RNA polymerase to carry out λN-mediated or cellular antitermination. In addition, we have determined whether the presence of the Rif^r mutations affected the antitermination defect of *nusA1* or the termination defect of *nusA10*(Cs) and *nusA11*(Ts). The phenotypes of the Rif^r mutations, summarized in Table 8, indicate that they affect both termination and antitermination processes involving NusA protein.

In the discussion below, we first analyze the nature of the altered interaction exhibited by Rif^r mutations with mutant *nusA* alleles. We then describe the antitermination phenotypes of the Rif^r mutations and consider the extent to which each of these phenotypes can be explained either by altered interaction with NusA or by the termination defects previously attributed to these alleles (Jin *et al.*, 1988). *In vitro* studies will be required to determine if the mutant proteins do have the altered

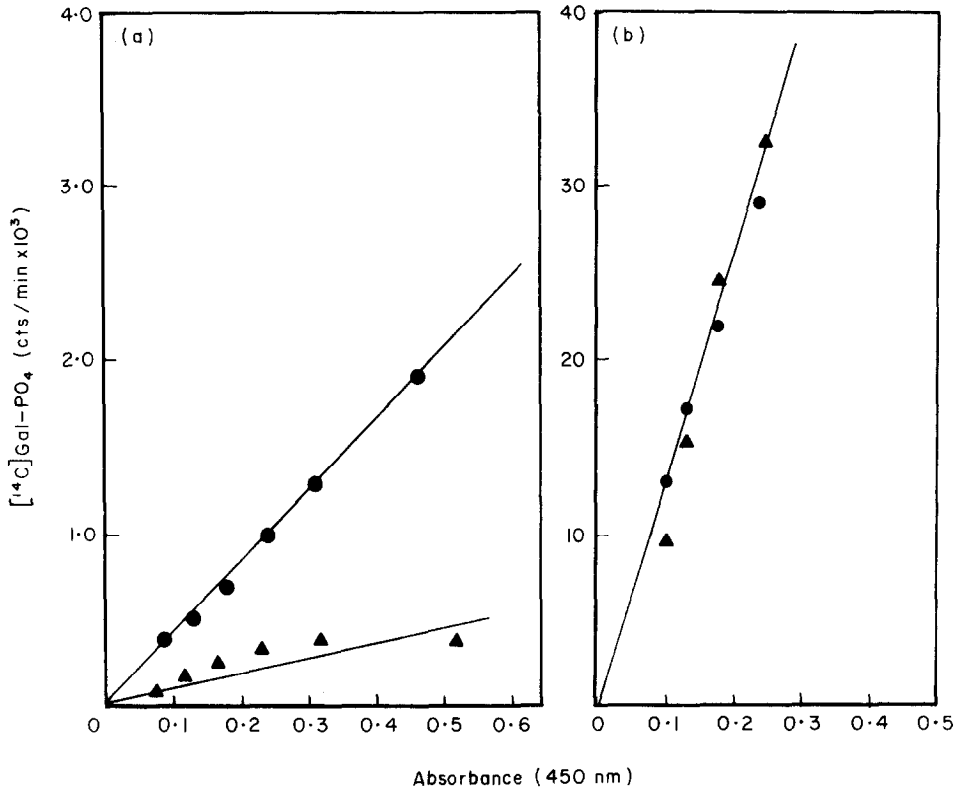


Figure 4. Comparison of *galK* expression in *nusA*$^+$ and *nusA10*(Cs) strains containing either plasmid pKG1810 or pKG1800. GalK levels in strains containing pKG1810 reflect extent of termination at T_{IS2} interposed between P_{gal} and *galK* in the pKG1810 while those in strains containing pKG1800 reflect initiation at P_{gal}. Wild-type (CAG8102) and *nusA10*(Cs) (CAG3844) cells containing plasmid pKG1810 (a) or pKG1800 (b) were grown at 37 °C in M9-fructose complete medium supplemented with ampicillin. The levels of galactokinase in *nusA*$^+$ (▲) and *nusA10*(Cs) (●) cells are corrected for plasmid copy number and plotted *versus* A_{450} (see Materials and Methods). A background of [^{14}C]Gal-PO$_4$ (600 cts/min) was subtracted from each experimental point.

interactions postulated here or whether some of the *in vivo* phenotypes result from other perturbations in cellular physiology.

(a) *Interaction of NusA and Rif*^{r} *RNA polymerase*

We find that many of the Rif^r alleles located at the distal end of the Rif region (see Table 8) suppress the termination defects of the *nusA10*(Cs) and the *nusA11*(Ts) alleles. This suggests that the Rif^r RNA polymerases encoded by these mutant alleles interact differently with the mutant NusA proteins than does wild-type RNA polymerase. If NusA binds to RNA polymerase in the Rif region then the mutational changes in the Rif^r mutants could directly affect the interaction between NusA and RNA polymerase and the effects we observe could reflect altered protein contacts between the two proteins. Mutations that suppress a phenotype by altering protein contacts are generally found to be allele-specific (Jarvik & Botstein, 1975). Our results indicate that of the seven alleles suppressing *nusA10*(Cs), only the suppression by *rpoB111* is allele-specific. Of the other six alleles suppressing *nusA10*(Cs), five also suppressed *nusA11*(Ts) and the remaining allele (*rpoB3406*) grew too poorly to be assayed in combination with *nusA11*(Ts). This genetic criterion suggests that most of the effects we observe may not be due to altered protein–protein contacts. As an alternative explanation, the interaction could be indirect; for example, the mutant RNA polymerase might interact differently with some signal in the transcript to change the conformation of the ternary complex and affect the activity of NusA. Regardless of whether the effect of the mutant RNA polymerase on NusA activity is direct or indirect, because NusA is involved in both termination and antitermination processes, such alterations could have profound effects on the transcription process.

(b) *N-mediated antitermination phenotypes of Rif*^{r} *mutations*

Three types of *rpoB* mutations affecting N-mediated antitermination have been described: (1) N-bypass mutations, which allow λ growth in the absence of N-mediated antitermination (Lecocq & Dambly, 1976); (2) NusC mutations, which restrict λ growth in otherwise wild-type cells (Friedman *et al.*, 1984); and (3) Snu mutations, which restrict λ growth in *nusA1* cells at low temperature (32 °C) (Sternberg, 1976; Baumann & Friedman, 1976). We have found all of these phenotypes among our Rif^r mutations and in

Table 8

Summary of the effects of Rif^r mutations on antitermination and their interaction with nusA *mutations*

rpoB allele	*rpoB*$^+$	*3445*	*101*	*8*	*113*	*148*	*3051*	*3595*	*2*	*3401*	*3402*	*114*	*3449*	*3443*	*3370*	*111*	*7*	*3406*
Amino acid residue affected		507-511	513	513	516	516	517	522	526	529	529	531	532	533	563	564	572	687
Suppressed termination defect in *nusA10*(Cs)												++	+++	+++	+	+	+	+
in *nusA11*(Ts)												++	+++	+++	+		+	
N bypass for λ plaque formation								+										
Inhibition of λ plaque formation in *nusA*$^+$																++		
Interfered with antitermination (A.T.) in *nusA1*		++		+	+	+										++		
Suppressed *nusA1* A.T. defect			++				+	+	+	+	+		+	+	++		+	+
Suppressed termination defect in *rho15*			++												++			
Defect in cellular antitermination (with pES3 system)												++	+++	++				

+, Has the phenotype indicated in the first column; ++, has stronger or the strongest effect on the phenotype indicated. For details, see the text.

addition, we describe another class of mutation: one which suppresses the *nusA1* defect, permitting λ growth at higher temperatures. We have assayed the effects of Rif^r mutations on N-mediated antitermination by determining the efficiency of λ plating and by quantifying N-dependent *galK* expression. These two assays showed excellent agreement. In addition, the use of both assays enabled us to determine that λ plaque formation was inhibited when antitermination was reduced to about 10% of that normally observed for *rpoB*$^+$ parental strains at 32°C (Tables 3 and 4).

One Rif^r mutant, *rpoB3595*, allowed λ plating in the absence of N and is therefore an N-bypass mutant. Interestingly, whereas expression from λP_R (assayed by λ growth) was N-independent, expression from λP_L (assayed by *galK* expression) was not. The *Rif501* mutation, whose known mutational change is identical to that in *rpoB3595* (Jin & Gross, 1988) also shows N-independent transcription from λP_R but not from λP_L (Lecocq & Dambly, 1976; D. J. Jin, data not shown). The termination defect of *rpoB3595* is probably responsible for the lack of N-dependence of this Rif^r mutant. Strains containing *rpoB3595* (or *rif501*) were defective in termination at every Rho-dependent and Rho-independent terminator we assayed (Jin *et al.*, 1988). Very likely, the terminators in the λP_L transcript are not ones that the *rpoB3595* (*rif501*) mutant RNA polymerase can readthrough efficiently; hence antitermination is required for readthrough transcription from λP_L.

Only one Rif^r mutant, *rpoB111*, exhibited a NusC phenotype, preventing λ growth in an otherwise wild-type strain at 32°C but not at 42°C. Although the plating phenotype is conditional, the defect in N-mediated antitermination is actually manifest at both temperatures as measured by GalK assay. However, the fivefold decrease in N-mediated antitermination at 42°C is not severe enough to prevent λ growth at high temperature. *rpoB111* was also the only Rif^r mutation to fit the genetic criteria for an allele-specific suppressor of mutant *nusA* alleles: it suppressed the termination defects of the *nusA10*(Cs) allele but not those of the *nusA11*(Ts) allele. The suppression assay for *nusA10*(Cs) was performed at 37°C while that for *nusA11*(Ts) was carried out at 30°C. One could imagine that the lack of suppression of *nusA11*(Ts) resulted from a termination defect of *rpoB111* at 30°C. This is not the case. Strains containing *rpoB111* show normal termination efficiency at these terminators in the presence of wild-type *nusA*$^+$ (D. J. Jin, unpublished data). If the *rpoB111* allele defines a protein–protein contact between NusA and RNA polymerase, then an altered contact between the two proteins could be responsible for the NusC phenotype exhibited by this allele.

Five Rif^r alleles (*rpoB111*, *rpoB3445*, *rpoB8*, *rpoB113*, and *rpoB148*) inhibit λ plating on *nusA1* strains at low temperatures. The fact that *rpoB111* affects antitermination on both *nusA*$^+$ and *nusA1* strains while the effect of the other alleles is limited to *nusA1* strains could simply reflect quantitative differences in the antitermination defect. The *rpoB111* mutation restricted antitermination in the *nusA1* strain much more severely than *rpoB3445*, the most restrictive of the four alleles affecting only *nusA1* strains. On the other hand, there may be a qualitative difference between the mechanism of inhibition by *rpoB111* and the other four alleles. The four alleles affecting antitermination only in *nusA1* strains could be having an effect that is possible only in the presence of the defective *nusA* allele. The idea that these mutations may have a qualitatively different effect from *rpoB111* is supported by their termination phenotypes. The four alleles whose effect is limited to the *nusA1* strain map near each other (Jin & Gross, 1988; see also Table 8) and increase termination at Rho-dependent terminators (Jin *et al.*, 1988). In

contrast, the *rpoB111* allele is actually defective in termination at Rho-independent terminators (Jin *et al.*, 1988). Possibly, enhanced termination by the mutant RNA polymerases decreases antitermination in strains with the defective *nusA1* allele without affecting antitermination in the wild-type strain. *rpoB111*, on the other hand, must affect antitermination in some other way, perhaps through altered interaction of RNA polymerase with NusA.

The class of RNA polymerase mutations partially suppressing the *nusA1* restriction of N-mediated antitermination at high temperatures has not been previously described. Two mutations, *rpoB101* and *rpoB3370*, suppress *nusA1* more than tenfold, while nine others suppress the defect several-fold. Of the 11 mutations suppressing the *nusA1* defect, six had previously been shown to decrease termination, and three to increase termination, while two others had no effect upon termination (Jin *et al.*, 1988). Thus, there does not seem to be a close correlation between these termination and antitermination phenotypes.

rpoB101 and *rpoB3370*, the two Rif^r mutations that suppress the *nusA1* phenotype most efficiently, are the only Rif^r mutations that suppress the termination defect of *rho15* (Das *et al.*, 1978; Jin *et al.*, 1988; Table 8). These two phenotypes may arise from the same defect in RNA polymerase. Either the alteration in RNA polymerase partially compensates for the defects in *nusA1* and *rho15* and bypasses the need for these proteins, or suppression results from altered interactions in the ternary complex. *In vitro* experiments indicate that RNA polymerase from *rpoB101* does not simply bypass the need for Rho. The mutant RNA polymerase does not terminate by itself. Instead, it restores the ability of Rho15 to hydrolyze ATP and terminate (Das *et al.*, 1978) suggesting that suppression results from altered interactions in the ternary complex. Because NusA is known to bind to RNA polymerase and modify its termination properties, it is appealing to consider the possibility that suppression of *rho15* might be mediated by altered interactions with NusA. Suppression of *rho15* could result from altered contacts between NusA and RNA polymerase, or from indirect effects, possibly mediated by altered interactions of the ternary complex with the transcript.

(c) *Cellular antitermination phenotypes of Rif*^{r} *mutations*

This study has identified three Rif^r mutations that cause a defect in cellular antitermination. These are the first RNA polymerase mutations affecting this process to be identified. These mutations alter amino acids 531 (*rpoB114*), 532 (*rpoB3449*) and 533 (*rpoB3443*) in the β polypeptide. Our assay indicates that each of these mutations decreases the ability of the cellular antitermination system to readthrough the strong *rrn*T1 and T_{IS2} terminators carried in plasmid pES3 (Table 6). The fact that these mutations are highly clustered and have similar effects upon cellular antitermination suggests that this region of RNA polymerase plays a crucial role in cellular antitermination.

We do not think that the plasmid pES3 vector necessarily provides an accurate measure of the extent to which cellular antitermination is altered. First, the *boxA* site in the plasmid is mutant, which decreases the efficiency of the antitermination system significantly (M. Cashel, data not shown). Second, the terminators in pES3 differ from those in the ribosomal RNA transcripts. The stable RNA transcript does not contain the IS*2* terminator and not all *rrn* operons contain the T1 terminator. The effects on antitermination we observed may well reflect the particular terminators chosen to assay this process. These differences may explain why mutations apparently inhibiting expression of stable RNA so severely are still viable.

The three Rif^r mutations that are defective in the cellular antitermination system involved in stable RNA synthesis could work either by decreasing antitermination or increasing termination. Our experiments suggest that the latter is the case. Although these Rif^r mutations do not affect termination at Rho-dependent or Rho-independent terminators (Jin *et al.*, 1988), they were the only Rif^r alleles that completely restored termination to strains containing the *nusA10*(Cs) or the *nusA11*(Ts) allele. It seems likely that the altered interaction of these Rif^r mutations at NusA-dependent terminators such as *rrnA* T1 uncovered in the mutant *nusA* strains is responsible, at least in part, for their antitermination defect.

In summary, we have determined that alterations in the Rif^r region of the β subunit of RNA polymerase have effects on both N-mediated antitermination and the cellular antitermination system involved in expression of stable RNA. Many alterations in this region of the protein also alter the phenotypes of several *nusA* alleles, suggesting an interaction, either direct or indirect, between this region of β and NusA. The effects of some of the Rif^r mutations on antitermination may be explained by the termination capabilities of RNA polymerase containing the mutant β subunit. Other effects on antitermination are not correlated with the termination phenotypes of the Rif^r alleles. These effects may be explained by altered interactions with NusA protein or with some other component of the antitermination apparatus.

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