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Transcription frequency modulates the efficiency of an attenuator preceding the *rpoBC* RNA polymerase genes of *Escherichia coli*: possible autogenous control

Keith L.Steward and Thomas Linn*

Department of Microbiology and Immunology, Faculty of Medicine, University of Western Ontario, London, Ontario N6A 5C1, Canada

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ABSTRACT

Expression of the *rpoBC* genes encoding the β and β' RNA polymerase subunits of *Escherichia coli* is autogenously regulated. Although previous studies have demonstrated a post-transcriptional feedback mechanism, complex transcriptional controls of *rpoBC* expression may also contribute. We show that an attenuator (*rpoBa*) separating the ribosomal protein (*rpl*) genes from the *rpoBC* genes in the *rplKAJLrpoBC* gene cluster is modulated in its efficiency in response to changes in the frequency of transcription initiated by promoters located upstream. A series of *rplJLrpoBa-lacZ* transcriptional fusions was constructed on lambda vectors in which transcription into the *rpoBa* attenuator was varied by using a variety of promoters with different strengths. β-galactosidase assays performed on monolysogens of the recombinant phage show that with transcription increasing over a 40-fold range, readthrough of *rpoBa* decreases from 61% to 19%. In contrast, two other well-characterized terminators show nearly constant efficiencies over a similar range of transcription frequencies. Using a set of phage P22 *ant* promoter variants with single-nucleotide changes in the promoter consensus sequences also demonstrates that the modulation of *rpoBa* function appears to be unrelated to the phenomenon of 'factor-independent antitermination' reported by others. The implications for autogenous control of RNA polymerase synthesis are discussed.

INTRODUCTION

The RNA polymerase of *E.coli* performs a central role in the expression of the cell's genome. Although the minimal core enzyme ($\beta\beta'\alpha_2$) is capable of elongating the mRNA transcript, one of several σ subunits is required along with core enzyme to accurately initiate transcription. Consequently, the cell's capacity for gene expression is largely determined by the concentration of active RNA polymerase (reviewed in 1). Several studies have indicated that the number of RNA polymerase molecules in the cell is closely regulated by an autogenous or feedback mechanism.

While the evidence is strong for a post-transcriptional mechanism for autogenous control of RNA polymerase synthesis (2–8), little is known about possible autogenous control mechanisms acting at the level of transcription. Earlier attempts to reveal transcriptional feedback have used conditions that interfere with the transcriptional activity of RNA polymerase, such as rifampicin treatment (9–11) or the shift of RNA polymerase temperature-sensitive mutants to a non-permissive temperature (12, 13). Such treatments make it difficult to determine if the observed effects are the consequence of regulatory compensation or side-effects of altered enzyme function.

The *rpoB* and *rpoC* genes encoding the β and β' subunits respectively of RNA polymerase are located at the distal end of the large *rplKAJLrpoBC* gene cluster, which also includes the genes that encode four ribosomal proteins (Figure 1). The α subunit is synthesized in excess over that required to assemble core enzyme (14–18), while the σ^{70} subunit is synthesized at approximately 0.4 the level of core enzyme, consistent with its transient catalytic role in transcription initiation (15). Therefore the regulation of RNA polymerase synthesis would appear to be governed by controls acting on the expression of *rpoB* and *rpoC*. Transcription of the gene cluster containing *rpoBC* exhibits a complex pattern (19–23). A strong promoter located in front of *rplJ* (*rplJp*) directs the transcription of the *rplJLrpoBC* genes while initiation at another strong promoter preceding *rplK* (*rplKp*) leads to transcription of not only the *rplKA* genes but also the downstream *rplJLrpoBC* genes. Although both promoters contribute to *rplJLrpoBC* transcription, the *rpoBC* genes are transcribed at about one fifth the frequency of the upstream *rplJL* genes (21). This differential transcription is due to an attenuator (*rpoBa*) located within the *rplL-rpoB* intercistronic region. *rpoBa* resembles a simple or factor-independent terminator in that it is capable of generating an RNA sequence with an 11-bp GC-rich stem-loop structure followed by a run of 5 uridine residues. Nevertheless, recent experiments suggest that NusA and NusG proteins increase the *in vitro* readthrough frequency of *rpoBa* (24).

* To whom correspondence should be addressed

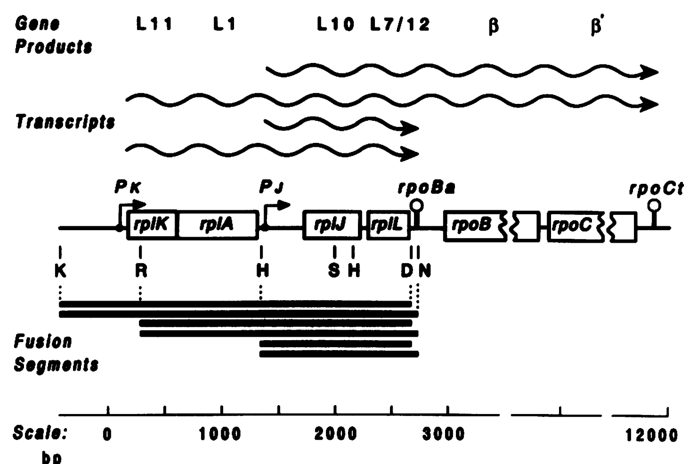

Figure 1. Organization and expression of the *rplKAJLrpoBC* ribosomal protein and RNA polymerase gene cluster. The nucleotide scale is according to Post *et al.* (69). The *rplKAJL* region is drawn to scale, with breaks drawn in the *rpoB* and *rpoC* genes to illustrate the contraction of the map. Bent arrows mark the location and direction of transcription from the *rplK* (P_K) and rplJ (P_J) promoters. Hairpins indicate the locations of the *rpoBa* attenuator and the *rpoCt* transcriptional terminator. Wavy arrows indicate the major *rpoBa*-terminated and readthrough transcripts. Solid bars indicate the DNA segments used in the construction of transcriptional fusions to *lacZ*. Restriction sites are drawn below the map; those corresponding to the endpoints of the fusion segments are drawn with lines extending to the solid bar ends. Restriction site abbreviations and nucleotide positions are as follows: D (*Dde*I, 2672), H (*Hin*dIII, 2154, and engineered at 1338 [see MATERIALS AND METHODS]), K (*Kpn*I, −428), N (*Nar*I, 2730), R (*Eco*RI, 280), and S (*Sma*I, 1986).

We have recently shown *in vivo* that driving transcription of *rplJLrpoBC* with both *rplKp* and *rplJp* as compared to either promoter alone, resulted in an alteration of *rpoBa* termination frequency (21). These results suggested that *rpoBa* efficiency might be modulated by the absolute frequency of transcription into the attenuator. In the current study we have employed single copy transcriptional fusions of the gene cluster to the *lacZ* gene to further test this possibility. By replacing the native *rplJp* promoter and upstream sequences with different promoters spanning a range of initiation frequencies, we demonstrate that the frequency of termination at *rpoBa in vivo* is directly influenced by the frequency of transcription into the attenuator. The implications for autogenous transcriptional control are discussed.

MATERIALS AND METHODS

Bacterial strains

TL11 (25) is a Δ*lacU169* derivative of HB101. MG4 (26) is a Δ*lacU169 recA56* derivative of MG1655. C600 is described in (27). DH5α [F⁻(ϕ80d*lac*ZΔM15) Δ*lacU169 endAl recAl hsdR17* (r_K^- m_K^+) *deoR supE44 thi-1* λ⁻ *gyrA96 relAl*] was obtained from BRL (Burlington, Ontario).

Recombinant DNA

Standard recombinant DNA techniques were used essentially as described by Sambrook, *et al.* (28). Transcriptional or operon fusions were constructed in the λTL61 transcriptional fusion vector (29). Insert DNA fragments and vector phage arms were produced by restriction endonuclease digestion of source DNAs and resolved by agarose gel electrophoresis. Appropriate bands were excised from the standard agarose or Nusieve GTG (FMC BioProducts, Rockland, ME) agarose gels and purified by the Geneclean process (Bio 101 Inc., La Jolla, Calif.) with final resuspension of the DNA in TE. When required, staggered ends were filled in using Klenow enzyme (28). Recombinant phage were recovered from ligation reactions by packaging *in vitro* and plating the reactions on fresh lawns of C600 as previously described (30). Recombinant plasmids were recovered from ligation reactions by transformation into TL11 or DH5α strains either with the $CaCl_2$ cell shock procedure (30) or by using frozen competent cells prepared as described by Hanahan (31). All phage and plasmid constructs were screened by extensive restriction endonuclease digestion analysis. Critical regions of some constructs were confirmed by dideoxy sequencing. Phage DNA was prepared from plate lysates by small scale (30) or mini-prep (32) methods, or a modification of the $ZnCl_2$ method (33). Plasmid DNA for screening was prepared by a modification of the method of Holmes and Quigley (34). Preparative amounts of plasmid were purified by the Qiagen method (Qiagen Inc., Chatsworth, CA).

Table 1. *lacZ* transcriptional fusions

Fusion	Promoter(s)	Downstream Sequences
PA217	P_{ant217}	–
PA219	P_{ant219}	–
KS200	P_{ant224}	–
PA285	P_{ant285}	–
KS160	P_{tac}	–
KS167	P_{ant217}	*rplJL*
KS194	P_{ant219}	*rplJL*
KS195	P_{ant224}	*rplJL*
KS169	P_{ant285}	*rplJL*
KS128	P_{tac}	*rplJL*
KS135	P_{tet}	*rplJL*
KS99	P_{trc}	*rplJL*
TL611	*rplJp*	*rplJL*
KS58	*rplKp* + *rplJp*	*rplJL*
KS76	*rplKp*	*rplJL*
KS168	P_{ant217}	*rplJLrpoBa*
KS189	P_{ant219}	*rplJLrpoBa*
KS166	P_{ant224}	*rplJLrpoBa*
KS170	P_{ant285}	*rplJLrpoBa*
KS123	P_{tac}	*rplJLrpoBa*
KS134	P_{tet}	*rplJLrpoBa*
KS97	P_{trc}	*rplJLrpoBa*
TL601	*rplJp*	*rplJLrpoBa*
KS59	*rplKp* + *rplJp*	*rplJLrpoBa*
KS77	*rplKp*	*rplJLrpoBa*
KS260	P_{ant217}	λt_{R2}
KS259	P_{ant219}	λt_{R2}
KS256	P_{ant224}	λt_{R2}
KS255	P_{ant285}	λt_{R2}
KS258	P_{tac}	λt_{R2}
KS213	P_{ant217}	*rrnBt2*
KS212	P_{ant219}	*rrnBt2*
KS217	P_{ant224}	*rrnBt2*
KS206	P_{ant285}	*rrnBt2*
KS219	P_{tac}	*rrnBt2*

Construction of transcriptional fusions in λTL61

Table 1 lists the single-copy *lacZ* transcriptional fusions that were constructed in the λTL61 vector.

lacZ transcriptional fusions containing different promoters. In this series of constructs each of the P_{ant} 217, 219, 224, and 285 promoters was isolated from pMS217, pMS219, pMS224, and

pMS285 on a 235-bp *Eco*RI(filled in)-*Hin*dIII fragment and attached upstream of *lacZ* carried on a *Hin*dIII-generated left arm of λTL93. The right phage arm in each case came from *Pml*I-digested λTL92, which is essentially λTL61 in which the *Xho*I site in the right arm has been eliminated. The corresponding phage are designated λPa217, λPa219, λKS200, and λPa285, respectively. λTL93 was constructed by ligating the 47-bp *Xba*I-*Bam*HI polylinker segment of pGEM7Zf(+) (Promega, Madison, WI) between the *Xba*I-generated left arm of λTL61 and the *Bam*HI-generated right arm of λTL91. λTL91 is essentially λTL61 that has been modified by elimination of the *Xho*I site in the right arm of the phage genome and replacement of the polylinker with a smaller version containing only *Eco*RI-*Sma*I-*Bam*HI recognition sites. A P_{tac}-containing right phage arm was isolated from *Eco*RI-digested λTAC1. λTAC1 was built by ligating P_{tac} as a 257-bp *Eco*RI-*Bam*HI(filled in) fragment of pKK223-3 (35) between the *Eco*RI-generated left arm of λTL601 (21) and *Pml*I-generated right arm of λTL61.

lacZ transcriptional fusions containing rplJL downstream of different promoters. In this series of constructs, the segment of *rplJL* extending from the engineered *Hin*dIII site at nucleotide (nt) 1338 to the *Dde*I site located 43 bp before the *rpoBa* attenuator was fused between the various promoters and the *lacZ* gene. Each of the P_{ant} 217, 219, 224, or 285 promoters, *tac, tet, trc* promoters, or *rplJp*, *rplKp* + *rplJp*, *rplKp* + *rplJpm* native promoters (21) was fused upstream of the *rplJL* segment to create λKS167, λKS194, λKS195, λKS169, λKS128, λKS135, λKS99, TL611, λKS58, and λKS76 respectively. *rplJpm* contains an engineered mutation in the promoter which reduces initiation to less than 10% of the wildtype *rplJp* (21); consequently, transcription from the *rplKp*+*rplJpm* combination is primarily from *rplKp*. The *rplJL* segment was reconstituted downstream of the promoters by using in all cases the *Sma*I-generated left arm of λTL611 (providing *rplL* and the distal portion of *rplJ* in addition to *lacZ* and the left arm of the λ genome) and either the 647-bp *Sma*I-*Hin*dIII fragment of pKS75 (providing the leader and proximal portion of *rplJ* for fusing with P_{tac} , P_{tet} , P_{trc}) or the 668-bp *Sma*I-*Xba*I fragment of pKS155 (providing the leader and proximal portion of *rplJ* for fusing with *ant* promoters). pKS155 contains the 647-bp *Sma*I-*Hin*dIII fragment of pKS75 (21) ligated between the *Sma*I and *Hin*dIII sites of pTL61T (29). The *ant* wildtype and mutant promoters were fused upstream of the *rplJL* segment by using the *Xba*I-generated right arms of λPa217, λPa219, λKS200, and λPa285. P_{tac} was attached upstream of the reconstituted *rplJL* segment as a 590-bp *Hin*dIII-*Xba*I fragment of pKS106 together with the *Xba*I-generated right arm of λTL61. pKS106 consists of the 579-bp *Hin*dIII(complete)-*Sal*I(partial) P_{tac}-containing fragment of pKK223-3 cloned between the *Hin*dIII and *Sal*I sites of pTL61T. P_{tet} was attached upstream of the *rplJL* segment using the *Eco*RI-generated right arm of λPT3 (essentially λTL61 carrying the *tet* promoter from pBR322) and a 50-bp *Eco*RI-*Hin*dIII adaptor obtained from the polylinker of pTL61T. The *trc* promoter was attached upstream of the *rplJL* segment as a 300-bp *Hin*dIII-*Eco*RI fragment of pKK223-3 together with the *Eco*RI-generated right arm of λTL25 (25). The construction of the λTL611, λKS58, and λKS76 fusions has previously been described (21).

lacZ transcriptional fusions containing rplJLrpoBa downstream of different promoters. In this series of constructs, the segment of *rplJLrpoBa* extending from the engineered *Hin*dIII site at nt 1338 to the *Nar*I site located 15 bp past *rpoBa* was fused between the various promoters and the *lacZ* gene. Each of the P_{ant} 217, 219, 224, and 285 promoters, or *tac, tet, trc* promoters, or *rplJp*, *rplKp* + *rplJp*, *rplKp* + *rplJpm* promoters was fused upstream of the *rplJLrpoBa* segment to create λKS168, λKS189, λKS166, λKS170, λKS123, λKS134, λKS97, λTL601, λKS59, and λKS77 respectively. The *rplJLrpoBa* segment was reconstituted downstream of the promoters by using in all cases the *Sma*I-generated left arm of λTL601 (providing *rpLrpoBa* and the distal portion of *rplJ* in addition to *lacZ* and the left arm of the λ genome) and either the 647-bp *Sma*I-*Hin*dIII fragment of pKS75 (providing the leader and proximal portion of *rplJ* for fusing with P_{tac} , P_{tet} , P_{trc}) or the 668-bp *Sma*I-*Xba*I fragment of pKS155 (providing the leader and proximal portion of *rplJ* for fusing with *ant* promoters). The *ant* wildtype and mutant promoters were fused upstream of the *rplJLrpoBa* segment by using the *Xba*I-generated right arms of λPa217, λPa219, λKS200, and λPa285. P_{tac} was attached upstream of the *rplJLrpoBa* segment as a 590-bp *Hin*dIII-*Xba*I fragment of pKS106 together with the *Xba*I-generated right arm of λTL61. P_{tet} was attached upstream of the *rplJLrpoBa* segment using the *Eco*RI-generated right arm of λPT3 and a 50-bp *Eco*RI-*Xba*I segment of the pTL61T polylinker as an adaptor. The *trc* promoter was attached upstream of the *rplJLrpoBa* segment as a 300-bp *Hin*dIII-*Eco*RI fragment of pKK233-3 (36) together with the *Eco*RI-generated right arm of λTL25. λTL601, λKS59, and λKS77 have previously been described (21).

lacZ transcriptional fusions containing λt_{R2} *downstream of different promoters.* In this series of constructs the λt_{R2} terminator corresponding to nt 40132−41364 of the λ genome was isolated from pKS252 on a 1256-bp *Eco*RI-*Bam*HI fragment and attached upstream of *lacZ* using the *Bam*HI-generated left arm of λTL61. pKS252 consists of the 1232-bp *Nde*I(filled in)-*Cla*I fragment of the λ genome ligated between the *Sma*I and *Cla*I sites of pGEM7Zf(+). The P_{ant} 217, 219, 224, and 285 promoters were joined upstream of λt_{R2} to produce λKS260, λKS259, λKS256, and λKS255 respectively, using the *Eco*RI-generated right arms of λPa217, λPa219, λKS200, and λPa285 respectively. P_{tac} used to construct λKS258 was provided by the right arm of *Eco*RI-digested λTAC1.

lacZ transcriptional fusions containing rrnBt2 downstream of different promoters. In this series of constructs the *rrnBt2* region corresponding to nt 6760−6842 as designated by Brosius *et al.*(37) was isolated as an 83-bp *Xba*I-*Eco*RI fragment from pRT2 and fused upstream of *lacZ* carried on the *Xba*I-generated left arm of λTL61, and downstream of each of the P_{ant} 217, 219, 224, 285 or *tac* promoters carried on *Eco*RI-generated right arms of λPa217, λPa219, λKS200, λPa285 or λTAC1 respectively; this yielded λKS213, λKS212, λKS217, λKS206, and λKS219 respectively. pRT2 was constructed by ligating the 83-bp *Bsa*HI-*Ear*I(filled in) fragment of pTL61T (containing *rrnBt2*) between the *Acc*I and *Xba*I(filled in) sites of pGEM11Zf(−) (Promega, Madison, WI).

Lysogenization and β-galactosidase assays

Monolysogens of each recombinant *lacZ* transcriptional fusion phage were recovered in MG4 as described previously (29). Lysogens were confirmed as monolysogens using the TER excision test (29, 38). Xactosidase assays were performed essentially as described by Miller (39), except that cultures were

grown in AB medium (40) supplemented with thiamine (2 μg/ml), 0.4% glucose, and 0.4% casamino acids. Cultures were grown to an A_{600} of about 0.1 to 0.2, then 30 to 500 μl of cells were permeabilized by the addition of 20 μl chloroform and 10 μl of 0.1% sodium dodecyl sulfate in a final volume of 800 μl. In the case of strain KS160, the culture was grown in the presence of 1mM isopropyl-β-D-thiogalactopyranoside (IPTG) to fully induce the *tac* promoter.

RESULTS

Replacing the native *rplJ* promoter with alternative promoters changes termination frequency at the *rpoBa* attenuator

We have previously demonstrated that whether transcription into *rpoBa* was driven by *rplKp* + *rplJp*, or either *rplKp* or *rplJp* alone, the level of transcription downstream of the attenuator did not vary in proportion, suggesting a possible modulation of termination frequency at *rpoBa*.

To directly test whether *rpoBa* efficiency was modulated by the frequency of transcription, we constructed a series of single-copy *rplJLrpoBa-lacZ* transcriptional fusions in which *rplJp* and the upstream sequences were replaced with various promoters that provided higher and lower rates of initiation. In all cases the promoters were attached via a *Hin*dIII site engineered at nt 1338 in the *rplrpo* sequence carried on pKS75 (21). Since the normal transcription start site of *rplJp* is nt 1346, these fusions position the promoters so that the *rplJ* leader remains essentially unchanged, although the new initiation sites and transcribed sequences upstream of nt 1338 will be different for each promoter. The P_{tac}, P_{trc}, P_{tet}, and P_{ant} fusions introduce an additional 31, 51, 29, and 166 bp respectively of transcribed sequence. Two constructs were built with each promoter: one contained the *rplJL* sequences extending from the engineered *Hin*dIII site at nt 1338 to the *Dde*I site located at nt 2672, which is 43 bp before the *rpoBa* termination site. β-galactosidase measurements for these constructs revealed the transcription frequency just before the attenuator. A second construct contained *rplJLrpoBa* sequences extending from the engineered *Hin*dIII site to the *Nar*I site at nt 2730, just 15 bp after the *rpoBa* termination site. β-galactosidase measurements from this second fusion revealed transcription frequencies after the attenuator.

Table 2 shows the results of β-galactosidase assays performed on the transcriptional fusions with the *trc*, *tac*, and *tet* promoters compared to fusions with *rplKp* and/or *rplJp*. The collection of promoters exhibited a 27-fold range of transcription frequencies (374 to 10207 β-galactosidase units) through the *rplJL* sequences as measured 43 bp before *rpoBa*. The corresponding transcription frequencies, measured 15 bp after *rpoBa*, only exhibited an approximate 9-fold range (202 to 1949 β-galactosidase units). As a result, calculated readthrough frequencies showed about a 3-fold variation from 54% for the weakest promoter to 19% for the strong *trc* promoter.

These results demonstrate that replacement of *rplJp* does not eliminate modulation of *rpoBa* termination frequency. In fact, the replacement of *rplJp* and upstream sequences with the alternative promoters shows that the modulation of *rpoBa* efficiency can be extended over an even greater range than that seen with the native promoters under these growth conditions.

Table 2. *rpoBa* termination frequencies when transcription is driven by the endogenous or alternative promoters

	Transcription Frequencies					
	Upstream		Downstream			
Promoter	Fusion	β-gal[a]	Fusion	β-gal[a]	TERM %[b]	RT %[c]
P_{trc}	KS99	10207	KS97	1949	81	19
P_{tac}	KS128	9482	KS123	1951	79	21
rplKp+*rplJp*	KS58	5967	KS59	1299	78	22
rplKp	KS76	4040	KS77	1077	73	27
rplJp	TL611	3497	TL601	1144	67	33
P_{tet}	KS135	374	KS134	202	46	54

[a]β-galactosidase activities were determined as described in Materials and Methods; all values represent the averages of at least 6 assays performed on 3 or more independent cultures and have standard deviations of 5% or less.
[b]Termination frequency in percent was calculated as 100×(1−(β-gal units downstream of attenuator ÷ β-gal units upstream of attenuator))
[c]Readthrough frequency in percent was calculated as 100×(β-gal downstream of attenuator ÷ β-gal units upstream of attenuator)

Transcription frequency dependent modulation of *rpoBa* efficiency does not depend on the initial transcribed region of the alternative promoters

The modulation of *rpoBa* termination frequency seen when *rplJp* is replaced with several alternative promoters appears to correlate directly with the rates of initiation from these promoters. An alternative explanation for this observation could be the phenomenon of factor-independent antitermination suggested by Telesnitsky & Chamberlin (41), and Goliger *et al.* (42). Results from these groups suggested that the efficiency of some terminators may be modulated by sequences located between +1 to +30 in the initial transcribed region of certain promoters. Therefore, the possibility remained that the different termination rates obtained for our fusions might be a consequence of differences among the initial transcribed regions of the fragments containing *rplJp*, *rplKp*, P_{tac}, P_{tet}, or P_{trc}. This question was particularly relevant since the *tac* promoter used in our constructs was one of the promoters reported in the above studies to show antitermination at some terminators.

To determine if *rpoBa* termination efficiency could be modulated while keeping sequences in the initial transcribed region constant, we constructed additional *rplJLrpoBa-lacZ* transcriptional fusions in which *rplJp* was replaced with either the P22 wild-type *ant* promoter or several of the single-basepair variants previously described (43). These P_{ant} variants contain identical transcribed regions but each differs from the wild-type *ant* promoter by a unique, single-basepair change in the non-transcribed −10 or −35 regions. Such single-basepair changes provide initiation rates that differ significantly from that of the wild type *ant* promoter. As with the previous fusions, each of the variant promoters was used to build two constructs: both constructs fused the promoter upstream of the engineered *Hin*dIII

Table 3. *rpoBa* termination frequencies when transcription is driven by P_{ant} promoter variants

	Transcription Frequencies					
	Upstream		Downstream			
Promoter	Fusion	β-gal[a]	Fusion	β-gal[a]	TERM %[b]	RT %[b]
P_{ant285}	KS169	6463	KS170	1341	79	21
P_{ant219}	KS194	2379	KS189	646	73	27
P_{ant224}	KS195	774	KS166	300	61	39
P_{ant217}	KS167	256	KS168	155	39	61

[a]β-galactosidase activities as described in Table 2.
[b]Termination and readthrough frequencies as described in Table 2.

site at nt 1338 of the *rplJL* sequence, but extended the *rplJL* sequence to either 43 bp before or 15 bp beyond *rpoBa*.

Table 3 shows the results of β-galactosidase assays performed on monolysogens of the fusion phage. The set of fusions containing the P_{ant} variants and *rplJL* sequences extending to just upstream of *rpoBa*, show a 25-fold range of transcription frequencies. β-galactosidase measurements for the corresponding *rpoBa*-containing fusions show a much smaller (9-fold) range of readthrough transcription. The calculated *rpoBa* readthrough rates for this set of fusions varied from 61% for the weakest *ant* promoter mutation to 21% for the wild-type *ant* promoter.

These observations are consistent with the previous fusions in that decreasing transcription frequency appears to lead to increasing readthrough of the *rpoBa* attenuator (Figure 2). Since these *ant* promoter fusions share identical transcribed regions, and differ only in their non-transcribed promoter regions and consequently their initiation rates, we conclude that the modulation of *rpoBa* efficiency is controlled by transcription frequency, rather than factor-independent antitermination mediated by initial transcribed sequences of different promoters.

Other terminators maintain constant terminator efficiency when transcribed from promoters of different strength

The current model for simple or intrinsic terminator function proposes that sequences of the terminator, when transcribed into mRNA, form a stem-loop structure followed by a run of uridine residues. Such a stem-loop structure is thought to pause the elongating RNA polymerase while the run of uridines destabilizes the RNA-DNA hybrid, causing release of the transcript (reviewed in 44). The probability of termination is thought to be determined by the combined stabilities of the RNA stem-loop, uridine-deoxyadenosine RNA-DNA hybrid, and the DNA-DNA hybrid in the transcription complex. Consequently, the implication is that terminator efficiency will usually be constant regardless of the frequency with which transcription complexes enter the terminator. A more recent model proposes that kinetic processes in addition to thermodynamic stability determine terminator location and efficiency (45, 46). Since, at least in the case of *rpoBa*, transcription frequency was shown to influence termination frequency, we wondered if other terminators might be similarly affected.

We tested two well-characterized simple terminators, the strong *rrnBt2* terminator of the *rrnB* ribosomal RNA operon (37), and the t_{R2} terminator of phage λ (47). A series of constructs were assembled in which an 83-bp fragment carrying *rrnBt2* was inserted between P_{tac} or each of the P_{ant} variants and *lacZ*. An analogous set of fusions was made with λt_{R2} carried on a 1256-bp fragment. To measure transcription frequencies in the absence of the terminator, each promoter by itself was fused upstream of *lacZ*.

Table 4 shows the results of β-galactosidase assays on these fusions, and Figure 2 graphically compares the results with those of *rpoBa*. The β-galactosidase measurements from the series of fusions containing each promoter alone fused upstream of *lacZ* shows that an approximate 42-fold range of transcription frequency was achieved. The corresponding β-galactosidase measurements from the series of fusions containing either the *rrnBt2* or λt_{R2} terminator failed to show a clear decrease in termination frequency in response to the 42-fold decrease in transcription. Therefore, the results at least from these two terminators, support the prediction of fixed terminator efficiency for simple terminators, irregardless of transcription frequency.

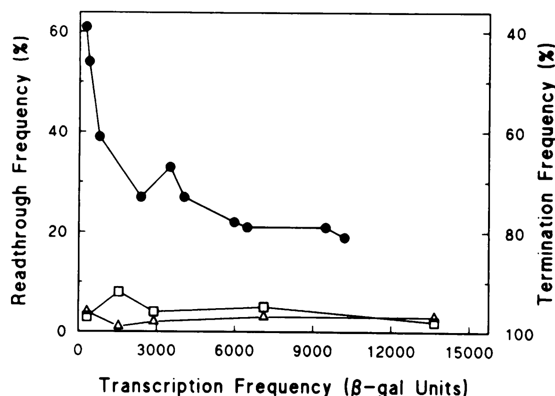

Figure 2. Modulation of rpoBa efficiency and constant efficiencies of the *rrnBt2* and λt_{R2} terminators. The transcription rate into the various terminators is plotted versus the readthrough or termination frequencies presented in Tables 2 through 4. In the case of the *rpoBa* curve (solid circles) the transcription rates corresponding to the β-galactosidase activity measured with different promoters placed upstream of the *rplJL* region which extends to the *DdeI* site 43 bp before *rpoBa*. For the *rrnBt2* (open triangles) and λt_{R2} (open squares) curves the transcription rates indicate the β-galactosidase levels produced by the promoters alone attached upstream of *lacZ*.

DISCUSSION

We have examined *in vivo* the ability of the *rpoBa* transcriptional attenuator to regulate the transcription of the *rpoBC* genes. The results show that the *rpoBa* attenuator is not fixed in its termination efficiency, but varies with the frequency of transcription initiated by promoters located in the upstream *rplKAJL* ribosomal protein genes. This modulation does not require the *rplK* or *rplJ* promoters per se or any sequences located upstream of *rplJp* since replacing these with heterologous promoters reproduces the modulated termination. Modulation of *rpoBa* efficiency appears to be unrelated to the factor-independent antitermination that was proposed by other groups to be mediated

Table 4. Termination frequencies of simple terminators when transcribed by alternative promoters

Promoter	Promoter Alone		+ *rrnBt2*			+ λt_{R2}		
	Fusion	β-gal[a]	Fusion	β-gal[a]	TERM %[b]	Fusion	β-gal[a]	TERM %[b]
P_{tac}	TAC1	13659	KS219	272	98	KS258	404	97
P_{ant285}	PA285	7113	KS206	391	95	KS255	241	97
P_{ant219}	PA219	2891	KS212	119	96	KS259	63	98
P_{ant224}	KS200	1539	KS217	124	92	KS256	23	99
P_{ant217}	PA217	323	KS213	10	97	KS260	14	96

[a]β-galactosidase activities as described in Table 2.
[b]Termination frequency as described in Table 2.

by sequences located in the initial transcribed region of some promoters. Furthermore, comparison of *rpoBa* function with that of *rrnBt2* and λt_{R2}, suggests that the modulation of termination efficiency by transcription frequency may not occur in other simple terminators. Our finding that the termination frequency of two well-characterized terminators is essentially constant when transcribed from a set of promoters spanning a 42-fold range of initiation rates is not surprising. Nevertheless, this question warranted examination since the assumption that terminators have fixed efficiencies has largely been intuitive, and has seldom been rigorously tested. Our results agree with those of Li *et al.* (48), which showed nearly constant termination frequencies (98%, 96.4%, and 98.5%) for the inverted 16S terminator when transcribed *in vivo* from the *tac*, *lac*, and *rrnGp*$_2$ promoters respectively. Together these studies show that P_{tac}-initiated transcripts are no more prone to antitermination at the *rrnBt2*, λt_{R2}, or inverted 16S terminators than are those from other promoters. These results indicate that although P_{tac} can confer factor-independent antitermination at the Te terminators of bacteriophages T7 and T3, as reported by Telesnitsky and Chamberlin (41), it does not appear to be a universal phenomenon at all terminators.

Our finding that *rpoBa* efficiency is modulated in response to transcription frequency, combined with evidence that the global level of transcription increases in proportion to the concentration of active RNA polymerase (49), argues that modulation of *rpoBa* efficiency may provide a transcriptional means for autogenously regulating the expression of *rpoB* and *rpoC*. Under the steady state growth conditions employed here, only one in five transcribing polymerase molecules initiated at the combination of the endogenous *rplK* and *rplJ* promoters continues past *rpoBa* into the *rpoBC* structural genes. This level of readthrough appears to be near the lower limit as increasing transcriptional frequency with stronger promoters has little effect on the termination frequency at *rpoBa*. However as transcription frequency decreases, the level of readthrough increases substantially. With the lowest level of transcription tested in this study, three out of every five transcription complexes encountering *rpoBa* read through the attenuator. Such a transcriptional mechanism may augment the previously demonstrated translational feedback control of *rpoBC* expression (2−8).

While some of our conclusions for modulation of *rpoBa* efficiency are consistent with the results of earlier work, the present study uses a more direct approach to address the issue. Previous studies examined conditions where the cell's overall transcription initiation capacity was restricted and reported that termination efficiency at *rpoBa* was altered. One method used in those experiments to limit initiation involved shifting to non-permissive temperatures, strains carrying temperature-sensitive mutations either directly in RNA polymerase subunit genes (12, 13) or in suppressor genes suppressing amber mutations in polymerase genes (50). Another method involved treating cells with rifampicin to directly impair the enzyme's transcription initiation activity. Both of these methods have the potential to alter the response of RNA polymerase to normal transcriptional regulatory signals, or to have pleiotropic effects on overall gene expression that may indirectly affect termination at *rpoBa*. Rifampicin for example, has been shown to alter the sensitivity of RNA polymerase to several different terminators in a non-specific fashion (10, 51), and to affect the level of DNA supercoiling (52), with the possible side-effects on transcriptional regulation that may entail (53). In contrast to these approaches, our use of single-copy *lacZ* transcriptional fusions, in which alternative promoters increase or decrease the normal rate of polymerase loading onto the *rplJLrpoBa* template, is not expected to alter the normal function and sensitivity of RNA polymerase to transcriptional control signals.

The observation that it is the transcription frequency which modulates *rpoBa* function suggests that transcriptional feedback may take the form of RNA polymerase in active transcription complexes rather than inactive enzyme. Mechanistically, how altered transcription initiation rates, and therefore changes in the frequency at which transcription complexes arrive at *rpoBa*, might alter termination at this site is not clear. Since the *rplK* and *rplJ* promoters were shown to be dispensable for this effect, and because other promoters could substitute in their place, the signals important for the modulation of termination apparently lie downstream of *rplJp*. The paradigm for promoter-distal signals that modulate termination is the *nut* site involved in N-mediated antitermination of phage λ, 21, and P22 (reviewed in 54). *nut* sites are composed of both a conserved sequence, *boxA*, and a region capable of forming a stem-loop structure in the RNA, *boxB*. The *nut* site appears to be the nucleation site for the formation of an elongation complex on the surface of RNA polymerase that contains the λ N protein and the host-encoded NusA, NusB, NusG, and S10 proteins (55, 56). Nut-like sequences have also been implicated in antitermination of transcription in the host's *rrn* (57; reviewed in 58) and *rpsU-dnaG-rpoD* operons (59). Moreover, NusA has been shown to either stimulate or suppress termination at a number of host operons (60−66) and nusB has been implicated in the processivity of transcription in *rrn* operons (67).

There is some preliminary evidence supporting a site- and/or factor-mediated antitermination system in the *rplJLrpoBC* operon. NusA mutants demonstrate a decreased level of transcription of *rpoB* with respect to *rplJL*, suggesting that the normal function of NusA is to decrease termination at the attenuator (68). Recent *in vitro* transcription experiments also show that both NusA and NusG increase readthrough at *rpoBa* in a dose-dependent manner (24). Interestingly, the *rpoCt* terminator located at the end of the operon seems to have in common with the *rrnGt* terminator the ability to stop antiterminated transcription from the *rrnG* promoter region (57). The demonstrated role of *rrnGt* in stopping antiterminated transcription from the *rrnG* operon suggests that *rpoCt* may play a similar role in the *rplKAJLrpoBC* gene cluster. It is notable that the same study also showed that, unlike *rpoCt*, the *rpoBa* attenuator is sensitive to antitermination when fused downstream of the *rrnG* antiterminator region. These collective findings are suggestive that there may indeed be site-mediated alteration of *rpoBa* termination frequency, but a direct demonstration is required by mapping such a site.

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