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Rifampin Resistance Mutations That Alter the Efficiency of Transcription Termination at the Tryptophan Operon Attenuator

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Rifampin-resistant mutants of *Escherichia coli* were isolated which had altered patterns of resistance or sensitivity to the inhibitory compounds 5-methyltryptophan and 5-methylanthranilate. The levels of tryptophan (*trp*) operon polypeptides in different rifampin-resistant mutants were elevated or reduced, in a manner consistent with their sensitivity to the two analogs. Complementation tests established that the mutations were in *rpoB*, the structural gene for the β subunit of ribonucleic acid polymerase. Introduction of these *rpoB* mutations into mutant strains which terminate transcription abnormally at the *trp* operon attenuator established that the *rpoB* mutations alter *trp* operon expression by increasing or decreasing transcription termination at the attenuator. The *rpoB* mutations affected transcription termination at the attenuator only in strains which were able to form what is thought to be a ribonucleic acid termination structure. These findings suggest that alteration of the β subunit of ribonucleic acid polymerase directly or indirectly affects ribonucleic acid polymerase's recognition of the transcription termination signal at the *trp* operon attenuator.

Transcription of the tryptophan (*trp*) operon of *Escherichia coli* is regulated at a promoter-operator site and at a transcription termination site called the attenuator (26). The attenuator is located in the transcribed region immediately preceding the structural genes of the operon (3, 12, 24). When DNA restriction fragments containing the initial segment of the operon are transcribed in vitro, most RNA polymerase molecules terminate transcription at the attenuator and form a stable RNA-DNA-polymerase complex (8, 13; Winkler and Yanofsky, manuscript in preparation). Single base-pair mutations in the attenuator region appreciably reduce transcription termination in vivo and in vitro (24, 31), indicating that RNA polymerase must recognize some feature of the transcript, template, or transcript-template complex as the termination signal. Various lines of evidence suggest that a base-paired structure in the RNA transcript is at least one component of the transcription termination signal (13, 17, 30, 31). Thus, mutations that disrupt base pairing in this structure, and, hence, its stability, reduce termination in vivo and in vitro (24, 31). In addition, substitution of ITP for GTP as substrate in the in vitro reaction eliminates termination at the attenuator (13). Presumably replacing G $\equiv$ C base pairs in the transcript by the weaker I = C base pairs destabilizes the base-paired region, and this eliminates termination.

Studies with RNA polymerase mutants have shown that polymerase alterations can markedly affect the efficiency of transcription termination in vivo and in vitro (6, 10, 16, 28). In particular, the studies of Neff and Chamberlin (16) have established that the polymerases of some Rif^r mutants behave aberrantly in transcription termination reactions. With their studies as a basis, we isolated rifampin-resistant mutants to determine whether polymerase alterations affect the efficiency of transcription termination at the *trp* attenuator. We have found that mutations to rifampin resistance may either increase or decrease the efficiency of transcription termination at the *trp* attenuator.

MATERIALS AND METHODS

The bacterial strains employed in this study are described in Table 1.

Selection of rifampin-resistant (Rif^r) strains. The procedure of Leive (14) was followed with minor modifications. Cultures of strains CY15001 (*trpR*), CY15006 (*trpR trpL29*), and CY15007 (*trpR trpL75*) were grown in L broth (15) to 10^9 cells per ml and centrifuged, and the cells were suspended in 2.5 ml of shaken at 37°C for 2 min, and 0.25 M EDTA (pH 8.1) was added to a final concentration of 1 mM. The suspensions were shaken at 37°C for an additional 2 min. The cells were collected by centrifugation and suspended in 0.5 ml of minimal medium (25). Approximately 10^9 and 2×10^9 cells were spread on plates of L-broth agar plus 100 μg of rifampin per ml. Multiple

TABLE 1. *Bacterial strain employed*

Strain[a]	Genotype	Characteristics
CY15001	*trpR*	Inactivated repressor, *trp* operon regulated by attenuation.
CY15002	*trpR ΔtrpE5*	*trpE* deletion, attenuator intact.
CY15003	*trpR Δ(trpED)24*	*trpED* deletion, attenuator intact.
CY15004	*trpR Δ(trpLD)102*	*trpLED* deletion, attenuator deleted.
CY15005	*trpR Δ(trpLE)1417*	*trpLE* deletion, attenuator deleted.
CY15006	*trpR trpL29*	Mutation at position 29 of the leader region, altering the start codon for the synthesis of the *trp* leader peptide; this mutation increases transcription termination at the attenuator in vivo (30, 31).
CY15007	*trpR trpL75*	Mutation at position 75 of the leader region; this mutation increases transcription termination at the attenuator in vivo (30, 31).
CY15008	*trpR trpL117-1*	Mutation at position 117 in the leader region; this mutation partially destabilizes a base-paired structure in the RNA transcript of the leader region and results in reduced termination at the attenuator in vivo and in vitro (24).
CY15009	*trpR trpT*(Ts)	A temperature-sensitive mutation affecting $tRNA^{Trp}$ that results in reduced termination at the attenuator in vivo (7, 27).
N01602	*arg leu metB gal lac rts* (λ)	A rifampin-sensitive strain carrying an *rpoB*-linked temperature-sensitive mutation, *rts*, that is complemented along with *rpoB*(Rif^r) by λ d *rif-6*.
CY15010	*trpR trpL29 rpoB2*	Rifampin-resistant mutant.
CY15011	*trpR trpL29 rpoB6*	Transductant of CY15006.
CY15012	*trpR trpL75 rpoB2*	Transductant of CY15007.
CY15013	*trpR trpL75 rpoB6*	Rifampin-resistant mutant.
CY15014	*trpR rpoB2*	Transductant of CY15001.
CY15015	*trpR rpoB6*	Transductant of CY15001.
CY15016	*trpR Δ(trpLD)102 rpoB2*	Transductant of CY15004.
CY15017	*trpR Δ(trpLD)102 rpoB6*	Transductant of CY15004.
CY15018	*trpR trpL117-1 rpoB2*	Transductant of CY15008.
CY15019	*trpR trpL117-1 rpoB6*	Transductant of CY15008.
CY15020	*trpR trpT rpoB2*	Transductant of CY15009.
CY15021	*trpR trpT rpoB6*	Transductant of CY15009.
CY15022	*trpR rpoB7*	Rifampin-resistant mutant of CY15001.
CY15023	*trpR rpoB8*	Rifampin-resistant mutant of CY15001.
CY15024	*trpR ΔtrpE5 rpoB7*	Transductant of CY15002.
CY15025	*trpR ΔtrpE5 rpoB8*	Transductant of CY15002.
CY15026	*trpR Δ(trpED)24 rpoB7*	Transductant of CY15003.
CY15027	*trpR trpL29 rpoB7*	Transductant of CY15006.
CY15028	*trpR trpL29 rpoB8*	Transductant of CY15006.
CY15029	*trpR trpL75 rpoB7*	Transductant of CY15007.
CY15030	*trpR trpL75 rpoB8*	Transductant of CY15007.
CY15031	*trpR trpL117-1 rpoB7*	Transductant of CY15008.
CY15032	*trpR trpL117-1 rpoB8*	Transductant of CY15008.
CY15033	*trpR Δ(trpLD)102 rpoB7*	Transductant of CY15004.
CY15034	*trpR Δ(trpLD)102 rpoB8*	Transductant of CY15004.
CY15035	*trpR Δ(trpLE)1417 rpoB7*	Transductant of CY15005.
CY15036	*trpR trpT rpoB7*	Transductant of CY15009.

[a] All strains except N01602 are derivatives of W3110 and carry the same *tnaA* allele.

starting cultures were used to insure sampling of independent events.

Detection of termination mutants. Rifampin-resistant mutants were patched onto master plates of L-broth agar plus rifampin (100 μg/ml) and replicated to 5MT agar (containing minimal agar, 0.2% glucose, 20 μg of DL-5-methyltryptophan per ml, and 0.2% acid-hydrolyzed casein) and MAA agar (containing minimal agar, 0.2% glucose, 100 μg of 5-methylanthranilate per ml, 50 μg of L-cysteine per ml, 40 μg of L-leucine per ml, and 40 μg of L-methionine per ml). The addition of the latter three amino acids appeared to enhance the distinction between sensitive and resistant colonies.

The responses of the various strains on 5MT agar and MAA agar at 30°C and 41°C are summarized in Table 2. Generally, 5MT resistance was scored at 41°C, whereas MAA resistance was scored at 30°C. Rifampin-resistant colonies that exhibited differences in resistance to 5MT or MAA were picked, purified, and grown for *trp* enzyme assays. The *rpoB* alleles of mutants with altered enzyme levels were transduced into their corresponding parental strains, and enzyme

TABLE 2. *Growth response of various strains upon replication to media containing 5-methyltryptophan or 5-methylanthranilate*

Strain	Relevant genotype	Incubation for 41 h at 30°C on[a]:			Incubation for 17 h at 41°C on[a]:		
		minimal	5MT	MAA	minimal	5MT	MAA
CY15001	*trpR*	+	+	−	+	+	±
CY15006	*trpR trpL29*	+	−	+	+	−	±
CY15007	*trpR trpL75*	+	−	+	+	−	±

[a] For composition of agars, see text.

assays were performed on the transductants to be certain that each Rif^r mutation was responsible for the *trp* enzyme level change.

Determination of *trp* enzyme levels. Each culture was grown for at least three generations in minimal medium containing 0.2% glucose, 0.05% acid-hydrolyzed casein, and 50 μg of L-tryptophan per ml. Cells were harvested at a density of 6×10^8 to 7×10^8 cells per ml, washed with saline, suspended in 0.1 M Tris-HCl (pH 7.8), and disrupted by sonic oscillation. Cell debris was removed, and the supernatant was assayed for *trp* enzymes by previously described procedures (5, 23). Strains with the *trpT*(Ts) allele (7, 27) were grown at 35°C, as were all strains with which they were compared. All other strains were grown at 37°C.

RESULTS

Selection of rifampin-resistant mutants with altered expression of the *trp* operon. *E. coli* mutant strains which have high levels of the five *trp* operon polypeptides will grow in the presence of 5-methyltryptophan but are inhibited by 5-methylanthranilate (30, 31). We assume that such cells establish a higher internal concentration of toxic 5-methyltryptophan when they synthesize it from 5-methylanthranilate than when they transport the 5-methyltryptophan from the culture medium. Thus, *trpR* mutants, mutants which lack a functional *trp* repressor, are normally resistant to 5-methyltryptophan and sensitive to 5-methylanthranilate. However, strains which are *trpR* and contain the *trpL29* or *trpL75* mutation are inhibited by 5-methyltryptophan and resistant to inhibition by 5-methylanthranilate (30). These strains have *trp* polypeptide levels only 20 to 25% of that of parental *trpR* strains. The decreased *trp* enzyme levels in these strains are due to increased transcription termination at the *trp* operon attenuator (30, 31). The characteristics mentioned above permit the detection of mutants of CY15006 (*trpR trpL29*) and CY15007 (*trpR trpL75*) that have become resistant to 5-methyltryptophan and mutants of the parental *trpR* strain, CY15001, that have become resistant to 5-methylanthranilate.

Cultures of strains CY15001 (*trpR*), CY15006 (*trpR trpL29*), and CY15007 (*trpR trpL75*) were plated on rifampin agar, and the resistant colonies which spontaneously developed were picked and patched onto master plates. The master plates were then replica plated to agar containing 5-methyltryptophan or 5-methylanthranilate (Fig. 1). Colonies phenotypically different from the plated strains were readily apparent. These colonies were picked and purified for further study.

***trp* enzyme levels in rifampin-resistant mutants and transductants.** Strains CY15010 (*trpR trpL29 rpoB2*) and CY15013 (*trpR trpL75 rpoB6*) were obtained by selecting rifampin-resistant, 5-methyltryptophan-resistant colonies, as described above (evidence that the Rif^r mutations are in *rpoB* will be presented in the next section). The *rpoB2* and *rpoB6* alleles were transduced into a variety of strains which were then grown for *trp* enzyme level determinations. The *rpoB2* and *rpoB6* alleles, when present in strains with *trpR* and either *trpL29* or *trpL75*, elevated *trp* operon expression about three- to fourfold (Table 3). This increase accounts for the phenotypic change in these strains of acquisition of resistance to 5-methyltryptophan. The *rpoB2* and *rpoB6* alleles have only a small effect on *trp* enzyme levels when introduced into the parental *trpR* strain, CY15001. They elevate enzyme levels only about 30% (see CY15014 and CY15015 in Table 3). That these *rpoB* alleles increase expression by reducing termination at the *trp* attenuator is shown by the data in Table 4. Deletion of the attenuator, inactivation of the attenuator by mutation, and introduction of a *trpT*(Ts) allele that relieves termination at the attenuator each prevents an observable effect of these *rpoB* alleles on *trp* operon expression. Rif^r mutants isolated in the same experiments which were not resistant to 5-methyltryptophan had *trp* polypeptide levels indistinguishable from those of the starting Rif^s strains, CY15006 and CY15007 (data not shown).

In other studies, we examined the effect of the *rpoB2* and *rpoB6* mutations on translational polarity. It is known that the decrease in distal gene expression in polar mutants of polygenic operons results from transcription termination (1). It was of interest, therefore, to determine whether the *rpoB* mutations that reduce transcription termination at the *trp* attenuator also

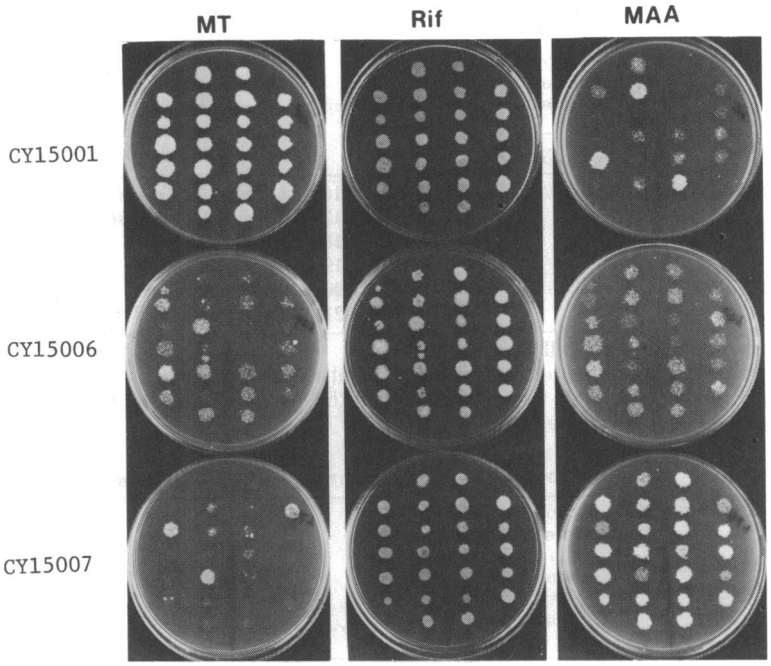

FIG. 1. *Replica plating of patched rifampin-resistant colonies (Rif) to media containing 5-methyltryptophan or 5-methylanthranilate. Colonies resistant to 5-methyltryptophan (MT) or 5-methylanthranilate (MAA) are evident. The three strains used are CY15001 (trpR), CY15006 (trpR trpL29), and CY15007 (trpR trpL75). Strain CY15001 is resistant to 5-methyltryptophan and sensitive to 5-methylanthranilate, whereas the reverse is true of the other two strains.*

TABLE 3. *Effects of Rif^r mutations on trp operon expression in strains containing the trpL29 and trpL75 mutations*

Strain	Relevant genotype[a]	% of parental enzyme value[b]	
		trpE	*trpD*
CY15001	Parental	100	100
CY15006	*trpL29*	27	26
CY15010	*trpL29 rpoB2*	68 (2.5)	81 (3.1)
CY15011	*trpL29 rpoB6*	83 (3.1)	80 (3.1)
CY15007	*trpL75*	14	14
CY15012	*trpL75 rpoB2*	59 (4.2)	58 (4.1)
CY15013	*trpL75 rpoB6*	58 (4.1)	60 (4.3)
CY15014	*rpoB2*	127	137
CY15015	*rpoB6*	121	141

[a] All strains have the same *trpR* allele.

[b] Average of values with two or more cultures; the fold increase over parent is within parentheses.

relieve polarity-associated termination. To test this possibility, we introduced the *rpoB2* and *rpoB6* alleles into *trpR* strains that have the strongly polar *trpC* nonsense mutation, *trpC315*(Am). We then assayed the levels of the polypeptide products of *trpE*, *trpD*, *trpB*, and *trpA*. The ratios of *trpB/trpE* activities and *trpA/trpD* activities (data not shown) indicated that the two *rpoB* alleles tested had no significant effect on the translational polarity associated with the *trpC315* mutation.

Mutations to rifampin resistance were also detected that had the opposite effect, namely, they decreased *trp* operon expression (Table 5). The *rpoB7* and *rpoB8* mutations detected in the CY15001 parental *trpR* strain decreased *trp* enzyme levels in the parent and when introduced into CY15002 (*trpR ΔtrpE5*), CY15003 (*trpR Δ(trpED)24*), CY15006 (*trpR trpL29*) and CY15007 (*trpR trpL75*), but not in CY15004 (*trpR Δ(trpLD)102*) or CY15005 (*trpR Δ(trpLE)1417*). These observations indicate that these Rif^r alleles also affect termination at the *trp* operon attenuator. The *rpoB7* and *rpoB8* alleles also decreased expression in strains with the *trpL117-1* mutation (CY15031, CY15032; Table 5), but did not depress and, in fact, slightly elevated *trp* enzyme levels in the CY15036 (*trpT*) strain.

Complementation analyses with rifampin-resistant mutants. Rifampin-resistant mutants are generally altered in *rpoB*, the structural gene for the β subunit of RNA polymerase (6, 10, 16, 28). To ascertain whether the rifampin-resistant mutants we isolated were of this type, we performed complementation analyses

TABLE 4. trp *enzyme levels in strains with Rif^r mutations*

Reference stain	Relevant genotype[a]	*trp* polypeptide level in reference strain (% of parental value)[b]		Test strain	Relevant genotype[a]	Relative *trp* polypeptide levels (% of the value for the corresponding reference strain)[c]			
		trpE or *B*	*trpD* or *A*			*trpE*	*trpD*	*trpB*	*trpA*
CY15004	*Δ(trpLD)102*	600	660	CY15016	*Δ(trpLD)102 rpoB2*			83	90
				CY15017	*Δ(trpLD)102 rpoB6*			82	83
CY15005	*trpL117-1*	530	450	CY15018	*trpL117-1 rpoB2*	84	93		
				CY15019	*trpL117-1 rpoB6*	83	86		
CY15009	*trpT*	570	610	CY15020	*trpT rpoB2*	99	110		
				CY15021	*trpT rpoB6*	94	86		

[a] All strains have the same *trpR* allele.
[b] Average of values for two or more cultures; the parental strain is CY15001.
[c] The reference strain values used in the calculation of the values in these last four columns are the values given in columns 3 and 4 of this table.

TABLE 5. *trp enzyme levels in strains with Rif^r mutations*

Reference strain	Relevant genotype	*trp* polypeptide level in reference strain (% of parental value)[a]		Test strain	Relevant genotype[b]	Relative *trp* polypeptide levels (% of the value for the corresponding reference strain)[c]			
		trpE or *B*	*trpD* or *A*			*trpE*	*trpD*	*trpB*	*trpA*
CY15001	Parental	(100)	(100)	CY15022	*rpoB7*	46	45	35	39
				CY15023	*rpoB8*	22	24		
CY15002	*ΔtrpE5*	133	135	CY15024	*ΔtrpE5 rpoB7*		43	48	
				CY15025	*ΔtrpE5 rpoB8*		21	25	
CY15003	*ΔtrpE24*	152	153	CY15026	*Δ(trpED)24 rpoB7*			27	26
CY15006	*trpL29*	39	30	CY15027	*trpL29 rpoB7*	40	32		
				CY15028	*trpL29 rpoB8*	18	22		
CY15007	*trpL75*	16	19	CY15029	*trpL75 rpoB7*	34			
				CY15030	*trpL75 rpoB8*	50	41		
CY15008	*trpL117-1*	443	433	CY15031	*trpL117-1 rpoB7*	56	58		
				CY15032	*trpL117-1 rpoB8*	35	34		
CY15004	*Δ(trpLD)102*	671	612	CY15033	*Δ(trpLD)102 rpoB7*			106	118
				CY15034	*Δ(trpLD)102 rpo B8*			99	92
CY15005	*Δ(trpLE)1417*	328		CY15035	*Δ(trpLE)1417 rpoB7*			139	
CY15009	*trpT*	662	745	CY15036	*trpT rpoB7*	152	157		

[a] Average of values with two or more cultures in most cases.
[b] All strains have the same *trpR* allele.
[c] The reference strain values used in the calculation of the values in these last four columns are the values given in columns 3 and 4 of this table.

with λ d *rif-6*, a defective phage containing *rpoB*$^+$ and a small segment of *rpoC* (11). Each rifampin-resistant allele was transduced into N01602, a strain with the *rpoB*-linked *rts* mutation, and an *rts*$^+$ Rif^r isolate was recovered. Each of these isolates was transduced to temperature insensitivity by using λ d *rif-6* (this phage also carries *rts*$^+$). The resulting colonies were tested and found to be rifampin sensitive, suggesting that the *rpoB*$^+$ allele of λ d *rif-6* complements each of the Rif^r mutations. Thus, the Rif^r mutations are probably within *rpoB*. In other tests, it was shown that each of the *rpoB* (Rif^r) alleles was cotransduced with *argE*$^+$ at a frequency of approximately 50%, the same frequency obtained with other *rpoB* mutants. On the basis of these findings, we conclude that all of the Rif^r mutations we have described are in *rpoB*. We have analyzed other Rif^r mutations of both types by the above tests and all appear to be in *rpoB*.

DISCUSSION

E. coli RNA polymerase terminates transcription in vitro at specific sites in DNA (4, 18–20). Rho factor is required for termination at some of these sites, whereas at others, termination occurs without this or any other accessory factor (18–20). Transcription termination sites consist of GC-rich regions of DNA that are immediately

followed by AT-rich segments within which termination occurs (18, 20). The GC-rich regions often exhibit dyad symmetry. It is thought that this symmetry reflects the stable base pairing of corresponding segments of the RNA transcript, and that such base-paired structures function as termination signals (2, 20, 21). How the rho factor participates in those termination events in which it is involved is not known. Nor is it known how release of the transcript and template from RNA polymerase is accomplished. Release occurs spontaneously in vitro at some termination sites, whereas at others, the rho factor mediates dissociation of the termination complex (4, 9, 18–20).

Mutant RNA polymerases have been described that are defective in termination in vivo and in vitro (6, 10, 16, 28). *rpoB* mutations in particular appear to affect the efficiency of the termination event, implicating the β subunit of RNA polymerase in recognition of the transcription termination signal (6, 10, 16, 28). Since the β subunit is believed to contain the nucleoside triphosphate binding site, perhaps it also interacts with the 3′ end of the growing or to-be-terminated transcript (22, 29). The spatial relationships of template, transcript, and polymerase in the termination complex have yet to be elucidated.

Studies on transcription termination at the *trp* attenuator and its regulation have served as the basis for the development of a model that attempts to account for these events (12, 17, 30). It is assumed that when RNA polymerase transcribes the initial segment of the *trp* operon, the transcript segment from nucleotides 110 to 130 forms a hydrogen-bonded structure (the termination structure) that is recognized by the transcribing polymerase as the transcription termination signal. The termination structure has been shown to form in vitro (13, 17), and is thought to form in vivo in cells that have an adequate supply of tryptophan. In cells that are starved of tryptophan, however, this structure presumably does not exist because a competing base-paired structure forms first and temporarily prevents the base pairing that generates the termination structure. We believe that the competing base-paired structure forms because the ribosome translating the initial segment of the transcript "stalls" over one of the adjacent tryptophan codons at nucleotides 54 to 59 in the transcript and that this allows formation of the competing structure (17, 31). Within the framework of this model, we can account for the characteristics of the different types of *trp* operon mutations used in the present study (17, 31). To explain the behavior of our rifampin-resistant mutants, we propose that two factors normally contribute to the observed frequency of termination in vivo. First, for RNA polymerase to terminate transcription, the transcript termination structure must form and be recognized by the transcribing polymerase. The frequency of formation of the termination structure undoubtedly varies under different environmental conditions and in different mutants. The second factor is the efficiency with which RNA polymerase terminates transcription when it encounters a termination structure. This is not known, but if we assume that every transcript forms a termination structure when the *trp* operon is transcribed in vitro, then the frequency of termination in vitro, 96 to 97% at 37°C (31; Winkler and Yanofsky, manuscript in preparation), would be the efficiency of termination. This interpretation is probably an oversimplification, however, since it is conceivable that the transcript termination structures of the wild type and of certain mutants are recognized with different efficiencies.

Rif^r mutations that lead to reduced transcription termination at the *trp* attenuator are readily detected in mutant strains CY15006 (*trpR trpL29*) and CY15007 (*trpR trpL75*), strains in which the in vivo termination frequency at the *trp* attenuator is abnormally high. We believe that termination is frequent in these strains because in vivo, the termination structure forms more often than in the wild-type strain (31). We interpret the Rif^r termination relief mutations as alterations of the β subunit of RNA polymerase that reduce the efficiency of termination whenever RNA polymerase encounters a transcript termination structure. Consistent with this interpretation is the finding that these Rif^r mutations had no effect on operon expression when introduced into attenuator deletion, attenuator point mutation, and *trpT*(Ts) strains, all of which are thought not to form the normal transcript termination structure. These Rif^r mutations had only a small effect when introduced into the parental *trpR* strain, CY15001, reducing transcription termination sufficiently to give only a 30% increase in expression. This finding is consistent with the interpretation that when the wild-type leader region is transcribed, the termination structure forms less often than in strains with the *trpL29* and *trpL75* mutations.

We have also detected Rif^r mutations that increase transcription termination at the *trp* attenuator. These mutations allow growth of *trpR* strains on 5-methylanthranilate. As expected, these mutations decreased expression in *trp* deletion attenuator-containing strains but did not do so in attenuator deletion strains or the *trpR*(Ts) strain (Table 5). Surprisingly, they decreased expression in strains with the *trpL29*,

trpL75, and *trpL117-1* mutations (Table 5). We surmise that this is the case because these Rif^r polymerases terminate transcription more efficiently than the wild-type polymerase when they encounter the *trpL29*, *trpL75*, and *trpL117-1* transcript termination structures. We cannot explain the 50% increase in operon expression in CY15036, the *trpR trpT*(Ts) *rpoB7* strain. However, of the strains tested, only this one was extraordinarily slow-growing on either rich or minimal medium. This behavior may indicate that the particular combination of mutations in this strain has some adverse effect.

Our findings and those of others, therefore, indicate that the β subunit of RNA polymerase participates directly or indirectly in the recognition of transcription termination signals. Our results also suggest that Rif^r *rpoB* mutations generally increase or decrease *trp* operon expression only under conditions where a termination structure is expected to form. This conclusion is consistent with our view that a base-paired segment of the RNA transcript of the *trp* operon functions as the termination signal.

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