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Bicyclomycin Sensitivity and Resistance Affect Rho Factor-Mediated Transcription Termination in the *tna* Operon of *Escherichia coli*

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The growth-inhibiting drug bicyclomycin, known to be an inhibitor of Rho factor activity in *Escherichia coli*, was shown to increase basal level expression of the tryptophanase (*tna*) operon and to allow growth of a tryptophan auxotroph on indole. The drug also relieved polarity in the *trp* operon and permitted growth of a *trp* double nonsense mutant on indole. Nine bicyclomycin-resistant mutants were isolated and partially characterized. Recombination data and genetic and biochemical complementation analyses suggest that five have mutations that affect *rho*, three have mutations that affect *rpoB*, and one has a mutation that affects a third locus, near *rpoB*. Individual mutants showed decreased, normal, or increased basal-level expression of the *tna* operon. All but one of the resistant mutants displayed greatly increased *tna* operon expression when grown in the presence of bicyclomycin. The *tna* operon of the wild-type drug-sensitive parent was also shown to be highly expressed during growth with noninhibitory concentrations of bicyclomycin. These findings demonstrate that resistance to this drug may be acquired by mutations at any one of three loci, two of which appear to be *rho* and *rpoB*.

Zwiefka et al. (24) found that the antibiotic bicyclomycin (bicozamycin), an inhibitor of the growth of several gram-negative bacterial species (24), interferes with the functions of Rho factor, a protein largely responsible for factor-dependent transcription termination (15, 16). They performed in vitro analyses demonstrating that bicyclomycin inhibited the poly (C)-stimulated ATPase activity of *Escherichia coli* Rho factor (24). They also isolated bicyclomycin-resistant mutants and showed that these mutants had alterations of Rho factor that conferred resistance to the drug both in vivo and in vitro (24). In other studies of bicyclomycin resistance in *E. coli*, a gene that conferred resistance to the drug when it was present on a multicopy plasmid was identified (2). The mechanism of action of the product of this gene is believed to be exclusion of the drug from the bacterial cell (2).

Rho-dependent transcription termination is an integral event in a variety of metabolic processes (15, 16). In *E. coli*, expression of the tryptophanase (*tna*) operon involves tryptophan-induced relief from Rho-mediated transcription termination in the leader region of the operon; i.e., tryptophan induction results in antitermination during transcription of the leader region of the operon (6, 17). Tryptophanase can also catalyze tryptophan formation from indole; thus, expression of the operon permits tryptophan auxotrophs to grow on indole as a substitute for tryptophan (22). Rho-mediated transcription termination is responsible for maintaining the low, basal-level expression of the *tna* operon that occurs when cells are grown in media lacking inducer (17). Rho also is responsible for the polar effects of nonsense mutations on downstream gene expression in polycistronic operons, such as the *trp* operon (7, 9). Introduction of a nonsense codon within one of the coding regions of a multigene operon, such as *trp*, results in premature termination of translation (23). Translation termination generates a distal mRNA segment that is ribosome free. If the exposed RNA segment has the appropriate sequence and length, Rho presumably binds at sites within this RNA segment and interacts with the transcribing RNA polymerase molecule, causing it to terminate transcription (7, 9).

In this report we describe genetic and regulatory studies designed to examine bicyclomycin action. We show that exposing bacterial cells to bicyclomycin increases basal-level expression of the *tna* operon and allows a tryptophan auxotroph to synthesize sufficient tryptophanase to grow on indole. The drug increased *tna* operon expression in noninducible *tna* mutants that have low operon expression. We also demonstrate that bicyclomycin relieves polarity in the *trp* operon of *E. coli*. We isolated bicyclomycin-resistant mutants and characterized them genetically and biochemically. The mutants were altered at any one of three loci, one of which appears to be *rho*. We measured basal-level and tryptophan-induced expression of the *tna* operon in our drug-resistant mutants in the presence and absence of bicyclomycin and observed a variety of interesting effects that have implications regarding Rho action. We also show that noninhibitory concentrations of the drug increase basal-level expression of the *tna* operon in wild-type cells.

MATERIALS AND METHODS

Bacterial strains. The bacterial and plasmid strains used in this study and their relevant characteristics are listed in Table 1. The principal strain used, SVS1144, has been described in detail elsewhere (18). This strain has the *lac* operon deleted and is a single-lysogen-carrying lambda SVS44 derivative (18). In this prophage *tnaA* and *lacZ* are fused in frame to give a translational fusion, *tna'-'lacZ*, that is under the control of the unaltered *tna* regulatory region. SVS1144 also has the intact *tna* operon.

Media and growth conditions. The minimal media used were Vogel and Bonner medium (20) and morpholinepropanesulfonic acid (MOPS) (18). For β-galactosidase assays (12) cells were grown in MOPS plus 1% acid-hydrolyzed casein (18). Vogel and Bonner minimal medium plus 0.2% glycerol plus 0.05% acid-hydrolyzed casein, with or without 5-bromo-4-chloro-3-indolyl-β-D-galactopyranoside (X-Gal) (40 μg/ml) or with or without 10 μg of indole per ml, was used in experiments with strain CY15602, double mutant CY15621 (*trpE9851-trpA38*), and strains PDG1114 and PDG1182. Vogel and Bonner minimal medium plus 0.5% glucose, 0.05% acid-hydrolyzed casein, 100 μg of bicyclomycin per ml, and 40 μg of X-Gal per ml was used for the selection of bicyclomycin-resistant mutants (Bc^r-1 to Bc^r-9). L broth agar plus tetracycline (15 μg/ml), rifampin (100 μg/ml), or bicyclomycin (100 μg/ml) was used in tests of genetic linkage.

Enzyme assays. β-Galactosidase assays were carried out with permeabilized

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TABLE 1. Bacterial strains and plasmids used

Strain[a] or plasmid	Relevant characteristic(s)	Source or reference(s)
Strains		
SVS1144	W3110 *bgl-551* Δ(*lac-argF*)*U169* (λSVS44)	18
CY15602	W3110 Δ*trpEA2*	This study
CY15611	Bc^r-1	This study
CY15612	Bc^r-2	This study
CY15613	Bc^r-3	This study
CY15614	Bc^r-4	This study
CY15615	Bc^r-5	This study
CY15616	Bc^r-6	This study
CY15617	Bc^r-7	This study
CY15618	Bc^r-8	This study
CY15619	Bc^r-9	This study
CY15010	*trpL29 rpoB2*	21
CY15620	SVS1144 *ilv*::Tn*10*	This study
CY15621	W3110 *trpE9851-trpA38*	8
PDG1114	λ*tnaC261 tnaA′-′lacZ*	6
PDG1182	λ*tnaC261*, *C275 tnaA′-′lacZ*	6
Plasmids		
p39	pBR322 containing *rho*, its promoter, and immediately adjacent regions	3, 13
pRL408	pBR322 containing *rpoB* driven by the *lacI*^{q} promoter	1, 10
pSS105	pBR322 containing the *nusG* segment of the chromosome	5, 19

[a] Bicyclomycin-resistant mutants were isolated in strain SVS1144.

cells as described by Miller (12). The units of β-galactosidase reported are Miller units (12).

RESULTS

Bicyclomycin increases basal-level expression of the *tna* operon. If bicyclomycin's mechanism of action is inhibition of the activity of Rho factor, then exposing sensitive cells to appropriate concentrations of the drug could lead to increased basal-level expression of the *tna* operon. To examine this possibility, we performed an agar plate analysis of *tna* operon expression. We used a group of strains, each of which contained a *tnaA′-′lacZ* translational fusion driven by the *tna* promoter-leader region (6, 17). Washed cells were suspended in 2.5 ml of agar, and the suspension (ca. 10^6/10 ml) was poured over a basal layer of agar. Both layers contained X-Gal as an indicator. A filter paper disk was placed in the center of each poured plate, and 0.05 ml of a 10-mg/ml solution of bicyclomycin in water was pipetted onto the disk. The plates were incubated at 37°C and were inspected daily for growth and X-Gal hydrolysis. Within 36 to 48 h growth covered the agar surface with the exception of a 1- to 2-cm zone of inhibition immediately surrounding the filter paper disk (not shown). The initial ring of growth just beyond the zone of inhibition was dark blue because of X-Gal hydrolysis; the intensity of the blue color was most pronounced just inside the zone of bacterial growth. On the control plates, lacking bicyclomycin, growth was uniform and there was no comparable X-Gal hydrolysis. This test was repeated with *tnaA′-′lacZ* fusions in strains in which the *tnaC* start codon was inactivated (PDG1182 [6]) or in which the regulatory Trp codon of *tnaC* was replaced by an Arg codon (PDG1114 [6]). These constructs do not respond to inducer; when tryptophan is present, expression of the mutant *tna* operon remains low, at the basal level (6). These constructs responded to bicyclomycin identically to the wild-type construct. In the presence of the drug there was marked X-Gal hydrolysis in the zone of growth closest to the filter paper disk, reflecting elevated *tna* operon expression (data not shown).

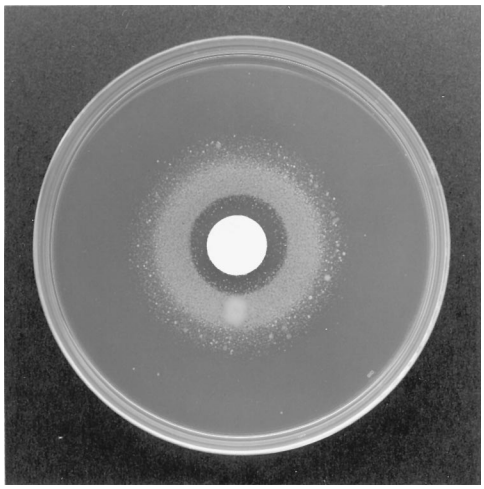

FIG. 1. Bicyclomycin activation of tryptophanase allows growth of a *trp* operon deletion strain on indole agar. Washed cells of strain CY15602 (Δ*trpEA2*) were plated on a glycerol–acid-hydrolyzed casein–indole agar plate, and a solution of bicyclomycin was pipetted onto a filter paper disk placed in the center of the agar surface. After 2 days of incubation a zone of growth was evident a few centimeters from the edge of the disk. Presumptive resistant mutant colonies may be seen within the zone of inhibition. Presumptive mutant colonies that are more responsive to the drug may be seen just beyond the ring of solid growth. For additional details, see Materials and Methods and Results.

In view of the above results, we examined the ability of the drug to permit a *tna*$^+$ strain that had the *trp* operon deleted to grow on indole agar. This strain normally will not grow on indole agar unless expression of the *tna* operon is induced by a tryptophan analog, such as 1-methyltryptophan (22). Indole alone does not induce expression of the wild-type *tna* operon. The experiment was performed as described above by pipetting a bicyclomycin solution onto a disk placed in the center of an indole agar plate spread with cells of the tryptophan auxotroph W3110 Δ*trpEA2* (CY15602). After 2 days of incubation we observed a ring of growth, 1 to 2 cm wide, several centimeters from the edge of the disk (Fig. 1). Beyond the circle of growth there was a clear zone (no growth) that extended to the edge of the plate. Evidently bicyclomycin relieved Rho-dependent termination in the *tna* operon in the absence of inducer and allowed production of sufficient tryptophanase to convert the supplied indole to tryptophan, which supported localized growth of the deletion strain. No growth was observed when a strain that also lacked the *tna* operon was employed (not shown).

Bicyclomycin relief of polarity. The phenomenon termed polarity is caused by Rho-dependent transcription termination in polygenic operons. Rho action follows premature translation termination at a nonsense codon introduced at an appropriate location within one of the coding regions of the operon (7, 9, 23). To determine if bicyclomycin would relieve polarity in the *trp* operon, we plated cells of the *trp* double nonsense mutant CY15621 (*trpE9851-trpA38*) on minimal agar containing indole. This strain is incapable of growth on indole because of the combined effects of the two nonsense mutations (8). The *trpE* mutation is polar and reduces *trpB* expression to approximately 5% of normal. The *trpA* mutation eliminates TrpA activation of the low level of TrpB protein that this strain produces. TrpB, when uncomplexed with TrpA, has only 3% of its maximal indole-to-tryptophan catalytic activity. A paper disk was placed in the center of an indole agar plate spread

TABLE 2. Genetic and regulatory characteristics of bicyclomycin-resistant mutants

Resistant mutant	No. of transductants scored (% cotransduction with gene)[a]		β-Galactosidase[b]			
	ilv	*rpoB*	Basal	+Bicyclomycin	+Trp	+DL-1-methyltryptophan
Control			1,810	ND[c]	22,730	15,400
Bc^r-1	148 (47)	50 (0)	2,030	3,110	7,680	7,200
Bc^r-3	56 (58)	180 (0)	2,940	14,390	17,400	15,040
Bc^r-6	111 (71)	23 (0)	7,350	11,830	19,060	16,110
Bc^r-7	109 (12?)	170 (0)	2,180	16,900	20,530	19,150
Bc^r-8	91 (63)	90 (0)	2,930	12,870	22,150	19,150
Bc^r-2	278 (0)	228 (75)	583	8,090	13,360	9,740
Bc^r-4	134 (0)	316 (100)	2,070	11,760	21,610	16,320
Bc^r-5	332 (0)	94 (100)	1,129	5,600	13,210	10,960
Bc^r-9	237 (0)	135 (95)	2,670	10,330	26,970	19,010

[a] Genetic linkage tests were performed as described in the text. Mutant Bc^r-7 was only moderately resistant to bicyclomycin; therefore, the indicated cotransduction value is only approximate.

[b] Cultures were grown in MOPS minimal medium plus 1% acid-hydrolyzed casein (17) with or without a supplement of bicyclomycin (50 μg/ml), L-tryptophan (100 μg/ml), or DL-1-methyltryptophan (20 μg/ml). At a Klett reading of approximately 70 (660 filter) cells were chilled, permeabilized, and assayed for β-galactosidase. The values presented are Miller units (12).

[c] ND, not determined. The growth of the parental strain is inhibited by 50 μg of bicyclomycin per ml; therefore, this strain could not be tested under the above-described conditions. At 20 μg of bicyclomycin per ml, the β-galactosidase level would be 12,000 (see Fig. 2).

with the double mutant, and 0.05 ml of a solution containing 10 mg of bicyclomycin per ml was pipetted onto this disk. After 2 days of incubation there was a marked ring of growth a few centimeters from the disk (not shown). Presumably, bicyclomycin inhibited Rho action, thereby preventing premature Rho-dependent transcription termination in the *trp* operon. This inhibition presumably led to an increase in the *trp* mRNA level, which in turn provided sufficient TrpB protein (despite the absence of TrpA) to permit growth on indole.

Isolation and characterization of bicyclomycin-resistant mutants. To investigate bicyclomycin action further, we isolated a set of bicyclomycin-resistant mutants. SVS1144, a strain with the wild-type *tnaA'-'lacZ* fusion, was grown overnight in broth containing the mutagen ethane methanesulfonate. The treated cells were washed, diluted, and plated on glucose agar containing bicyclomycin (100 μg/ml) plus X-Gal. Glucose was added to catabolite repress the *tna* operon, thereby reducing the intensity of the color of colonies of the test strain to light blue. After 2 days of incubation, resistant colonies appeared; the color of the colonies ranged from white to blue, reflecting nonexpression versus expression of the *tna* operon. A representative sample of colonies (picked on the basis of size predominantly) was picked and purified by streaking on bicyclomycin agar. These colonies were then examined in genetic and biochemical tests to determine if resistance was due to mutations at the *rho* locus and if transcription termination in the *tna* operon was affected.

The genetic analyses summarized in Table 2 indicate that some, but not all, of the nine resistant mutants were altered near the *ilv* operon. These are presumably *rho* structural or regulatory mutants. The others were altered at or near the *rpoB* locus and may have alterations that affect *rpoB* or a locus near *rpoB*. Linkage to *ilv* was tested in two ways. First, *ilv*::Tn*10* was transduced into each mutant, selecting for tetracycline resistance; resistant colonies were then tested for bicyclomycin resistance by streaking. Second, each mutant was used as donor in transduction experiments with the *ilv*::Tn*10* recipient, selecting Ilv$^+$, and the transductants were subsequently screened for bicyclomycin resistance. Results obtained with the two procedures were nearly identical, ruling out the possibility of double mutations. Linkage to *rpoB* was tested by transducing a rifampin resistance-conferring *rpoB* allele, from strain CY15010 (21), into each resistant mutant, selecting rifampin resistance (21). Rifampin-resistant transductants were then screened for bicyclomycin resistance. These genetic tests (Table 2) showed that the alterations responsible for bicyclomycin resistance in Bc^r-1, -3, -6, -7, and -8 map near the *ilv* locus, whereas those in Bc^r-2, -4, -5, and -9 map near the *rpoB* locus. The linkage of Bc^r-2 to *rpoB* was sufficiently different from that of the other mutants to raise the possibility that this mutant is not altered in *rpoB* but is altered at some nearby locus. To establish that the strains with the presumed *rpoB* mutations, Bc^r-4, Bc^r-5, and Bc^r-9, did not carry secondary mutations at the *rho* locus, wild-type *rho* was transduced into each of these strains and into Bc^r-2 and Bc^r-6. All transductants from each of the presumed *rpoB* mutants, and Bc^r-2, retained bicyclomycin resistance, indicating that their resistance is not partially due to a second mutation at the *rho* locus. Only Bc^r-6, the control *rho* mutant, gave bicyclomycin-sensitive transductants when wild-type *rho* was introduced.

Of the five mutants examined that had *ilv*-linked mutations that were presumably at the *rho* locus, one, Bc^r-6, had fourfold-elevated basal-level expression of the *tna* operon, while the other four were indistinguishable from the parental control strain (Table 2). By contrast, a different mutant, Bc^r-1, had somewhat reduced induced levels of *tna* operon expression. Of the four mutants with alterations at or near *rpoB*, two had basal and induced levels approximately one-third and one-half those of the control, respectively, and two had basal and induced levels comparable to those of the parental control strain (Table 2). These results demonstrate that mutations at either of at least two loci can confer bicyclomycin resistance and that some of these alterations have significant effects on expression of the *tna* operon, an operon regulated by Rho factor. The basal level of expression of the *tna* operon in our mutants correlated with the colony color observed on streaks on X-Gal agar of the initially isolated resistant mutants: white colonies had low basal-level expression, while blue colonies had normal or elevated expression.

Bicyclomycin increases basal-level expression of the *tna* operon of most resistant mutants. Since more than one locus was altered in our bicyclomycin-resistant mutants, it seems likely that the activities of different altered proteins can influence *E. coli*'s sensitivity to this drug. If this interpretation is correct, then some of our mutants might be expected to respond to bicyclomycin despite being resistant to the growth-

TABLE 3. Complementation of bicyclomycin-resistant mutants by plasmid p39 expressing Rho factor

Mutant	p39	Drug sensitivity[a]	β-Galactosidase[b]	
			Basal	+Trp
Control	−	Sens.	2,640	19,000
	+	Sens.	2,080	19,600
Bc^r-1	−	Resist.	1,660	11,500
	+	Sens.	1,490	12,700
Bc^r-2	−	Resist.	634	14,800
	+	Sl. sens.	521	10,820
Bc^r-3	−	Resist.	3,140	17,600
	+	Sl. sens.	2,560	17,300
Bc^r-4	−	Resist.	1,926	21,070
	+	Resist.	2,274	16,530
Bc^r-6	−	Resist.	8,740	18,800
	+	Resist.	3,080	15,000
Bc^r-7	−	Resist.	2,770	15,600
	+	Sl. sens.	2,054	12,400
Bc^r-8	−	Resist.	3,960	15,100
	+	Sens.	2,480	13,600

[a] Drug sensitivity was tested by streaking cells of strains with (+) and without (−) plasmid p39 on L broth agar containing 100 μg of bicyclomycin per ml. Streaks were scored as resistant (Resist.), sensitive (Sens.), or slightly sensitive (more sensitive than the plasmid-free parent in that colonies were smaller and sparser) (Sl. sens.).

[b] Cultures of strains with and without plasmid p39 were grown in MOPS minimal medium plus 1% acid-hydrolyzed casein (17) with or without a supplement of L-tryptophan (100 μg/ml). Ampicillin at 200 μg/ml was present in each culture of strains with p39. At a Klett reading of approximately 70 (660 filter) cells were chilled, permeabilized, and assayed for β-galactosidase. The values presented are Miller units (12). All mutants are derivatives of SVS1144 (see Table 1).

TABLE 4. Complementation of bicyclomycin-resistant mutants by plasmid pRL408 expressing RpoB

Mutant	pRL408	Drug sensitivity[a]	β-Galactosidase[b]	
			Basal	+Trp
Control	−	Sens.	2,530	25,400
	+	Sens.	860	19,700
Bc^r-1	−	Resist.	1,460	7,800
	+	Resist.	1,220	10,420
Bc^r-2	−	Resist.	650	16,700
	+	Resist.	404	11,580
Bc^r-4	−	Resist.	2,090	19,250
	+	Sl. sens.	861	12,650
Bc^r-5	−	Resist.	1,370	18,150
	+	Sl. sens.	801	11,270
Bc^r-6	−	Resist.	11,830	21,200
	+	Resist.	9,500	18,500
Bc^r-9	−	Resist.	2,983	22,500
	+	Resist.	932	11,210

[a] Drug sensitivity was tested by streaking cells of strains with (+) and without (−) plasmid pRL408 on L broth agar containing 100 μg of bicyclomycin per ml. Streaks were scored as resistant (Resist.), sensitive (Sens.), or slightly sensitive (more sensitive than the plasmid-free parent in that colonies were smaller and sparser) (Sl. sens.).

[b] Cultures of strains with and without plasmid pRL408 were grown in MOPS minimal medium plus 1% acid-hydrolyzed casein (17) plus 1 mM isopropyl-β-D-thiogalactopyranoside (IPTG) with or without a supplement of L-tryptophan (100 μg/ml). Ampicillin at 200 μg/ml was present in each culture of strains with pRL408. At a Klett reading of approximately 70 (660 filter) cells were chilled, permeabilized, and assayed for β-galactosidase. The values presented are Miller units (12). All mutants are derivatives of SVS1144 (see Table 1).

inhibiting properties of the drug. Accordingly, we grew our resistant mutants in the presence and absence of the drug and examined basal-level expression of the *tna* operon. It is evident from the results presented in Table 2 (+Bicyclomycin) that most of the mutants responded to the drug; in fact, they displayed levels of β-galactosidase approaching those of fully induced cultures. Thus, most of the resistant mutants retain sensitivity to the antitermination activity of the drug. Only one mutant, Bc^r-1, responded weakly to bicyclomycin (Table 2). Perhaps only this mutant, like previously described resistant mutants (24), produces a Rho factor that cannot bind the drug or binds it poorly. In each of the other mutants bicyclomycin may still bind to Rho and affect its activity. As shown below (see Fig. 2), in the wild type low levels of bicyclomycin induce high levels of *tna* operon expression.

Complementation tests. Complementation tests are likely to be ambiguous when one is dealing with mutants that produce a functional product, albeit altered. Thus, the relative concentrations of the mutant and wild-type forms of the protein are relevant, the question of what the wild-type protein normally does is relevant, and it is also important to know whether the protein functions as a component of a multimeric protein, such as Rho. Also relevant is whether the mutant form is defective or partially defective functionally and capable of competing with the wild-type form. These issues are further clouded in the present case because some of our mutants appear to form products that are more active than the wild-type protein. Despite these reservations, we thought it essential to examine the effects of expression of *rho*, *rpoB*, and *nusG*, present on multicopy plasmids, on the behavior of our mutants. We made no attempt to measure the levels of wild-type and mutant proteins in the various test strains and assumed that for Rho and RpoB the wild-type form of the product was present in excess over the mutant form. We used multicopy plasmids p39 (*rho*) (3, 13), pRL408 (*rpoB*) (1, 10), and pSS105 (*nusG*) (5, 19), kindly provided by Terry Platt, Robert Landick, and Max Gottesman, respectively. We introduced the plasmids into selected bicyclomycin-resistant strains, generally those presumed to be altered in the corresponding gene, and a few controls. We then compared the drug sensitivity of the plasmid-containing strains with that of their drug-resistant parents. We also measured basal and induced *tna* operon expression in these cultures grown in the absence and presence of tryptophan. Resistance versus sensitivity was assessed by examining the growth of streaks of plasmid-containing and plasmid-free strains on bicyclomycin agar. Most of the resistant strains became sensitive or slightly sensitive to bicyclomycin when they carried the appropriate plasmid (Tables 3 and 4; drug sensitivity). Thus, of the presumed *rho* mutants, when the *rho* plasmid was present, Bc^r-1, -3, -7, and -8 were more sensitive than their parents (Table 3). Bc^r-6, the most abnormal of this group, was not complemented by the *rho* plasmid in resistance tests, but this mutant was effectively complemented when basal-level expression of the *tna* operon was measured (Table 3). In addition, Bc^r-2, the genetically nondefined mutant, was more sensitive to the drug in the presence of the *rho* plasmid. The presence of the *rho* plasmid did not reverse the decreased basal-level expression of Bc^r-2. The *rho* plasmid did not affect resistance or basal-level expression of the *rpoB* mutant Bc^r-4 (Table 3). Bc^r-5 and Bc^r-9 were not tested. In the complementation tests performed with the *rpoB* plasmid (Table 4), mutants Bc^r-4 and -5 were more sensitive to the drug when they carried the plasmid. However, presumed *rpoB* mutant Bc^r-9 was not more sensitive when it carried the plasmid. The plasmid did not increase the drug sensitivity of the presumed *nusG* mutant, Bc^r-2. The *rpoB* plasmid was not tested on Bc^r-3, Bc^r-7, or Bc^r-8. In expression assays, most strains with the *rpoB* plasmid, including the wild-type control, displayed decreased basal

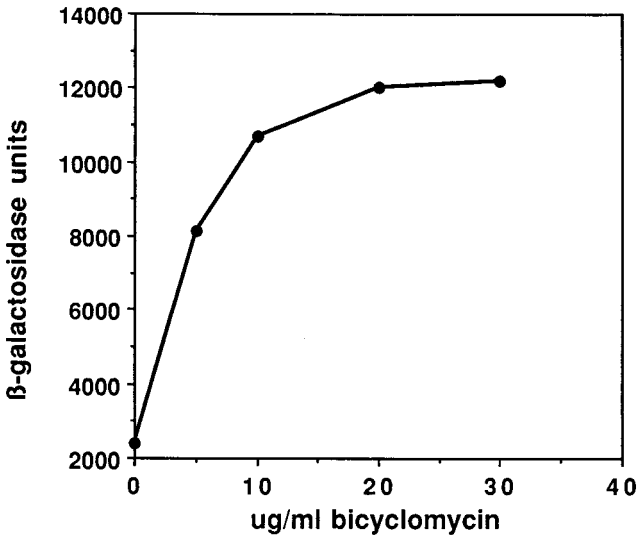

FIG. 2. Bicyclomycin increases basal-level expression of the *tna* operon. Cultures of parental strain SVS1144 were grown in MOPS minimal medium plus 1% acid-hydrolyzed casein plus the indicated level of bicyclomycin. At late log phase cells were harvested and assayed for β-galactosidase as described in Materials and Methods.

and/or induced levels of *tna* operon expression relative to those of their plasmid-free parents. An exception to this generalization was the response of Bc^r-1, which was not affected appreciably by the plasmid.

Complementation tests were also performed with plasmid pSS105, a plasmid that carries the genetic region including *nusG* (5, 19). The presence of this plasmid in mutants Bc^r-2, Bc^r-4, and Bc^r-8 had no effect on drug sensitivity or on basal-level expression of the *tna* operon (data not shown). In particular, it had no effect on Bc^r-2.

Bicyclomycin activation of the wild-type *tna* operon. Since bicyclomycin increased expression of the *tna* operon in most of our resistant mutants, we tested the ability of noninhibitory concentrations of the drug to activate *tna* operon expression in a wild-type, bicyclomycin-sensitive strain (SVS1144). Growth of this strain in MOPS minimal medium containing 1% acid-hydrolyzed casein was not inhibited by the presence of bicyclomycin at levels of 30 μg/ml or lower. SVS1144 cultures were grown with various concentrations of the drug, and β-galactosidase expressed from the *tnaA′-′lacZ* translational fusion operon was measured (Fig. 2). It is apparent that the β-galactosidase level increased with increasing bicyclomycin levels. The β-galactosidase activity attained was approximately 50% of the level reached in maximally induced cultures. Thus, bicyclomycin appears to be capable of interfering with Rho-dependent transcription termination in the *tna* operon in the wild type, at levels of the drug that do not adversely affect growth.

DISCUSSION

The drug bicyclomycin was shown to increase basal-level expression of the wild-type and mutant *tna* operons of *E. coli*. The drug presumably interferes with the action of Rho factor and thereby increases basal-level expression of the operon. Bicyclomycin addition also relieved the polar effects of a nonsense mutation in *trpE* on downstream gene expression in the *trp* operon of *E. coli*. The drug probably reduced the extent of polarity-induced Rho-mediated termination in the operon. Mutants resistant to the drug were isolated and characterized. Mapping studies suggested that the mutants have alterations in or near *rho* and *rpoB* and at a locus near *rpoB*, conceivably *nusG*. The genetic changes in several of these mutants altered their ability to regulate *tna* operon expression in the absence of the drug. In our initial set of nine mutants, five are believed to have alterations that affect *rho* synthesis or activity. One of these (Bc^r-1) exhibited lowered induced levels of *tna* operon expression, while another (Bc^r-6) had very high basal levels (Table 2). The other four mutants had alterations at or in the vicinity of *rpoB*. Two of these (Bc^r-2 and Bc^r-5) had lowered basal and induced levels of *tna* expression, suggesting that their alterations result in more-effective Rho action, at least in the *tna* operon. The other two resistant mutants had basal and induced levels of *tna* operon expression that were essentially indistinguishable from those of the wild type. Genetic and functional complementation tests with plasmids expressing Rho or RpoB provided support for the conclusion that many of our mutants do have alterations that affect Rho or RpoB levels or function (Tables 3 and 4). Interestingly and unexplainedly, the *rpoB* plasmid inhibited basal-level expression of the control and several of the mutants. Although complete explanations for the behavior of our resistance mutations will require in vitro analysis and further genetic characterization, some interesting tentative conclusions can be drawn. On the basis of prior work one might have expected that the most common mechanism of resistance to the drug would be loss or reduction of Rho's ability to bind bicyclomycin, alteration of the drug's uptake, or destruction of the drug (2, 24). However, although our sample was small, our resistant mutants (with the exception of Bc^r-1) retained their responsiveness to the drug. Thus, they exhibited appreciable increases in *tna* operon expression when grown with the drug (Table 2). In fact, in most of the resistant mutants, *tna* operon expression in the presence of the drug approached that of the fully induced wild-type control culture. Thus, although these mutants are drug resistant, their sensitivity to the antitermination activity of the drug appears to have been retained. These findings suggest that in these mutants Rho-dependent termination is still operable, at least in the *tna* operon.

The fact that mutations at or near the *rho* and *rpoB* loci confer drug resistance yet allow a response to the drug is consistent with many findings indicating that the products of these and other genes play a role in Rho-dependent termination (4, 11, 14–16, 19). Since mutations in *rho* can prevent drug binding (24), we assume that Rho is the primary target of the drug. Mutations in *rho* that confer resistance yet allow a response to the drug could alter the protein so that it can still perform all of its normal functions, despite the presence of the drug. Affinity for the drug might be reduced, or alternatively, tightly bound drug might be incapable of completely inhibiting a mutant Rho's action. In one of the presumed *rho* mutants, Bc^r-6, basal-level expression of the *tna* operon was appreciably elevated, suggesting that the mutation conferring drug resistance partially inactivated Rho.

Mutations in *rpoB* that confer bicyclomycin resistance could act by increasing the responsiveness of polymerase to Rho or the Rho-drug complex. Thus, a mutationally altered polymerase might be more likely to terminate at a Rho-dependent termination site; this has been observed with altered polymerases at Rho-independent termination sites (21). One of the resistant mutants, Bc^r-5, in fact showed increased termination activity in the absence as well as in the presence of inducer (Table 2). Two presumed *rpoB* mutants, Bc^r-4 and Bc^r-9, showed normal and induced levels of *tna* operon expression, and both responded to the presence of the drug by increasing

tna operon expression. The genetically unclassified mutant, Bc^r-2, showed what may be increased Rho action in that both the basal and induced levels of *tna* operon expression were reduced. The alteration in this mutant may make Rho factor more efficient as a transcription terminator at some sites of Rho action.

Taken together, our results are consistent with the current view that mutations in several genes can influence transcription termination events mediated by Rho (4, 11, 14–16, 19). Our findings are also consistent with the many known features of Rho action (15, 16). Our data further suggest that alterations affecting RpoB can influence Rho's action in mediating transcription termination in the leader region of the *tna* operon. Further characterization of the mutants described in this paper and isolation of additional mutants may result in identification of the protein domains in the respective proteins that participate in crucial protein-protein interactions or are influenced by such interactions and may define essential events in factor-dependent termination.

One of the more interesting implications of our findings is that the event responsible for drug sensitivity of wild-type *E. coli* appears to have a sensitivity to the drug different from that of *tna* operon expression. Thus, the *tna* operon can be activated near maximally, presumably by relief from Rho action, by noninhibitory concentrations of the drug. Also, drug-resistant mutants may or may not exhibit increased or decreased transcription termination in the *tna* operon. Continued studies of such mutants may lead to the identification of the crucial gene or genes that are adversely affected by the presence of bicyclomycin and its effects on Rho-mediated termination.

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