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Mutant RNA polymerase of *Escherichia coli* terminates transcription in strains making defective rho factor

(rifampicin-resistant mutation/*trp* termination/*rho* mutations)

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ABSTRACT **We have isolated a rifampicin-resistant mutant of *Escherichia coli* RNA polymerase that restores transcription termination in strains with a defective rho protein. In such strains, the mutant RNA polymerase terminates transcription at normally rho-dependent sites at the end of the *trp* operon, in bacteriophage λ, and within the *lac* operon. In addition, a strain with this mutant RNA polymerase remains viable with an amber mutation in *rho*, whereas a strain with wild-type RNA polymerase does not. These results suggest that the mutant RNA polymerase can terminate transcription at normally rho-dependent sites in the absence of rho.**

Termination of transcription in *Escherichia coli* occurs by at least two different mechanisms. In some cases a bacterial protein, rho protein, is necessary for transcription termination; in other cases, termination can take place in the absence of rho (1).

The most detailed studies of rho-dependent transcription termination events involve early mRNA synthesis of bacteriophage λ. The two early transcripts of phage λ have been shown to terminate at specific sites on the genome in the presence of rho (2–4). The N protein of λ appears to overcome termination at these sites *in vivo* and thus allow expression of distal genes (5). Transcription termination by rho is also believed to be the cause of mutational polarity in bacterial operons (6–8).

We have shown that a *rho* mutation *rho 201* (formerly *tsul*) eliminates transcription termination at the end of the *trp* operon (9). Lack of termination at the end of the *trp* operon can be detected by read-through into the adjacent genes of the *lac* openon in strain X8605 (Fig. 1). Strain X8605 has been constructed such that the *lac* operon has been brought close to the *trp* operon, but the transcription termination signal at the end of the *trp* operon, t_{trp}, has been left intact. The *lac* operon is not expressed in strain X8605 with a wild-type *rho* allele because the *lac* promoter is partially deleted.

The most detailed study of a rho-independent transcription termination signal is that of 6S RNA of phage λ. Genetic and biochemical evidence indicate that termination of 6S RNA is independent of rho (11–14). Also, termination of the early transcript of bacteriophage T7 occurs in the absence of rho in a purified transcription system (15).

Rho must play an essential role in *E. coli* growth because it is indispensable for cell viability (3, 16). The indispensability of rho might result from its involvement in normal transcription termination, as at the end of the *trp* operon. We have described (9) a rifampicin-resistant RNA polymerase mutant that substantially restores termination at the end of the *trp* operon in a strain with the *rho* mutation *rho201*. Here, we describe experiments that indicate that rho is rendered dispensable in a strain with such a RNA polymerase mutation. Our findings support the hypothesis that the mutant RNA polymerase can terminate transcription at normally rho-dependent sites in the absence of rho.

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MATERIALS AND METHODS

Bacterial Strains. Bacterial strains of *E. coli* K-12 used in this work are listed in Table 1. Since the previous publication (9) we have shown that the *rho201* mutation (formerly *tsul*) fails to complement with the *rho104* mutation that has been shown to affect *rho* protein (7). A *rho* amber carrying strain was a gift of M. Imai (16).

Media and Assays. These were as described (9). 5-Bromo-4-chloro-3-indolyl-β-D-galactoside, a noninducing substrate of β-galactosidase, is hydrolyzed to release modified indolyl moieties that dimerize to produce the blue dye indigo. On minimal agar containing this substrate, colonies that have high levels of β-galactosidase are deep blue, whereas colonies with low or no levels are pale blue or white, respectively. Isopropyl thiogalactoside is a nonmetabolizable inducer of the *lac* operon.

RESULTS

The *rpo203* Mutation Restores Termination at or near t_{trp} in a Strain with *rho201* Mutation. The *rho* mutation *rho201* eliminates transcription termination at the end of the *trp* operon (9). Thus, in strain X8605 with the *rho201* allele, expression of the *lac* operon derives almost entirely from read-through from the *trp* operon (Fig. 1). The *rpo203* mutation, like the *rpo123* mutation used in a previous study, is a spontaneously occurring rifampicin-resistant mutation that decreases expression of the *lac* operon in strain X8605 with the *rho201* mutation (Table 2). A few percent of all rifampicin-resistant mutant strains showed a decrease in *lac* expression. All experiments were performed after introducing the *rpo203* mutation into a new strain background by P1 transduction. Because the ability of the *rpo203* mutation to restore termination was never separated from the rifampicin-resistant phenotype by P1 transduction (in >100 transductants), we conclude that both phenotypes are probably due to a single mutation.

The decrease in expression of the *lac* operon by the *rpo203* RNA polymerase could result from decreased transcription initiation at the *trp* promoter, premature transcription termination within the *trp* or *lac* operon, or restored termination at the end of the *trp* operon. In order to distinguish among these possibilities, we examined the effect of the *rpo203* mutation on expression of the *lac* operon in strain XW211 (10). In this strain

Abbreviations: lac POZYA, operon for genes involved in the catabolism of lactose; *trp* EDCBA, operon for genes involved in the biosynthesis of tryptophan (21).

Table 1. Bacterial strains

Strain	Characteristics
CA85	HfrH, *lacZ* 14
EC-1	Hfr, Δ(*lac pro*)XIII, SuII$^+$
W3140	F$^-$, *trpR*, *val*^{R}, *lacZ* U118, *trpE*$^-$, *trpA*$^-$
X8605	F$^-$, *trpR*, Δ*lac* U169, *strA* Δ(*tonB-lacP*)

att80 trp

All strains were from the collection of J. Beckwith except W3140 which was from the collection of C. Yanofsky. The structure of the *trp–lac* region of the chromosome of strain X8605 is shown in Fig. 1.

the *lac* operon has been fused to the *trp* operon by deletion of all intervening DNA, including the terminus of the *trpA* gene (Fig. 1). It was found that the *rpo203* mutation had no effect on expression of the *lac* operon in strain XW211, even in the presence of the *rho201* mutation (Table 2). This indicates that the *rpo203* mutation does not affect initiation of transcription at the *trp* promoter or continuation of transcription within the *trp* or *lac* operons. Therefore, the *rpo203* mutation must restore termination in the region between the terminus of the *trpA* gene, and the *lac* operon in strain X8605 *rho201*.

Because the region between the terminus of the *trpA* gene and the *lac* operon in X8605 is fairly extensive (approximately 1800 base pairs), we wished to determine whether the mutant polymerase restored termination specifically at the rho-dependent termination signal at the end of the *trp* operon (t_{trp}) or elsewhere within that region. We have described (9) a class of mutations derived from strain X8605 which is genetically linked to the *trp* locus and which eliminates termination at the end of the *trp* operon. One such mutation, RT38, is a small deletion of about 150 base pairs thus partially defining t_{trp} (Fig. 1; unpublished data). The *rpo203* mutation was found to exert little effect on strains with the RT38 mutation and other similar mutations (Table 2). These results suggest that the RT38 deletion covers the site where the *rpo203* RNA polymerase terminates transcription in a strain with the mutation *rho201*. This means that the mutant RNA polymerase terminates transcription at or close to t_{trp} in a strain with the *rho201* mutation.

Other Phenotypes of the *rho201* Mutation Are Reversed by the *rpo203* Mutation. If the *rpo203* mutation restores proper termination at the end of the *trp* operon in a strain with defective rho (*rho201*), then other phenotypes of the *rho201* mutation might also be reversed by the *rpo203* mutation. Like certain other *rho* mutations (3, 4), the *rho201* mutation increases the plating efficiency of bacteriophage λ carrying an amber mutation in the N gene. Also, the *rho201* mutation suppresses mutational polarity in bacterial operons (9). Both of these phenotypes are reversed by the *rpo203* mutation (Table 3).

The *rpo203* Mutation Restores Termination at t_{trp} in Strains with Different *rho* Mutations. We have conceived of two general models to explain the reversal of *rho201* phenotypes by the *rpo203* mutation.

(*i*) The mutant RNA polymerase restores an interaction with altered rho protein, thereby restoring its ability to terminate transcription.

(*ii*) The mutant RNA polymerase can terminate transcription in the absence of rho protein.

If the *rpo203* RNA polymerase restores an interaction with the altered rho protein in a strain with the *rho201* allele, it might be possible to find other mutant alleles of *rho* whose effects are not reversed by the mutant RNA polymerase. Accordingly, the effect of the *rpo203* mutation was analyzed in strains carrying different *rho* mutations that eliminate termination at t_{trp}. The *rho202–rho205* mutations were isolated in the same manner as *rho201*, except that the mutagen used was *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine. Like the *rho201* mutation, these mutations are genetically linked to the *ilv* locus. The *rho102* and *rho103* mutations are suppressors of mutational polarity which map in the *rho* gene (6, 7). It was found that the *rpo203* mutation restored termination at t_{trp} in all strains tested that carried *rho* mutations (Table 4). The effect of the mutant RNA polymerase, therefore, does not appear to be limited to specific mutant alleles of the *rho* gene.

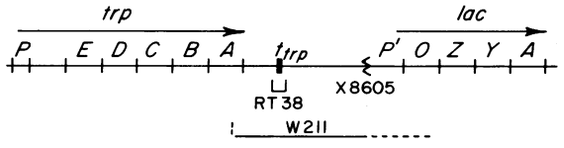

FIG. 1. Structure of the *trp–lac* region of the X8605 chromosome. The distance between the terminus of *trpA* and the X8605 fusion joint is approximately 1800 base pairs (9). RT 38 is a deletion of about 150 base pairs that removes t_{trp} (unpublished data). The position of this deletion with respect to the *trp* operon has not yet been determined. W211 is a *trp–lac* fusion deletion that cuts into the end of *trpA* on the end and into *lacP* on the other end (10).

Table 2. Expression of the *lac* operon in mutant strains of t_{trp} and *rpo203* derivatives

Strain	β-Galactosidase units*	Transacetylase levels†
X8605	30	1.5
X8605 *rho201*	300	70
X8605 *rho201, rpo203*	40	8
XW211	3000	100
XW211 *rpo203*	3000	84
XW211 *rho201*	2500	90
XW211 *rho201, rpo203*	2000	65
X8605 RT38‡	250	37
X8605 RT38, *rpo203*	160	35
X8605 RT17§	450	
X8605 RT17, *rpo203*	400	
X8605 RT87§	350	
X8605 RT87, *rpo203*	310	

All assays were performed twice.

* β-Galactosidase units are expressed according to Miller (17). Read-through mutants of X8605, like some other *trp–lac* fusion strains, have an unusually high ratio of *lacA* protein to *lacZ* protein. This could be due to a poor ribosome binding site for *lacZ* generated by the X8605 deletion (18).

† Transacetylase units are expressed as percentage of level in strain XW211, an efficient *trp–lac* fusion strain (10).

‡ RT38 is a deletion of t_{trp} of about 150 base pairs.

§ RT17 and RT87 are deletions of t_{trp} of about 500–700 base pairs (unpublished data).

Table 3. Effect of *rpo203* mutation on mutational polarity and λ N$^-$ plating efficiency

Strain*	Transacetylase levels†	λ N$^-$ plating efficiency‡
W3140	1	10^{-5}–10^{-6}
W3140 *rho201*	37	10^{-1}
W3140 *rho201, rpo203*	3	10^{-5}–10^{-6}

* The strains carry the *lacZ* U118 polar mutation.

† Transacetylase units are expressed as percentage of the value in an isogenic *lac*$^+$ control.

‡ Phage λ carries the N amber 17 mutation.

Table 4. Effect of *rpo203* mutation on termination at t_{trp} in strains carrying *rho* mutations

Strain	β-Galactosidase units*	Transacetylase levels†
X8605	30	1.5
X8605 *rho201*	300	70
X8605 *rho201, rpo203*	40	8
X8605 *rho202*	180	28
X8605 *rho202, rpo203*	38	7
X8605 *rho203,*	150	27
X8605 *rho203, rpo203*	25	4
X8605 *rho204*	—	93
X8605 *rho204, rpo203*	—	13
X8605 *rho205*	500	91
X8605 *rho205, rpo203*	60	15
X8605 *rho102*	90	10
X8605 *rho102, rpo203*	10	1.5
X8605 *rho103*	90	10
X8605 *rho103, rpo203*	10	1.5

* β-Galactosidase units are according to Miller (17).
† Transacetylase levels are expressed as percentage of the level in strain XW211.

Temperature Sensitivity of Two *rho* Mutant Strains Is Reversed by the *rpo203* Mutation. Two of the t_{trp} termination suppressor strains, *rho204* and *rho205*, have a temperature-sensitive phenotype, growing at 37° but not at 42°. This is consistent with previous findings that is indispensable for cell viability (3, 16). It was found that the *rpo203* mutation restores viability to both of these strains at the restrictive temperature.

The *rpo203* Mutation Restores Viability to a Strain with an Amber Mutation in rho. The results in the previous two sections are more consistent with the model that the mutant RNA polymerase can terminate transcription in the absence of rho. This model predicts that the *rpo203* mutation should suppress a totally defective *rho* mutation. An amber mutation in *rho* has been isolated which is suppressed by the amber suppressor *supF* (16). In the absence of *supF* or other amber suppressors, a strain carrying the amber mutation in *rho* is not viable. We have constructed strains to determine whether the *rpo203* mutation will allow a strain carrying the *rho* amber mutation to be viable in the absence of an amber suppressor.

The strains that were constructed, A, B, and C, are described in Table 5. All strains contained an amber mutation in *lacZ*, a φ80 prophage which carries the *supF* gene at the φ80 attachment site (*att80*), and a deletion spanning the *trp-tonB* loci, which is closely linked to *att80*. In addition, strain B carried the *rho* amber mutation, and strain C had both the *rho* amber mutation and the *rpo203* mutation. Because *supF* suppresses the *lacZ* amber mutation, these strains all give rise to blue colonies on minimal glucose agar containing 5-bromo-3-chloro-indolyl-galactoside and isopropyl thiogalactoside.

It is expected that *supF* is essential for viability of strain B, because of the amber mutation in *rho*, but not strain A, which has a wild-type *rho* allele. If the *rpo203* mutant RNA polymerase can terminate transcription in the absence of rho, *supF* might not be essential for viability of strain C. In order to determine whether *supF* is essential, strains A, B, and C were used as recipients in a conjugal cross with strain EC-1, an Hfr which transfers *att80* and *trp*$^+$ early, with the *trp*$^+$ marker distal. The mating was interrupted after 30 min so that the *rho*$^+$ marker of EC-1 could not be introduced, and *trp*$^+$ recombinants were selected on minimal glucose agar containing the dye. Most *trp*$^+$ recombinants of strain A were white on this medium

Table 5. Ability of strains to lose *supF*

	Strain	*trp*$^+$ / recombinants: White on XG agar	*trp*$^+$ / recombinants: Blue on XG agar
A	(*lac*Zam, φ80*supF*^{ts}, Δ*trp-tonB*) × Ec-1	390	52
B	(*lac*2am, φ80*supF*^{ts}, Δ*trp-tonB*, *rho*am) × EC-1	0	72
C	(*lac*Zam, φ80*supF*^{ts}, Δ*trp-tonB*, *rho*am, *rpo203*) × EC-1	230	30

Strains A, B, and C are derivatives of X8605. First, an *ilv met* derivative of X8605 was made *tonB*$^+$ (and hence Δ*lac*) by mating with EC-1 and selecting for recombinants on M63 glucose minimal agar in the absence of citrate. Next, the *lacZ* amber mutation Y14 was mated in by conjugation with CA85, selecting ability to utilize melibiose as sole carbon source (*lac* Y$^+$). Next, a *tonB-trp* deletion was isolated as described (19) in a strain containing a φ80 prophage which carries *supF*^{ts}. This deletion along with φ80 *supF*^{ts} was introduced into the above *lacZ* amber strain by P-1 transduction, selecting *lac*$^+$ (*supF*$^+$) and screening for the *trp-tonB* markers. A *rho* amber mutation (16) was introduced by cotransduction with *ilv*$^+$, screening for transductants that are temperature-sensitive for viability (due to the temperature-sensitive nature of *supF*). The *rpo203* mutation was introduced by cotransduction with *met*$^+$, screening for rifampicin resistance.

Conjugation with EC-1 was for 30 min, and *trp*$^+$ recombinants were selected on minimal glucose agar containing 5-bromo-4-chloro-3-indolyl-β-D-galactoside (XG) and isopropyl thiogalactoside at 37° (the permissive temperature for *supF*^{ts}). The frequency of *trp*$^+$ recombinants was about 1%.

and therefore had lost *supF* (Table 5). This is expected because of the close linkage between *trp* and *att80*. In contrast, all *trp*$^+$ recombinants of strain B retained *supF*, because its loss would result in the absence of rho protein which would be lethal to the cell. *trp*$^+$ recombinants of strain B that lost *supF* would simply not appear on the selective medium.

However, when strain C, carrying the *rpo203* mutation, was mated with EC-1, *trp*$^+$ colonies appeared that were white on the dye agar. These colonies were distinctly smaller than the blue *supF*$^+$ colonies. This result indicates that, in the presence of the *rpo203* mutation, the *supF* amber suppressor can be eliminated, even though the strain carries a *rho* amber mutation. The white colonies obtained in the cross with strain C were shown to have lost *supF* by their loss of β-galactosidase activity (<0.1% of that of the *supF* parent). These results suggest that the *rpo203* RNA polymerase can terminate transcription in the absence of rho.

DISCUSSION

We have described a rifampicin-resistant RNA polymerase mutant, *rpo203*, which reverses the effects of all mutations in the *rho* gene we have studied. Because the RNA polymerase mutant also allows a strain with an amber mutation in *rho* to be viable, we believe it is likely that the *rpo203* polymerase can terminate transcription in the absence of rho.

Although the above explanation appears most likely to us, there are alternative explanations for these results. For instance, it is possible that there is a low level of rho activity in the strain carrying the *rho* amber mutation. A low level of rho activity in this strain could come from infrequent insertion of an amino acid at the amber codon or from activity of the amber fragment itself. It may be that the mutant polymerase (*rpo203*) restores an interaction with the amber fragment in the *rho* amber-

carrying strain. This seems unlikely because the *rpo203* mutation also reverses the effects of all other *rho* mutation studied (*rho201-rho205*). Alternatively, the *rpo203* mutation might cause general misreading at amber codons. However, this does not appear to be the case, because the level of expression of *lacZ* in this strain, which also contains an amber mutation, is still low (<0.1%). Finally, it is possible that a low level or rho activity could be amplified if the mutant polymerase initiates transcription at the *rho* promoter at a higher rate than wild-type polymerase.

Our results suggest that termination by the *rpo203* polymerase in a strain with defective rho occurs at normally rho-dependent sites on the DNA. First, the *rpo203* mutation restores termination in several systems in which rho-dependent termination has been eliminated by mutation in *rho* (phage λ, mutational polarity of bacterial operons, and the end of the *trp* operon). Second, the termination site at the end of the *trp* operon for the *rpo203* polymerase in a strain with defective rho is at or close to the rho-dependent site, t_{trp}. A small deletion (about 150 nucleotides) removes both sites.

Transcription terminations signals that have been studied *in vitro* fall into two classes: those which require rho, and those which are rho-independent. It is possible that all termination is rho-dependent *in vivo*. However, it seems more likely that there are two kinds of termination signals, of which only one requires rho to effect termination. Our results suggest that the *rpo203* mutation alters polymerase so that it can terminate transcription at both types of signals in the absence of rho. Our results also suggest that the indispensability of rho is related to its role in transcription termination.

Purified rho protein has been shown to display an ATPase activity (8). There has been conjecture that rho may serve as a subunit in one or more cellular ATPases (3). Strains carrying the *rho* amber mutation and *rpo203* could provide insight into whether rho is involved in any cellular processes other than transcription termination. We have already observed some unusual phenotypes of this strain, such as failure to grow in rich media and a filamentous cellular shape. Whether these phenotypes result from imperfect transcription termination by the *rpo203* polymerase or reflect other cellular defects resulting from the absence of rho needs further exploration.

Finally, Chakrabarti and Gorini (20) have shown that certain streptomycin-resistant mutations allow F^+ strains of *E. coli* to plate bacteriophage T7. F^+ strains are normally nonpermissive for T7 growth. They found that a rifampicin-resistant mutation, *rpo123*, reversed the effect of the streptomycin-resistant mutation, making the host once again nonpermissive for T7 growth. In our hands, the *rpo123* mutation behaves in a manner similar to the *rpo203* mutation with respect to transcription termination. It is possible that increased transcription termination activity gives rise to the *rpo123* phenotype observed by Chakrabarti and Gorini.

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