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Escherichia coli RNA Polymerase Mutants That Enhance or Diminish the SOS Response Constitutively Expressed in the Absence of RNase HI Activity

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Escherichia coli rnhA **mutants lacking RNase HI chronically express the SOS response (T. Kogoma, X. Hong, G. W. Cadwell, K. G. Barnard, and T. Asai, Biochimie 75:89–99, 1993). Seventeen *rpoB*(Rif^r) mutant alleles, which encode altered β subunits of RNA polymerase, giving rise to resistance to rifampin, were screened for the ability to enhance or diminish constitutive expression of the SOS response in *rnhA* mutants. Two mutations, *rpoB3595* and *rpoB2*, were found to enhance the SOS response 5- and 2.5-fold, respectively, only when RNase HI is absent. These mutations rendered *rnhA* mutant cells very sensitive to broth; i.e., the plating efficiency of the double mutants was drastically reduced when tested on broth plates. Two mutations, *rpoB8* and *rpoB3406*, were found to diminish constitutive SOS expression in *rnhA* mutants by 43 and 30%, respectively. It was suggested that RNA polymerase may have a property that influences the size of DNA-RNA hybrids, the frequency of their formation, or both and that the property resides at least in part in the β subunit of the polymerase.**

Escherichia coli RNase HI, encoded by *rnhA*, is an endonuclease which specifically recognizes DNA-RNA hybrids and digests the RNA moiety of the duplex (for a review, see reference 2). Inactivation of RNase HI activity by *rnhA* mutations results in derepression of normally repressed origins (*oriK*s) of DNA replication (for a review, see reference 12). DNA replication originating from *oriK*s in *rnhA* mutants can sustain chromosome replication in the absence of normal DNA replication, which is initiated from *oriC*, the only active origin of replication in wild-type cells (14). The activation of *oriK*s in *rnhA* mutants strictly requires transcription. It has been postulated that a large DNA-RNA hybrid occasionally forms during transcription at certain sites on the chromosome and can be stabilized in the absence of RNase HI and that the resulting R loop, which consists of a DNA-RNA hybrid and the displaced single-stranded DNA, serves as an origin of opportunistic DNA replication (19).

rnhA339::*cat* mutants, which completely lack RNase HI, constitutively express the SOS response. This SOS expression can be further elevated both by a nutritional shift-up which enhances certain types of transcription and by introduction of *recD* or *polA* mutations which inactivate exonuclease V or DNA polymerase I, respectively (13). In the absence of exonuclease V and RNase HI as in *recD rnhA*::*cat* double mutants, homologous recombination functions become essential for cell survival. Thus, for example, *rnhA*::*cat recB*(Ts) mutants are temperature sensitive for growth (5, 13), because exonuclease V activity and RecBC-dependent recombination are temperature sensitive in the strains. It has been proposed that the single-stranded DNA associated with persisting R loops in *rnhA* mutant cells induces the SOS response and that the removal of the persisting R loops involves two DNA repair systems: one involving exonuclease V and DNA polymerase I and the other involving the homologous recombination repair pathway. These observations suggested that RNA polymerase might naturally generate DNA-RNA hybrids of considerable lengths during transcription, albeit infrequently (13).

Previously, we reported the isolation of *rpoB* mutations that enhance constitutive expression of the SOS response in *rnhA* mutants (13). Gross and colleagues cloned and characterized a set of *rpoB*(Rif^r) mutations which confer upon RNA polymerase resistance to rifampin, an inhibitor of transcription initiation. Nucleotide sequence analysis allowed the determination of the precise map positions of the mutations within a domain of the β subunit of RNA polymerase as well as the amino acid residues affected (8). These mutations are located in three distinct clusters in the center of the *rpoB* gene. The effects of the *rpoB*(Rif^r) mutations on transcription termination and antitermination have been examined (7, 10). In this study, we have screened these well-characterized *rpoB*(Rif^r) mutations for the property that either enhances or diminishes the chronic SOS expression caused by the *rnhA*::*cat* mutation.

Expression of the *sfiA*::*lacZ* operon fusion in *rpoB*(Rif^r) *rnhA* mutants. By P1 phage-mediated transduction, the 17 *rpoB*-(Rif^r) mutations (8) were introduced, with a linked Tn*10*, into strain AQ8136 (*rnhA339*::*cat*), which is lysogenized with a λ phage carrying an *sfiA*::*lacZ* operon fusion. Transduction with P1 phage grown on the original *rpoB8* strain gave two types of Rif^r transductants: one type grew much slower than the *rpoB*$^+$ strain did and lowered *sfiA*::*lacZ* expression in *rnhA*::*cat* strains, and the other type had the wild-type growth rate and did not affect *sfiA*::*lacZ* expression. Since *rpoB8* strains have been reported to grow slowly (8), the former type of transductants was chosen and characterized further in this study. The β-galactosidase activity that results from expression of the *sfiA*::*lacZ* operon fusion was determined as a measure of the induction of SOS response in each of the *rnhA rpoB* double mutants, as described previously (13). The presence of two mutations, *rpoB3595* and *rpoB2*, when combined with *rnhA*::*cat*, in strains gave about 5- and 2.5-fold-higher activities, respectively, than that of the *rpoB*$^+$ strain (Table 1). The *rpoB501* mutant, which was previously shown to carry the identical mutation as in the *rpoB3595* mutant (8), exhibited a very high *sfiA*::*lacZ* expression level (668.9 ± 17.2 U), but it

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TABLE 1. β-Galactosidase activities in *rpoB rnhA* double mutants

Strain[a]	Relevant genotype			β-Galactosidase activity[b] (Miller units, mean ± SEM [*n*])
	rnhA	*rpoB*	Other gene or plasmid	
AQ8107	+	+		55.6 ± 0.4 (2)
AQ8136	*339*	+		198.9 ± 5.6 (11)
AQ8656	+	*3595*		72.2 ± 19.3 (2)
AQ8622	*339*	*3595*		991.0 ± 28.9 (3)
AQ9118	*339*	*3595*	Δ*recA302*	15.1 ± 1.5 (2)
AQ9142	*339*	*3595*	*lexA3*	20.9 ± 1.1 (4)
AQ8687	*339*	*3595*	pSK760	49.3 ± 0.2 (2)
AQ8685	*339*	*3595*	pBR322	842.8 ± 2.4 (2)
AQ8654	+	*2*		65.4 ± 3.0 (2)
AQ8583	*339*	*2*		456.7 ± 16.5 (5)
AQ8660	*339*	*2*	pSK760	53.6 ± 0.1 (2)
AQ8658	*339*	*2*	pBR322	350.9 ± 13.2 (2)
AQ8627	*339*	*8*		114.4 ± 10.7 (7)
AQ8608	*339*	*3406*		131.3 ± 9.3 (5)

[a] All strains were derived from strain AQ8107 (GC4597) F⁻ Δ(*pro lac*) *pro rpsL* λp[*sfiA*::*lacZ cI*(Ind⁻)] (4).
[b] Cells were grown and assayed for β-galactosidase activity as described previously (13).

was significantly lower than that of the *rpoB3595* mutant. This is consistent with the suggestion that there seem to be additional mutations in the *rpoB501* allele (7). Similarly, the *rpoB180* mutant which was reported to contain the same mutation as that in the *rpoB2* mutant (9), expressed the operon fusion at a level (222.9 ± 2.9) only slightly above that of the *rpoB*⁺ strain, suggesting the presence of additional mutation(s) in this allele. Strains with the *rpoB8* and *rpoB3406* alleles registered the lowest levels of expression (Table 1). Strains with the remaining *rpoB* alleles had expression levels between 145 and 240 U (data not shown). In sum, only two alleles (*rpoB3595* and *rpoB2*) enhanced and two alleles (*rpoB8* and *rpoB3406*) significantly reduced constitutive expression of the operon fusion in *rnhA*::*cat* mutants.

Enhanced *sfiA*::*lacZ* expression depends on *recA*⁺ and *lexA*⁺. Derepression of LexA regulon genes depends on *recA*⁺ and *lexA*⁺. The Δ*recA302* and *lexA3*(Ind⁻) mutations completely blocked enhanced derepression of the operon fusion in *rnhA*::*cat rpoB3595* mutants (strains AQ9118 and AQ9142 in Table 1). These results indicated that the expression is under LexA control and suggested that the enhancement of expression occurs at the transcriptional level.

***rnhA*⁺ suppresses enhanced *sfiA*::*lacZ* expression in *rpoB3595* and *rpoB2* mutants.** The plasmid pSK760 is a pBR322 derivative carrying an *rnhA*⁺ gene, and cells carrying the plasmid overproduce RNase HI 15- to 20-fold (11). Introduction of pSK760 into *rnhA*::*cat rpoB3595* and *rnhA*::*cat rpoB2* mutants brought the *sfiA*::*lacZ* expression to the level of the *rnhA*⁺ *rpoB*⁺ strain, whereas pBR322 had only small effects (Table 1). Thus, RNase HI, when overproduced, completely suppresses the constitutive expression of the operon fusion in the *rnhA*::*cat rpoB3595* or *rnhA*::*cat rpoB2* double mutant. In fact, nearly complete suppression could be achieved with a single copy of *rnhA*⁺ (strains AQ8656 and AQ8654 in Table 1). This indicated that the *rpoB3595* or *rpoB2* mutation alone does not lead to a constitutive *sfiA*::*lacZ* expression. We conclude that enhanced *sfiA*::*lacZ* expression can be seen only when RNase HI is absent.

***rpoB2 rnhA* and *rpoB3595 rnhA* mutants are sensitive to broth.** The *rpoB2 rnhA*::*cat* double mutant could not grow well on Luria broth (LB) plates, and the plating efficiency of the mutant was 4×10^{-4} on LB plates, although it had near-normal plating efficiencies on minimal medium (M9G) plates with or without Casamino Acids (Table 2). The *rpoB3595 rnhA*::*cat* mutant was even more sensitive to broth; the plating efficiency was 5×10^{-6}. This mutant had poor plating efficiencies, even on minimal medium plates (Table 2). Introduction of an *sfiA*::Tn*10* mutation did not appreciably change the broth sensitivity (data not shown). Thus, the induction of the chromosomal *sfiA*⁺ gene encoding a cell division inhibitor (4) is not a major cause of the broth sensitivity. On the other hand, the *rpoB8 rnhA*::*cat* and *rpoB3406 rnhA*::*cat* mutants had only slightly decreased plating efficiencies on rich medium (Table 2). Active RNase HI, encoded either by a high-copy-number plasmid (pSK760) or by a single-copy chromosomal gene, completely suppressed the broth sensitivity of *rpoB2 rnhA*::*cat* and *rpoB3595 rnhA*::*cat* mutants (data not shown), as it did suppress *sfiA*::*lacZ* expression as described above. Thus, the broth sensitivity appears to stem from the condition that is caused in *rnhA*::*cat* cells by the *rpoB* mutations and that leads to high levels of *sfiA*::*lacZ* expression.

RpoB3595 RNA polymerase has been shown to have an accelerated elongation rate during transcription in vitro and in vivo (6) and is defective in rho-dependent transcription termination (7). RpoB2 polymerase is also defective in transcription termination (7). Since the elongation speed of a polymerase is related to the efficiency of termination (3, 6, 18), RpoB2 polymerase is also likely to be a fast polymerase. On the other hand, RpoB8 polymerase is a slow polymerase, and the elongation rate in vivo is 60% lower than that for the wild type (6). Therefore, it is possible that enhanced or decreased *sfiA*::*lacZ* expression in these *rpoB* mutants observed may simply be a direct effect of an increase or decrease in the overall rate of transcription of the *sfiA* gene caused by the altered elongation rates of the mutant RNA polymerases. Since the levels of *sfiA*::*lacZ* expression were determined as the specific activity of β-galactosidase, this explanation is possible only if mutant RNA polymerases differentially affect the

TABLE 2. Broth sensitivity of *rpoB rnhA* mutants[a]

Strain	Plating efficiency[b]		
	M9G	M9G + CAA	LB
AQ8136	0.938	1.15 ± 0.01	1.04 ± 0.079
AQ8583	0.950	0.814 ± 0.035	$4.14 \times 10^{-4} \pm 2.06 \times 10^{-4}$
AQ8622	1.32×10^{-3}	$7.11 \times 10^{-4} \pm 3.33 \times 10^{-4}$	$5.39 \times 10^{-6} \pm 9.22 \times 10^{-8}$
AQ8627	1.06 ± 0.06	0.731 ± 0.020	0.294 ± 0.043
AQ8608	0.393	0.637 ± 0.034	0.422 ± 0.064

[a] See Table 1 for the relevant genotypes of strains. Cells were grown in minimal medium, M9 glucose (M9G) supplemented with proline, and assayed for plating efficiencies on M9G, M9G with Casamino Acids (CAA) (0.2%), and LB plates, as described previously (13).
[b] The values are, with a few exceptions, the averages of at least two determinations ± standard error of the mean.

expression of *sfiA*. Alternatively, these alterations of mutant polymerases may affect expression of some genes, which in turn indirectly alters *sfiA* expression. The differences in *sfiA*::*lacZ* expression can be detected only in *rnhA* mutant cells (Table 1). Thus, these explanations require a mechanism by which the presence of active RNase HI negates the effect. There is no evidence to suggest that RNase HI is in any way directly involved in gene expression, however.

The enhanced expression of the *sfiA*::*lacZ* operon fusion is under LexA control and is seen only when RNase HI activity is absent (Table 1). Thus, it is possible that the *rpoB* mutations affect the amount of a signal for SOS induction generated in the absence of RNase HI. The increased or decreased amount of the SOS signal in turn increases or decreases the rates of expression of the LexA regulon genes including *sfiA*. In previous work (13), we demonstrated that the SOS response is constitutively expressed in cells lacking RNase HI and that the level of the expression can be elevated by stimulated transcription as in a nutritional shift-up. This stimulation by a nutritional shift-up is seen only when RNase HI is absent. Since RNase HI is an endonuclease specific to the RNA moiety of DNA-RNA hybrids (2), it is reasonable to postulate that the SOS-inducing signal derives from persisting R loops that are formed during or after transcription and are stabilized in the absence of RNase HI (13). The displaced single-stranded DNA in the R loops could directly act as a signal or the persisting R loop could block DNA replication leading to SOS induction (17). The above postulate implies that RNA polymerase naturally generates R loops during transcription of selected transcription units. The *rpoB*(Rif^r) mutations could directly or indirectly affect this property of RNA polymerase; *rpoB3595* and *rpoB2* mutants could generate an increased amount of DNA-RNA hybrids, and *rpoB8* and *rpoB3406* mutants could generate hybrids at reduced rates. The broth sensitivity phenotype displayed by *rpoB3595* and *rpoB2* mutants is consistent with this explanation; R loops generated at the accelerated rate during growth in rich medium would overwhelm the repair capacity of the double mutants. *rnhA* mutants that have defects in homologous recombination repair have been shown to be sensitive to broth (13).

An actively transcribing complex is postulated to normally contain only a very short stretch of DNA-RNA hybrid, up to 12 bp (for a review, see reference 20), but the size could be much shorter (15). I wish to suggest the possibility that certain transcription units could generate significantly larger hybrids in vivo and that *E. coli* RNA polymerase is at least partially responsible for this phenomenon. A similar suggestion has previously been made (1, 16). The results of this study further suggest that the determinant of the property resides at least in part in the β subunit of the polymerase.

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