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Effect of *dnaA* and *rpoB* Mutations on Attenuation in the *trp* Operon of *Escherichia coli*

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The rate of synthesis of tryptophan synthetase was found to be increased by heat inactivation of the *dnaA* protein in three *dnaA* mutants temperature sensitive for initiation of DNA replication. The effect of the *dnaA* mutations was dependent upon the presence of an intact attenuator in the tryptophan operon. The activity of the mutated *dnaA* protein at the tryptophan attenuator and its activity as initiator for chromosome replication decreased gradually with increasing temperature. Two *rpoB* mutations that suppress the temperature defect of the *dnaA46* mutation in initiation of replication were tested for effects on attenuation in the tryptophan operon. One of the *rpoB* mutations caused increased transcription termination at the attenuator independent of the *dnaA* allele, whereas the other mutation had no effect. Expression of the histidine and threonine operons, which are also regulated by attenuation, was unaffected by the *dnaA* mutations.

Several temperature-sensitive mutations (1, 6, 7, 18, 39) and a few amber mutations (21, 34) map in the *dnaA* gene of *Escherichia coli*. The phenotype of the *dnaA* mutants shows that the gene product is essential for initiation of chromosome replication. The *dnaA* protein seems to be involved early in the initiation process, i.e., before or during the transcriptional step mediated by RNA polymerase (14, 22, 33, 43). The *dnaA* protein probably also plays a role in control of chromosome replication (16, 20). Secondary mutations can suppress the temperature-sensitive phenotype of *dnaA* mutants (2, 4, 35, 40). Many of these suppressor mutations map in the *rpoB* gene, which encodes the β subunit of RNA polymerase (2, 4, 35); some *rpoB* mutations have been shown to act in combination with some, but not other, *dnaA* mutations (2, 35). This allele specificity strongly suggests that the *dnaA* protein interacts with the RNA polymerase during initiation of replication.

Two observations suggest that the *dnaA* protein might be involved in termination of transcription: one *rpoB* mutation, isolated as a suppressor of the DnaA(Ts) phenotype (2), was fortuitously found to affect the expression of the *trp* operon; two other *rpoB* alleles, isolated for their enhancement of termination at the *trp* attenuator (42), were shown by Schaus and coworkers to act as *dnaA* suppressors (35).

We show here that three different *dnaA*(Ts) mutations increase expression of the *trp* operon at high temperature, but have no effect on expression of the *his* and *thr* operons. Genetic evidence is presented to support the conclusion that the effect is exerted at the attenuator, indicating that the *dnaA* protein is active in termination of transcription; we suggest that it may be required for an essential transcription termination event in initiation of replication at the chromosomal origin. We have also studied two *rpoB* suppressor mutations and found that only one had the effect on *trp* attenuation described above; the other had no effect.

MATERIALS AND METHODS

Bacterial strains. The strains used in this study are described in Table 1.

Growth media and genetic methods. Cultures were grown in AB minimal medium (8) or in M63 minimal medium (27) with 0.2% glucose, 1 μg of thiamine per ml, and 20 μg of tryptophan per ml. For growth of strains TC175 and TC176 this medium was supplemented with 1% Casamino Acids to obtain similar growth rates for the two strains. For all other strains the minimal medium was supplemented with an amino acid mixture giving a final concentration of 20 μg of the aromatic amino acids per ml, 100 μg of serine, leucine, and arginine per ml, and 50 μg of the other amino acids, except cysteine, per ml. In some experiments tyrosine or histidine was omitted depending on the enzyme assays to be carried out in addition to the measurements of tryptophan synthetase (TSase).

Preparation of P1 lysates and P1 transductions were carried out as described by Miller (27).

Deo$^+$ transductants were selected on AB minimal plates supplemented with 0.2% thymidine as the carbon source, and Tna$^+$ transductants were selected on

TABLE 1. *E. coli* K12 strains

Strain	Genetic markers[a]	Origin
NF880	*thi relA tonA argR trpR rpsL lacZp51 lacZpUV5*	N. Fiil
FH115	*thi thr leu thyA lacY mal bglR deoC*	*dnaA*$^+$ *bglR ilv*$^+$ derivative of CRT46 (18)
FH116	*thi thr leu thyA lacY mal bglR deoC dnaA46*	*bglR ilv*$^+$ derivative of CRT46
TC175	*thi thr leu thyA lacY mal bglR trpR*	P1(NF880) × FH115, DeoC$^+$
TC176	*thi thr leu thyA lacY mal bglR trpR dnaA46*	P1(NF880) × FH116, DeoC$^+$
TC2	*thi thr leu thyA lacY mal bglR deoC dnaA46 rpoB902*	(2)
TC17	*thi thr leu thyA lacY mal bglR deoC dnaA46 rpoB917*	(2)
TC152	*thi argH metB his trp pyrE lac xyl ilv tsx rpsL uhp tna*	MM301 Mal$^+$ and cured of phage P1 (26)
TC186	*thi argH metB his trp pyrE lac xyl ilv tsx rpsL uhp dnaA46 bglR*	P1(FH116) × TC152, Tna$^+$
CM740	*thi metE his trp mtl ara gal lac tsx rpsL dnaA5*	K. von Meyenburg (17)
CM905	*thi metE his trp mtl ara gal lac tsx rpsL dnaA167* (λ^+)	K. von Meyenburg (17)
S11	*thi lysA29 ilv*::Tn*5 argE*::Tn*10 rpsE relA lacZpUV5 lacZ* (Am)	S. Brown
TC456	*thi* Δ(*trpED24*) *trpR tna*	C. Yanofsky (5)
TC460	*thi* Δ(*trpED*)*24 trpR*	P1(TC186) × TC456, Tna$^+$
TC462	*thi* Δ(*trpED*)*24 trpR dnaA46*	P1(TC186) × TC456, Tna$^+$ T^s
TC480	*thi* Δ(*trpED*)*24 trpR dnaA5*	P1(CM740) × TC456, Tna$^+$ T^s
TC481	*thi* Δ(*trpED*)*24 trpR dnaA167*	P1(CM905) × TC456, Tna$^+$ T^s
TC516	*thi* Δ(*trpED*)*24 trpR argE*::Tn*10*	P1(S11) × TC460, Tet^r
TC519	*thi* Δ(*trpED*)*24 trpR argE*::Tn*10 dnaA46*	P1(S11) × TC462, Tet^r
TC528	*thi* Δ(*trpED*)*24 trpR rpoB902*	P1(TC2) × TC516, Arg^{+b}
TC529	*thi* Δ(*trpED*)*24 trpR dnaA46 rpoB902*	P1(TC2) × TC519, Arg$^+$
TC532	*thi* Δ(*trpED*)*24 trpR rpoB917*	P1(TC17) × TC516, Rif^r, Arg^{+c}
TC534	*thi* Δ(*trpED*)*24 trpR dnaA46 rpoB917*	P1(TC17) × TC519, Rif^r, Arg$^+$
TC457	*thi* Δ(*trpLD102*) *trpR tna*	C. Yanofsky (5)
TC465	*thi* Δ(*trpLD102*) *trpR*	P1(TC186) × TC457, Tna$^+$
TC467	*thi* Δ(*trpLD*)*102 trpR dnaA46*	P1(TC186) × TC457, Tna$^+$ T^s
TC478	*thi* Δ(*trpLD*)*102 trpR dnaA5*	P1(CM740) × TC457, Tna$^+$ T^s
TC482	*thi* Δ(*trpLD*)*102 trpR dnaA167*	P1(CM905) × TC457, Tna$^+$ T^s
TC520	*thi* Δ(*trpLD*)*102 trpR argE*::Tn*10*	P1(S11) × TC465, Tet^r
TC530	*thi* Δ(*trpLD*)*102 trpR rpoB902*	P1(TC2) × TC520, Arg^{+b}
TC536	*thi* Δ(*trpLD*)*102 trpR rpoB917*	P1(TC17) × TC520, Rif^r, Arg^{+c}

[a] Genetic symbols are as described by Bachmann and Low (3).

[b] The *rpoB902* mutation is a rifampin-resistant *dnaA* suppressor allele. The presence of the *rpoB902* allele could therefore be scored independently of the *dnaA46* mutation.

[c] Spontaneous Rif^r derivatives of strains TC516, TC519, and TC520 were used, and the Arg$^+$ transductants were screened for introduction of the Rif^s *rpoB917* allele. The presence of the *rpoB917* allele was verified by growing P1 lysates on strains TC532 and TC536 and transducing the *dnaA46* strain WM448 (6) to MetB$^+$.

AB minimal plates supplemented with 0.1% tryptophan as the carbon source. The *trpR* marker was scored on minimal plates containing 100 μg of 5-methyl-tryptophan per ml (27). Rifampin-resistant mutants were selected on LB plates (27) containing 100 μg of rifampin per ml.

Preparation of samples for enzyme assays. Samples corresponding to 10 to 15 ml of cells at an optical density at 450 nm (OD_{450}) of 1 were transferred from exponentially growing cultures into chloramphenicol (200 μg/ml, final concentration) at 0°C. The cells were harvested, washed with TM buffer (10 mM Tris-hydrochloride [pH 7.8] and 5 mM $MgCl_2$) and suspended in TM buffer to an OD_{450} of approximately 10. Part of the sample was stored at 4°C in TM buffer and used to determine TSase and histidinol-phosphate phosphatase activities, and part was stored as pellets at −20°C and used to determine homoserine dehydrogenase and threonine synthetase activities. The optical density of the final cell suspension was read after appropriate dilution in TM buffer, and in some experiments protein concentration was determined by the method of Lowry et al. (25).

TSase assay. Cell suspensions were diluted in TM buffer to give a suitable enzyme activity (1 to 4 U per assay), and 0.3 ml of cells was shaken thoroughly with 30 μl of toluene and then incubated at 37°C for 1 h to evaporate the toluene. TSase activity, requiring the *trpA* and *trpB* gene products, was determined as described by Smith and Yanofsky (36). The time course of disappearance of indole was followed by transferring 75-μl samples of reaction mixture into 1.5 ml of modified Ehrlichs indole reagent (28) every 5 or 10 min. Absorption at 540 nm was read after incubation at room temperature for at least 20 min to allow full development of the color. One unit was defined as

the enzyme activity which consumed 0.1 μmol of indole in 20 min (36). The amount of TSase per ml of the culture was calculated from activity of TSase/(milliliters × OD_{450}).

Histidinol-phosphate phosphatase activity. Histidinol-phosphate phosphatase activity was determined by the method of Ely (11).

Homoserine dehydrogenase assay. The frozen cells were thawed, suspended in 150 μl of buffer containing 10 mM Tris-hydrochloride (pH 7.6), 0.4 M KCl, 4 mM $MgCl_2$, 2 mM dithiothreitol, and 2 mM ethylene glycol-bis(β-aminoethyl ether)-*N*,*N*-tetraacetic acid, and sonicated in an ice water bath. Cell debris was removed by 15 min of centrifugation at 16,000 rpm at 20°C. Cell extracts were kept at room temperature. Protein concentration was determined by the method of Lowry et al. (25), and homoserine dehydrogenase activity was determined as described by Patte et al. (32).

Threonine synthetase assay. Cell extracts were prepared and enzyme activity was measured as described by Daniel (9). The substrate for the assay, L-[^{14}C]homoserine phosphate, was a gift from J. Daniel.

RESULTS

Effect of *dnaA* mutations on the expression of the *trp* operon. The *trp* operon is regulated both by a repressor-operator system and by attenuation (41). To study the effects of *dnaA* and *rpoB* mutations on attenuation in the *trp* operon, the following experiments were carried out in *trpR* strains.

Figure 1 shows a temperature shift experiment with strains TC175 (*dnaA*$^+$) and TC176 (*dnaA46*). Expression of the *trp* operon was followed by measuring the activity of TSase. The differential rates of synthesis were determined from the slopes in the plot of TSase units per milliliter versus OD_{450} of the culture. At 30°C the *dnaA46* mutant showed a twofold higher rate of synthesis than did the *dnaA*$^+$ strain. The temperature shift to 42°C caused a 1.6-fold reduction in the rate of synthesis in the wild-type strain, whereas the shift produced a 50% increase in the mutant strain, resulting in a 4.5-fold difference at 42°C between the two strains.

This experiment suggested that the *dnaA* protein may play a role in attenuation because the heat inactivation of the *dnaA* protein in the mutant strain TC176 increased the differential rate of synthesis of TSase.

To distinguish this possibility from an effect on initiation of transcription we introduced the *dnaA46*, *dnaA5*, and *dnaA167* mutations into a pair of strains carrying the *trpLD102* and *trpED24* deletions. The *trpLD102* deletion removes the entire attenuator structure, but retains an intact *trp* promoter and operator; deletion *trpED24* begins in the 11th codon of the *trpE* gene and thus leaves the *trp* leader region intact (5). The *dnaA5* and *dnaA167* alleles were included to test whether the effect observed with the strain carrying the *dnaA46* allele was characteristic of *dnaA*(Ts) mutations in general.

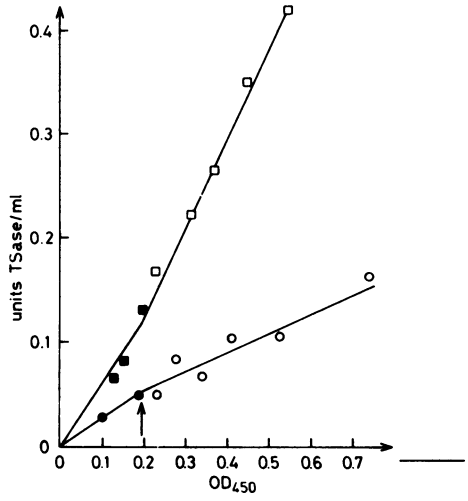

FIG. 1. The *dnaA46* mutation causes overproduction of TSase. The two strains TC175 (*dnaA*$^+$) and TC176 (*dnaA46*) were grown exponentially at 30°C in AB medium supplemented with glucose, Casamino Acids, and thymidine. The cultures were shifted to 42°C at the optical density indicated by the arrow. Samples for determination of TSase activity (see the text) were taken at intervals. Symbols: (●) TC175, 30°C; (○) TC175, 42°C; (■) TC176, 30°C; (□) TC176, 42°C. Doubling times were 67 min at 30°C and 35 min at 42°C for strain TC175 and 72 min at 30°C and initially 41 min at 42°C (for the first 1.5 doublings) for strain TC176.

Table 2 shows the differential rate of synthesis of TSase before and after shifts from 30 to 42°C in these sets of isogenic strains. The growth medium (glucose minimal medium supplemented with amino acid mixture) used for all experiments with these strains gave nearly identical growth rates for the *dnaA*$^+$ and *dnaA*(Ts) strains at permissive temperature. The cultures were monitored for two generations after the shift to nonpermissive temperature. The growth rates of the *dnaA*(Ts) mutant strains were close to that of the *dnaA*$^+$ strains for the first mass doubling after the shift (Table 2). Since the replication time of the chromosome is nearly identical to the doubling time (approximately 35 min), the immediate stop of initiation of replication in the mutants upon the shift should begin to affect the concentration of genes located near the terminus, like the *trp* operon, only about one doubling time later.

The experiments in Table 2 show that the expression of the *trp* operon was unaffected by temperature and by the presence of the *dnaA*(Ts) mutations in the strains lacking the attenuator Δ(*trpLD*)*102*. The temperature shift to 42°C with the strains still carrying the attenua-

TABLE 2. Effect of *dnaA* mutations on *trp* operon attenuation[a]

Strain	Relevant genotype	t_D (min)[b]		Rate of TSase synthesis			
				Differential[c]		Relative[d] (%)	
		30°C	42°C	30°C	42°C	30°C	42°C
TC465	Δ(*trpLD*)*102 dnaA*$^+$	80	37	5.8	5.8	100	100
TC478	Δ(*trpLD*)*102 dnaA5*	86	(46)	5.6	6.5	97	112
TC467	Δ(*trpLD*)*102 dnaA46*	82	(42)	5.6	5.6	97	97
TC482	Δ(*trpLD*)*102 dnaA167*	87	(39)	5.6	4.7	97	85
TC460	Δ(*trpED*)*24 dnaA*$^+$	74	33	0.89	0.31	15	5
TC480	Δ(*trpED*)*24 dnaA5*	77	(40)	1.22	1.49	21	26
TC462	Δ(*trpED*)*24 dnaA46*	76	(35)	1.20	1.25	21	22
TC481	Δ(*trpED*)*24 dnaA167*	75	(40)	0.96	0.96	17	17

[a] The strains were grown exponentially at 30°C in AB medium supplemented with glucose and amino acid mixture lacking histidine. At an OD_{450} of 0.25, half of the culture was shifted to 42°C, and six samples for determination of TSase were taken within two mass doublings. Samples from the 30°C culture were taken at OD_{450} values of 0.25, 0.45, and 0.6. The differential rate of synthesis of TSase was determined from a plot of TSase per milliliter against OD_{450} at the time of sampling.

[b] t_D, Mass doubling time. The value for the *dnaA*(Ts) strains (given within parentheses) is the initial doubling time at 42°C.

[c] Differential rate of TSase synthesis given in units/(milliliters × OD_{450}).

[d] Given relative to strain TC465 at 30°C.

tor Δ(*trpED*)*24* caused a threefold reduction in the rate of synthesis of TSase in the *dnaA*$^+$ strain, whereas the rate in the *dnaA*(Ts) mutant strains was unaffected or slightly stimulated.

The effect of the temperature shift was more pronounced with the Δ(*trpED*)*24 dnaA*$^+$ strain than that observed with the *trp*$^+$ *dnaA*$^+$ strain TC175 (Fig. 1). This difference is probably not caused by the *trpED24* deletion, but is due to differences in the genetic background as we have seen a 2.6-fold reduction, similar to that of the Δ(*trpED*)*24* strain, in another *dnaA*$^+$ *trpR* strain carrying an intact *trp* operon (data not shown). In the Δ(*trpED*)*24* background the *dnaA46* mutation caused only a slight increase in the rate of TSase synthesis at 30°C, whereas a twofold increase was observed in the *dnaA46* strain TC176 (Fig. 1). This is probably due to the different degree of temperature sensitivity conferred by the mutation in the two genetic backgrounds: 30°C is a true permissive temperature for the Δ(*trpED*)*24* strain, but not for strain TC176, judged from a comparison of growth rates (Fig. 1 and Table 2) and DNA/mass ratio (data not shown) in wild-type and mutant strains.

The differential rate of synthesis of TSase has been expressed as units per OD_{450}. Control experiments, determining the amount of protein by the method of Lowry et al. (25), showed that the ratio of protein to OD_{450} was constant over the shift, and that the *dnaA46* strain had a slightly higher ratio than did the wild-type strain (the difference was less than 10%).

Figure 2 shows a shift from 30 to 39°C with three of the *trpED24* deletion strains. In the *dnaA*$^+$ strain (TC460) the effect of this shift was delayed when compared with the shifts to 42°C; the rate of TSase synthesis decreased within half a mass doubling after the shift. In the two *dnaA*(Ts) strains the shift resulted in an immediate increase in the rate of synthesis, which then dropped to near the preshift value at the time when the rate had decreased in the wild-type strain.

The temperature effect on the function of the *trp* attenuator was studied further by using intermediate growth temperatures. Figure 3 shows the differential rate of synthesis of TSase as a function of growth temperature for the *trpED24* deletion strains carrying the *dnaA*$^+$ and *dnaA46* alleles. In the *dnaA*$^+$ strain the rate of synthesis decreased gradually with increasing temperature, indicating that the efficiency of termination at the attenuator increased with increasing temperature. In the *dnaA46* strain the rate of synthesis seemed to be virtually independent of temperature; therefore, the rate of synthesis relative to that in the *dnaA*$^+$ strain increases as a function of growth temperature, indicating that attenuation is reduced in parallel with the heat inactivation of the *dnaA46* protein in the initiation of replication (16).

Effect of two *rpoB* suppressor mutations on attenuation in the *trp* operon. Two spontaneous *rpoB* mutations isolated as suppressors of the temperature sensitivity of the *dnaA46* mutant (2) were tested for effects on *trp* attenuation. The two *rpoB* mutations (*rpoB902* and *rpoB917*) were introduced into the *trpED24* and *trpLD102* deletion strains by P1 transduction (Table 1).

Neither of the *rpoB* mutations affected the rate of synthesis of TSase in the strain with the attenuator deletion, indicating that initiation of

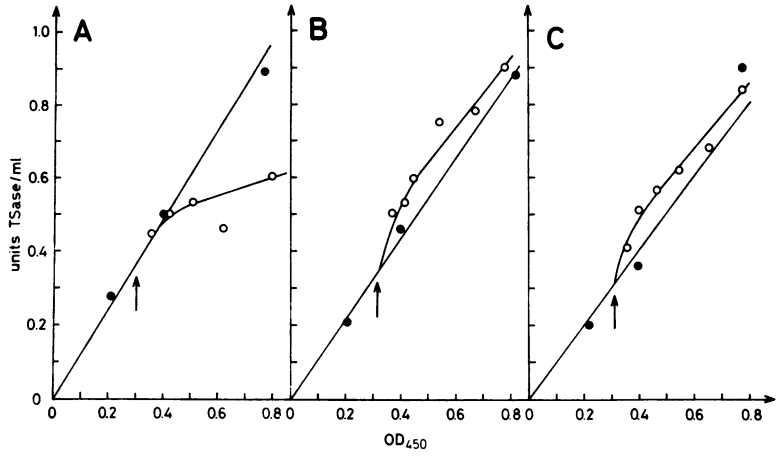

FIG. 2. Effect of the *dnaA46* and *dnaA5* alleles on synthesis of TSase during a shift from 30 to 39°C. The three strains TC460, TC462, and TC480 were grown exponentially at 30°C in M63 minimal medium supplemented with glucose and amino acid mixture lacking tyrosine. Part of the cultures were shifted to 39°C at the OD_{450} indicated by the arrow. Symbols: (●) 30°C, (○) 39°C. Panels: (A) strain TC460 (*dnaA*$^+$); (B) strain TC462 (*dnaA46*); (C) strain TC480 (*dnaA5*).

transcription at the *trp* promoter was normal (Table 3). The presence of the *rpoB902* mutation reduced the rate of synthesis about twofold in the *trpED24* deletion strain, which carries the intact attenuator. The other mutation, *rpoB917*, had no detectable effects on *trp* operon expression.

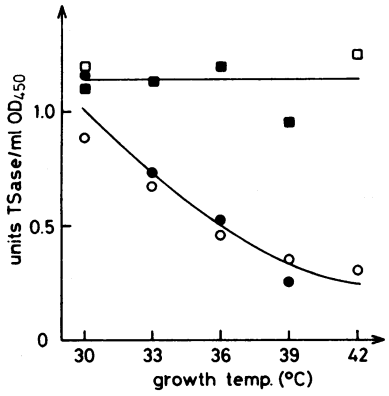

FIG. 3. Differential rate of synthesis of TSase in the *dnaA*$^+$ and *dnaA46* strains as a function of growth temperature. Symbols: (○, ●) strain TC460 (*dnaA*$^+$); (□, ■) strain TC460 (*dnaA46*); (○, □) cultures grown in AB minimal medium supplemented with glucose and amino acid mixture lacking histidine; (●, ■) cultures grown in M63 medium supplemented with glucose and amino acid mixture lacking tyrosine. The determinations at 30, 33, and 36°C were carried out on cultures in balanced growth, as was one of the 39°C determinations for strain TC460. Three to four samples were taken at intervals of one generation from each culture. The values at 39 and 42°C were determined from temperature shift experiments.

To determine whether the *rpoB* mutations could suppress the effect of the *dnaA46* mutation on *trp* attenuation, the *rpoB902* and *rpoB917* alleles were also introduced into the *trpED24 dnaA46* strain (TC462). Table 4 shows the results of temperature shift experiments carried out with these four strains. In the *dnaA*$^+$ *rpoB902* strain the shift to 42°C resulted in the same threefold reduction in the rate of synthesis of TSase as in the *rpoB*$^+$ strain (Table 2). At 42°C the *rpoB902* mutation had no effect on the rate of synthesis of TSase in the attenuator deletion strain (data not shown). In the double mutant, *dnaA46 rpoB902*, the separate effects of the two mutations seemed to be additive: the rate was reduced at 30°C due to the *rpoB902* mutation, and the heat inactivation of the *dnaA46* protein upon the shift to 42°C was reflected in a virtually unchanged rate instead of the threefold reduction in the *dnaA*$^+$ strain. The two *rpoB917* strains had synthesis rates similar to those of the parental *rpoB*$^+$ strains at both temperatures.

Effect of the *dnaA* mutations on other attenuators. Preliminary experiments to test whether the *dnaA* mutations affected other operons regulated by attenuation were carried out in parallel with the experiments described above.

The activity of histidinol-phosphate phosphatase, the product of the *hisB* gene, was assayed in the samples from the experiments described in Table 2. The *dnaA5* and *dnaA46* strains had somewhat elevated levels of the enzyme at 30°C (1.4- and 1.8-fold that of the *dnaA*$^+$ strain). The differential rate of synthesis was, however, unaffected by the temperature shift.

Expression of the *thr* operon was measured as

TABLE 3. Effect of *rpoB* mutations on *trp* operon attenuation[a]

Strain	Relevant genotype	Doubling time (min)	Relative rate of TSase synthesis[b] (%)
TC465	Δ(*trpLD*)*102 rpoB*$^+$	85	100
TC530	Δ(*trpLD*)*102 rpoB902*	98	117
TC536	Δ(*trpLD*)*102 rpoB917*	85	117
TC460	Δ(*trpED*)*24 rpoB*$^+$	73	8.8
TC528	Δ (*trpED*)*24 rpoB902*	80	3.8
TC532	Δ(*trpED*)*24 rpoB917*	72	8.8

[a] The strains were grown exponentially at 31°C in AB medium supplemented with glucose and amino acid mixture lacking tyrosine. The activity of TSase was determined in samples taken at OD_{450} values 0.15, 0.3, and 0.6.

[b] Given relative to strain TC465, which in this experiment was 4 U/(ml × OD_{450}).

the activity of homoserine dehydrogenase. Two genes in *E. coli*, *thrA* and *metM*, code for homoserine dehydrogenases. However, the *metM* gene contributes less than 5% of the total activity under normal growth conditions, and it is repressed by methionine (31). The differential rate of synthesis of homoserine dehydrogenase was determined in shift experiments like those in Fig. 2. No differences between the mutant and wild-type strains were observed. The activity of threonine synthetase, the gene product of *thrC*, was also measured in one of the experiments. The *dnaA* mutations did not affect the synthesis of this enzyme either.

DISCUSSION

Three independently isolated mutations, *dnaA5*, *dnaA46*, and *dnaA167*, caused increased expression of the *trp* operon at high temperature. The effect was seen in a strain with a wild-type *trp* operon and in a strain in which most of the *trpE* gene and about two-thirds of the *trpD* gene were deleted [Δ(*trpED*)*24*] (5, 37). The effect of the *dnaA* mutations was lost, however, in a strain in which most of the leader peptide, the attenuator region as well as the *trpE* gene and most of the *trpD* gene had been deleted [Δ(*trpLD*)*102*] (5, 37). This indicates that the *dnaA* mutations do not affect initiation of transcription at the *trp* promoter or the efficiency of translation of the *trpBA* genes; they probably interfere with termination at the attenuator.

A weak promoter for the *trpCBA* genes, *trpp2*, is located in the distal part of the *trpD* gene (19, 29) and thus is probably present in all of the strains used. Normally this promoter contributes at most 2% of the total *trpCBA* message in a *trpR* strain (29). The experiments described here do not exclude the possibility that the heat inactivation of the *dnaA* protein in the mutant strains dramatically increases the initiation of transcription from this promoter. However, to account for the three- to fourfold increase in the rate of synthesis of the enzyme in the Δ(*trpED*)*24* strain, the efficiency of this secondary promoter would have to increase about 200 times. This would produce only a 20% increase in the amount of enzyme in the Δ(*trpLD*)*102* strain, which is within the limits of accuracy of our measurements. Preliminary experiments with plasmids carrying the *trp* promoter and attenuator region fused to either the *tet* gene or the *lacZ* gene showed that the *dnaA46* mutation affected synthesis of these gene products to nearly the same extent as TSase in the strain with the *trpED24* deletion (T. Atlung and E. Clausen, unpublished data). We therefore conclude that the action of the *dnaA* protein occurs upstream from the first codons of the *trpE* gene. The effect of the *dnaA*(Ts) mutations on *trp* attenuation indicates that the wild-type *dnaA* protein plays a role in termination of transcription at the *trp* attenuator, and that this activity is heat labile in the mutant gene products. Alternatively, the presence of a wild-type *dnaA* protein might be required for the decrease in readthrough at the *trp* attenuator with increasing temperature. The possibility that the mutated *dnaA* proteins have acquired a temperature dependent antitermination activity at the *trp* atten-

TABLE 4. Combined effect of the *dnaA46* and *rpoB* mutations on expression of the *trp* operon[a]

Strain	Relevant genotype	Doubling time (min)		Relative rate of TSase synthesis[b] (%)	
		31°C	42°C	31°C	42°C
TC528	Δ(*trpED*)*24 dnaA*$^+$ *rpoB902*	72	36	4.5	1.4
TC529	Δ(*trpED*)*24 dnaA46 rpoB902*	78	38	4.7	4.2
TC532	Δ(*trpED*)*24 dnaA*$^+$ *rpoB917*	68	35	10.2	3.9
TC534	Δ(*trpED*)*24 dnaA46 rpoB917*	70	43	16.3	19.5

[a] The strains were grown exponentially at 31°C in AB medium supplemented with glucose and amino acid mixture. At an OD_{450} of 0.2 part of the culture was shifted to 42°C. Samples were taken and treated as described in footnote *a* of Table 2.

[b] Given relative to the rate observed with strain TC465 in Table 3.

uator seems unlikely in view of the very similar effect of the three different *dnaA*(Ts) mutations.

The *his* and *thr* operons are also regulated by attenuation (10, 13), and heat inactivation of the *dnaA* protein in the *dnaA* mutants had no effect on the synthesis of the corresponding enzymes. The *dnaA* protein thus seems to play no role at the *his* and *thr* attenuators, indicating that its role in *trp* attenuation is due to some particular feature of this attenuator.

Farnham and Platt (12) reported that read-through at the *trp* attenuator decreases with increasing temperature in a purified in vitro transcription system. We found that read-through at the attenuator [the Δ*(trpED)24* strain relative to the Δ*(trpLD)102* strain] in vivo decreased from 15 to 5% between 30 and 42°C (in the *dnaA*$^+$ strain). The presence of the *dnaA*(Ts) mutations increased read-through from 5 to 20% at 42°C. Thus, about 80% of the RNA polymerases still terminate at the *trp* attenuator in vivo in the absence of active *dnaA* protein. Termination of transcription is very efficient in vitro in the absence of any factors (23). This indicates that the *dnaA* protein is not needed for recognition of the termination signal; rather, it may modulate some step in the intricate attenuation mechanism, which depends on a coupling of transcription and translation (41). For instance, the *dnaA* protein might affect pausing of the RNA polymerase in the leader region (12, 41) or the release of the transcript from the template.

Two *rpoB* mutations, isolated as suppressors of the temperature-sensitive phenotype of a *dnaA46* strain (2), differed in the sense that one affected *trp* attenuation and the other did not. In strains carrying the *rpoB902* mutation read-through at the attenuator was decreased twofold independent of temperature (30 or 42°C) and of the *dnaA* allele (*dnaA*$^+$ or *dnaA46*). The *rpoB902* mutation is similar to the *rpoB7* and *rpoB8* mutations, isolated by Yanofsky and Horn (42): they all confer rifampin resistance, decrease read-through at the *trp* attenuator, and are *dnaA* suppressors (as shown for the latter two by Schaus et al. [35]).

The role of the *dnaA* protein in *trp* attenuation suggests to us that the *dnaA* protein and the RNA polymerase may interact to terminate transcription at the chromosomal origin of replication. The efficiency of the *dnaA46* protein in *trp* attenuation and its activity as an initiator of DNA replication (16, 38) depend in similar ways on temperature. When acting to initiate replication, the *dnaA* protein is probably part of a multiprotein complex, as suggested by the large number of *dnaA* suppressor loci (2, 40). It is therefore not surprising that two *rpoB* suppressor alleles can differ in the way their products interact with the *dnaA* protein when it acts either as initiator of replication or as modulator of attenuation. Finally, the *dnaA* protein is required for the autonomous replication of a 422-base-pair DNA segment encompassing the minimal chromosomal origin (24, 30) and containing at least one in vivo functional transcription terminator (15). We are at present investigating the function of this terminator in initiation of replication and its possible interaction with the *dnaA* protein.

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