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Influence of Chromosome Structure on the Frequency of *tonB trp* Deletions in *Escherichia coli*

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The frequency of *tonB trp* deletions varies in different strains and substrains of *Escherichia coli*. Studies with chromosomal hybrids constructed by transducing various segments of the *cysB-trp-sulII* region from K-12(Ymel) into K-12(W3110) indicate that the characteristic low deletion frequency of K-12(Ymel) is determined largely by the (genetic) structure of the *trp-sulII* region of the chromosome. Transduction of the *trp* region from K-12(W3110) or K-12(Ymel) into strain B has little effect on the frequency of *tonB trp* deletions in that strain. When *tonB trp* deletions occur at 42 C rather than at 37 C, there is a significant reduction in the frequency of deletions in all strains examined except K-12(Ymel) and hybrids exhibiting a Ymel deletion pattern. The magnitude of this temperature effect in different K-12 strains increases proportionally with the frequency of *tonB trp* deletions at 37 C. At 42 C the frequency of *tonB trp* deletions in all K-12 strains approaches the low frequency observed for Ymel at 37 or 42 C. In contrast, spontaneous deletions in another region of the genome which simultaneously result in resistance to phages T7 and λ and in proline auxotrophy (*tfrA pro* deletions) occur at a constant frequency regardless of growth temperature or the structure of the chromosome in the *trp* region. Two mutants of strain KB30 obtained after treatment with nitrosoguanidine show very low *tonB trp* deletion frequencies. The alterations in both mutants map in the *trp* region of the chromosome. These studies indicate that the structure of the *cysB-trp-sulII* region is responsible for many of the characteristic deletion frequencies observed.

Spontaneous deletion of the *tonB-trp* region of the *Escherichia coli* chromosome can be rapidly and accurately detected by selecting for resistance to coliphages T1 or ϕ80*vir* and colicins V and B (*tonB* mutants) and scoring for tryptophan auxotrophy (*tonB trp* deletions; see references 2 and 13).

The average frequency of *tonB trp* deletions in bacterial cultures has been shown to vary in different strains and substrains of *E. coli* and to be affected by the growth temperature. For example, Spudich et al. (13) reported that, although the frequency of spontaneous *tonB* mutants in *E. coli* B and in several K-12 strains was approximately the same at 37 C, the frequency of *tonB trp* deletions in strain B was threefold higher than in K-12(W3110#290) and 35- to 40-fold higher than in K-12(Ymel). When a growth temperature of 42 C was employed, a sharp reduction was observed in the frequency of total *tonB* mutants produced in both B and K-12(W3110#290). In each case, this decrease could be accounted for by a reduction in *tonB trp* deletions, i.e., the frequency of *tonB*(*trp*$^+$) mutants remained relatively constant regardless of the temperature. In the same study, experiments with K-12-B hybrid strains, constructed by transducing the *cysB-trp-sulII* region from strain B into K-12, indicated that the structure of the chromosomal region containing the *trp* operon could alter significantly the *tonB trp* deletion frequency. As a result of these observations, the present study was initiated to investigate further the role of chromosome (genetic) structure in determining the frequency of *tonB trp* deletions. The findings obtained indicate that the structure of the *cysB-trp-sulII* region determines many of the characteristic *tonB trp* deletion frequencies and influences the variations in deletion frequencies observed at different temperatures.

MATERIALS AND METHODS

Bacterial strains. Most strains employed in this study were described previously (13). Other strains used are described in the text or in footnotes to the tables. The structures of the *cysB-trp-sulII* regions of the chromosomes of the hybrid strains W3110#290 and KB30 are diagrammed in Fig. 1.

Media. The minimal agar medium utilized was that of Vogel and Bonner (15) containing 15 g of agar per liter. When required, the minimal agar medium was supplemented with the following per liter: acid-hydrolyzed casein, 10 g; L-histidine, 30 mg; L-tryptophan, 20 mg; and indole, 10 mg. Proline-free agar medium is minimal agar supplemented with the 17 common L amino acids (30 mg/liter each) with proline omitted. Nutrient agar plus cysteine (CysNA; 30 mg/liter) was used as a complete solid medium and L broth (12) was used as a complete liquid medium.

Preparation of colicins and bacteriophage lysates. Colicins B and V were prepared as described by Spudich et al. (13). Lysates of P1*kc* and P1*vir* on *E. coli* K-12 (12) and P1*bt* on *E. coli* B (8) were prepared as previously reported. Coliphages ϕ80*vir*, λ*vir*, and T1 were grown on *E. coli* K-12(W1485). T7 was grown on *E. coli* B.

Determination of the frequency of tonB trp and tfrA pro deletions. The frequency of *tonB* and *tonB trp* deletion mutants in stationary-phase bacterial cultures was determined as described previously (13), except that ϕ80*vir* (in place of T1) was used with colicins B and V to select *tonB* mutants in cultures of *E. coli* K-12. Throughout this study, *tonB* mutants were selected by spreading portions of bacterial cutlures (up to 5×10^8 cells) on CysNA plates with ca. 10^9 particles of ϕ80*vir* (or T1 for *E. coli* B) and 10^9 to 10^{10} particles of the colicin B and V preparation and incubating the plates for 1 to 2 days at 37 C. Gratia (7) suggested that resistance to coliphage T1, colicin B, and colicin V might be determined by distinct genes. If this is true, then all the *tonB* mutants selected in this way must be deletion mutants. In this paper only *tonB trp* mutants are scored as deletions.

The frequency of *tfrA* and *tfrA pro* deletion mutants (4, 14) in stationary-phase bacterial cultures was determined by plating 10^8 to 3×10^8 cells (grown from <100 cells per ml in L broth) on minimal acid-hydrolyzed casein agar with $>10^9$ particles each of T7 and λ*vir*. The plates were incubated at 37 C for 2 days. The resulting *tfrA* mutants were replicated to proline-free agar plates, and the replicates were incubated a further 24 hr. Pro^- colonies were scored as *tfrA pro* deletions. Under these conditions, the fraction of proline auxotrophs among *tfrA* mutants ranged from 8 to 54% in different experiments with *E. coli* K-12(W3110). *TfrA pro* deletions cannot be accurately detected by this procedure in cultures of strains which produce large numbers of mucoid colonies.

The temperature effect on deletion frequency was assayed as described by Spudich et al. (13). In general, the bacterial cultures were grown from a small inoculum to stationary phase in L broth at 37 or 42 C and then exposed to colicins and phage in the usual manner at 37 C. Expression of *tonB* mutations is not affected by a shift in growth temperature.

Construction of W3110-Ymel hybrids. W3110 strains carrying genetic material from Ymel in the *cysB-trp-suIII* region of the chromosome (WY-hybrids) were constructed in the following ways. WY-hybrids of classes 1, 2, and 3 (*see* Table 2) were constructed by transducing W3110#290(*cysB* his_{am} *suIII*$^-$) with P1 phage grown on YmelA23(*trpA suIII*$^+$). Selection was for Cys^+ or Su^+ (by suppression of his_{am}). The regions

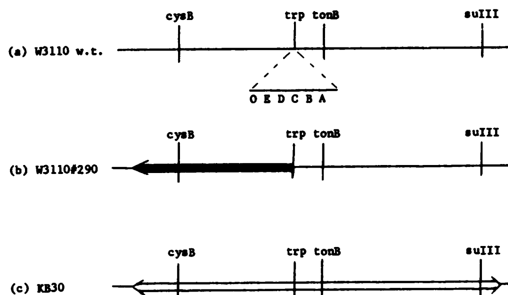

FIG. 1. *Structure of the trp region of the chromosome of E. coli W3110 w.t., W3110#290, and KB30. The chromosomal material originated from: W3110 w.t.,* ___*; Ymel,* ▬*; or B,* ▭*. Arrows indicate that the exact crossover points are unknown.*

of Ymel material in W3110 were determined by scoring for the unselected markers (and assuming the minimum number of crossovers). Class 4 hybrids were constructed by transducing YmelE5927(*trpE suIII*$^+$) into W3110A9813(*trpA suIII*$^-$) and selecting for Trp^+. Trp^+Su^+ recombinants were identified by spotting each hybrid strain with lysates of T4 nonsense mutants.

Mixing experiments. Each *tonB trp* deletion strain and corresponding nondeletion strain was grown to stationary phase (10^9 to 3×10^9 cells per ml) in L broth at 37 C. The cultures were then mixed in fresh L broth (0 C) to give the initial deletion cell (D) to total viable cell (T) ratios desired. (D was varied from 10^3 to 10^6 cells per ml in different experiments, whereas T remained constant at 10^7 cells per ml.) Five-milliliter portions of the mixed cultures were shaken in tubes in rotary baths at 37 and 42 C. The cultures were permitted to grow to stationary phase (seven to eight generations). Triplicate samples of the initial and stationary cultures were diluted appropriately in saline and plated on CysNA (to determine the total number of viable cells) and on CysNA with ϕ80*vir* and colicins V and B (to determine the number of *tonB trp* deletion cells). The results are expressed as a ratio of the fraction of deletion cells in the initial mixed cultures to the fraction of deletion cells in the stationary-phase cultures. A ratio of 2 represents a 50% decrease in the relative number of *tonB trp* deletion mutants following the seven- to eight-generation growth period.

Selection of Del^- mutants. An overnight L broth culture of KB30, grown at 37 C from <100 cells per ml, was diluted to ca. 10^7 cells per ml in fresh L broth and grown at the same temperature to mid-log phase. Four milliliters of this culture was centrifuged, and the cells were treated with *N*-methyl, *N'*-nitro-*N*-nitrosoguanidine (NNG; Aldrich Chemical Co., Milwaukee, Wis.) either by suspending them in 5 ml of 0.1 M sodium citrate buffer (*p*H 5.5) containing 100 μg of NNG per ml and incubating the suspension at 37 C for 1 hr or by suspending the cells in 5 ml of 0.05 M tris(hydroxymethyl)aminomethane-maleate buffer (*p*H 6.0) containing 100 μg of NNG per ml and incubating the suspension at 37 C for 30 min. Mutagenesis was terminated by diluting the cells 100-fold in 0.05 M potassium phosphate buffer (*p*H 7.0). The suspensions were then further diluted in the same buffer and spread

on CysNA at a concentration calculated to give 100 to 200 colonies per plate. After 2 to 3 days of incubation at 37 C, the colonies were replicated to CysNA spread with ϕ80*vir* and colicins V and B (as described above). Colonies which failed to produce microcolonies on the CysNA-ϕ80*vir*-colicin plates were picked from the master plates, purified by restreaking, and examined for the frequency of occurrence of *tonB trp* deletions. Del⁻ 1 was isolated by J. A. Spudich.

RESULTS

TonB trp deletion patterns in W3110-Ymel hybrid strains. Spudich et al. (13) observed that, when the *cysB-trp-suIII* region of the *E. coli* B chromosome was transduced into a K-12 strain (W3110#290), hybrids (KB strains) were obtained which produced *tonB trp* deletions at a frequency 10- to 20-fold greater than the frequency observed for either the donor or the recipient. Furthermore, over 80% of the *tonB* mutants in cultures of these hybrid strains were Trp⁻. The pattern and frequency of *tonB trp* deletions in various strains of *E. coli* at 37 C and at 42 C are summarized in Table 1. By transducing more precisely defined segments of the *E. coli* B chromosome into K-12, it was possible to pinpoint the region between *cysB* and *trp* as the only segment of the B chromosome required to produce this new deletion pattern. With these experiments in mind, we asked the question: Is it possible to transform the deletion pattern characteristic of W3110 w.t. to the pattern observed for strain Ymel simply by altering the genetic structure of the chromosome in the vicinity of the *trp* operon? Preliminary results indicated that the frequency of total *tonB* mutants in W3110 w.t. is reduced to the value observed for Ymel when chromosomal material from Ymel is introduced into the *cysB-trp* region of W3110 w.t. (e.g., W3110#290). However, this change in chromosome structure had no effect on the percentage of those *tonB* mutants which were Trp⁻. To establish whether the exceptionally low relative Trp⁻ frequency in strain Ymel is also determined by a particular segment of the chromosome in this region, we constructed (by P1 transduction) W3110 strains carrying different segments of Ymel chromosomal material in the vicinity of the *trp* operon (WY-hybrids; Table 2). The *tonB trp* deletion frequencies were determined at 37 C for 92 recombinants from 4 recombinant classes (*see* Table 2). Only deletion patterns corresponding to the patterns characteristic of strains W3110 w.t., W3110#290, and Ymel were observed. The data of Table 2 indicate that, in general, the percentage of transductants displaying a Ymel deletion pattern increases as Ymel genetic material is introduced into the *trp-suIII* region of the W3110 chromosome. This finding suggests that the genetic structure of the *trp-suIII* region of the Ymel chromosome must be at least partially responsible for the reduced fraction of Trp⁻ mutants in this strain. The W3110-Ymel system, therefore, appears to involve two genetic sites: one in the *cysB-trp* region which affects the frequency of total *tonB* mutants and the other in the *trp-suIII* region which affects the relative frequency of Trp⁻ mutants. However, although hybrid classes 1 and 2 were constructed by replacing a Ymel *cysB-trp* region with another Ymel *cysB-trp* re-

TABLE 1. *tonB trp deletion patterns in various strains of Escherichia coli*[a]

Strain	37 C			42 C			*tonB trp* deletion frequency ratio (37 C/42 C)
	tonB/ viable cell[b]	Per cent Trp⁻	*tonB trp* deletion/ viable cell[b]	*tonB*/ viable cell[b]	Per cent Trp⁻	*tonB trp* deletion/ viable cell[b]	
B	2.9	81	2.4	0.6	56	0.34	7.0
K-12 W3110 w.t.	6.0	27	1.6	1.5	12	0.18	9.0
Ymel	2.2	8[c]	0.18	3.6	11	0.40	0.5
W3110#290	2.7	26	0.7	1.6	10	0.16	4.4
KB30	18	80	14.5	3.2	53	1.7	8.5
Del⁻ 1[d]	3.0	19	0.6	5.2	17	0.9	0.7
Del⁻2	5.7	74	4.2	—[e]	—	—	

[a] Values for total *tonB* mutants, per cent Trp⁻, and *tonB trp* deletion mutants for each strain are averages of four or more independent determinations.

[b] Figures shown to be multiplied $\times 10^7$.

[c] Spudich et al. (13) reported that only about 2% of the *tonB* mutants of strain Ymel were Trp⁻. Since the relative frequency of Trp⁻ mutants is very low in this strain, the variance in these values might be related to the number of independent frequency determinations considered in each study. Alternatively, the discrepancy in these values might be due to the use of ϕ80*vir* and colicins in the present study rather than T1 and colicins to select *tonB* mutants.

[d] The original Del⁻ strain was temperature sensitive at 42 C. Del⁻ 1 is a spontaneous revertant selected at 42 C. Both strains exhibit the same *tonB trp* deletion pattern at 37 C.

[e] Not determined.

TABLE 2. *tonB trp deletion patterns in WY-hybrids*

Hybrid class[a]	Region from Ymel (*cysB* *trp* *sulII*)	No. examined	Per cent of transductants exhibiting the *tonB trp* deletion pattern[b] of		
			W3110 w.t.	W3110 #290	Ymel
1	————	25	8	28	64
2	——	32	41	56	3
3	—— —	25	0	12	88
4	——	10	0	0	100

[a] Hybrid classes 1, 2, and 3 were constructed by crossing strain Ymel A23(*trpA sulII*$^+$) into W3110#290(*cysB* his_{am} *sulII*$^-$) and hybrid class 4 by crossing Ymel E5927(*trpE sulII*$^+$) into W3110 A9813(*trpA sulII*$^-$). Details of procedure are described in Materials and Methods.

[b] Characteristic *tonB trp* deletion patterns of W3110 w.t., W3110#290 and Ymel are shown in Table 1.

gion, a number of the recombinants (8% and 41%, respectively) exhibit a W3110 w.t. deletion pattern. These results suggest that still other factors may be involved in establishing these deletion patterns.

It has been observed that the frequency of total *tonB* mutants in strains displaying a W3110 w.t. deletion pattern is considerably more variable than the frequency in strains displaying other patterns. For example, the standard deviations (and range of values) obtained from 20 independent frequency determinations on WY-hybrids exhibiting W3110 w.t., W3110#290, and Ymel deletion patterns were $6.0 \pm 3.68 \times 10^{-7}$ (2.40 to 13.9×10^{-7}), $2.7 \pm 0.74 \times 10^{-7}$ (1.04 to 3.66×10^{-7}), and $2.2 \pm 0.72 \times 10^{-7}$ (0.86 to 3.78×10^{-7}), respectively. As a result of this variability, the average *tonB* frequency values for W3110 w.t. fluctuated when examined on different days. This phenomenon can be eliminated by growing these strains at 42 rather than at 37 C (see later) or by introducing genetic material from strain B or Ymel into the *cysB-trp* region (e.g., KB30 and W3110#290). The reason for this variability is unknown.

Effect of chromosome structure on the frequency of tonB trp deletions in E. coli B. To determine whether chromosome structure in the *trp* region of *E. coli* B alters the frequency of deletions in this strain, chromosomal material from strains exhibiting very high (KB30) and very low (Ymel) *tonB trp* deletion frequencies were transduced into a B strain carrying a deletion of the entire *trp* operon (B *trp*^{delAE}). Trp$^+$ recombinants were then examined for deletion frequencies significantly different from those of strain B. The results of these experiments are summarized in Table 3. The introduction of chromosomal material from Ymel into the *cysB-trp-sulII* region of *E. coli* B (experiment 3) reduced slightly both the frequency of total *tonB* mutants and the fraction of those which are Trp$^-$. However, the average *tonB trp* deletion frequency in these hybrid strains was reduced only about twofold. None of the deletion frequencies of the individual strains examined varied substantially from the average values. The *tonB trp* deletion frequencies of the 20 BK-hybrids constructed from KB30 (experiment 2) were also relatively constant, with an average frequency comparable to the control (experiment 1).

Since the recipient in these transduction crosses (B *trp*^{delAE}) lacked a marker linked to the *trp* operon, it was impossible to determine (for the BK-hybrids) the extent of the B chromosome near *trp* replaced by K-12 genetic material. If a change in the frequency of *tonB trp* deletions requires the modification of a large chromosomal region, such a structure might be expected to arise only rarely in this cross. One might argue, therefore, that the failure to detect a hybrid strain exhibiting a deletion frequency greatly different from *E. coli* B might be due to the fact that many recombinant classes were not examined. The following experiment was devised to examine a large number of BK-hybrids for abnormally high deletion frequencies. A B *trp*^{delAE} strain was transduced to Trp$^+$ with a P1 lysate grown on W3110#290 (the strain originally used as a *recipient* in the construction of the KB-hybrids). Approximately 800 of the Trp$^+$ recombinants were replicated to CysNA plates previously spread with T1 and colicins V and B. After incubation for 24 hr at 37 C, 23 colonies produced a large number of microcolonies on the T1-colicin plates.

TABLE 3. *Frequency of tonB trp deletions in BK-hybrids*[a]

Expt	Donor	Recipient	No. examined	Avg *tonB*/viable cell[b]	Avg per cent Trp$^-$	Avg *tonB trp* deletions/viable cell
1	B	B *trp*^{delAE}	8	3.0 ± 0.58	78 ± 6.4	2.3 ± 0.57
2	KB30	B *trp*^{delAE}	20	2.8 ± 0.83	80 ± 5.0	2.2 ± 0.73
3	Ymel	B *trp*^{delAE}	10	1.8 ± 0.71	53 ± 9.0	1.0 ± 0.42
4	W3110#290	B *trp*^{delAE}	23	4.5 ± 2.8	44 ± 9.9	2.0 ± 1.5

All crosses were mediated by P1 transduction. P1*kc* was grown on strains KB30, Ymel, and W3110 #290; P1*bt* was grown on strain B. In each case selection was for Trp$^+$.

[b] Figures shown to be multiplied $\times 10^7$.

The corresponding colonies were picked from the master plates, purified by restreaking, and examined for frequency of occurrence of *tonB trp* deletions. None of the 23 hybrid strains examined showed more than a twofold increase in deletion frequency. Furthermore, although the average frequency of total *tonB* mutants was somewhat higher in the BK-hybrids (experiment 4) than in strain B (experiment 1), the average frequency of *tonB trp* deletions in these strains was the same. In the reciprocal cross (B---->W3110#290 trp^{delAE}), three Trp^+ transductant colonies which grew well when replicated to the T1-colicin plates were examined. All three KB-hybrid strains exhibited *tonB trp* deletion frequencies at least 20-fold greater than the frequency observed for W3110#290. In addition, approximately 90% of the *tonB* mutants of these strains were Trp^-. These experiments suggest that the structure of the chromosome in the vicinity of the *trp* operon has considerably less influence on the frequency of *tonB trp* deletions in strain B than it has in strain K-12.

Relationship between chromosome structure and temperature effect on tonB trp deletions. Experiments by Spudich et al. (13) indicated that the frequency of *tonB trp* deletions in *E. coli* B, K-12(W3110), and KB-hybrids was reduced when the cultures were grown at 42 C rather than at 37 C (Table 1). Subsequent experiments, however, have revealed that the deletion frequency of strain K-12(Ymel) is not decreased at the high temperature. In fact, the deletion frequency in Ymel is often slightly higher at 42 C (Tables 1, 4, and 5). To establish whether the occurrence of a temperature effect on deletion frequencies is related to the structure of the chromosome in the *cysB-trp-suIII* region, the *tonB trp* deletion frequency for various WY-hybrids exhibiting different deletion patterns was determined at 37 C and at 42 C (Table 4). All hybrid strains exhibiting a deletion pattern similar to W3110 w.t. or W3110#290 (*see* Table 1) showed a reduced deletion frequency at the high temperature (Table 4).

TABLE 4. *Effect of growth temperature on the frequency of tonB trp deletions in WY-hybrids*

Hybrid class[a]	Strain	*tonB trp* deletion pattern[b]	*tonB trp* deletion frequency ratio (37 C/42 C)
1	WY42	W3110 w.t.	7.6
	WY60	W3110#290	3.6
	WY62	Ymel	0.6
2	WY79	W3110 w.t.	7.7
	WY11	W3110#290	5.1
	WY43	Ymel	0.6
3	WY38	W3110#290	3.0
	WY39	Ymel	0.9

[a] Chromosome structure of the *trp* region of each hybrid class is diagrammed in Table 2.

[b] Different *tonB trp* deletion patterns are described in Table 1.

None of the hybrid strains exhibiting a Ymel deletion pattern displayed a comparable temperature effect, despite the fact that these strains were constructed in the same background.

In general, the magnitude of this temperature effect in the different strains increased proportionally with the frequency of *tonB trp* deletions at 37 C, i.e., the higher the frequency of deletions at 37 C the greater the temperature effect. At 42 C, the frequency of *tonB trp* deletions in all hybrid strains approached the low frequency observed for Ymel at 37 C or at 42 C.

To examine further the relationship between chromosome structure in the *cysB-trp-suIII* region and the effect of high growth temperature on the generation of *tonB trp* deletions, the following experiment was performed. A Ymel trp^{delAE} strain was transduced to Trp^+ with a P1 lysate grown on KB30. Ten transductants were purified by restreaking, and the *tonB trp* frequency of each was determined at 37 C and at 42 C. Only two categories of deletion frequencies were observed. Eight of the transductants showed a deletion frequency and pattern identical to the

TABLE 5. *Effect of growth temperature on the frequency of tonB trp deletions in KB30-Ymel hybrid strains*[a]

Donor	Recipient	No. examined	Transductants showing: High *tonB trp* deletion frequency[b] — No.	High: Avg frequency at 37 C	High: Avg frequency at 42 C	Low *tonB trp* deletion frequency[b] — No.	Low: Avg frequency at 37 C	Low: Avg frequency at 42 C
KB30	Ymel trp^{delAE}	10	8	14.2 ± 2.1	2.60 ± 0.83	2	0.19 ± 0.02	0.21 ± 0.02
Ymel	KB30 trp^{delAE}	10	2	0.80 ± 0.04	0.23 ± 0.07	8	0.13 ± 0.09	0.30 ± 0.18

[a] All hybrid strains were constructed by P1 transduction. Selection was for Trp^+.

[b] *tonB trp* deletion frequencies are expressed as the number of *tonB trp* deletion mutants per 10^7 viable cells in the bacterial cultures.

donor (KB30), and the deletion frequency of these strains was reduced at the high growth temperature (Table 5). The other transductants exhibited a deletion frequency and pattern characteristic of the recipient (Ymel). Neither of these strains showed a temperature effect on deletions (Table 5). From the reciprocal cross (Ymel--> KB30 *trp*^{delAE}), again only two categories of *tonB trp* deletion frequencies were observed for the Trp$^+$ recombinants (Table 5). In this case, however, 2 of the 10 hybrid strains examined exhibited deletion frequencies and patterns characteristic of W3110#290 (*see* Table 1). This deletion pattern is commonly observed among W3110 hybrid strains constructed with genetic material from Ymel in the *cysB-trp* region of the chromosome (13; Fig. 1; Table 2). The other eight transductants exhibited the deletion pattern of the donor (Ymel). A temperature effect on deletions was observed only for the strains showing the W3110#290 deletion frequency.

To eliminate the possibility that the temperature effect on *tonB trp* deletions was the result of selection against the deletion mutants at the high temperature, reconstruction experiments were performed with mixed cultures of *tonB trp* deletion and nondeletion strains (Table 6). All the deletion strains examined grew more slowly in mixed cultures at 37 C than did the corresponding nondeletion strains. However, this slight effect on the growth of the deletion strains was not enhanced at the high temperature in strains showing a temperature effect (e.g., KB30). Therefore, it is unlikely that the reduced number of *tonB trp* mutants in liquid cultures grown at 42 C is the result of a preferential selection against the deletion mutants.

If a specific configuration or sequence in the *cysB-trp-suIII* region of the *E. coli* chromosome is required for this temperature effect on *tonB trp* deletions, deletions occurring at other sites on the chromosome might not exhibit a temperature effect. Accordingly, we determined for strains W3110 w.t. and KB30 the frequency of spontaneous deletion of the *tfrA* region of the chromosome which simultaneously results in resistance

TABLE 6. *Effect of temperature on the growth of mixtures of tonB trp deletion and nondeletion strains*[a]

Expt	Nondeletion strain	*tonB trp* deletion strain	Mixing ratios[b] (D/T)	No. of independent determinations	Initial D/T ratio / Final D/T ratio: 37 C	Initial D/T ratio / Final D/T ratio: 42 C	42 C/37 C
1	KB30	KB30 *trp*^{delAE}	10^{-1} to 10^{-4}	8	1.35 ± 0.39	1.33 ± 0.44	0.99
2	Ymel	Ymel *trp*^{delAE}	10^{-1} to 10^{-3}	6	1.48 ± 0.80	1.22 ± 0.49	0.83
3	KBY3[c]	KBY *trp*^{delAC}	10^{-1}	1	1.38	2.28	1.65
			10^{-3}	1	1.51	1.57	1.04
4	WY62[d]	WY62 *trp*^{delAE}	10^{-1}	1	1.55	1.69	1.09
			10^{-3}	1	1.48	2.13	1.44
5	KBY3	KBY3 *tonB*(Trp$^+$)[e]	10^{-1}	1	1.11	1.35	1.22
			10^{-3}	1	0.96	1.57	1.64

[a] Details of procedure are described in Materials and Methods.

[b] Mixing ratios are expressed as the number of *tonB trp* deletion cells per milliliter (D) divided by the total number of viable cells per milliliter (T).

[c] Strain KBY3 is a KB30-Ymel hybrid (*see* Table 5) with Ymel genetic material replacing at least the *trpE-tonB* region of KB30. This strain exhibits a Ymel deletion pattern.

[d] Strain WY62 is a W3110-Ymel hybrid of class 1 type (*see* Tables 2 and 4). This strain exhibits a Ymel deletion pattern.

[e] It is not known whether the *tonB* mutation is the result of a deletion or a point mutation.

TABLE 7. *Frequency of tfrA pro deletions at 37 C and at 42 C*[a]

Strain	Growth temp	*tfrA*/viable cell[b]	Per cent Pro$^-$	*tfrA pro* deletions/viable cell[b]
W3110 w.t.	37 C	2.0 ± 0.13	31 ± 12.7	0.62 ± 0.26
	42 C	1.8 ± 0.17	34 ± 11.6	0.62 ± 0.25
KB30	37 C	1.6 ± 0.19	30 ± 3.0	0.48 ± 0.21
	42 C	1.9 ± 0.28	28 ± 1.8	0.53 ± 0.28

[a] Values for total *tfrA* mutants, per cent Pro$^-$, and *tfrA pro* deletion mutants are averages of seven or more independent determinations.

[b] Figures shown to be multiplied × 10^7.

to phages T7 and λ and in proline auxotrophy. The data of Table 7 indicate that the frequency of *tfrA pro* deletions is constant regardless of the growth temperature or the structure of the *cysB-trp-suIII* region of the chromosome.

Mutations which decrease the frequency of tonB trp deletions. As the result of a mutant hunt for strains unable to generate deletions, two mutants of KB30 were obtained after nitrosoguanidine treatment which showed low *tonB trp* deletion frequencies (Table 1). Strain Del$^-$1 showed a reduction in both the frequency of total *tonB* mutants and the percentage of those which were Trp$^-$, whereas in strain Del$^-$2 only the frequency of total *tonB* mutants was reduced. In addition, Del$^-$1 no longer displayed a temperature effect on *tonB trp* deletions when the cultures were grown at 42 C. To determine whether the mutational changes responsible for these effects were in the vicinity of the *trp* operon, strains Del$^-$1 and Del$^-$2 were used as donors in a P1-mediated cross with KB30 trp^{delAE}. Trp$^+$ recombinants were selected and examined for the frequency of occurrence of *tonB trp* deletions. From the Del$^-$1 cross, 32/32 Trp$^+$ recombinants showed the low deletion frequency characteristic of the donor (Table 8). This suggests that the mutational change in Del$^-$1 is within or closely linked to the region deleted from the recipient. Results of the Del$^-$2 cross were somewhat more complex. *TonB trp* deletion frequencies of the 27 strains examined ranged from 4.7 to 28.2 $\times 10^{-7}$. The individual deletion frequencies roughly fell into two classes: 14 displayed frequencies similar to KB30 (average 15.5 $\times 10^{-7}$, range 10.2 to 28.2 $\times 10^{-7}$), and 13 displayed frequencies more characteristic of the Del$^-$ donor (average 6.9 $\times 10^{-7}$, range 4.7 to 8.4 $\times 10^{-7}$). These data suggest that the mutation(s) characterizing Del$^-$2 also maps in the vicinity of the *trp* operon.

To determine whether the genetic alterations in Del$^-$1 and Del$^-$2 influence the frequency of deletions in another region of the chromosome, the frequency of *tfrA pro* deletions was determined in these strains. The frequency of *tfrA pro* deletions in Del$^-$1 and Del$^-$2 was indistinguishable from the frequency of these deletions in other W3110 strains.

DISCUSSION

In the present and in a previous study (13), many observations on the frequency of appearance of *tonB trp* deletions in different strains of *E. coli* suggest that the structure of the chromosome in the vicinity of the *trp* operon influences the generation of these deletions. Analyses with hybrid strains indicate that the replacement of certain segments of the *cysB-trp-suIII* region of the chromosome of one strain with genetic material from another strain can alter *tonB trp* deletion frequencies and deletion patterns, and, in some cases, the position of the deletion termini. For example, only a segment of chromosome approximately midway between *cysB* and *trp* from *E. coli* B is needed in K-12(W3110) to produce the extraordinarily high deletion frequency and unique deletion pattern characteristic of KB hybrids (type 2). Furthermore, ca. 95% of these deletions have one terminus between *cysB* and *trpE* (13). In the present study, an examination of *tonB trp* deletion patterns in different W3110-Ymel hybrids suggests that two distinct chromosomal regions are involved in determining the characteristic deletion patterns of these strains. The *cysB-trp* chromosomal segment appears to influence specifically the frequency of total *tonB* mutants, whereas the *trp-suIII* segment determines the relative frequency of Trp$^-$ mutants. One possible interpretation of these findings is that introduction of foreign chromosomal material near the *trp* operon creates or removes base sequences complementary to nearby regions, thus altering the configuration of this chromosomal segment. Alterations in the structure of the chromosome in this region might determine the different deletion patterns. Consistent with this interpretation are the effects of mutations in the

TABLE 8. *Partial mapping of the mutations in the Del$^-$ strains*[a]

Donor	Recipient	No. examined	Transductants showing: KB30 *tonB trp* deletion frequency[b]		Del$^-$ *tonB trp* deletion frequency[b]	
			No.	Avg frequency	No.	Avg frequency
KB30	KB30 trp^{delAE}	20	20	15.4 ± 4.2	0	
Del$^-$1	KB30 trp^{delAE}	32	0		32	0.5 ± 0.34
Del$^-$2	KB30 trp^{delAE}	27	14	15.5 ± 4.3	13	6.9 ± 1.2

[a] All crosses were mediated by P1 transduction. P1*kc* was grown on KB30 and Del$^-$2; P1*vir* was grown on Del$^-$1. Selection was for Trp$^+$.

[b] *tonB trp* deletion frequencies are expressed as the number of *tonB trp* deletion mutants per 10^7 viable cells in the bacterial cultures.

vicinity of the *trp* operon and elevated growth temperatures on the frequency of *tonB trp* deletions. Structural and environmental changes such as these might modify the structure of the chromosome in this region by decreasing the stability of abnormally paired chromosomal regions. However, if such deletions are generated as a consequence of pairing of complementary base sequences, it is clear that the *rec* system is not required (1, 6, 10, 13).

When *tonB trp* deletions occur at 42 C, the frequency in all *E. coli* K-12 strains examined is near the basal value observed for K-12(Ymel) at 37 or 42 C. In addition, strain Del$^-$1, which no longer exhibits a temperature effect on *tonB trp* deletion frequency (Table 1), also displays a deletion frequency similar to Ymel (ca. twofold higher). These findings suggest that the generation of *tonB trp* deletions in *E. coli* K-12 involves two deletion systems: (i) a temperature-sensitive system active in strains exhibiting deletion frequencies greater than Ymel and (ii) a temperature-insensitive system active in all K-12 strains. The observations that the temperature-sensitive *tonB trp* deletion generating system can be detected in strain Ymel after modifying the structure of the *cysB-trp-sulII* segment of the chromosome and that temperature sensitivity can be eliminated from strain KB30 by mutational changes near *trp* indicate that this deletion system recognizes specific chromosomal configurations. It is conceivable that the temperature effect on *tonB trp* deletion frequency could result from the inactivation (at the high temperature) of a protein (enzyme?) involved in the generation of deletions (13). However, this hypothetical protein —if present—must act only in response to certain chromosomal configurations, since the frequency of *tfrA pro* deletions in K-12 (W3110) as well as the frequency of *tonB trp* deletions in K-12 (Ymel) are unaffected by a change in growth temperature.

Recently we found that mutants of *E. coli* deficient in deoxyribonucleic acid (DNA) polymerase activity in vitro (Pol$^-$mutants; see references 5 and 9) exhibit an increased *tonB trp* deletion frequency (Coukell and Yanofsky, Nature, *in press*). The effect of *pol* mutations on the frequency of *tonB trp* deletions appears to be independent of the structure of the chromosome in the *cysB-trp-sulII* region since: (i) the Pol$^-$ mutations do not map near *trp* (9; J. Gross, *personal communication*), (ii) *polA1* does not influence the occurrence or the magnitude of the temperature effect on *tonB trp* deletions (Coukell and Yanofsky, *unpublished data*), and (iii) *pol A1* does not alter the position of the termini of *tonB trp* deletions in the *trp* operon (Coukell and Yanofsky, Nature, *in press*). It has been reported (3, 11) that the sensitivity of Pol$^-$ mutants to ultraviolet irradiation is related to their inability to complete the final step in excision repair, the rejoining of the repaired segment to the parental DNA. As a result of these findings, we suggested that the high frequency of *tonB trp* deletions observed in Pol$^-$ mutants may result from an increased number of unrepaired single-strand breaks in DNA (Coukell and Yanofsky, Nature, *in press*).

On the basis of these observations, it seems reasonable to conclude that the frequency of deletion of the *tonB* region of the chromosome is determined by mechanisms involving at least two components. One component is the structure of the chromosome in the *cysB-trp-sulII* region, and the second component may be visualized tentatively as a "nick-generating" system. If the primary structure of this chromosomal region and the environmental conditions in the cell permit this segment to assume an abnormal configuration, nicks in this region might arise more frequently or be repaired less efficiently than nicks in regions less complex in structure. Therefore, the frequency of occurrence of nicks in different regions of the chromosome would determine whether deletions in a particular region occur at a low frequency or whether the region becomes a "hot-spot" for deletion mutations.

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