Available online at www.sciencedirect.com
SCIENCE DIRECT®
Biochemical and Biophysical Research Communications 323 (2004) 197–203
BBRC
www.elsevier.com/locate/ybbrc

Role of the RuvAB protein in avoiding spontaneous formation of deletion mutations in the *Escherichia coli* K-12 endogenous *tonB* gene

Kazumi Mashimo[a], Yuki Nagata[a], Masakado Kawata[a], Hiroshi Iwasaki[b], Kazuo Yamamoto[a,*]

[a] *Department of Biomolecular Sciences, Graduate School of Life Sciences, Tohoku University, Sendai 980-8577, Japan*
[b] *Graduate School of Integrated Science, Yokohama City University, Yokohama 230-0045, Japan*

Received 29 July 2004
Available online 28 August 2004

Abstract

The endogenous *tonB* gene of *Escherichia coli* was used as a target for spontaneous deletion mutations which were isolated from *ruvAB*$^-$, *recG*$^-$, and *ruvC*$^-$ cells. The rates of *tonB* mutation were essentially the same in *ruv*$^+$, *ruvAB*$^-$, *recG*$^-$, and *ruvC*$^-$ cells. We analyzed *tonB* mutants by sequencing. In the *ruv*$^+$, *recG*$^-$, and *ruvC*$^-$ strains, the spectra were different from those obtained from the *ruvAB*$^-$ cells, where deletions dominated followed by IS insertions, base substitutions, and frameshifts, in that order. We then analyzed the *tonB-trp* large deletion, due to simultaneous mutations of the *trp* operon, and found that the frequency in *ruvAB*$^-$ was higher than those in *ruv*$^+$, *recG*$^-$, and *ruvC*$^-$ cells. To characterize deletion formation further, we analyzed all the *tonB* mutants from one colicin plate. Seven deletions were identified at five sites from the 45 *tonB* mutants of *ruv*$^+$ cells and 24 deletions at 11 sites from the 43 *tonB* mutants of *ruvAB*$^-$ cells. Thus, the *ruvAB*$^-$ strain is a deletion mutator. We discuss the role of RuvAB in avoiding deletions.

Keywords: Spontaneous mutagenesis; *tonB* gene; Deletion; *ruvAB*; *recG*; *ruvC*; Replication fork collapse

Homologous genetic recombination is an essential process for the repair of damaged DNA and for the generation of genetic diversity in all living organisms. Studies of recombination protein from *Escherichia coli* have helped to define each stage of the recombination process and to elucidate the molecular interactions that occur during DNA pairing, strand exchange, and the resolution of recombination intermediates [1].

During the late stages of recombination in *E. coli*, intermediates made by RecA-mediated homologous pairing and strand exchange, which are called Holliday junctions, are processed into mature recombinants by the RuvA, RuvB, and RuvC proteins [2,3]. RuvA and RuvB proteins act together to provide activities for unwinding and rewinding of duplex DNA at the junction that promotes branch migration [4,5]. In this reaction, RuvA binds specifically to the Holliday junctions and targets the assembly of RuvB, a hexameric ring AAA$^+$ ATPase that provides the driving force for the branch migration reaction [4,6–8]. Holliday junction resolution is mediated by the RuvC endonuclease, a homodimer that introduces nicks into symmetrical sites at Holliday junction [9–12]. The resulting nicked duplexes are subsequently repaired by DNA ligase.

Ruv mutants were first isolated as mutants sensitive to DNA damaging agents such as UV and mitomycin C [13]. Although *ruv* single mutants were only moderately defective in homologous recombination, the association of *ruv* with *recG* mutants [14] resulted in cells that were severely defective in recombination and very

* Corresponding author. Fax: +81 22 217 5053.
E-mail address: yamamot@mail.tains.tohoku.ac.jp (K. Yamamoto).

0006-291X/$ - see front matter © 2004 Elsevier Inc. All rights reserved.
doi:10.1016/j.bbrc.2004.08.078

sensitive to DNA-damaging agents. The results thus demonstrated that *ruv* gene products are involved in recombination and that *ruv* can be partly replaced by *recG*. The deduced primary structure of RecG possesses DNA and RNA helicase motif. RecG can dissociate synthetic Holliday junction and promote the migration of Holliday junction formed by RecA protein in a manner similar to RuvAB [15].

Recent studies further indicate the role of RuvAB during DNA replication. In bacterial cells, replication forks often encounter DNA damage that is known to inactivate forks. Stalled forks lead to a specific reaction called replication fork reversal; at blocked forks, annealing of the nascent strands, and pairing of the template strands result in a four-way junction [16–18]. RuvAB stabilizes this four-way junction to serve as a substrate for RecBCD digestion, and the digested ends can then be incorporated into the chromosome by recombination [19].

The molecular mechanisms responsible for generating deletions are not well understood. In *E. coli*, the *xonA* (*sbcB*) mutation was shown to increase the frequency at which deletions formed in the *lacZ* target gene on the plasmid [20]. Using a plasmid system for the analysis of deletions, Yamaguchi et al. [21] demonstrated that the frequency of deletions is increased by the *sbcA* mutation. Albertini et al. [22] demonstrated that the frequency is decreased by the *recA* mutation in the *lacI* target gene on the F factor. Finally, Ikeda and Ukita [23] observed that *recJ* mutations alter the sites and frequency of deletions during the formation of *λbio* phages following DNA damage, and *recQ* mutations show an increase in the frequency of deletions during the formation of *λbio* phages [24]. These results strongly suggest that genetic recombination is involved in the formation of deletions.

During the past several years, we have developed a system, using the *E. coli* endogenous *tonB* gene as a target, to study deletions systematically [25–27]. The *tonB* gene is located 4.6 kb counterclockwise from the *trp* operon at about 28 min on the linkage map (Fig. 1). Thus, a system for the detection of long deletion mutations in the *tonB-trp* region could be developed [25,28,29]. Using this system, we investigated the formation of spontaneous deletions and found that mutations in *recA* [26], *recQ*, and *recJ* [30] did not produce any increase or decrease in the rate of deletion mutations or particular types of deletion mutations in the *tonB* gene or in the *tonB-trp* region compared with wild-type strains. To interpret the differences among our results and those mentioned above, we argued that distinct mechanisms for deletion formation were operating in plasmid systems and in the chromosome system [30].

To try to understand the mechanisms by which deletions form, we have further characterized the spectra of *tonB* mutations and *tonB-trp* region deletions in *ruvAB*-, *recG*-, and *ruvC*-defective *E. coli* strains. We found that mutations in *recG* and *ruvC* did not produce any increase in the rate of deletion mutations or an increase in particular types of deletion mutations either in the *tonB* gene or in the *tonB-trp* region compared with wild-type strains. In contrast, strain *ruvAB* produced an increase in the rate of deletion mutations in *tonB* mutants as well as *tonB-trp* region deletion. From these results, deletions are avoided by the RuvAB protein.

Materials and methods

Bacterial strains and plasmids. The *E. coli* K12 strains TM31 (*ruv*$^+$) [25], KM3 (*ΔruvAB*::Km), KM4 (*ΔrecG*::Km), and KM5 (*ΔruvC*::Km), which are derivatives of AB1157 (*ruv*$^+$) [31], were used for collecting mutations. TM31, KM3, KM4, and KM5 carry the Cm^r gene (*cat*), located about 1.6 kb upstream of the *tonB* gene on a chromosome (Fig. 1; [25]). The *ΔruvAB*::Km allele, *ΔrecG*::Km allele, and *ΔruvC*::Km allele were derived from strains HRS3403 [32], HRS2000 [32], and HRS1200 [32], respectively. The colicinogenic *E. coli* strain CA18 carries a colicin B factor [29].

Reagents and media. L broth, L agar, and phosphate buffer were prepared as described previously [33,34]. Minimal medium (MM) agar,

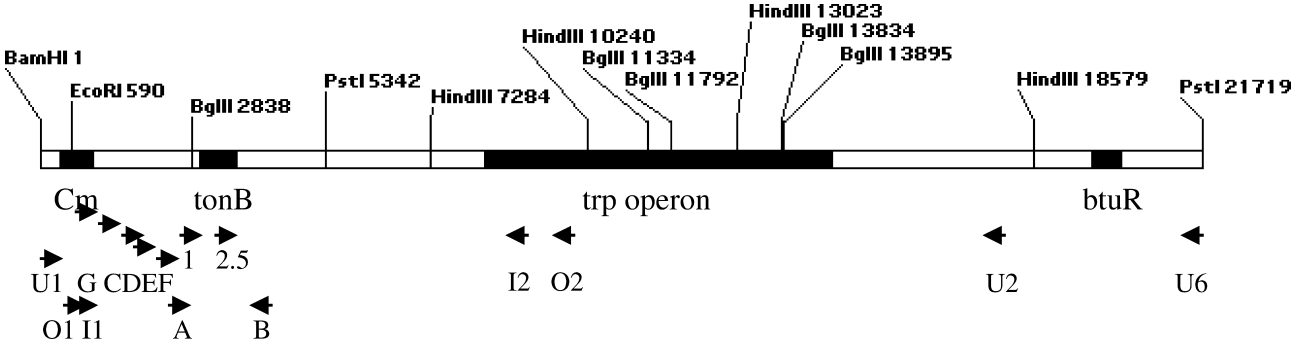

Fig. 1. Map of the *E. coli tonB-trp* region and the location of the primers for PCR-amplification and sequencing [25,30]. Relevant restriction sites with nucleotide numbers of the 21,755 bp DNA sequences in which one is the first G of the GATC *Bam*HI site are included. With this numbering *cat* is from bp 377 to 1036, *tonB* from bp 2993 to 3727, *trp* from bp 14,864 to 8334, and *btuR* from bp 19,683 to 20,253. Arrows below the map indicate the primers for amplification and sequencing which were described previously [30].

used for testing for tryptophane auxotrophy, contained M56 salts [33] with or without tryptophane supplementation (20 μg/ml). Chloramphenicol (Cm; 30 μg/ml), ampicillin (Amp; 50 μg/ml) or tetracycline (Tet; 10 μg/ml) was included, if necessary, in L broth, L agar or MM agar.

tonB mutant and tonB-trp deletion mutant selection. For the *tonB* mutation assay, independent colonies of TM31, KM3, KM4, and KM5 were grown in 2.5 ml L broth at 37 °C overnight. Then, samples of these cultures were plated on colicin plates and colonies were scored as colicin-B-resistant (ColB^r) mutants after 48 h incubation. Viable cells were scored on L agar after 18 h incubation. ColB^r mutations have been shown to be associated with tryptophane auxotrophy [29]. The association of tryptophane auxotrophy with ColB^r, which we define as a *tonB-trp* deletion mutant, was checked by restreaking 100 independent ColB^r colonies on MM agar with or without tryptophane. To analyze the *tonB* mutation and *tonB-trp* deletion mutant, only one ColB^r and tryptophane auxotroph was chosen from each colicin plate and MM agar plate with tryptophane, respectively, an approach that ensured that all mutants analyzed were of an independent origin. The DNA fragment including the mutant *tonB* gene or *tonB-trp* region deletion was amplified by a polymerase chain reaction (PCR) using appropriate primers (Fig. 1) from a genomic DNA that had been extracted from the mutants. Mutant sequences were determined by the dideoxy chain termination method using an automated sequencer.

Mutation rate. Mutation of ColB^r was determined for 10 independent cultures of TM31 (*ruv*$^+$), KM3 (*ΔruvAB*), KM4 (*ΔrecG*), and KM5 (*ΔruvC*) in 2.5 ml L broth after overnight growth. For assays scoring ColB^r, independent cultures were directly plated onto colicin plates. ColB^r colonies were scored after 48 h incubation at 37 °C. Total viable cells were determined by serial dilution with phosphate buffer, followed by plating on L agar. Mutation rates, expressed as mutations per cell per generation, were calculated by the method of the median [35]. The rate at which an overnight culture produced at least one *tonB-trp* deletion mutant was estimated from the fluctuation test data by assuming that all the *tonB-trp* deletion mutations occur randomly following the Poisson distribution [36]. The χ^2 test was used to examine differences in the mutation rate among the strains, *ruv*$^+$, *ruvAB*$^-$, *recG*$^-$, and *ruvC*$^-$. A value of $P < 0.05$ was regarded as significant.

Results

Effect of ruvAB, recG, and ruvC on efficiency of tonB mutations

We first measured the spontaneous mutation rate of TM31 (*ruv*$^+$), KM3 (*ΔruvAB*), KM4 (*ΔrecG*), and KM5 (*ΔruvC*), which were selected as the ColB^r phenotype. The ColB^r mutation rate of TM31 was 3.03×10^{-8}, KM3 was 4.43×10^{-8}, KM4 was 4.07×10^{-8}, and KM5 was 1.80×10^{-8} (Table 1).

tonB mutation spectrum

A total of 49 independent ColB^r mutated clones from KM3 (*ruvAB*$^-$), 71 from KM4 (*recG*$^-$), and 59 from KM5 (*ruvC*$^-$) were collected and used for DNA sequencing. Analysis yielded a mutant sequence in 49, 71, and 59 of these clones, respectively. For comparison, their distributions by class are listed in Table 1 along with previously published results from *ruv*$^+$ strain TM31 [25]. The most frequent mutational event in both the *recG* and *ruvC* strains was an IS insertion followed by a base substitution, deletions, and frameshift, in that order. These spectra did not differ among the *ruv*$^+$, *recG*, and *ruvC* strains. In this case, a χ^2 test showed that deletions did not differ among the *ruv*$^+$ strain (10 deletions among 51 *tonB* mutants analyzed), *recG* strain (10/71), and *ruvC* (8/59) (degrees of freedom = 6, $\chi^2 = 2.37$, $P = 0.883$). The most frequent mutational event in *ruvAB* strain was a deletion followed by a IS insertion, base substitution, and frameshift, in that order. In this case, deletion in the *ruv*$^+$ strain (10/51), *ruvAB* strain

Table 1
Spontaneous endogenous *tonB* mutations

Class of mutation[a]	Number detected[b]			
	TM31 (wt)[c]	KM3 (*ruvAB*)	KM4 (*recG*)	KM5 (*ruvC*)
Deletion	10 (0.59)	23 (2.08)	10 (0.57)	8 (0.24)
Frameshift	3 (0.18)	3 (0.27)	6 (0.34)	5 (0.18)
−Frameshift	2	2	5	5
+Frameshift	1	1	1	0
Base substitution	14 (0.83)	7 (0.63)	15 (0.86)	10 (0.31)
Transversion	6	5	11	6
Transition	8	2	4	4
IS elements	22 (1.31)	15 (1.36)	39 (2.24)	36 (1.09)
Miscellaneous	2 (0.12)[d]	1 (0.09)[e]	1 (0.06)[f]	0 (—)
Total	51	49	71	59
Mutation rate ($\times 10^8$)	3.03 ± 0.40	4.43 ± 1.62	4.07 ± 1.02	1.80 ± 0.51

[a] In this table, −frameshift means the deletion of one or two bases. More than a three-base deletion is defined as a deletion. +Frameshift means the addition of one or two bases.
[b] Parentheses ($\times 10^8$) indicate mutation rate of each subclass.
[c] Data, except for mutation rate, from Kitamura et al. [25].
[d] GC → TG (nucleotides 3500 and 3501) and one 4-base addition (bold letters) at sequence GC (nucleotides 3040 and 3041) to G**GTTT**C.
[e] Insertion of *oppF* at nucleotide 2022.
[f] Insertion of *yciA* at nucleotide 3472.

(23/49), *recG* strain (10/71), and *ruvC* (8/59) was significantly different (degrees of freedom = 6, $\chi^2 = 24.5$, $P < 0.001$).

Table 2 shows the 23 deletions identified from the *ruvAB* strain, ranging in size from 373 to 15,990 bp, 10 from the *recG* strain, ranging in size from 5 to 12,702 bp, and 8 from the *ruvC* strain, ranging in size from 11 to 6249 bp, together with four previously identified deletions from the *ruv*$^+$ strain, ranging in size from 94 to 15,792 bp [25]. The results indicate that the *ruvAB*

Table 2
Location and types of deletion mutations among *tonB* mutants in *ruv* strains

Position[a]	Sequence[b]	Size (bp)	Incidence
TM31 (wild-type)			
2624–18,416	ATCCCG[CAGCC···TTCGC]GCTCCAG	15,792	1
2906–3163	CAAAAA[**TG**ACATT···CC]**TG**CTG	258	1
2907–5329	AAAAAT[**GA**CATTT···TG]**GAC**GC	2423	1
3645–3738	GCAGAT[GGCGTT···AAGTCA]AAAGCCT	94	1
KM3 (*ruvAB*)			
1011–3387	GTACT[GCGAT···GAAAA]TACGG	2377	1
1196–3485	TTTAC[CGGTT···TAATC]AGCCGC	2290	1
1408–3026	GTT[**GC**AGGAT···CTC]**GC**CGCTT	1619	1
1840–5761	ATGC[**AAAAT**CCA···AGCG]**AAAAT**ATT	3922	1
1903–5371	CAACA[ACAAA···GGTGA]CATAT	3469	1
2019–3116	ATCC[**AACT**CTG···TATTG]**AACT**ACCT	1098	1
2177–3994	GTAGC[CCAGT···GTCAC]TACGC	1818	1
2177–10,178	GTAGC[CCAGT···CGGCA]TAGTT	8002	1
2329–3304	GTACC[TCAGG···CAAAA]CCGAA	976	1
2364–13,214	CACAT[CATAT···GTAAA]TCACC	10,851	1
2378–3184	AAATA[CAGAT···CACAA]GCCGT	807	1
2489–9096	CTATT[CAGAA···CTTCT]TTGCG	6608	1
2489–4482	TATT[**C**AGAAC···TTTA]**C**CCAGC	1994	1
2605–18,594	TAAC[**GG**TTGA···TAAA]**G**ATAGT	15,990	1
2752–4283	CTATC[AGCTT···GGCAT]GTGGC	1532	1
2964–4086	GCATT[TAAAA···GTCAC]CATTG	1123	1
2995–4854	GAAAT[GATTA···CAACA]TATAC	1860	1
3176–4580	GAA[**CC**GCCAC···CTT]**CC**ATTTA	1405	1
3207–4303	GGAGC[CGGTG···GTAGA]TATAG	1097	1
3496–3970	AGTA[**T**CCGGC···GCCG]**T**AAGAA	475	1
3653–6225	TTAT[**G**AGCCG···AACC]**G**TTCAT	2573	1
3680–4052	AAT[**AT**CCTGT···GAC]**AT**TAACC	373	1
3692–4132	ATATC[CTGTT···ACAAG]ATCGC	441	1
KM4 (*recG*)			
2274–4698	AAAC[**A**ATAGT···GATA]**A**GCACA	2425	1
2352–15,053	CTGA[**A**TATGC···GATT]**A**ATTGT	12,702	1
2751–3470	TAT[**CA**GCTTT···GCG]**CA**TTAAG	720	1
2757–2999	CTT[**TG**TCTTG···TTA]**TG**ACTTC	243	1
2817–3270	GTAAA[GTACG···GTGGT]CATTG	454	1
3068–5321	TTCAT[GGTGC···CTGAA]AAAGT	2254	1
3442–3531	CC[**GGTTA**CCAGT···CA]**GGTTA**AAGTT	90	1
3556–3567	GTTAC[GCCGG···GGTCG]CGTGG	12	1
3570–3574	TCGCG[TGGAT]AACGT	5	1
3628–3033	CAG[**CCCCTGGA**···CTT]**CCCCTGG**C	406	1
KM5 (*ruvC*)			
1175–3498	GCCGC[TTATG···TATCC]GGCAC	2324	1
1752–3176	GAAA[**C**TAATA···GAAC]**C**GCCAC	1425	1
2043–5762	GG[**AAA**ATATC···GA]**AAA**TATTT	3720	1
3040–3054	CCTG[**G**CCGAC···TTTC]**G**GTCTG	15	1
3118–7080	ATTGA[ACTAC···ATTCG]GCTTG	3963	1
3260–9508	GAA[**GC**ACCGG···CAC]**GC**GACCA	6249	1
3446–3456	CGGTT[ACCAG···GTGGC]TTCAG	11	1
3514–3529	CAGGC[ATTGC···AAGGG]CAGGT	15	1

[a] Numbering is in accordance with Fig. 1 in which 2993 is the first A of the ATG start and 3727 is the last A of the TAA end.

[b] The precise positions of the breakpoints in brackets in those cases involving direct repeats are ambiguous and could lie anywhere within the repeated sequences (bold letters). The breakpoint shown represents only one possibility.

strain forms relatively longer deletions than the *ruv*$^+$, *recG*, and *ruvC* strains.

Concerning base substitution mutations and frameshift mutations, we observed 7, 15, and 10 base substitutions and 3, 6, and 5 frameshifts among 49, 71, and 59 *tonB* mutants in the *ruvAB*, *recG*, and *ruvC* strains, respectively. Characteristics of base substitutions and frameshifts in the *ruvAB*, *recG*, and *ruvC* strains were essentially the same as those in the *rec*$^+$ strains (data not shown, but available at the web site as Tables S1 and S2, http://www.biology.tohoku.ac.jp/~kazuo/mashimo/).

tonB-trp deletion mutation in the ruvAB, recG, and ruvC strains

We next determined the spectra of *tonB-trp* long deletion mutations. To obtain the *tonB-trp* deletion, the association of ColB^r with tryptophane auxotrophy was checked by re-streaking 100 independent ColB^r colonies from one colicin plate on MM agar with or without tryptophane. The distance between the *tonB* gene and *trp* operon is 4.6 kb (Fig. 1). From this analysis, 52 colicin plates among 58 colicin plates for KM3 (*ruvAB*), 24 colicin plates among 34 colicin plates for KM4 (*recG*), and 43 colicin plates among 100 colicin plates for KM5 (*ruvC*) gave at least one tryptophane auxotroph. In other words, 6 cultures for *ruvAB*, 10 for *recG*, and 57 for *ruvC* did not give any tryptophane auxotrophs. As shown in Table 3, the rate of *tonB-trp* deletion was 1.00×10^{-9} for the *ruvAB*, 4.97×10^{-10} for the *recG*, and 1.01×10^{-10} for the *ruvC* strain. We estimated the *tonB-trp* deletion frequency in the TM31 (*ruv*$^+$) strain as 1.03×10^{-10}. Thus, as far as *tonB-trp* is concerned and the region is on a chromosome, deletions occurred more frequently in the *ruvAB* strain than the *ruv*$^+$, *recG*, and *ruvC* strains (degrees of freedom = 3, $\chi^2 = 55.1$, $P < 0.001$).

We determined the location and types of 52 *tonB-trp* deletions from *ruvAB* strain, 24 from *recG*, strain and 43 from *ruvC* strain. The results were compared with 12 previously published *tonB-trp* deletion results recovered from the *ruv*$^+$ strain [26]. Although occurrences of *tonB-trp* deletions in *ruvAB* and *ruv*$^+$ were different, the location and types of *tonB-trp* deletions were essentially the same (data not shown, but available as Table S3 at the web site, http://www.biology.tohoku.ac.jp/~kazuo/mashimo/).

Characterization of tonB mutations from one colicin plate

To further demonstrate the weak deletion mutator phenotype of the *ruvAB* strain, all the ColB^r mutants from one colicin plate were sequenced. After an appropriate dilution, we obtained 46 ColB^r colonies for the *ruv*$^+$ strain and 45 ColB^r colonies for the *ruvAB* strain. DNA sequencing of ColB^r mutants of *ruv*$^+$ identified 7 deletions at 5 sites, 9 frameshifts at 5 sites, 13 base substitutions at 10 sites, and 16 IS insertions at 9 sites in the *tonB* gene. We could not identify any mutation in the *tonB* gene from one ColB^r mutant. Among seven deletions, ranging in size from 11 to 16,110 bp, from the *ruv*$^+$ strain, those at nucleotides 2880–7853 and 3458–3468 were detected twice (Table 4). DNA sequencing of ColB^r mutants of *ruvAB* identified 24 deletions at 11 sites, 7 frameshifts at 3 sites, 10 base substitutions at 2 sites, and 2 IS insertions at 2 sites in the *tonB* gene. We could not identify any mutation in the *tonB* gene from two ColB^r mutants. Among 24 deletions, ranging in size from 520 to 16,110 bp, from the *ruvAB* strain, that at nucleotides 2508–3562 was detected 14 times (Table 4). Thus, during DNA replication in the *ruvAB* strain, replication slippage can occur even in the early stage of cell division, thus resulting in a higher occurrence of deletion mutations compared to the *ruv*$^+$ strain.

Discussion

In this experiment, we studied spontaneous deletion mutations in the endogenous *tonB* gene and found a significant increase in the *ruvAB* compared with *ruv*$^+$ strain. In *recG* and *ruvC* compared with the *ruv*$^+$ strain, the frequency of deletion mutations was not increased.

We first observed 23 *tonB* deletions among 49 *tonB* mutants from the *ruvAB* strain, giving a deletion rate of 2.08×10^{-8}, which is 3.5-fold different from the rate for *ruv*$^+$, 0.59×10^{-8} (Table 1). Concerning the *tonB-trp*

Table 3
TonB-trp region deletion rate

ruv genotype	Total number of cultures	ColB^r-rate ($\times 10^8$)	Number of cultures w/o a trp mutant	Average number of cells in colony	Deletion rate per cell per generation[a]
ruv$^+$	66	3.03 ± 0.40	47	3.30×10^9	1.03×10^{-10}
ruvAB	58	4.43 ± 1.62	6	2.26×10^9	1.00×10^{-9}
recG	34	4.07 ± 1.02	10	2.46×10^9	4.97×10^{-10}
ruvC	100	1.80 ± 0.51	57	5.56×10^9	1.01×10^{-10}

[a] The fraction of cultures having no *trp*$^-$ mutants among 100 ColB^r colonies was used to calculate the *tonB-trp* region deletion rate by the method of the mean [36]. The mean number of viable cells producing at least one *tonB-trp* region deletion mutant per tube, mN, can be given by the equation $P(0) = e^{-mN}$, where $P(0)$ is the fraction of dishes containing no *tonB-trp* mutants, m is the mutation frequency per replication, and N is the mean final cell count per culture.

Table 4
Location and types of *tonB* deletion mutation from one colicin dish in the *ruvAB* strain

Position[a]	Sequence[b]	Size (bp)	Incidence
TM31 (*ruv*$^+$)			
2211–18,320	AATT[**T**TGTTT···AAAC]**T**GGATT	16,110	1
2880–7853	ATGCA[**TAAA**GTA···TTTT]**TAAA**ATTA	4974	2
3329–3441	ACAG[**G**AGCAG···AGCC]**G**GTTAC	113	1
3458–3468	TGGCT[TCAGGACCACG]CGCAT	11	2
3628–4376	TGAA[**A**AATGC···CAAAT]**A**TTTTG	749	1
KM3 (*ruvAB*)			
1343–3335	GGAT[**C**GGGTA···CAGC]**C**AAAAC	1993	1
1790–12,354	CTACT[GTGGG···GAACC]AGATT	10,565	1
1970–3061	CAAG[**A**AAGAC···CTGC]**A**TTCAT	1092	1
2211–18,320	AATT[**T**TGTTT···AAAC]**T**GGATT	16,110	1
2508–3562	AATG[**G**TTTCA···GGAT]**G**GTCGC	1055	14
2936–12,126	CTTGC[CTTATTG···CTATA]AACAC	9191	1
2937–17,892	TTGCC[**TTA**TTGAA···TCT]**TTA**CGGTA	14,956	1
3176–3837	CGAA[**C**CGCCA···GTCG]**C**TTTAT	662	1
3375–7070	CGCAT[**CA**CCGTT···AAG]**CA**TAGAT	3696	1
3528–4047	TGAAG[GGCAG···GCCAA]TATCC	520	1
3586–4686	AAAT[**C**CTCTC···AACC]**C**AGCTA	1101	1

[a] Numbering is in accordance with Fig. 1 and Table 2.
[b] The precise positions of the breakpoints in brackets in those cases involving direct repeats are ambiguous and could lie anywhere within the repeated sequences (bold letters). The breakpoint shown represents only one possibility.

deletion rate, we calculated it to be 1.00×10^{-9} for *ruvAB*, which is 10-fold higher than that of *ruv*$^+$, 1.03×10^{-10} (Table 3). When all ColB^r colonies on the dish, after appropriate dilution, were analyzed by DNA sequencing, 24 deletions at 11 sites in the *ruvAB* strain compared to 7 deletions at 5 sites in the *ruv*$^+$ strain were counted (Table 4). Among 11 sites, one particular ColB^r mutant was counted 14 times (Table 4), indicating the deletion occurred early in the generation. These results indicated that the *ruvAB* strain is a deletion mutator.

RuvAB protein is known to bind RecA-mediated Holliday junction, during the late stages of homologous recombination, to promote branch migration [4,5]. We have shown in this study that the frequency of deletions increased in the *ruvAB* strain. It was demonstrated previously that deletions in the endogenous *tonB* gene are not dependent of *recA*, suggesting a role for RuvAB in replication slippage processes but not recombination processes for deletion formation.

As mentioned in the introduction, RuvAB binds a four-way junction formed at the stalled replication forks in a RecA-independent manner. During leading strand synthesis, slippage occurs in the nascent strand forming a bulged structure in the template strand. If the bulge is too large to prevent replication fork movement, RuvAB can stabilize fork reversal and serve double-strand ends to a substrate for RecBCD, The RecBCD nuclease then digests double-stranded ends to a χ-sequence. During these processes, the bulged structure will be removed, avoiding the formation of large deletions in wild-type cells.

The RecG helicase was proposed to act at blocked replication forks as RuvAB [37]. However, RecG helicase is highly specific for forks at which a protruding 5′ end is formed by progression of the lagging strand rather than the leading strand, and does not act at structures that mimic canonical replication forks [38]. In our mutation assay, although *ruvAB* showed a weak deletion mutator phenotype, *recG* did not (Tables 1–3). These results may indicate that RecG cannot process the bulged structure in the leading strand.

Previously we argued that the *E. coli* DNA polymerase I (PolI) Klenow domain can process the replication slip bulge which may be formed during lagging strand synthesis [27]. Actually, in the PolI Klenow-deficient strain, the deletion rate increased 20- to 50-fold compared with *pol*$^+$ parental strain. In a Klenow null mutant, we found that, among 21 deletions at 12 sites, all except one was less than 100 bp in size [27]. In a Klenow amber mutant, Agemizu et al. [39] found that, among 39 deletions at 20 sites, 7 consisted of less than 100 bp. In the *ruvAB* strain, among 23 deletions at 23 sites, we observed no deletions less than 100 bp in size (Table 2). These results may suggest that the replication slip bulge is processed by two mechanisms; one is PolI Klenow-dependent processing which removes the short bulge formed during lagging strand synthesis, the other is RuvAB-dependent processing which removes the large bulge formed during leading strand synthesis.

Acknowledgment

This work was supported by Grants-in-Aid for Scientific Research from the Ministry of Education, Culture, Sports, Science and Technology, Japan.

References

[1] S.C. Kowalczykowski, D.A. Dixon, A.K. Eggleston, S.D. Lauder, W.M. Rehrauer, Biochemistry of homologous recombination in *Escherichia coli*, Microbiol. Rev. 58 (1994) 401–465.

[2] H. Shinagawa, H. Iwasaki, Processing the Holliday junction in homologous recombination, Trends Biochem. Sci. 21 (1996) 107–111.

[3] S.C. West, Processing of recombination intermediates by the RuvABC proteins, Annu. Rev. Genet. 31 (1997) 213–244.

[4] H. Iwasaki, M. Takahagi, A. Nakata, H. Shinagawa, *Escherichia coli* RuvA and RuvB proteins specifically interact with Holliday junctions and promote branch migration, Genes Dev. 6 (1992) 2214–2220.

[5] I.R. Tsaneva, B. Muller, S.C West, ATP-dependent branch migration of Holliday junctions promoted by the RuvA and RuvB proteins of *E. coli*, Cell 69 (1992) 1171–1180.

[6] C.A. Parsons, I. Tsaneva, R.G. Lloyd, S.C. West, Interaction of *Escherichia coli* RuvA and RuvB proteins with synthetic Holliday junctions, Proc. Natl. Acad. Sci. USA 89 (1992) 5452–5456.

[7] H. Iwasaki, Y.W. Han, T. Okamoto, T. Ohnishi, M. Yoshikawa, K. Yamada, H. Toh, H. Daiyasu, T. Ogura, H. Shinagawa, Mutational analysis of the functional motifs of RuvB, an AAA+ class helicase and motor protein for Holliday junction branch migration, Mol. Microbiol. 36 (2000) 528–538.

[8] K. Yamada, N. Kunishima, K. Mayanagi, T. Ohnishi, T. Nishino, H. Iwasaki, H. Shinagawa, K. Morikawa, Crystal structure of the Holliday junction migration motor protein RuvB from *Thermus thermophilus* HB8, Proc. Natl. Acad. Sci. USA 98 (2001) 1442–1447.

[9] H.J. Dunderdale, F.E. Benson, C.A. Parsons, G.J. Sharples, R.G. Lloyd, S.C. West, Formation and resolution of recombination intermediates by *E. coli* RecA and RuvC proteins, Nature 354 (1991) 506–510.

[10] H. Iwasaki, M. Takahagi, T. Shiba, A. Nakata, H. Shinagawa, *Escherichia coli* RuvC protein is an endonuclease that resolves the Holliday structure, EMBO J. 10 (1991) 4381–4389.

[11] R.J. Bennett, H.J. Dunderdalem, S.C. West, Resolution of Holliday junctions by RuvC resolvase: cleavage specificity and DNA distortion, Cell 74 (1993) 1021–1031.

[12] M. Ariyoshi, D.G. Vassylyev, H. Iwasaki, H. Nakamura, H. Shinagawa, K. Morikawa, Atomic structure of the RuvC resolvase: a Holliday junction-specific endonuclease from *E. coli*, Cell 78 (1994) 1063–1072.

[13] N. Otsuji, H. Iyehara, Y. Hideshima, Isolation and characterization of an *Escherichia coli ruv* mutant which forms nonseptate filaments after low doses of ultraviolet light irradiation, J. Bacteriol. 117 (1974) 337–344.

[14] R.G. Lloyd, Conjugational recombination in resolvase-deficient *ruvC* mutants of *Escherichia coli* K-12 depends on *recG*, J. Bacteriol. 173 (1991) 5414–5418.

[15] R.G. Lloyd, G.J. Sharples, Dissociation of synthetic Holliday junctions by *E. coli* RecG protein, EMBO J. 12 (1993) 17–22.

[16] M. Seigneur, V. Bidnenko, S.D. Ehrlich, B. Michel, RuvAB acts at arrested replication forks, Cell 95 (1998) 419–430.

[17] B. Michel, Replication fork arrest and DNA recombination, Trends Biochem. Sci. 2 (2000) 173–178.

[18] B. Michel, M.J. Flores, E. Viguera, G. Grompone, M. Seigneur, V. Bidnenko, Rescue of arrested replication forks by homologous recombination, Proc. Natl. Acad. Sci. USA 98 (2001) 8181–8188.

[19] G. Grompone, M. Seigneur, S.D. Ehrlich, B. Michel, Replication fork reversal in DNA polymerase III mutants of *Escherichia coli*: a role for the beta clamp, Mol. Microbiol. 44 (2002) 1331–1339.

[20] N.D. Allgood, T.J. Silhavy, *Escherichia coli xonA* (*sbcB*) mutants enhance illegitimate recombination, Genetics 127 (1991) 671–680.

[21] H. Yamaguchi, K. Hanada, Y. Asami, J.I. Kato, H. Ikeda, Control of genetic stability in *Escherichia coli*: the SbcB 3′-5′ exonuclease suppresses illegitimate recombination promoted by the RecE 5′–3′ exonuclease, Genes Cells 5 (2000) 101–109.

[22] A.M. Albertini, M. Hofer, M.P. Calos, J.H. Miller, On the formation of spontaneous deletions: the importance of short sequence homologies in the generation of large deletions, Cell 29 (1982) 319–328.

[23] T. Ukita, H. Ikeda, Role of the *recJ* gene product in UV-induced illegitimate recombination at the hot spot, J. Bacteriol. 178 (1996) 2362–2367.

[24] K. Hanada, T. Ukita, Y. Kohno, K. Saito, J. Kato, H. Ikeda, RecQ DNA helicase is a suppressor of illegitimate recombination in *Escherichia coli*, Proc. Natl. Acad. Sci. USA 94 (1997) 3860–3865.

[25] K. Kitamura, Y. Torii, C. Matsuoka, K. Yamamoto, DNA sequence changes in mutations in the *tonB* gene on the chromosome of *Escherichia coli* K12: insertion elements dominate the spontaneous spectra, Jpn. J. Genet. 70 (1995) 35–46.

[26] N. Uematsu, C. Matsuoka, Y. Agemizu, E. Nagoshi, K. Yamamoto, Asymmetric crossing over in the spontaneous formation of large deletions in the *tonB-trp* region of the *Escherichia coli* K-12 chromosome, Mol. Gen. Genet. 261 (1999) 523–529.

[27] Y. Nagata, K. Mashimo, M. Kawata, K. Yamamoto, The Roles of Klenow processing and flap processing activities of DNA polymerase I in chromosome instability in *Escherichia coli* K12 strains, Genetics 160 (2002) 13–23.

[28] N.C. Franklin, Extraordinary recombinational events in *Escherichia coli*. Their independence of the rec^+ function, Genetics 55 (1967) 699–707.

[29] Y. Ishii, S. Kondo, Spontaneous and radiation-induced deletion mutations in *Escherichia coli* strains with different DNA repair capacities, Mutat. Res. 16 (1972) 13–25.

[30] K. Mashimo, M. Kawata, K. Yamamoto, Roles of the RecJ and RecQ proteins in spontaneous formation of deletion mutations in the *Escherichia coli* K12 endogenous *tonB* gene, Mutagenesis 18 (2003) 355–363.

[31] P. Howard-Flanders, R.P. Boyce, L. Theriot, Three loci in *Escherichia coli* K-12 that control the excision of pyrimidine dimers and certain other mutagen products from DNA, Genetics 53 (1966) 1119–1136.

[32] K. Ishioka, A. Fukuoh, H. Iwasaki, A. Nakata, H. Shinagawa, Abortive recombination in *Escherichia coli ruv* mutants blocks chromosome partitioning, Genes Cells 3 (1989) 209–220.

[33] T. Watanabe, T. Nunoshiba, M. Kawata, K. Yamamoto, An in vivo approach to identifying sequence context of 8-oxoguanine mutagenesis, Biochem. Biophys. Res. Commun. 284 (2001) 179–184.

[34] E. Yamamura, T. Nunoshiba, T. Nohmi, K. Yamamoto, Hydrogen peroxide-induced microsatellite instability in the *Escherichia coli* K-12 endogenous *tonB* gene, Biochem. Biophys. Res. Commun. 306 (2003) 570–576.

[35] D.E. Lea, C.A. Coulson, The distribution of the numbers of mutants in bacterial populations, J. Genet. 49 (1949) 264–285.

[36] S.E. Luria, M. Delbrück, Mutations of bacteria from virus sensitivity to virus resistance, Genetics 28 (1943) 491–511.

[37] P. McGlynn, R.G. Lloyd, Modulation of RNA polymerase by (p)ppGpp reveals a RecG-dependent mechanism for replication fork progression, Cell 101 (2000) 35–45.

[38] P. McGlynn, R.G. Lloyd, Rescue of stalled replication forks by RecG: simultaneous translocation on the leading and lagging strand templates supports an active DNA unwinding model of fork reversal and Holliday junction formation, Proc. Natl. Acad. Sci. USA 98 (2001) 8227–8234.

[39] Y. Agemizu, N. Uematsu, K. Yamamoto, DNA sequence analysis of spontaneous *tonB* deletion mutations in a *polA1* strain of *Escherichia coli* K12, Biochem. Biophys. Res. Commun. 261 (1999) 584–589.