Deletion Formation Between the Two *Salmonella typhimurium* Flagellin Genes Encoded on the Mini F Plasmid: *Escherichia coli ssb* Alleles Enhance Deletion Rates and Change Hot-Spot Preference for Deletion Endpoints

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ABSTRACT

Deletion formation between the 5′-mostly homologous sequences and between the 3′-homeologous sequences of the two *Salmonella typhimurium* flagellin genes was examined using plasmid-based deletion-detection systems in various *Escherichia coli* genetic backgrounds. Deletions in plasmid pLC103 occur between the 5′ sequences, but not between the 3′ sequences, in both RecA-independent and RecA-dependent ways. Because the former is predominant, deletion formation in a *recA* background depends on the length of homologous sequences between the two genes. Deletion rates were enhanced 30- to 50-fold by the mismatch repair defects, *mutS, mutL* and *uvrD,* and 250-fold by the *ssb-3* allele, but the effect of the mismatch defects was canceled by the Δ*recA* allele. Rates of the deletion between the 3′ sequences in plasmid pLC107 were enhanced 17- to 130-fold by *ssb* alleles, but not by other alleles. For deletions in pLC107, 96% of the endpoints in the *recA*$^+$ background and 88% in Δ*recA* were in the two hot spots of the 60- and 33-nucleotide (nt) homologous sequences, whereas in the *ssb-3* background >50% of the endpoints were in four- to 14-nt direct repeats dispersed in the entire 3′ sequences. The deletion formation between the homeologous sequences is RecA-independent but depends on the length of consecutive homologies. The mutant *ssb* allele lowers this dependency and results in the increase in deletion rates. Roles of mutant SSB are discussed with relation to misalignment in replication slippage.

DELETION is one of major DNA rearrangements in genomes of both prokaryotes and eukaryotes and plays important roles in gene differentiation, gene fusion and genome evolution. In bacteria, sequence analysis of deleted regions has revealed some important factors for deletion formation. Most deletions occur between two tandem repeated sequences even if the repeats are only a few nucleotides long (ALBERTINI *et al.* 1982). Repeats with perfect homology cause deletion more efficiently than those with partial homology (ALBERTINI *et al.* 1982; WHORISKEY *et al.* 1991), and deletion rates increase in proportion to the length of repeated sequences unless it is beyond 100 bp (BI and LIU 1994). Palindromic or quasi-palindromic sequences have been detected in the deleted sequences between direct repeats (ALBERTINI *et al.* 1982; GLICKMAN and RIPLEY 1984), and palindromic inserts enhance rates of deletion between flanking sequences more efficiently than do nonpalindromic inserts (DASGUPTA *et al.* 1987; TRINH and SINDEN 1991). The size of inserts also affects deletion rates; as the distance between direct repeats increases, deletion rates exponentially decrease (BI and LIU 1994; CHÉDIN *et al.* 1994; LOVETT *et al.* 1994). These results indicate that three structural factors direct deletion formation: the length of direct repeats, the distance between them, and stable secondary structure between them. On the other hand, *Escherichia coli* genes, whose mutant alleles enhance deletion rates between short direct repeats, have been reported. These are the *bglY* gene encoding histone-like protein H-NS (HULTON *et al.* 1990), the *topB* (*mutR*) gene encoding topoisomerase III (DIGATE and MARIANS 1989), and the *sbcB* gene encoding exonuclease I (KUSHNER *et al.* 1971). The products of the first two genes seem to function in maintaining a certain DNA structure, but null mutations of these genes are required for enhancement of deletion rates (LEJUNE and DANCHIN 1990; YI *et al.* 1988; WHORISKEY *et al.* 1991; SCHOFIELD *et al.* 1992). Some mutant alleles of the *sbcB* gene are *xonA* (ALLGOOD and SILHAVY 1991). The mechanism for enhancement by these mutant alleles of deletion formation has not yet been clarified.

To obtain new insight into the mechanism for deletion formation, we have constructed deletion-detection systems using a mini-F plasmid and two flagellin genes. *Salmonella typhimurium* has two flagellin genes, *fliC* and *fljB* (IINO 1977; SANDERSON and HURLEY 1987), which encode quite different antigenic determinants, but share nucleotide sequences in other regions. Thus it is inferred that the *fljB* gene is probably derived by duplication from the *fliC* gene, which exists in most enteric bacteria. Flagellin genes consist of the 5′-constant, central variable and 3′-constant regions (WEI and JOYS 1985; OKAZAKI *et al.* 1993). The central variable region contains the above antigenic determinant (JOYS

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1985) and is permissive to various kinds of mutations including short deletions (NEWTON *et al.* 1991; T. MUKAIHARA, unpublished data) so long as the correct reading frame is retained. In contrast, both constant regions encode flagellin terminals essential for secretion and assembly (HOMMA *et al.* 1987) and are highly conserved. As one of genetic events between the two flagellin genes, gene conversion of the *fliC* (antigenic determinant i) gene into *fliC* (e,n,x) has been reported (OKAZAKI *et al.* 1993), where the central variable region of *fliC*(i) is nonreciprocally replaced with the corresponding region of the *fljB* gene (antigenic determinant e,n,x). The conversion endpoints between the two genes are found to lie in the 5′- and 3′-constant regions, each sharing high homology between the two genes. However, detailed sequence comparison between the two flagellin genes, *fliC*(i) and *fljB*(1,2), that were used in this experiment reveals that the two 3′-constant sequences (~570 bp), although they share 79.6% homology, contain ~100 mismatches in the maximum homology alignment; the maximum length with consecutive homology is 80 bp. On the other hand, the 5′-constant sequences (513 bp), with 97.9% homology, contain only nine mismatches and the maximum homologous sequence is 284 bp. This indicates that the 5′-constant sequences are mostly homologous and the 3′-constant sequences are homeologous.

In this paper we report deletion formation between the 5′-mostly homologous sequences and between the 3′-homeologous sequences of the two flagellin genes. The results show that deletion formation between the 5′ sequences is mostly RecA-independent and that between the 3′ sequences is thoroughly RecA-independent, but in either case deletion formation depends on the length of consecutive homologies. Defects in the mismatch repair and *mutD* (*dnaQ*) genes enhance deletion formation between the 5′ sequences but not between the 3′ sequences. Mutant *ssb* alleles remarkably enhance deletion rates in both cases and two alleles also alter hot-spot preference for endpoints of deletions between the 3′-homeologous sequences. We discuss mechanisms for the enhancement of deletion rates with relation to the involvement of mutant SSB.

MATERIALS AND METHODS

Strains, phage and plasmids: *E. coli* strains used are listed in Table 1. Strains EJ2081 and MV1184 were used as hosts for various intermediates during the construction of deletion-detection plasmids. Strain EJ2848, the parent into which each of various mutations was transduced, is a derivative of W3110 (LEDERBERG and LEDERBERG 1953) and was constructed as follows. *zai*::Tn*10* of strain ME8282 was transduced into strain ME6060 to yield a strain (EJ2845) with *lacI3* Δ*lacZ lacY*$^+$ *zai*::Tn*10*, which was transduced into W3110 by selecting for Tet^r. A Tet^s derivative was positively selected from the transductant (MALOY and NUNN 1981). The Tet^s clone isolated was transduced with *fliC*::Tn*10* from strain EJ1556 and a Tet^s derivative of the transductant was isolated as above, yielding strain EJ2848. EJ2848 derivatives were constructed by transduction with a mutant allele linked to a Tn element; mutant transductants were identified by elevated UV-sensitivity in cases of *ssb* and *recA* alleles and by an increase in spontaneous Rif^r mutations for *mut* alleles. *S. typhimurium* strain SJ2353 [*ah1-fliC*(i) *fljB*(1,2)], expressing only the phase 2 flagellar antigen, was used as a DNA donor for cloning the *fljB* gene. P1*vir* was used for transduction (ENOMOTO and STOCKER 1974). Plasmids pTN1105 (NOHNO *et al.* 1986), pUC119 (VIEIRA and MESSING 1987), pHSG398 and pHSG399 (TAKESHITA *et al.* 1987) were used for plasmid construction. pMC1871 (SHAPIRA *et al.* 1983) and pUC-4K (VIEIRA and MESSING 1982) were donors of the *lacZ* and *neo* genes, respectively. pBR322 (BOLIVAR *et al.* 1977) was the cloning vector for the *fljB* gene.

Media: Luria-Bertani (LB) medium (MILLER 1992) was used for cultivation of all strains. Nutrient semisolid medium for the motility test was as previously described (ENOMOTO *et al.* 1983). H medium and minimal A medium (MinA) were as described (MILLER 1992). XPG medium (MILLER 1992) was solid (1.5% agar) MinA medium containing glucose (2 g/liter), phenyl-β-D-galactopyranoside (P-Gal) (0.5 g/liter) and 5-bromo-4-chloro-3-indolyl-β-D-galactopyranoside (X-Gal) (40 mg/liter). Lactose minimal medium contained lactose (2 g/liter) and X-Gal (40 mg/liter) in MinA buffer. Tetracycline (Tet) (Sigma Chemical Co.), chloramphenicol (Cm) (Sigma) and kanamycin (Km) (Meiji Seika Co.) were used at final concentrations of 20 mg/liter, 12.5 mg/liter and 10 mg/liter, respectively.

DNA manipulation: Preparation of genomic DNA and the cloning procedure were described previously (BIRNBOIM and DOLY 1979; TOMINAGA *et al.* 1991). Isolation of plasmid DNA, restriction analysis of cloned fragments and other recombinant DNA techniques followed standard methods (SAMBROOK *et al.* 1989). Restriction endonucleases, T4 DNA ligase and alkaline phosphatase were purchased from Takara Shuzo Co.

Cloning of the *fljB* gene and plasmid construction: The *fljB* gene has previously been cloned in this laboratory. Genomic DNA of *S. typhimurium* strain SJ2353 was digested with *Sal*I, ligated into pBR322, and transformed into *E. coli* Δ*fliC* strain EJ2081. Transformants streaked on semisolid medium were selected for motility. Plasmid pTH101, isolated from one of the motile transformants, carried the entire *fljBA* operon as in plasmid pTY102 with the *fljBA* operon from a different serotype (HANAFUSA *et al.* 1993). The nucleotide sequence of the *fljB* open reading frame has been reported (VANEGAS and JOYS 1995). The 3.7-kb *Sal*I-*Eco*RI fragment of pTH101 was subcloned to the pUC119 polylinker to yield pCB105B (Figure 1A). A 1.7-kb *Stu*I-*Nsp*I fragment of pCB105B was subcloned to pUC119 to yield pBZ110 (Figure 1A), which carries the *fljB* gene truncated 87 bp from its 3′ end. This fragment, named *fljB* Δ87, can be excised as the *Xho*I-*Sal*I cassette. Plasmid pHI301 (Figure 1B), which was made by recloning the *Hind*III-fragment of pHI101 (OKAZAKI *et al.* 1993) into pHSG399, carries the 4.5-kb *Hin*dIII fragment with the *S. typhimurium fliC* and *fliD* genes. A 1.4-kb *Hin*cII-*Nsp*I fragment with most of the *fliC* gene was subcloned from pHI301 into the pHSG397 polylinker to yield pCZ105 (= pCZ106 without *lacZ*), in which the promoter sequences, 38 bp of the 5′ end, and 87 bp of the 3′ end of the *fliC* gene, are truncated. Then, the 3.1-kb *Bam*HI fragment with the 5′-truncated *lacZ* gene of plasmid pMC1871 was inserted in-frame into the *Bam*HI site downstream of the truncated *fliC* gene to yield pCZ106 with the *fliC*′-′*lacZ* fusion (Figure 1B). The fusion, Δ38 *fliC*′-′*lacZ*, can be excised as the 4.5-kb *Sal*I-*Xho*I cassette. Plasmid pCZ107 whose *fliC* gene was truncated for the entire 5′-constant sequence (603 bp) was made from pCZ106; the 3.9-kb *Sca*I-*Kpn*I fragment of pCZ106, in which the *Sca*I site is unique

TABLE 1

***E. coli* strains used**

Strain	Relevant genotype	Reference, source, or construction
CS72	*ssb-1 zjc*::Tn*10*	SCHMELLIK-SANDAGE and TESSMAN (1990)
CS196	*ssb-3 zjc*::Tn*10*	SCHMELLIK-SANDAGE and TESSMAN (1990)
CSH116	*mutD5 zae*::Tn*10*	MILLER (1992)
CSH126	Δ(*srl-recA*) *306 srl-301*::Tn*10-84*	MILLER (1992)
GW3733	*mutL218*::Tn*10*	PANG *et al.* (1985)
GW3773	*mutH471*::Tn*5*	G. WALKER
ME6060	*lacI3* Δ*lacZ lacY*$^+$	National Institute of Genetics, Japan
ME8282	*zai*::Tn*10*	National Institute of Genetics, Japan
ME8324	*ssb-113 zjb*::Tn*10*	National Institute of Genetics, Japan
ME8488	*uvrD260*::Tn*5*	National Institute of Genetics, Japan
MV1184	Δ(*lac-proAB*) Δ(*srl-recA*) *306*::Tn*10*/F′ *lacI*^{q} *lacZ*ΔM15	SAMBROOK *et al.* (1989)
RDK1695	*mutS201*::Tn*5*	LUISI-DELUCA *et al.* (1989)
W3110	Wild type	LEDERBERG and LEDERBERG (1953)
EJ1556	*fliC*::Tn*10*	This laboratory
EJ2081	Δ*fliC* Δ*pin recA56*	OKAZAKI *et al.* (1993)
EJ2848	*lacI3* Δ*lacZ lacY*$^+$ Δ*fliC*	This work
EJ2849	EJ2848 Δ(*srl-recA*) *306 srl-301*::Tn*10-84*	P1(CSH126) × EJ2848 → Tet^r
EJ2851	EJ2848 *mutS201*::Tn*5*	P1(RDK1695) × EJ2848 → Km^r
EJ2852	EJ2848 *uvrD260*::Tn*5*	P1 (ME8488) × EJ2848 → Km^r
EJ2855	EJ2848 *mutS201*::Tn*5* Δ(*recA-srl*) *306 srl-301*::Tn*10-84*	P1 (CSH126) × EJ2851 → Tet^r
EJ2856	EJ2848 *uvrD260*::Tn*5* Δ(*recA-srl*) *306 srl-301*::Tn*10-84*	P1(CSH126) × EJ2852 → Tet^r
EJ2879	EJ2848 *ssb-113 zjb*::Tn*10*	P1 (ME8324) × EJ2848 → Tet^r
EJ2881	EJ2848 *ssb-1 zjc*::Tn*10*	P1 (CS72) × EJ2848 → Tet^r
EJ2882	EJ2848 *ssb-3 zjc*::Tn*10*	P1 (CS196) × EJ2848 → Tet^r
EJ2886	EJ2848 *ssb-113*	EJ2879 Tet^s
EJ2896	EJ2848 *mutD5 zae*::Tn*10*	P1(CSH116) × EJ2848 → Tet^r
EJ2901	EJ2848 *ssb-113* Δ (*recA-srl*) *306 srl-301*::Tn*10-84*	P1 (CSH126) × EJ2886 → Tet^r
EJ2917	EJ2848 *mutL218*::Tn*10*	P1(GW3733) × EJ2848 → Tet^r
EJ2918	EJ2848 *mutH471*::Tn*5*	P1 (GW3773) × EJ2848 → Km^r

to *fliC* and the *Kpn*I site lies downstream of *lacZ*, was inserted into the pUC119 polylinker. The fusion Δ603 *fliC′-′lacZ* can also be excised as the *Sal*I-*Xho*I cassette. Mini F plasmid pTN1102, which contains *ori2* and the *repE* and *sopABC* genes, was constructed as the recipient for the above cassettes by deleting the 2.7 kb-*Xho*I fragment from pTN1105 (Figure 2A). The two cassettes, *fljB* Δ87 and Δ38 *fliC′-′lacZ*, were inserted one by one into the *Sal*I site of pTN1102 in the same transcriptional direction. The 1.2-kb *Sal*I fragment with the *neo* gene from plasmid pUC-4K was inserted between the two cassettes in the transcriptional direction opposite to that of the genes in the cassettes, yielding plasmid pLC103 (Figure 2B). Likewise, the cassette Δ603 *fliC′-′lacZ* was used in place of the cassette Δ38 *fliC′-′lacZ* to yield plasmid pLC107 (Figure 2C).

Isolation of Lac$^+$ revertants: The standard method (MILLER 1992) was used with partial modifications. Strain EJ2848 and its derivatives, transformed with pLC103 or pLC107, were grown overnight at 37° in LB broth containing Cm. Cells, collected and washed with MinA buffer, were plated after appropriate dilution on lactose minimal plates. To titer the viable cells, aliquots were plated on LB plates. Minimal plates were incubated at 37° for 4 days, and the number of Lac$^+$ colonies were counted. For the papillation assay, appropriate dilutions were spread on XPG plates to yield 200–400 colonies per plate, incubated at 37° for 5 days, and papillae formation was examined. Papillae, each isolated from a single colony, were streaked on lactose minimal plates or H plates with X-Gal to isolate single colonies.

Sequencing analysis of deletion endpoints: The 0.9-kb *Hin*dIII-*Bam*HI fragment (Figure 4A) of each deletion plasmid from pLC107 was subcloned into pHSG398, and sequencing reactions were performed using this plasmid and Dye Terminator Cycle Sequencing (Applied Biosystems). Products were analyzed by the model 373A automatic DNA sequencer (Applied Biosystems).

The nucleotide sequence of the *fliC* gene used here is registered under the accession number D13689.

RESULTS

Detection systems for deletions between the two flagellin genes: To examine the mechanism for deletion formation between the 5′-mostly homologous sequences and between the 3′-homeologous sequences of the two tandem oriented flagellin genes *fliC*(i) and *fljB*(1,2), we constructed two plasmids pLC103 and pLC107, which carry the *fljB*, *neo* (Kan^r) and *fliC′-′lacZ* genes on a mini-F plasmid pTN1102 (Figure 2A). In pLC103 (Figure 2B), the 3′ end of the *fljB* gene is truncated, both the 5′ and 3′ ends of the *fliC* gene are truncated, and the *lacZ* gene is fused in frame to the 3′ end of the *fliC* gene. The fusion gene is not expressed because it lacks a promoter, but translation of read-through transcripts from p_{fljB} promoter might occur. To prevent this, the *neo* gene is inserted between the two flagellin genes in the direction opposite to the tran-

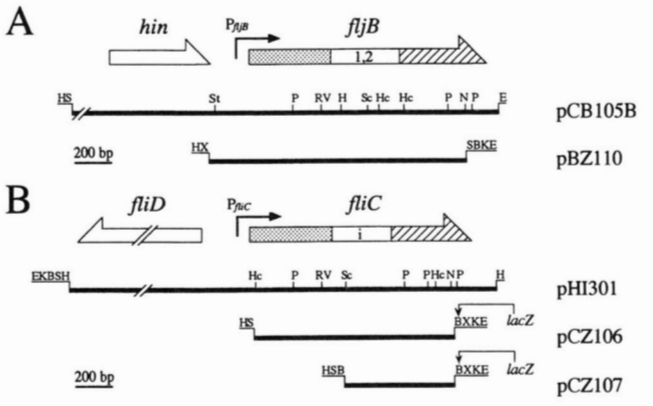

FIGURE 1.—Cloned fragments with the *S. typhimurium* flagellin genes and subclones used as cassettes for construction of deletion-detection plasmids. (A) pCB105B carries the 3.7-kb *Sal*I-*Eco*RI fragment, genes on which are diagrammatically shown on the top. pBZ110 was used as *fljB* Δ87 cassette. (B) pHI301 carries the 4.5-kb *Hin*dIII fragment with the *fliC* and *fliD* genes (top). pCZ106 and pCZ107 have the *lacZ* gene fused in frame to their 5′ ends and were used as Δ38 *fliC′-′lacZ* and Δ603 *fliC′-′lacZ* cassettes, respectively. A hooked arrow on the top of each figure shows the promoter and direction of transcription of each gene. Stippled, white, and striped segments of the flagellin genes depict the 5′-constant, central variable and 3′-constant regions, respectively. Thick lines indicate cloned fragments and flanking thin lines show the polylinker sequences of parental plasmids. Abbreviation for restriction sites: B, *Bam*HI; E, *Eco*RI; H, *Hin*dIII; K, *Kpn*I; N, *Nsp*I; P, *Pst*I; RV, *Eco*RV; S, *Sal*I; Sc, *Sca*I; X, *Xho*I.

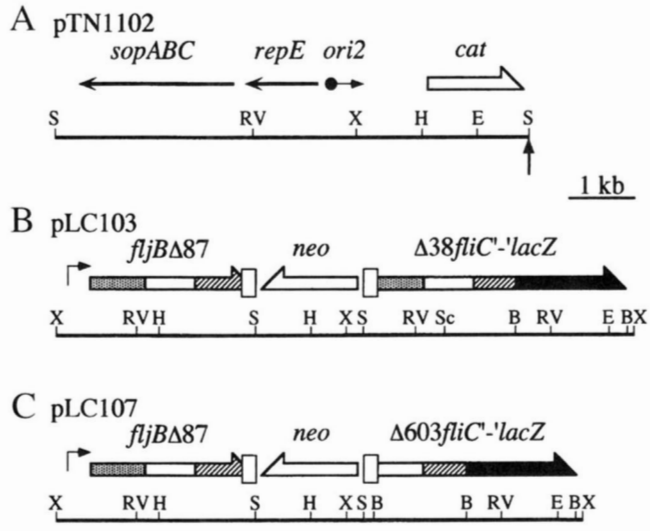

FIGURE 2.—Diagrams of vector plasmid pTN1102 and the two deletion-detection plasmids derived from the vector. (A) Plasmid pTN1102. Replication starts unidirectionarily from *ori2*. The deletion units (B and C) were inserted into the *Sal*I site shown by the vertical arrow. (B) Plasmid pCL103. Two cassettes *fljB* Δ87 and Δ38 *fliC′-′lacZ*, excised respectively from plasmids pBZ110 and pCZ106 (Figure 1), were inserted one by one into the *Sal*I site of pTN1102 and the *neo* cassette was then inserted into the *Sal*I site between the above two cassettes (see MATERIALS AND METHODS for detail). (C) Plasmid pCL107. Construction procedure was the same as pLC103, but cassette Δ603 *fliC′-′lacZ* was used in place of Δ38 *fliC′-′lacZ*. Solid thick arrows show the *lacZ* gene and other patterns in the flagellin genes are the same as in Figure 1. Open rectangles at the 5′ or the 3′ end of flagellin genes depict the truncation. Abbreviation for restriction sites are the same as in Figure 1.

scriptional direction of the fusion gene. Transcripts from the *neo* gene in this orientation contain 10 stop codons (OKA *et al.* 1981). Plasmid pLC107 (Figure 2C) has the same construction as pLC103 but the large truncation of the entire 5′ sequence of the *fliC* gene. Because of these truncations, the number of mismatches between the 5′ sequences of the two flagellin genes in pLC103 decreases from nine to four, and the longest homologous sequence between the two 3′ sequences decreases from 80 to 60 nucleotides. pLC103 and pLC107 were designed to detect deletions mainly between the 5′ sequences and only between the 3′ sequences, respectively. When an in-frame deletion occurs between the two flagellin genes, the fusion gene is transcribed from p_{fljB} promoter and simultaneously the *neo* gene is deleted.

Isolation and characterization of deletions: Strain EJ2848 (Δ*lacZ recA*$^+$), transformed with pLC103, was incubated on XPG plates with Cm at 37°. After 4–5 days, a few Lac$^+$ papillae appeared in most colonies. Thirty-six papillae, each from a single colony, were restreaked onto X-gal plates, and a blue single colony from each clone was examined. Three colonies (8%) were resistant to Km and the rest were sensitive. Electrophoresis showed that plasmids from the three Km^r colonies are larger, and those from the rest are smaller, than the parental plasmid (Figure 3B). Restriction analysis of these plasmids showed that one of the two *Eco*RI fragments of pLC103, which is 8.1 kb long and contains the *fljB*, *neo* and *fliC′-′lacZ* genes, is only 5.5 kb in all the 33 small plasmids examined. In addition, the two *Hin*dIII sites, each unique to the central region of the *fljB* gene and to the *neo* gene, disappear, resulting in one large *Hin*dIII fragment (Figure 3). These results indicate that in the small plasmids deletions occur between the 5′ sequences of the two flagellin genes. The three large plasmids all contained three *Eco*RI fragments of 8.1, 6.4, and 5.5 kb, and the 6.4-kb band was thick. Hence the large plasmid was probably a heterodimer of the parental plasmid (8.1 + 6.4) and the small plasmid (5.5 + 6.4) described above. As expected, pLC103 in the *recA*$^+$ background was deleted preferentially between the two 5′ sequences with longer homologous sequences rather than those in the 3′-sequences.

Deletions between the two 3′ sequences in pLC107 were examined in strain EJ2848. As with pLC103, three (8%) of 36 blue colonies isolated from Lac$^+$ papillae were Km^r and the rest were Km^s. The plasmids from the Km^r colonies were larger, and those from the rest were smaller, than the parent. Restriction analysis of the 33 small plasmids showed that the parental 7.5-kb *Eco*RI fragment is only 5.5 kb, the smallest *Hin*dIII fragment (1.4 kb) disappears, and the largest 10.1-kb *Hin*dIII fragment changes to 9.5 kb (Figure 4). These

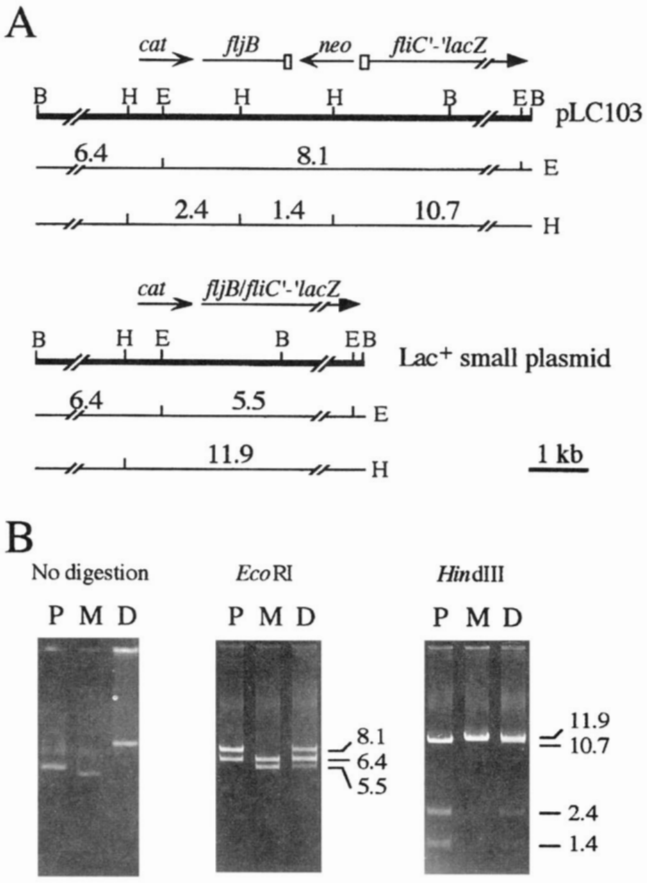

FIGURE 3.—Restriction analysis of Lac$^+$ plasmids from pLC103 in the *recA*$^+$ background. (A) Restriction maps of pLC103 and the Lac$^+$ small plasmid. Genes carried by each plasmid are shown above the restriction map and a rectangle at the end of each flagellin gene depicts the truncation. The size of restriction fragments is shown under the map. (B) Electrophoretic analysis of the parental (P), monomer (M, small Lac$^+$) and dimer (D, large Lac$^+$) plasmids. The restriction enzyme used for digestion is shown on the top of each panel and the size of fragments is shown on the right of each panel. Abbreviation for restriction sites are the same as in Figure 1.

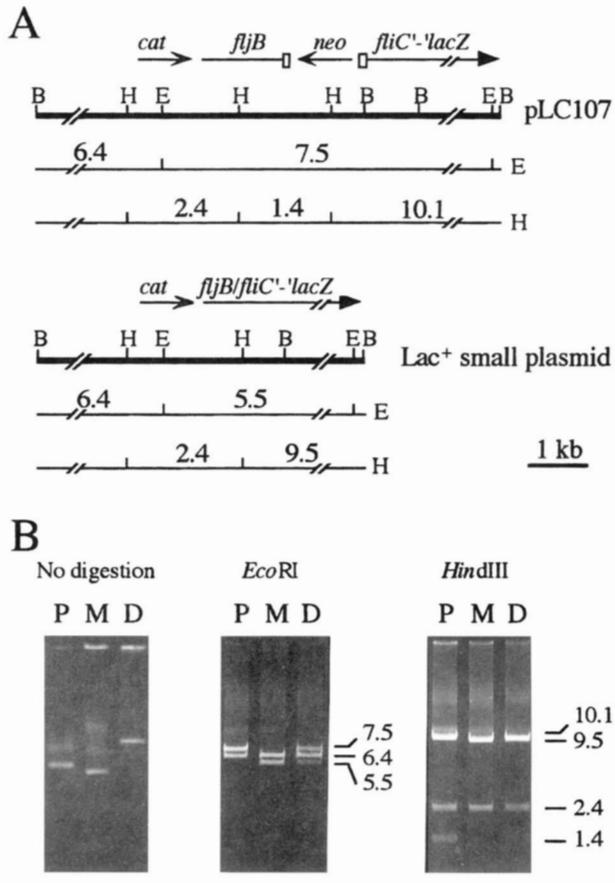

FIGURE 4.—Restriction analysis of Lac$^+$ plasmids from pLC107 in the *recA*$^+$ background. (A) Restriction maps of pLC107 and the Lac$^+$ small plasmid. Genes carried by each plasmid are shown above the restriction map and the size of restriction fragments is shown under the map. (B) Electrophoretic analysis of the parental (P), monomer (M, small Lac$^+$) and dimer (D, large Lac$^+$) plasmids. Explanation is the same as in Figure 3.

results indicate that the small plasmids lack the *Hin*dIII site unique to the *neo* gene and hence deletion endpoints lie in the 3′ sequences of the two flagellin genes as expected. The large plasmids contained the three *Eco*RI fragments as in the large plasmids from pLC103, indicating that these large plasmids also are heterodimers of the parent and deletion plasmids. The occurrence of heterodimers is commonly seen in other plasmid-based deletion-detection systems even in *recA* backgrounds (DIANOV *et al.* 1991; MAZIN *et al.* 1991; LOVETT *et al.* 1993; BI *et al.* 1995).

Host factors affecting deletion rates: To examine host factors that affect deletion rates, Lac$^+$ papillation with pLC103 was examined in various genetic backgrounds. These were defects that affect DNA repair (*lexA3, mutD5, mutS, mutL, mutH, mutT, mutY, uvrC, uvrD*), recombination (*recA, recBC, recE, recN, recO*) and replication (*ssb-113*). Increased papillation occurred in the six strains, *mutS, mutL, mutH, uvrD, mutD5* and *ssb-113*, whereas the other strains showed the same papillation level as the wild-type strain (data not shown). Moreover, colonies of the *mutS, mutL, uvrD* and *ssb-113* strains produced blue (Lac$^+$) sectors probably due to deletions occurring at an early stage of colony growth. Such sectors were not observed in the wild-type, *mutH* and *mutD5* strains (data not shown). To quantitate deletion rates, Lac$^+$ reversion rates in the above six strains showing elevated papillation, as well as the *recA*$^+$ and Δ*recA* strains, were measured on lactose minimal plates (Table 2). In the *recA*$^+$ and Δ*recA* strains, the rates of Lac$^+$ reversion were not significantly different ($2–3 \times 10^{-6}$/cell), indicating that most of the deletion formation between the two 5′ sequences is RecA-independent. The rates in the *mutH* and *mutD5* strains slightly increased, while in the *mutS, mutL, uvrD* and *ssb-113* strains the rates dramatically increased; among them the *ssb-113* strain showed the highest rate (133-fold).

The Lac$^+$ reversion rate with pLC107 was also measured in the various genetic backgrounds as above (Ta-

TABLE 2

Deletion rates in plasmids pLC103 and pLC107 in various genetic backgrounds

Strain	Mutation	pLC103		pLC107	
		No. of Lac$^+$ revertants	Ratio	No. of Lac$^+$ revertants	Ratio
EJ2848	*recA*$^+$	2.7 ± 1.2	1.0	0.8 ± 0.4	1.0
EJ2849	Δ*recA306*	2.2 ± 0.9	0.8	0.5 ± 0.2	0.6
EJ2851	*mutS201*::Tn*5*	86.3 ± 10.4	32.0	0.7 ± 0.2	0.9
EJ2917	*mutL218*::Tn*10*	92.5 ± 63.5	34.3	0.8 ± 0.3	1.0
EJ2918	*mutH471*::Tn*5*	10.7 ± 2.7	4.0	0.5 ± 0.1	0.6
EJ2852	*uvrD260*::Tn*5*	109.0 ± 34.0	40.4	0.9 ± 0.3	1.0
EJ2896	*mutD5*	9.8 ± 1.9	3.6	0.7 ± 0.2	0.9
EJ2879	*ssb-113*	360.9 ± 108.8	133.7	50.6 ± 5.2	63.3
EJ2881	*ssb-1*	125.8 ± 12.0	46.6	13.6 ± 3.9	17.0
EJ2882	*ssb-3*	670.4 ± 86.2	248.3	106.9 ± 28.3	133.6

Strain EJ2848 and its derivatives (Table 1), transformed with plasmid pLC103 or pLC107, were grown overnight at 37° in LB broth, and ~10^6 cells from each strain were plated on a lactose minimal plate after appropriate dilution. For each strain, three plates were used in each experiment and at least three experiments were carried out. The number of Lac$^+$ revertants per 10^6 cells and the standard deviation are shown. Mutants *ssb-1*, *ssb-3* and *ssb-113* are temperature-sensitive, but their plating efficiency was the same at 30° and 37°.

ble 2). The rates in the *recA*$^+$ and Δ*recA* strains decreased to 23–30% of the rates with pLC103, but the rates in both strains were not significantly different, suggesting that deletion formation in pLC107 also is RecA-independent. However, the major difference between the two detection systems was that deletion rates in pLC107 were greatly enhanced only by *ssb-113*. These results suggest that deletions enhanced by the *ssb* allele in both plasmids are generated by the same mechanism, and this mechanism is different from that of the deletions enhanced by the *mut* and *uvrD* alleles.

To determine whether the deletion plasmids produced in the *ssb* background were the same in structure as those produced in other backgrounds, Lac$^+$ plasmids isolated from the *ssb*, *mutS*, *uvrD* and Δ*recA* strains were analyzed by electrophoresis. Lac$^+$ plasmids derived from pLC103 and pLC107 in the *ssb-113* strain contained 17% and 8% heterodimers, respectively, which were not significantly different from the proportions obtained from the *recA*$^+$ strain as above. Structure analysis by restriction enzymes of the deletion plasmids from each parent showed that each of the monomer and dimer deletion plasmids isolated from the *ssb* strain has the same structure as that from the *recA*$^+$ strain (data not shown). Deletion plasmids isolated from each parent in the three other strains also showed the same structure as those from the *recA*$^+$ strain.

To test whether the enhanced deletion formation by *ssb-113* is allele-specific, Lac$^+$ reversion rates in two other *ssb* mutant strains were examined (Table 2). The *ssb-1* and *ssb-3* alleles enhanced deletion rates in both plasmids. The *ssb-3* allele was most effective and enhanced deletion rates 250-fold in pLC103 and 130-fold in pLC107 over wild-type levels.

Effect of the Δ*recA* allele on the enhanced deletion formation: Defects in the mismatch repair genes are known to promote RecA-mediated homologous recombination (ARTHUR and LLOYD 1980; FEINSTEIN and LOW 1986; RAYSSIGUIER *et al.* 1989) and so, deletions enhanced by these defects in pLC103 might be catalyzed by RecA protein. To examine this possibility, double mutants, *mutS* Δ*recA*, *uvrD* Δ*recA* and *ssb-113* Δ*recA*, were constructed, and Lac$^+$ reversion rates in pLC103 were examined (Table 3). The deletion rates in the *mutS* and *uvrD* backgrounds decreased to the wild-type level in the double mutants with Δ*recA*, whereas the deletion rate in the *ssb-113* strain was only slightly diminished by Δ*recA*. These results suggest that the *mutS* and *uvrD* defects enhance deletion rates through activation of the RecA pathway, but enhancement by *ssb-113* is RecA-independent.

Distribution of deletion endpoints: To investigate the mechanism for the enhanced deletion formation in the *ssb* strains, deletions of pLC107, isolated from each of the *recA*$^+$, Δ*recA* and *ssb-3* backgrounds, were examined in detail. As described above, the 3′ sequences of the two flagellin genes show a number of short homologous sequences in the maximum homology alignment. Such homologies also appear in alignments other than in the maximum homology. Suppos-

TABLE 3

Effect of the Δ*recA* allele on enhanced deletion rates in pLC103

Strain	Mutation	No. of Lac$^+$ revertants	Ratio
EJ2848	*recA*$^+$	2.7 ± 1.2	1.0
EJ2849	Δ*recA306*	2.2 ± 0.9	0.8
EJ2855	*mutS201*::Tn*5* Δ*recA306*	1.6 ± 0.5	0.6
EJ2856	*uvrD260*::Tn*5* Δ*recA306*	4.2 ± 1.6	1.6
EJ2901	*ssb-113* Δ*recA306*	261.4 ± 64.1	96.8

The number of Lac$^+$ revertants per 10^6 cells ± SD is shown. Experimental procedures were the same as shown in Table 2.

TABLE 4

Spectra of deletion endpoints in pLC107 in the three genetic backgrounds

Size of direct repeats (nt)	No. of isolates		
	recA$^+$	Δ*recA306*	*ssb-3*
60	18 (0.75)	12 (0.50)	7 (0.29)
33	5 (0.21)	9 (0.38)	4 (0.17)
14a			1 (0.04)
14b			1 (0.04)
12	1 (0.04)		6 (0.25)
11a		1 (0.04)	1 (0.04)
11b			1 (0.04)
11c		1 (0.04)	
8			1 (0.04)
6		1 (0.04)	1 (0.04)
4			1 (0.04)

Twenty-four deletions of pLC107, isolated from each genetic background, were examined by sequencing the 0.9-kb *Hin*dIII-*Bam*HI fragment (Figure 4A) from each plasmid. Direct repeats between the 3′ sequences of the two flagellin genes and their positions are shown in Figure 5. Percentages are shown in parentheses.

ing that deletion in pLC107 occurs by replication slippage, deletion events start with annealing between short homologous sequences (or direct repeats) somewhere in the two 3′ sequences. To ascertain whether the annealing occurs in the specific alignment between the two 3′ sequences or in different alignment in each event, the size of the fragments that must contain deletion junctions was examined for deletion plasmids from the three genetic backgrounds. When deletions occur in different alignment in each plasmid, various fragments with different sizes will be detected. Twenty-four deletion plasmids from each background were analyzed by electrophoresis after digestion with *Hin*dIII and *Bam*HI: the same 0.9-kb fragment as shown in Figure 4A was detected in the digests of all the plasmids tested (data not shown). This suggests that all the deletions result from the annealing between short homologous sequences recognized in the specific alignment like the maximum homology alignment, regardless of the genetic background. Next, to see whether the occurrence of these deletions is dependent on the length of consecutive homology, the *Hin*dIII-*Bam*HI fragment of 24 deletion plasmids from each background were sequenced, and all the deletion endpoints were determined (Table 4 and Figure 5). For deletions from the *recA*$^+$ background, most endpoints (96%) were detected in the two hot spots, the homologous sequences of the 60 and 33 nucleotides. These are the two sequences with the greatest extent of homology. This also was the case for deletions isolated from the Δ*recA* background (Table 4 and Figure 5). These results indicate that the deletion pathway in pLC107 is RecA-independent but depends on the length of consecutive homologies between the two 3′ sequences. On the other hand, endpoints of the deletions from the *ssb-3* background were widely dispersed in the entire 3′-sequences (Table 4 and Figure 5). Half (46%) of them were still located in the two hot spots, but another half were in 4- to 14-nt direct repeats. These results show that the *ssb-3* allele lowers the role of consecutive homology in deletion formation and, as a result, increases deletions between short direct repeats.

DISCUSSION

Deletions in plasmid pLC103 all occurred between the two 5′ sequences, which has longer homologous sequences than do the 3′ sequences, and deletion rates were not significantly different between the *recA*$^+$ and Δ*recA* backgrounds (Table 2). These results suggest that the deletion in pLC103 is generated by both RecA-independent and RecA-dependent mechanisms, but the former is more active and catalyzes deletion by recognizing the length of homologous sequences. However, deletion rates were notably enhanced by *mutS, mutL,* and *uvrD* defects (Table 2), which are known to promote the RecA pathway of recombination (ARTHUR and LLOYD 1980; FEINSTEIN and LOW 1986; RAYSSIGUIER *et al.* 1989). RecA was also involved in deletion in pLC103 because the double mutants, *mutS* Δ*recA* and *uvrD* Δ*recA*, restored deletion rates to the Δ*recA* or the wild-type level (Table 3). MutS and MutL proteins prevent strand exchange during synapsis catalyzed by RecA if mismatches exist between the two strands (WORTH *et al.* 1994). The two 5′-constant sequences (513 nucleotides each) in pLC103 contain four mismatches that separate this region into the five homologous sequences ranging from 34 to 284 bp long. In the mismatch repair defective backgrounds, these mismatches are ignored and as the result, the RecA pathway would be able to direct deletion over the entire 5′ sequences. A mutant allele of the *uvrD* gene induces the SOS pathway (LLOYD 1983), suggesting that induced RecA protein or some other protein in the SOS pathway may be involved in the deletion formation in this background. Deletion rates in pLC107 were 30 and 23% of the rates in pLC103 in the *recA*$^+$ and Δ*recA* backgrounds, respectively, and were not enhanced by the defects in the mismatch repair genes (Table 2). The two 3′ sequences (about 570 nt each) in pLC107 contain ~100 mismatches, which make consecutively homologous sequences much shorter than those of the 5′ sequences in pLC103. This explains why pLC107 would have a lower rate of the class of deletions that depends on the homology length. A number of mismatches dispersed over the 3′ sequences in pLC107 might also prevent the involvement of the RecA pathway even in the mismatch repair defective backgrounds. Therefore, it can be concluded that deletion formation in pLC107 is completely RecA-independent. The slight enhancement of the deletion rate in pLC103 in the *mutH* and *mutD* (*dnaQ*) background

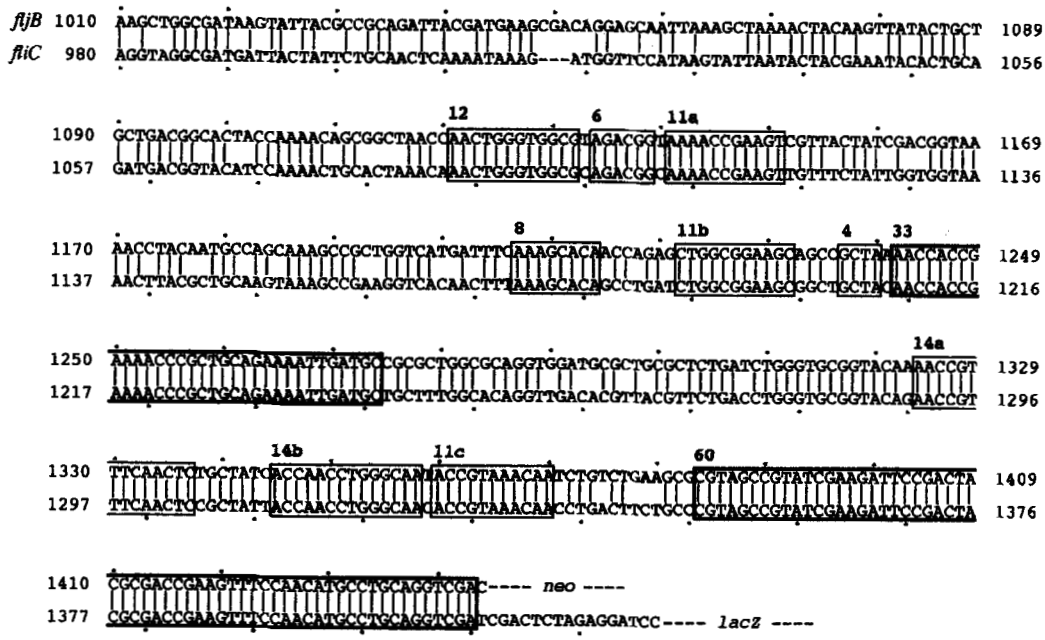

FIGURE 5.—Distribution of deletion endpoints in pLC107. The two 3′ sequences from the *fljB* and *fliC* genes are shown in the maximum homology alignment. Boxed sequences indicate direct repeats in which deletion endpoints mapped, and the number on each box shows the size of direct repeats and corresponds to that shown in Table 4. The two deletion hot spots of 60 and 33 nucleotides are boxed with thick lines. The last 12 nucleotides (positions 1432–1443 of the top strand and positions 1399–1410 of the bottom strand) in the 60-nucleotide hot spot are derived from the polylinkers of vector plasmids.

(Table 2) seems to be caused by the RecA pathway as it was only observed in pLC103.

Conjugational recombination between *E. coli* and *S. typhimurium*, which are 20% divergent in DNA sequence, is greatly enhanced by the defects in mismatch repair (RAYSSIGUIER *et al.* 1989). This result seems to be inconsistent with the result with pLC107 (Table 2). However, the above divergence is only an estimate for the entire chromosomes of the two strains but not for the surrounding sequences of recombination junctions in the intergeneric crosses. It is plausible that heterologies between the two strains are not uniform in the entire chromosomes but are localized. The two sequences that are involved in the intergeneric recombination might have only a small number of mismatches, which are recognizable by the mismatch repair system. Recently the defects in the Dam-dependent mismatch repair pathway have been shown to enhance RecA-independent deletion formation between short tandem repeats (101 bp) with four mismatches (LOVETT and FESCHENKO 1996). This also is inconsistent with our results. The two 5′ sequences of pLC103 have two dam (GATC) sites and a lower rate of mismatches than do the above 101-bp repeats, but the mismatch repair effect in pLC103 is RecA-dependent (Tables 2 and 3). One of major differences between the two deletion-detection systems is the existence of an intervening sequence: the above system has no such sequence while pLC103 has the 1.2-kb sequence. Short tandem repeats without any intervening sequence might easily cause replication slippage in a different way from strand transfer mediated by RecA, resulting in a high rate of deletion in the mismatch repair defective backgrounds. The 3′ sequences of pLC107 have too many heterologies in addition to the intervening sequence, which might preclude the involvement of the mismatch repair pathway as well as the RecA pathway.

In both plasmids the three *ssb* alleles greatly enhanced deletion rates (Table 2). In pLC103 the effect of the *ssb-113* allele was RecA-independent (Table 3). These results indicate that the *ssb* alleles exert their effect on deletion formation without RecA function; that is, there is a distinct RecA-independent deletion pathway that is promoted by the mutant *ssb* alleles.

Excision of transposons is independent of RecA and transposases and is considered to be the genetic event equivalent to deletion (EGNER and BERG 1981). Transposon excision is also stimulated by the *mutS, mutL, mutH, uvrD, mutD5,* and *ssb-113* defects (LUNDBLAD and KLECKNER 1985). However, the RecA-independent deletion in pLC107 is not affected by defects in mismatch repair and so the mechanism for deletion formation in this plasmid should be different from that for transposon excision.

Analysis of deletion endpoints in pLC107 revealed the two hot spots (Table 4 and Figure 5). These hot spots included almost all the endpoints of the deletions in the *recA*$^+$ and Δ*recA* backgrounds, indicating again that the deletion pathway is RecA-independent but depends on the length of consecutive homologies. Deletion endpoints in pLC103 were not analyzed by sequencing, but most of them are probably located in

the longest homologous sequence (284 nt). In both plasmids, the regions that are deleted are >2 kb long and contain the *neo* gene (Figure 3). These regions do not contain any palindromic sequence capable of producing a stable secondary structure and hence the effect of secondary structure on deletion formation (ALBERTINI *et al.* 1982; GLICKMAN and RIPLEY 1984; DASGUPTA *et al.* 1987; TRINH and SINDEN 1993) can be neglected in these plasmids. Therefore, the only structural factor to influence the deletion formation is the length of homologous sequences or of direct repeats. On the other hand, deletion endpoints in the *ssb-3* background were dispersed over the entire 3′ sequences (Table 4 and Figure 5), and only 49% of the endpoints were in the above two hot spots. The rest of the endpoints were in the direct repeats from four to 14 nt long. This shows that the hot-spot specificity that depends on the homology length is changed by the *ssb* allele. In the *ssb-113* background, where deletion enhancement was 50% lower than that in the *ssb-3* background (Table 2), 73% of the endpoints were still located in the two hot spots (data not shown). The increase in deletion rates seems to be related to the decrease in the hot-spot specificity.

A few *E. coli* mutants that stimulate deletion between short direct repeats have been reported. In a *topB* strain, deletion of a 1.3-kb segment is stimulated 10-fold, but deletion endpoints are always in a hot spot (YI *et al.* 1988). In some *xonA* strains, the rates of deletion of a 460-bp segment are enhanced 100-fold and 90–95% of the endpoints are in a 17-bp hot spot with four mismatches; in the *xonA*$^+$ strain only 20% of the endpoints are in the same hot spot (ALLGOOD and SILHAVY 1991). These changes are clearly different in the hot-spot specificity in pLC107 caused by the *ssb-3* allele. To ascertain this difference, deletion in pLC107 needs to be examined in the *topB* and *xonA* strains.

All the three *ssb* alleles used in this experiment cause temperature sensitivity, UV sensitivity, and lack of SOS induction (MEYER and LAINE 1990). The degree of UV sensitivity is *ssb-1* < *ssb-113* < *ssb-3* (SCHMELLIK-SANDAGE and TESSMAN 1990), which is parallel to the degree of the enhancement of deletion rates (Table 2). The excision repair pathway, requiring SSB protein, might have some interaction with the deletion pathway. SSB protein (18.9 kDa), functioning as tetramers, is a multifunctional protein involved in replication, recombination and repair (MEYER and LAINE 1990). SSB-1 is shown to lose DNA-binding activity at 45° (WILLIAMS *et al.* 1984), but *in vitro* ssDNA-binding activity of SSB-113 is shown to be the same as the wild-type SSB (CHASE *et al.* 1984). Therefore, the decrease in ssDNA-binding activity seems to be independent of the mechanism for deletion enhancement. It is reported that SSB-113 cannot interact with χ subunit, one of the members of γ-complex that plays a role in lagging strand synthesis in the DNA Pol III holoenzyme (KELMAN and O'DONNELL 1995). In the *ssb-113* background, replication of the leading and lagging strands of plasmid pLC107 may be out of balance, resulting in stalled replication forks that may increase the possibility of replication slippage, leading to deletions. If this is the case, most deletions in pLC107 will have endpoints in the same hot spots because only the time of an early stage (slippage) that triggers deletion formation is extended by stalled replication, resulting in only a quantitative change of deletion formation. However, SSB-113 changes the hot-spot preference for deletion endpoints and SSB-3 exerts a more striking effect, indicating that the mutant SSBs function at a late stage in deletion formation. The wild-type SSB seems to prevent annealing between short homologies caused by misalignment or seems to dissociate such misaligned short sequences in cooperation with other proteins such as a nuclease or a helicase. In both cases, the mutant SSB increases deletions between short homologies. Annealing between longer homologeis in misalignment may escape from recognition or dissociation by the wild-type SSB, resulting in the deletion hot spots observed in the *recA*$^+$ and Δ*recA* strains.

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