A *recB recC sbcB recJ* Host Prevents *recA*-Independent Deletions in Recombinant Cosmid DNA Propagated in *Escherichia coli*

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Segments of DNA are deleted from recombinant cosmid DNAs with high frequency during propagation in standard *recA Escherichia coli* hosts. An attempt has been made to derive an appropriate strain of *E. coli*, suitable for cosmid cloning, in which such deletions do not occur. We examined the effects of a series of host recombinational mutations on the deletion process, using six independent recombinant cosmids that carry inserts of mouse, Chinese hamster, or human DNA. Various *E. coli* host cells carrying the recombinant cosmids were cultured serially in liquid medium, and the recombinant cosmid DNAs were extracted from the host cells and analyzed by agarose gel electrophoresis and by gene transfer of the DNAs into cultured mammalian cells. Of the mutations examined, only a *recB recC sbcB recJ* (or *recN*) quadruple combination of host mutations prevented the deletion of DNA segments. The recombinant cosmid DNAs propagated in *E. coli* hosts that carried this combination of mutations were functionally as well as structurally intact. We propose that the *recJ* (and/or *recN*) gene is involved in some aspect of the events that lead to deletions of cosmid DNA in a *recB recC sbcB* genetic background.

Construction of DNA libraries in which the total genomes of specified organisms are stably maintained and represented is a prerequisite for studies of gene structure and function. Although most of the total sequence of various genomes has been found in libraries constructed with lambda bacteriophage vectors or plasmid vectors, some specific sequences have been found to be lost during the construction of the libraries and their propagation in *Escherichia coli* hosts, while other sequences have been found to have undergone rearrangement (for reviews, see references 32 and 40). These phenomena are probably due to deletion events in recombinant DNA which occur during propagation in *E. coli* hosts.

During cosmid cloning, we have encountered the serious problem that deletions in recombinant cosmid DNAs occur at very high frequency during propagation in standard *recA E. coli* hosts in liquid culture. Several other groups have also reported such deletion events (8, 38, 41). In the present study, we examined the effects of a series of host recombinational mutations on cosmid deletion events, using six independent cosmids that carry insert DNAs derived from mouse, Chinese hamster, or human genomic DNAs. We found that a *recB recC sbcB recJ* (or *recN*) quadruple combination of mutations in *E. coli* hosts prevented the cosmid deletion events and that the recombinant cosmid DNAs propagated in an *E. coli* host that carries such a combination of mutations were structurally and functionally intact.

MATERIALS AND METHODS

Bacterial strains. The bacterial strains used in this study are listed in Table 1.

Media. Luria-Bertani broth (LB) was used routinely for growth of bacterial cultures. Cells were plated on LB that contained 1.2% Bacto-Agar (Difco Laboratories, Detroit,

Mich.) (LB agar plates). Mouse L cells deficient in thymidine kinase activity (Ltk⁻ cells) (18) were cultured in Falcon plastic dishes (Becton Dickinson Labware, Oxnard, Calif.) in modified (37) Eagle minimum essential medium supplemented with 10% calf serum.

Enzymes. Restriction enzymes and T4 DNA ligase were obtained from Takara Shuzo Co., Kyoto, Japan.

Plasmid and cosmid DNAs. Plasmid DNA and deletion derivatives of recombinant cosmid DNA were introduced into *E. coli* cells by the modified calcium chloride procedure (30). Plasmid and cosmid DNAs were prepared by the boiling (15) or the alkaline lysis (2) method. A 2.0-kilobase (kb) *Eco*RI-*Sal*I segment (pJ765) or a 3.7-kb *Hind*III-*Sal*I segment (pJ763) carrying the wild-type *recJ* gene (28) (gifts from A. J. Clark) and a 19-kb *Bam*HI segment carrying the wild-type *recB* and *recC* genes (35) (a gift from G. R. Smith) were recloned with the pACYC184 plasmid vector (7) (a gift from H. Shinagawa) by standard methods (29).

Recombinant cosmid clones. We constructed a cosmid library by using complete *Bam*HI digests of mouse L-cell genomic DNA and vector pDC1 by the standard method (29), with modifications, and isolated three recombinant cosmids, cMB7, cMB15, and cMB30, each of which carries single *Bam*HI fragments of 40 to 50 kb (M. Ishiura, H. Ohashi, T. Uchida, and Y. Okada, Anal. Biochem., in press). Three recombinant cosmids, cCEF2-7, cCEF2-9, and cHEF2-12, were constructed with the pDC104 vector and carry toxin resistance genomic EF2 genes isolated from Chinese hamster ovary cells (cCEF2-7 and cCEF2-9) and human fibroblasts (cHEF2-12), respectively (Ishiura, et al., unpublished data). cCEF2-7 and cCEF2-9 probably contain the same allelic as well as 10- and 15-kb nonoverlapping regions, respectively. These six recombinant cosmids were designated as "test cosmids" for convenience. The test cosmids were propagated in *E. coli recA* strain DH1. Cosmid vectors pDC1 and pDC104 carry a unique *Bam*HI site as a

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TABLE 1. *E. coli* strains

Strain[a]	Relevant genotype	Source (reference)
AB1157	*rec+*	B. J. Bachmann (12)
AB2463	*recA13*	B. J. Bachmann (17)
JC5547	*recA13 recB21 recC22*	A. J. Clark
JC5519	*recB21 recC22*	A. J. Clark (39)
JC3881	*recB21 recC22 recF143*	A. J. Clark
JC9239	*recF143*	A. J. Clark, B. J. Bachmann (16)
JC13031	*recJ153*	A. J. Clark (27)
JC13032	*recJ154*	A. J. Clark
RDK1541	*recO1504::Tn5*	R. Kolodner (20)
JC11445	*sbcA23*	A. J. Clark
JC11451	*sbcB15*	A. J. Clark
JC7623[b]	*recB21 rec22 sbcB15*	A. J. Clark (16)
JC15329	*recB21 recC22 sbcB15 Δ(recA-srlR)306::Tn10*	A. J. Clark
JC8111	*recB21 recC22 sbcB15 recF143*	A. J. Clark (16)
JC10967	*recB21 recC22 sbcB15 recF144*	A. J. Clark
JC7994	*recB21 recC22 sbcB15 rec-1*	A. J. Clark (16)
JC12182	*recB21 recC22 sbcB15 recJ77*	A. J. Clark (27)
JC8141	*recB21 recC22 sbcB15 rec-146*	A. J. Clark (16)
JC8151	*recB21 recC22 sbcB15 recJ147*	A. J. Clark (16)
JC8161	*recB21 recC22 sbcB15 recJ148*	A. J. Clark (16)
JC12190	*recB21 recC22 sbcB15 recJ153*	A. J. Clark (16)
JC12186	*recB21 recC22 sbcB15 recJ154*	A. J. Clark (16)
JC12166	*recB21 recC22 sbcB15 recJ284::Tn10*	A. J. Clark (27)
JC7912	*recB21 recC22 sbcB15 recK149*	A. J. Clark (16)
SP226	*recB21 recC22 sbcB15 recN261*	R. G. Lloyd (34)
SP231	*recB21 recC22 sbcB15 recN262*	R. G. Lloyd (34)
RDK1531	*recB21 recC22 sbcB15 recO1504::Tn5*	R. Kolodner (20)
RDK1563	*recB21 recC22 sbcB15 recO1504::Tn5*	R. Kolodner (20)
KD2179	*recB21 recC22 sbcB15 recQ1801*	H. Nakayama (33)
FB154	*recB21 recC22 sbcB15 ruv-52*	R. G. Lloyd (25)
FB155	*recB21 recC22 sbcB15 ruv-54*	R. G. Lloyd (25)
FB156	*recB21 recC22 sbcB15 ruv-59*	R. G. Lloyd (25)
JC9388	*recB21 recC22 sbcA23*	A. J. Clark
JC13021	*recB21 recC22 sbcA23 recJ153*	A. J. Clark (27)
JC13024	*recB21 recC22 sbcA23 recJ154*	A. J. Clark (27)
CES200	*recB21 recC22 sbcB15 hsdR*	C. Shurvinton and F. Stahl (32)
CES201	*recB21 recC22 sbcB15 Δ(recA-srlR)306::Tn10 hsdR*	C. Shurvinton and F. Stahl
DB1161	*recB21 recC22 sbcB15 recA56 hsdR hsdM*	A. R. Wyman and D. Botstein
HB101	*recA13 hsdS20*	Our stock (4, 5)
490A	*recA hsdR hsdM*	T. Nishimoto (38)
DH1	*recA1 hsdR17*	D. Hanahan (14)
ED8767	*recA56 hsdS3*	B. J. Bachmann (31)
MI1	*ΔrecA hsdR hsdM*	Our stock
MI2	*Δ(recA-srlR)306::Tn10 hsdR514*	Our stock

[a] With the exception of strains HB101, 490A, DH1, ED8767, MI1, and MI2, all strains used were derivatives of *E. coli* K-12 AB1157, which has the following markers: *thr-1 ara-14 leuB6 Δ(gpt-proA)62 lacYI tsx-33 supE44 galK2 hisG4 rpsL31 kdgK51 xyl-5 mtl-1 argE3 thi-1*. AB1157 and most of its derivatives lack the Rac prophage that carries the *sbcA* and *recE* genes and so have no potential for expressing the *recE* gene. The *sbcA23* strains listed are Rac+ derivatives of AB1157 and express the wild-type *recE* gene. The additional mutations in each strain are as follows: JC11451, Su⁻; JC11445, *his-60*; FB154, FB155, and FB156, *eda-51::Tn10*; JC9388, Su⁻ *his-60*; CES200, Su⁻ *thr::Tn10*; CES201, Su⁻ *thr+ leu+*; DB1161, *end srl::Tn10*.

[b] JC7623 and its derivatives (probably) have the additional mutation *sbcC201* (26).

cloning site, and pDC104 also carries a 2.0-kb *Pvu*II segment which contains the thymidine kinase (TK) gene from herpes simplex virus type 1 (HSV-1) (Ishiura et al., in press).

Assay of deletions in recombinant cosmids. DNAs of the six test cosmids (cMB7, cMB15, cMB30, cCEF2-7, cCEF2-9,

and cHEF2-12) were packaged into phage particles by a packaging reaction in vitro (29), using an Amersham packaging kit (Amersham Corp., Arlington Heights, Ill.), and were introduced into various strains of *E. coli*. Transductant cells carrying the recombinant cosmids were selected on LB agar plates that contained 7.5 to 30 μg of ampicillin per ml. Twenty independent ampicillin-resistant colonies for each test cosmid were inoculated into 2 ml of LB that contained 7.5 to 30 μg of ampicillin (LB-Ap medium) per ml and incubated at 37°C for 24 h with shaking at 175 rpm. We designated these cultures "cultures 0" for convenience. Recombinant cosmid DNAs were prepared from cultures 0 and analyzed by electrophoresis through a 0.8% agarose gel run in buffer that contained 40 mM Tris-acetate buffer (pH 8.0) and 2 mM EDTA (29). Ten intact clones without any apparent deletions were selected for each test cosmid; a mixture of 0.1-ml samples of cultures 0 of the 10 clones was inoculated into 1 liter of LB-Ap medium in a 3-liter flask and incubated at 37°C with shaking for 24 h, as described above, to obtain culture 1. A 1-ml sample of each first culture was inoculated into 1 liter of fresh LB-Ap medium and incubated as above. Serial cultures were continued in this way until the cultures 15 were obtained. To detect possible deletions, recombinant cosmid DNA was prepared from 1-ml samples of each culture from 0 to 15 and analyzed by agarose gel electrophoresis, as described above. The number (0 to 15) of the culture in which a deletion was first detected was taken as the "stability index" of the text cosmid propagated in the specified strain of *E. coli*.

Gene transfer. Recombinant cosmid DNA was introduced into Ltk⁻ cells by the calcium phosphate coprecipitation method (13), with modifications (18), and transformant cells expressing the HSV-1 TK gene were selected in modified Eagle minimum essential medium supplemented with 10% calf serum and a mixture of hypoxanthine, aminopterin, and thymidine (HAT medium [23]), as described previously (18). Transformant cells expressing both the HSV-1 TK gene and the toxin resistance human EF2 gene were selected in HAT medium that contained 50 ng of exotoxin A from *Pseudomonas aeruginosa* per ml. The colonies were stained with crystal violet as described previously (18) and then counted.

Biohazards. Experiments were carried out according to the guidelines established by the Japanese Committee for Recombinant DNA Research.

RESULTS

Effects of host recombinational mutations on deletions in recombinant cosmids. Experiment 1 in Table 2 summarizes the effects of host recombinational mutations on deletions in recombinant cosmids propagated in *E. coli*. We examined the effects of *recF*, *rec-151*, *recJ*, *recO*, *sbcA*, and *sbcB* mutants, a *recB recC* mutant, a *recA recB recC* mutant, and a *recB recC recF* mutant of *E. coli*. None of these mutants increased the stability index over that in the *rec+* strain. We also examined various standard *recA* mutant strains such as HB101, 490A, ED8767, and DH1, which have been widely used as hosts for cosmid cloning, and two strains with a *recA* deletion mutation, MI1 and MI2. None of these *recA* mutants had any effect on the stability index. AB1157 and its derivatives lack the Rac prophage that carries the *sbcA* and *recE* genes and so have no potential for expressing the *recE* gene. *sbcA* mutations result in expression of the *recE* gene product (exonuclease VIII) (for reviews, see reference 36). Neither the expression nor the absence of the *recE* gene affected the stability index.

TABLE 2. Deletions in cMB15 cosmid DNA propagated in various *E. coli* strains[a]

Expt	Strain	Relevant genotype	Stability index[b]
1	AB1157	*rec⁺*	3
	AB2463	*recA13*	3
	JC5547	*recA13 recB21 recC22*	3
	JC5519	*recB21 recC22*	2
	JC3881	*recB21 recC22 recF143*	2
	JC9234	*recF143*	2
	JC7994	*rec-151*	2
	JC13031	*recJ153*	2
	JC13032	*recJ154*	2
	RDK1541	*recO1504::Tn5*	2
	JC11445	*sbcA23*	2
	JC11451	*sbcB15*	1
	HB101	*recA13 hsdS20*	2
	490A	*recA hsdR hsdM*	2
	DH1	*recA1 hsdR17*	4
	ED8767	*recA56 hsdS20*	2
	MI1	Δ*recA hsdR hsdM*	1
	MI2	Δ*recA hsdR514*	2
2	JC7623	*recB21 recC22 sbcB15*	1
	DB1161	*recB21 recC22 sbcB15 recA56*	1
	JC15329	*recB21 recC22 sbcB15* Δ*recA*	1
	JC8111	*recB21 recC22 sbcB15 recF143*	1
	JC10967	*recB21 recC22 sbcB15 rec-144*	2
	JC7994	*recB21 recC22 sbcB15 rec-151*	2
	JC8141	*recB21 recC22 sbcB15 recJ146*	1,1
	JC8151	*recB21 recC22 sbcB15 recJ147*	2,4
	JC12190	*recB21 recC22 sbcB15 recJ153*	>15
	JC12186	*recB21 recC22 sbcB15 recJ154*	>15
	JC7912	*recB21 recC22 sbcB15 recK149*	1
	SP226	*recB21 recC22 sbcB15 recN261*	2
	SP231	*recB21 recC22 sbcB15 recN262*	>15
	RDK1531	*recB21 recC22 sbcB15 recO1504::Tn5*	1
	RDK1563	*recB21 recC22 sbcB15 recO1504::Tn5*	2
	KD2179	*recB21 recC22 sbcB15 recQ1*	2
	FB154	*recB21 recC22 sbcB15 ruv-52*	1
	FB155	*recB21 recC22 sbcB15 ruv-54*	1
	FB156	*recB21 recC22 sbcB15 ruv-59*	2
3	JC12190	*recB21 recC22 sbcB15 recJ153*	>15
	JC12186	*recB21 recC22 sbcB15 recJ154*	>15
	JC7623	*recB21 recC22 sbcB15*	1
	JC5519	*recB21 recC22*	2
	JC11451	*sbcB15*	1
	JC13031	*recJ153*	2
	JC13032	*recJ154*	3
	JC13021	*recB21 recC22 sbcA23 recJ153*	1,1
	JC13015	*recB21 recC22 sbcA23 recJ154*	1
	JC9388	*recB21 recC22 sbcA23*	1

[a] DNA of test cosmid cMB15 was introduced into various strains of *E. coli* by phage infection. Each set of transductant cells was cultured, with daily serial passages, in LB-Ap medium at 37°C with shaking. To detect possible deletions, recombinant cosmid DNA was prepared from each culture from 0 to 15 and analyzed by agarose gel electrophoresis.

[b] The numerical designation of the culture in which a deletion was first detected. Data from one or two independent experiments are shown.

Deletions in test cosmid cMB15 propagated in *recB recC sbcB recJ* (or *recN*) strains. A *recB sbcB* mutant, a *recB recC sbcB* mutant, and a *recB recC sbcB recF* mutant of the *E. coli* host have been reported to prevent deletions in some kinds of recombinant DNA (3, 11, 21, 32, 40). Therefore, we examined the effects of additional single mutations in a *recB recC sbcB* genetic background on cosmid deletion events (experiment 2 in Table 2).

The additional single mutations examined were mutations in the *recA*, *recF*, *rec-151*, *recJ*, *recK*, *recN*, *recO*, *recQ*,

TABLE 3. Effects of introduction of the cloned *recJ* gene into strain JC12186 on deletions in cMB15 cosmid DNA[a]

Plasmid introduced	Stability index[b]
None	>15, >15
pACYC184	>15, >15
pJ765	0, 1
pJ763	0, 1

[a] Strain JC12186 and its derivatives carrying only pACYC184, the 2.0-kb *Eco*RI-*Sal*I segment that carries the wild-type *recJ* gene cloned in pACYC184 (pJ765), or the 3.7-kb *Hin*dIII-*Sal*I segment carrying the wild-type *recJ* gene cloned in pACYC184 (pJ763) were used as hosts. JC12186 cells carrying both test cosmid cMB15 and the indicated plasmids were cultured in LB that contained 20 µg of ampicillin and 5 µg of chloramphenicol per ml. Other conditions were as described in Table 2, footnote *a*.

[b] Two independent experiments.

and *ruv* genes which have been reported to be involved in the RecF recombinational pathways in *E. coli* (36). Among the additional single mutations examined, only the *recJ* (JC12186 and JC12190) and the *recN* (SP231) mutations prevented deletions, at least as far as culture 15, whereas the other additional mutations tested, *recA*, *recF*, *rec-151*, *recK*, *recO*, *recQ*, and *ruv*, did not affect the stability index. The ability of single *recJ* or *recN* mutations to prevent the cosmid deletion events in the *recB recC sbcB* genetic background differed for various *recJ* or *recN* mutations (experiment 2 in Table 2): three additional single mutations, *recJ153*, *recJ154*, and *recN262*, prevented the deletion, whereas four analogous mutations, *recJ146*, *recJ147*, *recJ148*, and *recN261*, did not, indicating that only very specific *recJ* or *recN* mutations are effective in preventing the deletion events.

***recJ* mutations prevent deletions only in the *recB recC sbcB* genetic background.** For further characterization of the role in cosmid deletion events of each gene in the quadruple combination of host mutations, we examined the effects of a series of mutations related to the quadruple combination of mutations described above. Experiment 3 in Table 2 summarizes the results.

recJ mutants (JC13031 and JC13032), a *sbcB* mutant (JC11451), and a *recB recC* mutant (JC5519) did not affect the stability index, while a *recB recC sbcB* mutant (JC7623) slightly decreased the stability index. The *recJ* mutations were able to prevent deletion events only in a *recB recC sbcB* genetic background (JC12186 and JC12190). In the quadruple combination of mutations, replacement of the *sbcB* mutation by an *sbcA* mutation (JC13015 and JC13021) resulted in the loss of inhibition of deletions. The involvement of the *recJ* gene in deletion events was also demonstrated by the observation that introduction of the wild-type *recJ* gene into the *recB recC sbcB recJ* strain JC12186 restored the occurrence of deletion events (Table 3). In spite of extensive efforts, we have been unable to introduce a stable derivative of plasmid pACYC184, which carries the wild-type *recB* and *recC* genes, into JC12186 cells; in all transformants examined, large deletions resulting in loss of most of the *recB* and *recC* genes were observed in the plasmid.

***recB recC sbcB recJ* strains prevent deletions events in all recombinant cosmids examined.** To determine whether the protective effect of the quadruple combination of host mutations is general for all recombinant cosmids, we examined other test cosmids (Table 4).

The *recB recC sbcB recJ* strains JC12186 and JC12190 inhibited deletion events in all cosmids tested, although the extent of the inhibition differed from cosmid to cosmid

TABLE 4. Inhibition of deletions in various recombinant cosmids by the quadruple combination of mutations in the host[a]

Strain	Relevant genotype	Stability index[b]					
		cMB7	cMB15	cMB30	cHEF2-12	cCEF2-7	cCEF2-9
AB1157	rec+	1, 1	3, 3	1, 1	3, 3	13, 13	8, 10
HB101	recA13	1, 1	3, 3	3, 3	4, 4	>15, >15	>15, >15
AB2463	recA13	2, 2	2, 2	1, 1	4, 5	—	—
JC12190	recB21 recC22 sbcB15 recJ153	>15	>15, >15	12, >15	—[c]	—	—
JC12186	recB21 recC22 sbcB15 recJ154	10, 10	>15, >15	8	>15, >15	>15	>15
SP231	recB21 recC22 sbcB15 recN262	>15, >15	>15, >15	12, >15	4, 5	>15, >15	>15

[a] Other conditions were as described in Table 2, footnote *a*.
[b] One or two independent experiments.
[c] —, Not determined.

(stability indices varied from 8 to >15). The *recB recC sbcB recN* strain SP231 prevented deletions in five test cosmids, cMB7, cMB15, cMB30, cCEF2-7, and cCEF2-9, but not in cHEF2-12. *recA13* mutants AB2463 and HB101 did not prevent deletion events in four test cosmids, namely, cMB7, cMB15, cMB30, and cHEF2-12, which occurred in *rec+* strain AB1157. Two test cosmids carrying the Chinese hamster EF2 gene, cCEF2-7 and cCEF2-9, were stably propagated even in *rec+* (AB1157) and *recA13* (AB2463 and HB101) strains.

Recombinant cosmid DNAs propagated in the *recB recC sbcB recJ* strain. Figure 1A shows the electrophoretic patterns on agarose gel of the DNA from test cosmid cMB15 which had been propagated in JC12186, JC12190, or SP231. The cMB15 cosmid DNAs propagated in the *recB recC sbcB recJ* strains JC12186 and JC12190 or in *recB recC sbcB recN* strain SP231 were apparently intact. After digestion of these DNAs with *Bam*HI, two clear bands which corresponded to the intact vector and insert DNA, respectively, were detected. On the other hand, various bands that corresponded

to deletions were detected in the cMB15 cosmid DNAs propagated in *rec+* (AB1157) or *recA13* (AB2463) control strains.

Figure 1B shows the bands of DNA from the other test cosmids after agarose gel electrophoresis. The cMB7 and cMB30 cosmid DNAs were also apparently propagated intact in the *recB recC sbcB recJ* strain JC12186 until cultures 9 and 7, respectively (Fig. 1B; see also Table 4). The cHEF2-12 test cosmid is composed of seven *Bam*HI fragments of 2.0, 2.3, 3.3, 4.5, 5.9, 6.2 (vector portions), and 17 kb, and the 17-kb fragment carries the toxin resistance human EF2 gene. The cHEF2-12 cosmid was stably maintained in JC12186 up to culture 15 without any apparent structural rearrangement: DNA bands separated by agarose gel electrophoresis before and after digestion with *Bam*HI were the same at the cultures 1 and 15. In contrast, the cHEF2-12 test cosmid showed deletions at culture 4 during propagation in *recA13* strain HB101 (Table 4 and Fig. 1B). Mapping experiments with restriction enzymes showed that most deletions in the cHEF2-12 cosmid occurred at several
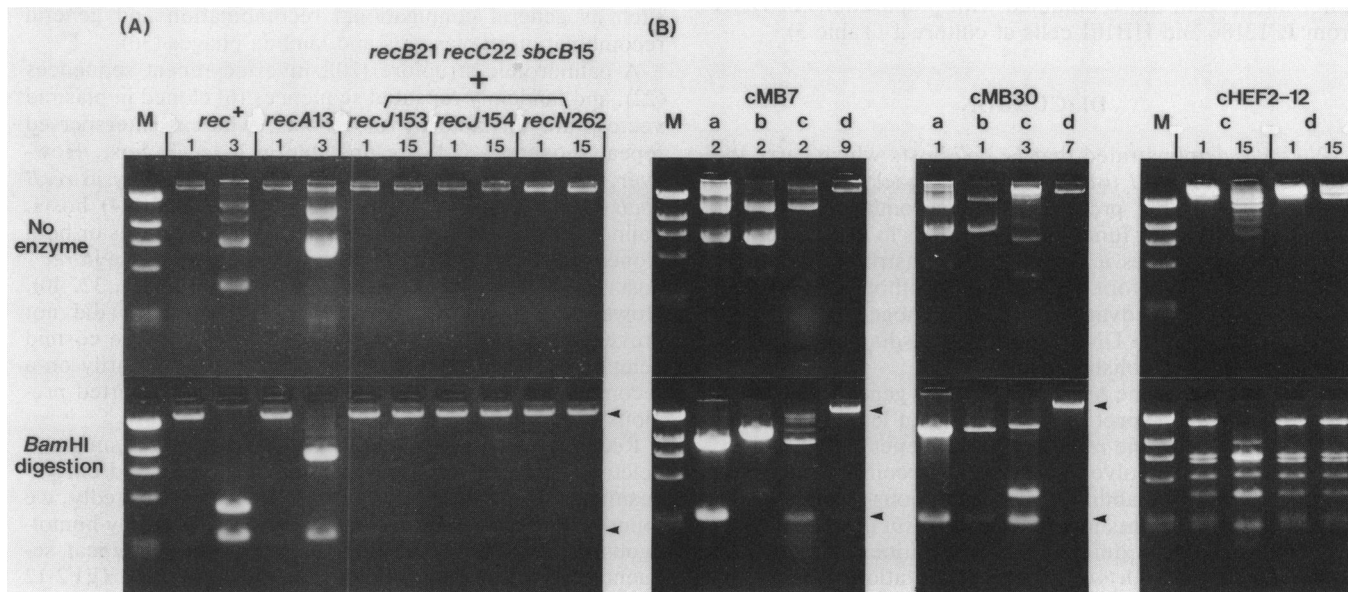

FIG. 1. Electrophoretic analyses on agarose gels of cosmid DNAs propagated in various strains of *E. coli*. Four test cosmids, cMB7, cMB15, cMB30, and cHEF2-12, were propagated in various strains of *E. coli* as described in Table 4, footnote *a*. After the serial cultures indicated, recombinant cosmid DNA was prepared and analyzed, directly or after digestion with *Bam*HI, by electrophoresis on agarose gels. M, Size markers from *Hind*III digests of lambda phage DNA. Arrowheads indicate the positions of the intact vector and intact insert DNAs, respectively; numbers indicate at which cultures cosmid DNAs were extracted. (A) cMB15. *E. coli* strains: *rec+*, AB1157; *recA13*, AB2463; *recB21 recC22 sbcB15 recJ153*, JC12190; *recB21 recC22 sbcB15 recJ154*, JC12186; *recB21 recC22 sbcB15 recN262*, SP231. (B) cMB7, cMB30, and cHEF2-12. *E. coli* strain: a, AB1157; b, AB2463; c, HB101(*recA13*); d, JC12186.

TABLE 5. Functional assay of cHEF2-12 cosmid DNAs propagated in HB101 nad JC12186 by gene transfer
of the DNAs into cultured mammalian cells[a]

Strain	DNA prepn from cells at culture:	No. of transformant colonies per dish[b]								PA[r]/TK[+] (%)[c]
		TK[+]				TK[+] + PA[r]				
		Dish 1	Dish 2	Avg	%[d]	Dish 1	Dish 2	Avg	%	
HB101	1	868	1,024	946	100	421	519	470	100	50
HB101	15	972	1,160	1,066	113	1	1	1	0	0.1
JC12186	1	696	980	838	89	479	518	498	106	59
JC12186	15	836	896	866	92	376	384	380	81	44

[a] The cHEF2-12 cosmid was propagated in HB101 or JC12186 as described in Table 2, footnote a, and cHEF2-12 DNA was prepared from the indicated culture by the alkaline lysis method (2) and transferred to L cells deficient in thymidine kinase activity, at a dose of 100 ng per dish, by the modified calcium phosphate coprecipitation method. Transformant cells expressing the HSV-1 TK gene were selected in HAT medium, and transformant cells expressing both the HSV-1 TK gene and the toxin resistance human EF2 gene were selected in HAT medium that contained 50 ng of *P. aeruginosa* (PA) toxin.
[b] TK[+], HAT-resistant colonies; TK[+] + PA[r], simultaneously HAT- and PA toxin-resistant colonies.
[c] Ratio of TK[+] + PA[r] to TK[+].
[d] Percentage of value for culture 1 of HB101.

hot spots in the insert portions and resulted in the complete elimination of the EF2 gene (data not shown).

To determine whether the cHEF2-12 cosmid propagated in *recB recC sbcB recJ* strain JC12186, which seemed to be structurally intact, was also functionally intact, we assayed the activities of selective marker genes, the HSV-1 TK gene and the toxin resistance human EF2 gene, which are carried on the vector arm and on the insert, respectively, by gene transfer into Ltk⁻ cells. We used the cHEF2-12 cosmid propagated in HB101 showing severe deletions as a control (Table 5). With a preparation of DNA from HB101 cells at culture 15, we were unable to obtain any transformants that expressed the EF2 gene, although we obtained many transformants that expressed the TK gene. In contrast, with a preparation of DNA from JC12186 cells at the 15th culture, we obtained transformants that expressed the EF2 gene, and the numbers of transformants that expressed the TK gene and those that expressed both the TK and EF2 genes did not differ much from those obtained with preparations of DNA from JC12186 and HB101 cells at culture 1 (Table 5).

DISCUSSION

We have demonstrated that *E. coli* hosts which carry the *recB recC sbcB recJ* (or *recN*) genetic background can be used for the stable propagation of recombinant cosmids without structural or functional alterations in those cosmids. This finding provides a clue for the construction of more suitable host strains for cosmid cloning, although these hosts suffer from the disadvantage of nonhomogeneous growth, low yields of cosmid DNA, and low transduction efficiency (Ishiura et al., unpublished data).

We propose that the *recJ* (and/or *recN*) gene function(s) is involved in some aspect of the events that lead to deletions of cosmid DNA in the *recB recC sbcB* genetic background. These genes are involved in the RecF recombination pathway in *E. coli*. The ability of *recJ* mutations to prevent the cosmid deletion events in the *recB recC sbcB* genetic background differed for different *recJ* mutations. Lovett and Clark have identified two classes of mutations in the *recJ* gene (27): class 1 alleles (*recJ153* and *recJ154*), which produce a strong mutant phenotype; and class 2 alleles (*recJ146*, *recJ147*, and *recJ148*), which produce a weak or temperature-sensitive mutant phenotype. Consistent with the phenotypes of these *recJ* mutations, all class 1 alleles examined here prevented the cosmid deletion events, while all class 2 alleles did not. This relationship also suggests the

direct involvement of the *recJ* gene in cosmid deletion events in the *recB recC sbcB* genetic background. More direct evidence for the involvement of the *recJ* gene was obtained by the introduction of the wild-type *recJ* gene into a *recB recC sbcB recJ* host. The introduction of the *recJ* gene completely restored the occurrence of deletion events in a *recB recC sbcB recJ* host.

Among the additional single mutations examined in the *recB recC sbcB* genetic background, including *recF*, *rec-151*, *recJ*, *recK*, *recN*, *recO*, *recQ*, and *ruv* mutations, all of which have been reported to be involved in the RecF recombination pathway (36), only *recJ* and *recN* mutations prevented the cosmid deletion events. *recJ* mutations in an otherwise wild-type genetic background or in a *recB recC sbcA* genetic background had no effect on the deletion events. Because of these unique characteristics of the cosmid deletion events, it is not easy to correlate such events directly with other recombinational events in *E. coli*, such as general conjugational recombination and general recombination in plasmids and lambda phages (36).

A palindromic structure (10), inverted repeat sequences (22), and tandemly repeated sequences (6) cloned in plasmid vectors are unstable in *recA* hosts, whereas interspersed repeat sequences (24) are unstable in a *recE⁺* host. However, some of these features are maintained stably in *recB sbcB* (11), *recB recC sbcB recF* (3), or *recE* (24) hosts. Palindromic structures or inverted repeat sequences or both cloned in lambda phage vectors, which are unstable in *rec⁺* hosts, are maintained in *recB recC sbcB* hosts (21, 32, 40). However, hosts with these genetic backgrounds did not prevent the cosmid deletion events. Therefore, the cosmid deletion events described here must depend primarily on a recombination system(s) different from those reported previously.

Recently, we analyzed the DNA sequences that surround deletion junctions in cosmids, using cHEF2-12 and cMB15 cosmids propagated in *recA* host HB101. Unexpectedly, we found that cosmid deletion events were mediated by homologous recombination between two short direct repeat sequences (Ishiura et al., unpublished data). In the cHEF2-12 cosmid, most deletions were found to be mediated by homologous recombination between two human *Alu* repeat sequences arrayed in tandem. Therefore, it appears that a *recB recC sbcB recJ* quadruple combination of host mutations prevents homologous recombination between short direct repeat sequences which are separated from each other by more than 20 kb. This combination of mutations may also

inhibit the *recA*-independent deletion events in plasmids which involve a short direct repeat sequence (1, 9, 19).

Recently, Lovett and Clark cloned the *recJ* gene and identified the RecJ protein (28). However, its function(s) in recombination in *E. coli* remains to be resolved. We expect that, by specifying a sequence(s) or structure(s) that is supposedly involved in the high-frequency deletion events in cosmids, we shall be able to shed light on the function of the RecJ protein in recombination.

ACKNOWLEDGMENTS

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