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Identification and Genetic Analysis of *sbcC* Mutations in Commonly Used *recBC sbcB* Strains of *Escherichia coli* K-12

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Evidence is presented to show that *Escherichia coli* JC7618, JC7621, and JC7623, previously regarded as having a *recB recC sbcB* genotype, carry an additional mutation in a new gene designated *sbcC* at minute 9 on the standard genetic map. In the absence of the *sbcC* mutation these strains are sensitive to mitomycin C and have a reduced efficiency of recombination. Cultures of *recBC sbcB* (*sbcC*$^+$) strains grow slowly, contain many inviable cells, and rapidly accumulate fast-growing variants due to mutation of *sbcC*. *sbcC* has been identified on recombinant plasmids and tentatively located by Tn*1000* mutagenesis to a 0.9-kilobase DNA section between *proC* and *phoR*.

Studies of the conjugational process of genetic exchange in *Escherichia coli* have identified an array of genes (*recA*, *recB*, *recC*, *recE*, *recF*, *recJ*, *recN*, *recQ*, *ruv*, *sbcB*) involved in recombination between homologous DNA molecules (6, 8, 15, 20, 25 29, 32, 33, 37). The product of *recA* is indispensible and plays a vital catalytic role in the formation of recombination intermediates (35). *recB* and *recC* specify subunits of an ATP-dependent DNA exonuclease (11) and helicase (40) that is also able to recognize and cleave DNA at specific sequences referred to as Chi sites (38). The absence of RecBC enzyme in *recB* or *recC* mutants reduces the efficiency of recombination to about 1% of normal (44). However, this deficiency can be alleviated by mutations in *sbcB* that abolish exonuclease I (18, 19) or, in certain strains, by mutations (*sbcA*) of the cryptic Rac prophage that lead to synthesis of exonuclease VIII, the *recE* product (3, 10, 41). Recombination in both cases still requires RecA protein but otherwise proceeds by mechanisms that are genetically distinguishable from that operating in *recBC*$^+$ strains (6, 7, 26, 27).

The identification of recombination-proficient revertants of *recBC* strains in which recombination is restored indirectly by suppressor mutations proved an important milestone in the analysis of recombination mechanisms. Revertants of this type have been used widely in the investigation of recombination between or within plasmids (9), in phage lambda (39), and during DNA repair (43). Recombination in the *sbcB* revertant in particular has been investigated in detail and shown to require the products of *recF*, *recJ*, *recN*, *recQ*, and *ruv*, none of which is essential in the presence of RecBC nuclease (15, 20, 25, 29, 32, 33). Many of these studies used one of the original revertant isolates (JC7623) defined as carrying a single suppressor mutation in *sbcB* (19). In this paper we present evidence to show that this strain and other similar *sbcB* revertants also carry a mutation in a gene designated *sbcC* and that this additional mutation is necessary for the full suppression of the *recBC* mutant phenotype.

MATERIALS AND METHODS

Strains and plasmids. The *E. coli* K-12 strains used are described in Table 1. Genotype designations are those described by Bachmann (2) except for *sbcC* which is designated here as a conditional suppressor of *recB* and *recC* mutations depending on the status of the *sbcB* locus. The *sbcC* mutations associated with the *sbcB* strains JC7623, JC7621, and JC7618 are designated as *sbcC201*, *sbcC202*, and *sbcC203* respectively. Figure 1 shows the location of *sbcC* and other genes relevant to this study. The abbreviation MC is used for mitomycin C. Superscripts r for resistance or s for sensitive are used with phenotypic symbols to describe the response of strains to antibiotics. Plasmids pJP71 and pJP77 carry the *proC-phoR* region of the *E. coli* chromosome cloned in the pACYC184 vector (42) and confer resistance to chloramphenicol. pBR322 is described by Maniatis et al. (30) and confers resistance to ampicillin and tetracycline.

Media. LB broth and agar have been described previously (24) and unless stated otherwise contained sodium chloride at a final concentration of 0.5 g/liter. Strains carrying plasmids were grown routinely in LB medium with suitable antibiotic selection—20 μg of tetracycline per ml or 25 μg of ampicillin or chloramphenicol per ml. 56/2 salts were used as minimal medium and was supplemented as described previously (23, 24). Agar plates containing 5-bromo-4-chloro-3-indolyl phosphate (Xp) at 40 μg/ml were used to score the alkaline phosphatase constitutive phenotype of *phoR* mutants. Mitomycin C was incorporated into LB agar at concentrations of 0.2 and 0.5 μg/ml for sensitivity tests.

General methods for strain construction, measuring growth rate (doubling time) in LB broth by following the A_{650}, and determining sensitivity to UV light and mitomycin C have been described previously (21, 24).

Matings and transductions. The procedures used for mating in liquid medium and for transduction with phage P1 *vir* were as described before (23, 24, 36), except that strains were grown in LB broth containing 10 g of sodium chloride per liter. Matings were terminated by blending samples in 3 ml of molten water agar supplemented with 0.05 ml of LB broth, before plating. Zygotic induction of lambda prophage in crosses with the lysogenic Hfr GY2200 was measured by incorporating a suitable indicator strain into the overlays and scoring plaques on LB maltose agar. Streptomycin was used at 100 μg/ml to counterselect donor strains. In matings with recipient strains carrying pACYC184, pJP71, or pJP77 the donor strains used carried pACYC184, and chloramphenicol was present in the media used for growth, mating, and transconjugant selection.

Enzymes and DNA analysis. Restriction endonucleases and T4 DNA ligase were purchased from Bethesda Research

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TABLE 1. *E. coli* K-12 strains

Strain	Genotype	Source or reference
AB1157	F⁻ *thi-1 his-4 proA2 argE3 thr-1 leuB6 ara-14 lacY1 galK2 xyl-5 mtl-1 supE44 tsx-33 rpsL31*	1
AB2463	As AB1157 but *recA13*	1
AB2470	As AB1157 but *recB21*	1
JC5489	As AB1157 but *recC22*	1
JC7623	As AB1157 but *recB21 recC22 sbcB15 sbcC201*	19
JC7621	As JC7623 but *sbcB13 sbcC202*	19
JC7618	As JC7623 but *sbcB10 sbcC203*	19
JC8679	As AB1157 but *recB21 recC22 sbcA23*	10
N1116	As AB1157 but *thyA*	Trimethoprim selection
N1349	F⁻ *thi-1 metE70 leuB6 proC32 cysC43 lacZ36 ara-14 mtl-1 xyl-5 recB21 sbcB15 sbcC207 rpsL109 rpsE2015*	P1.AB2470 × KL266 (24) to Thy⁺ then × JC9223 to His⁺ and (?) segregation to MC⁻
N2208	As JC7623 but *purE85*::Tn*10*	P1.N3005 × JC7623 to Tet^r
N2215	As JC7623	KL226 × N2208 to Pur⁺
N2216	As JC7623 but *sbcC*⁺	KL226 × N2208 to Pur⁺ MC^s
N2239	As JC7621 but *pro*⁺	KL226 × JC7621 to Pro⁺
N2255	As JC7623 but *pro*⁺	KL226 × JC7623 to Pro⁺
N2271	As JC7623 but *thr*⁺ *leu*⁺ *proA*⁺ *proC29 ara*⁺	χ342 × N2255 to Thr⁺ Leu⁺ Pro⁻
N2272	As N2271 but *sbcC*⁺	χ342 × N2255 to Thr⁺ Leu⁺ Pro⁻ MC^s
N2273	As JC7621 but *thr*⁺ *leu*⁺ *proA*⁺ *proC29 ara*⁺	χ342 × N2239 to Thr⁺ Leu⁺ Pro⁻
N2275	As N2273 but *sbcC*⁺	χ342 × N2239 to Thr⁺ Leu⁺ Pro⁻ MC^s
N2306	As JC7623 but *his-221*::Tn*10* *sbcB*⁺	P1.N3008 × JC7623 to Tet^r MC^s
N2308	As JC7623 but *phoR79*::Tn*10*	P1.K797 × JC7623 to Tet^r
N2309	As JC7623 but *phoR79*::Tn*10* *sbcC*⁺	P1.K797 × JC7623 to Tet^r MC^s
N2312	As N2271 but *phoR79*::Tn*10* *proC*⁺ *sbcC*⁺	P1.K797 × N2271 to Tet^r MC^s
N2314	As JC7621 but *phoR79*::Tn*10*	P1.K797 × JC7621 to Tet^r
N2315	As JC7621 but *phoR79*::Tn*10* *sbcC*⁺	P1.K797 × JC7621 to Tet^r MC^s
N2317	As N2273 but *phoR79*::Tn*10* *proC*⁺ *sbcC*⁺	P1.K797 × N2273 to Tet^r MC^s
N2351[a]	As N1116 but *phoR79*::Tn*10* *sbcC201*	P1.N2308 × N1116 to Tet^r
N2361	As AB1157	P1.AB2470 × N1116 to Thy⁺
N2362	As AB1157 but *recB21*	P1.AB2470 × N1116 to Thy⁺
N2363	As AB1157 but *recC22*	P1.JC5489 × N1116 to Thy⁺
N2364	As N2351	P1.AB2470 × N2351 to Thy⁺
N2365	As N2351 but *recB21*	P1.AB2470 × N2351 to Thy⁺
N2366	As N2351 but *recC22*	P1.JC5489 × N2351 to Thy⁺
N2375	As AB1157 but *his*⁺ *sbcB*?	JC9223 × N2361 to His⁺
N2376	As N2362 but *his*⁺	JC9223 × N2362 to His⁺
N2377	As N2363 but *his*⁺	JC9223 × N2363 to His⁺
N2378	As N2362 but *his*⁺ *sbcB15*	JC9223 × N2362 to His⁺
N2379	As N2363 but *his*⁺ *sbcB15*	JC9223 × N2363 to His⁺
N2380	As N2378 but *sbcC205*	Spontaneous MC^r segregant
N2381	As N2379 but *sbcC206*	Spontaneous MC^r segregant
N3005	As W3110 but *purE85*::Tn*10*	λNK55 × W3110 to Tet^r
N3008	As W3110 but *his-221*::Tn*10*	λNK55 × W3110 to Tet^r
N3033	As W3100 but *lacZ98*::Tn*10*	λNK55 × W3110 to Tet^r
W3110	F⁻ IN(*rrnD-rrnE*)[b]	1
K797	F⁻ *phoR79*::Tn*10* *purE41 his-53 metB65 xyl-14 rpsL97 cycA1 cycB2*? *tsx-63 tonA32*? *aroA357 ilv-227 lacY29*	CGSC 6456[c]
KL226	HfrC (PO2A) *relA1 tonA22 pit-10 spoT1*	CGSC 4311
W1895	HfrC (PO2A) *metB1 relA1 spoT1*	CGSC 2332
AB259	Hfr (Hayes, PO1) *thi-1 relA1*	K. B. Low
JC9223	Hfr (PO44) *sbcB15 phoA526*	A. J. Clark
χ342	Hfr (Cavalli, PO2A) *proC29 metB1 relA1*	CGSC 4515
GY2200	Hfr (Hayes, 3000) *thi-1 mal* (λ *ind*⁻)⁺	R. Devoret
KL548	F(128) *proAB*⁺ *lacI3 lacZ183/ recA1* Δ(*pro-lac*) $_{XIII}$ *rpsE xyl mtl*	K. B. Low
NH4104	F(42) *lac*⁺/*uvrA6 proA2 leu-8 thi thr-4 ara-14 lac-1 his*	K. B. Low

[a] The *sbcC201* genotype of N2351 was confirmed by the fact that when a P1 lysate grown on this strain was used to transduce JC7623 to Tet^r 200 of 200 transductants examined were *phoR* but none was MC^s.

[b] IN, Inversion.

[c] CGSG, *E. coli* Genetic Stock Center, Yale University, New Haven, Conn.

Laboratories, Cambridge Biotechnology Laboratories, or Amersham International and were used as directed by the suppliers. Plasmids were maintained in strain AB2463 (*recA13*), and plasmid DNA for transformations and restriction analysis was prepared by the rapid alkaline-sodium dodecyl sulfate lysis procedure of Ish-Horowicz and Burke (16). pBR322 DNA was purified by centrifugation in cesium chloride-ethidium bromide density gradients for use in cloning experiments. DNA restriction fragments were resolved by electrophoresis on 0.6 to 0.8% (wt/vol) agarose slab gels.

RESULTS

Identification and genetic location of *sbcC*. Mutations in *recB* or *recC* that abolish the activity of RecBC nuclease reduce the efficiency of recombinant formation in conjugational and transductional crosses, increase sensitivity to UV light, ionizing radiation, and mitomycin C, and lead to the segregation of inviable cells (lethal sectoring) in liquid culture (5, 14, 44). Strains JC7618, JC7621, and JC7623 (Table 1) are recombination-proficient derivatives of a *recB21 recC22* double mutant (JC5519 [3, 19]) selected on the basis of their resistance to mitomycin C (3, 19). The suppression of *recBC* in these strains has, until now, been attributed to a

mutation of *sbcB* since introduction of *sbcB*$^+$ restores the mutant phenotype (19, 41). However, we have found that when these strains are crossed with Hfr strain KL226 about 50% of the Pro$^+$ (*rpsL*) recombinants selected after short matings are MC^s. Since the Hfr does not transfer *sbcB* or *recBC* as early markers (Fig. 1) and is itself MC^r, the segregation of MC^s recombinants suggested the possibility of a second suppressor mutation in this genetic background.

A series of further matings and P1 transductions (Tables 2 and 3; Fig. 2) established that the locus segregating in these crosses lies between *proC* and *phoR* (Fig. 1) and that the allele responsible for sensitivity to mitomycin C is present in all of the *sbcB*$^+$ strains tested, including AB1157, AB2470, and JC5489, the genetic background to the immediate *recB21 recC22* ancestor of JC7618, JC7621, and JC7623 (19). From these results, it is clear that the MC^r phenotype of strains JC7618, JC7621, and JC7623 must be due to the combined effects of two suppressor mutations, one in *sbcB* and one in a new gene which we designate *sbcC*. In the case of JC7618, the distinction between *sbcC*$^+$ and *sbcC* derivatives is not as clear as it is with JC7621 (Fig. 2) or JC7623. We have no explanation for this difference other than to suggest that it may reflect the properties of different *sbcC* mutations.

The *sbcC* suppressor mutation appears to be restricted to the *sbcB* class of MC^r revertants of a *recBC* strain. No MC^s segregants were observed among the Pro$^+$ recombinants from matings with Hfr KL226 when the recipient was the *recBC sbcA* strain JC8679. Presumably, the *sbcA* mutation alone is sufficient in this strain to confer a fully MC^r phenotype.

Effect of *sbcC* on growth and viability. The data presented in Table 4, part a, show that *sbcC*$^+$ (MC^s) derivatives of strains JC7621 and JC7623 grow more slowly than the isogenic *sbcC* derivatives. Broth cultures of these strains contain cells that are unusually variable in length, with some 10 to 20% growing into filaments approximately 5 to 20 times the normal size. In addition, up to 70% of the cells present failed to form colonies on either LB agar or 56/2 agar plates. The colonies formed on LB agar in particular are distinctly

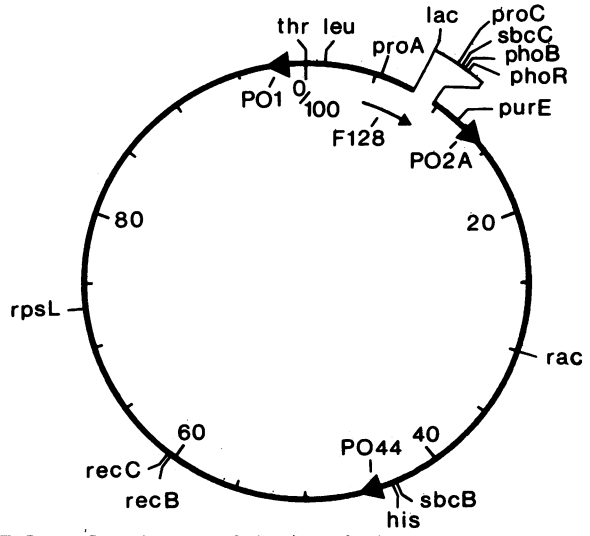

FIG. 1. Genetic map of the *E. coli* chromosome showing the location of relevant genes. The *lac-phoR* region is expanded for ease of presentation and shows *sbcC* at minute 9 on the basis of the current map (2). Points of origin (PO) and directions of transfer of the Hfr strains used are indicated by arrowheads.

TABLE 2. Segregation of MC^s progeny in crosses with *recBC sbcB* recipients

Donor	Recipient	Selection	No. analyzed	% MC^s
(a) Conjugations				
KL226	JC7623	Pro$^+$	350	44.5
KL226	JC7621	Pro$^+$	150	48.0
KL226	JC7618	Pro$^+$	140	65.7
W1895	JC7621	Pro$^+$	48	77.1
χ342 (*proC*)	N2239 (JC7621 *pro*$^+$)	Thr$^+$ Leu$^+$	448	20.0[a]
χ342 (*proC*)	N2255 (JC7623 *pro*$^+$)	Thr$^+$ Leu$^+$	250	21.2[a]
(b) Transductions				
N3005 (*purE*::Tn*10*)	JC7623	Tet^r	100	0
N3033 (*lacZ*::Tn*10*)	JC7623	Tet^r	100	2.0
K797 (*phoR*::Tn*10*)	JC7623	Tet^r	221	78.3
K797 (*phoR*::Tn*10*)	JC7618	Tet^r	100	90.0
K797 (*phoR*::Tn*10*)	JC7621	Tet^r	300	67.3
K797 (*phoR*::Tn*10*)	AB1157 (*rec*$^+$ *sbc*$^+$)	Tet^r	50	0
W3110	N2271 (JC7623 *proC*)	Pro$^+$	200	76.0
KL226	N2271 (JC7623 *proC*)	Pro$^+$	200	72.0
AB1157	N2271 (JC7623 *proC*)	Pro$^+$	100	77.0
JC7623	N2271 (JC7623 *proC*)	Pro$^+$	100	0
JC7621	N2271 (JC7623 *proC*)	Pro$^+$	100	0
W3110	N2273 (JC7621 *proC*)	Pro$^+$	200	78.5
KL226	N2273 (JC7621 *proC*)	Pro$^+$	200	74.5
AB1157	N2273 (JC7621 *proC*)	Pro$^+$	300	72.0
AB2470	N2273 (JC7621 *proC*)	Pro$^+$	100	70.0
JC5489	N2273 (JC7621 *proC*)	Pro$^+$	100	74.0
KL226	N1349 (*recB sbcB*)	Pro$^+$	100	80.0

[a] All of the MC^s recombinants were Pro$^-$.

smaller than those of *sbcC* mutants and tend to have ragged edges, presumably as a result of lethal sectoring. A typical feature of the growth on LB agar is the frequent appearance of an MC^r variant that forms large colonies and which is indistinguishable from *recBC sbcB sbcC* strains. Genetic analysis (data not shown) revealed that these fast-growing variants have indeed regained a mutation in *sbcC*. The growth advantage of the *sbcC* variant means that it accumulates very rapidly during serial subculture of *recBC sbcB* strains in LB broth (Table 5). Cultures of *recBC sbcB* strains grown in LB broth were therefore streaked routinely on LB agar, and only those giving low numbers of fast-growing colonies of *sbcC* variants were used in experiments.

During the course of these studies we noticed that the MC^s phenotype of *recBC sbcB* strains as detected on LB agar could be alleviated to some extent by increasing the sodium chloride concentration to 10 g/liter. However, this was due to a general improvement in growth, and the strains remained MC^s relative to *recBC sbcB sbcC* or *rec*$^+$ strains grown under the same (high-salt) conditions, although higher concentrations of mitomycin C were needed to see the

TABLE 3. Mapping of *sbcC* by three-factor transductional crosses[a]

Recipient (*proC sbcC*)	Selected marker	No. tested	% Inheritance of unselected markers							
			sbcC$^+$ *phoR*$^+$	*sbcC*$^+$ *phoR*	*sbcC phoR*$^+$	*sbcC phoR*	*sbcC*$^+$ *proC*$^+$	*sbcC*$^+$ *proC*	*sbcC proC*$^+$	*sbcC proC*
N2271	*proC*$^+$	500	14.2	33.8	49.6	2.4				
N2273	*proC*$^+$	300	33.7	34.7	31.3	0.3				
N2271	*phoR*::Tn*10*	222					59.0	8.1	4.1	28.8
N2273	*phoR*::Tn*10*	428					65.4	9.6	5.4	19.6

[a] The P1 donor was K797 (*phoR*::Tn*10 sbcC*$^+$). *phoR*::Tn*10* was selected on LB agar plus tetracycline. All Tet transductants were *phoR* as judged by their blue growth on 56/2 agar containing Xp. *sbcC*$^+$ progeny were scored by their MC^s phenotype.

difference clearly. *sbcC* derivatives of *recBC sbcB* strains were still detectable on high-salt LB agar as fast-growing (large colony) variants.

Deletion of *sbcC*. The fact that the *proC-phoR* interval (Fig. 1) is devoid of genes essential for growth enabled us to devise a test to see if a MC^r phenotype could be restored to *recBC sbcB* strains by deletion of *sbcC*. For this purpose, we used strains carrying Tn*10* inserted in *phoR*. The presence of Tn*10* should increase the incidence of deletion (17). Its insertion in *phoR* provides the additional advantage that the colonies formed on Xp agar are deep blue in color due to the constitutive synthesis of alkaline phosphatase (4, 42). Deletions arising from the Tn*10* insertion and extending into *proC* would eliminate *phoB* and therefore should give rise to alkaline phosphatase-negative (white) colonies (4, 42).

Several single colonies of the *recBC sbcB pro*$^+$ *sbcC*$^+$ *phoR*::Tn*10* strains N2312 and N2317 were restreaked on LB agar. As usual, MC^r colonies of fast-growing variants were obtained at a high frequency. Of 66 independent variants of strain N2312 examined, 22 were blue on Xp plates and Pro$^+$, while 42 were white, of which 2 were Pro$^-$. Similarly, of 64 MC^r variants of strain N2317, 16 were blue and Pro$^+$, while 48 were white, of which 9 were Pro$^-$. The strains that remain blue on Xp plates probably carry point mutations in *sbcC* since they retain the parental phenotype in other respects. We assume that the Pro$^-$ derivatives carry *phoR-proC* deletions. The remaining MC^r derivatives presumably carry deletions through *phoB* that extend at least as far as *sbcC* (Fig. 1). From these results, we conclude that mutations in *sbcC* improve viability and restore resistance to mitomycin C by inactivating the *sbcC* product rather than by changing its function.

Recombination in *recBC sbcB sbcC*$^+$ strains. The data presented in Table 4, part a, show that introduction of *sbcC*$^+$ into *recBC sbcB sbcC* mutants reduces their capacity for recombination in crosses with Hfr donors. However, the strains remain considerably more proficient in recombination than a *recB* single mutant. From these results it is clear that mutation of *sbcB* alone significantly improves the recombination efficiency of a *recBC* mutant. But it is equally clear that the additional mutation of *sbcC* is necessary to allow the high level of recombination that is associated with strains JC7621 and JC7623. Similarly, transductional recombination in *recBC sbcB* strains was very inefficient compared with *recBC sbcB sbcC* strains (Table 6).

Reconstruction of *recBC sbcB* and *recBC sbcB sbcC* strains. The data presented so far demonstrate that strains JC7618, JC7621, and JC7623, which were selected as MC^r revertants of a *recB recC* double mutant, have acquired two additional mutations, one in *sbcB* and another in *sbcC*. These mutations probably arose in that order since although *recBC sbcB* strains are MC^s they readily segregate MC^r (*sbcC*) variants. If this explanation for the origin of these MC^r revertants is correct, then it should be possible to reconstruct similar strains by suitable combination of the same four mutations.

We first constructed *rec*$^+$, *recB21*, and *recC22* strains in *sbcC*$^+$ (AB1157) and *sbcC201* (N2351) genetic backgrounds. By comparing the six constructs, we were able to conclude that mutation of *sbcC* has no effect on recombination in a *rec*$^+$ strain and does not suppress the recombination defi-

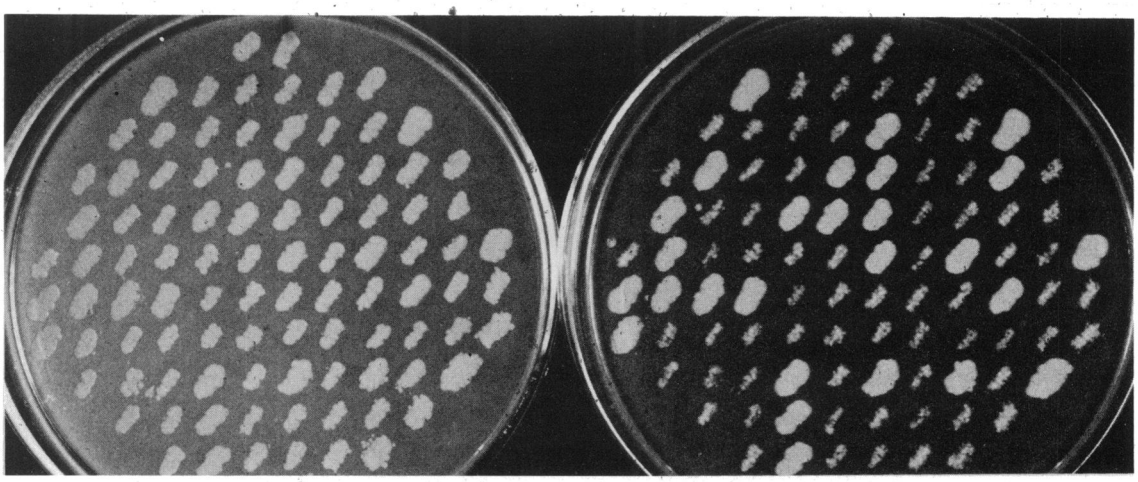

FIG. 2. Photograph showing MC^s progeny from a P1.K797 × JC7621 cross. Pro$^+$ transductant colonies were inoculated in a regular array on supplemented 56/2 agar (selective for Pro$^+$) and incubated for 18 h before replica plating on LB agar with (right) or without (left) mitomycin C at a final concentration of 0.2 μg/ml in the plate agar. The replica plates were incubated for a further 20 h before being photographed.

TABLE 4. Effect of *sbcC* on growth rate, viability, and recombination[a]

Strain no.	Relevant genotype or plasmid	Growth rate (min)	Viability	Relative number of transconjugants			
				× KL548	× KL226	× AB259	× GY2200
(a) AB1157	*rec*$^+$ *sbc*$^+$	25	0.98	1.0	1.0	1.0	1.0
AB2470	*recB*		0.29	0.27	0.0018	0.0016	0.89
N2215	*recBC sbcB sbcC*		0.58	0.67	0.43	0.58	0.54
N2216	*recBC sbcB*		0.27	0.22	0.036	0.031	0.92
N2308	*recBC sbcB sbcC*	34	0.52	0.56	0.51	0.47	0.58
N2309	*recBC sbcB*	48	0.28	0.26	0.059	0.071	0.58
N2314	*recBC sbcB sbcC*	38	0.57	0.58	0.50	0.72	0.95
N2315	*recBC sbcB*	56	0.27	0.22	0.017	0.029	1.10
(b) AB1157	pACYC184	35		1.0	1.0	1.0	
AB1157	pJP71			1.86	2.86	3.50	
AB1157	pJP77	30		1.83	2.82	3.52	
JC7623	pACYC184	43		1.0	1.0	1.0	
JC7623	pJP71		0.26	0.14	0.0072	0.0058	
JC7623	pJP77	70	0.31	0.19	0.0060	0.0098	
JC7621	pJP71		0.19	0.097	0.013	0.0012	
JC7621	pJP77		0.23	0.14	0.0096	0.0098	

[a] Viability was measured in the cultures used for matings and is the fraction of the total cells able to form colonies on LB agar. Similar values were obtained with 56/2 agar. For matings, donor and recipient strains were grown to approximately 2×10^8 cells per ml before mixing in a ratio of 1:10. Mating was for 30 (KL548), 40 (KL226, AB259), or 60 (GY2200) min, and selection was for Pro$^+$ (KL548, KL226), Thr$^+$ Leu$^+$ (AB259), or λ infective (zygotic induction) (GY2200) centers. Transconjugant yields with AB1157 were 1.6×10^7 (KL548), 5.7×10^6 (KL226), 7.3×10^6 (AB259), or 4.5×10^6 (GY2200) per ml of the mating mixtures. These values were two- and four-fold-lower respectively with the AB1157 and JC7623 pACYC184 derivatives. All values are averages of two to five independent matings.

ciency of *recB* or *recC* mutants (Table 7, part a). Furthermore, the *recB sbcC* and *recC sbcC* double mutants remained just as sensitive to mitomycin C as the *recB* and *recC* single mutants.

We next attempted to introduce *sbcB15* into each of these strains by mating with Hfr strain JC9223. As a control we crossed JC9223 with N2306, a *recBC sbcC sbcB*$^+$ derivative of JC7623. His$^+$ (*rpsL*) recombinants were selected after a mating of 30-min duration. (The Rac effect [see references 10 and 21] in this cross increases the yield of recombinants obtained with *recBC* receipients.) All of the recombinants obtained with the *rec*$^+$ recipients N2361 and N2364 that were tested were MC^r. In the control cross with N2306, 94% of the recombinants were just as resistant. This indicated that these had inherited *sbcB15*, as was to be expected from the proximity of *sbcB* to *his* (Fig. 1). Similarily, 96% of the recombinants obtained with the *recB sbcC* and *recC sbcC* strains N2365 and N2366 had a fully MC^r phenotype, which implies that these too had inherited *sbcB15*.

In contrast, all of the recombinants obtained with the *recB* and *recC* single mutants N2362 and N2363 appeared to be MC^s. However, on closer examination about 80% of these were seen to be slightly more resistant than the others on plates containing the lower (0.2 μg/ml) of the two mitomycin C concentrations used in the test. If these were the recombinants that had inherited *sbcB15*, then on the basis of previous experience with known *recBC sbcB* strains they should segregate MC^r variants due to mutation of *sbcC*. Ten independent His$^+$ recombinants of both N2362 and N2363 with this very weakly resistant phenotype were purified, and a single colony from each was streaked on LB agar. In every case, some large colonies appeared against a background of small colonies. Furthermore, when some of the small colonies were restreaked they also gave large-colony variants. All of these large-colony variants were MC^r. No such variants were observed on plates streaked with the His$^+$ recombinants initially scored as fully MC^s clones. Two of the MC^r variants, one derived from N2362 and one from N2363 (N2380 and N2381, respectively) were mated with Hfr KL226. In each case, 50% of the Pro$^+$ (*rpsL*) recombinants obtained regained a MC^s phenotype.

From these observations we conclude that the weakly MC^r recombinants of N2362 and N2363 isolated initially from the cross with Hfr JC9223 had indeed inherited *sbcB15* and that these were able to segregate fully resistant variants by further mutation of *sbcC*. This conclusion is supported by the relative recombination proficiency of the various strains

TABLE 5. Accumulation of *sbcC* mutants in subcultures of *recBC sbcB* strains[a]

Expt	% Fast-growing *sbcC* mutants	
	N2309 (*recBC sbcB15*)	N2315 (*recBC sbcB13*)
1	5.9	1.0
2	17.2	4.4
3	51.7	0.3
4	12.8	15.3
5	18.1	3.3

[a] For each experiment a single colony was inoculated into LB broth, and the culture was grown to saturation overnight (approximately 2×10^9 cells per ml). A sample was diluted 40-fold in fresh broth and grown to a cell density of 2×10^8 per ml before various dilutions were plated on LB agar. After incubation overnight, large colonies of MC^r cells were scored as *sbcC* mutants.

TABLE 6. Effect of *sbcC* on transduction[a]

Recipient strain	Pro$^+$ transductants per 10^9 PFU
N2271 (*recBC sbcB sbcC*)	1,104
N2272 (*recBC sbcB*)	23
N2273 (*recBC sbcB sbcC*)	1,949
N2275 (*recBC sbcB*)	59

[a] The P1 donor was AB1157.

TABLE 7. Recombination in reconstructed strains carrying combinations of *recB*, *recC*, *sbcB*, and *sbcC* mutations[a]

Strain no.	Relevant genotype	Relative no. of transconjugants		
		× KL548	× KL226	× AB259
(a) N2361	*rec*$^+$ *sbc*$^+$	1.0	1.0	1.0
N2362	*recB21*	0.28	0.0012	0.0009
N2363	*recC22*	0.25	0.0067	0.0065
N2364	*sbcC201*	0.84	0.78	0.73
N2365	*recB21 sbcC201*	0.16	0.0003	0.0003
N2366	*recC22 sbcC201*	0.20	0.0013	0.0051
(b) N2375	*rec*$^+$ *sbcC*$^+$ *sbcB*?	1.0	1.0	
N2376	*recB21*	0.16	0.0016	
N2377	*recC22*	0.24	0.0056	
N2378	*recB21 sbcB15*	0.17	0.029	
N2379	*recC22 sbcB15*	0.21	0.048	
N2380	*recB21 sbcB15 sbcC205*	0.48	0.62	
N2381	*recC22 sbcB15 sbcC206*	0.57	0.63	

[a] Mating times and transconjugants selected are as in Table 4, part a. The yields of transconjugants for N2361 and N2375 were approximately the same as those for AB1157 in Table 4, part a.

constructed (Table 7, part b). The *recB21 sbcB15 sbcC201* and *recC22 sbcB15 sbcC201* strains constructed were as recombination proficient as the spontaneous *sbcC* segregants N2380 and N2381 (data not shown).

These reconstructions support the explanation given above for the original derivation of strains JC7618, JC7621, and JC7623 as MC^r revertants of a *recB21 recC22* double mutant. Since *recBC sbcB* strains are slightly resistant to mitomycin C, *sbcB* mutants of the *recBC* ancestor (JC5519) would have been able to grow a little on the mitomycin C agar used in the original selection (3, 19). This would have provided an opportunity for further mutation of *sbcC*, particularly since mitomycin C is a mutagen, and allowed for the outgrowth of MC^r colonies.

Identification of the cloned *sbcC*$^+$ gene. The *proC-phoR* region of the chromosome has been cloned into the pACYC184 vector (42) to give plasmids pJP71 and pJP77 (Fig. 3). These plasmids should therefore carry *sbcC*$^+$. Since mutation of *sbcC* appears to exert its effect by inactivating the gene product, *sbcC*$^+$ should be dominant to *sbcC*, and pJP71 or pJP77 derivatives of strains JC7621 and JC7623 should therefore resemble the *sbcC*$^+$ recombinants obtained from genetic crosses. This appears to be the case. The plasmid strains were MC^s, grew very slowly, were deficient in recombination, and in liquid culture accumulated inviable cells and 20 to 30% long filaments (Table 4, part b). pACYC184 also reduced the growth rates and increased sensitivity to mitomycin C, but the effects were very modest and were not associated with reduced recombination efficiency. The pJP71 and pJP77 derivatives segregated plasmid-free clones very rapidly in the absence of chloramphenicol. Apart from their Cam^s phenotype, these were detectable on LB agar as large colonies of MC^r cells. Colonies of this type were rare (<0.01%) as long as chloramphenicol selection was maintained. We conclude that pJP71 and pJP77 both carry *sbcC*$^+$.

pJP71 and pJP77 derivatives of strains JC7621 and JC7623 were UV sensitive whereas *sbcC*$^+$ recombinants (plasmid-free) were not (Table 8), except in the sense that growth of the irradiated cells was more severely retarded than usual. This difference suggests that increased synthesis of the *sbcC* product has an antagonistic effect on the survival of the irradiated *recBC sbcB* cells. We also noted that the pACYC184 vector increased UV sensitivity, but the effect

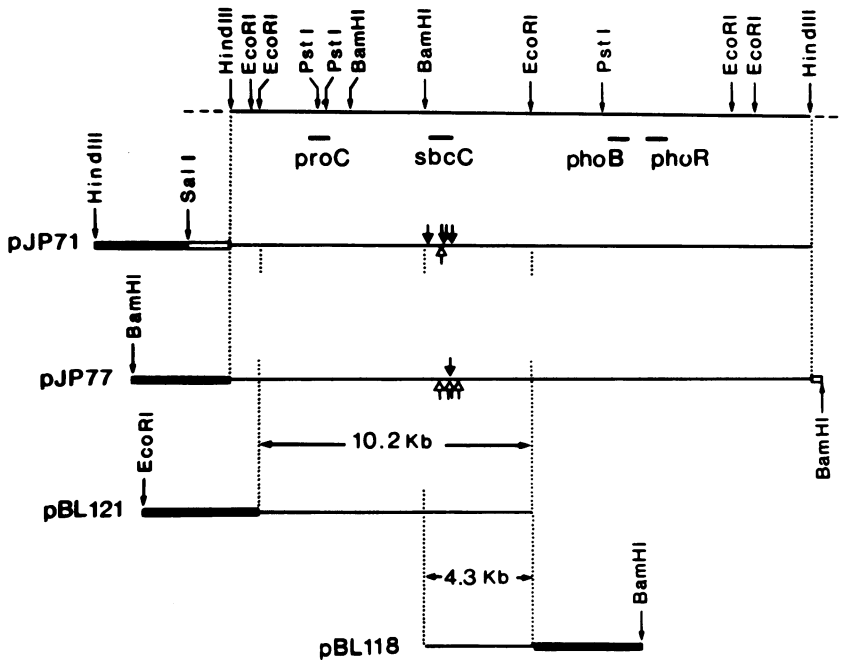

FIG. 3. Restriction map of the *proC-phoR* region of the *E. coli* chromosome (top line) and recombinant plasmids carrying *sbcC*$^+$. The map is compiled from the data of Hadley et al. (13), Tommassen et al. (42), and this work. The vector plasmids are indicated by heavy lines, and only those restriction sites used for inserting chromosomal DNA are shown. Open boxes in pJP71 and pJP77 are noncontiguous fragments of chromosomal DNA (42). The Tn*1000* insertions that define *sbcC* are in the γδ orientation (↓) or the δγ orientation (↑). pBL120 (not shown) is the same as pBL121 except that the 10.2-kb insert is in the reverse orientation.

TABLE 8. Survival of UV-irradiated *sbcC*$^+$ and *sbcC* strains[a]

Strain no.	Relevant genotype or plasmid	Relative no. of surviving colonies after a UV dose (J m^{-2}) of:				
		0	5	10	20	60
N2215	*recBC sbcB sbcC*	1.0	0.95		0.27	0.12
N2216	*recBC sbcB*	1.0	0.66		0.23	0.09
N2308	*recBC sbcB sbcC*	1.0	0.99		0.65	0.29
N2309	*recBC sbcB*	1.0	0.55		0.43	0.18
N2314	*recBC sbcB sbcC*	1.0	0.71		0.21	0.064
N2315	*recBC sbcB*	1.0	0.41		0.14	0.05
AB1157	pACYC184	1.0	0.95	0.32	0.10	
AB1157	pJP71	1.0	0.9	0.23	0.07	
AB1157	pJP77	1.0	0.69	0.29	0.14	
JC7623	pACYC184	1.0	0.69	0.15	0.05	
JC7623	pJP71	1.0	0.0073	0.0029	0.0004	
JC7623	pJP77	1.0	0.0067	0.0033	0.0016	
JC7621	pJP71	1.0	0.008	0.0004	0.0002	
JC7621	pJP77	1.0	0.0067	0.0002		

[a] Cells were irradiated on the surface of LB agar plates, and the colonies of survivors were scored after 18 h, except for *sbcC*$^+$ strains and *sbcC* mutants carrying pJP71 or pJP77, for which the colonies on irradiated plates grew very slowly and required incubation for 48 h before they could be scored. Plasmid strains were maintained at all times in the presence of chloramphenicol.

was much less and was observed with both wild-type and *recBC sbcB sbcC* strains.

The location of *sbcC* in the pJP plasmids was determined by using the Tn*1000* (γδ) mutagenesis procedure of Guyer (12). pJP71 and pJP77 were transformed into the F *lac* strain NH4104, and independent Cam^r transformants were used as donors in matings with strain JC7623. Plasmids mobilized as a result of cointegrate formation and which carried Tn*1000* insertions that inactivate *sbcC* were expected to allow expression of the *sbcC* mutant phenotype of the recipient. Approximately 25% of the Cam^r (*rpsL*) transconjugants selected on LB agar formed large colonies rather like the fast-growing *sbcC* variants of *recBC sbcB* strains. Plasmids extracted from these large colony variants were transformed into the *recA* mutant AB2463, and the Cam^r transformants were used to provide DNA for further analysis. The plasmids from 10 independent isolates were cleaved with *Eco*RI and *Bam*HI in single and double digests, and the fragments were analyzed by electrophoresis. Each plasmid contained an insertion equivalent to the 5.7 kilobases (kb) of Tn*1000*. The insertion sites spanned a 0.9-kb section of DNA within the 4.3-kb *Bam*HI-*Eco*RI fragment of the chromosomal insert (Fig. 3) which defines a possible location for the *sbcC* coding region. This would agree with the mapping of *sbcC* to the *proC-phoR* interval. However, it is possible that the insertions define some locus other than *sbcC* whose inactivation improves plasmid stability. Three of the pJP77 mutant derivatives were therefore transformed into JC7623. The transformants grew with a doubling time of 50 to 52 min in LB broth, and the cultures contained cells which showed very little evidence of filamentation, which suggests that the plasmid *sbcC* gene is inactive. JC7623 tranformed with a pJP77 derivative carrying Tn*1000* inserted in the 4.7-kb *Eco*RI fragment (Fig. 1) grew with a doubling time of 75 min, and 20 to 30% of the cells were long filaments.

The 10.2-kb *Eco*RI fragment carrying *proC* and *sbcC* was cloned from pJP71 into the *Eco*RI site of pBR322 in both orientations to give the recombinants pBL120 and pBL121 (Fig. 3). A further plasmid. pBL118, carrying *sbcC* was constructed by replacing the small *Bam*HI-*Eco*RI fragment of pBR322 with the 4.3-kb *Bam*HI-*Eco*RI fragment from pJP71. While these plasmids were stable in strains AB2463 and AB1157, we were unable to obtain transformants with JC7623 or JC7621, although plasmid uptake appeared reasonably efficient since we could detect a good yield of abortive colonies on plates selective for Amp^r. There was no difficulty in obtaining normal transformant colonies with the pBR322 vector. Since the presence of multiple copies of *sbcC*$^+$ has been shown to reduce the growth rate of these strains, and the copy number of pBR322 is much higher than that of the pACYC184 vector, we assume that the inability to recover stable transformants of *recBC sbcB* strains is at least partly due to the further amplification of the *sbcC* product. However, a more detailed analysis of *sbcC* will be necessary to confirm this conclusion and eliminate the possibility that an adjacent gene or DNA sequence may be deleterious beyond a certain number of copies.

DISCUSSION

The results presented demonstrate that *recB recC sbcB* strains such as JC7623 and other similar recombination-proficient derivatives of *recB recC* double mutants isolated on the basis of their resistance to mitomycin C are in fact *recB recC sbcB sbcC* quadruple mutants. We showed that the presence of the *sbcC* mutation in these strains is essential if the *sbcB* mutation is to fully suppress the mitomycin C sensitivity, lethal sectoring, and recombination deficiency otherwise associated with the *recBC* genotype. Reconstruction experiments revealed that mutation of *sbcC* has no obvious effect on the phenotype of *rec*$^+$, *recB*, or *recC* strains, from which it is clear that mutation of *sbcB* is the critical factor needed for the restoration of recombination and DNA repair activity in the absence of the RecBC enzyme, at least in strains lacking the Rac prophage. Nevertheless, current models (7, 27, 28) based on what were considered to be studies with *recBC sbcB* strains will have to be revised to accommodate *sbcC*.

The discovery that suppression of the mitomycin C sensitivity of *recBC* strains depends on mutation of *sbcC* as well as *sbcB* provides an explanation for the original observation (3, 41) made with *recB* or *recC* single mutants in the AB1157 (Rac$^-$) genetic background that the only recombination-proficient derivatives obtained by direct selection for MC^r colonies were *rec*$^+$ revertants. Similarily, it explains why almost all of the MC^r colonies selected from a *recB recC* double mutant lysogenic for the cryptic Rac phage were *sbcA* mutants (3, 41). In both cases the events observed were those requiring a single mutation rather than the two needed to give the *sbcB* (*sbcB sbcC*) class of revertant.

A further corollary to this work is that it explains why previous attempts by one of us to construct *recB sbcB* strains by genetic crosses (R. G. Lloyd, unpublished data) were the cause of some concern in that they failed to generate progeny with the expected phenotype. One exception gave the ancestor (N1349) to strain N1394 (22). N1349 has now been shown to carry a mutation of *sbcC* (Table 2, part b) and presumably arose as a fast-growing segregant of a *recB sbcB* recombinant from the initial cross. This ability of constructed *recB sbcB* strains to segregate fast-growing variants has also been reported recently by Masters et al. (31). The authors also noted that these variants were much more proficient in transductional recombination than the slow-growing *recB sbcB* strain.

The observations reported here provide few clues as to the possible function of the *sbcC* product. Recombination in strains established herein as *recBC sbcB sbcC* mutants is known to require activation of *recN* and *ruv*, both of which

specify functions normally regulated by the LexA protein as components of the SOS response (26, 28, 43). Increased synthesis of the 60,000-dalton RecN protein, which has been implicated in the repair of DNA double-strand breaks (33; S. M. Picksley and R. G. Lloyd, unpublished data), seems particularly vital in this respect (34). It is possible that the *sbcC*$^+$ product interferes with some such inducible activity by limiting gene expression or by inhibiting the gene product. This might be consistent with the observation that while *recBC sbcB* (*sbcC*$^+$) strains are sensitive to mitomycin C they are resistant to UV light. Another possibility arising from the filamentation of *recBC sbcB* (*sbcC*$^+$) strains is that mutation of *sbcB* increases expression of the SOS-induced inhibitor of cell division specified by *sulA* (43) and that mutation of *sbcC* helps to reduce the steady-state level of this inhibitor. Accordingly, cell division in *recBC sbcB* strains may be more vulnerable to inhibition as a result of damage to DNA or the transfer of single-stranded DNA during conjugation. However, this hypothesis would predict that *sulA* mutations should be able to act like *sbcC* suppressor mutations. We observed no such mutations during the course of these studies. Further analysis of *sbcC* and investigation of the cloned gene product should help to provide answers to these and other questions raised as a result of this work.

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