GENETIC ANALYSIS OF MUTATIONS INDIRECTLY SUPPRESSING *recB* AND *recC* MUTATIONS[1]

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ABSTRACT

Mutations in *sbcB* inactivate exonuclease I and suppress the UV-sensitive, mitomycin-sensitive, recombination-deficient phenotypes associated with *recB* and *recC* mutations. Mapping experiments have located *sbcB* about 0.4 minutes from the *his* operon at 38.0 on the standard map of *E. coli*. This places *sbcB* between *supD* and *his*. A four-point cross shows that *sbcB* lies between P2 *attH* and *his*. P2 eduction deleting the *his* operon beginning with P2 *attH* also deletes *sbcB* and produces the expected exonuclease I deficiency and suppression of *recB*$^-$. The occurrence of chemical-mutagen-induced and spontaneous mutations indirectly suppressing *recB*$^-$ and *recC*$^-$ is examined. Three lines of strains produce only *sbcA* mutations while only *sbcB* mutations occur in a fourth line. Explanations for this behavior are proposed in light of the ability of the first three lines to express *sbcB* mutations which they inherit by transduction.

MUTATIONS in the *recA* gene of *Escherichia coli* K12 virtually abolish the ability to perform recombination following conjugation or transduction (CLARK 1967). In crosses with various Hfr strains progeny formed by recombination arise from zygotes to which the *recA*$^+$ allele has been transferred (CLARK 1967; WILLETTS, CLARK and Low 1969). Progeny formed from zygotes not receiving the *recA*$^+$ allele are most often formed by repliconation; i.e., by inheriting autonomous F′ elements (Low 1968). *recB* or *recC* mutants, on the other hand, perform substantial amounts of residual recombination even though the conjugational zygotes have not received the *recB*$^+$ or *recC*$^+$ alleles (CLARK 1967; Low 1968).

Since this residual recombination might result from a pathway of recombination alternative to that involving *recB* and *recC*, revertants of *recB* and *recC* mutants were examined for indirect suppression. Two different kinds of indirect suppression were found. The first appeared to result from the appearance of an ATP-independent DNAase produced by mutations called *sbcA*$^-$ (BARBOUR *et al.* 1970). The second resulted from the loss of exonuclease I activity produced by mutations called *sbcB*$^-$ (KUSHNER *et al.* 1971). In a previous publication (KUSH-

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TABLE 1

*Characteristics of bacterial strains**

Strain Number	*recB*	*recC*	*sbcB*	*str*	*arg*	*his*	*thr*	*leu*	*trp*	*ilv*	*pro*	*thy*	*thi*	*met*	Other markers	Reference
1. F⁻ strains																
AB1157	+	+	+	31	E3	4	1	6	+	+	A2	+	1	+		KUSHNER *et al.* 1971
JC5519	21	22	+	31	E3	4	1	6	+	+	A2	+	1	+		WILLETTS and CLARK 1969
JC7617	21	22	9	31	E3	4	1	6	+	+	A2	+	1	+		KUSHNER *et al.* 1971
JC7619	21	22	11	31	E3	4	1	6	+	+	A2	+	1	+		KUSHNER *et al.* 1971
JC7620	21	22	12	31	E3	4	1	6	+	+	A2	+	1	+		KUSHNER *et al.* 1971
JC7623	21	22	15	31	E3	4	1	6	+	+	A2	+	1	+		KUSHNER *et al.* 1971
JC7644	21	22	15	31	E3	+	1	6	+	+	A2	+	1	+		KUSHNER *et al.* 1971
JC4693	+	+	+	321	+	318	+	—	E9829	+	+	+	—	+	*supD*+	KUSHNER *et al.* 1971
JC7735	21	+	+	321	+	+	+	—	E9829	+	+	+	—	+	*supD*⁻	This paper
2. Hfr strains																
KL98	+	+	+	S	+	+	+	+	+	+	+	+	—	+	PO53	gift of BROOKS LOW
JC5491	21	22	+	S	+	+	300	+	+	318	+	+	—	+	PO45	WILLETTS and CLARK 1969
AB259	+	+	+	S	+	+	+	+	+	+	+	+	—	+	PO1	Standard Hayes Hfr
KL96B	+	+	+	S	+	+	+	+	+	+	+	+	—	+	*supD*⁻, PO44	HOFFMAN and WILHELM 1970
JC7756	+	+	+	S	+	+	+	+	+	+	+	+	—	+	*supD*⁻, PO44	P2 lysogen of KL96B
3. F′ strain																
JC2429	+	+	+	309	G6	A323/1	+	+	+	+	+	+	+	B1	*recA1*, F30	BASTARRACHEA and CLARK 1968
JC7705	+	+	15	31	E3	A323/4	1	+	+	+	A2	282	1	+	F30	This paper

* Gene symbols are those used by TAYLOR (1970). Point of origin designations (PO53 etc.) were obtained from B. BACHMANN.

NER *et al.* 1971) we indicated that *sbcB* mutations were 30–50% cotransducible with *his* and that *sbcB*$^+$ was carried by the F30 episome. In this work we provide data which indicate the location of *sbcB* relative to *his* and flanking markers and a method of producing *sbcB* deletion mutations. We also discuss the occurrence of *sbcA* mutations.

MATERIALS AND METHODS

Bacterial strains: The strains used in this study are all derivatives of *E. coli* K12 and are listed in Table 1. Map positions of the relevant genetic markers are shown in Figure 1. P2 phage was obtained from Dr. MELVIN SUNSHINE.

Nomenclature conforms to the recommendations of DEMEREC *et al.* (1966) except that the minus sign is used with the gene symbol to indicate a general mutant allele when the specific allele number is not required. The gene symbols are those recorded by TAYLOR (1970) with the exception of *sbc*, which stands for a set of genes involved in the indirect suppression of *recB21* or *recC22*. Phenotypic abbreviations are as follows: Rec, recombination; UV, ultraviolet irradiation; Mit, mitomycin; His, histidine; Trp, tryptophan; Leu, leucine; Pro, proline; TL, threonine leucine; Arg, arginine; IV, isoleucine-valine; DES, diethylsulfate; "—", requiring when used with abbreviations of amino acids and deficient when used with Rec; "+", independence when

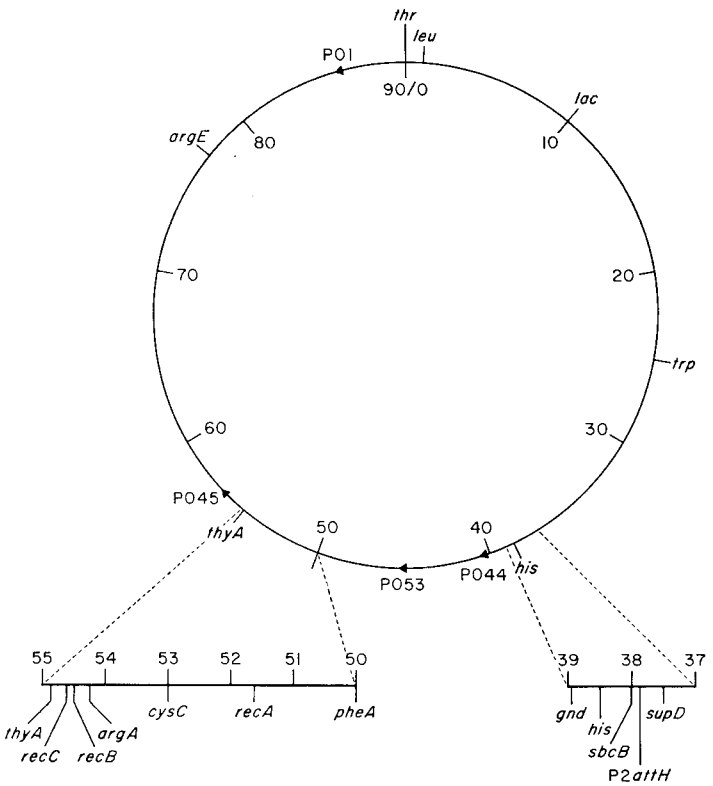

FIGURE 1.—Linkage map of *Escherichia coli* K12 showing genetic markers relevant to this work. The map is according to TAYLOR (1970) and gene symbols conform to DEMEREC *et al.* (1960). Points of origin of Hfr strains are as cited by BARBARA BACHMANN (personal communication).

used with abbreviations of amino acids and proficient when used with Rec; R, resistance; S, sensitivity.

Methods: The procedures and media for conjugational and transductional crosses and for the discrimination of Rec$^-$ from Rec$^+$ strains have been described previously (CLARK 1967; CLARK and MARGULIES 1965; WILLETTS, CLARK and LOW 1969). Acridine curing was performed as described by BASTARRACHEA and WILLETTS (1968). Enzyme assays for exonuclease I were performed as described by KUSHNER *et al.* (1971) and for both exonuclease V, and the ATP-independent nuclease appearing in *sbcA*$^-$ strains as described by BARBOUR and CLARK (1970). Thymine-requiring mutants were isolated by the use of trimethoprim (STACEY and SIMSON 1965).

Diethysulfate (DES) treatment: A saturated solution of DES was made by shaking 0.1 ml DES in 5 ml of minimal medium lacking a carbon source and allowing the undissolved DES to settle. 0.1 ml of a fully grown L broth culture of the strain to be mutagenized was inoculated into the mixture without removing the DES. After 15 min incubation at 37°C an aliquot of 0.1 ml was removed, added to 10 ml of L broth and shaken overnight at 37°C. Appropriate dilutions were plated on supplemented minimal media.

Eduction: P2 treatment to obtain His$^-$ eductants was performed as described by SUNSHINE and KELLEY (1971). When *sbcB*$^-$ eductants were desired the method was modified by plating appropriate dilutions on Luria agar containing mitomycin C at a concentration of 1.0 microgram per ml rather than on the low histidine media used to detect the His$^-$ eductants.

Detection of sbcB$^-$ *and* recB$^-$ *mutations:* The presence of *sbcB*$^-$ mutations in a *rec*$^+$ strain was tested for by growing P1 on the strain in question and using the lysate to transduce a *his*$^-$ *recB*$^-$ *recC*$^-$*sbcB*$^-$ recipient to His$^+$. Cotransduction of *sbcB*$^-$ with *his*$^+$ caused no change in the UV^{R}Mit^{R}Rec$^+$ phenotype of the recipient. The presence of *reB*$^-$ in a strain containing *sbcB*$^-$ was tested for by growing P1 on the strain and using the lysate to transduce an *argA*$^-$ *rec*$^+$ *sbcB*$^+$ recipient to Arg$^+$. Cotransduction of *recB*$^-$ with *argA*$^+$ yielded a UV^{S}Mit^{S}Rec$^-$ phenotype.

RESULTS

Location of sbcB: The strains which carry the *sbcB* mutations are multiply marked and are of genotype *recB*$^-$ *recC*$^-$ *sbcB*$^-$. Phenotypically these strains are Rec$^+$ and give rise to Rec$^-$ progeny by inheritance of the *sbcB*$^+$ allele. Since the *recB* and *recC* cistrons are near *thyA* (EMMERSON and HOWARD-FLANDERS 1967; WILLETTS and MOUNT 1969), we can locate the *sbcB* gene on the chromosome by crossing the triple mutant with an appropriate Hfr, selecting for inheritance of widely spaced markers and screening for those which become Rec$^-$ because of their unselected inheritance of *sbcB*$^+$. The Hfr JC5491 is appropriate because it carries the *recB21* and *recC22* mutations and transfers them one to two min from its point of origin PO45 (Figure 1) and because it is Rec$^-$ by virtue of its nonsuppressed (i.e. *sbc*$^+$) genotype. When His$^+$ [IV$^+$] recombinants sired by JC5491 were selected about 70% were found to have inherited the Rec$^-$ phenotype. Since the recipients were Rec$^+$ phenotypically but *recB*$^-$*recC*$^-$*sbcB*$^-$ genotypically, the only way they could inherit the Rec$^-$ phenotype was by inheriting *sbcB*$^+$ from the donor. In parallel crosses of the Hfr KL98, which is *recB*$^+$*recC*$^+$ but transfers these cistrons late in conjugation, and our *recB*$^-$*recC*$^-$*sbcB*$^-$ strains we found between 65% and 82% coinheritance of *sbcB*$^+$ with *his*$^+$.

The strong linkage between *his* and *sbcB* observed in conjugational crosses led us to suspect that cotransduction of the two might be observed. AB259 was assumed to be a suitable wild-type, i.e. *his*$^+$ *sbcB*$^+$, strain. P1 grown on this donor strain were used to transduce four *his*$^-$*sbcB*$^-$*recB*$^-$*recC*$^-$ strains to His$^+$ and Pro$^+$. As shown in Table 2 between 51% and 62% of the His$^+$ transductants had

TABLE 2

Cotransduction of* sbcB+ *with* his+

Recipient	Genotype	Transduction frequency† His+	Pro+	Number tested His+	Pro+	Number UV^S Mit^S Rec$^-$ His+	Pro+	Percent *sbcB*+ cotransduction His+	Pro+
AB1157	*recB*+ *recC*+ *sbcB*+	6.2	10.4	100	91	0	0	—	—
JC5519	*recB21 recC22 sbcB*+	0.3	<0.04	8	—	8	—	—	—
JC7617	*recB21 recC22 sbcB9*	7.6	13.0	100	97	51	0	51	<1
JC7619	*recB21 recC22 sbcB11*	4.7	5.2	100	98	52	0	52	<1
JC7620	*recB21 recC22 sbcB12*	3.8	4.9	94	86	57	0	60	<1
JC7623	*recB21 recC22 sbcB15*	12.0	6.8	100	95	62	0	62	<1

* P1 virus was grown on AB259 (*his*+ *sbcB*+); the transduction was performed as described in WILLETTS, CLARK and LOW (1969).

† Number of transductants per 10^6 input phage.

inherited the *sbcB*+ allele as judged by their Rec$^-$ Mit^S and UV^S behavior. No Rec$^-$ UV^S could be found among the approximately 100 Pro+ transductants per strain tested indicating no detectable cotransduction with *pro* and ruling out various hypotheses to explain the Rec$^-$His+ transductants which do not assume cotransduction.

To determine whether *sbcB* lies to the right or left of *his*, we resorted to a series of transductional crosses using the *supD* marker already mapped on the right side of *his* (HOFFMAN and WILHELM 1970). *supD* suppresses certain amber mutations, among which are a *trp* amber mutation and a *leu* amber mutation present in a Su$^-$Sm^R strain JC4693. The *leu* amber mutation in JC4693 was detectably suppressed by *supD*$^-$ only in the presence of streptomycin but this did not prevent our selection for *supD*$^-$ by selecting for Trp+Leu+ transductants. JC4693 was *recB*+ *sbcB*+ however so we first constructed a *recB*$^-$*sbcB*$^-$ derivative in three steps: (1) selection of a *thyA*$^-$ spontaneous mutant by the trimethoprim method (STACEY and SIMSON 1965), (2) cotransduction of *sbcB15* with *his*+ from a *his*+*sbcB15* donor, JC7644, made by transduction of *his*+ into our primary *sbcB15* mutant strain, and (3) cotransduction of *recB21* with *thyA*+. The resulting strain, JC7722, had all the desired properties for use in our mapping experiments except that it was *his*+. Consequently a *his*$^-$ mutant was obtained by the use of DES and tests were made to ensure that the *his* mutation derived was not suppressible by *supD*$^-$ and that the strain retained both *sbcB15* and *recB21*.

The resulting strain, JC7729, was used as a recipient in a transductional cross with the *his*+*sbcB*+*supD*$^-$ strain KL96b as a donor. *his*+ inheritance was detected by the His+ phenotype, *supD*$^-$ inheritance by the Trp+Leu+ phenotype and *sbcB*+ inheritance by the Rec$^-$ phenotype. When His+ transductants were selected 35% inherited *sbcB*+ and 10% inherited *supD*$^-$ indicating that *sbcB* is closer to *his* than *supD* is to *his* but not that *sbcB* and *supD* are on the same side of *his*. When Trp+Leu+ (i.e. *supD*$^-$) transductants were selected an average of 5.5% (102/1869) inherited *sbcB*+ and 3.9% (74/1869) inherited *his*+ indicating that *sbcB* is closer to *supD* than *his* is to *supD* and the order is *his–sbcB–supD*.

To measure more closely the location of *sbcB* between *his* and *supD* we used

TABLE 3

Cotransduction frequencies obtained in four-point transductional crosses

Selected marker*	Selected phenotype	Unselected donor markers				Percent *sbcB*+ among (P2)+ recombinants
		his+	*sbcB*+	(P2)+	*supD*-	
his+	His+	—	51%	48%	2%	81%
supD-	Trp+ Leu+	2%	6%	26%	—	16%

* P1 was grown on JC7756 which is *his*+ *sbcB*+ (P2)+ *supD*-. JC7729 was the recipient and was *his*- *sbcB*- (P2)- *supD*+. In addition JC7729 carried *recB21* and an amber mutation in both *trpE* and *leu*. Forty-nine transductants of each genotype were tested.

one of the P2 attachment sites (P2 *attH*) which lies between *his* and *supD*. A P2 lysogen of KL96b was isolated and the location of the prophage near *his* was verified. This strain JC7756 was used as a transductional donor with JC7729, the same recipient we used above. Both His+ and Trp+Leu+ transductants were selected. Forty-nine of each were streaked and single colonies were tested for unselected characters. Table 3 shows that the results are in agreement with the order *his–sbcB–*(P2)*–supD*. The four-point cross when analyzed as a series of two-point crosses shows *sbcB*+ more closely linked to *his* than the P2 prophage and less closely linked to *supD* than the P2 prophage. When analyzed as three-point crosses then 81% of *his*+ (P2)+ transductants inherit *sbcB*+ while only 16% of (P2)+ *supD*- transductants inherit *sbcB*+. Thus it is clear that *sbcB* lies between *his* and the P2 attachment site (Figure 1).

Deletion of sbcB: The location of *sbcB* has been confirmed by making use of the phenomenon of P2 eduction. SUNSHINE and KELLY (1971) have found that strains of *E. coli* K12 lysogenic for P2 at P2 *attH* spontaneously give rise to a small fraction of non-lysogenic, histidine-requiring cells. The deleted portion of the chromosome always starts with P2 prophage on one end while the other end is located beyond the *gnd* locus. The process is termed eduction and the resulting deletion-containing strains are called eductants. If *sbcB*+ does lie between the P2 attachment site and *his* as indicated by our transductional studies then eductants should have lost the *sbcB*+ gene.

To test this prediction we made P2 eductants from JC7735 which has the genotype *his*+*sbcB*+*recB21 supD*-*leu*- amber *trp*- amber and the phenotype His+ Rec-Mit^{S}Leu+Trp+. Advantage was taken of the fact that one does not need to start with P2 lysogens to obtain eductants but can isolate them from the bacterial growth in the center of P2 plaques. P2 *old*- was used since the *old*+ product kills *recB*- or *recC*- strains (LINDAHL *et al.* 1970).

The results show that 14 of 4500 colonies tested were His- eductants; all 14 were Mit^R as expected if the *sbcB*+ gene had also been deleted. At the same time Mit^R (i e. *sbcB*-) eductants were selected directly by plating on complex media containing mitomycin C. Of 392 Mit^R colonies picked all were His-Mit^{R}UV^R Trp+Leu+. Thus P2 eductants have lost *his* and *sbcB* but not *supD*.

Enzyme assays for exonuclease I were performed on two eductants, JC7749 selected as His- and JC7750 selected as Mit^R and both were found to be exonuclease I deficient. These two strains were also tested for their recombination

ability in crosses with the Hfr JC158. Both produced as many Lac$^+$ [Ser$^+$Sm^R] recombinants as did their *rec*$^+$ *sbcB*$^+$ ancestor, JC4693, and its *recB21 sbcB15* derivative, JC7729.

Effect of episome carrying sbcB: Because *sbcB* is so closely linked to *his* it seemed likely that the *sbcB* gene might be present on F*his* plasmids. F30 carries *hisA323* which complements the *his-4* mutation in the AB1157 series of strains. F30 was transferred into the *rec*$^+$ and *rec*$^-$ strains and into a series of suppressed *rec*$^-$ strains by crossing with an appropriate F30 donor, JC2429. The presence of another *sbc*$^+$ gene does not affect the phenotype of either the *recB*$^+$*recC*$^+$*sbcB*$^+$ or *recB*$^-$*recC*$^-$*sbcB*$^+$ strains. However, *recB*$^-$*recC*$^-$*sbcB*$^-$ strains are converted to a UV^{s}Mit^{s}Rec$^-$ phenotype indicating both that *sbcB*$^+$ is present on the episome and that it is dominant. *sbcB9*, *sbcB11*, *sbcB12* and *sbcB15* were all found to be recessive by this method.

Nonspecificity of suppression: Since *sbcB* mutations have been obtained only in strains which are *recB21 recC*$^+$ or *recB21 recC22* it is possible that these combinations of *rec* alleles are the only ones which can be suppressed by *sbcB*$^-$. This possibility was tested by constructing strains which contain other *recB* alleles and an *sbcB*$^-$ gene. P1 lysates on strains which were *recB58* or *recB60* were used to transduce a *thyA*$^-$*his*$^-$*sbcB15* strain which also contained the F30 episome. The resulting Thy$^+$ transductants which had received *recB*$^-$ were phenotypically UV^{s}Mit^{s}Rec$^-$ because of the presence of the dominant *sbcB*$^+$ gene on the episome. When the episome was eliminated by use of acridine orange, the phenotypes became UV^{R}Mit^{R}Rec$^+$ showing that *recB58* and *recB60* are suppressed by *sbcB15*. Transfer of F30 back into the cured *recB*$^-$ *sbcB15* strains converts them to the UV^{s}Mit^{s}Rec$^-$ phenotype. In the same fashion a *recB*$^+$*recC22 sbcB15* strain was constructed. Its phenotype is UV^{R}Mit^{R}Rec$^+$ indicating that *recC22* is suppressed by *sbcB15*.

Occurrence of suppressor mutations: Mutations causing indirect suppression of the *recB* and *recC* genes have been obtained in strains of at least four different genetic backgrounds. We can characterize the strains and their backgrounds as follows: (1) Endo I$^-$ to stand for a line carrying an *endA* mutation and consequently endonuclease I deficient (DURWALD and HOFFMANN-BERLING 1968), (2) Su$^-$ to stand for a line carrying amber *leu* and *trp* mutations in the absence of amber-suppressing alleles, (3) Hfr to stand for a line derived from the Hfr JC5029, and (4) 1157 to stand for a line derived from the multiply auxotrophic F$^-$ AB1157. Table 4 summarizes the data concerning the occurrence of backmutations and suppressor mutations among Mit^R revertants selected from these strains with or without exposure to a mutagen. Single *recB21* or *recC22* derivatives of the Endo I genetic background were previously shown to revert to Mit^R by indirect suppression (BARBOUR *et al.* 1970), and here we indicate that the same is true for *recB*$^-$*recC*$^+$ derivatives in the Su$^-$ and Hfr background. By contrast single *recB21* or *recC22* derivatives with the 1157 background revert chiefly by backmutation. When Mit^R revertants of a *recB21 recC22* derivative with the 1157 background are selected only suppressed revertants occur and all of these carry an *sbcB* mutation which cotranduces with *his* and cause exonuclease I deficiency

TABLE 4

Occurrence of suppressor mutations and backmutations in Mit^R revertants of recB21 *and* recC22 *derivatives of four different genetic backgrounds*

Genetic background	*rec* allele *recB*	*recC*	Number of revertants tested	Genotype of Mit^R revertants* *recB*$^+$ *recC*$^+$	*sbcA*$^-$	*sbcB*$^-$
Endo I$^-$	—	+	2†	0	2	0
	+	—	2†	0	2	0
	+	—	2†	0	2	0
Su$^-$	—	+	6‡	1	5	0
Hfr	—	+	1§	0	1	0
	—	—	7‖	0	7	0
1157	—	+	3¶	2	0	1
	+	—	3¶	3	0	0
	—	—	7‖	0	0	7

* The presence of *recB*, *recC*, *sbcA* and *sbcB* mutations was tested for genetically and enzymatically.
† One of each pair of revertants was induced by EMS; the other, by NTG.
‡ All of these mutants were induced by EMS.
§ Spontaneous mutant.
‖ One revertant was spontaneous; the others were induced by EMS.
¶ One revertant was spontaneous; one was induced by EMS and one by NTG.

(KUSHNER *et al.* 1971). By contrast all indirectly suppressed Mit^R revertants isolated from strains with the Endo I$^-$, Su$^-$ or Hfr backgrounds carry an *sbcA* mutation which does not cotransduce with *his* and results in high levels of ATP-independent nuclease activity. Thus there is a clear difference between the 1157 strains and the strains with three other genetic backgrounds both in the type of suppressor which occurs and in the frequency of suppressor mutations as opposed to backmutations.

DISCUSSIONS

Three- and four-point transductional crosses place the *sbcB* gene between *his* and the *E. coli* K12 P2 *attH*. The distance from *his* to *sbcB* can be calculated by means of a formula relating cotransductional frequency and distance as follows: c.f. $= (1 - d/l)^3$ where c.f. is the cotransductional frequency expressed as a fraction, l is the length of the transducing fragment expressed in whatever units are convenient and d is the distance between selected and unselected marker (WU 1966). We assume that l is 6.5×10^7 daltons (the size of the P1 chromosome) or 2.3 min of distance as the *E. coli* map is generally related to its chromosome size of 2.5×10^9 daltons. Then by averaging the cotransduction frequencies of *sbcB* to *his* shown in Table 2 (0.56) we calculate that the distance between these two markers is about 0.4 min.

This location has been confirmed by the isolation of eductants in which the chromosomal region from P2 *attH* through *gnd* is deleted. This deletion invariably takes out both the *his* operon and *sbcB* thus making eductants both His$^-$ and Exo I$^-$. The deletions also invariably suppress the UV^{S}Mit^S phenotypes conferred by *recB21*. Two of the eductants were tested and found to be Rec$^+$ indicating

suppression of the Rec⁻ phenotype as well. This result confirms the hypothesis of KUSHNER *et al.* (1971) that the absence of exonuclease I rather than the presence of a mutationally altered enzyme is responsible for the suppression of the *recB* mutation. Recently we reported the existence of *xonA* mutations inactivating exonuclease I and lying very close to *sbcB* (KUSHNER, NAGAISHI and CLARK 1972). The phenotype of *recB⁻recC⁻xonA⁻* strains is $UV^{R}Mit^{R}Rec^{-}$ indicating that *xonA* mutations suppress the UV^{S} and Mit^{S} of *recB⁻recC⁻* but not their Rec⁻ property. By contrast *sbcB* mutations suppress all three phenotypes. Since the eductants tested were Rec⁺ as well as $Mit^{R}UV^{R}$ they must genetically be *sbcB⁻xonA⁻* or *sbcB⁻xonA⁺*. At present we can not determine which.

Considerable evidence has been adduced to demonstrate indirect suppression of *recB* and *recC* mutations by both *sbcA* and *sbcB* mutations (BARBOUR *et al.* 1970; KUSHNER *et al.* 1971; TEMPLIN and CLARK, unpublished results). Intragenic suppression is ruled out by the occurrence of both types of suppressors in *recB⁻recC⁻* double mutants and by the lack of cotransduction between the original mutations and their suppressors. Informational suppression is ruled out by the lack of detectable suppression of UAG, UAA or UGA mutant phages. Furthermore exonuclease V activity determined by *recB* and *recC* is not restored in either *sbcA* or *sbcB* mutants, a fact incompatible with either intragenic or informational suppression. In this paper we show that one *recC* and three *recB* mutations are suppressed by *sbcB15* demonstrating the nonallele-specific behavior of the indirect suppression.

sbcA and *sbcB* mutations appear to suppress *recB* and *recC* mutations by different means. *sbcA⁻* strains show higher levels of an ATP-independent nuclease than shown by *sbcA⁺* strains (BARBOUR *et al.* 1970); in addition *sbcA⁻* strains contain exonuclease I (KUSHNER *et al.* 1971). *sbcB⁻* strains do not show higher levels of ATP-independent nuclease activity than *sbcB⁺* strains; in addition *sbcB⁻* strains do not contain exonuclease I (KUSHNER *et al.* 1971). This difference led us to expect that the suppressed mutants isolated from any given strain would fall into both *sbcA* and *sbcB* groups. Instead when we have looked for spontaneously occurring or mutagen-induced revertants, we have found no *sbcA* mutants from strains of the 1157 genetic background and no *sbcB* mutants from strains of Endo I⁻, Su⁻, and Hfr background. One possibility to explain this is that *sbcB* mutations would be unexpressed in the three genetic backgrounds in which they do not appear to occur as primary mutants. We have, however, shown previously (KUSHNER *et al.* 1971) that *sbcB15* transduced to an Endo I⁻, a Su⁻ and an Hfr strain confers exonuclease I deficiency on these three strains. Another possibility is that the absence of exonuclease I in these three genetic backgrounds would not indirectly suppress *recB* or *recC* mutations. In this paper we have shown however that *sbcB15* when transduced into the Su⁻ background can suppress *recB21* and we have used this suppression in order to map the *sbcB* mutation relative to the *his* operon and P2 *attH*. Furthermore we have shown that *sbcB* deletion mutations which suppress *recB21* occur in the Su⁻ background by a process known as P2 eduction.

It is still possible that the Endo I⁻, Su⁻, and Hfr strains we have used all carry

a tandem duplication of *sbcB*$^+$ which is lost by transduction from the 1157 strains which do not carry this duplication. This, however, does not explain why *sbcA* mutations apparently fail to occur in the 1157 background. Since the *sbcA* mutation may be looked upon as a regulatory mutation derepressing a structural gene, *recE*, which determines an ATP-independent nuclease activity (CLARK 1971a,b), the lack of occurrence of *sbcA* mutations in the 1157 line of strains may therefore indicate that these strains are *recE*$^-$.

In fact there is a highly speculative line of reasoning which indicates that the 1157 series of strains may be *sbcA*$^-$ as well as *recE*$^-$. This speculation depends upon the behavior of the gene *rac*$^+$ which is located near *trp* (TAYLOR 1970) and was discovered by Low (personal communication). *rac*$^+$ when transferred to zygotes of *recB*$^-$ or *recC*$^-$ derivatives of the 1157 line suppressed the recombination deficiency of these zygotes and permitted nearly wild-type frequencies of recombinants for distal markers to be produced. When *rac*$^+$ is transferred to zygotes of *recB* or *recC* mutants of other genetic backgrounds the activation of recombination is not observed (Low, personal communication). This suggests that the *rac*$^+$ gene can be zygotically derepressed in the 1157 line only, and not in other lines of strains. These other lines may be *sbcA*$^+$ like the Endo I$^-$, Su$^-$, and Hfr lines we have used. If we hypothesize that *rac* and *recE* are two symbols for the same gene, then we expect that *rac* would be repressed by *sbcA*$^+$. Since only the 1157 lines allows zygotic derepression of *rac* we would then expect the 1157 line to be *sbcA*$^-$.

Thus we feel that the 1157 line of strains may be both *sbcA*$^-$ and *recE*$^-$ (i.e. *rac*$^-$) while other strains may be *sbcA*$^+$*recE*$^+$. If true this would fit quite well with Low's hypothesis (personal communication) that *rac* occurs on an active or cryptic prophage. Presumably the regulator gene for *rac* would also occur on the same prophage; hence we can explain the genotype of 1157 strains by hypothesizing that they lack this crucial prophage. Since lambdoid phages carry a *redX* gene which determines an ATP-independent exonuclease involved in phage recombination (SHULMAN *et al.* 1970) it is therefore attractive to suspect that the *recE* (i.e. *rac*) nuclease presumably derepressed by *sbcA* mutations will be found to possess substrate specificities and activities similar to those of the lambda exonuclease. This is even more attractive when it is recognized that the lambda exonuclease will catalyze bacterial recombination (Low, personal communication) and that the *recE* enzyme will apparently catalyze lambda recombination (UNGER, ECHOLS and CLARK 1972).

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