# Physical Analysis of Spontaneous and Mutagen-Induced Mutants of *Escherichia coli* K-12 Expressing DNA Exonuclease VIII Activity

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Manuscript received January 25, 1989
Accepted for publication February 28, 1990

ABSTRACT

We have mapped the extents of two deletion *sbcA* mutations which result in production of DNA exonuclease VIII (ExoVIII). One mutation, *sbcA8*, deletes about 140 kb of DNA which includes most of the Rac prophage and the *trg* gene. Western blot analysis shows that the protein produced is larger than wild type ExoVIII. The nucleotide sequence shows that a translational gene fusion has occurred. The N-terminal 294 codons of *recE* have been deleted and the remaining C-terminal codons have been fused to the N-terminal portion of another reading frame we call *sfcA*. Analysis of the protein sequence encoded by *sfcA* shows an 83% similarity with rat and mouse NADP-linked malic enzyme. We discuss the possibility that *sfcA* is identical to *maeA* which encodes NAD-linked malic enzyme from *Escherichia coli*. Restriction nuclease analysis of a second deletion, *sbcA81*, by Southern blot technique indicates that about 105 kb of DNA have been deleted and a transcriptional gene fusion has occurred between *recE* and the regulatory region of an *E. coli* chromosomal gene. We also examined eight other *sbc* mutations that result in ExoVIII production. Five have no effect on restriction nucleotide fragment sizes detected by complementarity to λ rev as probe. These are presumed point mutations. Three seem to produce additional restriction nucleotide fragments complementary to λ rev. The possible nature of these *sbc* mutations is discussed.

MUTATIONS suppressing *recB21* and/or *recC22*, called *sbc* mutations, have been detected in four different genetic backgrounds of *Escherichia coli* K-12. Such backgrounds can be nicknamed to avoid writing the complete genotype each time they are referred to. In this case the backgrounds were designated EndoI$^-$, Hfr, Su$^-$ and 1157 (TEMPLIN, KUSHNER and CLARK 1972). In the first three genetic backgrounds the *sbc* mutations lead to detectable levels of exonuclease VIII (ExoVIII), an ATP-independent DNA exonuclease not found in the original strains (BARBOUR *et al.* 1970; TEMPLIN, KUSHNER and CLARK 1972). The authors interpreted this to indicate that the structural gene for ExoVIII (called *recE*) had become expressed as a result of the suppressor mutation. In the fourth background (1157) *sbc* mutations do not result in *recE* expression. KAISER and MURRAY (1979) found that several strains with the 1157 background lacked a 27-kb element of DNA present in the other backgrounds. Where it is present, this element is partly complementary to and can recombine with phage lambda (GOTTESMAN *et al.* 1974; KAISER and MURRAY 1979). The element comprises the region from 29.6 to 30.2 on the *E. coli* genetic map (FOUTS *et al.* 1983; WILLIS *et al.* 1983), and is the defective prophage Rac (LOW 1973). Rac carries the *recE* gene (GILLEN *et al.* 1977; KAISER and MURRAY 1979) and *oriJ* (DIAZ and PRITCHARD 1978; DIAZ, BARNSLEY and PRITCHARD 1979). Thus the 1157 background carries a deletion mutation which removes *recE* and hence cannot host *recE*-expressing suppressor mutations.

Because the *sbc* suppressors fell into the *recE*-expressing and nonexpressing groups, TEMPLIN, KUSHNER and CLARK (1972) called the first *sbcA* and the second *sbcB*. *sbcB* was found to be the structural gene for DNA exonuclease I (KUSHNER, NAGAISHI and CLARK, 1972, 1974). *sbcA*, however, has not been so easy to characterize because different types of mutations lead to expression of *recE*, possibly by different mechanisms. Of five *sbcA* mutations examined, KAISER and MURRAY (1980) found that two, *sbcA1* and *sbcA23*, showed no change in Rac detectable by the Southern blot method and presumed them to be point mutations. *sbcA1* was later found to be cis-acting and dominant to *sbcA*$^+$ (FOUTS *et al.* 1983) indicating the possibility that it alters an operator or promoter for *recE*. One mutation, *sbcA8*, consisted of a large deletion of most of Rac, including *attR* and the immunity repressor region (KAISER and MURRAY 1980). Because the leftward promoter and operator, presumed to control *recE* transcription by analogy with phage λ, were also absent, KAISER and MURRAY (1980) hypothesized that

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*sbcA8* fused *recE* to a promoter from a gene outside Rac. To explain recessiveness of *sbcA8* to *sbcA*$^+$ (LLOYD and BARBOUR 1974), KAISER and MURRAY (1980) further hypothesized that an operator and a repressor gene controlled the promoter to which *recE* was fused. Furthermore, they hypothesized that *sbcA8* had deleted the repressor gene. Two final mutations, *sbcA50* and *sbcA51* were associated with *Eco*RI nuclease digestion fragments of Rac which could have been derived from tandemly arranged multiple copies of Rac, either on the chromosome or in the cytoplasm, or from monomeric circular copies of excised prophage (KAISER and MURRAY 1980). Additional unusual *Eco*RI fragments of Rac made it seem possible that small deletions occurred in some of the copies of Rac in the *sbcA51* strain (KAISER and MURRAY 1980). The physical nature of *sbcA50* and *sbcA51* could not be conclusively determined partly because of the numerous copies of Rac apparently present and partly because neither *sbcA*$^+$ strain from which the two mutants were derived was examined.

In this paper we extend the studies of KAISER and MURRAY (1980) by determining that *sbcA8* is a translational fusion of part of *recE* with part of another gene approximately 140 kb distant. We also determine the characteristics of another deletion, *sbcA81*, and hypothesize that this produces a transcriptional fusion of *recA* to a promoter about 105 kb distant. Additionally we examine the Rac prophage in mutants carrying *sbcA1*, *sbcA23*, and six other *sbcA* mutations not examined by KAISER and MURRAY (1980). Finally we discuss the appropriateness of labeling as *sbcA* all mutations which suppress *recB* and *recC* mutations and lead to ExoVIII activity.

## MATERIALS AND METHODS

**Bacterial strains, abbreviations and general genetic methods:** All bacterial strains analyzed in this work are derivatives of *Escherichia coli* K-12. The derivations and relevant genotypic markers of most are listed in Table 1. Genotype symbols are those used by BACHMANN (1983). Question marks indicate that allele and plasmid numbers have not been stated or that there is a question about the presence of an allele. We also used DH1, *recA1*, *endA1* *gyrA96* *thi-1* *hsdR17* *supE44* *relA1*(?) F$^-$λ$^-$ (MANIATIS, FRITSCH and SAMBROOK 1982) and JM103, de (*lac-proAB*) *thi-?* *supE?* *rpsL?* *endA?* *sbcB15* *hsdR4* [F? *traD36* *proAB*$^+$ *lac*$^q$ *de*(*lacZ*)M15], [P1] (MESSING 1983). Tet abbreviates tetracycline and MitC abbreviates mitomycin C. Superscripts R and S indicate resistance and sensitivity respectively. Transduction procedures were described by WILLETTS and MOUNT (1969). λrev Sam7 cI857 (GOTTESMAN *et al.* 1974) was obtained from M. GOTTESMAN as lysogen N2262.

**Isolation of bacterial DNA and detection of restriction fragments complementary to λrev DNA:** Isolation of bacterial chromosomal DNA, *in vitro* labeling of λrev with $^{32}$P, conditions for restriction endonuclease digestion of chromosomal DNA, and methods for the separation of fragments of agarose gel electrophoresis were as previously described (GILLEN, WILLIS and CLARK 1981). Restriction fragments were transferred to nitrocellulose filter paper by the procedure of SMITH and SUMMERS (1980). Hybridization of radioactive λrev probe DNA to the immobilized restriction fragments was by the method of WAHL, STERN and STARK (1979).

**Sequencing the endpoints of *sbcA8*:** Samples of 1.8 μg chromosomal DNA from JC5412 (*sbcA8*) and 1.2 μg pBR322 DNA were digested separately with *Hin*dIII, mixed, treated with T4 DNA ligase and used to transform JC5519 (*recB21recC22*) to resistance to ampicillin (50 μg/ml) and mitomycin (1 μg/ml). Six transformants were obtained of which one, JC15901, was stably MitC$^R$ UV$^R$ and Rec$^+$. This transformant contained a 12.4-kb plasmid having an 8.1-kb *Hin*dIII fragment inserted into the *Hin*dIII site of pBR322. The plasmid was named pSKM1. pSKM2 was constructed by digesting pSKM1 DNA with *Eco*RI and *Bam*HI, treating the mixture with ligase and using it to transform DH1 to ampicillin resistance. Subsequent restriction enzyme analysis of plasmid DNA from five of the transformants revealed one 5.2 kb plasmid which contained a 1.2 kb *Eco*RI-*Bam*HI fragment inserted between the *Eco*RI and *Bam*HI sites of pBR322. The strain containing pSKM2 was named JC15903.

pSKM1 and pSKM2 were used as sources of DNA to make M13 bacteriophage strains for sequencing (Figure 1a). Most phages were made by digesting plasmid DNA with one or two restriction nucleases, adding the mixture to appropriately digested phage DNA, treating the mixture with ligase, transfecting JM103 with the mixture and screening surviving cells in colorless plaques for the desired phage by restriction digests of DNA minipreps. Techniques used were those of MESSING (1983). Enzymes used were those whose sites mark the termini of cloned fragments (Figure 1a). Phages JCM15911 to JCM15916 were derived from pSKM1 and M13 mp8. Phages JCM15919 to JCM15923 were derived from pSKM2 and M13 mp8. JCM15927 was constructed from pSKM2 and M13 mp9. Finally, phages JCM15658 and JCM15659 were derived respectively from gel-isolated 505 bp and 684 bp *Bam*HI-*Eco*RI fragments of JCM15914 RF DNA and M13 mp8.

Template ssDNA generated from the above phages was employed in the chain termination method (SANGER *et al.* 1977) to obtain the sequence. The extents of each insert actually sequenced are illustrated in Figure 1b. The universal primer was used except where noted. Three oligonucleotide primers were also used. prJC22 has the sequence 5′ ACGATCGGCAATGCCCAT 3′. It has two mismatches to 5′ ACCGATCGGAATGCCCAT 3′ from coordinates 1000 to 983 of the final sequence because it was designed with reference to an earlier inaccurate version of the sequence. prJC23 has the sequence 5′ TTGAGGAGTTAAAATGAA 3′ corresponding to coordinates 172 to 189 and prJC27 has the sequence 5′ TCTGCTTTATACTTG 3′ corresponding to coordinates 389 to 403 of the final sequence.

## RESULTS

**Deletion mutations:** KAISER and MURRAY (1980) showed that an *sbcA8* strain JC5412 carried a deletion of most of Rac. We have confirmed their observation and have also determined the amount of Rac deleted by *sbcA81* in strain JC9625. Figure 2a shows a Southern blot of DNA from each mutant and its wild type ancestor digested with *Eco*RI. Southern blots of *Hin*dIII-cut DNA and *Bam*HI-cut DNA are shown respectively in Figure 3a and Figure 3b. λrev DNA

**TABLE 1**

**Bacterial strains**

| Strain | Sex | Genetic background | Relevant genotype | | | Reference |
|---|---|---|---|---|---|---|
| | | | *sbcA* | RAC | Other | |
| AB1157 | F$^-$ | 1157 | Deletion | − | —[a] | BACHMANN (1972) |
| JC5519 | F$^-$ | 1157 | Deletion | − | —[b] | WILLETTS and CLARK (1969) |
| JD8675 | F$^-$ | 1157 | *23* | + | —[c] | GILLEN, WILLIS and CLARK (1981) |
| JC8679 | F$^-$ | 1157 | *23* | + | —[c] | GILLEN, WILLIS and CLARK (1981) |
| JC4695 | F$^-$ | Su$^-$ | + | + | —[d] | TEMPLIN, KUSHNER and CLARK (1972) |
| JC7608 | F$^-$ | Su$^-$ | *19* | + | —[d] | TEMPLIN, KUSHNER and CLARK (1972) |
| JC7609 | F$^-$ | Su$^-$ | *20* | + | —[d] | TEMPLIN, KUSHNER and CLARK (1972) |
| JC9625 | F$^-$ | Su$^-$ | *81* | de-81[e] | —[d] | GILLEN (1974) |
| KL16 | Hfr(P045) | Hfr | + | + | —[f] | BACHMANN (1972) |
| JC5029 | Hfr(P045) | Hfr | + | + | —[g] | BACHMANN (1972) |
| JC5412 | Hfr(P045) | Hfr | *8* | de-8[e] | —[h] | BACHMANN (1972) |
| JC5491 | Hfr(P045) | Hfr | + | + | —[i] | WILLETTS and CLARK (1969) |
| JC7659 | Hfr(P045) | Hfr | *21* | + | —[i] | TEMPLIN, KUSHNER and CLARK (1972) |
| JC7660 | Hfr(P045) | Hfr | *22* | + | —[i] | TEMPLIN, KUSHNER and CLARK (1972) |
| JC7661 | Hfr(P045) | Hfr | *23* | + | —[i] | TEMPLIN, KUSHNER and CLARK (1972) |
| JC5172 | F$^-$ | EndoI$^-$ | *2* | + | —[j] | BARBOUR *et al.* (1970) |
| JC5174 | F$^-$ | EndoI$^-$ | *1* | + | —[j] | BARBOUR *et al.* (1970) |
| JC5176 | F$^-$ | EndoI$^-$ | *6* | + | —[k] | BARBOUR *et al.* (1970) |
| JC6720 | F$^-$ | EndoI$^-$ | + | + | —[k] | CAPALDO-KIMBALL and BARBOUR (1971) |
| JC6722 | F$^-$ | EndoI$^-$ | + | + | —[j] | CAPALDO-KIMBALL and BARBOUR (1971) |

[a] The genotype of AB1157 is *thr-1 ara-14 leuB6 proA2 lacY1 tsx-33 supE44 galK2 his-4 rpsL31* (Sm$^R$) *mtl-1 xyl-5 argE3 thi-1.*
[b] Same as AB1157 except *recB21 recC22.*
[c] Same as JC5519 except JC8675 is *his$^+$ recE$^+$* and JC8679 is *his-328 recE$^+$.*
[d] The mutant genotypes of JC4695 and its derivatives are *his-318 leu-307 trpE9829 rpsL321, thi-1 recB21 sup$^+$.*
[e] The letters "de" are used in place of an upper case Greek delta to indicate deletion The hyphen stands for a parenthetical expression indicating the length of the deletion. For de-81 the expression is "(*rcC-trg*)" and for de-8 the expression is "(*recE-sfcA*)."
[f] The mutant genotype of KL16 is *thi-1, rel-1,* λ$^-$.
[g] Same as KL16 except *thr-300, ilv-318, spc-300.*
[h] Same as JC5029 except *recB21.*
[i] Same as JC5029 except *recB21 recC22.*
[j] Genotype is F$^-$ lambda? Rac+ *deo-27 lacZ4 supE44 gal-44 endA1 recB21 thi-1* and the *rfa* allele from *E. coli* C [ΦX$^s$] (CAPALDO-KIMBALL and BARBOUR 1971; B. J. BACHMANN, personal communication).
[k] Same as JC6722 except *recC22* (In addition to *recB21*).

was used as a probe. This phage carries about 8 kb derived from the ends of Rac prophage as shown by the shaded areas in the restriction map of Rac and flanking chromosome regions (Figure 4a). Comparing the restriction map with the bands visible in the DNA of the mutants shows that those from the middle and right hand part of the Rac prophage are missing, *e.g.*, the 13.6- and 4.3-kb *Eco*RI fragments, the 4.0 and 26.5-kb *Hin*dIII fragments and the 13.0- and 7.1-kb *Bam*HI fragments. By contrast bands from the left hand part of Rac prophage are retained, *e.g.*, the 2.8-kb *Eco*RI and 3.6-kb *Bam*HI fragments.

The approximate location of one endpoint of each deletion of Rac can be deduced from the band pattern to lie between an *Eco*RI and a *Hin*dIII cleavage site in the left hand portion of the phage (bold arrows in Figure 4a). In JC5412 DNA the 2.8-kb *Eco*RI fragment is retained but the 7.6-kb *Hin*dIII fragment changes to an 8.1-kb fragment (Figures 2a and 3a). In JC9625 DNA the 2.8-kb *Eco*RI fragment is retained (Figure 2a) but the 7.6 kb *Hin*dIII fragment changes to the mobility of a 16.5 kb fragment (Figure 3a). These changes have been incorporated in a restriction map of the two deletion mutant chromosomes (Figure 4, b and c). An approximate location for the other endpoint of each deletion was deduced by examining an *Eco*RI *Hin*dIII *Pst*I restriction map of 10 min (about 400 kb) of the chromosome from *trp* to *man* (BOUCHÉ 1982) for the appropriate spacing of *Eco*RI and *Hin*dIII restriction sites. Southern blots of *Pst*I digested DNAs from the mutant and wild type strains were essentially consistent with the restriction maps as drawn in Figure 4 (data not shown). *sbcA8* was deduced to be a deletion of about 140 kb (Figure 4b) and *sbcA81* a deletion of about 105 kb (Figure 4c). These deductions lead to two predictions both of which have been experimentally confirmed.

First of all both deletions should have removed *trg*, a gene lying at 247 kb (BOUCHÉ *et al.* 1982) from the zero point on the map of BOUCHÉ (1982). To test this we transduced the two deletion strains with P1 grown on strain RP3342, a *trg-2::*Tn*10* mutant (HARAYAMA, PALVA and HAZELBAUER 1979). Table 2 shows that neither deletion strain produced Tet$^R$ transductants while their point *sbcA* mutant relatives (see below) did. This indicates that homologous DNA for approxi-

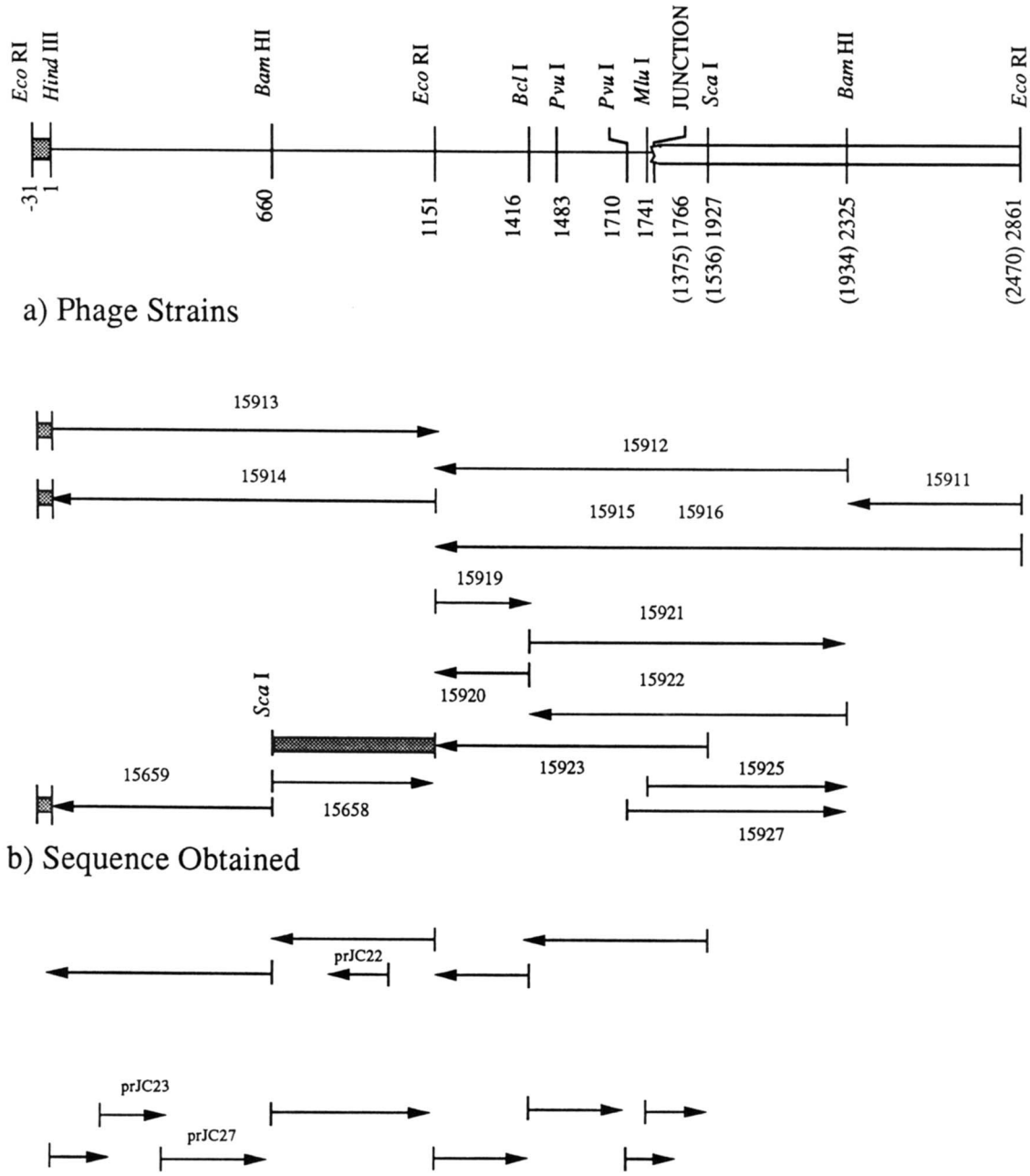


FIGURE 1.—M13 phage strains and their use in determining the nucleotide sequence of *sbcA8*. A 2892-bp fragment bounded by *Eco*RI sites from pSKM1 is shown in the upper part of the figure for reference. Relevant restriction enzyme sites and deletion junction site are marked. A shaded box indicates pBR322 DNA. The line is *E. coli* DNA and the empty box is Rac DNA. Coordinates refer to the first base of the *Hin*dIII recognition site as coordinate 1. Numbers in parentheses refer to coordinate system for Rac prophage sequence containing *recE* obtained by CHU, TEMPLIN and CLARK (1989). (a) M13 phage strains: Arrows indicate extent of fragment subcloned into M13 vectors. The arrowhead indicates orientation of insertion into M13 vectors, where the arrowhead indicates the end of the fragment distal to the universal primer site. Above or below the arrows are phage numbers (minus the JCM prefix) denoting the phage containing this portion of DNA. JCM15923 was created by inserting an approximately 1.3-kb *Sca*I fragment from pSKM2 containing about 0.5 kb of pBR322 DNA into appropriately digested M13mp8 RF DNA. (b) Nucleotide sequence obtained from phage strains. Arrows indicate extents sequenced in a 5′ to 3′ direction, where the arrowhead symbolized the sequence most distal from the primer site. Arrows labeled above by prJC22, prJC23, or prJC27 indicate that these sequences were obtained by utilizing these primers. Otherwise, the universal primer was utilized.

mately 1 min (40 kb) on both sides of *trg* is missing from the deletion mutants. We also compared the transduction recipient-abilities of the *sbcA8* and *sbcA23* strains and found that they inherited *ilv*$^+$ from the same P1 lysate at about equal frequencies (Table 2).

The second prediction is that plasmids carrying fragments deduced to be deleted should not hybridize with restriction nuclease-digested DNA from the mutants. Figure 5 shows that this prediction was verified. Three plasmids were used which contained DNA hybridizable to restriction fragments spanning more

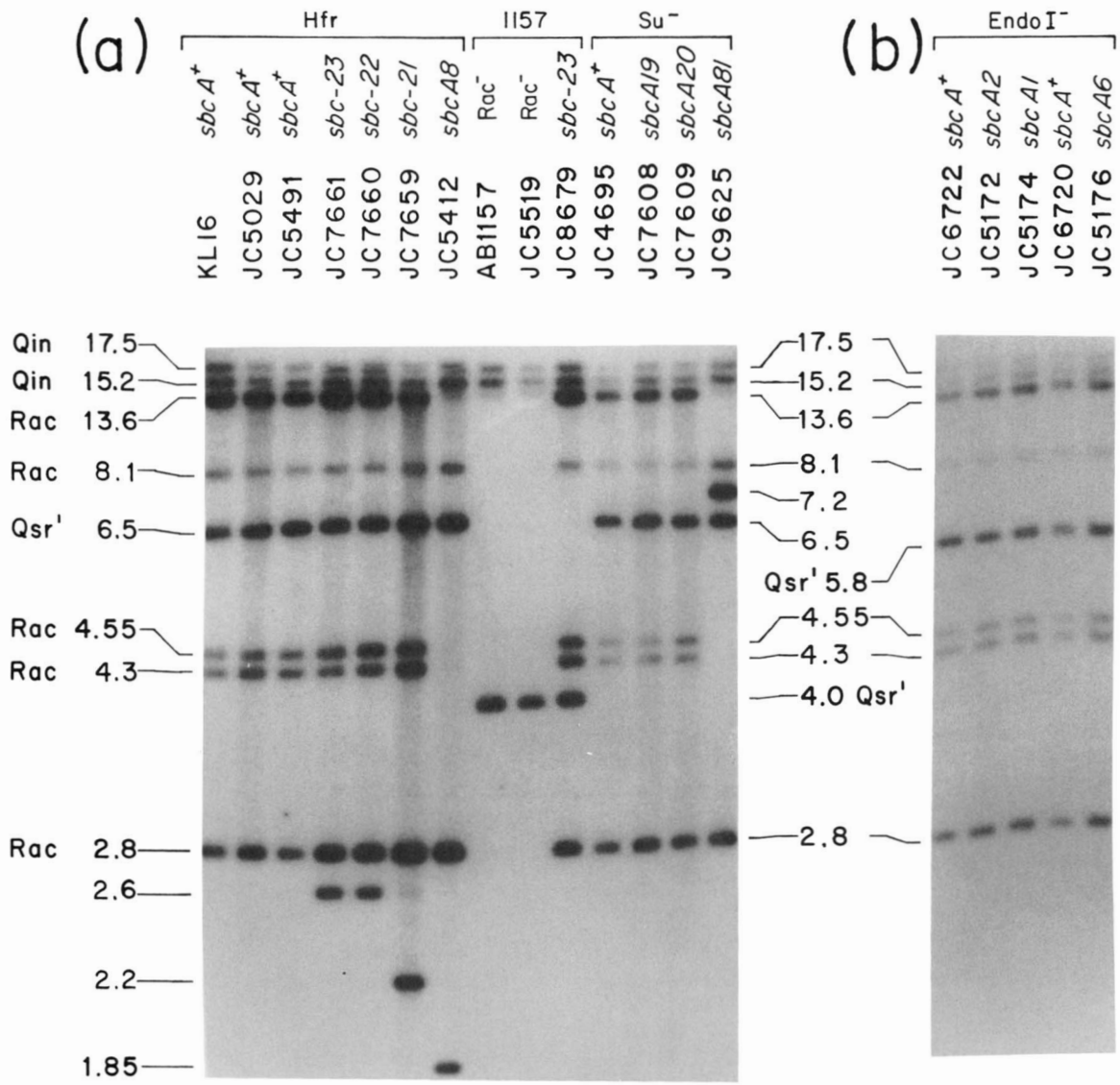


FIGURE 2.—Autoradiographs showing *Eco*RI generated fragments of chromosomal DNA that contain homology to $^{32}$P-labeled λrev DNA. The strains analyzed are listed. The fragment sizes are given in kb. The *Eco*RI fragments which belong to Rac are 13.6 kb, 8.1 kb, 4.55 kb, 4.3 kb, and 2.8 kb. Fragments which belong to two other prophages (KAISER 1980; ESPION, KAISER and DAMBLY-CHAUDIERE 1983) are as follows: Qsr′–13.8 kb (not indicated), 6.5 kb (Hfr and Su$^-$ genetic backgrounds), 5.8 kb (EndoI$^-$ background), 4.0 kb (1157 background), and Qin—15.2 kb, 17.5 kb.

than 60 kb of the chromosome in the region concerned. *sbcA81* DNA has lost part of the DNA corresponding to a 13.4-kb *Eco*RI and a 6.9-kb *Hin*dIII fragment hybridizable to pLN54. Thus the deletion endpoint is between the *Hin*dIII sites at 267.47 and 274.37 on the map of BOUCHÉ (1982). *sbcA8* DNA has lost not only the fragments lost by *sbcA81* DNA but several others as well, retaining only some fragments hybridizable to pBS20. This puts the deletion endpoint between 299.20 and 310.05 on the map of BOUCHÉ (1982).

**Gene fusions deduced for both *sbcA8* and *sbcA81*:** The nucleotide sequence has been determined for the 2.45-kb *Eco*RI-*Hin*dIII fragment of Rac within which lie the endpoints of *sbcA8* and *sbcA81* (CHU, TEMPLIN and CLARK 1989). The N-terminal half (approximate) of *recE* occupies the left hand 1982 nucleotides of this fragment, as the maps in Figure 4 are drawn. Because *sbcA8* reduces the size of the *Eco*RI fragment to 1.85 kb, one endpoint must lie within the coding portion of *recE*. Because no *Hin*dIII or *Pst*I sites can lie in this *Eco*RI fragment, given the sizes of *Hin*dIII and *Pst*I fragments observed (Figure 3a, and data not shown), and because the *Bam*HI site approximately 540 nucleotides from the *Eco*RI site is not deleted, we can further refine the deduction to predict that the endpoint lies between 167.12 and 168.43, using the map coordinates of BOUCHÉ (1982, see the black box in Figure 4b). These are the equivalents of codons 41 and 441 of *recE*, respectively, indicating that *sbcA8* is likely to be a translational gene fusion.

The same type of calculation was done for *sbcA81*.

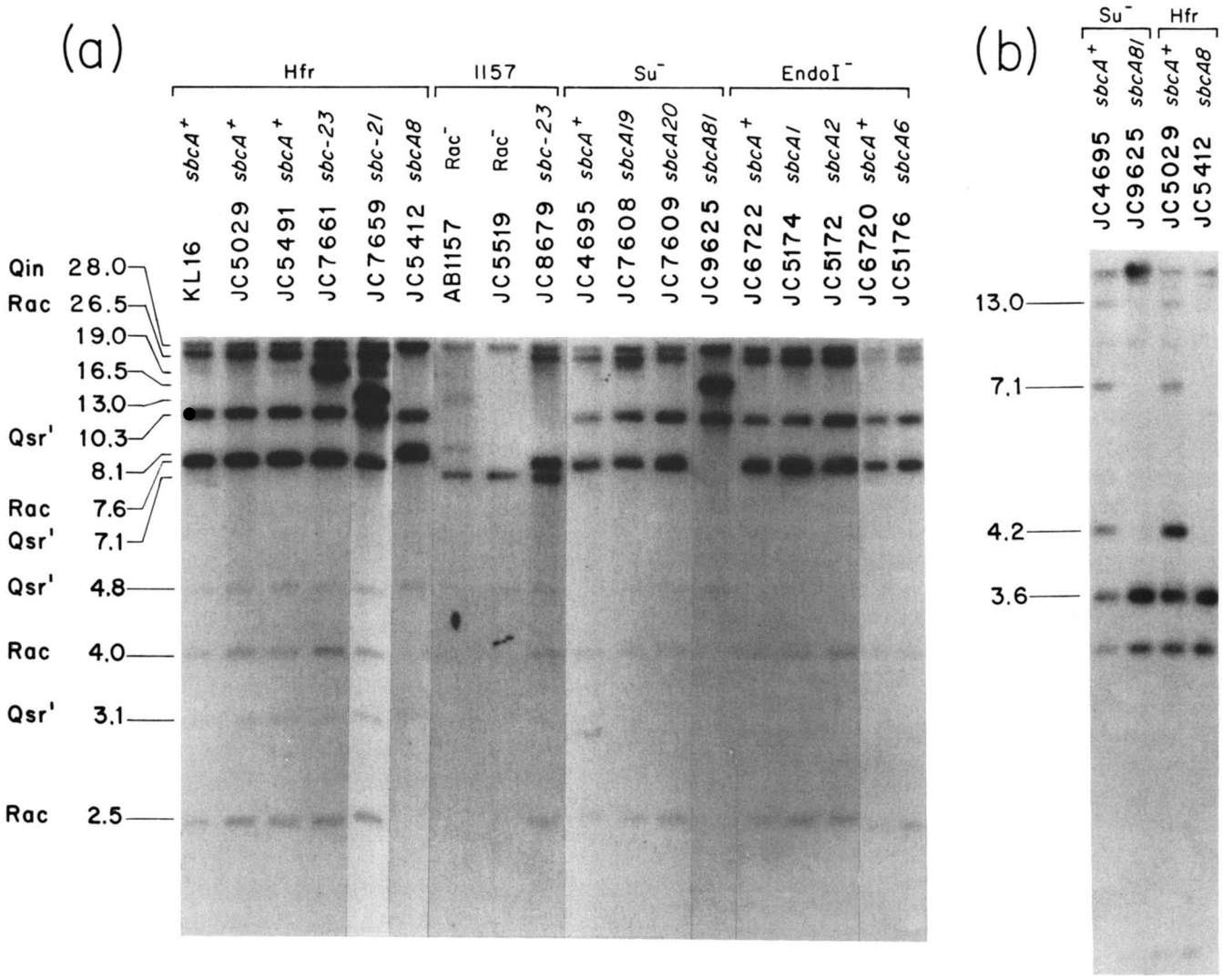


FIGURE 3.—(a) Composite autoradiograph showing *Hin*dIII generated chromosomal DNA fragments that are homologous to $^{32}$P-labeled λrev DNA. Fragment sizes are given in kb and the strains analyzed are listed at the top. The prophage fragment sizes are as follows: Rac—26.5 kb, 7.6 kb, 4.0 kb, 2.5 kb; Qin–28.0 kb; Qsr′–10.3 kb (Hfr and Su⁻ backgrounds), 9.6 kb (EndoI⁻ background, not indicated), 7.1 kb (1157 background), 4.8 kb and 3.1 kb. (b) Autoradiograph showing λrev homologous *Bam*HI restriction fragments. Strains analyzed are listed at the top and the sizes of Rac prophage restriction fragments are indicated in kb.

In this case the range within which the endpoints must fall is quite small because *Hin*dIII and *Eco*RI sites, which must be deleted, lie within 0.15 kb of a *Pst*I site that must be retained (BOUCHÉ 1982). Since the new *Eco*RI fragment formed by *sbcA81* was measured at 7.2 kb (Figure 2a) and since the distance from the retained *Pst*I to *Eco*RI site is 4.8 kb (BOUCHÉ 1982), 2.4 ± 0.15 kb must be contributed by the *Eco*RI-*Hin*dIII fragment of Rac. This is more than enough to include the entire amino terminal coding portion of *recE* (CHU, TEMPLIN and CLARK 1989) and leads to the deduction that *sbcA81* is a transcriptional gene fusion.

**Nucleotide sequence of *sbcA8*:** The 8.1-kb *Hin*dIII fragment containing endpoints of *sbcA8* (Figure 4b) was cloned as described in MATERIALS AND METHODS, using pBR322 as vector. The resulting plasmid pSKM1 was able to suppress recombination deficiency and UV and mitomycin C sensitivity of *recB* and *recC* mutations. This not only showed that pSKM1 contained a functional (presumed hybrid) *recE* gene but that expression of this gene on the multicopy plasmid was not prevented by the chromosomal repressor hypothesized by KAISER and MURRAY (1980). The sequences of two portions of this fragment were determined by the strategy outlined in MATERIALS AND METHODS. One portion was the 1.85-kb *Eco*RI fragment containing the endpoints of *sbcA8* (Figure 4b); the other was the approximately 1.1-kb *Eco*RI-*Hin*dIII fragment between the bold arrows in Figure 4b. The combined sequence is presented in Figure 6. The first 1765 bp are not contained in the portion of Rac previously sequenced (CHU, TEMPLIN and CLARK 1989). The remainder of the sequence is identical to the *recE* sequence beginning at *recE* codon 294. This is within the range predicted in the previous section.

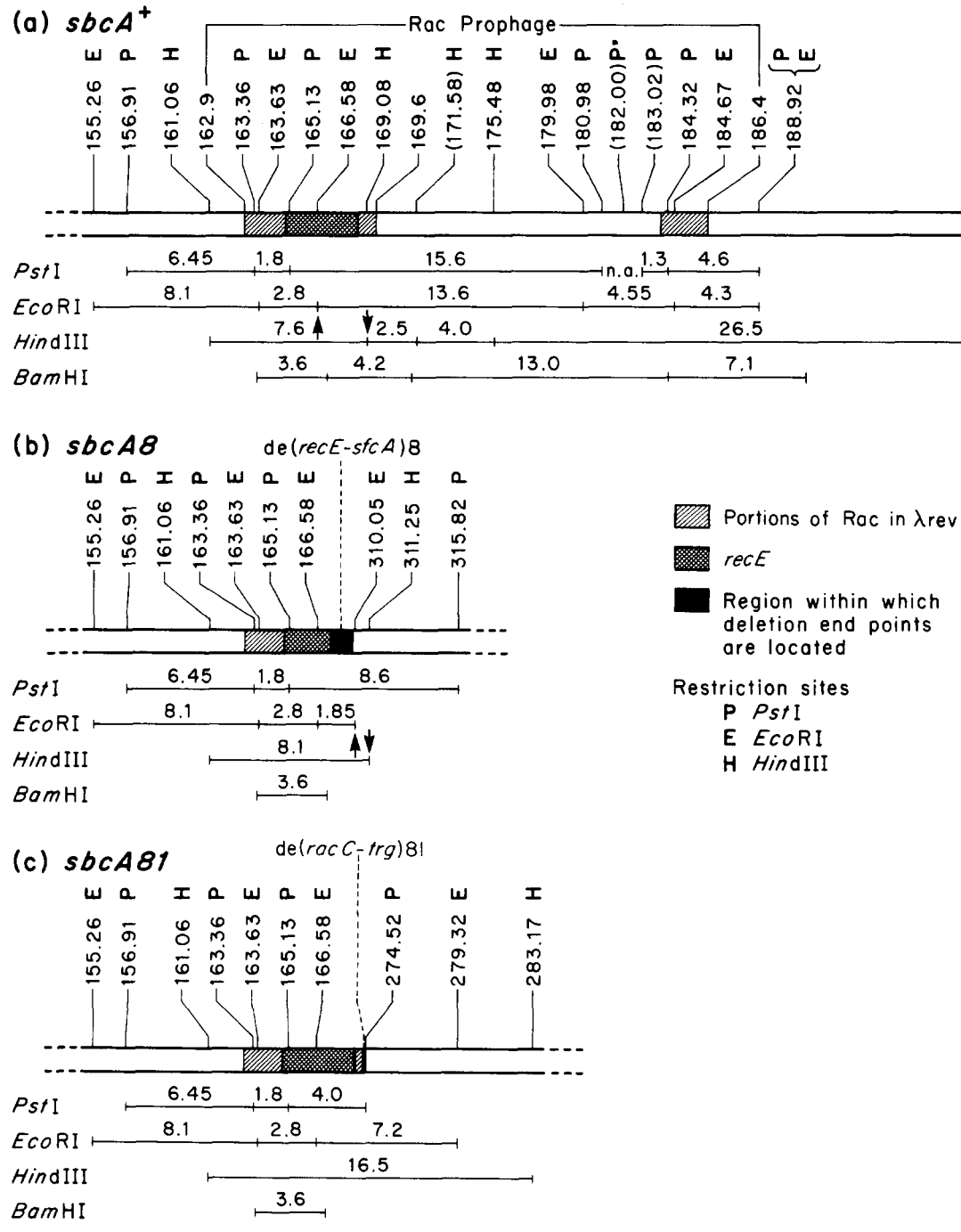


FIGURE 4.—Physical map of chromosomal DNA near the Rac prophage showing restriction sites for *Eco*RI, *Hin*dIII, and *Pst*I. Coordinates are taken from the map of BOUCHÉ (1982). Coordinates of the Rac prophage are deduced from the work of WILLIS *et al.* (1983). Shaded boxes indicate the extent of Rac DNA found in λrev. Solid boxes indicate an area of uncertainty for the deletion end points. Lines underneath the maps indicate the length of restriction fragments detected in the strains carrying *sbcA* alleles; numbers are the sizes measured in kb. Only those restriction sites detectable by Southern blot using λrev as probe are shown; further sites distal to Rac (*e.g.*, E at 314.6 in b and E at 280.97 in c) would be undetectable. Location of the *Bam*HI sites is taken from the work of WILLIS *et al.* (1983). (a) KL16 (*sbcA*$^+$) and JC4695 (*sbcA*$^+$), (b) JC5412 (*sbcA8*) and (c) JC9625 (*sbcA81*). n.a. means not available. Arrows are explained in text.

Fused to the *recE* reading frame is an ORF of 447 codons. Within the first 67 codons of this ORF are 3 ATG (Met) and 2 GTG (Val) codons at which translation could potentially initiate (Figure 6). We think the most likely initiation codon is the ATG at nucleotides 479-81 because the sequence 5′AAGAGT3′ terminates 7 bp upstream, matches in 4 out of 6 positions with the Shine-Dalgarno sequence (SHINE and DALGARNO 1974) and hence could be a ribosome binding site. We call the ORF from this ATG codon *sfcA*. Translation of *sfcA* would substitute 429 codons for 293 codons of *recE*. The differential 136 codons would be expected to add about 14.5 kDa in mass to *recE* protein. A Western blot of protein extracted

TABLE 2

**Absence of Tet$^R$ transductants implies inability by deletion *sbcA* mutants to inherit *trg-2*::Tn*10* following P1 transduction**

| Selected property[a] | Hfr background[b] | | Su$^-$ background[c] | |
|---|---|---|---|---|
| | *sbcA8* | *sbcA23* | *sbcA81* | *sbcA20* |
| Tet$^R$ | <0.4 | 51 | <0.4 | 10 |
| Ilv$^+$ | 47 | 140 | ND[d] | ND |

Numbers of transductants are measured per $10^6$ input phage plaque-forming units.

[a] A P1 lysate was produced on strain RP3342. Tet$^R$ (tetracycline resistance) implies inheritance of *trg-2*::Tn*10* and Ilv$^+$ (isoleucine and valine independence) implies inheritance of the wild-type allele of *ilv-318*.

[b] Strains JC5412 and JC7661 were recipients.

[c] Strains JC9625 and JC7609 were recipients.

[d] Not done.

from *sbcA8* mutant JC5412 shows a protein of about 150 kDa compared to protein from an *sbcA* point mutant of about 140 kDa (data not shown). This confirms our finding of an unequal substitution and is consistent with the putative initiation codon at nucleotides 479-81 but does not rule out translation initiation at one of the other potential initiation codons.

**Possible identity of *sfcA*:** We screened 4206 translated nucleotide sequences of GENBANK (December 1987) using the tfastp program (with parameter "ktup" set at two) to detect similarities to *sfcA*. Two showed very high optimized scores indicating a high degree of similarity. Both were NADP-linked malic enzymes (EC 1.1.1.40), one from mouse liver (MAGNUSON *et al.* 1986), the other from rat liver (BAGCHI *et al.* 1987). They showed 43% identical residues to the 429 amino acids encoded by *sfcA* (data not shown). If conservative amino acid changes were accepted, the sequences were 83% similar (data not shown). Only four small gaps in the pairs of sequences were necessary to achieve this degree of similarity.

In *E. coli* two malic enzymes have been reported: NADP-linked malic enzyme (EC 1.1.1.40) and NAD-linked malic enzyme (EC 1.1.1.38) (KATSUKI *et al.* 1967). These enzymes have been purified (SPINA, BRIGHT and ROSENBLOOM, 1970; YAMAGUCHI, TOKUSHIGE and KATSUKI 1973) and their amino acid compositions determined. Table 3 shows a comparison of *sfcA* product with the two *E. coli* malic enzymes. Lower amounts of lysine, histidine, proline, alanine and valine and higher amounts of tyrosine and tryptophan make *sfcA* protein more similar to the NAD-linked enzyme. *E. coli* mutants lacking each of the enzymes have been detected and characterized phenotypically (HANSEN and JUNI 1975) but mapping the mutant genes was not reported. The genes encoding NAD-linked and NADP-linked malic enzymes are known as *maeA* and *maeB*, respectively (B. BACHMANN, personal communication). We hypothesize that *sfcA* is identical to *maeA*.

Identification of *sfcA* as *maeA*, the gene for NAD-linked malic enzyme, must be considered provisional partly because we do not know how large a protein the wild type allele encodes. If it is really *maeA*, then only about eighteen codons would have been deleted and the amino acid composition would not be expected to change very much from that shown in Table 3. If, however, it is really *maeB* then about 113 codons would have been deleted. In this case the low amounts of five amino acids might be redressed by the missing codons but the already large amounts of tyrosine and tryptophan might increase. Consequently we favor *maeA* as the true identity of *sfcA*.

**Point mutations:** Those *recE*-expressing mutants which show no differences from their ancestors in DNA fragments complementary to λrev are likely to carry *sbcA* point mutations, although very small deletions or insertions (<50 bp) or internal Rac inversions would not produce detectable fragment size changes and cannot be ruled out. Two Su$^-$ background mutants (JC7608[*sbcA19*], JC7609[*sbcA20*]) and three EndoI$^-$ background mutants (JC5172[*sbcA2*], JC5174[*sbcA1*] and JC5176[*sbcA6*]) fall into this category and show all of the Rac restriction fragments containing homology to λrev (Figure 2, a, b and c). The results with JC5174 are identical to those obtained by KAISER and MURRAY (1980). All five mutants in this group were obtained after mutagenesis with NTG (*N*-methyl-*N'*-nitro-*N*-nitrosoguanidine) or EMS (ethyl methanesulfonate), which is consistent with our hypothesis that they carry *sbcA* point mutations.

**Other mutants:** KAISER and MURRAY (1980) observed an extra 2.45-kb *Eco*RI band with DNA from *sbcA23* mutant JC7661 and hypothesized that it was derived by juxtaposition of *attL* and *attR* elements of the Rac prophage. We have also examined DNA from JC7661 and find an extra band which we measure to be 2.6 kb (Figure 2a). In addition we analyzed JC7661 and JC5491 DNAs with *Hin*dIII and found that JC7661 DNA contains an extra fragment with a size of approximately 19 kb (Figure 3a). This fragment could also have been formed by juxtaposition of the two ends of the prophage. KAISER and MURRAY (1980) pointed out that DNA from JC8679, a transconjugant inheriting Rac prophage and *sbcA23* from JC7661, did not show the extra 2.6-kb *Eco*RI band. We have confirmed that (Figure 2a) and have additionally shown the absence of the 19-kb *Hin*dIII band (Figure 3a). JC8679, however, is a second generation transconjugant. It was derived by a second conjugation event in which *his-328* was transmitted to JC8675, the original Rac$^+$ *sbcA23* transconjugant (GILLEN, WILLIS and CLARK 1981). We have found that DNA from JC8675 cleaved with *Eco*RI or *Hin*dIII also lacks the 2.6-kb and 19-kb bands (data not shown).

JC7661, whose DNA contains the extra 2.6-kb *Eco*RI and 19-kb *Hin*dIII bands, was derived along

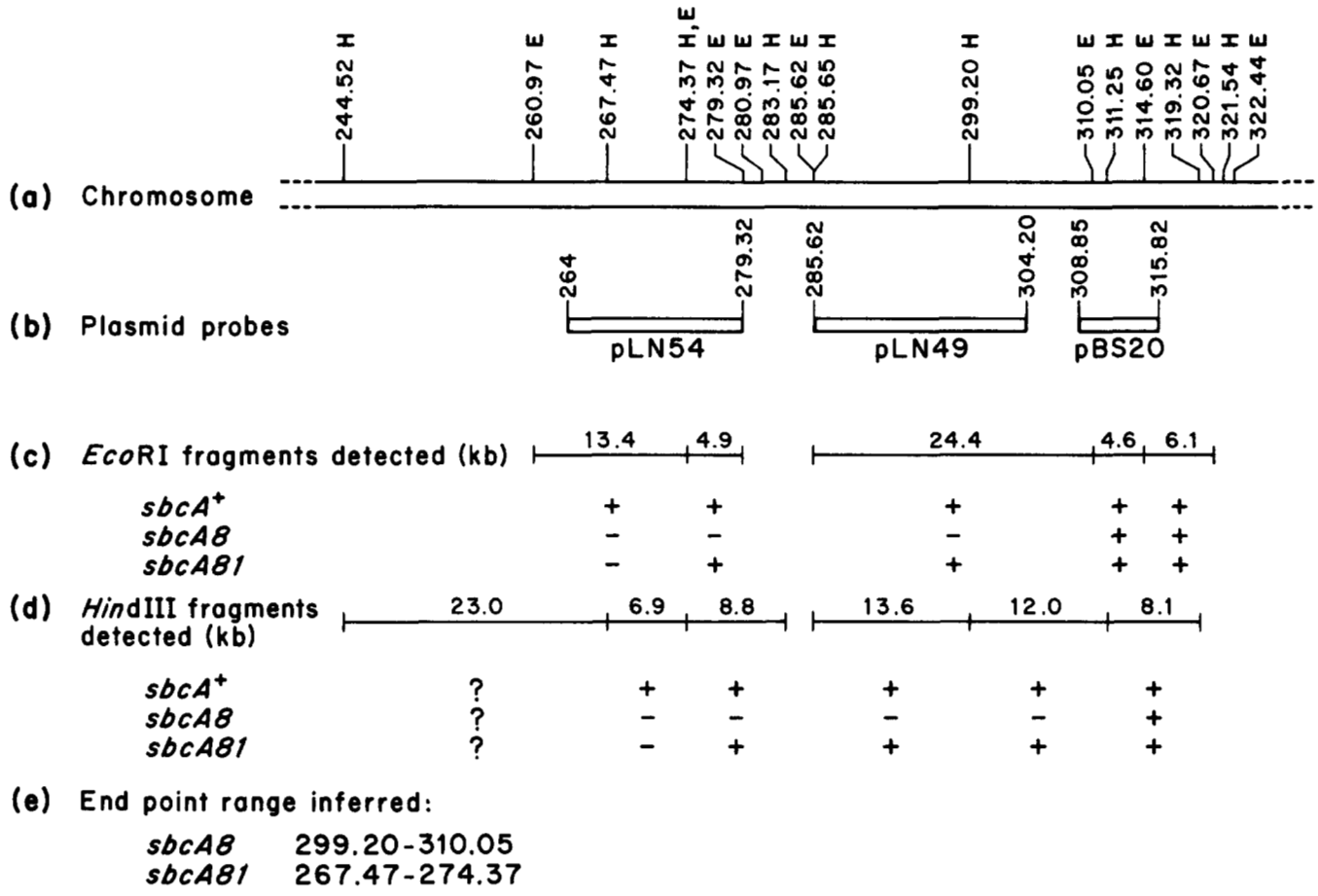


FIGURE 5.—Summary of data on the endpoints of deletion mutations *sbcA8* and *sbcA81*. (a) Diagram of the region of the chromosome tested. This region includes the right hand portions of the maps in Figure 4, b and c. Coordinates of restriction sites are taken from BOUCHÉ (1982). H stands for *Hin*dIII and E stands for *Eco*RI. More restriction sites are shown here than in corresponding places in Figure 4, b and c. This is intended to be a complete restriction map while Figure 4, b and c are probe-dependent Southern blot fragment maps. (b) The names of the plasmid probes are listed under rectangles indicating the chromosomal region carried. Plasmids pLN54 and pLN49 are derivatives of pBR325 (BOLIVAR 1978) and were provided by J. P. BOUCHÉ in strains LN1175 and LN1170, respectively. pBS20 is also derived from pBR325 and was supplied by J. P. BOUCHÉ in the C600 background. (c) and (d) Sizes (in kb) of the restriction fragments detected by the plasmid probes are listed above a line which shows the extent of the fragment on the map in (a). + and − indicate (respectively) that the fragment was or was not detected in restriction digests of DNA from the strain listed at the left. ? indicates that the expected fragment was observed only indistinctly with wild type DNA so that we cannot be certain of its absence in mutant DNA. DNA was isolated from JC5029 (*sbcA*$^+$), JC5412 (*sbcA8*), JC4695 (*sbcA*$^+$) and JC9625 (*sbcA81*). (e) Coordinates indicate the restriction sites shown in (a) between which the deletion endpoints must lie based on the evidence in (c) and (d).

with five other strains by mutagen treatment of Hfr strain JC5491 (TEMPLIN, KUSHNER and CLARK 1972). We have tested *Eco*RI cleaved DNA from JC7660, another mutagen-induced mutant and find it has the same extra 2.6-kb *Eco*RI band (Figure 2a). The same extra *Eco*RI band is present in DNA from JC7659, an *sbcA* mutant of JC5491 selected for MitC$^R$ after no mutagenic treatment (Figure 2a); however in this case the 2.6-kb band is weak and a much stronger extra 2.2-kb band is present (Figure 2a). *Hin*dIII digests of JC7659 DNA likewise show a relatively weak 19-kb and a new strong 13-kb band (Figure 3a).

The presence of the extra bands in JC7661 DNA was interpreted by KAISER and MURRAY (1980) to indicate either of three conditions of Rac DNA: (1) tandemly arranged multiple copies in the chromosome, (2) circular or linear multimers in the cytoplasm, and (3) circular monomers in the cytoplasm. They thought the first most likely while we prefer the second and third for reasons presented in the DISCUSSION. At this point, however, we need to point out that JC5491 DNA does not show these extra bands (Figures 2a and 3a). Thus we have no evidence that ancestral *sbcA*$^+$ strain JC5491 has a tandem array of chromosomal Rac prophages, in contradiction to the reference to our data made by KAISER and MURRAY (1980, see p. 560 second column). We think it is likely that the extra bands in JC7659, JC7660 and JC7661 are in some way related to the *sbcA* mutations each has suffered.

## DISCUSSION

Expression of ExoVIII activity is accomplished by a variety of mutations. Thinking that a simple repression system might be operating (BARBOUR *et al.* 1970; TEMPLIN, KUSHNER and CLARK (1972)) called these

**A**

```
  1 AAGCTTCTGA TAACGACATC CTCAGTGATA TCTACCAGCA AACGATCAAT TATGTGGTCA GTGGCCAGCA CCCTACGCTT

 81 TAAGGTGCTA TGCTTGATCG GCAACCTAAT TTAGGGGTTT AGCACGTGTT TCTTCGCTAC GGCGATGTTG TCCTTAAAAC

161 TAGCTACAGG ATTGAGGAGT TAAAATGAAA TCGAACCGTC AGGCACGTCA TATTCTTGGA CTGGACCATA AAATTTCTAA

241 CCAGCGCAAA ATAGTTACCG AAGGTGACAA ATCCAGCGTA GTAAATAACC CAACCGGCAG AAAACGCCCC GCTGAAAAGT

321 AATTCATAAC CATCAGTCCT CAATGACGAT T AAACACCATTGC CTGC GCAATGGTGTTT TTGTTTTTAT CTGCTTTATA

                                  *** Ser Lys Asp Asp Lys Ser Pro Pro Gly Met Asp Ile Gln Lys Arg Val
400 CTTGAGGCCG ACGCCCTGGC GG TAA AGC AAA GAC GAT AAA AGC CCC CCA GGG ATG GAT ATT CAA AAA AGA GTG

    Ser Asp Met Glu Pro Lys Thr Lys Lys Gln Arg Ser Leu Tyr Ile Pro Tyr Ala Gly Pro Val Leu Leu
473 AGT GAC ATG GAA CCA AAA ACA AAA AAA CAG CGT TCG CTT TAT ATC CCT TAC GCT GGC CCT GTA CTG CTG

    Glu Phe Pro Leu Leu Asn Lys Gly Ser Ala Phe Ser Met Glu Glu Arg Arg Asn Phe Asn Leu Leu Gly
542 GAA TTT CCG TTG TTG AAT AAA GGC AGT GCC TTC AGC ATG GAA GAA CGC CGT AAC TTC AAC CTG CTG GGG

    Leu Leu Pro Glu Val Val Glu Thr Ile Glu Glu Gln Ala Glu Arg Ala Trp Ile Gln Tyr Gln Gly Phe
611 TTA CTG CCG GAA GTG GTC GAA ACC ATC GAA GAA CAA GCG GAA CGA GCA TGG ATC CAG TAT CAG GGA TTC

    Lys Thr Glu Ile Asp Lys His Ile Tyr Leu Arg Asn Ile Gln Asp Thr Asn Glu Thr Leu Phe Tyr Arg
680 AAA ACC GAA ATC GAC AAA CAC ATC TAC CTG CGT AAC ATC CAG GAC ACT AAC GAA ACC CTC TTC TAC CGT

    Leu Val Asn Asn His Leu Asp Glu Met Met Pro Val Ile Tyr Thr Pro Thr Val Gly Ala Ala Cys Glu
749 CTG GTA AAC AAT CAT CTT GAT GAG ATG ATG CCT GTT ATT TAT ACC CCA ACC GTC GGC GCA GCC TGT GAG

    Arg Phe Ser Glu Ile Tyr Arg Arg Ser Arg Gly Val Phe Ile Ser Tyr Gln Asn Arg His Asn Met Asp
818 CGT TTT TCT GAG ATC TAC CGC CGT TCA CGC GGC GTG TTT ATC TCT TAC CAG AAC CGG CAC AAT ATG GAC

    Asp Ile Leu Gln Asn Val Pro Asn His Asn Ile Lys Val Ile Val Val Thr Asp Gly Glu Arg Ile Leu
887 GAT ATT CTG CAA AAC GTG CCG AAC CAT AAT ATT AAA GTG ATT GTG GTG ACT GAC GGT GAA CGC ATT CTG

    Gly Leu Gly Asp Gln Gly Ile Gly Gly Met Gly Ile Pro Ile Gly Lys Leu Ser Leu Tyr Thr Ala Cys
956 GGG CTT GGT GAC CAG GGC ATC GGC GGG ATG GGC ATT CCG ATC GGT AAA CTG TCG CTC TAT ACC GCC TGT

    Gly Gly Ile Ser Pro Ala Tyr Thr Leu Pro Val Val Leu Asp Val Gly Thr Asn Asn Gln Gln Leu Leu
1025 GGC GGC ATC AGC CCG GCG TAT ACC CTT CCG GTG GTG CTG GAT GTC GGA ACG AAC AAC CAA CAG CTG CTT

    Asn Asp Pro Leu Tyr Met Gly Trp Arg Asn Pro Arg Ile Thr Asp Asp Glu Tyr Tyr Glu Phe Val Asp
1094 AAC GAT CCG CTG TAT ATG GGC TGG CGT AAT CCG CGT ATC ACT GAC GAC GAA TAC TAT GAA TTC GTT GAT

    Glu Phe Ile Gln Ala Val Lys Gln Arg Trp Pro Asp Val Leu Leu Gln Phe Glu Asp Phe Ala Gln Lys
1163 GAA TTT ATC CAG GCT GTG AAA CAA CGC TGG CCA GAC GTG CTG TTG CAG TTT GAA GAC TTT GCT CAA AAA

    Asn Ala Met Pro Leu Leu Asn Arg Tyr Arg Asn Glu Ile Cys Ser Phe Asn Asp Asp Ile Gln Gly Thr
1232 AAT GCG ATG CCG TTA CTT AAC CGC TAT CGC AAT GAA ATT TGT TCT TTT AAC GAT GAC ATT CAG GGC ACT

    Ala Ala Val Thr Val Gly Thr Leu Ile Ala Ala Ser Arg Ala Ala Gly Gly Gln Leu Ser Glu Lys Lys
1301 GCG GCG GTA ACA GTC GGC ACA CTG ATC GCA GCA AGC CGC GCG GCA GGT GGT CAG TTA AGC GAG AAA AAA

    Ile Val Phe Leu Gly Ala Gly Ser Ala Gly Cys Gly Ile Ala Glu Met Ile Ile Ser Gln Thr Gln Arg
1370 ATC GTC TTC CTT GGC GCA GGT TCA GCG GGA TGC GGC ATT GCC GAA ATG ATC ATC TCC CAG ACC CAG CGC

    Glu Gly Leu Ser Glu Glu Ala Ala Arg Gln Lys Val Phe Met Val Asp Arg Phe Gly Leu Leu Thr Asp
1439 GAA GGA TTA AGC GAG GAA GCG GCG CGG CAG AAA GTC TTT ATG GTC GAT CGC TTT GGC TTG CTG ACT GAC
```

FIGURE 6A

**B**

```
     Lys Met Pro Asn Leu Leu Pro Phe Gln Thr Lys Leu Val Gln Lys Arg Glu Asn Leu Ser Asp Trp Asp
1508 AAG ATG CCG AAC CTG CTG CCT TTC CAG ACC AAA CTG GTG CAG AAG CGC GAA AAC CTC AGT GAC TGG GAT

     Thr Asp Ser Asp Val Leu Ser Leu Leu Asp Val Val Arg Asn Val Lys Pro Asp Ile Leu Ile Gly Val
1577 ACC GAC AGC GAT GTG CTG TCA CTG CTG GAT GTG GTG CGC AAT GTA AAA CCA GAT ATT CTG ATT GGC GTC

     Ser Gly Gln Thr Gly Leu Phe Thr Glu Glu Ile Ile Arg Glu Met His Lys His Cys Pro Arg Pro Ile
1646 TCA GGA CAG ACC GGG CTG TTT ACG GAA GAG ATC ATC CGT GAG ATG CAT AAA CAC TGT CCG CGT CCG ATC

     Val Met Pro Leu Ser Asn Pro Thr Ser Arg Val Glu Ala Thr Pro Gln Asp | Ile Ile Ala Glu Asn Lys
1715 GTG ATG CCG CTG TCT AAC CCG ACG TCA CGC GTG GAA GCC ACA CCG CAG GAC | ATT ATC GCT GAA AAT AAA

     Pro Pro Phe Ser Val Phe Arg Asp Lys Phe Ile Thr Met Pro Gly Gly Leu Asp Tyr Ser Arg Ala Ile
1784 CCG CCC TTT TCT GTT TTC CGC GAC AAA TTC ATC ACC ATG CCT GGC GGG CTG GAT TAT TCC CGC GCC ATC

     Val Val Ala Ser Val Lys Glu Ala Pro Ile Gly Ile Glu Val Ile Pro Ala His Val Thr Glu Tyr Leu
1853 GTG GTT GCG TCC GTA AAA GAA GCA CCA ATT GGG ATC GAG GTC ATC CCC GCG CAC GTC ACT GAA TAT CTG
```

FIGURE 6.—DNA sequence of the region containing *sbcA8*. The entire sequence of the 1.07-kb *Hin*dIII-*Eco*RI fragment followed by a partial sequence of the 1.85-kb *Eco*RI fragment up to the deletion junction plus 156 bp of *recE* sequence is presented 5′ and 3′. The first base of the *Hin*dIII recognition site was arbitrarily designated as coordinate 1, resulting in *Eco*RI sites at coordinates 1151 and 2861. Nucleotide coordinates are provided on the sides of the sequence. The ORF containing the *sfcA-RecE* fusion protein is shown translated above the DNA sequence. Five potential translational start sites are illustrated by bold lettering of Met or Val. *** indicates a termination codon. The fusion site is represented as a vertical line between *sfcA* (up to nucleotide 1765) and *recE* sequences (nucleotide 1766 and beyond). A dyad symmetry is represented by bold lettering of the DNA sequence. The locations of primers prJC22, prJC23, and prJC27 are illustrated by underlining of the DNA sequence prJC22 corresponds with two mismatches to the sequence complementary to that underlined from coordinates 1000-983. prJC23 and prJC27 correspond to sequences underlined from 172–189 and 389–403, respectively.

**TABLE 3**

**Comparison of the translation product from the partial sequence of *sfcA* with *E. coli* malic enzymes**

| | Number of residues per subunit | | | Percent of total residues | | |
|---|---|---|---|---|---|---|
| Amino acid | NADP-linked enzyme | NAD-linked enzyme | *sfcA* product | NADP-linked enzyme | NAD-linked enzyme | *sfcA* product |
| Lys | 39 | 17 | 18 | 7.2 | 3.9 | 4.2 |
| His | 12 | 5 | 6 | 2.2 | 1.0 | 1.4 |
| Arg | 28 | 22 | 26 | 5.2 | 4.8 | 6.1 |
| Asx | 46 | 46 | 49 | 8.6 | 10.4 | 11.3 |
| Glx | 58 | 59 | 53 | 10.7 | 13.3 | 12.3 |
| Thr | 21 | 23 | 23 | 3.8 | 5.1 | 5.4 |
| Ser | 27 | 23 | 20 | 5.1 | 5.2 | 4.7 |
| Cys | 8 | 5 | 5 | 1.4 | 1.1 | 1.2 |
| Tyr | 11 | 14 | 14 | 2.0 | 3.0 | 3.3 |
| Phe | 18 | 17 | 17 | 3.2 | 3.8 | 4.0 |
| Trp | 1 | 7 | 4 | 0.2 | 1.5 | 0.9 |
| Pro | 33 | 24 | 23 | 6.1 | 5.5 | 5.4 |
| Gly | 39 | 33 | 31 | 7.2 | 7.3 | 7.2 |
| Ala | 59 | 34 | 23 | 11.0 | 7.7 | 5.4 |
| Val | 47 | 30 | 30 | 8.7 | 6.7 | 7.0 |
| Met | 16 | 14 | 13 | 2.9 | 3.0 | 3.0 |
| Ile | 34 | 32 | 31 | 6.3 | 7.2 | 7.2 |
| Leu | 45 | 42 | 43 | 8.2 | 9.5 | 10.0 |
| Total | 542 | 447 | 429 | 100.0 | 100.0 | 100.0 |

*sbcA* mutations, as if they affected one regulatory gene. KAISER and MURRAY (1980) found that some *sbcA* mutations (*sbcA8*, *sbcA23*, *sbcA50* and *sbcA51*) affected the structure of the Rac prophage, as expected for deletions and tandem multiplications, while *sbcA1* did not affect the structure, as expected for a point mutation. In this paper we have confirmed and extended these results by showing that *sbcA1*, *sbcA2*, *sbcA6*, *sbcA19* and *sbcA20* fall into the point mutant class, and by showing that *sbcA8*, *sbcA21*, *sbcA22*, *sbcA23* and *sbcA81* affect the structure of the Rac prophage. The main purpose of this discussion is to examine the evidence to see whether or not the common gene symbol *sbcA* for all these mutations is appropriate.

If a simple repression system were operative one might expect to find both operator and repressor gene mutations which derepress *recE*. FOUTS *et al.* (1983) found that *sbcA1* and *sbcA6* were dominant and *cis*-acting to *sbcA*$^+$. Both are characteristics expected of operator and not repressor mutations (JACOB and MONOD 1961; JACOB, ULLMANN and MONOD 1964). Thus FOUTS *et al.* (1983) hypothesized that *sbcA* was an operator of *recE* (*i.e.*, *recEo*) although they could not rule out other possibilities such as that *sbcA1* and *sbcA6* create new promoters not repressed by the hypothesized Rac repressor.

The basic nomenclature question can then be restated as follows. Is there any evidence that some mutations which are called *sbcA* mutations do not affect the operator of *recE*? For point mutations *sbcA2*, *sbcA19* and *sbcA20* there is no such evidence. Mutations *sbcA8* and *sbcA81*, although large deletions rather than point mutations, delete the operator of *recE* and hence are justifiably called *sbcA* mutations even though such nomenclature is not standard and lacks important information. Mutations *sbcA21*, *sbcA22* and *sbcA23* differ from the others in that they

produce new 2.6-kb *Eco*RI and 19-kb *Hin*dIII bands. For these mutations the question becomes whether or not the new bands signify that a sequence other than the operator of *recE* has been affected.

The new 2.6-kb *Eco*RI band was first detected by KAISER and MURRAY (1980) in DNA from *sbcA23* Hfr strain JC7661 and in DNA from *sbcA50* and *sbcA51* strains. *sbcA50* is a spontaneous mutation which occurred in a *thyA⁻polA1* F⁻ strain which was transduced to *thyA⁺recB21* (STRIKE and EMMERSON 1972). *sbcA51* is a mutation selected for conferring resistance to methyl methane sulfonate on a *recB21* HfrH derivative (STRIKE and EMMERSON 1974). Whether *sbcA51* is spontaneous or mutagen induced is thus open to question. KAISER and MURRAY (1980) suggested that the new 2.6-kb *Eco*RI band in the three *sbcA* mutants was due to tandem multiplication of Rac prophage in the chromosome although they advanced two other possibilities. We have adopted as working hypothesis the other possibilities because they involve excised prophage which could undergo limited multiplication in the cytoplasm. This is consistent with our hypothesis that the presence of the extra bands reflects coordinate derepression of *recE* and *int(xis)* genes of Rac so that there is a high frequency of prophage excision. Excised prophage might multiply somewhat because of the *oriJ* sequence (DIAZ and PRITCHARD 1978; DIAZ, BARNSLEY and PRITCHARD 1979).

The nomenclature question then becomes whether coordinate derepression of *recE* and *int(xis)* could be due to operator mutations in one genetic background and not another. The answer is "yes" if there are differences in the Rac prophage in different backgrounds. For example, equal amounts of *recE* transcription could result in different amounts of downstream *int(xis)* transcription if an intervening transcription terminator were less powerful in one background than another. There is, however, an alternative possibility, namely that *sbc* mutations which cause coordinate derepression of *recE* and *int(xis)* may lie in the repressor gene rather than the *recE* operator. Since prophage excision is not complete, we would expect that the mutant repressors would retain some activity. As a result we think it preferable to leave open the possibility that *sbc-21*, *sbc-22*, *sbc-23*, *sbc-50* and *sbc-51* are not in *sbcA* by substituting a hyphen for the cistron designation in their symbols.

Regardless of the gene which they affect, *sbc-22* and *sbc-23* are likely to be point mutations because they do not affect the internal structure of Rac. The same may not be true for the spontaneous mutant JC7659 carrying *sbc-21* because strong 2.2-kb *Eco*RI and 13-kb *Hin*dIII bands are found in addition to the 2.6-kb *Eco*RI and 19-kb *Hin*dIII bands. We are puzzled by this result, but our working hypothesis is that *sbc-21*, as a spontaneous mutant, may have been produced by transposition of an element of the approximate size of Tn*1000* (GUYER 1978). Accordingly the extra bands would include transposon DNA. If confirmed by further experiment, *sbc-21* would join the class of mutation expressing *recE* by insertion, which contains fourteen Tn*5* and IS*50* insertion mutations (FOUTS *et al.* 1983; WILLIS *et al.* 1983). Alternative hypotheses, also under consideration, are that the extra bands represent either an aberrantly excised form of Rac or deletion mutants of normally excised Rac which replicate more from *oriJ*.

In apparent contradiction we have found here that *sbc-23*, after transmission from its original Hfr genetic background to the AB1157 genetic background, is not associated with the extra bands of DNA. To explain this we note that the cross involved transfer of the Rac prophage into a naive cytoplasm and that Rac⁺ *sbc-23* recombinants were very rare (GILLEN 1974). We assume that JC8679 and its predecessor JC8675 carry a spontaneous mutation inactivating the *int* or *xis* genes in addition to *sbc-23*. We have not yet been able to test our assumption.

The authors are grateful to J. P. BOUCHÉ for his gift of plasmids and information relative to them and the restriction site map; STEVEN SANDLER for improvements in Figure 1; WARREN GISH and CONRAD HALLING for help with computer-aided analysis of sequence; and LESLIE SATIN and MURTY MADIRAJU for aid in the sequencing of *sbcA8*. C.C.C. was supported through the Department of Genetics at the University of California at Berkeley by National Institutes of Health (NIH) Predoctoral Institutional Training Grant GM07127. This work was supported by grant NP-237 from the American Cancer Society and NIH grant AI05371 from the National Institute of Allergy and Infectious Diseases.

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Communication editor: J. R. ROTH