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Plasmid Recombination by the RecBCD Pathway of *Escherichia coli*

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Previously, we demonstrated that exonuclease I-deficient strains of *Escherichia coli* accumulate high-molecular-weight linear plasmid concatemers when transformed with plasmids carrying the chi sequence (5′-GCTGGTGG-3′) (M. M. Zaman and T. C. Boles, J. Bacteriol. 176:5093–5100, 1994). Since high-molecular-weight linear DNA is believed to be the natural substrate for RecBCD-mediated recombination during conjugation (A. J. Clark and K. B. Low, p. 155–215, *in* K. B. Low, ed., *The Recombination of Genetic Material*, 1988), we analyzed the recombination frequencies of chi$^+$ and chi^0 plasmids in *sbcB* strains. Here, we report that chi sites stimulate plasmid recombination frequency by 16-fold in *sbcB* strains. Chi-stimulated plasmid recombination is dependent on RecBCD but is independent of RecF pathway genes. The distribution of recombination products suggests that high-molecular-weight linear plasmid DNA is a substrate for RecBCD-mediated recombination. Surprisingly, our data also suggest that chi$^+$ plasmids also recombine by the RecBCD pathway in *rec*$^+$ *sbcB*$^+$ cells.

The RecBCD enzyme of *Escherichia coli* is required by the major recombination pathway of conjugational and generalized transductional recombination (3, 35). RecBCD is also the major enzyme responsible for the degradation of linear double-stranded DNA in vivo (34, 39). From in vitro experiments using purified RecBCD, it was observed that the preferred substrates for RecBCD processing are linear double-stranded DNA molecules (17, 19). RecBCD enzyme's dual roles as recombinase and exonuclease are regulated by its interaction with the chi sequence. RecBCD exonuclease activity is attenuated by chi sites in the substrate (6, 7, 9, 10, 41), and RecBCD-dependent recombination is greatly stimulated in the vicinity of chi sites (2, 12, 21, 24).

Previous work has shown that, in contrast to conjugational recombination, plasmid recombination is not reduced by *recBC* mutations (18, 27). Instead, plasmid recombination is dependent on the *recA*, *recF*, *recJ*, *recO*, *ssb*, and *topA* gene products (5, 15, 16, 18, 22, 28), which comprise a subset of the RecF pathway of conjugational recombination.

We have previously observed that in *sbcB* (exonuclease I-deficient) strains, plasmids carrying the chi sequence produce high-molecular-weight linear concatemeric plasmid DNA (HMW linear plasmid DNA) (41). In contrast, chi^0 plasmids produce HMW linear plasmid DNA only if additional mutations are present in the genes coding for the RecBCD enzyme (4, 23, 30, 41). On the basis of these data, we proposed that chi sites on the HMW linear plasmid DNA allow productive chi-RecBCD interactions leading to the attenuation of the RecBCD nuclease activity, in agreement with the in vivo observations of Dabert et al. (6, 7) and with the in vitro observations of Dixon and Kowalczykowski (9, 10).

In this work, we have investigated chi effects on plasmid recombination frequency in *sbcB* strains. We have also analyzed the products of chi-dependent and chi-independent plasmid recombination to shed additional light on the recombination pathways. Our findings suggest that (i) recombination of chi-containing plasmids in *sbcB* strains occurs predominantly by the RecBCD pathway, (ii) HMW linear plasmid DNA is a substrate for RecBCD-mediated plasmid recombination, and (iii) chi$^+$ plasmids can recombine by the RecBCD pathway even in wild-type *rec*$^+$ strains.

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MATERIALS AND METHODS

Bacterial strains and growth conditions. Bacterial strains used in this study are listed in Table 1. Transformations were carried out by the $CaCl_2$ procedure (33), and recombination assays were performed the following day. Transformants were selected on Luria-Bertani (LB) medium (10 g of tryptone per liter, 5 g of yeast extract per liter, and 5 g of NaCl per liter) supplemented with the appropriate antibiotics (100 μg of ampicillin per ml for plates, 50 μg of ampicillin per ml for liquid medium, and 25 μg of kanamycin per ml for both plates and liquid medium). All cultures were grown at 37°C.

Plasmids. pNK1213 is a pACYC184 derivative carrying the mini-Tn*10* (Km^r) transposon of pNK862 (20). A 983-bp Km^r gene fragment was excised from pNK1213 with *Dra*III and *Fnu*4HI and inserted between the *Eco*RI and *Nhe*I sites of pBR322 (by using linkers) to create pAN1 (see Fig. 1). A derivative of pAN1, pACB1, carrying an inactive 3′-truncated Km^r gene, was created by deleting the 300-bp *Hin*dIII-*Nhe*I fragment of pAN1. The 727-bp *Cla*I-*Nhe*I fragment of pAN1, which contains the 3′ two-thirds of the Km^r gene, was cloned into the *Ava*I site of pACB1 to create the chi^0 recombination substrate pTCB76. pTCB80 is a chi$^+$ derivative of pTCB76 constructed by inserting a chi-containing double-stranded oligonucleotide (formed by annealing 5′-GATCCTCTAGAGCTGGTGGG-3′ and 5′-GATCCCCACCAGCTCTAGAG-3′) into the *Bam*HI site between the two Km^r fragments. The chi site of pTCB80 is oriented clockwise, 5′-GCTGGTGG-3′, in the drawing shown in Fig. 1. The oligonucleotide also carries an *Xba*I restriction site to facilitate restriction analysis and screening. All constructs were prepared in the *recA* deletion strain, JC10287. For recombination assays, pure monomeric samples of supercoiled pTCB76 and pTCB80 were gel purified by an agarase method (Gelase, Epicentre Technologies).

Recombination assay. Strains were transformed with the test plasmid pTCB80 (chi$^+$) or pTCB76 (chi^0), and Ap^r transformants were identified on LB plates containing ampicillin (LB+AMP). Six independent Ap^r colonies from each transformation were grown to mid-log phase in liquid LB+AMP medium. Serial 10-fold dilutions of each culture were prepared, and 5-μl droplets of each serial dilution were plated and allowed to dry without spreading on LB+AMP and LB+AMP plus kanamycin (KM) plates. After overnight growth at 37°C, the number of colonies in each dilution drop was counted. To minimize statistical fluctuations, only dilutions containing more than 5 colonies and less than 50 colonies were counted. The number of Ap^r and Ap^r Km^r colonies per ml in the original LB+AMP culture was calculated from the colony counts and dilution factors of the drop used. The values obtained from the LB+AMP plates give the total concentration of plasmid-carrying cells (Km^r and Km^s) in the original culture, whereas the values from the LB+AMP+KM plates give the concentrations of cells carrying recombinant products.

TABLE 1. *E. coli* strains

Strain[a]	Relevant genotype	Source
AB1157	*rec*$^+$	A. J. Clark
RDK1899	*sbcB15*	S. T. Lovett
JC7623	*recB15 recC22 sbcB15*	A. J. Clark
RDK1792	*recD1013*	S. T. Lovett
JC8814	*sbcB15 recF143*	S. T. Lovett
STL1671	*sbcB15* del(*recA*)	S. T. Lovett
JC10287	del(*srlR-recA*)*304*	A. J. Clark
JC8111	*recB15 recC22 sbcB15 recF*	R. D. Kolodner
STL1273	*recJ284 sbcB15*	S. T. Lovett

[a] All strains contained mutations *argE3 his-4 leu-6 proA2 thr-1 thi-1 rpaL31 galK2 lacY1 ara-14 xyl-5 mtl-1 supE44 kdgK51.*

Km^r plasmid analysis. For each strain, Km^r recombination products produced from chi$^+$ and chi^0 substrate plasmids were analyzed by gel electrophoresis. Approximately 10 Km^r colonies were analyzed per strain, obtained by picking 1 to 2 colonies from each of the six dilution spots used in the recombination frequency assays. Selected colonies were grown to saturation in LB+KM liquid cultures (2 ml), and plasmid DNA was isolated by the alkaline lysis method (33). The size of the recombination products was determined by comparing the electrophoretic mobility of supercoiled plasmid samples with supercoiled plasmid standards (in agarose gels). The standards used were as follows: pGEMEX, 3,995 bp (31); pUC19, 2,686 bp (40); pBR322, 4,361 bp (38); pTCB45, 8,999 bp (constructed by opening pRDK41 [11] at one *Bam*HI site and inserting a 277-bp *Pvu*II-*Dra*III λ DNA fragment containing λ *cos* [unpublished data]); and pACYC184, 4,244 bp (32). For this series of standards, supercoiled plasmid mobility is a logarithmic function of plasmid size (data not shown). Since some products could not be distinguished on the basis of size alone, the plasmids were further characterized by restriction mapping with *Xba*I, *Bam*HI, and *Cla*I, enzymes that produce unique sets of restriction fragments from each substrate and product. Diagrams of the substrates and products are shown in Fig. 1 and the diagnostic restriction fragments are listed in Table 2. *Xba*I and *Bam*HI cut only once in pTCB80 and in pTCB76, respectively, but not in the monomeric recombinant product, pAN1. *Cla*I cuts once in pTCB76, pTCB80, and the recombinant monomer produced from these plasmids (see Table 2). *Cla*I digestion of both pAN1 and p(7.8) will produce only 3.9-kb fragments, whereas digestion of p(9.4) will produce a 5.5-kb fragment in addition to the 3.9-kb fragment. Digestion of the 11-kb pTCB80 dimer with *Cla*I will produce only 5.5-kb fragments, whereas digestion of p(11-T) with the same enzyme will result in 3.9- and 7.1-kb fragments. Similarly, digestion of a pTCB76 trimer (16.5-kb) with *Cla*I will result in only 5.5-kb fragments, whereas digestion of p(16) will result in 3.9-, 5.5-, and 7.1-kb fragments.

In some mapping experiments, only small amounts of plasmid were available, and the analysis was performed by blot hybridization as previously described (41). For use as a probe, pAN1 was linearized with *Pst*I and labeled with ^{32}P by random priming (13, 14).

TABLE 2. Restriction analysis of recombination products

Recombinant plasmid	Enzyme	Observed fragment(s) (kb)[a]	
		Chi^0	Chi$^+$
pAN1	*Cla*	3.9	3.9
	Bam	N.S.	N.D.
	Xba	N.D.	N.S.
p(7.8)	*Cla*	3.9	3.9
	Bam	N.S.	N.D.
	Xba	N.D.	N.S.
p(9.4)	*Cla*	3.9, 5.5	3.9, 5.5
	Bam	9.4	N.D.
	Xba	N.D.	9.4
p(11-T)	*Cla*	3.9, 7.1	N.F.
	Bam	9.4, 1.6	N.F.
	Xba	N.D.	N.F.
p(16)	*Cla*	3.9, 5.5, 7.1	N.F.
	Bam	1.6, 5.5, 9.4	N.F.
	Xba	N.D.	N.F.

[a] N.S., no sites on plasmid; N.D., restriction site on plasmid not determined; N.F., plasmid not found.

Statistical analysis of Km^r product distributions. The data set used for Fig. 4 was analyzed to obtain the number of occurrences of each type of recombinant product. For each strain-substrate combination, the occurrence data were grouped into two classes. The first class was the sum of occurrences of pAN1 and p(7.8), and the second was the sum of occurrences of p(9.4), p(11-T), and p(16). The grouped data were used in contingency χ^2 analyses (2×2 tables, one degree of freedom, with Yates correction factor) to test the independence of the chi$^+$ and chi^0 product distributions for each strain (36). The numbers of colonies and plasmids obtained from each strain-plasmid combination are as follows: *sbcB* strain-chi$^+$ substrate, 21 independent recombinant products from 10 colonies; *sbcB* strain-chi^0 substrate, 10 products from 10 colonies; *sbcB recF* strain-chi$^+$ substrate, 26 products from 10 colonies; *sbcB recF* strain-chi^0 substrate, 8 products from 8 colonies; *sbcB recJ* strain-chi$^+$ substrate, 17 products from 8 colonies; *sbcB recJ* strain-chi^0 substrate, 10 products from 10 colonies; *sbcB* del(*recA*) strain-chi$^+$ substrate, 10 products from 10 colonies; *sbcB* del (*recA*) strain-chi^0 substrate, 11 products from 10 colonies; *rec*$^+$ strain-chi$^+$ substrate, 10 products from 10 colonies; and *rec*$^+$ strain-chi^0 substrate, 8 products from 8 colonies.

RESULTS

Chi effect on plasmid recombination frequencies in *sbcB* mutants. We used an assay that requires intramolecular recombination within an interrupted tandem duplication to reconstitute a functional plasmid Km^r gene. The plasmid recombination frequency assay is described in detail in Materials and Methods. Briefly, cells of the test strain were transformed with the recombination substrates, and transformants were selected on LB+AMP plates. Ten colonies from each plasmid-strain combination were grown to mid-log phase in liquid medium, serially diluted with LB medium, and plated on either LB+AMP plates to determine the number of plasmid-carrying cells or LB+AMP+KM plates to determine the number of cells carrying recombinant products.

The design of the recombination substrates is shown in Fig. 1 (pTCB80, chi$^+$). A fragment containing the 5′ two-thirds of a Km^r gene is separated from a fragment containing the 3′ two-thirds of the gene by 1,200 bp of the pBR322 Tc^r region. Recombination events within the duplicated Km^r sequences will reconstitute an intact Km^r gene, resulting in the formation of pAN1 (Fig. 1). pTCB80 (chi$^+$) was created from pTCB76 (chi^0) by insertion of a single chi site 200 bp downstream (clockwise as drawn in Fig. 1) of the 5′ Km^r fragment. The chi sequence is oriented 5′-GCTGGTGG-3′ in the clockwise direction as shown in Fig. 1.

Using these recombination substrates in the assay described above, we found that chi stimulates plasmid recombination frequency by 16-fold in the *sbcB* strain (Fig. 2). This stimulation was dependent on *recA* function as seen by comparing recombination frequencies in *sbcB* and *sbcB* del(*recA*) strains. Chi stimulation was not decreased by mutations in *recF* and *recJ*, suggesting that the RecF pathway is not responsible for the effect. No chi effect was observed in the *recBC sbcB* strain or in the *recD* strain, in agreement with the previous proposals that chi acts through direct interaction with the RecBCD enzyme (9, 10). Taken together, these results suggest that chi-stimulated plasmid recombination in the *sbcB* background occurs predominantly via the RecBCD pathway.

In *recBC sbcB* cells, chi$^+$ and chi^0 substrates recombine at frequencies similar to those for chi$^+$ substrates in *sbcB* cells, suggesting a possible mechanistic similarity. However, recombination of our substrates in the *recBC sbcB* cells is strongly dependent on *recF* function (Fig. 2), demonstrating that different recombination pathways are utilized in the two strains. The *recF* dependence of plasmid recombination in the *recBC sbcB* strain has been noted previously by many workers (5, 23, 27).

Plasmids carrying directly repeating DNA sequences undergo *recA*-independent recombination (1, 8, 25, 26, 29). In

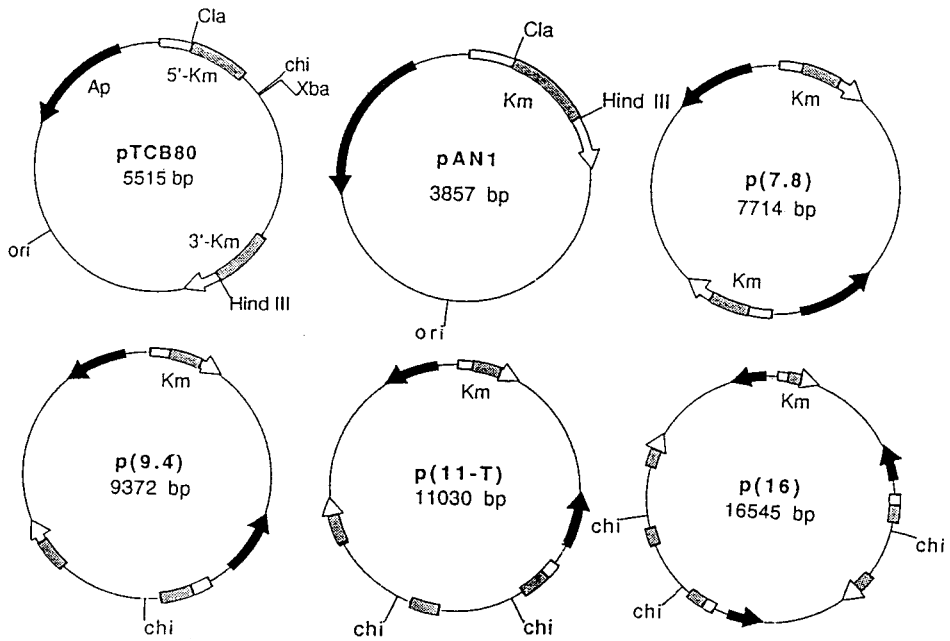

FIG. 1. Plasmid recombination substrates and products. pTCB80 is a derivative of pBR322. pTCB80 carries two fragments from the Km^r gene of Tn*903*. A fragment containing the 5′ two-thirds of this gene is separated from a fragment containing the 3′ two-thirds of the Km^r gene by 1,200 bp of the pBR322 Tc^r region. The two Km^r fragments share a 400-bp region of homology. Recombination events within that region remove the intervening pBR322 sequence and thereby reconstitute a functional Km^r gene. The chi site of pTCB80 was inserted into the unique *Bam*HI site which is 200 bp downstream of the 5′-Km^r fragment and 1 kb upstream of the 3′-Km^r fragment. The chi site is oriented clockwise 5′-GCTGGTGG-3′ as drawn here. pTCB76 (not shown) is identical to pTCB80 except that it lacks a chi site. Recombination products observed in this study included the monomeric recombinant pAN1, homodimeric recombinant p(7.8), heterodimeric recombinant p(9.4), dimeric triplication product p(11-T), and heterotrimeric recombinant p(16). Recombination products derived from pTCB76 (chi^0) were identical to the products shown except that they lacked chi sites.

agreement with these observations, Fig. 2 shows that significant levels of recombination are observed from both chi$^+$ and chi^0 substrates in del(*recA*) and del(*recA*) *sbcB* strains (Fig. 2). *recA*-independent recombination is stimulated sevenfold by the *sbcB* mutation [Fig. 2; chi^0 substrates, *sbcB*, and del(*recA*) *sbcB*].

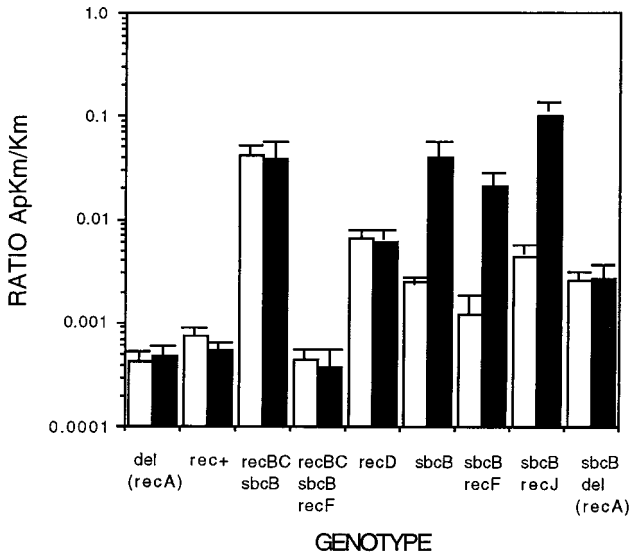

FIG. 2. Recombination assays using pTCB76 and pTCB80. For each strain-plasmid combination, six independent transformants were grown in LB+AMP medium to mid-log phase. Serial dilutions of the cultures were plated on LB+AMP and LB+AMP+KM agar plates, and the number of colonies per dilution was counted. These values were extrapolated to zero dilution in order to calculate the number of Ap^r and Ap^r Km^r cells per ml in the original LB+AMP culture. Solid bars, chi$^+$ substrate (pTCB80); open bars, chi^0 substrate (pTCB76). Error bars indicate one standard error of the mean.

Analysis of recombinant products. If different recombination pathways are used by chi$^+$ and chi^0 plasmids in *sbcB* strains, different Km^r recombinants might be produced. To test this possibility, the structures of Km^r products generated from chi$^+$ and chi^0 substrates were determined. The analysis was carried out in two steps. First, plasmid size was determined from the electrophoretic mobility of supercoiled samples by using appropriate supercoiled plasmid size markers. Second, the plasmids were characterized by restriction mapping, since some of the possible products could not be distinguished on the basis of size alone. Restriction analysis of the Km^r products is described in detail in Materials and Methods.

Figure 1 illustrates the structures of the Km^r recombination products that were observed. pAN1 is the simplest monomeric Km^r product. p(7.8) is a homodimer of pAN1. p(9.4) is a heterodimer of pAN1 and the initial substrate. p(11-T) is a complex heterodimer composed of one unit of pAN1 and one unit of the recombination substrate that carries a tandem duplication of the pBR322 region that separates the 5′-Km^r and 3′-Km^r fragments. p(16) is a heterotrimer composed of one copy of the p(11-T) dimer joined to one copy of the recombination substrate.

Figure 3 shows representative gels displaying Km^r plasmids recovered from 10 independent recombinant colonies following transformation of *sbcB*, *sbcB recF*, *sbcB* del(*recA*), and *rec*$^+$ strains with the substrate plasmids. Figure 4 graphically illustrates the distribution of Km^r plasmid species recovered from these strains and also from the *sbcB recJ* strain. In Fig. 4, the ordinate values are the fraction of Km^r colonies in which a particular Km^r plasmid species is found. The recombinant product distributions are chi-dependent for the *sbcB*, *sbcB recJ*,

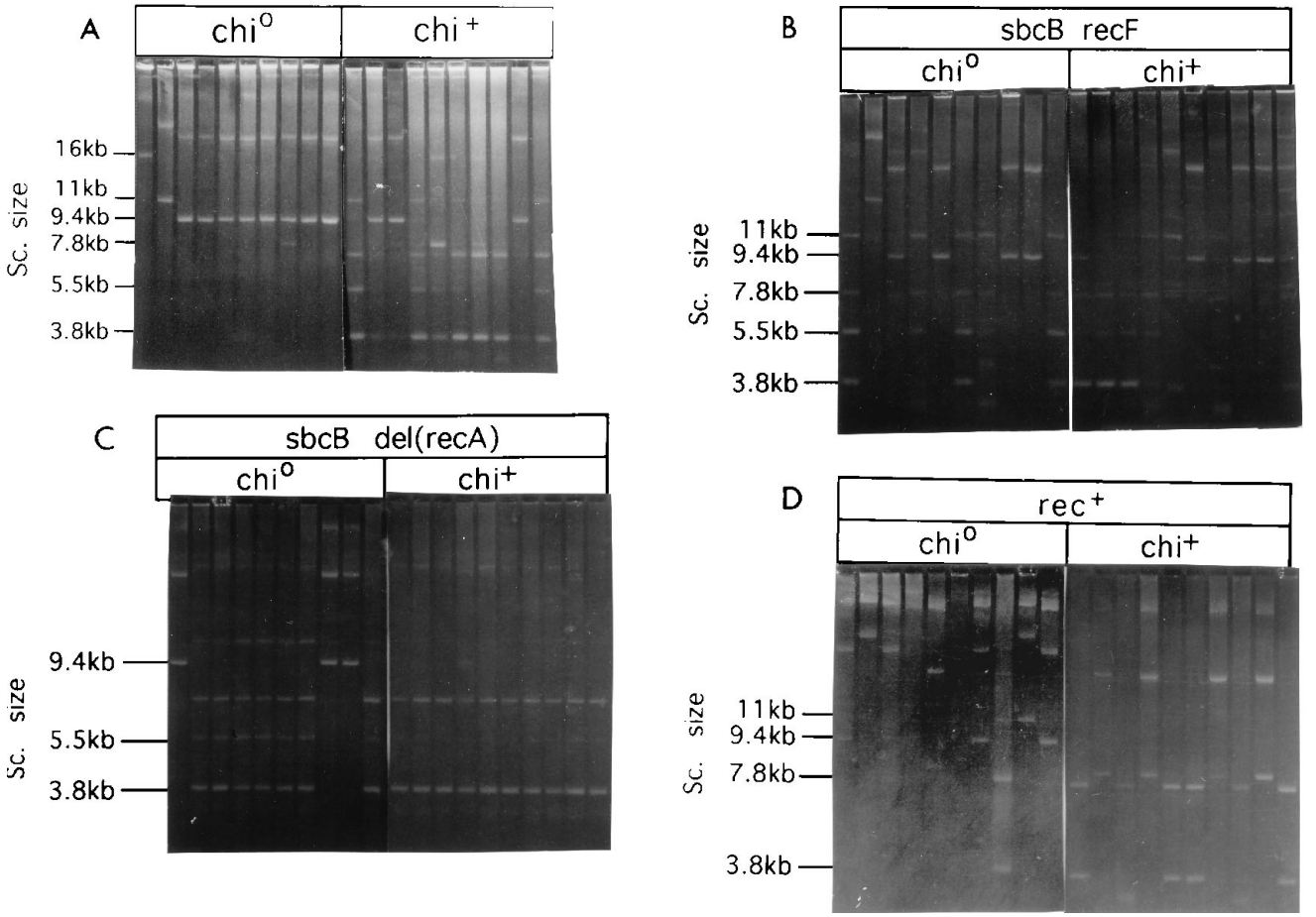

FIG. 3. Km^r plasmid products obtained from *rec*$^+$ cells and *sbcB* mutants. Plasmid DNA from Km^r progenies of *sbcB* (a), *sbcB recF* (b), *sbcB* del (*recA*) (c), and (d) *rec*$^+$ (AB1157) cells harboring pTCB76 or pTCB80 were isolated by the alkaline lysis procedure and run in the supercoiled state on agarose gels. For each strain, approximately 10 Km^r colonies from each chi^0 and chi$^+$ derivative were tested. The sizes of the species were determined by comparison with supercoiled plasmid markers (Sc.) as described in Materials and Methods.

*sbcB recF*4 and *rec*$^+$ strains. In these backgrounds, chi$^+$ substrates were converted exclusively to recombinant monomer pAN1, homodimer p(7.8), and heterodimer p(9.4). The chi^0 substrates were converted principally to the heterodimer p(9.4) and, at lower frequencies, the monomer pAN1, the complex heterodimer p(11-T), and heterotrimer p(16). Although the data sets are small, contingency χ^2 analysis shows that the chi-dependent differences in the product distributions in the *sbcB* strain are statistically significant ($P < 0.001$; see Materials and Methods). For the *sbcB recF* and *sbcB recJ* product distributions, the differences are not statistically significant at the 5% level.

Another difference between chi$^+$ and chi^0 recombinations in the *sbcB*, *sbcB recF*, and *sbcB recJ* strains is that recombinant colonies derived from chi$^+$ substrates always contained more than one type of recombinant product, whereas recombinant colonies derived from chi^0 transformants only contained one Km^r species (Fig. 3 and 4). We discuss possible interpretations of this observation in the following section.

In the *sbcB* del(*recA*) strain, the product distributions are not chi-dependent, suggesting that the same recombination process operates on both types of substrate plasmid. The recombination frequencies of the chi$^+$ and chi^0 substrates were also very similar in this strain (Fig. 2).

Taken together, the data discussed in this section demonstrate that in all of the *recA*$^+$ *sbcB* backgrounds tested, a significant fraction of the chi$^+$ substrates recombine by a process that differs, at least in part, from the process used by chi^0 substrates. These findings are consistent with the conclusion of the previous section that chi$^+$ substrates recombine by the RecBCD pathway in *sbcB* strains, whereas chi^0 substrates use a different pathway.

Plasmid recombination by the RecBCD pathway in *rec*$^+$ *sbcB* strains. The distributions of recombination products from the *rec*$^+$ strain AB1157 are also chi-dependent and similar to those of the *sbcB* strain (Fig. 3 and 4). The difference between the chi$^+$ and chi^0 distributions in the *rec*$^+$ strain is statistically significant by contingency χ^2 analysis ($P < 0.01$). This surprising finding suggests that the chi$^+$ and chi^0 substrates recombine by different pathways in this strain, despite the fact that they have similar recombination frequencies (Fig. 2). By analogy with the *sbcB* results discussed in the previous section, the data suggest that a significant fraction of chi$^+$ plasmid recombination in *rec*$^+$ cells is mediated by the RecBCD pathway, even in the absence of *sbcB* mutations.

DISCUSSION

The RecBCD pathway mediates chi$^+$ plasmid recombination. We have previously observed that chi$^+$ plasmids in *sbcB*

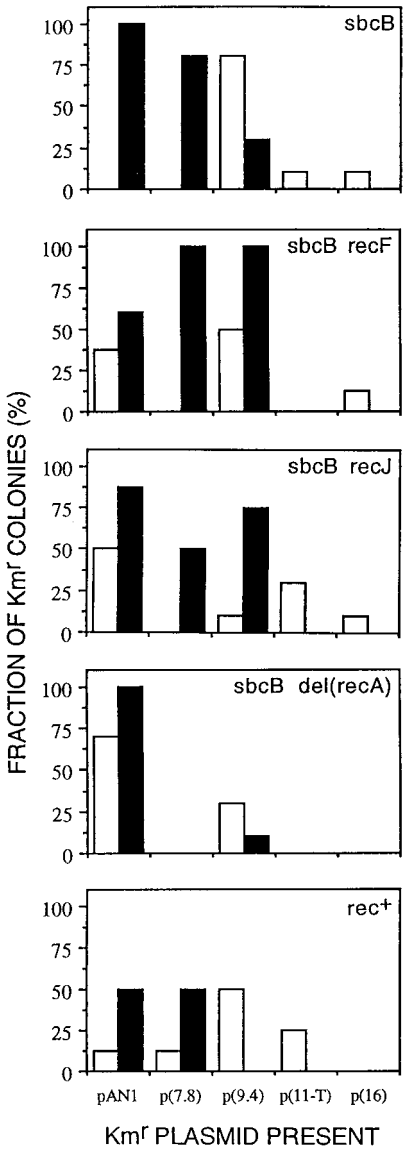

FIG. 4. Frequencies of Km^r plasmid species derived from pTCB76 or pTCB80 in different genotypes. Open bars represent Km^r plasmids derived from chi^0 plasmids; solid bars represent Km^r plasmids derived from chi^+ plasmids. The value on the vertical axis indicates the fraction of Km^r colonies which contained a particular Km^r plasmid species. Approximately 10 colonies of chi^0 or chi^+ Km^r derivatives from each strain were tested. The numbers of plasmids and colonies analyzed are listed in Materials and Methods.

strains produce HMW linear plasmid DNA (41), a substrate which should be efficiently utilized by the RecBCD enzyme. We therefore investigated whether chi^+ plasmids can recombine by the RecBCD pathway. Chi stimulates plasmid recombination frequency by 16-fold in the *sbcB* strain (Fig. 2). This stimulation is dependent on *recBC* and *recA* and is independent of *recF* and *recJ* (Fig. 2). Analysis of recombination products (Fig. 3 and 4) also supports the conclusion that chi^+ and chi^0 plasmids recombine by different pathways in $recA^+$ $recBCD^+$ *sbcB* strains. The simplest interpretation of these data is that chi^+ plasmids recombine via the RecBCD recombination pathway, whereas chi^0 plasmids do not.

The frequency of chi-independent plasmid recombination in the *recBC sbcB* strain is quantitatively similar to that of chi^+ plasmid recombination in the *sbcB* strain (Fig. 2). This similarity raises the possibility that attenuation of RecBCD activity by chi^+ plasmids in the *sbcB* strain allows plasmid recombination to proceed by the RecBCD-independent RecF pathway, just as in *recBC sbcB* strains. While the simplest form of this hypothesis is ruled out by the observation that mutations in two key RecF pathway genes, *recF* and *recJ*, dramatically reduce *recBC sbcB* plasmid recombination (Fig. 2), we cannot exclude the possibility that other RecF pathway genes may participate in the chi-dependent pathway of *sbcB* strains.

In the *sbcB* strain, the major chi^0 products are the p(9.4) heterodimer and the more complex p(11-T) and p(16) products. The latter two plasmids contain tandem duplications of the overlapping Km^r gene sequences and the intervening sequence from the parental pBR322 vector (Fig. 1). Such "triplication" products have been observed previously in studies of *recA*-independent recombination (25, 26). This similarity suggests that a significant fraction of the chi-independent recombination events in our study may have been produced by that *recA*-independent pathway, a suggestion supported by the relatively high recombination frequencies of the del(*recA*) strains (Fig. 2).

The role of *sbcB* in plasmid recombination. Chi^+ plasmid recombination in $recA^+$ *sbcB* strains always results in the formation of multiple recombinant plasmid species per cell (Fig. 3 and 4). In contrast, no evidence for multiple products was found for chi^0 substrates in the same strains. Since recombination is rare even in *sbcB* cells with chi^+ substrates (less than 5% of all plasmid-carrying cells have recombinant plasmids) (Fig. 2), it is unlikely that the multiple products are derived from independent events. To explain this apparent contradiction, we propose that the rate-limiting step for the production of Km^r recombinants is the formation of a recombinogenic DNA substrate that can be processed to yield multiple products. This is clearly suggestive of a multimeric species such as HMW linear plasmid DNA or rolling circle replication intermediates. Our previous discovery of chi-mediated HMW linear plasmid DNA in *sbcB* strains (41) provides direct support for this hypothesis.

Chi^0 plasmid recombination frequency was also stimulated by mutations in *sbcB* (Fig. 2). A *recA* deletion did not reduce recombination of this substrate in the *sbcB* background (Fig. 2), suggesting that chi^0 plasmid recombination in this strain is primarily *recA* independent. However, chi^0 substrates yield different product distributions in *sbcB* and del(*recA*) *sbcB* strains. For this reason, it appears that chi^0 plasmid recombination in *sbcB* strains proceeds by *recA*-independent and *recA*-dependent pathways. Since the combined frequency from both pathways is very similar to the frequency of *recA*-independent plasmid recombination in the *sbcB* del(*recA*) strain, we believe that the two pathways share a common rate-limiting step. Although our data are insufficient to determine whether this rate-limiting step precedes or follows recombination, we favor models in which the *sbcB* mutation allows increased production of a recombination precursor, since this hypothesis best explains the features of chi^+ recombination discussed in the preceding paragraph.

In summary, the observation that *sbcB* mutations stimulate both chi^+ and chi^0 plasmid recombination (Fig. 2) suggests that exonuclease I normally suppresses formation of aberrant replication structures which can be utilized as substrates by several recombination pathways, a hypothesis that has been previously proposed by others (3, 4). The distribution of products from the chi^+ substrate (Fig. 3 and 4) implicates linear concatemeric plasmid DNA molecules, or the rolling circle

plasmid replication forms that can produce them, as candidates for the proposed recombinogenic substrates.

Plasmid recombination by the RecBCD pathway in *rec*$^+$ (AB1157) strains. A particularly surprising observation is that the chi$^+$ product profile differs markedly from the chi^0 product profile in the *rec*$^+$ strain, AB1157 (Fig. 4). The product profiles of the *rec*$^+$ strain resemble those of the *sbcB* strain, suggesting that the RecBCD pathway can operate on plasmid substrates even in *rec*$^+$ (*sbcB*$^+$) strains. To date, previous studies have not demonstrated chi effects on plasmid recombination. Indeed, the chi effect on product distribution (Fig. 3 and 4) is our only evidence for two plasmid recombination pathways in this strain, since there is no chi effect on plasmid recombination frequency (Fig. 2). The lack of a quantitative chi effect in *rec*$^+$ cells suggests again the possibility that RecBCD and non-RecBCD pathways may compete for a common rate-limiting precursor that can be used with equal efficiency by either pathway. By analogy with the arguments of the previous section, we speculate that this rate-limiting step may be the same one that is normally suppressed by the *sbcB* gene product, exonuclease I.

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