Genetic and Physical Analysis of Plasmid Recombination in *recB recC sbcB* and *recB recC sbcA Escherichia coli* K-12 Mutants

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ABSTRACT

The effect of mutations in known recombination genes (*recA, recB, recC, recE, recF, recJ, recN, recO, recQ* and *ruv*) on intramolecular recombination of plasmids was studied in *recB recC sbcB* and *recB recC sbcA Escherichia coli* mutants. The rate of recombination of circular dimer plasmids was at least 1000-fold higher in *recB recC sbcB* or *recB recC sbcA* mutants as compared to wild-type cells. The rate was decreased by mutations in *recA, recF, recJ, recO, ruv* or *mutS* in *recB recC sbcB* mutants, and by mutations in *recE, recN, recO, recQ, ruv* or *mutS* in *recB recC sbcA* mutants. In addition to measuring the recombination rate of circular dimer plasmids, the recombination-mediated transformation of linear dimer plasmids was also studied. Linear dimer plasmids transformed *recB recC sbcB* and *recB recC sbcA* mutants 20- to 40-fold more efficiently than wild-type cells. The transformation efficiency of linear dimer plasmids in *recB recC sbcB* mutants was decreased by mutations in *recA, recF, recJ, recO, recQ* or *lexA* (*lexA3*). In *recB recC sbcA* mutants the transformation efficiency of linear dimers was decreased only by a *recE* mutation. Physical analysis of linear dimer- or circular dimer-transformed *recB recC sbcB* mutants revealed that all transformants contained recombinant monomer genotypes. This suggests that recombination in *recB recC sbcB* cells is very efficient.

RECOMBINATION of circular dimer plasmids has been most extensively studied in wild-type and *recD* mutants where, of the known recombination genes, the recombination requires *recA, recF, recJ* and *recO* and *recA, recB, recC* and *recJ*, respectively. In *recB recC sbcA* mutants, recombination of plasmids is at least 20-fold higher as compared to wild-type cells and is unique in that it is *recA* (and *recF*) independent (FISHEL, JAMES and KOLODNER 1981; LABAN and COHEN 1981; JAMES, MORRISON and KOLODNER 1982; COHEN and LABAN 1983; SYMINGTON, MORRISON and KOLODNER 1985). Of the tested recombination genes (*recA, recE* and *recF*), *recE* is the only one required for plasmid recombination in *recB recC sbcA* mutants (COHEN and LABAN 1983; SYMINGTON, MORRISON and KOLODNER 1985). The effect of various mutations on plasmid recombination in *recB recC sbcB* mutants has not yet been reported.

The observation that linear dimer plasmids transformed *recB recC sbcA* mutants as well as circular dimer plasmids led to the analysis of the intramolecular recombination of linear dimer plasmids (SYMINGTON, MORRISON and KOLODNER 1985). These molecules appear to cyclize by recombination in order to be established as autonomously replicating plasmids in cells. Such substrates do not recombine efficiently in wild-type or *recB recC* mutant strains, presumably because the rate of generating mature recombinants is low. However, linear dimer plasmids do recombine efficiently in *Escherichia coli* strains containing either *recD* mutations (LOVETT, LUISI-DELUCA and KOLODNER 1988) or *recB recC sbcA* mutations (SYMINGTON, MORRISON and KOLODNER 1985). As with the recombination of circular dimer plasmids, the recombination of linear dimer plasmids requires the *recE* gene product and is independent of *recA* and *recF* function in *recB recC sbcA* mutants (SYMINGTON, MORRISON and KOLODNER 1985). The requirement for other genes has not been tested. In addition, the recombination of linear dimer plasmids has never been tested in *E. coli* strains containing *recB recC sbcB* mutations where the RecF pathway is thought to act with high efficiency (CLARK 1973, 1974, 1980; LOVETT and CLARK 1983; CLARK *et al.* 1984).

This paper reports the effect of mutations in known recombination genes (*recA, recE, recF, recJ, recN, recO, recQ* and *ruv*) on the rate of recombination of circular dimer plasmids and on recombination-mediated transformation of linear dimer plasmids in *recB recC sbcA* and *recB recC sbcB* mutants. In addition, the effect of *mutS* (inactivates a mismatch repair protein; SU and MODRICH 1986; MODRICH 1987) and *lexA3* (encodes a noncleavable LexA repressor; LITTLE *et al.* 1980) mutations were tested in these two genetic backgrounds. The effect of the *mutS* mutation was studied to determine if the formation of heteroduplex DNA is an intermediate in the recombination of the two *tet* alleles prior to the formation of a functional *tet* gene.

The *lexA3* mutation was studied to determine if the failure to properly regulate a LexA-regulated gene such as *recQ* (IRINO, NAKAYAMA and NAKAYAMA 1986), *recN* (SHURVINTON and LLOYD 1982) or *ruv* (PICKSLEY, LLOYD and BUCKMAN 1984) affects plasmid recombination. A structural analysis of the plasmid products purified from *recB recC sbcB* mutants that were transformed with either circular or linear dimer plasmid has been performed for comparison with a similar analysis previously performed in wild-type cells and *recB recC sbcA* mutants (DOHERTY, MORRISON and KOLODNER 1983; SYMINGTON, MORRISON and KOLODNER 1985). This data provides insight into the mechanism of recombination in the various genetic backgrounds studied.

MATERIALS AND METHODS

Bacterial strains, media and general methodology: The bacterial strains and relevant genotypes are listed in Table 1. The plasmids used in the recombination studies were maintained in JC10287 (CSONKA and CLARK 1979), a *recA304 E. coli* mutant derived from AB1157 (pRDK41), or in RDK1518, a *thyA* derivative of JC10287 (pRKD69). L-Broth supplemented with 50 μg/ml thymidine and 0.2% glucose was used routinely to grow bacterial cultures (SYMINGTON, MORRISON and KOLODNER 1985). Minimal salts medium consisted of 56/2 (WILLETTS, CLARK and LOW 1969) or VB (VOGEL and BONNER 1956) salts with 0.2% glucose, 0.5 μg/ml thiamine and 50 μg/ml amino acid supplements. Solid media contained 1.5% agar (Difco Laboratories, Detroit, MI). When appropriate, ampicillin (Ap; Totacillin-N, Beecham Laboratories, Bristol, TE), kanamycin (Km; kanamycin sulfate, QUAD Pharmaceuticals, Indianapolis, IN), tetracycline (Tc; tetracycline HCl, Sigma, St. Louis, MO), and rifampicin (Rf; Sigma) were added to final concentrations of 100, 30, 20, and 100 μg/ml, respectively. Transformations, P1 transductions, and replica plate tests for recombination proficiency and UV sensitivity were carried out as described (KOLODNER, FISHEL and HOWARD 1985; LOVETT, LUISI-DELUCA and KOLODNER 1988). The mutator phenotype associated with *mutS* strains was tested by spotting 25 μl of an exponentially growing culture on an LB/Rf plate.

Preparation and analysis of plasmid DNA: Preparative quantities of plasmid DNA were purified by the BIRNBOIM and DOLY (1979) method followed by two cycles of CsCl/ethidium bromide density gradient centrifugation. This DNA was used for all transformation and recombination assays. For the structural analysis of plasmid products (see Table 4) small scale plasmid preparations were prepared as follows. JC7623 cells were transformed with pRDK69 or *Xba*I-digested pRKD69. Individual Ap^r colonies were inoculated into 3 ml LB + glucose + Ap and aerated overnight at 37°. Plasmid DNA was prepared from 1.4 ml of this culture by the boiling method (HOLMES and QUIGLEY 1981) except that sometimes the DNA was further purified by extraction with phenol and precipitation with ethanol. The structure of each plasmid was then determined by restriction mapping with *Xho*I and *Pst*I essentially as previously described (SYMINGTON, MORRISON and KOLODNER 1985). Restriction endonucleases were purchased from New England Biolabs (Beverly, MA) and used according to the instructions of the manufacturer. Agarose gel electrophoretic separation of DNA fragments followed the procedure of BOLIVAR *et al.* (1977).

TABLE 1

Bacterial strains[a]

Strain	Relevant genotype	Source
A. AB1157 derivatives		
AB1157	Wild type[b]	A. J. CLARK
JC5519	*recB21 recC22*	A. J. CLARK
JC7623	*recB21 recC22 sbcB15 sbcC201*	A. J. CLARK
JC8679	*recB21 recC22 sbcA23*	A. J. CLARK
JC10287	Δ(*srlR-recA*)*304*	A. J. CLARK
RDK1518	Δ(*srlR-recA*)*304 thyA*	This laboratory
B. JC7623 derivatives (*recB recC sbcB* background)		
JC7906	*recJ153*	A. J. CLARK
JC7940	*recA142*	A. J. CLARK
JC8111	*recF143*	A. J. CLARK
JC9383	*sbcA23*	A. J. CLARK
RDK1530	*recN1502*::Tn*5*	This laboratory[c]
RDK1531	*recO1504*::Tn*5*	This laboratory[c]
RDK1645	*ruvB9*	This study[d]
RDK1649	*recQ1*	This study[e]
RDK1654	*lexA3*	This study[f]
RDK1695	*mutS201*::Tn*5*	This study[g]
C. JC8679 derivatives (*recB recC sbcA* background)		
JC8691	*recE159*	A. J. CLARK
JC9604	*recA56*	A. J. CLARK
JC9610	*recF143*	A. J. CLARK
JC13021	*recJ153*	A. J. CLARK
RDK1657	*recN1502*::Tn*5*	This study[h]
RDK1658	*recO1504*::Tn*5*	This study[i]
RDK1693	*recQ1*	This study[j]
RDK1696	*mutS201*::Tn*5*	This study[k]
RDK1713	*ruvB9*	This study[l]

[a] All strains are derivatives of *E. coli* K12.

[b] Other markers are F$^-$ *thr-1 leuB6 thi-1 lacY1 galK2 ara-14 xyl-5 mtl-1 proA2 his-4 argE3 rpsL31*(Sm^r) *tsx-33 supE44 kdgK51.*

[c] KOLODNER, FISHEL and HOWARD (1985).

[d] A KdgK$^+$ derivative of JC7623 (RDK1632; KOLODNER, FISHEL and HOWARD 1985) was transduced to Tc^r with P1 grown on JC14297 (AB1157 *ruvB9 zea-3*::Tn*10 eda-50*). This strain was then transduced to Eda$^+$ (growth on glucuronate) with P1 grown on AB1157 and screened for Tc^s UV^s.

[e] A *metE163*::Tn*10* derivative of JC7623 (RDK1630) was transduced to Met$^+$ with P1 grown on KD2196 (*recQ1*; NAKAYAMA *et al.* 1984) and screened for UV^s.

[f] A *malE*::Tn*10* derivative of JC7623 (RDK1653) was transduced to Mal$^+$ with P1 grown on DM49 (*his-4 argE3 leuB6 proA2 thr-1 thi-1 rpsL31 galK2 lacY1 ara-14 xyl-5 mtl-1 kdgK51 supE44 tsx-33 lexA3*) and screened for UV^s.

[g] JC7623 was transduced to Km^r with P1 grown on ES1574 (*his-4 argE3 leuB6 proA2 thr-1 thi-1 rpsL31 galK2 lacY1 ara-14 xyl-5 mtl-1 kdgK51 supE44 tsx-33 mutS201*::Tn*5*) and screened for the *mutS* mutator phenotype as described in MATERIALS AND METHODS.

[h] JC8679 was transduced to Km^r with P1 grown on RDK1540 (*recN1502*::Tn*5* in AB1157). *recN* was confirmed by backcross to JC7623.

[i] JC8679 was transduced to Km^r with P1 grown on RDK1541 (*recO1504*::Tn*5* in AB1157) and screened for Rec$^-$ UV^s. *recO* was confirmed by backcross to JC7623.

[j] A *metE163*::Tn*10* derivative of JC8679 (RDK1691) was transduced to Met$^+$ with P1 grown on KD2196 (*recQ1*; NAKAYAMA *et al.* 1984). *recQ* was confirmed by backcross to RDK1630.

[k] JC8679 was transduced to Km^r with P1 grown on ES1574 and screened for the *mutS* mutator phenotype as described in MATERIALS AND METHODS.

[l] A KdgK$^+$ derivative of JC8679 (RDK1701) was transduced to Tc^r *eda-50* with P1 grown on JC14297 (AB1157 *ruvB9 zea-3*::Tn*10 eda-50*). This strain (RDK1712) was transduced to Eda$^+$ (growth on glucuronate) with P1 grown on RDK1542 (AB1157 *kdgK*$^+$ *ruvB9*) and screened for Tc^s UV^s.

Plasmid recombination tests: The dimer plasmid pRDK41 was used to study the recombination of circular plasmids. A circular dimer of pBR322, pRDK41 confers Ap^r to a cell and contains one copy each of the *tet-10* and *tet-14* mutant alleles (DOHERTY, MORRISON and KOLODNER 1983). Fluctuation test analysis was used to determine the rate of production of Tc^r progeny after transformation of a strain with 44 ng of pRDK41; this was done essentially as described (FISHEL, JAMES and KOLODNER 1981; JAMES, MORRISON and KOLODNER 1982; LOVETT, LUISI-DELUCA and KOLODNER 1988). Briefly, transformants were selected on LB + Ap at 37°. Entire single Ap^r colonies were resuspended in 1 ml of minimal salts, the cell suspension was then serially diluted and 25 μl of each dilution was spotted onto LB + Tc and LB + Ap plates. For each strain, 11 individual Ap^r transformants were analyzed in this way. The fluctuation test was performed one to three times for each strain tested. Recombination rates were calculated by the method of LEA and COULSON (1949) as previously described (LOVETT, LUISI-DELUCA and KOLODNER 1988). Using this method, $r_0 = M(1.24 + \ln M)$ where r_0 is the median number of Tc^r recombinants among the 11 cultures and M is the average number of recombinants per culture. M was solved by interpolation and then used to calculate the recombination rate, $r = M/N$ where N is the final average concentration of Ap^r cells in each 1-ml cell suspension. Two- to threefold decreases in rates or frequencies were not considered significant because these were in the range of variation observed with the methods used.

The dimer plasmid pRDK69 was used to study the recombination of linear plasmid dimers. A derivative of pRDK41, pRDK69 confers Ap^r to a cell and contains an *Xba*I linker insertion in the *Bam*HI site closest to the *tet-14* mutation of pRDK41 (SYMINGTON, MORRISON and KOLODNER 1985). Linear dimer plasmid transformation efficiencies were determined using HPLC-purified, *Xba*I-digested pRDK69 as described (LOVETT, LUISI-DELUCA and KOLODNER 1988). Undigested pRDK69 was used as the circular dimer control in the transformations. Competent *E. coli* cells were prepared and transformed essentially as described (WENSINK *et al.* 1974) with each transformation mix containing 56 ng of linear or circular plasmid DNA. The frequency of co-transformation by two different plasmids under these conditions was less than 1%. Transformation efficiency is the number of Ap^r transformants per ml of culture relative to the total number of viable cells per ml of culture. The L/C reported is the transformation efficiency of the linear dimer relative to that of the circular dimer analog.

RESULTS

Transformation by linear dimers: The transformation efficiency of the linear dimer, *Xba*I-digested pRDK69, in various derivatives of *recB recC sbcB* and *recB recC sbcA E. coli* mutants is presented in Tables 2 and 3, respectively. The results are presented as the relative transformation efficiency (L/C), or the transformation efficiency of the linear dimer (L) with respect to the transformation efficiency of the circular dimer counterpart (C). Linear dimers transformed wild-type (AB1157) cells 26-fold and *recB recC* mutants 50-fold less efficiently than circular dimers (legends to Tables 2 and 3, and SYMINGTON, MORRISON and KOLODNER 1985). However, linear dimers transformed *recB recC sbcB* (JC7623, Table 2) and *recB recC sbcA* (JC8679, Table 3) mutants almost as efficiently (*recB recC sbcB*), or more efficiently (*recB recC sbcA*) than circular dimer plasmids. In the *recB recC sbcB* mutant (Table 2), the relative transformation efficiency (L/C) was decreased by *recA*, *recF*, *recJ*, *recO* and *recQ* mutations. In addition, the noncleavable LexA repressor mutation, *lexA3*, in the *recB recC sbcB* mutant, reduced the relative transformation efficiency 15-fold. Presumably this reflects the requirement for a LexA-regulated gene like *recQ*. There was little or no effect on the relative transformation efficiency (L/C) by *recN*, *ruv*, *mutS* or *sbcA* when present in a *recB recC sbcB* mutant (Table 2). In contrast, in the *recB recC sbcA* (JC8679) genetic background only a *recE* mutation (*recE159*) significantly lowered the relative transformation efficiency (Table 3). There was little to no effect on the relative transformation efficiency (L/C) by *recA*, *recF*, *recJ*, *recN*, *recO*, *recQ*, *ruv* or *mutS* mutations in this genetic background (Table 3).

Recombination of circular dimers: The fraction of Tc^r bacteria in an Ap^r colony was measured after transformation of various derivatives of *recB recC sbcB* (JC7623) and *recB recC sbcA* (JC8679) mutants with the circular dimer, pRDK41. The results are tabulated as the circular recombination rate, r, in Tables 2 and 3, respectively. The recombination rate of circular dimer plasmids was increased 700-fold in *recB recC sbcB* (Table 2) mutants and 1200-fold in *recB recC sbcA* (Table 3) mutants with respect to the recombination rate in wild-type (AB1157) cells. Note that previous studies have reported recombination frequencies rather than recombination rates (recombination frequency per generation). In a *recB recC sbcB* genetic background the recombination rate of circular dimer plasmids was decreased by *recA* and *mutS* mutations and to a lesser, but still significant, extent by *recF*, *recJ*, *recO* and *ruv* mutations. There was little to no effect on the enhanced recombination rate observed in *recB recC sbcB* mutants by a *recN* or *recQ* mutation. In the *recB recC sbcA* genetic background (JC8679; Table 3) the recombination rate of circular dimer plasmids was decreased by *recE*, *recN*, *recO*, *recQ*, *ruv* and *mutS* mutations. Mutations in *recA* or *recF* had little to no effect on the enhanced recombination rate. In addition, a *recJ* mutation (in a *recB recC sbcA* mutant) enhanced the recombination rate 3-fold above the rate observed in *recB recC sbcA* mutants (JC13021 *vs.* JC8679; Table 3).

Structure of recombination products: The structure of the plasmid DNA purified from an Ap^r colony after transformation of *recB recC sbcB* mutants (JC7623) with circular or linear dimer plasmid DNA was analyzed by restriction mapping. The results are presented in Table 4. All transformants analyzed were selected only by growth in ampicillin. This type of plasmid analysis has previously been reported for plas-

TABLE 2

Plasmid dimer recombination rates and linear dimer transformation efficiencies in *recB recC sbcB* derivatives

Strain	Relevant genotype	Relative transformation efficiency $(L/C)^a$	Relative circular recombination rate $(r)^a$
JC7623	*recB21 recC22 sbcB15*	1.0	1.0
JC7940	*recB21 recC22 sbcB15 recA142*	0.014	≤0.0081
JC8111	*recB21 recC22 sbcB15 recF143*	0.0066	0.17
JC7906	*recB21 recC22 sbcB15 recJ153*	0.014	0.095
RDK1530	*recB21 recC22 sbcB15 recN1502*::Tn*5*	0.45	0.35
RDK1531	*recB21 recC22 sbcB15 recO1504*::Tn*5*	0.0040	0.046
RDK1649	*recB21 recC22 sbcB15 recQ1*	0.039	0.90
RDK1645	*recB21 recC22 sbcB15 ruvB9*	0.51	0.030
RDK1654	*recB21 recC22 sbcB15 lexA3*	0.066	0.45
RDK1695	*recB21 recC22 sbcB15 mutS201*::Tn*5*	0.89	0.0030
JC9383	*recB21 recC22 sbcB15 sbcA23*	0.51	1.34

[a] L/C is the transformation efficiency of the linear dimer plasmid relative to the transformation efficiency of the circular dimer plasmid analog. Transformation efficiency is the number of Ap^r transformants per ml of culture relative to the total number of viable cells per ml of culture. r is the recombination rate as described in MATERIALS AND METHODS (plasmid recombination tests). L/C and r for the parent strains are:

JC7623 (*recB21 recC22 sbcB15*) $L/C = 0.87$ $r = 1.05 \times 10^{-3}$
JC5519 (*recB21 recC22*) $L/C = 0.020$ $r = 6.47 \times 10^{-5}$
AB1157 (wild type) $L/C = 0.038$ $r = 1.47 \times 10^{-6}$

TABLE 3

Plasmid dimer recombination rates and linear dimer transformation efficiencies in *recB recC sbcA* derivatives

Strain	Relevant genotype	Relative transformation efficiency $(L/C)^a$	Relative circular recombination rate $(r)^a$
JC8679	*recB21 recC22 sbcA23*	1.0	1.0
JC9604	*recB21 recC22 sbcA23 recA56*	1.75	0.89
JC8691	*recB21 recC22 sbcA23 recE159*	0.0036	0.060
JC9610	*recB21 recC22 sbcA23 recF143*	1.07	0.54
JC13021	*recB21 recC22 sbcA23 recJ153*	0.88	2.7
RDK1657	*recB21 recC22 sbcA23 recN1502*::Tn*5*	1.46	0.045
RDK1658	*recB21 recC22 sbcA23 recO1504*::Tn*5*	0.48	0.016
RDK1693	*recB21 recC22 sbcA23 recQ1*	1.14	0.068
RDK1713	*recB21 recC22 sbcA23 ruvB9*	0.50	0.026
RDK1696	*recB21 recC22 sbcA23 mutS201*::Tn*5*	1.19	0.088

[a] The transformation efficiency (L/C) and circular recombination rate (r) are as described in the footnote to Table 2. L/C and r for the parent strains are:

JC8679 (*recB21 recC22 sbcA23*) $L/C = 1.37$ $r = 1.84 \times 10^{-3}$
JC5519 (*recB21 recC22*) $L/C = 0.020$ $r = 6.47 \times 10^{-5}$
AB1157 (wild type) $L/C = 0.038$ $r = 1.47 \times 10^{-6}$

mid DNA isolated from transformants of both wild-type cells (DOHERTY, MORRISON and KOLODNER 1983) and *recB recC sbcA* mutants (SYMINGTON, MORRISON and KOLODNER 1985), and restriction maps of all the monomeric recombination products (pBR322, wild-type *tet* gene; pRDK35, *tet-10* allele; pRDK39, *tet-14* allele; and, double mutant, *tet-10/tet-14* alleles) observed are described elsewhere (Figure 3 in SYMINGTON, MORRISON and KOLODNER 1985). The physical analysis revealed that the plasmid DNA isolated from each of the 56 Ap^r transformants examined after transformation with the circular dimer consisted of primarily recombinant circular monomers. The amount of circular dimer DNA was estimated to be <1% of the total DNA based on mobility in an agarose gel and visualization after EthBr staining.

The results from the restriction mapping analysis results of the recombination products obtained after transformation of *recB recC sbcB* mutants with circular dimer pRDK69 DNA are tabulated in Table 4. The restriction pattern observed represents a distinct *tet* genotype (wild type, *tet-10*, *tet-14* or *tet-10/tet-14*). 39% of the DNA samples analyzed by restriction endonuclease digestion yielded a pattern corresponding to a single monomeric unit and 61% of the DNA samples analyzed contained mixed monomer genotypes. Since *recB recC sbcB* mutants resolved the circular dimers almost exclusively to monomers, the mixed genotype class most likely represents the presence of different monomeric plasmids rather than

TABLE 4

Structural analysis of plasmid DNA purified from individual Ap^r transformants of JC7623 (*recB recC sbcB*)[a]

Substrate DNA	N[b]	Recombination products observed (%)[c]				
		Single monomer genotypes				Mixed monomer genotypes
		Wild type	*tet-10*	*tet-14*	*tet-10/ tet-14*	
pRDK69	56	3.6	25	8.9	1.8	61[d]
pRDK69/*Xba*I	64	4.7	72	0	7.8	16[e]

[a] Entire individual Ap^r colonies were inoculated into 5 ml LB/Ap and grown overnight with aeration at 37°. Plasmid DNA was purified by the boiling method as described.

[b] The number of transformants tested.

[c] The structures of the observed plasmid DNA products are as described previously. Wild type, *tet-10*, and *tet-14* refer to the *tet* allele of pBR322, pRDK35, and pRDK39, respectively. A *tet-10/tet-14* refers to a double mutant.

[d] *tet-10* + *tet-14* (14%); *tet-10* + *tet-14* + *tet-10/tet-14* (20%); *tet-10* + *tet-10/tet-14* (16%); wild type + *tet-14* (1.8%); wild type + *tet-10* + *tet-10/tet-14* (5.4%); wild type + *tet-10/tet-14* (1.8%); and *tet-14* + *tet-10/tet-14* (1.8%).

[e] *tet-10* + *tet-10/tet-14* (9.4%); *tet-10* + wild type (3.1%); *tet-10/tet-14* + an unknown species (3.1%).

dimers of mixed *tet* genotypes. After digestion with *Xho*I and *Pst*I, 64% of the single monomeric recombinant products yielded 0.78-kb and 3.59-kb fragments. These results are consistent with the *Xho*I + *Pst*I digestion pattern of pRDK35 which is a pBR322-derived monomer containing a *tet-10* mutation. Of the remaining single monomer genotypes observed, 23% yielded fragment sizes of 2.02 kb and 2.34 kb, 9.1% yielded 4.36-kb linear molecules that were resistant to digestion with *Xho*I, and 4.5% yielded fragment sizes of 0.78 kb, 1.25 kb and 2.34 kb after digestion with *Xho*I and *Pst*I. These results are consistent with the *Pst*1 + *Xho*I digestion pattern of pRDK39 (a pBR322-derived monomer containing a *tet-14* mutation), pBR322 (wild-type *tet* gene), and a pBR322 monomer derivative containing both the *tet-10* and *tet-14* mutations (double mutant) in a single *tet* gene, respectively. The structure of the monomer plasmids present in the mixed genotype classes was determined by this method and the data are present in the footnote to Table 4. The combinations of monomers observed in the mixed genotype class can not be explained by simple intramolecular crossing over suggesting that either multiple recombination events occurred during the growth of the colony or that heteroduplex regions were often formed and processed during recombination. The effect of *mutS* on circular dimer plasmid recombination supports this latter idea.

Of the monomer genotypes observed, 12.5% contained a wild-type *tet* gene. Since the fluctuation test (as described in the preceding section) only measures the rate of production of wild-type (*tet*) recombinants and the physical analysis of the plasmid products revealed that 88% of the recombinant containing clones analyzed did not contain a wild-type *tet* gene, the recombination rate must be at least 10-fold higher. Even with a recombination rate of approximately 1%, it was surprising that all of the DNA recovered from a 3-ml culture was recombinant and was often (40% of the time) only a single recombinant. Similar experiments were performed with *recB recC sbcA* mutants, which have similar recombination rates, yet mostly all of the plasmid DNA was present in an unrecombined form (SYMINGTON, MORRISON and KOLODNER 1985; R. KOLODNER, unpublished data). Possible explanations for the apparent discrepancy between the physical analysis and the fluctuation test analysis results include: (1) the fluctuation test underestimates the recombination rate because the rate is so high, (2) segregation or selection results in selective maintenance of recombinant monomeric plasmids or (3) recombination continues to occur while the cells are in stationary phase during propagation of the cultures. Additional experiments will be required to distinguish these and other possibilities.

The structure of the plasmids obtained from an Ap^r colony after transformation of *recB recC sbcB* mutants with the linear plasmid dimer, pRDK69/*Xba*I, was also studied, and the results from the restriction mapping analysis are given in Table 4. All of the transformants analyzed contained only circular monomer plasmids. Of the total recombination products observed, 72% contained the single monomer genotype of pRDK35 (*tet-10* single mutant). In addition, 7.8% and 4.7% of the recombination products observed contained the single monomer genotypes of a double *tet-10/tet-14* mutant and wild-type pBR322, respectively. No single monomer genotype of pRDK39 (*tet-14*) was observed. 16% of the recombination products were mixed monomer genotypes containing some combination of the *tet-10* (pRDK35), the *tet-10/tet-14* (double mutant) and the wild-type (pBR322) *Xho*I/*Pst*I restriction enzyme pattern. Again, no pRDK39 monomer genotype (*tet-14* single mutant) was observed in the mixed monomer genotype group. These results on the recombination of *Xba*I-digested pRDK69 in JC7623 (*recB recC sbcB*) are similar to previously obtained results on the recombination of the same linear dimer in JC8679 (*recB recC sbcA*; SYMINGTON, MORRISON and KOLODNER 1985). A similar distribution of the various recombinant monomer genotypes was observed suggesting a similar recombination mechanism. However, the plasmids obtained were circular monomers in *recB recC sbcB* cells, and in *recB recC sbcA* cells the products consisted of a series of circular oligomers having a single repeating recombinant monomeric unit (SYMINGTON, MORRISON and KOLODNER 1985). This suggests that in *recB recC sbcB* mutants, plasmids either do not form higher order

oligomers or that such oligomers recombine into monomers significantly faster than in *recB recC sbcA* strains.

DISCUSSION

Previous studies have identified four distinct genetic backgrounds in *E. coli* strains where it has been insightful to study genetic recombination. These genetic backgrounds are wild-type, *recB recC sbcA*, *recB recC sbcB* and *recD* (CLARK 1973, 1974, 1980; SMITH 1988). Extensive genetic analysis of plasmid recombination has been reported for the wild type (FISHEL, JAMES and KOLODNER 1981; JAMES, MORRISON and KOLODNER 1982; COHEN and LABAN 1983; KOLODNER, FISHEL and HOWARD 1985; SYMINGTON, MORRISON and KOLODNER 1985) and the *recD* (LOVETT, LUISI-DELUCA and KOLODNER 1988) genetic backgrounds. The goal of the present study was to extend this analysis to *recB recC sbcA* and *recB recC sbcB* mutants.

It was previously demonstrated that linear plasmid dimers undergo efficient recombination after transformation into *recD* mutants and *recB recC sbcA* mutants but not in wild-type strains or *recB recC* mutants (SYMINGTON, MORRISON and KOLODNER 1985; LOVETT, LUISI-DELUCA and KOLODNER 1988). The results presented here demonstrate that linear plasmid dimers also undergo efficient intramolecular recombination after transformation into *recB recC sbcB* mutants. The evidence for this is that (1) transformation was efficient, (2) the recovery of transformants required the function of five recombination genes and (3) all transformants contained only recombinant plasmid DNA molecules. The distribution of recombinants obtained was the same as was obtained with an isogenic *recB recC sbcA* mutant. This suggests the mechanism of recombination may be similar in both genetic backgrounds even though the genetic requirements for recombination were different.

As was the case for *recD* mutants (BIEK and COHEN 1986; LOVETT, LUISI-DELUCA and KOLODNER 1988) and *recB recC sbcA* mutants (FISHEL, JAMES and KOLODNER 1981; LABAN and COHEN 1981; JAMES, MORRISON and KOLODNER 1982; COHEN and LABAN 1983; SYMINGTON, MORRISON and KOLODNER 1985) but not wild-type strains, circular plasmids recombined at high rates in *recB recC sbcB* mutants. Structural analysis of the recombinant DNA molecules obtained suggested the fluctuation method underestimated the recombination rate and that the rate of recombination could be at least as high as 1% per generation. The high rate of recombination of circular dimer plasmids in *recB recC sbcB* mutants, *recB recC sbcA* mutants and *recD* mutants is 100 (*recD*) to 1,000 (*recB recC sbcB* and *recB recC sbcA*) -fold higher than in wild-type *E. coli* strains and correlates with the ability of linear dimer plasmids to undergo recombination in these mutants. Presumably, the reason that linear dimer plasmids do not undergo recombination in wild-type strains is that the rate of generating mature recombinants is low and the DNA is degraded before it recombines. This extreme enhancement of circular dimer plasmid recombination rates in *recB recC sbcB* mutants, *recB recC sbcA* mutants, *recD* mutants and *recB recC* mutants is not observed with other types of recombination (BARBOUR *et al.* 1970; TEMPLIN, KUSHNER and CLARK 1972; CLARK 1973; GILLEN and CLARK 1974; CHAUDHURY and SMITH 1984; LLOYD and THOMAS 1984; LLOYD, PORTON and BUCKMAN 1988; LOVETT, LUISI-DELUCA and KOLODNER 1988). Although it is unclear what is responsible for the enhancement, inhibitory effects by the RecBCD protein, altered regulation of required gene products or other factors may be involved.

The data presented here permit a comparison of the effects of mutations in all presently described recombination genes on plasmid recombination in *recB recC sbcB* and *recB recC sbcA* mutants. A summary of these results is presented in Table 5. Data obtained with other substrates and in other genetic backgrounds is also presented in Table 5 and will be discussed below. The data are complex and presumably reflect differences in the structure of the recombination substrates, the mechanisms by which the substrates recombine and the gene products functionally available in the different strains to interact with the substrates.

In the *recB recC sbcB* mutant strain the two types of plasmid recombination showed both common and unique genetic requirements. This may suggest that the gene products required by both events reflect similar steps in recombination whereas the unique genetic requirements reflect unique steps. With respect to the *mutS* requirement for the recombination of circular dimer plasmids but not linear dimers, this difference could reflect the known involvement of *mutS* in mismatch repair (SU and MODRICH 1986; MODRICH 1987). Formation of a wild-type *tet* gene during a heteroallelic cross has been postulated to involve gene conversion (DOHERTY, MORRISON and KOLODNER 1983) and such events could require *mutS*-dependent mismatch repair. However, cyclization of a linear dimer plasmid by recombination need not require gene conversion to yield a recombinant and therefore may not require *mutS*. The requirement of *recA* in both processes probably indicates a requirement for catalyzed homologous pairing (RADDING 1982) while the requirement for *lexA* for linear dimer plasmid recombination could reflect the induction of a *lexA*-regulated gene during the transformation process (LITTLE and MOUNT 1982). The nature of the other genetic requirements is unclear at this time.

The genetic requirements for circular dimer and

TABLE 5

Summary of known recombination genes required for specific recombination events in four distinct genetic backgrounds of *E. coli*[a]

Genetic background	Intramolecular plasmid recombination		Conjugation-mediated recombination[b]	UV sensitivity[b]
	Circular	Linear		
Wild type	*recA*		*recA*	*recA*
	recF		*recB*	*recB*
	recJ		*recC*	*recC*
	recO			*recF*
				recO
				ruv
recD	*recA*	*recA*	*recA*	*recA*
			(*recB*)[c]	(*recB*)[c]
	recC	*recC*	*recC*	*recC*
	(*recJ*?)[d]	*recJ*	*recJ*	*recF*
		recN		*recJ*
		recO		*ruv*
recB recC sbcB	*recA*	*recA*	*recA*	*recA*
	recF	*recF*	*recF*	*recF*
	recJ	*recJ*	*recJ*	*recJ*
	recO	*recO*	*recN*	*recN*
	ruv	*recQ*	*recO*	*recO*
	mutS		*recQ*	*recQ*
			ruv	*ruv*
recB recC sbcA	*recE*	*recE*	*recA*	*recA*
	recN		*recE*	*recE*
	recO		*recF*	*recF*
	recQ		*recJ*	*recJ*
	ruv		*recO*	*recO*
	mutS		*recQ*	*ruv*

[a] The references for the data summarized in this table are: CLARK and MARGULIES 1965; EMERSON and HOWARD-FLANDERS 1967; WILLETTS, CLARK and LOW 1969; BARBOUR *et al.* 1970; TEMPLIN, KUSHNER and CLARK 1972; HORII and CLARK 1973; CLARK, VOLKERT and MARGOSSIAN 1979; CLARK 1980; FISHEL, JAMES and KOLODNER 1981; GILLEN, WILLIS and CLARK 1981; JAMES, MORRISON and KOLODNER 1982; LLOYD, PICKSLEY and PRESCOTT 1983; LOVETT and CLARK 1983; CLARK *et al.* 1984; LLOYD, BENSON and SHURVINTON 1984; LOVETT and CLARK 1984; NAKAYAMA *et al.* 1984; KOLODNER, FISHEL and HOWARD 1985; SYMINGTON, MORRISON and KOLODNER 1985; LLOYD, BUCKMAN and BENSON 1987; LLOYD, PORTON and BUCKMAN 1988; LOVETT, LUISI-DELUCA and KOLODNER 1988; this study.

[b] The effect of *mutS* on conjugation-mediated recombination and UV survival has not been tested except in an otherwise wild-type genetic background where *mutS* was hyper-rec and UV[r] (FEINSTEIN and LOW 1986).

[c] A *recB recD* double mutant has not been constructed. Because *recB21* is polar on *recD* (ADMUNDSEN *et al.* 1986) and *recB21* mutants are defective in conjugal recombination and UV repair, we presume that conjugal recombination and UV repair in *recD* mutants are *recB*-dependent (LOVETT, LUISI-DELUCA and KOLODNER 1988). *recB21* mutants have not been tested for plasmid recombination, but we consider it likely that plasmid recombination in *recD* mutants is *recB*-dependent.

[d] A *recD* mutant containing the best *recJ* allele (*recJ284*::Tn*10*; LOVETT and CLARK 1984) was not tested for the ability to recombine circular dimer plasmids since it is tet[r]. A *recD* mutant containing a weaker *recJ* allele (*recJ77*) was tested and showed a twofold reduction in the recombination of circular dimers (LOVETT, LUISI-DELUCA and KOLODNER 1988).

linear dimer plasmid recombination in *recB recC sbcA* mutants are strikingly different and suggest two different recombination mechanisms. The differential requirement for *mutS* could reflect the mechanistic difference suggested above for recombination in *recB recC sbcB* mutants and is consistent with recent observations suggesting that plasmid recombination in *recB recC sbcA* mutants involves gene conversion (KOBAYASHI and TAKAHASHI 1988; YAMAMOTO *et al.* 1988). The common requirement for *recE* could indicate a requirement for the 5′ to 3′ exonuclease activity of the *recE* gene product (KUSHNER, NAGAISHI and CLARK 1974; JOSEPH and KOLODNER 1983); however, recent studies have suggested that the *recE* gene product may be multifunctional (LUISI-DELUCA, CLARK and KOLODNER 1988; C. C. CHU, A. TEMPLIN and A. J. CLARK, unpublished data). A partial analysis of the genetic requirements for other types of plasmid recombination has been reported and the results support two general properties of plasmid recombination in *recB recC sbcA* mutants. First, recombination of an inverted duplication on a plasmid (YAMAMOTO *et al.* 1988) and recombination between two plasmids encoding *tet* alleles that are similar to the alleles used here (JAMES, MORRISON and KOLODNER 1982) were *recA*-independent. This is similar to both circular dimer plasmid recombination and the recombination-mediated transformation of linear dimer plasmids. The only exception to the *recA* independence of plasmid recombination in *recB recC sbcA* mutants is the recombination between two incompatible plasmids containing large insertion or deletion mutations (LABAN and COHEN 1981). Whether some other gene product replaces the function of the *recA* gene product in *recB recC sbcA* mutants is not known. Second, recombination of an inverted duplication on a plasmid required *recE*, *recF* and *recJ* (*recN*, *recO*, *ruv* and *recQ* were not tested; YAMAMOTO *et al.* 1988) and recombination between monomer plasmids was *recF* independent (other mutations were not tested; JAMES, MORRISON and KOLODNER 1982). The COHEN and LABAN (1983) study reported that the recombination between two incompatible monomer plasmids containing large insertion or deletion mutations was *recF*-dependent. The circular dimer plasmid recombination described in this study was *recF* and *recJ* independent, but did require many other RecF pathway genes. These results indicate that changes in the structure of a recombination substrate alters the recombination mechanism.

The effect of mutations in all presently known *E. coli* recombination genes on two types of plasmid recombination, conjugal recombination and UV repair has been tested in wild-type, *recB recC sbcA*, *recB recC sbcB* and *recD* strains (Table 5). Similar experiments have been performed with other recombination

substrates; however, a complete genetic survey is not available for these substrates and consequently the data are not presented in Table 5. Historically, recombination genes were grouped into pathways according to whether they were required for conjugal recombination in wild-type, *recB recC sbcA* or *recB recC sbcB* strains (CLARK 1973, 1980). These recombination pathways were called the RecBC, the RecE and the RecF pathway, respectively. The presently available data suggest that some revision of this idea is necessary. First, regardless of what "pathway" is functioning the exact gene products required depends on the structure of the substrate studied. Second, the RecE and RecF "pathways" may not be as different as originally thought since some types of recombination are quite similar in both *recB recC sbcA* and *recB recC sbcB* mutants (Table 5). However, there appears to be at least three "types" of recombination and recombinational repair. First, there is *recB- recC*-dependent recombination that occurs in wild-type strains (conjugal recombination, UV repair, P1 transduction and recombination of λ *red gam* phages; CLARK and MARGULIES 1965; EMERSON and HOWARD-FLANDERS 1967; WILLETS, CLARK and LOW 1969; UNGER, ECHOLS and CLARK 1972; CLARK 1973; FISHEL, JAMES and KOLODNER 1981; JAMES, MORRISON and KOLODNER 1982; LLOYD and THOMAS 1984) and *recD* mutants (plasmid recombination, conjugal recombination, UV repair and P1 transduction; LLOYD, PORTON and BUCKMAN 1988; LOVETT, LUISI-DELUCA and KOLODNER 1988). In addition, in some cases recombination and repair can require additional gene products besides *recA*, *recB* and *recC* (LLOYD, PORTON and BUCKMAN 1988; LOVETT, LUISI-DELUCA and KOLODNER 1988). Second, there is recombination that depends on some, but not always all of the RecF pathway family of genes (*recF*, *recJ*, *recN*, *recO*, *recQ* and *ruv*) which can occur in wild-type, *recB recC sbcA* and *recB recC sbcB* strains depending on the substrate tested (HORII and CLARK 1973; CLARK, VOLKERT and MARGOSSIAN 1979; GILLEN, WILLIS and CLARK 1981; LLOYD, PICKSLEY and PRESCOTT 1983; LOVETT and CLARK 1983; LLOYD, BENSON and SHURVINTON 1984; LOVETT and CLARK 1984; NAKAYAMA *et al.* 1984; PICKSLEY, ATTFIELD and LLOYD 1984; KOLODNER, FISHEL and HOWARD 1985; LLOYD, BUCKMAN and BENSON 1987; this study). Finally, there is recombination of linear dimer plasmids and possibly recombination of λ *red gam* phages (GILLEN and CLARK 1974) in *recB recC sbcA* mutants which only requires *recE*. This analysis does not suggest a role for each recombination gene within a grouping of genes. However, it provides information about the genetic interaction of different recombination genes during recombination and it provides information about what types of recombination substrates could be used in biochemical and genetic studies to elucidate the role that each recombination protein plays.

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APPENDIX

Several of the mutant derivatives of the *recB recC sbcA* genetic background that were constructed for the studies presented in the accompanying paper had not previously been tested for their ability to perform conjugal recombination or repair UV damage to DNA. Analysis of these processes is required to

TABLE 6

Analysis of conjugal recombination events and UV survival in *recB recC sbcA* mutants

Strain[a]	Relevant genotype	Relative conjugational inheritance[b] × JC158[c] Leu$^+$ [Ser$^+$ Sm^r]	Relative conjugational inheritance[b] × RDK1911[d] λ plaques	Viability (%)	UV survival (20 J/m^2) (%)
JC8679	*recB21 recC22 sbcA23*	1	1	65	17
RDK1657	*recB21 recC22 sbcA23 recN1502*::Tn*5*	0.60	0.95	27	16
RDK1658	*recB21 recC22 sbcA23 recO1504*::Tn*5*	0.026	0.86	17	0.0031
RDK1693	*recB21 recC22 sbcA23 recQ1*	0.22	1.0	40	3.3
RDK1713	*recB21 recC22 sbcA23 ruvB9*	0.94	0.95	51	0.037

[a] The strains are described in detail in Table 1.
[b] The frequency for conjugational inheritance for JC8679 with JC158 was 14%; with RDK1911 the frequency was 7.7%.
[c] CLARK (1963).
[d] LOVETT, LUISI-DELUCA and KOLODNER (1988).

better characterize these mutant derivatives. In addition, the data obtained will aid in the interpretation of how these gene products interact in the previously defined recombination pathways. In this appendix, the results from conjugational recombination and UV-survival analysis in various *recB recC sbcA E. coli* derivatives are presented. The experiments were performed exactly as previously described (LOVETT, LUISI-DELUCA and KOLODNER 1988) and the results are presented in Table 6. In the *recB recC sbcA* background, only the *recO* mutation significantly reduced (38-fold) conjugational recombination as well as decreased UV-survival (300-fold). The *recQ* mutation had a smaller reduction (5-fold) on both conjugational recombination and UV-survival. The *recN* and *ruv* mutations had little to no effect on conjugational recombination; however, the *ruv* mutation decreased UV-survival 30-fold. Recent studies (LLOYD, BUCKMAN and BENSON 1987) on the effect of mutations in *recN*, *recO* and *ruv* on conjugational recombination are generally in agreement with this study. The small differences are probably due to differences in the alleles tested (*recN* and *ruv*). These results are incorporated in the summary table (Table 5) in the main text.