# Genetic Dissection of the Biochemical Activities of RecBCD Enzyme

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ABSTRACT

RecBCD enzyme of *Escherichia coli* is required for the major pathway of homologous recombination following conjugation. The enzyme has an ATP-dependent DNA unwinding activity, ATP-dependent single-stranded (ss) and double-stranded (ds) DNA exonuclease activities, and an activity that makes a ss DNA endonucleolytic cut near Chi sites. We have isolated and characterized ten mutations that reduced recombination proficiency and inactivated some, but not all, activities of RecBCD enzyme. One class of mutants had weak ds DNA exonuclease activity and lacked Chi-dependent DNA cleavage activity, a second class lacked only Chi-dependent DNA cleavage activity, and a third class retained all activities tested. The properties of these mutants indicate that the DNA unwinding and ss DNA exonuclease activities of the RecBCD enzyme are not sufficient for recombination. Furthermore, they suggest that the Chi-dependent DNA cleavage activity or another, as yet unidentified activity or both are required for recombination. The roles of the RecBCD enzymatic activities in recombination and exclusion of foreign DNA are discussed in light of the properties of these and other *recBCD* mutations.

RecBCD enzyme, also called exonuclease V (EC 3.1.11.5), plays several important roles in bacterial DNA metabolism. Although this enzyme is widely distributed in Gram-positive and Gram-negative bacteria (for a review see TELANDER-MUSKAVITCH and LINN 1981), it has been most thoroughly studied in *Escherichia coli.* The properties of *E. coli recBCD* mutants lacking the enzyme reveal that RecBCD enzyme is important for homologous recombination, recovery from DNA damage, maintenance of cell viability, and exclusion of foreign (restricted) DNA (for a review see TAYLOR 1988). *E. coli recB* and *recC* null mutants are deficient in recombination following conjugation or generalized transduction (EMMERSON 1968). They are more sensitive than wild-type *E. coli* to DNA damaging agents such as X rays, UV light, and mitomycin C (EMMERSON 1968; WILLETTS and CLARK 1969; BARBOUR *et al.* 1970). Cultures of *recBC* null mutants contain a large fraction of cells (up to 90%) that fail to form visible colonies (CAPALDO-KIMBALL and BARBOUR 1971). This phenomenon, called lethal sectoring, results in slower growth of *recBC* mutant colonies than wild-type colonies. DNA cut by the *E. coli* K-12 restriction enzyme or DNA that is UV-damaged is converted to acid-soluble nucleotides primarily by RecBCD enzyme (CLARK *et al.* 1966; WILLETTS and CLARK 1969; SIMMON and LEDERBERG 1972). Likewise, the DNA of phage T4 gene 2 mutants is rapidly degraded by RecBCD enzyme (OLIVER and GOLDBERG 1977).

The enzymatic activities of RecBCD enzyme are also complex. The enzyme unwinds DNA, hydrolyzes DNA, and hydrolyzes ATP (for a review see TAYLOR 1988). Linear, but not circular, double-stranded (ds) DNA is unwound with the production of single-stranded (ss) DNA loops that travel along the DNA (TAYLOR and SMITH 1980). Linear ds and ss DNA is hydrolyzed by RecBCD enzyme to acid-soluble oligonucleotides; circular ss DNA is slowly cut, but circular ds DNA is refractory to RecBCD enzyme (BUTTIN and WRIGHT 1968; GOLDMARK and LINN 1970). Endonucleolytic cleavage occurs at high frequency when the enzyme encounters the Chi recombinational hotspot sequence 5′G-C-T-G-G-T-G-G3′ on linear ds DNA; this cleavage occurs only during DNA unwinding, and only when the Chi site is encountered as the enzyme moves 3′ to 5′ (relative to the Chi sequence as written here) (PONTICELLI *et al.* 1985; TAYLOR *et al.* 1985). ATP hydrolysis is required to supply energy for DNA unwinding and the subsequent ds DNA hydrolysis and Chi-dependent cleavage. ss DNA hydrolysis also requires ATP (GOLDMARK and LINN 1972; EICHLER and LEHMAN 1977), possibly to activate the enzyme allosterically.

One way to elucidate the mechanism by which RecBCD enzyme promotes recombination, repair, and viability is to determine which of its multiple enzymatic activities are required for each of the physiological roles of the enzyme. Mutants lacking some but not all RecBCD enzymatic activities are crucial for this approach. For example, we have previously shown that extracts of *recBCD* mutants, lacking the intracellular localized stimulation of recombination by Chi (hotspot activity), do not cleave DNA at Chi sites

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**TABLE 1**

***E. coli* strains**[a]

| Strain designation | Alternate designation | Genotype | Source or reference[b] |
|---|---|---|---|
| AFT227 | NK5661 | *thyA lacZ2900*::Tn*10* λ⁻ F⁻ | (3) |
| AFT228 | NK2992 | *argA81*::Tn*10* λ⁻ F⁻ | (1) |
| AFT325 | | *thyA argA21 recF143 hisG4 met rpsL31 galK2 xyl-5* λ⁻ F⁻ | (1) |
| S501 | C600 | *thr-1 leu-6 thi-1 lacY1 tonA21 supE44* λ⁻ F⁻ | (1) |
| S665 | 594 | *lac-3350 galK2 galT22 rpsL179* λ⁻ F⁻ | (1) |
| S727 | KL96 | *thi-1 relA1* λ⁻ (Hfr P044) | (1) |
| S741 | JC9387 | *thr-1 leu-6 thi-1 lacY1 galK2 ara-14 xyl-5 proA2 hisG4 argE3 rpsL31 tsx-33 mtl-1 recB21 recC22 sbcB* λ⁻ F⁻ | (1) |
| S927 | JC5488 | *thr-1 leu-6 thi-1 lacY1 galK2 ara-14 xyl-5 proA2 hisG4 argE3 rpsL31 tsx-33 mtl-1 supE44 thyA* λ⁻ (F'15 *thyA*⁺ *recBCD*⁺ *argA*⁺) | (1) |
| S928 | JC5497 | *recA ton lac his trp rpsL rpsE thyA* λ⁻ (F'15 *thyA*⁺ *recB21 argA*⁺) | (1) |
| V66 | | *argA21 recF143 hisG4 met rpsL31 galK2 xyl-5* λ⁻ F⁻ | (1) |
| V67 | | As V66, plus *recB21* | (1) |
| V68 | | As V66, plus *recC73* | (1) |
| V78 | | *argA81*::Tn*10 recC73* λ⁻ (F'15 *thyA*⁺ *recC73 argA*⁺) | (1) |
| V182 | | As V186 plus (pDWS2) | T of V186 |
| V186 | AC113 | Δ(*thyA-argA*)*232* IN(*rrnD-rrnE*)1 λ⁻ F⁻ | (2) |
| V222 | | As V66 plus *recD1013 argA*::Tn*10* | AC207 × AFT325 |
| V376 | AC291 | Δ(*thyA-argA*)*235* (*Ap*$^R$) *recF143 hisG4 met rpsL31 galK2 xyl-5* λ⁻ F⁻ | (2) |
| V793 | | Δ(*thyA-argA*)*232* IN(*rrnD-rrnE*)1 λ⁻ (F'15 *thyA*⁺ *recBCD*⁺ *argA88*) | (4) |
| V894 | | Δ(*thyA-argA*)*232* IN(*rrnD-rrnE*)1 λ⁻ (F'15 *thyA*⁺ *recB2109 argA*⁺) | (4) |
| V967 | | *argA21 recB2109 recF143 hisG4 met rpsL31 galK2 xyl-5* λ⁻ F⁻ | V894 × AFT325 |
| V1282 | | *hisG4 met rpsL31 recF143 galK2 xyl-5* Δ(*recB-argA*)*250* λ⁻ F⁻ | (5) |
| V1295 | | As S741 plus *thyA argA81*::Tn*10* | S of V1307 |
| V1307 | | As S741 plus *argA81*::Tn*10* | AFT228 × S741 |
| V1312 | | As V186 plus (F'15 *thyA*⁺ *recB2152 argA*⁺) | (6) |
| V1313 | | As V1282 plus (F'15 *thyA*⁺ *recB2152 argA*⁺) | V1312 → V1282 |
| V1318 | | As V186 plus *lacZ2900*::Tn*10* | AFT227 × V186 |
| V1327 | | As V186 plus (F'15 *thyA*⁺ *recBCD*⁺ *argA*⁺) | (7) |
| V1328 | | As V186 plus (F'15 *thyA*⁺ *recB21 argA*⁺) | (7) |
| V1329 | | As V186 plus (F'15 *thyA*⁺ *recC73 argA*⁺) | (7) |
| V1330 | | As V186 plus (F'15 *thyA*⁺ *recD1013 argA*⁺) | (8) |

[a] Additional strains listed in Tables 3–11 are derivatives of the strains listed here.

[b] T, transformation; S, spontaneous derivative; A × B, P1 mediated transduction in which A is the donor and B is the recipient; A → B, result of mating where A is the donor and B is the recipient; (1) SCHULTZ, TAYLOR and SMITH (1983); (2) CHAUDHURY and SMITH (1984b); (3) LUNDBLAD *et al.* (1984); (4) Treatment of S927 with nitrosoguanidine and mating with V186 (see MATERIALS AND METHODS); (5) A *thyA* derivative of V66 was transduced to Thy⁺ with P1 grown on a Δ(*rec-argA*) strain thought to contain Δ(*recC-argA*)*234* (CHAUDHURY and SMITH 1984b), but the resultant transductant was *recC*⁺, *recB*, *argA* (complementation data not shown). The Δ(*recB-argA*)*250* deletion may be the same as another deletion allele in the deletion collection of CHAUDHURY and SMITH (1984b); (6) See MATERIALS AND METHODS; (7) Strains described in (1) mated to strain V186; (8) V222 × V793 and transfer of the resulting F'15 *thy*⁺ *recD1013 arg*⁺ to V186.

(SCHULTZ, TAYLOR and SMITH 1983; PONTICELLI *et al.* 1985); these observations indicate that Chi-dependent cleavage is required for Chi hotspot activity (for a review see SMITH 1987). To extend this approach to other aspects of RecBCD enzyme function, we have isolated additional classes of mutants differentially altered in the enzyme's activities.

## MATERIALS AND METHODS

**Bacterial and phage strains:** These are listed in Tables 1 and 2, respectively, with their genotypes and sources. The genotype *recBCD*⁺ indicates *recB*⁺ *recC*⁺ *recD*⁺. Genotypes such as *recB21* imply *recB21 recC*⁺ *recD*⁺ unless explicitly stated. The genotype *recBCD*‡ indicates a mutation in the *recB*, *recC* or *recD* gene conferring a ‡ phenotype (CHAUDHURY and SMITH 1984a). The designation *rec*⁺ indicates a strain that is *recA*⁺ *recBCD*⁺.

**Media:** LB broth and agar plates, tryptone broth (TB) and agar plates, minimal medium (OMBG) and suspension medium (SM) have been previously described (CHENG and SMITH 1989). OMB-lactose is OMBG with 0.4% lactose replacing the glucose. BBL agar is the same as tryptone agar with trypticase (BBL Microbiology Systems, Cockeysville, Maryland) substituted for tryptone (DIFCO, Detroit, Michigan). Minimal media were supplemented with required

**TABLE 2**

**Phage strains and plasmids**

| Strain number | Genotype | Source or reference |
|---|---|---|
| 801 | $\lambda^+$ | —[a] |
| 872 | λ *b1453 cI857* | — |
| 873 | λ *b1453* $\chi^+$*76 cI857* | — |
| 1081 | λ *susJ6 b1453 cI857* $\chi^+$*D123* | — |
| 1082 | λ *b1453* $\chi^+$*D123 susR5* | — |
| 1083 | λ *susJ6 b1453* $\chi^+$*76 cI857* | — |
| 1084 | λ *b1453* $\chi^+$*76 susR5* | — |
| | T4 | — |
| | T4 gene *2 amN51* | — |
| | P1 *vir-1* | — |
| | P2 *vir-1* | — |

| Plasmid number | Genotype of insertion[b] | Source or reference |
|---|---|---|
| pBR322 | None present | BOLIVAR *et al.* (1977) |
| pDWS2 | + | PONTICELLI *et al.* (1985) |
| pSA21 | *recB21* | AMUNDSEN *et al.* (1986) |
| pSMT1 | *recB2109* | C[c] of V894 |
| pSMT15 | *rec-2123* | NG[d] of V182 |
| pAMN3 | *rec-2140* | NG[d] of V182 |
| pAMN8 | *recC2145* | NG[d] of V182 |
| pAMN11 | *rec-2148* | *NG[d] of V182* |
| pAMN13 | *recB2150* | *NG[d] of V182* |
| pSA10 | *recB2152* | *See* MATERIALS AND METHODS |
| pSA40 | recB2153 | NG[d] of V182 |
| pSA46 | recB2154 | NG[d] of V182 |
| pSA120 | recB2155 | NG[d] of V182 |

[a] Described in SCHULTZ, TAYLOR and SMITH (1983).

[b] The *E. coli* chromosomal fragment inserted into pBR322 is the 18.5 kb *Bam*HI fragment containing the *thyA-recC-ptr-recB-recD-argA* region (SASAKI *et al.* 1982; DYKSTRA, PRASHER and KUSHNER 1984; AMUNDSEN *et al.* 1986).

[c] Transfer of the chromosomal *recB* allele to the plasmid following transformation of strain V894 with UV-irradiated pDWS2 DNA, according to the procedure of CHATTORAJ *et al.* (1984).

[d] Treatment of strain V182 with nitrosoguanidine, extraction of the mutagenized pDWS2 plasmid DNA, and transformation of nonmutagenized strain V186. For details, see MATERIALS AND METHODS.

amino acids at 20 μg/ml. Thymine (50 μg/ml) was added to all media used for *thyA* mutants. Drug-resistant transformants were selected and grown in media containing ampicillin (100 μg/ml) or tetracycline (25 μg/ml). Mitomycin C resistance was measured on LB plates containing mitomycin C (1 μg/ml).

**Mutagenesis:** A bacterial culture (strain S927 or V182) growing exponentially at 37° in LB broth was harvested by centrifugation, washed once in citrate buffer (0.1 M Na-citrate, pH 5.6) and resuspended in an equal volume of citrate buffer. Nitrosoguanidine (Sigma, St. Louis, Missouri) was added to a final concentration of 200 μg/ml. (Nitrosoguanidine was dissolved in a small amount of acetone and then diluted with water to 2 mg/ml.) The culture was incubated at 37° for 20 min. The cells were harvested by centrifugation, washed twice in citrate buffer, and resuspended in LB broth at one-half the original concentration. The culture was incubated at 37° for 2 hr, at which time the density of the culture had approximately doubled. The mutagenized culture was further manipulated as described below.

In the first method for mutant isolation, strain S927, containing F'15 *thyA*$^+$ *recBCD*$^+$ *argA*$^+$, was mutagenized and mixed with an equal number of cells of strain V186, containing the Δ(*thyA-argA*)*232* deletion, growing exponentially in LB broth. The mixture was incubated for 1 hr at 37°, and samples were plated on OMBG agar plates to select derivatives of V186 containing the mutagenized F'15. The mated mixture in LB broth was kept in the refrigerator for up to one month, with negligible loss of titer, for subsequent platings. Colonies were screened as described in RESULTS.

In the second method for mutant isolation, strain V182, containing plasmid pDWS2 with the *thyA*$^+$-*recBCD*$^+$-*argA*$^+$ region from the *E. coli* chromosome, was mutagenized, and plasmid DNA was extracted from it by the alkaline lysis method (MANIATIS, FRITSCH and SAMBROOK 1982). In some experiments strain V186 [Δ(*thyA-argA*)*232*] was transformed with this DNA (MANIATIS, FRITSCH and SAMBROOK 1982), and Amp$^R$ transformants were selected on LB agar plates containing ampicillin (100 μg/ml). Small colonies were screened as described in RESULTS. In other experiments strain V1282 [Δ(*recB-argA*)*250 his-4 rpsL31*] was transformed with the mutagenized plasmid DNA, and Amp$^R$ transformants were selected on LB agar ampicillin plates. After overnight incubation at 37°, the colonies on these plates were screened for recombination-proficiency following Hfr conjugation as described in RESULTS.

**Screening for phage sensitivity:** Phage spot tests were done as previously described (SCHULTZ, TAYLOR and SMITH 1983). When several bacterial strains were tested for sensitivity to a single phage the following modification was used. Freshly grown cultures (50 μl) of bacteria in TB maltose were mixed with an equal volume of BBL top agar and spotted on a BBL agar plate; about ten candidates were tested on each plate. After the top agar hardened, about 10 μl of phage (about $10^4$ pfu/ml) was placed on the spots.

The phages used to distinguish *recBCD* null mutants from *recBCD* mutants retaining some RecBCD enzymatic activities are the following. Phage T4 gene *2* mutants fail to make plaques on *recBCD*$^+$ cells but do make plaques on *recBCD* null mutants (OLIVER and GOLDBERG 1977). These authors hypothesized that binding of the gene *2* protein to the ends of T4 DNA in the viral particle protects the T4 DNA, after injection into the cell, from RecBCD enzyme's ds DNA exonuclease activity.

Phage λ Red$^-$Gam$^-$ mutants are affected by RecBCD enzyme in two contrasting ways (for a review see SMITH 1983). In the absence of the λ Gam$^+$ inhibitor protein, RecBCD enzyme blocks the transition from θ form (CAIRNS) replication to σ form (rolling circle) replication. θ replication produces only monomeric circles, which cannot be packaged unless they recombine to produce dimeric forms. In the absence of the λ Red$^+$ recombination pathway, recombination occurs primarily by the *E. coli* RecBCD pathway, which is enhanced by Chi sites. Therefore, on lawns of *recBCD*$^+$ cells λ Red$^-$Gam$^-$ phage lacking Chi ($\chi^0$) make small plaques, and those containing Chi ($\chi^+$) make large plaques. On lawns of *recBCD* mutants in which Chi is not activated by RecBCD enzyme, the $\chi^0$ and $\chi^+$ phages make plaques of the same size. These plaques are large on lawns of *recBCD* mutants in which rolling circle replication is not blocked, since concatemers resulting from such replication are readily packaged. λ Red$^-$Gam$^-$ ($\chi^0$ and $\chi^+$) plaques are invisible or tiny on lawns of recombination-deficient mutants that are able to block rolling circle replication, since packageable DNA is produced neither by recombination nor by replication. This situation occurs in (*recBCD*$^+$) *recA* null mutants and in two classes of special (*recA*$^+$) *recBCD* mutants described here.

Phages P1 and P2 fail to make plaques on *recBC* null mutants, although the reasons are unclear. P1 also fails to make plaques on *recA* mutants (ZABROVITZ, LEGEV and

COHEN 1977) but does make plaques on recombination-proficient *recBCD* mutants (SCHULTZ, TAYLOR and SMITH 1983; CHAUDHURY and SMITH 1984a). This result suggests that growth of P1 reflects the recombination-proficiency of the host cell. P2 makes plaques on *recA* mutants and on *recBCD* mutants containing RecBCD nuclease activity but not on those lacking nuclease activity (SIRONI 1969; SCHULTZ, TAYLOR and SMITH 1983; CHAUDHURY and SMITH 1984a). This result suggests that growth of P2 reflects RecBCD nuclease activity.

**Isolation of *recB2152*:** This mutation arose unexpectedly during an attempt to transfer *recB21* from the plasmid pSA21 to F'15 *argA88*. Strain V793, containing F'15 *argA88*, was mated with strain AFT325 (*rpsL thyA argA21*); a Thy$^+$[Str$^R$] exconjugant was purified and transformed to Amp$^R$ with plasmid pSA21 (*thyA*$^+$ *recB21 argA*$^+$). This transformant, containing the *recB21* plasmid, the *argA* mutant F'15, and *recBCD*$^+$ on the chromosome, was mated with strain V186, containing Δ(*thyA-argA*)*232* on the chromosome. Differential plating of this mixture showed the frequency of transfer of each genetic element (F'15 and pSA21). The *thy*$^+$ character (on F'15 *thy*$^+$) was transferred to strain V186 at a frequency of about $10^{-1}$, the *thy*$^+$ *arg*$^+$ character (on F'15 *thy*$^+$ *arg*$^+$ recombinants) at a frequency of $8 \times 10^{-5}$, and the Amp$^R$ character (on pSA21) at a frequency of about $10^{-6}$ (data not shown). From the plating on OMBG agar plates (selecting Thy$^+$Arg$^+$), 24 colonies were tested: all were Amp$^S$, 17 had a Rec$^+$ phenotype by phage tests, and 7 had the phenotype of *recB2109* by phage tests (see Table 4). These 7 strains readily transferred the *thyA*$^+$, *argA*$^+$, and *rec* mutant characters to strain V1282, containing Δ(*recB-argA*)*250*. This result indicated that the *rec* mutant character, designated *recB2152*, and the *thyA*$^+$ and *argA*$^+$ characters were on F'15. The *recB2152* mutation was transferred to the chromosome by P1-mediated transduction from strain V1312 to strain AFT325 (*thyA argA*) by selecting Thy$^+$; 8/12 Thy$^+$ transductants inherited *recB2152*, and one was designated V1314. The *recB2152* mutation was transferred to plasmid pDWS2 by the method of COMEAU and INOUYE (1988). DNA from strain V1314 was digested with *Bam*HI, DNA from plasmid pDWS2 was digested with *Sma*I, and DNA from plasmid pBR322 (the parent of pDWS2) was digested with *Bam*HI. [see SASAKI *et al.* (1982) and AMUNDSEN *et al.* (1986) for the locations of these restriction sites.] The DNAs were mixed in the approximate weight ratios of 2:1:1, respectively, denatured, annealed and ligated according to COMEAU and INOUYE (1988). The DNA was used to transform strain V186 to Thy$^+$ Arg$^+$. Of 29 transformants tested, 8 had the *recB2152* character; one was designated strain V1354.

The origin of the *recB2152* mutation is obscure. Although its phenotype is similar to that of *recB2109* (see RESULTS), it is evidently an independent mutation since it differs from *recB2109* in at least one property (see Figure 1). We entertained the possibility that *recB2152* resulted from imprecise excision of the 1.4-kb insertion sequence in *recB21* (unpublished data quoted in AMUNDSEN *et al.* 1986). Plasmid DNA preparations from strains V1354 (*recB2152*) and V182 (*rec*$^+$) were digested with *Bam*HI, *Pst*I, *Sal*I and *Hin*dIII analyzed on ethidium bromide-stained agarose gels. Chromosomal DNA preparations from strains V1314 (*recB2152*), V66 (*rec*$^+$), and V67 (*recB21*) were digested with *Bam*HI and compared by SOUTHERN (1975) analysis using plasmid pDWS2 DNA radioactively labeled as a probe. The restriction patterns of *recB2152* DNA were indistinguishable from those of *rec*$^+$ DNA (data not shown). Any DNA insertion associated with *recB2152* must be less than about 300 bp. Attempts to repeat the isolation of *recB2152* have produced only *rec*$^+$ and *recB21* derivatives.

**Transfer of *rec* alleles between F'15, plasmid and chromosome:** *rec* alleles were transferred from F'15 to the chromosome by P1-mediated transduction, with selection for Thy$^+$ or Arg$^+$. Except for *recB2152* (see above), *rec* alleles isolated on F'15 were transferred from the chromosome to plasmid pDWS2 by the method of CHATTORAJ *et al.* (1984). *rec* mutant strains were transformed to Amp$^R$ with UV-irradiated pDWS2 (*recBCD*$^+$); approximately 1/200 Amp$^R$ transformants had the *rec* mutant character. Plasmid DNA from these strains produced only Thy$^+$ Arg$^+$ *rec* mutant transformants of strain V186 [Δ(*thyA-argA*)*232*].

*rec* alleles isolated on mutagenized pDWS2 were transferred from the plasmid to the chromosome by the method of COSLOY and OISHI (1973). Strain V1295 (*thyA recC22 recB21 argA81*::Tn*10 sbcB15*) was transformed with plasmid DNA linearized with *Eco*RI, which cuts the plasmid only once, in the pBR322 vector DNA. Among the selected Thy$^+$ Arg$^+$ transformants (about 10 per μg of DNA), about half were Amp$^S$ and were deemed to have the linear plasmid DNA replacing the chromosomal *thyA-argA* region. Since the *sbcB* mutation in these transformants suppresses many *recBCD* mutant phenotypes (KUSHNER, NAGAISHI and CLARK 1972; our unpublished observations), P1 was grown on these strains and used to transduce strain AFT325 (*thyA argA21 sbcB*$^+$) to Thy$^+$ Arg$^+$. These transductants allowed unambiguous testing of the *rec* mutant allele derived from the plasmid.

**λ crosses, Chi activity measurement and Hfr conjugations:** Recombination proficiencies and Chi activities were measured as described by SCHULTZ, TAYLOR and SMITH (1983).

**Preparation of cell-free extracts and enzyme assays:** Cells were grown in LB broth to about $3 \times 10^8$/ml. Crude extracts were made as described by TOMIZAWA and OGAWA (1972) and assayed for ATP-dependent solubilization of ds (native) or ss (boiled) $^3$H-labeled T7 DNA as described by EICHLER and LEHMAN (1977). Except where noted, 25 μM ATP was used in the ds DNA assays and 50 μM ATP in the ss DNA assays. Each assay included two or three protein concentrations that gave a linear relationship between solubilized DNA and protein assayed. In extracts of cells containing the *recBCD*$^+$ genes on a plasmid, the ATP-dependent nuclease activity was >90% of the total ds DNA nuclease activity and >50% of the total ss DNA nuclease activity. Assays for DNA unwinding activity and Chi-dependent DNA cleavage activity used "improved" conditions as described by PONTICELLI *et al.* (1985) with the following exceptions. The DNA substrate was the 930-bp *Sal*I-*Dde*I fragment of pBR322 ($\chi^0$ or $\chi^+$*F225*) labeled at the *Dde*I 3′ end. After reaction, the products were analyzed without denaturation on 0.5% agarose-4% polyacrylamide or 5% polyacrylamide gels followed by autoradiography.

**Assay for precise excision of Tn*10*:** Precise excision of Tn*10* from *lacZ* was measured as described by FOSTER *et al.* (1981). Several isolated colonies of derivatives of strain V1318 (for plasmid *rec* alleles) or AFT227 (for chromosomal *rec* alleles) bearing *lacZ2900*::Tn*10* were picked and grown in 1 ml of LB broth for approximately 8 hr (to about $2 \times 10^9$ cells/ml). Samples were plated on LB agar plates (for total viable cell determination) and on OMB-lactose agar plates (for Lac$^+$ cell determination). Table 9 reports the number of cultures measured and the mean and range of the Lac$^+$ frequencies.

**Assay of phage T4 and T4 *2*$^-$ DNA degradation in infected cells:** Phages T4 and T4 *2*$^-$ were labeled with [$^3$H]

thymidine during growth in strain V68 (*recC73*). Bacteria were grown to approximately $5 \times 10^8$ cells/ml in the medium described by RHOADES, MACHATTIE and THOMAS (1968) with 3-[*N*-morpholino]propanesulfonic acid (MOPS) replacing Tris. The medium was then supplemented with 5 μCi [$^3$H]thymidine/ml and 100 μg adenine/ml, and phage were added at a multiplicity of infection (MOI) of 2.5–3.5. The culture was incubated at 37° without shaking for 20 min to allow phage adsorption and then with vigorous shaking until the bacteria began to lyse. Chloroform was added, and bacterial debris removed by centrifugation. The phage was precipitated by addition of polyethylene glycol (8000) to 10%, collected by centrifugation, resuspended in SM, and purified on a CsCl step gradient. Phage were titered on strains V66 (*rec*$^+$) and V68 (*recC73*). The yield was $10^9$–$10^{10}$ purified phage per ml of infected culture, with a specific activity of $10^{-3}$–$10^{-4}$ dpm per plaque-forming unit.

Phage DNA degradation was measured by infecting bacterial strains carrying plasmids with *recBCD* wild-type or mutant alleles. Bacteria were grown in TB broth plus ampicillin and maltose (0.1%) to a concentration of $5 \times 10^8$ cells/ml and infected at an MOI of 3–5. At 30 min, samples of the culture were measured for trichloroacetic acid-soluble material as described by SILVERSTEIN and GOLDBERG (1976).

## RESULTS

We used methods that would allow us to rapidly screen mutagenized cultures for *recBCD* mutants. To focus on *recBCD* mutations, we mutagenized cells containing the *recBCD* genes either on an F′ factor or on a plasmid. The mutagenized F′ factors or plasmids were transferred to a nonmutagenized *recBCD* deletion mutant. Individual colonies were screened for phenotypes associated with the loss of *recBCD* function–small colony-formation and mitomycin C-sensitivity in some cases and recombination-deficiency following Hfr conjugation in another case. Mutant candidates were then screened for intracellular RecBCD enzymatic activities by testing for their ability to support the growth of various phages (see MATERIALS AND METHODS). This step allowed us to identify candidates that retained some RecBCD enzymatic activities and to eliminate *recBC* null mutants, which lack all RecBCD enzymatic activities and hence give little information on the roles of the individual activities. The mutants reported here were chosen for complete analysis because they demonstrated reproducible phenotypes that were markedly different from those of wild-type, null, or *recBCD*‡ strains. Extracts of interesting mutants were assayed for RecBCD enzymatic activities.

**Isolation of *recB2109* on F′15:** As detailed in MATERIALS AND METHODS, strain S927 (F′15 *thyA*$^+$ *recBCD*$^+$ *argA*$^+$) was mutagenized with nitrosoguanidine, the F′15 episome was transferred to non-mutagenized strain V186 [Δ(*thyA-argA*)*232*], and small colonies indicative of *recBC* mutants were found at a frequency of about 5%. About 300 small colonies were picked to TB broth in microtiter wells, incubated overnight, and transferred with a metal-pronged replicator to LB agar containing mitomycin C (1 μg/ml). One hundred seventeen clones were mitomycin C-sensitive (MC$^S$). These clones were purified, grown in TB broth, and tested for phage sensitivity (see MATERIALS AND METHODS and Table 4). 35 clones had phenotypes different from *recB* or *recC* null mutants and different from *recBCD*$^+$ strains. One clone, strain V894 whose mutation was designated *recB2109*, had a unique phenotype: on this strain, phage T4 *2*$^-$ formed no visible plaques, while phage λ Red$^-$Gam$^-$ ($\chi^0$ or $\chi^+$) formed large plaques. Strain V894 was as sensitive to MC as an isogenic *recB21* (null) mutant. (For clarity we shall use throughout this paper the *rec* gene designations based on the complementation data presented later.)

**Isolation of *rec-2123*, *2140*, *C2145*, *2148* and *B2150* on plasmid pDWS2:** As detailed in MATERIALS AND METHODS, strain V182, containing plasmid pDWS2 with the *thyA*$^+$-*recBCD*$^+$-*argA*$^+$ region of the *E. coli* chromosome inserted into plasmid pBR322, was mutagenized with nitrosoguanidine. Plasmid DNA was extracted and used to transform strain V186 [Δ(*thyA-argA*)*232*] to ampicillin-resistance (Amp$^R$). About 3% of these transformant colonies, on LB agar with ampicillin, were distinctly smaller than the majority. About one-half of these small colonies were stably MC$^S$ and Amp$^R$ after purification. The MC$^S$ Amp$^R$ clones were grown in TB broth and tested for phage sensitivity. Clones with phenotypes different from those of *recB* or *recC* null mutants or of *recBCD*$^+$ strains were kept for further analysis.

In the first screen, 54 MC$^S$ clones were tested for sensitivity to phage T4 *2*$^-$ as described in MATERIALS AND METHODS. 10 candidates were judged to be T4 *2*$^-$ sensitive. Further tests for sensitivity to other phages (λ Red$^-$Gam$^-$, P1, and P2) and to MC revealed one *recBCD*$^+$ clone, four *recB* or *recC* null mutants, and five mutants with unusual phenotypes. One of these five mutants, strain V1148 whose mutation was designated *rec-2123*, allowed only minute plaque formation by λ Red$^-$Gam$^-$ ($\chi^0$ or $\chi^+$).

In the second screen, from an independent mutagenesis, 210 stable MC$^S$ Amp$^R$ clones were obtained from 550 small colonies picked from about 22,000 Amp$^R$ transformants. Phage sensitivity tests on the 210 MC$^S$ Amp$^R$ clones revealed 33 clones with wild-type phenotype, 104 clones with *recB* or *recC* null phenotype, 13 clones with *recD* (‡, double dagger) phenotype (CHAUDHURY and SMITH 1984a), 26 clones forming such poor lawns that the phage tests were unreliable, and 34 with unusual phenotypes. In the last class were mutants V1227 (*rec-2140*), V1268 (*recC2145*), V1271 (*rec-2148*), and V1273 (*recB2150*) whose mutations were designated as indicated. Their phenotypes are described below.

**Isolation of *recB2153, B2154, B2155* on plasmid pDWS2:** The third mutant search used the mutagenized pDWS2 DNA from the second screen, but recombination-deficient (Rec$^-$) mutants were sought directly by testing for recombination after mating with an Hfr. In this search, strain V1282 [Δ(*recB-argA*) *his rpsL*] was transformed to Amp$^R$ and plated on LB agar with ampicillin. Among the 5500 Amp$^R$ transformants examined, 146 made small colonies, and they were patched onto fresh LB ampicillin plates. After overnight growth, these patches were replicated to an OMBG-streptomycin-methionine minimal agar plate spread with about $10^7$ cells of strain S727 [*rpsL*$^+$ *his*$^+$ (Hfr P044)]. After 2 days of incubation at 37°, the plates were examined for His$^+$[Str$^R$] recombinants. Seventy-five of the mutants were Rec$^-$, and 20 others were judged to be less recombination proficient than a *rec*$^+$ control. The remaining large colonies on the LB-ampicillin plates were replicated directly to minimal plates spread with strain S727. 104 clones appeared to be Rec$^-$ or to have reduced recombination proficiency; after purification and retesting, 24 were Rec$^-$. The 119 Rec$^-$ candidates (95 among the initial small colonies and 24 among the initial large colonies) were purified and retested. 27 clones either were not stably Amp$^R$ or were Arg$^-$. Phage sensitivity tests of the remaining 92 Rec$^-$ candidates revealed 18 clones with a wild-type phenotype, 64 with *recB* or *recC* null phenotype, and 10 with unusual phenotypes. In the last class were mutants V1353 (*recB2153*), V1351 (*recB2154*) and V1352 (*recB2155*) whose mutations were designated as indicated. These three mutants were identified among the small colony formers. Their phenotypes are described below.

**Mitomycin C sensitivity and cell viability:** Qualitative assays of these phenotypes were used to isolate the mutants described here: small colony formation appears to reflect a low cell viability, and reduced or no growth on solid medium containing MC reflects sensitivity to MC. These phenotypes were quantitated as described in Table 3. In the presence of MC (1 μg/ml) the efficiency of plating (E.O.P.) of *rec*$^+$ cells was 0.65, while with one exception the E.O.P. of the new *rec* mutants, like the *recB21* null mutant, was $<10^{-3}$; the *rec-2140* mutant had an E.O.P. of 0.1. The viability of *rec* mutant cells, measured as colony-forming units/ml divided by the optical density of a growing culture, was less than that of *rec*$^+$ cells; except for *recB2153*, none of the new *rec* mutants had viabilities quite as low as the *recB21* mutant. These quantitative results are consistent with the qualitative behavior of the mutants noted during their isolation.

**Phage growth in *rec* mutants:** Several *E. coli* phages grow differently in *recBCD* mutants than in *rec*$^+$ cells (see MATERIALS AND METHODS). These differences were exploited during the isolation of the *rec* mutants described here. The abilities of these phages to form plaques on the *rec* mutants are shown in Table 4. T4 *2*$^-$ failed to form visible plaques or had a low E.O.P. on all of the new *rec* mutants, although this phage grew well on the *recB21* null mutant. These results indicate that the new *rec* mutants retain at least one RecBCD enzyme activity.

TABLE 3

**Mitomycin C-sensitivity and cell viability of *E. coli rec* mutants**

| *E. coli* strain[a] | *rec* allele | E.O.P. on mitomycin C[b] | Relative cell viability[c] |
|---|---|---|---|
| V182 | + | 0.65 | 1 |
| V653 | *B21* | <0.002 | 0.31 |
| V1200 | *B2109* | <0.001 | 0.60 |
| V1204 | *2123* | <0.001 | 0.43 |
| V1232 | *2140* | 0.1 | 0.73 |
| V1268 | *C2145* | <0.001 | 0.85 |
| V1271 | *2148* | <0.001 | 0.48 |
| V1273 | *B2150* | <0.001 | 0.81 |
| V1354 | *B2152* | <0.001 | 0.47 |
| V1373 | *B2153* | <0.001 | 0.21 |
| V1374 | *B2154* | <0.001 | 0.39 |
| V1375 | *B2155* | <0.001 | 0.41 |

[a] Strains are transformants of strain V186 [Δ(*thyA-argA*)*232*] with the indicated *rec* allele present on a derivative of plasmid pDWS2.

[b] Cultures grown overnight to saturation in LB broth were diluted and plated on LB agar plates with or without mitomycin C (1 μg/ml). The efficiency of plating (E.O.P.) is the titer on plates with mitomycin C divided by that on plates without mitomycin C.

[c] Cultures growing exponentially in LB broth at $OD_{650}$ of about 0.5 were diluted and plated on LB agar plates. The relative cell viability is the number of colony-forming units (cfu) per ml divided by the OD of that culture, normalized by the value for strain V182, which gave $5.9 \times 10^8$ cfu/ml at an $OD_{650}$ of 0.46 measured in a 1-cm cuvette.

The plaque sizes of λ Red$^-$Gam$^-$ ($\chi^+$ and $\chi^0$) phages on the new *rec* mutants differed from those on *rec*$^+$ cells. Plaque sizes were increased in some cases and decreased in others, indicating that some aspect of RecBCD enzyme activity was altered in the mutants. Major differences in the behavior of $\chi^0$ and $\chi^+$ λ Red$^-$Gam$^-$ phages (E.O.P. and plaque size) were detected with only one mutant (*recB2150*). This result indicates that *recB2150* retains Chi activation and suggests that the other *rec* mutants do not activate Chi. These suggestions were, with one exception (*recB2153*), confirmed by additional genetic and biochemical tests described later.

With respect to plaque formation by phages P1 and P2, most of the *rec* mutants had neither the *rec*$^+$ nor the *recB21* (null) phenotype. These results, like the preceding ones, imply the retention of some but not all of the activities of RecBCD enzyme in the mutants.

**Recombination proficiency and Chi activity:** Recombination proficiency of the *rec* mutants was measured in two situations–following conjugation with an Hfr and during mixed infection with λ Red$^-$Gam$^-$ phages (Tables 5 and 6). By both measures the mu-

**TABLE 4**

**Phage plaque formation on *E. coli rec* mutants**

| *E. coli* strain[a] | *rec* allele | λ Red⁻ Gam⁻ χ⁰ | λ Red⁻ Gam⁻ χ⁺ | T4 2⁻ | P1 | P2 |
|---|---|---|---|---|---|---|
| V182 | + | 1[b] S[c] | 1[b] L[c] | 0.005[b] | +[d] | +[d] |
| V653 | *B21* | 0.65 L | 0.61 L | 1 | − | − |
| V1200 | *B2109* | 1.0 L | 0.65 L | 0.004 | − | − |
| V1204 | *2123* | 0.19 VT | 0.17 VT | 0.01 | − | + |
| V1232 | *2140* | 1.3 S | 0.96 S | 0.007 | ± | − |
| V1268 | *C2145* | 0.023 T | 0.005 T | <0.001 | − | + |
| V1271 | *2148* | <0.001 − | <0.001 − | <0.001 | − | + |
| V1273 | *B2150* | <0.001 − | 0.49 T | <0.001 | + | + |
| V1354 | *B2152* | 0.9 L | 0.8 L | 0.006 | − | − |
| V1373 | *B2153* | 0.3 S | 0.3 S | 0.01 | ± | ± |
| V1374 | *B2154* | 0.5 VT | 0.4 VT | 0.03 | − | ± |
| V1375 | *B2155* | 0.5 VT | 0.4 VT | 0.04 | − | ± |

[a] Strains are transformants of strain V186 [Δ(*thyA-argA*)*232*] with the indicated *rec* allele present on a derivative of plasmid pDWS2.

[b] Data are the titers of a stock of the indicated phage on the indicated *E. coli* strain on BBL agar plates divided by the titer on strain V182 (for λ Red⁻Gam⁻ phage numbers 872 and 873; see Table 2) or on strain V653 (for T4 *2*⁻ phage).

[c] L, large plaques; S, small plaques; T, tiny plaques; VT, very tiny plaques; −, no visible plaques. λ⁺ made large plaques on all strains listed. T4⁺ made medium plaques on all strains listed; when present, plaques of T4 *2*⁻ were medium-sized.

[d] Dilute suspensions of phage were spotted on lawns of the indicated bacteria on BBL agar plates. +, abundant plaques; ±, few or tiny plaques; −, no visible plaques.

tants were, to various degrees, recombination deficient (Rec⁻). Conjugational crosses were conducted with recipients harboring the *rec* mutations on a multicopy plasmid (and a *recBCD* deletion on the chromosome) and also, in some cases, with the *rec* mutations on the chromosome. The *rec* mutations resulted in similar reductions in both configurations (Table 5). *recB2109*, *rec-2140*, *recC2145*, *recB2152*, *recB2153* and *recB2154* were about as Rec⁻ as the *recB21* null mutation: recombinant frequencies were reduced 100-fold or more. *recB2150* was the least Rec⁻: recombinant frequencies were reduced 2–4-fold. The other *rec* mutations showed intermediate reductions in recombinant frequencies.

Recombination of λ Red⁻Gam⁻ phages was also reduced in the *rec* mutants (Table 6). Red⁻Gam⁻ mutants of λ were used to eliminate the λ Red recombination pathway and the λ Gam inhibitor of the RecBCD enzyme (for a review see SMITH 1983). *recB2109*, *recC2145* and *recB2152* were nearly as Rec⁻ as *recB21*. *recB2150* was the least Rec⁻. *rec-2123* appeared more strongly Rec⁻ in λ Red⁻Gam⁻ crosses than in the Hfr conjugational cross. With this possible exception, the *rec* mutations in comparison to *recB21* were approximately equally Rec⁻ by the two measures. These results show that one or more activities required for recombination are inactivated in the *rec* mutants.

The activity of the recombinational hotspot Chi was also measured in the λ Red⁻Gam⁻ crosses (Table 6). *rec*⁺ cells produced a high Chi activity (5.0), reflecting more exchanges in an interval with a Chi site than in the same interval without Chi. The *recB21* null mutant produced no detectable Chi activity (0.98): a ratio of unity indicates equality of exchange in an interval with and without Chi. Seven of the *rec* mutants produced no significant Chi activity. *rec-2148* and *recB2150* produced slight, but significant, Chi activity (values of about 2.0), and *recB2153* produced full Chi activity. These results, with one exception (*rec-2148*), parallel those found by a more direct test of Chi activation–cutting of DNA at Chi by the mutant RecBCD enzymes (see below).

**ATP-dependent exonuclease activities:** Two activities of RecBCD enzyme digest linear DNA to acid-soluble oligonucleotides: one activity is active on ds DNA, and the other on ss DNA. Both activities were measured in crude extracts of the *rec* mutants (Table 7). The *recB21* mutation rendered both activities undetectable. The ds DNA exonuclease activity was also undetectable in extracts of *rec-2123* and *rec-2148*. The *recB2109* and *recB2152* extracts had only about 5% of the ds DNA exonuclease activity of *recBCD*⁺ extracts. There was greater activity, 15–40% of that in *recBCD*⁺ extracts, in *rec-2140*, *recB2154* and *recB2155* extracts, and nearly full activity in *recC2145*, *recB2150* and *recB2153* extracts. The ss DNA exonuclease activity was undetectable in extracts of *recB21* and *rec-2123* but in all other extracts was within a factor of about 2 of the *recBCD*⁺ activity.

Four mutations resulted in differential inactivation or reduction of the ss and ds DNA exonuclease activities. Under standard assay conditions extracts of *recB2109* and *recB2152* demonstrated very weak ds DNA exonuclease activity but nearly full ss DNA exonuclease activity. In extracts of *rec-2140* and *rec-2148* the ds DNA exonuclease activity was reduced more than the ss DNA exonuclease activity. The

**TABLE 5**

**Conjugational recombination in *E. coli rec* mutants**

| *E. coli* strain | *rec* allele | His⁺[Str^R] recombinant frequency (%)[a] Mean | Range | *n* | Relative frequency[b] |
|---|---|---|---|---|---|
| Plasmid-borne *rec* alleles[c] | | | | | |
| V1342 | + | 3.8 | 1.4–5.6 | 5 | 1 |
| V1343 | *B21* | 0.0067 | 0.005–0.014 | 4 | 0.0017 |
| V1344 | *B2109* | 0.0041 | 0.0015–0.009 | 3 | 0.0011 |
| V1345 | *2123* | 0.33 | 0.28–0.41 | 3 | 0.086 |
| V1346 | *2140* | 0.036 | 0.018–0.047 | 3 | 0.009 |
| V1347 | *C2145* | 0.039 | 0.0082–0.087 | 3 | 0.011 |
| V1348 | *2148* | 0.33 | 0.29–0.34 | 3 | 0.086 |
| V1349 | *B2150* | 1.0 | 0.3–1.7 | 3 | 0.26 |
| V1383 | *B2152* | 0.009 | 0.007–0.010 | 3 | 0.002 |
| V1353 | *B2153* | 0.001 | 0.0009–0.002 | 3 | 0.0002 |
| V1351 | *B2154* | 0.041 | 0.03–0.07 | 3 | 0.010 |
| V1352 | *B2155* | 0.052 | 0.04–0.06 | 3 | 0.013 |
| Chromosome-borne *rec* alleles[d] | | | | | |
| AFT325 | + | 4.9 | 3.5–7.6 | 7 | 1 |
| V1252 | *B21* | 0.027 | 0.001–0.059 | 7 | 0.006 |
| V967 | *B2109* | 0.021 | 0.005–0.068 | 5 | 0.004 |
| V1296 | *C2145* | 0.27 | 0.04–0.59 | 3 | 0.055 |
| V1292 | *B2150* | 2.5 | 0.73–4.4 | 3 | 0.51 |
| V1314 | *B2152* | 0.015 | 0.01–0.02 | 2 | 0.003 |
| V1356 | *B2153* | 0.01 | 0.009–0.04 | 4 | 0.002 |
| V1360 | *B2154* | 0.048 | 0.045–0.051 | 2 | 0.010 |
| V1363 | *B2155* | 0.064 | 0.057–0.071 | 2 | 0.013 |

[a] His⁺[Str^R] recombinant frequencies were determined after mating with donor strain S727 [*his*⁺ (Hfr P044)] as described in MATERIALS AND METHODS. Data are the number of His⁺[Str^R] recombinants per donor cell, corrected for viability of the recipients; the means and ranges are reported for *n* independent matings.

[b] Mean recombinant frequency for the indicated strain divided by that for the *rec*⁺ strain.

[c] Strains are transformants of strain V376 [Δ(*thyA-argA*)*235* (Ap^r) *his rpsL*] with the indicated *rec* allele present on a derivative of plasmid pDWS2.

[d] Strains are Thy⁺Arg⁺ transductants of strain AFT325 (*thyA argA his rpsL*) with the indicated *rec* allele present on the chromosome.

**TABLE 6**

**λ Red⁻Gam⁻ recombination and Chi activity in *E. coli rec* mutants**

| *E. coli* strain[a] | *rec* alelle | J⁺R⁺ recombinant frequency (%)[b] Mean | Range | *n* | Chi activity[c] Mean | Range | *n* |
|---|---|---|---|---|---|---|---|
| V182 | + | 10.3 | 8.0–13.8 | 10 | 5.0 | 4.6–5.4 | 4 |
| V653 | *B21* | 0.91 | 0.14–2.0 | 8 | 0.97 | 0.95–0.99 | 3 |
| V1200 | *B2109* | 1.2 | 0.8–1.6 | 4 | 0.90 | 0.86–0.93 | 2 |
| V1204 | *2123* | 0.3 | 0.1–0.5 | 4 | 1.2 | 1.04–1.26 | 2 |
| V1232 | *2140* | 2.3 | 1.9–2.5 | 6 | 1.2 | 0.93–1.4 | 3 |
| V1268 | *C2145* | 1.3 | 0.17–2.5 | 6 | 1.1 | 0.89–1.25 | 3 |
| V1271 | *2148* | 2.6 | 2.0–3.7 | 6 | 1.8 | 0.98–2.95 | 3 |
| V1273 | *B2150* | 3.3 | 2.4–4.0 | 6 | 2.1 | 2.0–2.1 | 3 |
| V1354 | *B2152* | 1.2 | 1.2–1.3 | 4 | 0.92 | 0.90–0.94 | 2 |
| V1373 | *B2153* | 1.1 | 0.9–1.3 | 4 | 5.3 | 5.2–5.4 | 2 |
| V1374 | *B2154* | 1.8 | 1.5–2.0 | 4 | 1.2 | 1.1–1.3 | 2 |
| V1375 | *B2155* | 1.5 | 1.2–1.7 | 4 | 1.0 | 0.92–1.1 | 2 |

[a] Strains are transformants of strain V186 [Δ(*thyA-argA*)*232*] with the indicated *rec* allele present on a derivative of plasmid pDWS2.

[b] The frequency of J⁺R⁺ recombinants in n independent cross lysates of λ phages 1081 and 1082 or of 1083 and 1084 was measured by plating on strain 594 (*sup*⁺) for J⁺R⁺ recombinant phage titer and on strain C600 (*supE*) for total phage titer. Data are the mean and range of these frequencies for cross lysates produced from each strain.

[c] Chi activity was measured by the method of STAHL and STAHL (1977) in crosses between λ phages 1081 and 1082 (cross 1) and between phages 1083 and 1084 (cross 2) (see Table 2 for genotypes). Chi activity = $\sqrt{(t/c)_1 \div (t/c)_2}$, where $(t/c)$ is the ratio of turbid to clear plaques from cross 1 or cross 2, among J⁺R⁺ recombinants selected on strain 594 (*sup*⁺).

residual ds DNA exonuclease activity in *recB2109*, *recB2152* and *rec-2140* may reflect DNA unwinding (see below) plus ss DNA exonuclease activity.

The amount of ds DNA exonuclease activity detected in extracts of *recB2109* and *recB2152* depended on the condition of the assay. The *recB2109* mutation apparently increases the enzyme's $K_M$ for ATP (A. EGGLESTON and S. KOWALCZYKOWSKI, personal communication). The *recB2152* mutation had a similar effect (Figure 1). The ds DNA exonuclease activity of wild-type enzyme in crude extracts was maximal with 25 μM ATP but was inhibited about 50% by 0.1–10 mM ATP (EICHLER and LEHMAN 1977). In contrast, *recB2109* extracts had little ds DNA exonuclease activity in the presence of 25 μM ATP but had 30–50% of the specific activity of wild-type extracts at 1–10 mM ATP. Extracts of *recB2152* also only weakly degraded ds DNA unless the ATP concentration was raised to 5–10 mM. Cell-free extracts of other *recBCD* mutants (*recB21*, *recD1011*, *recD1013*, *recD1014*, *rec-*

**TABLE 7**

**Exonuclease activity in extracts of *E. coli rec* mutants**

| *E. coli* strain[a] | *rec* allele | Relative dsDNA exonuclease[b] Mean | Range | *n* | Relative ssDNA exonuclease[c] Mean | Range | *n* |
|---|---|---|---|---|---|---|---|
| V182 | + | ≡1 | 0.83–1.69 | 6 | ≡1 | 0.65–1.20 | 6 |
| V653 | *B21* | <0.03 | | 5 | <0.2 | | 6 |
| V1200 | *B2109* | 0.04 | 0.03–0.06 | 9 | 0.88 | 0.76–1.10 | 4 |
| V1204 | *2123* | <0.03 | | 6 | <0.2 | | 6 |
| V1232 | *2140* | 0.14 | 0.13–0.20 | 4 | 0.47 | 0.35–0.53 | 3 |
| V1268 | *C2145* | 0.74 | 0.52–1.40 | 4 | 0.41 | 0.35–0.53 | 4 |
| V1271 | *2148* | <0.03 | | 10 | 0.35 | 0.29–0.47 | 10 |
| V1273 | *B2150* | 0.70 | 0.58–0.81 | 2 | 0.71 | 0.47–1.00 | 4 |
| V1354 | *B2152* | 0.04 | 0.03–0.05 | 6 | 0.53 | 0.35–0.59 | 6 |
| V1373 | *B2153* | 0.58 | 0.23–1.00 | 6 | 1.70 | 1.50–2.00 | 3 |
| V1374 | *B2154* | 0.43 | 0.40–0.43 | 4 | 1.90 | 1.90 | 2 |
| V1375 | *B2155* | 0.34 | 0.27–0.42 | 4 | 1.60 | 1.20–2.10 | 2 |

[a] Strains are transformants of strain V186 [Δ(*thyA-argA*)*232*] with the indicated *rec* allele present on a derivative of plasmid pDWS2.

[b] Native $^3$H-labeled T7 DNA was used as substrate. Data are the specific activities (units of ATP-dependent nuclease activity/mg of extract protein) of *n* extracts of the indicated strain divided by that of V182 (930 units/mg).

[c] Boiled $^3$H-labeled T7 DNA was used as substrate. Data are the specific activities of *n* extracts of the indicated strain divided by that of V182 (170 units/mg).

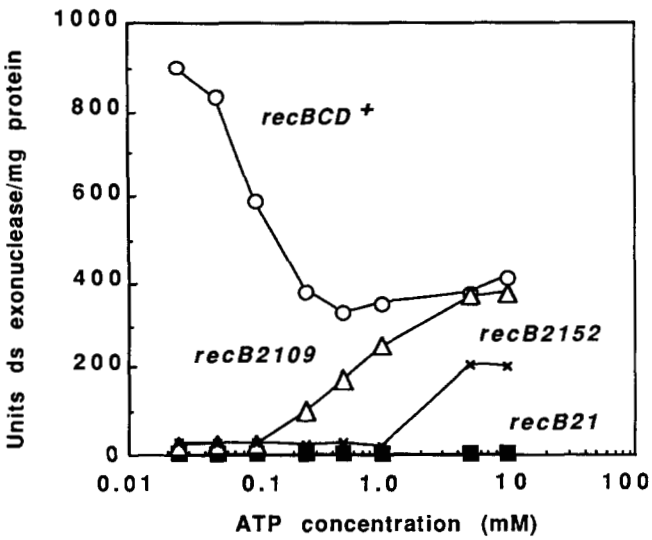


FIGURE 1.—Effect of ATP concentration on ds DNA exonuclease activity in cell-free extracts of *E. coli recBCD* mutants with plasmid-borne alleles. Cell-free extracts were made and assayed for ATP-dependent solubilization of ds [$^3$H]-T7 DNA as described in MATERIALS AND METHODS. The ATP concentration of the reaction is indicated. Data are the specific activities (units of RecBCD enzyme ds DNA exonuclease/mg protein; EICHLER and LEHMAN 1977) observed at each ATP concentration. The strains and the *rec* alleles tested were V182 (*recBCD*$^+$), V1200 (*recB2109*), V1354 (*recB2152*) and V653 (*recB21*); all are transformants of strain V186 [Δ(*thyA-argA*)*232*].

*2123* and *rec-2148*) lacking ds DNA exonuclease activity at 25 μM ATP failed to degrade ds DNA at any ATP concentration tested (Fig. 1; data not shown).

**ds DNA exonuclease activity in phage-infected cells:** To assess the activity of mutant RecBCD enzymes inside the cell, we measured the degradation of T4 *2*$^-$ [$^3$H]DNA following infection of the mutants. T4 *2*$^-$ DNA, but not T4 *2*$^+$ DNA, is rapidly converted to acid-soluble material in *recBCD*$^+$ cells, but neither is significantly degraded in *recBCD* null mutants (SILVERSTEIN and GOLDBERG 1976). In our experiments, after infection with T4 *2*$^+$ less than 12% of the adsorbed DNA was rendered acid soluble in 30 min, with the exception of one mutant (*recC2145*; Table 8). After infection with T4 *2*$^-$ 20–75% of the adsorbed DNA was degraded in *recBCD*$^+$, *rec-2140*, *recC2145*, *recB2150*, *recB2153*, *recB2154* and *recB2155* cells. Crude extracts of these mutants also had high ds DNA exonuclease activity (Table 7). In contrast, only low levels (<10%) of T4 *2*$^-$ adsorbed DNA was degraded in *recB21*, *recB2109*, *rec-2123*, *rec-2148* and *recB2152* cells, which had low levels of ds DNA exonuclease activity in crude extracts. There is, therefore, a good correlation of the two measures of RecBCD enzyme ds DNA exonuclease activity. There is less correlation, however, of the intracellular DNA degradation and the ss DNA exonuclease activities (Tables 7 and 8). These results suggest that the ss DNA exonuclease activity is not solely responsible for the degradation of infecting T4 *2*$^-$ DNA.

TABLE 8

**Degradation of T4 and T4 *2*$^-$ DNA in infected cells**

| *E. coli* strain[a] | *rec* allele | Percent phage DNA degraded[b] T4 | T4 *2*$^-$ |
|---|---|---|---|
| V182 | + | 8.7 | 62 |
| V653 | *B21* | 3.1 | 4.2 |
| V1200 | *B2109* | 2.9 | 8.1 |
| V1204 | *2123* | 2.2 | 7.9 |
| V1232 | *2140* | 3.3 | 30 |
| V1268 | *C2145* | 17 | 77 |
| V1271 | *2148* | 4.4 | 6.4 |
| V1273 | *B2150* | 11 | 75 |
| V1354 | *B2152* | 6.2 | 7.7 |
| V1373 | *B2153* | 9.6 | 70 |
| V1374 | *B2154* | 1.9 | 28 |
| V1375 | *B2155* | 6.2 | 21 |

[a] Strains are transformants of strain V186 [Δ(*thyA-argA*)*232*] with the indicated *rec* allele present on a derivative of plasmid pDWS2.

[b] DNA degradation was measured as described in MATERIALS AND METHODS. Percent phage DNA degraded is the amount of acid-soluble radioactive material present 30 min after infection divided by the amount of radioactive material adsorbed 5 min after infection. The amount of acid-soluble radioactive material in each phage preparation in the absence of bacteria (7–9%) was subtracted. Each value is the mean of three separate infections.

**DNA unwinding and Chi cutting activities:** A third activity of RecBCD enzyme unwinds linear ds DNA, and a fourth activity cuts one DNA strand near Chi during unwinding. These two activities are conveniently measured with a linear ds DNA fragment labeled with $^{32}$P at one end of the strand containing the Chi sequence (5′G-C-T-G-G-T-G-G 3′) (SMITH *et al.* 1981a; PONTICELLI *et al.* 1985). After brief reaction, the unwound ss DNA product and the products of Chi-dependent DNA strand cleavage are detected by gel electrophoresis and autoradiography.

Extracts from all of the new *rec* mutants unwound linear ds DNA, although the *recB21* null extract did not (Fig. 2). Quantitation of this activity is complicated by non-RecBCD nuclease activities in the extract, which destroy the unwound product; the fraction of the unwinding activity remaining in the mutants therefore cannot be reliably estimated.

With respect to Chi cutting activity, the *rec* mutants fell into two classes (Fig. 2). One class had no detectable activity: *recB2109*, *rec-2123*, *rec-2140*, *recC2145*, *rec-2148*, *recB2152*, *recB2154* and *recB2155*. The other class had strong Chi cutting activity: *recB2150* and *recB2153*. Quantitation of Chi cutting activity is also complicated by nucleolytic degradation of the product. With one exception, these results are concordant with the assay for Chi genetic activity in λ Red$^-$Gam$^-$ crosses (Table 6). The latter two mutants (*recB2150* and *recB2153*) had both Chi cutting activity in extracts and Chi genetic activity in crosses, whereas the *recB2109*, *rec-2123*, *rec-2140*, *recC2145*, *recB2152*,

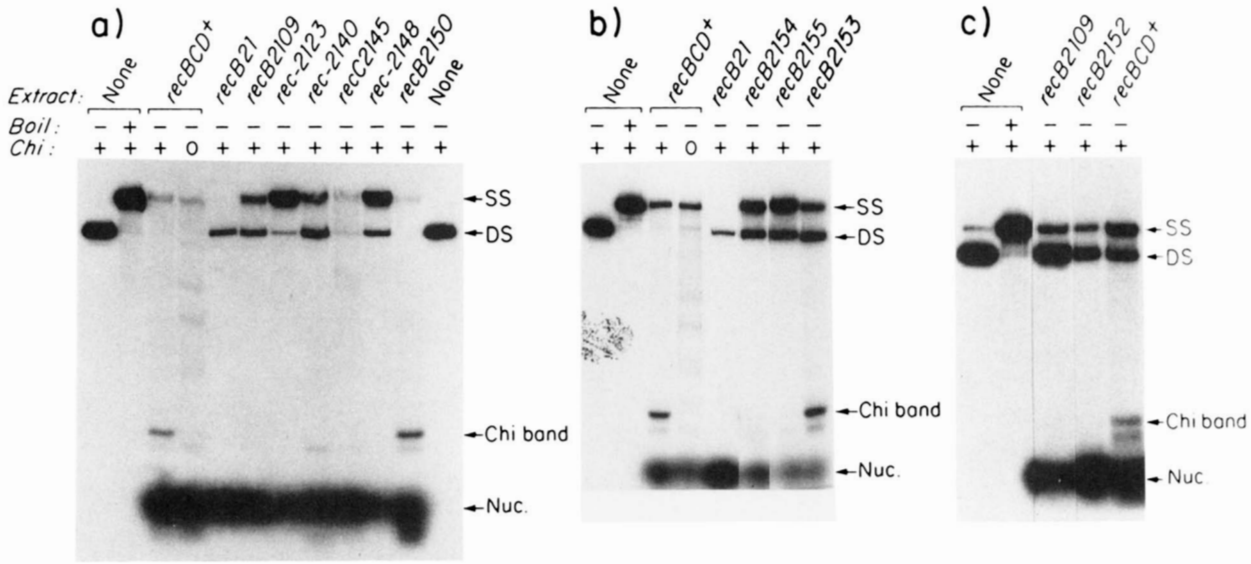


FIGURE 2.—Detection of DNA unwinding and Chi-dependent cleavage in cell-free extracts of *E. coli rec* mutants. Extracts were prepared from derivatives of strain V186 [Δ(*thyA-argA*)*232*] with plasmids carrying the indicated *rec* alleles. DNA substrates derived from pBR322 with (+) or without (0) Chi and labeled at the 3′ end with $^{32}P$ were prepared as described in MATERIALS AND METHODS. Two fmol of DNA were reacted at 37° with 10 μg (7 μg in panel c) of cell-free extract for 15 sec (5 sec in panel b and 60 sec in panel c). Reactions were stopped by the addition of EDTA and sodium dodecyl sulfate and heated at 65° for 5 min or boiled where indicated. The products of the reaction were analyzed on a 5% polyacrylamide gel (4% polyacrylamide-0.5% agarose in panels b and c) as described in MATERIALS AND METHODS. The positions of unwound ss DNA (SS), ds DNA (DS), degraded DNA (Nuc.), and the major product of Chi cleavage (Chi band) are indicated. A less intense product in reactions in which Chi was cut is also visible below the major Chi cleavage product. (a) First two and last lanes, no extract added to DNA. Remaining lanes, extracts from V182 (*recBCD*$^+$), V653 (*recB21*), V1200 (*recB2109*), V1204 (*rec-2123*), V1327 (*rec-2140*), V1268 (*recC2145*), V1271 (*rec-2148*), V1273 (*recB2150*). (b) First two lanes, no extract. Remaining lanes, V182 (*recBCD*$^+$), V653 (*recB21*), V1374 (*recB2154*), V1375 (*recB2155*), and V1373 (*recB2153*). (c) First two lanes, no extract. Remaining lanes, V967 carrying *recB2109* on the chromosome, V1313 carrying F′15 *recB2152*, and strain V793 carrying F′15 *argA88 recBCD*$^+$.

*recB2154* and *recB2155* mutants had neither activity. The *rec-2148* mutant had low Chi genetic activity but no detectable Chi cutting activity; the *rec-2148* mutant enzyme may be unstable in extracts.

**Precise excision of transposon Tn*10*:** Transposon Tn*10*, with 1.4-kb inverted repeats at its ends, can excise precisely and restore the function of the gene into which it had inserted. The frequency of this excision is increased, relative to that in *recBCD*$^+$ cells, in certain *recB* and *recC* mutants with the phenotype designated Tex (LUNDBLAD *et al.* 1984; SCHULTZ, TAYLOR and SMITH 1983). *recB* and *recC* null mutants do not have the Tex phenotype (LUNDBLAD *et al.* 1984). We measured the frequency of precise excision as the frequency of reversion of the *lacZ2900*::Tn*10* mutation. Only two mutants, *rec-2148* and *recB2150* had significantly elevated frequencies of excision (Table 9). The frequency in *rec-2148* cells was similar to that in the previously described Tex mutants, *recC343* and *recB344*; excision was 20–40 times more frequent than in *recBCD*$^+$ cells. Excision in the *recB2150* mutant was even higher: 160–180 times more frequent than in *recBCD*$^+$ cells. We also determined that the previously isolated *recD1009* mutant (CHAUDHURY and SMITH 1984a; AMUNDSEN *et al.* 1986) did not have the Tex phenotype. These results show that the *rec* mutants are differentially altered with respect to some activity of RecBCD enzyme, but the nature of that activity remains obscure (see DISCUSSION).

**Haploid derivatives of the *rec* mutants and complementation analysis:** We attempted to transfer the *rec* mutations from the F′15 episome or the pDWS2 plasmid, on which they were isolated, to the chromosome. These haploid derivatives would reveal the phenotypes of the mutations when the *recBCD* genes are at the dosage found in wild-type cells, and they would allow complementation analyses with F′15 bearing *rec* null mutations at gene dosages similar to those of the chromosomal *rec* mutations. As described in MATERIALS AND METHODS, the *recB2109* mutation was transferred from the F′15 episome to the chromosome by P1-mediated transduction. Complementation analysis of *recB2152* was performed with *recB2152* on the F′15 episome, on which it was isolated. The other *rec* alleles, isolated on plasmid pDWS2, were transferred to the chromosome by transformation with linearized plasmid DNA (COSLOY and OISHI 1973). With *recC2145*, *recB2150*, *recB2154* and *recB2155* we obtained derivatives (Thy$^+$ Arg$^+$ transductants of strain AFT325; see MATERIALS AND METHODS) with phenotypes (MC and phage sensitivities; see Tables 3 and 4) indistinguishable from those of the strains bearing these *rec* mutations on the plasmid. The *recB2153* derivatives supported slightly larger

**TABLE 9**

**Precise excision of Tn*10* in *E. coli rec* mutants**

| *E. coli* strain | *rec* allele | Frequency of precise excision[d] Mean × $10^9$ | Range × $10^9$ | *n* | Relative frequency[b] |
|---|---|---|---|---|---|
| Plasmid-borne *rec* alleles[c] | | | | | |
| V1319 | + | 1.9 | 0–9.4 | 5 | 2.3 |
| V1320 | *B21* | <0.5[d] | 0 | 5 | <0.7 |
| V1321 | *B2109* | 0.66 | 0–3.3 | 5 | 0.8 |
| V1322 | *2123* | 2.6 | 0–7.0 | 5 | 3.2 |
| V1323 | *2140* | 2.0 | 0–10 | 5 | 2.4 |
| V1324 | *C2145* | <0.3[d] | 0 | 5 | <0.3 |
| V1325 | *2148* | 20 | 9–36 | 5 | 24 |
| V1326 | *B2150* | 150 | 0–700 | 5 | 180 |
| V1376 | *B2153* | <0.2[d] | 0 | 5 | <0.1 |
| V1377 | *B2154* | 0.6 | 0–2 | 5 | 0.3 |
| V1378 | *B2155* | 0.2 | 0–1 | 5 | 0.1 |
| Chromosome-borne *rec* alleles[c] | | | | | |
| AFT227 | + | 0.82 | 0–6.3 | 11 | 1 |
| AFT229 | *C343* | 31 | 20–43 | 8 | 38 |
| AFT231 | *B344* | 17 | 7.7–36 | 8 | 21 |
| V1333 | *D1009* | 1.2 | 0–4.1 | 6 | 1.4 |
| V1334 | *B2109* | <0.9[d] | 0 | 6 | <1.1 |
| V1335 | *C2145* | <0.5[d] | 0 | 4 | <0.6 |
| V1336 | *B2150* | 130 | 80–260 | 13 | 160 |

[a] The frequency of Lac$^+$ cells in *n* independent cultures was measured by plating on OMB-lactose agar plates (for Lac$^+$ cfu) and on LB agar plates (for total cfu). Data are the mean and range of these frequencies for each strain.

[b] Mean Lac$^+$ frequency for the indicated strain divided by that for strain AFT227 (haploid *rec*$^+$).

[c] Strains are transformants of strain V1318 [Δ(*thyA-argA*)*232 lacZ2900*::Tn*10*] with the indicated *rec* allele present on a derivative of plasmid pDWS2.

[d] No Lac$^+$ colonies were observed. The upper limit of the frequency was calculated by assuming three Lac$^+$ colonies.

[e] Strains are Thy$^+$ transductants of strain AFT227 (*lacZ2900*::Tn*10*) with the indicated *rec* allele present on the chromosome.

**TABLE 10**

**Complementation analysis of *recB2109*, *recC2145* and *recB2150***

| *E. coli* strain[a] | *rec* allele[b] Chromosome | F′ | Relative E.O.P. of λ Red$^-$Gam$^-$ $\chi^{0}$[c] | Chi activity[d] |
|---|---|---|---|---|
| V66 | + | − | 1 | 4.6 |
| V67 | *B21* | − | 0.73 | 1.3 |
| V68 | *C73* | − | 0.84 | 1.6 |
| V222 | *D1013* | − | 0.73 | 1.1 |
| V967 | *B2109* | − | ND[e] | 0.92 |
| | *B2109* | *B21* | ND | 1.2 |
| | *B2109* | *C73* | ND | 5.4 |
| | *B2109* | *D1013* | ND | 2.5 |
| V1296 | *C2145* | − | <0.001 | 0.94 |
| | *C2145* | + | 0.94 | 6.1 |
| | *C2145* | *B21* | 1.0 | 6.1 |
| | *C2145* | *C73* | <0.001 | 1.1 |
| | *C2145* | *D1013* | 0.82 | 6.7 |
| V1292 | *B2150* | − | 0.09 | 2.0 |
| | *B2150* | + | 0.88 | 5.2 |
| | *B2150* | *B21* | 0.18 | 2.2 |
| | *B2150* | *C73* | 0.75 | 4.0 |
| | *B2150* | *D1013* | 0.80 | 6.3 |

[a] Strains are derivatives of strain AFT325 (*thyA argA21*).

[b] The indicated chromosomal allele was transduced into strain AFT325 by selection for Thy$^+$. The indicated F′ allele was introduced by mating with strain V1327 (F′ *recBCD*$^+$), V1328 (F′*recB21*), V1329 (F′*recC73*), or V1330 (F′*recD1013*) by selection for Arg$^+$. −, no F′ present.

[c] Data are the titers of a stock of λ strain 872 (*b1453 cI857*) on the indicated *E. coli* strain divided by that on *E. coli* strain V66.

[d] Chi activity was determined in the crosses λ1081 × λ1082 and λ1083 × λ1084, as described in footnote *c* to Table 6.

[e] ND, not determined.

plaque-formation by λ Red$^-$Gam$^-$ and demonstrated somewhat reduced Chi activity in λ Red$^-$Gam$^-$ crosses than did the strain with *recB2153* on the plasmid (data not shown). With *rec-2123*, *rec-2140* and *rec-2148*, however, we obtained only derivatives with the *recBC* null phenotype, even though 12 to 16 transformants were examined. We infer that these alleles have a null phenotype when expressed at the level of a chromosomal allele, but a non-null, non-wild-type phenotype when overexpressed on the plasmid, although this has not been directly tested by cloning of the presumptive haploid alleles.

We conducted complementation analyses with the seven mutations which, when transferred to the chromosome, manifested the non-null mutant phenotype. F′15 episomes bearing *recBCD*$^+$ or *recB*, *C* or *D* null alleles were introduced into these strains, and complementation with respect to two phenotypes was quantitated (Table 10). λ Red$^-$Gam$^-$ $\chi^0$ formed no or extremely tiny plaques on *recC2145*, *recB2150*, *recB2153*, *recB2154* and *recB2155* mutants (Table 4). Introduction of F′15 *recBCD*$^+$, *recB21* or *recD1013*, but not *recC73*, restored high E.O.P. of this phage on the *recC2145* mutant. This result indicates that *recC2145* is recessive and lies in the *recC* gene. A similar analysis indicates that *recB2150*, *recB2153*, *recB2154* and *recB2155* lie in the *recB* gene (Table 10 and additional data not shown). Chi activity was undetectable in the *recB2109*, *recC2145*, *recB2152*, *recB2154* and *recB2155* mutants and was low in the *recB2150* mutant (Table 6). Assays for Chi activity in appropriate merodiploids showed that *recB2109*, *recB2150*, *recB2152*, *recB2154* and *recB2155* complemented *recC73* and *recD1013* but not *recB21*, whereas *recC2145* complemented *recB21* and *recD1013* but not *recC73* (Table 10 and additional data not shown). Recombination proficiency as measured by λ vegetative crosses was restored to *recB2153* by *recC73* and *recD1013*, but not by *recB21* (data not shown). These results indicate that *recB2109*, *recB2150*, *recB2152*, *recB2153*, *recB2154* and *recB2155* lie in the *recB* gene and that *recC2145* lies in the *recC* gene. The results also show that the seven *rec* alleles are recessive.

## DISCUSSION

We have described the isolation and characterization of 10 mutations that inactivate some, but not all,

'ND, not determind.**TABLE 11**

**Summary of *recBCD* mutant properties**

| Class | Mutations | DNA unwinding | ds exo | ss exo | Chi cutting | Chi activity | Rec[a] | T4 $2^{-}$[b] | λ Red$^{-}$Gam$^{-}$[b] | Tn*10* hyperexcision | Reference[c] |
|---|---|---|---|---|---|---|---|---|---|---|---|
| I | *B2109, B2152* | + | −[d] | + | − | − | − | − | ++ | − | (1) |
| II | *C2145, B2154, B2155* | + | + | + | − | − | − | − | ± | − | (1) |
| III | *B2150* | + | + | + | + | + | ± | − | ± | + | (1) |
| III | *B2153* | + | + | + | + | + | − | − | ± | − | (1) |
| * | *C1002, C1003, C1004* | + | + | + | − | − | ± | − | ± | + | (2) |
| Tex | *B344, C343* | + | + | + | ± | ± | + | − | ± | + | (2, 3) |
| ‡ | *D, C1010* | − | − | − | − | − | + | + | ++ | − | (4, 5) |
| null | *B21, C22, C73, 232* | − | − | − | − | − | − | + | ++ | − | (1–7) |
| Wild type | + | + | + | + | + | + | + | − | + | − | (1–7) |

The properties listed for each class differ slightly, in some cases, from mutant to mutant. For details see the cited references. +, activity present; ±, activity reduced; −, activity absent.

[a] Recombination-proficiency in Hfr conjugations, P1-transductions, or λ Red$^{-}$Gam$^{-}$ crosses.

[b] Plaque-formation by the indicated phage. ++, large plaques; +, medium plaques; +, small or few plaques; −, no visible plaques; ±, few tiny or no visible plaques.

[c] (1) This study (see Tables 4–9 and Figures 1 and 2); (2) SCHULTZ, TAYLOR and SMITH (1983); (3) LUNBLAD *et al.* (1984); (4) CHAUDHURY and SMITH (1984a); (5) AMUNDSEN *et al.* (1986); (6) WILLETTS and CLARK (1969); (7) CHAUDHURY and SMITH (1984b).

[d] At high (>1 mM) ATP concentrations nearly full activity is detected (Figure 1).

of the biochemical activities of RecBCD enzyme. The properties of these mutants allow inferences about the role of RecBCD enzyme in recombination and the exclusion of foreign DNA.

***recBCD* mutant isolation:** The methods used here allowed the facile isolation of rare *recBCD* mutant types. After nitrosoguanidine mutagenesis, about 3% of the *recBCD* genes had a detectable mutation. Transfer of the mutagenized F'15 or plasmid with the *recBCD* genes to unmutagenized, *recBCD*-deletion cells permitted us to concentrate on *recBCD* mutations, rather than other mutations resulting in the phenotypes screened (small colony formation, mitomycin C-sensitivity, and altered phage sensitivity). We did find, however, a few mutations producing small colony formation or mitomycin C-sensitivity that were not on the F' or plasmid (data not shown); these mutations may have resulted from untargeted mutagenesis of the chromosome after introduction of the mutagenized F' or plasmid (WALKER 1984). Mutagenesis of the plasmid carrying the *recBCD*$^{+}$ region efficiently yielded non-null *recBCD* mutants: among the small colony-forming clones (about 3% of the total), 10–15% had a non-null *recBCD* mutation. The methods described here, and variations of them, should yield additional novel *recBCD* mutants.

**Dissection of RecBCD enzymatic activities:** We view the mutants described here as falling into three classes, each with a different set of RecBCD enzymatic activities affected and a different set of phenotypes, which are summarized in Table 11. Class I mutants (*recB2109* and *recB2152*) had, under standard assay conditions, greatly reduced ds DNA exonuclease and Chi-dependent DNA strand cleavage activities, but they retained DNA unwinding activity and ss DNA exonuclease activity. These mutants were recombination-deficient, had no detectable Chi activity, and supported large plaque-formation by λ Red$^{-}$Gam$^{-}$ phage. Class II mutants (*recC2145*, *recB2154* and *recB2155*) lacked Chi cutting activity but retained ds and ss DNA exonuclease and DNA unwinding activities. These mutants were recombination deficient, had no detectable Chi activity, and severely restricted the growth of λ Red$^{-}$Gam$^{-}$ phage. Three other mutants, *rec-2123*, *rec-2140* and *rec-2148*, shared all of the class II phage test and genetic phenotypes, but cell-free extracts of them had little ds DNA exonuclease activity (Table 7). Class III mutants (*recB2150* and *recB2153*) retained all RecBCD enzymatic activities tested; they had reduced recombination-proficiency, but, among the residual recombinants, there was partial to full Chi activity. These mutants partially restricted the growth of λ Red$^{-}$Gam$^{-}$. The *recB2150* and *recB2153* mutants differed in two respects: *recB2150* had the Tex phenotype (see below), whereas *recB2153* did not, and *recB2153* was about 100 times more recombination-deficient than *recB2150*.

The retention of some RecBCD enzymatic activities and the elimination of others in the mutants described here demonstrate that the activities can be dissected and suggest that they lie in separate, but perhaps overlapping, domains. Class I mutants demonstrate that DNA unwinding can be separated from ds DNA hydrolysis. This separation of activities can also occur biochemically, as $Ca^{2+}$ ions block DNA hydrolysis but not DNA unwinding (ROSAMOND, TELANDER and LINN 1979; TAYLOR and SMITH 1980). These mutants also suggest that the ds DNA exonuclease and ss DNA exonuclease activities of RecBCD enzyme can be uncoupled. Under standard assay conditions, only very weak ds DNA exonuclease activity was detected in cell-free extracts of *recB2109* and *recB2152* despite

nearly *recBCD*$^+$ levels of ss DNA exonuclease activity. This result suggests that the majority of the ds DNA exonuclease activity of the wild-type enzyme is not simply the combined result of DNA unwinding and ss DNA exonuclease activity. The residual solubilization of ds DNA by *recB2109* and *recB2152* mutant extracts may, however, be due to the combined activities of DNA unwinding and ss DNA exonuclease. Class II mutants demonstrate that the Chi-independent nuclease activity can be separated from Chi cutting. These two nuclease activities may lie in separate domains, or the class II mutations may block Chi recognition, which in the wild-type enzyme may stimulate the sole nuclease domain. Class III mutants suggest that an as yet unidentified RecBCD enzymatic activity not examined here is required for recombination. The proposal for separate domains would be strengthened if the amino acids altered in each mutant class were found to be clustered.

**Role of RecBCD enzyme in recombination:** Class I mutants demonstrate that DNA unwinding is not sufficient for recombination. The recombination-deficiency of these mutants supports the hypothesis that RecBCD enzyme must produce ss DNA ends as substrates for RecA and SSB protein-promoted strand exchange (reviewed in SMITH 1987, 1988). Unwinding can produce the required ss DNA but not the ends. The recombination-deficiency of these mutants argues against models in which the ss DNA loops produced by RecBCD enzyme invade ds DNA, aided by RecA protein without strand cleavage by RecBCD enzyme (see, *e.g.*, ROSENBERG 1987)

Class II mutants suggest that DNA strand cleavage at Chi is required for recombination, as predicted by a model of recombination proposed earlier (SMITH *et al.* 1981b; SMITH 1987). The lack of both Chi cutting activity and Chi genetic activity by class II mutants provides further evidence that Chi cutting is essential for Chi genetic activity. This conclusion has previously been drawn from the coordinate loss of the two activities in mutants altered in RecBCD enzyme or in Chi sites (reviewed in SMITH 1987). It has been suggested (CHENG and SMITH 1984, 1987) that recombination in the absence of Chi depends upon low level cutting of Chi-like sites by RecBCD enzyme. If class II mutants lack this activity as well, they would be predicted to be strongly recombination-deficient, as observed (Tables 5 and 6).

The *recB2153* mutant (class III, as defined in the preceding section) retained all tested enzymatic activities, yet it was recombination-deficient. This result indicates that wild-type RecBCD enzyme has an unknown activity required for recombination or that qualitative alterations in one or more of the known RecBCD enzymatic activities limits recombination proficiency. The hypothetical activity may be the cleavage of D-loops or the resolution of Holliday junctions, as predicted by certain models of recombination (see, *e.g.*, FAULDS *et al.* 1979; SMITH *et al.* 1981b; ROSENBERG 1987). Although there is some evidence that RecBCD enzyme has both of these activities (WIEGAND *et al.* 1977; TAYLOR and SMITH 1990), their relevance to recombination is unclear. For example, RecBCD enzyme resolves cruciforms (which mimic Holliday junctions) only if enzyme molecules can approach the junction along two open-ended arms (TAYLOR and SMITH 1990), but during intracellular recombination of λ, a single open-ended arm appears sufficient for RecBCD enzyme-promoted recombination (STAHL *et al.* 1982). Another possibility is that a single RecBCD enzyme molecule can resolve a Holliday junction in whose formation it directly participates, as predicted by one model of recombination (SMITH *et al.* 1981b). The possibility that class I and II mutants lack the hypothetical activity missing in the *recB2153* (class III) mutant, as well as Chi cutting activity, precludes a firm conclusion that class I and II mutants are Rec$^-$ solely because they lack Chi cutting. The *recB2150* mutant also retained all tested enzymatic activities and was recombination deficient, though not as severely deficient as *recB2153*. The *recB2150* mutant may have a reduced amount of the hypothetical activity missing in *recB2153*, or it may be altered in a different activity. The recombination deficiency of these two mutants may stem from a qualitative alteration of one or more of the remaining activities. For example, reduced processivity of DNA unwinding would show enzymatic activity as measured here (Figure 2) but might be insufficient to promote recombination in the cell.

**Exclusion of T4 *2*$^-$ and λ Red$^-$Gam$^-$ mutants by RecBCD enzyme:** Class I mutants blocked plaque formation by T4 *2*$^-$ mutants (Table 4) but produced little acid-soluble material following T4 *2*$^-$ infection (Table 8). The combined activities of DNA unwinding and ss DNA exonuclease were apparently not sufficient to solubilize T4 *2*$^-$ DNA in infected cells. This suggests that DNA unwinding can block this phage's growth. Although T4 *2*$^-$ DNA is rapidly degraded to acid-soluble products after infection of *recBCD*$^+$ cells (45% in 1.5 min; LABEDAN and GOLDBERG 1979), solubilization may occur subsequent to unwinding (TAYLOR 1988) and unwound T4 DNA may be nonfunctional. The greatly reduced ds DNA exonuclease activity inside the cell is apparently due to the *recB2109* and *recB2152* mutant enzymes' altered $K_M$ for ATP. ds DNA exonuclease activity in crude extracts of *recB2109* and *recB2152* cells was near the *recBCD*$^+$ level at 1–10 mM ATP but was about 25-fold less at 25 μM ATP, the standard condition for RecBCD ds DNA exonuclease assays (EICHLER and LEHMAN 1977). These results suggest that the ATP concentra-

tion inside the cell is below 1 mM although by other measures the total ATP concentration in *E. coli* has been estimated to be about 3 mM (MATTHEWS 1972). This concentration is sufficient to support DNA unwinding in cell-free extracts of *recB2109* and *recB2152* (data not shown).

The ability of class II and III mutants, but not class I mutants, to block plaque formation by λ Red$^-$Gam$^-$ mutants suggests that the ds DNA exonuclease activity of RecBCD enzyme is required to block rolling-circle replication of λ. Plaque formation by λ Red$^-$Gam$^-$ mutants requires either RecA-RecBCD-promoted recombination or rolling circle replication (see SMITH 1983). Class I, II and III *recBCD* mutants are deficient for λ Red$^-$Gam$^-$ recombination (Table 6); plaque formation on these mutants, therefore, reflects rolling-circle replication. Our observations with these mutants support the suggestion that RecBCD enzyme's nuclease activity blocks λ rolling-circle replication (ENQUIST and SKALKA 1973). As expected from these conclusions, λ Red$^-$Gam$^-$ formed large plaques on a *recA56 recB2109* (class I) mutant (data not shown).

It is not clear why RecBCD enzyme's unwinding activity can block T4 *2*$^-$ growth but not λ Red$^-$Gam$^-$ growth. We found only low or undetectable levels of intracellular T4 *2*$^-$ DNA degradation or ds DNA exonuclease activity in extracts of two class II-like mutants (*rec-2123* and *rec-2148*) that blocked plaque formation by λ Red$^-$Gam$^-$ phage (Tables 4, 7 and 8). The basis of the block of λ Red$^-$Gam$^-$ phage growth is not known for these mutants. One or more of the mutants' enzymatic activities may be unstable or altered. For example, *rec-2148* had slight Chi activity in λ vegetative crosses (Table 6), but cell-free extracts failed to detectably cut at Chi (Figure 2). The RecBCD enzymes of these mutants might also unwind DNA in a qualitatively different manner that is able to block both T4 *2*$^-$ and λ Red$^-$Gam$^-$ phage growth. The mutants' RecBCD ds DNA exonuclease activity might degrade DNA to a form that is not acid-soluble and therefore undetectable in either of the assays used in this study.

**Comparison with other *recBCD* mutants:** The properties of the *recBCD* mutants described here offer further insights into previously described *recBCD* mutants.

The *recBCD*‡ mutants lack the *recD* subunit or have an altered *recC* subunit that may not properly interact with the *recD* subunit (AMUNDSEN *et al.* 1986). The ‡ mutants are Rec$^+$ but have no detectable Chi activity or nuclease activity (CHAUDHURY and SMITH 1984a). Since *recBCD* deletion mutants are Rec$^-$ (CHAUDHURY and SMITH 1984b), the ‡ mutants must retain some aspect of RecBCD enzyme activity. CHAUDHURY and SMITH (1984a) suggested that the remaining activity was the DNA unwinding activity. Direct assays for this activity in cell-free preparations have, however, given conflicting results (S. K. AMUNDSEN, unpublished results; A. F. TAYLOR and S. D. KOWALCZYKOWSKI personal communications; PALAS and KUSHNER 1990). A comparison of the properties of class I and ‡ mutants suggest that ‡ mutants lack intracellular DNA unwinding activity. Extracts of class I mutants have DNA unwinding activity (Figure 2) yet the cells are Rec$^-$ and block T4 *2*$^-$. The ‡ mutants fail to block T4 *2*$^-$ growth and cell extracts of them fail to unwind DNA (Tables 4–6 and 11; CHAUDHURY and SMITH 1984a). If ‡ mutants had RecBCD enzyme unwinding activity, we would expect T4 *2*$^-$ growth to be blocked in the ‡ mutants. We infer, therefore, that the ‡ mutants do not unwind DNA inside the cell in a manner qualitatively similar to that in *recBCD*$^+$ cells. The enzymatic activity retained by ‡ mutants is unknown, as is the mechanism by which the ‡ mutant enzyme promotes recombination.

Class II mutants resemble certain *recC** mutants described by SCHULTZ, TAYLOR and SMITH (1983). The *recC** mutants *recC1002*, *recC1003* and *recC1004* lack Chi genetic activity and Chi cutting activity but retain the DNA unwinding and ds DNA exonuclease activities (SCHULTZ, TAYLOR and SMITH 1983; PONTICELLI *et al.* 1985; A. F. TAYLOR and N. H. MCKITTRICK, personal communication). These mutants have reduced recombination proficiency (3–5-fold reduction in λ Red$^-$Gam$^-$ crosses and 5–15-fold reduction in Hfr conjugations); *recBC* null mutants manifested 30 and 300-fold reductions, respectively, in comparable crosses (SCHULTZ, TAYLOR and SMITH 1983). λ Red$^-$Gam$^-$ $\chi^0$ or $\chi^+$ phages form small to very tiny plaques on these *recC** mutants, as they do on the class II mutants. The properties of these *recC** mutants and the class II mutants are readily accounted for by their lack of cutting at Chi sites and the assumption that the mutants have residual but variable abilities to cut at Chi-like sites.

*recBCD* mutants of the Tex class were isolated on the basis of their increased frequency of precise excision of transposon Tn*10* (LUNDBLAD *et al.* 1984). Two of the mutants described here–*rec-2148* and *rec-B2150*–and the *recC** mutants mentioned above also have the Tex phenotype (Table 9; SCHULTZ, TAYLOR and SMITH 1983). One suggested explanation for the Tex phenotype is that during DNA unwinding by RecBCD enzyme, the inverted repeats at the ends of Tn*10* anneal to form a cruciform (LUNDBLAD *et al.* 1984). RecBCD enzyme or another nuclease might cleave the cruciform to excise the transposon, or replication might jump over the cruciform "stem" to produce a daughter strand devoid of the transposon. The Tex phenotype might result from decreased nucleolytic destruction of the cruciform by the altered RecBCD enzyme, thereby allowing another activity to

produce DNA devoid of the transposon. This suggestion is weakened by the lack of the Tex phenotype in class I mutants, which unwind DNA but lack nuclease activity. We found that the one ‡ mutant tested, *recD1009*, did not have the Tex phenotype (Table 9). The molecular basis of the Tex phenotype remains unknown.

The results presented here suggest that DNA unwinding by RecBCD enzyme is not sufficient to promote recombination and that the cutting of DNA at Chi sites is required for recombination proficiency in *recBCD*$^+$ cells. In addition, RecBCD enzyme may have another, yet unidentified, activity that plays a role in recombination. Alternatively qualitative differences between the wild-type and mutant enzymes studied here may account for the recombination deficiencies. Further dissection of the structural and functional complexities of RecBCD enzyme may identify the role of other RecBCD enzymatic activities in recombination and recovery from DNA damage. Precise mapping of the amino acid changes in the mutants reported here as well as additional mutations derived by chemical or site-directed mutagenesis may be useful in this analysis.

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