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Salmonella recD Mutations Increase Recombination in a Short Sequence Transduction Assay

LYNN MIESEL* AND JOHN R. ROTH

Department of Biology, University of Utah, Salt Lake City, Utah 84112

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We have identified *recD* mutants of *Salmonella typhimurium* by their ability to support growth of phage P22 *abc* (anti-RecBCD) mutants, whose growth is prevented by normal host RecBCD function. As in *Escherichia coli*, the *recD* gene of *S. typhimurium* lies between the *recB* and *argA* genes at min 61 of the genetic map. Plasmids carrying the *Salmonella recBCD*$^+$ genes restore ATP-dependent exonuclease V activity to an *E. coli recBCD* deletion mutant. The new *Salmonella recD* mutations (placed on this plasmid) eliminate the exonuclease activity and enable the plasmid-bearing *E. coli* deletion mutant to support growth of phage T4 gene *2* mutants. The *Salmonella recD* mutations caused a 3- to 61-fold increase in the ability of a recipient strain to inherit (by transduction) a large inserted element (MudA prophage; 38 kb). In this cross, recombination events must occur in the short (3-kb) sequences that flank the element in the 44-kb transduced fragment. The effect of the *recD* mutation depends on the nature of the flanking sequences and is likely to be greatest when those sequences lack a Chi site. The *recD* mutation appears to minimize fragment degradation and/or cause RecBC-dependent recombination events to occur closer to the ends of the transduced fragment. The effect of a recipient *recD* mutation was eliminated if the donor P22 phage expressed its Abc (anti-RecBC) function. We hypothesize that in standard (high multiplicity of infection) P22-mediated transduction crosses, recombination is stimulated both by Chi sequences (when present in the transduced fragment) and by the phage-encoded Abc protein which inhibits the host RecBCD exonuclease.

The *recB*, *recC*, and *recD* genes of *Escherichia coli* and *Salmonella typhimurium* encode subunits of exonuclease V and provide a major bacterial recombination function (1, 4, 23, 25, 26, 33, 39, 61). This enzyme also contributes to maintenance of cell viability and DNA repair. The RecBCD enzyme has several in vitro activities: a highly processive ATP-dependent double-stranded exonuclease and helicase, an ATP-dependent single-stranded exonuclease, and an ATP-stimulated endonuclease. The enzyme recognizes a specific DNA base sequence (Chi) and responds by mediating exchanges nearby (32, 58; reviewed in references 29, 49, and 53).

E. coli strains that carry a *recB* or *recC* null mutation are deficient for all of the identified RecBCD enzymatic activities and display multiple phenotypic defects (30, 31, 33, 57). Because of their deficiency in DNA damage repair, *recBC* mutants are sensitive to mitomycin and UV light; they also show reduced cell viability and reduced transductional and conjugational recombination (12, 23). *E. coli* strains that carry *recD* mutations lack all exonuclease activity but retain the ATP-dependent helicase (1, 9, 29, 41, 43). These mutants show only a slight elevation of recombination ability (9, 34). Although *recD* mutants are recombination proficient, the recombination that they perform is independent of Chi sites, and the exchanges are localized near double-stranded ends (9, 55, 56).

A model which integrates the biochemical and genetic properties of the RecBCD enzyme suggests that this enzyme promotes DNA strand exchange (15, 50). (Figure 1 shows this model applied to transductional recombination.) The enzyme enters duplex DNA at a double-strand break (52, 54). Driven by ATP hydrolysis, RecBCD moves through DNA unwinding and cutting to yield oligonucleotides (26). When the enzyme encounters a properly oriented Chi site (5′-GCTGGTGG-3′), it responds by losing its exonuclease activity (13, 15, 51). After encountering Chi, the enzyme continues to unwind DNA, producing a single-stranded end that can serve as a substrate for RecA-mediated strand exchange (15, 45, 50; for reviews, see references 29 and 45.) According to this model, the RecBCD enzyme must interact with Chi and turn off its antirecombinagenic exonuclease activity before recombination can occur (15, 45, 51).

It has been proposed that *recD* mutations increase the recombination frequency by inactivating the RecBCD exonuclease, thereby preserving the recombination substrate (45, 51). However, a *recD* mutation confers only a twofold stimulation of recombination when its effect is scored by conjugal or standard transduction crosses (34). We propose that this effect is small because in standard conjugal and transduction assays, the recombination substrate (the DNA sequence flanking the selected marker) is long. For P22-mediated transduction, about 22 kb flank each side of the selected base pair; only a small piece of DNA needs to be incorporated into the recipient chromosome. (Studies of Ebel-Tsipis et al. [19] have shown that only 20 to 25% of a transduced DNA fragment becomes integrated as double-stranded DNA into the recipient chromosome [Fig. 1].) The length of the flanking sequences ensures that RecBCD-mediated degradation does not limit recombinant formation because much of the DNA is dispensable and multiple Chi sites may be included. Chi sites are present in *E. coli* DNA at an average frequency of one site per 4 kb (5).

We describe *recD* mutants in *S. typhimurium* and a new transductional recombination assay that is more sensitive to the RecBCD exonuclease. The assay tests for a recipient strain's ability to inherit a large inserted element (MudA; Ap^r) by P22-mediated transduction. MudA inheritance requires that the transduced fragment (44 kb) include the entire element (38 kb). To incorporate the element into the recipient chromosome, recombination must occur within the short sequences (average of 3 kb) that flank each side of the element. We find

* Corresponding author. Phone: (801) 581-3618. Fax: (801) 585-6207.

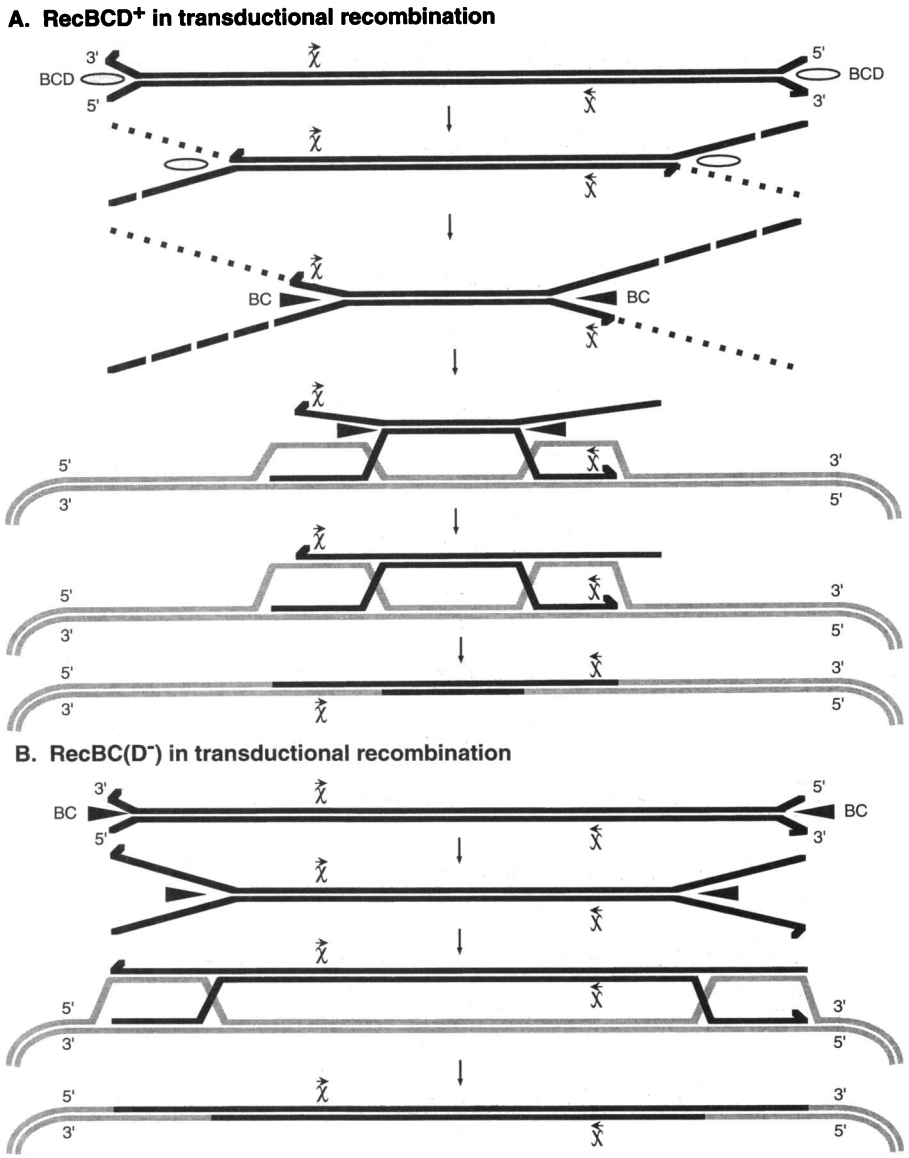

FIG. 1. Model for RecBCD in transductional recombination. (This model is derived from references 15, 16, 19, 45, and 51). (A) RecBCD$^+$ recombination. The RecBCD enzyme (designated by an oval) enters the transduced DNA fragment (heavy black line) and moves through it unwinding and degrading the DNA. (RecBCD degrades the 3′ strand more extensively than the 5′ strand [15, 16].) Encounter with a Chi stimulatory sequence (designated χ) turns off the enzyme's exonuclease activity. (The exonuclease-deficient enzyme is designated by a triangle.) The enzyme continues unwinding the DNA, making a single-stranded end that can efficiently synapse with homologous sequences in the recipient chromosome (gray lines). Subsequent resolution events incorporate the double-stranded transduced DNA into the recipient chromosome by an unknown mechanism. Because of RecBCD degradation, only a small piece of the transduced fragment is incorporated into the recipient chromosome. (B) RecBC$^+$ RecD$^-$ recombination. In *recD* mutants, the RecBC enzyme has no nuclease activity; it unwinds the transduced DNA without degradation and immediately stimulates exchanges near the ends of the transduced fragment. Most of the transduced fragment is incorporated into the recipient chromosome.

that recipient strains with a *recD* mutation have a greatly enhanced ability to inherit the MudA element. Our results are consistent with the idea that the RecBCD exonuclease degrades the ends of transduced fragments and this activity significantly limits inheritance of a large sequence element which must remain intact to be inherited. We suggest that P22 transductional recombination events are stimulated by Chi sequences in the donor fragment which stop fragment degradation and by the phage P22 Abc (anti-RecBCD) function which inhibits the host exonuclease. The importance of Chi sequences to P1 transduction has been demonstrated previously (17). Our results are consistent with the idea that the RecBCD exonuclease activity is antirecombinagenic.

MATERIALS AND METHODS

Bacterial and phage strains. Strains of bacteria and phage are listed in Table 1. Plasmids and their sources are listed in Table 2. Tn*10*dCm and Tn*10*dTc are transposition-defective derivatives of Tn*10*. Tn*10*dTc refers to the Tn*10* Δ16 Δ17 Tc^r

TABLE 1. Bacterial and phage strains used

Strain	Relevant characteristic or genotype	Source (reference)
Bacterial strains[a]		
LT2		Laboratory collection
TT146	*argA1832*::Tn*10*	Laboratory collection
TT7692	*hisD9953*::MudA	Laboratory collection
TT7701	*hisH9962*::MudA	Laboratory collection
TT8371	*thr-469*::MudA	Laboratory collection
TT9521	*pyrE2678*::MudA	Laboratory collection
TT9533	*pyrF2690*::MudA	Laboratory collection
TT10423	LT7 *proAB47*/F'128 *pro*$^+$ *lac*$^+$ *zzf-1831*::Tn*10*dTc	Laboratory collection (22)
TT10427	LT2/pNK972 (Tn*10* transposase)	Laboratory collection (3, 22)
TT10508	*cysA1585*::MudA	Laboratory collection
TT10604	LT7 *proAB47*/F'128 *pro*$^+$ *lac*$^+$ *zzf-1836*::Tn*10*dCm	Laboratory collection (22)
TT11587	*argA1936*::Tn*10*dCm	Laboratory collection
TT13229	*recB497*::MudJ	Laboratory collection (35)
TT13857	*hsdL6 hsdA29 galE496 metA22 metE551 ilv-452 rpsL120 xyl-404* H1-b *nml* H2-*enx* (Fels2)$^-$/pCDK30	Laboratory collection (18)
TT16812	*recD541*::Tn*10*dCm	This study
TT16813	*recD542*::Tn*10*dCm	This study
TT16814	*recD543*::Tn*10*dTc	This study
TT17223	*recB546*::Tn*10*dCm	This study
TT17224	*recB497*::MudJ *argA1832*::Tn*10*	This study
TT17227	*argA1915*::MudJ/pNHM10	This study
TT17238	*E. coli* Δ232(*thyA-argA*) IN1 (*rrnD-rrnE*)/pNHM10	G. R. Smith (8, 37)
TT17239	*E. coli* Δ232(*thyA-argA*) IN1(*rrnD-rrnE*)/pLM12	This study
TT17240	*E. coli* Δ232(*thyA-argA*) IN1(*rrnD-rrnE*)/pLM10	This study
TT17241	*E. coli* Δ232(*thyA-argA*) IN1(*rrnD-rrnE*)/pLM13	This study
TT17242	*E. coli* Δ232(*thyA-argA*) IN1(*rrnD-rrnE*)/pLM11	This study
TT17384	*recD547*::Tn*10*dTc	This study
TT17385	*recD548*::Tn*10*dTc	This study
TT17386	*recB549*::Tn*10*dTc	This study
TT17387	*recB550*::Tn*10*dTc	This study
TT17388	*recB551*::Tn*10*dTc	This study
TT17389	*recD552*::Tn*10*dCm	This study
TT17390	*recD553*::Tn*10*dCm	This study
TT17496	*hisG618 recD541*::Tn*10*dCm	This study
TT17497	*hisG618* (P22-19)	This study
TT17498	*hisG618 recD541*::Tn*10*dCm (P22-19)	This study
TT17499	*hisG618 cysA1585*::MudA	This study
TT17500	*hisG618 cysA1585*::MudA *recD541*::Tn*10*dCm	This study
TT17501	*hisG618* Δ763[*eut-171**MudA* cysA-1585*]	This study
TT17502	*hisG618* Δ763[*eut-171**MudA* cysA-1585*] *recD541*::Tn*10*dCm	This study
TT17503	*hisG618 cysA1585*::MudA (P22-19)	This study
TT17504	*hisG618 cysA1585*::MudA *recD541*::Tn*10*dCm (P22-19)	This study
TT17505	*hisG618* Δ763[*eut-171**MudA* cysA-1585*] (P22-19)	This study
TT17506	*hisG618* Δ763[*eut-171**MudA* cysA-1585*] *recD541*::Tn*10*dCm (P22-19)	This study
TR7043	*hsdL6 hsdA29 galE496 metA22 metE551 ilv-452 rpsL120 xyl-404* H1-b *nml* H2-*enx* (Fels2)$^-$	B. A. D. Stocker
TR7097	*E. coli* Δ232(*thyA-argA*) IN1(*rrnD-rrnE*)	G. R. Smith (8)
TR7098	*hsdL6 hsdA29 galE496 metA22 metE551 ilv-452 rpsL120 xyl-404* H1-b *nml* H2-*enx* (Fels2)$^-$/pNHM9	This study
TR7099	*hsdL6 hsdA29 galE496 metA22 metE551 ilv-452 rpsL120 xyl-404* H1-b *nml* H2-*enx* (Fels2)$^-$/pAFT13	This study
TR7100	*hsdL6 hsdA29 galE496 metA22 metE551 ilv-452 rpsL120 xyl-404* H1-b *nml* H2-*enx* (Fels2)$^-$/pDWS2	This study
TR7101	*hsdL6 hsdA29 galE496 metA22 metE551 ilv-452 rpsL120 xyl-404* H1-b *nml* H2-*enx* (Fels2)$^-$/pAC5	This study
TR7109	*hisG618*	Laboratory collection (10)
Phages		
P22 105-1	*HT105-1 int-201*	Laboratory collection (47)
P22 H5	*c2*$^-$	Laboratory collection
P22 Δ327	Δ327(*abc1 abc2*) *h21 c1-7*	A. Poteete (24)
P22 13$^-$	Gene *13am c1-7*	S. Casjens
P22-19	*sieA44 Ap68tpfr49*	M. Susskind
P22-31	Wild-type P22	
P22-184	Δ327(*abc1 abc2*) (isogenic to P22-31)	N. Benson (24)
T4 gene 2$^-$	Gene 2$^-$*am* N51	G. R. Smith (40)
T4$^+$	Wild type	G. R. Smith (40)

[a] All bacterial strains are derived from *S. typhimurium* LT2 unless otherwise noted.

TABLE 2. Plasmids used and their sources

Plasmid	Genes and alleles carried[a]	Species origin	Source (reference)
pDWS2	*thyA recBCD argA*	*E. coli*	G. R. Smith (1)
pCDK30	*recBD argA*	*E. coli*	S. R. Kushner (18)
pNHM9	*thyA recBC*	*S. typhimurium*	G. R. Smith (37)
pAFT13	*recD*	*E. coli*	G. R. Smith (1)
pAC5	*thyA recBC recD1001 argA*	*E. coli*	G. R. Smith (1)
pNHM10	*thyA recBCD argA*	*S. typhimurium*	G. R. Smith (37)
pLM10	*thyA recBC recD541*::Tn*10*dCm *argA*	*S. typhimurium*	This study
pLM11	*thyA recBC recD542*::Tn*10*dCm *argA*	*S. typhimurium*	This study
pLM12	*thyA recBC recD543*::Tn*10*dTc *argA*	*S. typhimurium*	This study
pLM13	*thyA recC recB546*::Tn*10*dCm *recD argA*	*S. typhimurium*	This study

[a] All plasmids are pBR322 derivatives except pAFT13, which was derived from pUC18.

element constructed by Way et al. (60), and Tn*10*dCm refers to the Tn*10*dCam element constructed by Elliott and Roth (22). Phage MudA is a transposition-defective derivative of the Mud1 (Ap *lac*) phage which is about 38 kb in length (6, 27).

Media and chemicals. Methods for preparing media have been described elsewhere (14). Minimal medium was E medium (14, 59) and was supplemented with 0.2% glucose. The rich medium was Luria broth (LB) (36). Super broth was LB with a twofold increase yeast extract and tryptone and supplemented with 0.2% glucose, thymine (0.3 mM), and E salts (at the standard concentration for minimal medium). Solid media contained BBL agar (1.5% [wt/vol]). Nutritional supplements to minimal media were added at the final concentrations described previously (14). Antibiotic concentrations were as follows: tetracycline hydrochloride, 16 μg/ml in rich medium and 10 μg/ml in minimal medium; kanamycin sulfate, 50 μg/ml in rich medium and 100 μg/ml in minimal medium; ampicillin, 100 μg/ml in rich liquid medium for plasmid selection and 35 μg/ml in rich medium for chromosomal markers; and chloramphenicol, 20 μg/ml in rich medium and 5 μg/ml in minimal medium. The chromogenic β-galactosidase substrate 5-bromo-4-chloro-3-indolyl-β-D-galactopyranoside (X-Gal; Bachem) was used at a final concentration of 25 μg/ml. All antibiotics and chemicals were purchased from Sigma Chemical Co. unless otherwise noted. The buffer HEPES (*N*-2-hydroxyethylpiperazine-*N*′-2-ethanesulfonic acid) was purchased from Calbiochem.

Transductional methods. Most transduction crosses were mediated by an *int-201* mutant derivative of the high-frequency generalized transducing phage P22 105-1 (47). Recipient cells were grown overnight in rich medium to 2×10^9 to 4×10^9 CFU/ml, and a 0.1-ml aliquot was mixed with 0.1 ml of transducing phage (10^8 to 10^9 PFU/ml); the mixture was spread directly onto solid selective medium. When resistance to kanamycin or chloramphenicol was selected, transduction mixtures were plated on nonselective medium and grown overnight before replica printing to selective medium. Transductant clones were purified and freed of phage by single-colony isolation on green indicator plates (7). Phage sensitivity was tested by cross-streaking cells with the clear-plaque P22 mutant H5.

When transduction frequency was quantitated, repair of the 28-bp *hisG618* deletion (10) (present in all recipients) was used as a control to normalize the quality of donor lysates. Some recipient strains were lysogenic for a P22 phage (P22-19) which is defective in superinfection exclusion (*sieA*) and antigen conversion. Cells with the P22-19 prophage can be superinfected with P22 and transduced, but host killing during transduction is prevented by repression of superinfecting phage genomes. Recipient strains were grown to 4×10^8 cells per ml in LB, and 0.5 ml of this growing culture was combined with 0.5 ml of phage suspension. After phage adsorption at 37°C for 30 min, 0.2-ml aliquots of the transduction mixture were spread onto a selective medium for most crosses. For selecting Ap^r in crosses with MudA donor strains, each cross was repeated five times and transductants were counted after 20 h incubation at 37°C. For selection of His^+ and Cys^+ prototrophs, each cross was repeated three times and transductants were counted following 60 h of incubation at 37°C. Transduction frequencies are expressed as the average number of transductants per PFU of transducing lysate.

Isolation of Tn*10*dTc and Tn*10*dCm insertions near the *argA* gene. The transposition-defective Tn*10* derivatives Tn*10*dTc and Tn*10*dCm were used for localized insertional mutagenesis of the *argA* region. Pools of random Tn*10*dCm or Tn*10*dTc insertion mutants were generated by Tom Doak as described previously (14, 22). The insertions were transposed from an F′ DNA fragment that was P22 transduced into the recipient strain; the donor strain was either TT10604 (for F′128 *zzf-1836*::Tn*10*dCm) or TT10423 (for F′128 *zzf-1831*::Tn*10*dTc). The transduction recipient (strain TT10427) provided transposase from its plasmid, pNK972. Each transductant colony (Cm^r or Tc^r) was formed by an independent transposition event in the recipient chromosome. Approximately 10^5 of these transductants were combined to form a pool that was used as a host to prepare a phage P22-transducing lysate. The Tn*10*dCm insertion pool was used as a donor to transduce chloramphenicol resistance into strain TT146 (*argA*::Tn*10*). Conversely, the lysate from the Tn*10*dTc pool was used to transduce strain TT11587 (*argA*::Tn*10*dCm) to tetracycline resistance. Transductants with an insertion mutation cotransducible with the original *argA* mutation were identified as those that lost the recipient arginine auxotrophy and drug resistance phenotypes when they acquired the donated drug resistance.

UV survival assay. Serial dilutions of overnight cultures were spread on LB plates and irradiated with UV light. After irradiation, plates were covered with aluminum foil and incubated at 37°C for 48 h before counting of surviving colonies.

Transfer of plasmids from *E. coli* to *S. typhimurium*. Plasmids prepared from *E. coli* strains were transferred to a restriction-deficient, modification-proficient *S. typhimurium* strain (TR7043) by electrotransformation using a Bio-Rad Gene Pulser as instructed by the manufacturer for *E. coli* transformation. Plasmids were transferred between *Salmonella* strains by P22-mediated transduction crosses.

Construction of plasmids with *recD* insertions. The *recD* and *recB* insertion mutations were transduced onto a pBR322 plasmid that includes the *Salmonella argA*$^+$-to-*thyA*$^+$ region (plasmid pNHM10 (Ap^r *argA*$^+$ *recBCD*$^+$ *thyA*$^+$; obtained from G. R. Smith). The transductional recipient (TT17227) carried

an *argA*::MudJ (Kn^r Lac$^+$) insertion and plasmid pNHM10. The new *rec*::Tn*10*dTc and *rec*::Tn*10*dCm insertion mutants were the transduction donors, and tetracycline (or chloramphenicol) resistance was selected. Since the *recD* gene is immediately adjacent to the *argA* gene, most transductants that inherited the *rec* mutation in the chromosome lost the recipient *argA*::MudJ insertion (and become phenotypically Kn^s Lac$^-$). Transductants that inherited the *recD*::Tn*10*dTc (or *recD*::Tn*10*dCm) insertion on the plasmid retained the chromosomal *argA*::MudJ (Kn^r Lac$^+$) insertion and are phenotypically Arg$^+$ as a result of the plasmid's *argA* gene. These plasmids were transduced into a wild-type strain, selecting for inheritance of the *bla* (Ap^r) gene of plasmid pBR322. Plasmids with a *rec* insertion mutation conferred an unselected Tc^r or Cm^r phenotype. (The Kn^r phenotype of the *argA*::MudJ element in the donor chromosome was never cotransmitted in these crosses.) Plasmids were maintained in *recA* mutant strains and were introduced by electrotransformation into an *E. coli* deletion mutant lacking the entire *argA-recBCD-thyA* region (TR7097). The resulting *E. coli* strains had phenotypes expected of *recD* or *recB* mutants (see Table 5 and Results).

Preparation of ^{3}H-labeled phage P22 DNA. A 100-ml culture of strain LT2 was grown to 100 Klett units (4×10^8 to 5×10^8 cells per ml) at 37°C in E glucose medium and was then infected with phage P22 (*13am c1-7*) at a multiplicity of infection (MOI) of 5. Infected cells were incubated for 20 min with shaking prior to the addition of 1 mCi of [^{3}H]thymidine (ICN), after which the infection was allowed to proceed for 2 h. Cells were concentrated by centrifugation and lysed with chloroform, and cell debris was removed by centrifugation. Phage particles were concentrated by centrifugation for 1 h at 18,000 rpm in a Sorvall RC-5 centrifuge; phage DNA was purified from the particles with Qiagen X500 columns, using the supplier's protocol for bacteriophage lambda DNA purification (we eliminated the polyethylene glycol precipitation step). The ^{3}H-labeled DNA was diluted to 0.1 nmol of bp per μl and had a specific activity of approximately 60,000 cpm/nmol of bp.

Cell extracts for exonuclease assays. Cells were grown in 500 ml of super broth with ampicillin added to the medium of strains harboring a plasmid. Each culture was grown to stationary phase (5 to 6 h of incubation at 37°C following inoculation with 2 ml of an overnight broth culture). Approximately 3 to 4 g of cells was harvested and suspended at 4 ml/g in buffer A (10 mM HEPES [pH 7.5], 20% sucrose, 0.1 mM dithiothreitol, 0.1 mM EDTA). The cells were frozen and stored at −70°C. Frozen cells were thawed at room temperature and lysed at 4°C or in an ice bath by the following procedure. A 1/10 volume of lysozyme (10 mg/ml) in buffer A was added to thawed cells and gently mixed. After 5 min, EDTA (5 mM), dithiothreitol (1 mM), and NH_4Cl (200 mM) were added sequentially to generate the final concentrations indicated in parentheses. After 25 min of gentle mixing, an equal volume of 1% Brij 58 in buffer A was added. After gentle mixing for 30 min, the extract was sonicated with three 2-min bursts. Cell debris was removed by centrifugation (123,400 × *g* for 90 min at 4°C). The extracts were stored at 4°C, and the exonuclease activity was assayed within 5 days after preparation. (The protocol for cell extract preparation was provided by Richard S. Myers.)

Assay of ATP-dependent double-stranded exonuclease. The ATP-dependent exonuclease assay is derived from that of Eichler and Lehman (20). The reaction mixture (0.3 ml) contained HEPES (pH 7.5; 50 mM), dithiothreitol (1 mM), albumin (1 mg/ml), $MgCl_2$ (10 mM), KCl (100 mM), glycerol (10% [vol/vol]), ATP (500 μM), and 10 μl of cell extract. The reaction was started with the addition of DNA substrate and 1 nmol of bp of ^{3}H bacteriophage P22 DNA (specific activity, 60,000 cpm/nmol of bp) and was allowed to proceed for 20 min at 37°C. The reaction was stopped by the addition of 0.2 ml of calf thymus DNA (0.1-mg/ml stock solution) and chilling on ice for 10 min. To precipitate large DNA pieces, 0.3 ml of 15% trichloroacetic acid was added, and the mixture was chilled on ice for at least 15 min. Material insoluble in trichloroacetic acid was removed by centrifugation (14,000 rpm, 15 min at room temperature in an Eppendorf model 5415 centrifuge). The radioactivity of 0.3 ml of supernatant was determined by mixing with 5 ml of Beckman Ready Safe scintillation fluid and counting with a Beckman model LS1800 scintillation counter. (The protocol for the exonuclease assay was provided by Richard S. Myers.)

Total protein concentration of cell extracts was determined with the Coomassie protein assay reagent from Pierce Chemical Co.

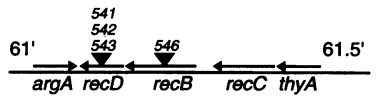

FIG. 2. The *Salmonella* genetic map of the *recB*, *recC*, and *recD* genes which are located at min 61 (references 35 and 48 and this study). Triangles and allele numbers indicate the new insertion mutations described in this study.

RESULTS

Isolation of Tn*10*dTc and Tn*10*dCm insertions near the *argA* gene. From genetic mapping in *E. coli* (1, 4, 41) and sequence data for *Salmonella* sp. (48), the *Salmonella recD* gene of is known to be adjacent to the *argA* gene (Fig. 2). To isolate a *recD* mutation, we first isolated mutants with insertions near the *argA* gene and then screened these mutants for a *recD* defect.

Transducing phage stocks were prepared on a large pool of random Tn*10*dTc insertions and a second pool of Tn*10*dCm insertions. These phage preparations were used to transduce an *argA* recipient to Tc^r or Cm^r (see Materials and Methods), and transductants which had coinherited the donor Arg$^+$ phenotype were saved. These transductants must each carry an insertion (from the pool) that lies near the *argA* gene. These *argA*-linked insertion mutations were tested for phenotypes expected of a *recD* mutation.

Identification of *recBCD* mutations. Potential *recB*, *recC*, and *recD* mutants were identified by their ability to support growth of phage P22 *abc* mutants. The bacteriophage P22 Abc protein inhibits the RecBCD exonuclease activity, thereby protecting the P22 genome from degradation (42). The phage P22 *abc* mutant forms pinpoint plaques on a *recBCD*$^+$ host but forms large plaques on a *recBC* mutant host (which lacks exonuclease) (24). Since *recD* mutants are also deficient in exonuclease activity, we expected that they would support improved growth of phage P22 *abc* mutants. A *recB* or *recC* mutant is expected to be UV sensitive (23), while the *recD* mutants described in *E. coli* (previously called *recB*‡ mutants) are UV resistant (1, 4, 9). Initially *recD* mutants were identified as those which supported growth of P22 *abc* mutants and were resistant to UV irradiation.

Of 291 *argA*-linked Tn*10*dTc insertions, 3 had a RecBC phenotype (large plaques of P22 Abc$^-$, UV sensitivity; *recB549*, *-550*, and *-551*) and 4 had a RecD phenotype (large plaques of P22 Abc, UV resistance; *recD543*, *-544*, *-547*, and

TABLE 3. Three-factor cross: relative order of *Salmonella recB-recD-argA* genes

Selected marker (no. tested)	Donor[a]	*recB*$^+$ (Kn^s)	*recD*::Tn*10*dCm	*argA*$^+$ (Tc^s)		No. with indicated phenotype	Inferred recombination events[b]
	Recombination site	I	II	III	IV		
	Recipient[a]	*recB*::MudJ (Kn^r)	*recD*$^+$ (Cm^s)	*argA*::Tn*10* (Tc^r)			
		Transductant phenotype[b]					
Arg$^+$ (500)		Kn^s	Cm^r	Tc^s		367	I, IV
		Kn^r	Cm^r	Tc^s		112	II, IV
		Kn^r	Cm^s	Tc^s		17	III, IV
		Kn^s	Cm^s	Tc^s		4	I, II and III, IV
Cm^r (100)		Kn^s	Cm^r	Tc^s		68	I, IV
		Kn^r	Cm^r	Tc^r		7	II, III
		Kn^r	Cm^r	Tc^s		24	II, IV
		Kn^s	Cm^r	Tc^r		1	I, III

[a] The donor was TT16813 (*recD542*::Tn*10*dCm), and the recipient was TT17224 (*recB497*::MudJ *argA1832*::Tn*10*).
[b] Recombination events are inferred to have occurred within the indicated regions (I, II, III, and IV) to give the recombinants of each phenotypic class.

-548). Of 294 Tn*10*dCm insertions in the *argA* region, 1 had the RecBC phenotype (*recB546*) and 5 had the phenotype expected for *recD* mutants (*recD541*, *-542*, *-545*, *-552*, and *553*). One of the putative *recB* mutants (*recB546*) and three of the *recD* mutants (*recD541*, *-542*, and *-543*) were characterized further.

Genetic mapping of *recD* insertions with respect to *argA* and *recB*. The relative gene order for the *recBCD* region of both *E. coli* and *S. typhimurium* is shown in Fig. 2 (1, 4, 35, 48, 61). Two- and three-factor transduction crosses showed that the insertions with the RecD phenotype are located between *argA* and *recB* mutations and are very closely linked to the *argA* locus as would be expected for *recD* mutations (Table 3). (Data for two-factor crosses are not shown.)

Complementation of *recD* insertion mutations with plasmids. Well-characterized plasmids carrying the *recBCD* genes from *E. coli* or *S. typhimurium* were introduced into the new *Salmonella* mutants to test for complementation (see Materials and Methods). (Plasmids were obtained from G. R. Smith and S. R. Kushner.) The plasmid-bearing strains were tested for the ability to support growth of P22 *abc* mutants (Table 4). The three putative *recD* mutants (TT16812, TT16813, and TT16814) are complemented by plasmids that contain a functional *recD* gene of *E. coli* but are not complemented by a plasmid with a mutant *recD* allele. A *Salmonella* strain carrying the *recB497*::MudJ insertion (TT13229) is complemented by a plasmid containing the *recB* and *recD* genes but not by a plasmid which contains the *recB* and *recC* genes. These data suggest that the *recB497*::MudJ mutation causes both a RecB and a RecD defect due to a polar effect on expression of the downstream *recD* gene. The *recB546*::Tn*10*dCm insertion mutant is complemented by a plasmid carrying the *recBC* genes, suggesting that this insertion does not eliminate *recD* gene expression.

***Salmonella recD* mutations show standard *recD* phenotypes when tested in *E. coli*.** The phenotypes of the new *Salmonella* mutations were tested in *E. coli* to permit comparison with the original *E. coli* mutants isolated in that organism. The *Salmonella recD* mutations described above were moved onto plasmid pNHM10, which carries the entire *Salmonella argA-recBCD-thyA* region, and were then transferred to an *E. coli recBCD* deletion mutant (TR7097) (37). *E. coli* strains carrying the *Salmonella recBCD* genes and mutations (TT17238 to TT17242) were tested for UV sensitivity and the ability to support growth of phage T4 gene *2* mutants. This mutant T4 phage lacks a function that protects its genome from the RecBCD nuclease, so it cannot form a plaque on a *recBCD*$^+$ host but can grow on *recBC* and *recD* mutants (8, 9, 40). The plasmid-bearing strains were also tested for RecBCD exonuclease activity in cell extracts (Materials and Methods). Strains of *E. coli* with *recB*, *recC*, or *recD* mutations are defective for the ATP-dependent double-stranded exonuclease activity present in wild-type cells (1, 2).

As shown in Table 5, an *E. coli recBCD* deletion strain with the plasmid-borne *Salmonella* wild-type *recBCD* region is UV^r and does not support growth of a T4 gene *2* mutant. The new *Salmonella recB* and *recD* mutations allow growth of a T4 gene *2* mutant. The new *recB* mutation causes UV sensitivity, whereas the new *recD* mutations do not.

Table 5 shows the ATP-dependent nuclease activities of extracts prepared from the *E. coli* deletion mutant with plasmids carrying the *Salmonella recBCD* genes. Extracts pre-

TABLE 4. Complementation of *rec* mutants with recombinant plasmids

Salmonella recipient	Inferred phenotype	Plaques formed by P22 Abc$^-$ on *Salmonella* strains carrying *E. coli* plasmids[a]					
		No plasmid	pCDK30 (*recBD*$^+$)	pNHM9 (*recBC*$^+$)	pAFT13 (*recD*$^+$)	pDWS2 (*recBCD*$^+$)	pAC5 (*recBC*$^+$ *recD*)
LT2 (Rec$^+$)	B$^+$ C$^+$ D$^+$	Tiny	None	Tiny	None	Not tested	Not tested
TT13229 (*recB497*::MudJ)	B$^-$ C$^+$ D$^-$	Large	Tiny	Large	Large	Not tested	Not tested
TT17223 (*recB546*::Tn*10*dCm)	B$^-$ C$^+$ D$^+$	Large	Tiny	Tiny	Large	Tiny	Tiny
TT16812 (*recD541*::Tn*10*dCm)	B$^+$ C$^+$ D$^-$	Large	Tiny	Large	Tiny	Tiny	Large
TT16813 (*recD542*::Tn*10*dCm)	B$^+$ C$^+$ D$^-$	Large	Tiny	Large	Tiny	Tiny	Large
TT16814 (*recD543*::Tn*10*dTc)	B$^+$ C$^+$ D$^-$	Large	Tiny	Large	Tiny	Tiny	Large

[a] This phage (P22 Δ327) is described in Table 1. The isogenic phage strain, P22 *c1-7* (Abc$^+$), formed large plaques on all hosts listed (data not shown). Plasmids used are described in Table 2.

TABLE 5. Phenotypes of an *E. coli recBCD* deletion mutant with *S. typhimurium recBCD* genes and insertion mutations

Strain	Relevant genotype[a]	Phage T4 plaque		UV sensitivity	Nuclease activity[b]	
		Gene 2^+	Gene 2^-		−ATP	+ATP
TR7097	Δ232(*thyA-recBCD-argA*)	+	+	Sensitive	37	11
TT17238	Δ232(*thyA-recBCD-argA*)/pNHM10 (*recBCD*$^+$)	+	−	Resistant	26	510
TT17241	Δ232(*thyA-recBCD-argA*)/pLM13 (*recB546*::Tn*10*dCm)	+	+	Sensitive	44	11
TT17239	Δ232(*thyA-recBCD-argA*)/pLM12 (*recD543*::Tn*10*dTc)	+	+	Resistant	39	16
TT17240	Δ232(*thyA-recBCD-argA*)/pLM10 (*recD541*::Tn*10*dCm)	+	+	Resistant	36	13
TT17242	Δ232(*thyA-recBCD-argA*)/pLM11 (*recD542*::Tn*10*dCm)	+	+	Resistant	35	12

[a] All strains are derivatives of *E. coli* TR7097, and the plasmids are derived from pNHM10, which carries *Salmonella argA*$^+$, *recB*$^+$, *recC*$^+$, *recD*$^+$ and *thyA*$^+$ genes. Individual *Salmonella rec* mutations were crossed onto pNHM10 as described in Materials and Methods. Complete genotypes of the plasmids are given in Table 2.

[b] Assayed in cell extracts and expressed as picomoles of trichloroacetic acid-soluble [^{3}H]DNA fragments released per milligram of protein. Assay conditions are described in Materials and Methods.

pared from both the *recD* and the *recB* mutants lack the ATP-dependent exonuclease activity which is present in extracts of a *recBCD*$^+$ strain (Table 5).

Effects of *recD* mutations on transductional recombination in *S. typhimurium*. Four types of P22 transduction events were assayed: (i) inheritance of sequences that repair a 28-bp recipient *his* deletion, (ii) inheritance of a 38-kb donor insertion (MudA), (iii) inheritance of sequences that repair a recipient insertion mutation, and (iv) inheritance of sequences that repair a large recipient deletion.

(i) Transductional repair of a 28-bp deletion and inheritance of a 38-kb donor insertion. These experiments test for the inheritance of *his*$^+$ sequences that repair a 28-bp deletion mutation (*hisG618*) and the inheritance of the MudA (Ap^r) element (38 kb) inserted at several points in the donor chromosome (Fig. 3). To repair the *his* deletion, only 28 bp of DNA need to be incorporated into the recipient chromosome. The fragments transduced by phage P22 are 44 kb in length. Thus, the required exchanges can occur anywhere within approximately 22 kb of DNA that flank the selected 28 bp (Fig. 3A).

The MudA element is about 38 kb in length and shares no sequence homology with the recipient chromosome (6). Successful transductional inheritance of this element requires transmission of a donor fragment that includes the entire element. In the recipient, genetic exchanges must occur within the approximately 3 kb of flanking DNA at the ends of the transduced fragment (Fig. 3B). Since very short DNA sequences flank the MudA element in the transduced fragment, we expected that DNA degradation by RecBCD exonuclease might reduce MudA inheritance more than it limits repair of the small *his* deletion.

We have used MudA elements at several chromosome positions to test the effects of different flanking sequences. Each transduction donor carries a MudA insertion in an otherwise wild-type chromosome. For each donor strain, we individually tested the ability of a single lysate to transmit the 38-kb MudA element (Ap^r) and a His$^+$ phenotype to isogenic *recD* and *recD*$^+$ recipients (TR7109 and TT17496; Table 6). The Ap^r (MudA) crosses (approximate MOI of 1) were performed with greater phage multiplicities than the His$^+$ crosses (approximate MOI of 0.01) because the large element is infrequently transmitted by a single transduced fragment (28).

In the initial crosses, a recipient *recD* mutation caused only a modest increase in inheritance of both His$^+$ and MudA (Ap^r) markers (Table 6). The *recD* mutant recipient typically inherited the His$^+$ phenotype about twofold more frequently than did the isogenic *recD*$^+$ recipient (Table 6). Inheritance of a MudA element was also stimulated rather little in a *recD* mutant (for each of the insertion positions tested) (Table 6).

(ii) Evidence that phage functions prevent the effect of a *recD* mutation. The modest stimulation of transduction caused by a *recD* mutation could be to due to the phage P22 Abc

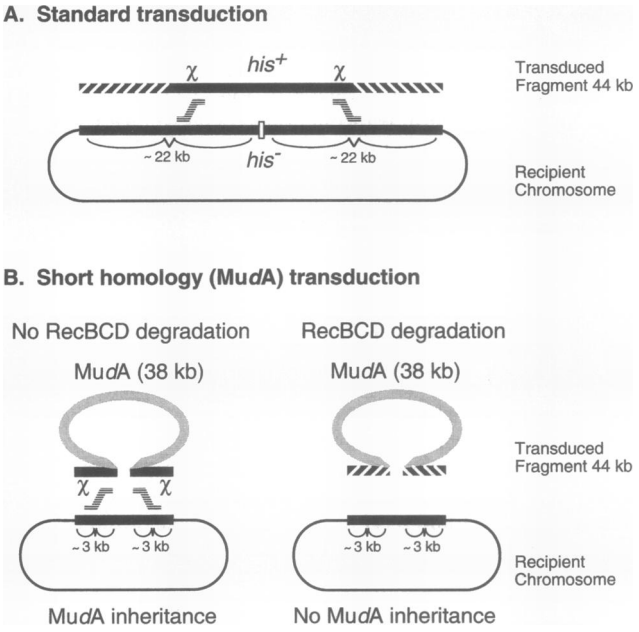

FIG. 3. His$^+$ and MudA (Ap^r) transductional recombination assays. Heavy black lines denote sequences of identity between the transduced fragment and the recipient chromosome. Degradation of the transduced fragment is indicated by white slashes. (A) Standard P22 transduction assay. Inheritance of a *his*$^+$ gene is selected. The entire transduced fragment (44 kb) is homologous to the recipient chromosome, and recombination events can occur at any site flanking the *his*$^+$ gene (about 22 kb). Degradation by the RecBCD enzyme does not limit incorporation of a *his*$^+$ gene, because much of the fragment is dispensable and Chi sites (χ) protect the fragment from RecBCD degradation. (B) Short homology transduction assay. Inheritance of the large inserted element MudA (Ap^r; denoted by the gray loop) is selected. The MudA element occupies 38 kb of the transduced fragment, so the flanking sequences (homologous to the recipient chromosome) are at the ends of the transduced fragment and are approximately 3 kb. Inactivation of the RecBCD exonuclease allows more efficient incorporation of these elements (left). The RecBCD exonuclease can rapidly degrade the homologous sequences and prevent incorporation of these elements (right).

TABLE 6. Effects of a recipient *recD* mutation on MudA (Ap^r) and His$^+$ transduction frequencies in nonlysogenic recipient strains[a]

Donor (Mud location [mm])	Inheritance of *his*$^+$ gene			Inheritance of MudA		
	No. His$^+$ (10^4)[b]		Fold stimulation by *recD* mutation	No. Ap^r (10^7)[b]		Fold stimulation by *recD* mutation
	recD$^+$ recipient	*recD* recipient		*recD*$^+$ recipient	*recD* recipient	
thr (0)	0.45	0.83	1.9	2.7 (70)	7.6	2.8
pyrF (33)	0.59	0.85	1.4	9.4	16.0	1.7
hisD (42)	0.39	0.73	1.9	1.5 (60)	5.3 (40)	3.4
hisH (42)	0.28	0.41	1.5	3.6 (30)	6.8	1.9
cysA (50)	1.2	2.3	2.0	1.1	1.7	1.6
pyrE (79)	0.39	0.62	1.6	2.4	3.1	1.3

[a] The recipients in these crosses are TR7109 (RecD$^+$) and TT17496 (RecD$^-$). These strains are His$^-$ Ap^s; all genotypes are listed in Table 1. The crosses are described in Results and were performed as described in Materials and Methods. For His$^+$ selections, the MOI was between 0.01 and 0.04. For Ap^r selections, the MOI was between 1 and 4.

[b] Number of transductants per PFU. Methods for data analysis are described in Materials and Methods. The standard deviations were 20% or less of the frequency of transductants unless noted by percentages in parentheses.

function which inhibits the RecBCD exonuclease. These crosses were performed with the phage concentrations equal to the cell concentration (approximate MOI of 1), and many transduced cells were infected by an intact phage genome. Each phage genome expresses the Abc protein (an inhibitor of the RecBCD exonuclease) as an early gene product. If the RecBCD exonuclease were inhibited in all crosses, the *recD* mutant and *recD*$^+$ strains would not be expected to show distinguishable phenotypes. The *recD*$^+$ phenotype should become distinguishable if one eliminated Abc expression and allowed the RecBCD exonuclease to act. This possibility was supported by use of a lysogenic recipient whose phage repressor would prevent expression of superinfecting phage genes.

In a recipients with a P22 prophage (TT17497 and TT17498), a *recD* mutation caused a substantial stimulation of inheritance of the MudA element (Table 7). The stimulatory effect of a *recD* mutation varied with the particular donor used, but for some MudA (Ap^r) elements, the effect was very large (Table 7). As before, a *recD* mutant typically inherited a His$^+$ phenotype only about twofold more frequently than the isogenic *recD*$^+$ recipient (Table 7).

(iii) Effect of flanking sequence on inheritance of MudA may be due to Chi sequence. The effect of a *recD* mutation on MudA inheritance varies widely (3.7- to 61-fold), depending on the chromosomal position of the MudA element. Thus, the nature of the sequences flanking the MudA element appears to influence the extent to which a recipient *recD* mutation increases MudA inheritance.

Even MudA elements located near each other can show distinct effects of a recipient *recD* mutation. Two of the donor strains used have MudA elements in the *his* operon; one is within the *hisD* gene, and the other is 4 kb away in the *hisH* gene. In lysogenic recipients, inheritance of the *hisH*::MudA element is increased 12-fold by a *recD* mutation; inheritance of the *hisD*::MudA element is increased approximately 4-fold. This result suggests that the DNA sequences which immediately flank the elements influence the effect of a *recD* mutation. Chi sites are a likely factor.

A Chi site is located (within the *hisB* gene) about 2 kb from the *hisD* insertion and is oriented so that it would activate a RecBCD complex entering one end of the fragment which carried the *hisD*::MudA insertion. There is no Chi sequence on the other side of the *hisD*::MudA insertion within the 2 kb of known sequence on that side. The Chi site in the *hisB* gene is improperly oriented to stimulate inheritance of the *hisH* insertion. There is no properly oriented Chi sequence on either side of the *hisH*::MudA element (within 2 kb of known sequence on the right and 5 kb on the left).

(iv) Effect of P22 Abc function on transduction frequency. We hypothesized that the phage P22 Abc function, expressed by infecting phage, might inhibit the RecBCD exonuclease and this might have minimized the difference between the *recD* mutant and *recD*$^+$ recipients in our initial crosses. This possibility was tested by using transducing phage which carried an *abc* mutation. The *abc* mutant phage, P22-184, carries a deletion of the *abc1* and *abc2* genes, Δ327 (38), and is isogenic to the *abc*$^+$ strain, P22-31. (The original P22 Δ327 phage mutant was obtained from Anthony Poteete; the *abc* deletion was crossed into the *c*$^+$ phage strain [P22-31] by Nick Benson. Phages P22-184 and P22-31 lack the high-frequency transduc-

TABLE 7. Effects of a recipient *recD* mutation on MudA (Ap^r) and His$^+$ transduction frequencies in lysogenic recipient strains[a]

Donor (Mud location [min])	Inheritance of *his*$^+$ gene			Inheritance of MudA		
	No. His$^+$ (10^4)[b]		Fold stimulation by *recD* mutation	No. Ap^r (10^7)[b]		Fold stimulation by *recD* mutation
	recD$^+$ recipient	*recD* recipient		*recD*$^+$ recipient	*recD* recipient	
thr (0)	1.8	3.9	2.2	0.64 (40)	19.0	29.0
pyrF (33)	1.7	3.1	1.8	0.6 (50)	37.0	61.0
hisD (42)	1.9	4.0	2.1	2.8 (30)	10.0	3.7
hisH (42)	0.89	1.8	2.0	1.5 (40)	18.0	12.0
cysA (50)	2.6	4.6	1.8	2.7 (40)	48.0	18.0
pyrE (79)	1.8	3.3	1.8	0.98	9.0	9.3

[a] The recipients in these crosses are TT17497 (RecD$^+$) and T17498 (RecD$^-$). Both strains are His$^-$ Ap^s and carry the P22-19 prophage, which represses infecting phage functions. All genotypes are listed in Table 1. For His$^+$ selections, the MOI was between 0.01 and 0.04. For Ap^r selections, the MOI was between 1 and 4.

[b] Number of transductants per PFU. Methods for data analysis are described in Materials and Methods. The standard deviations were 20% or less of the frequency of transductants unless noted by percentages in parentheses.

TABLE 8. RecD transduction stimulation using P22 Abc$^+$ and Abc$^-$ transducing phage[a]

Donor phage	Recipient strain	Relevant recipient genotype	Inferred Abc phenotype	Inheritance of *his*$^+$ gene		Inheritance of MudA (Ap^r)	
				No. His$^+$ (10^5)[b]	Fold stimulation by *recD* mutation	No. Ap^r (10^8)[b]	Fold stimulation by *recD* mutation
P22-31 (Abc$^+$)	TR7109	*recD*$^+$	Abc$^+$	1.2		2.0	
	TT17496	*recD*	Abc$^+$	1.9	1.6	2.2	1.0
	TT17497	*recD*$^+$ (P22-19)	Abc$^-$ (repressed)	1.8		0.37	
	TT17498	*recD* (P22-19)	Abc$^-$ (repressed)	3.4	1.9	4.7	13.0
P22-184 (Abc$^-$)	TR7109	*recD*$^+$	Abc$^-$ (mutant)	1.0		1.4	
	TT17496	*recD*	Abc$^-$ (mutant)	1.6	1.6	8.7	6.4
	TT17497	*recD*$^+$ (P22-19)	Abc$^-$ (mutant and repressed)	2.8		0.55	
	TT17498	*recD* (P22-19)	Abc$^-$ (mutant and repressed)	4.7	1.7	8.8	16.0

[a] The donor strain is TT9521 (*pyrE2678*::MudA); phage genotypes are given in Table 1. All recipients in these crosses are isogenic and carry the 28-bp *hisG618* deletion; their genotypes are listed in Table 1. Crosses were performed at MOIs of 11 (Abc$^-$) and 13 (Abc$^+$) for Ap^r selections and 0.11 (Abc$^-$) and 0.13 (Abc$^+$) for His$^+$ selections. Methods for quantitative transduction crosses and data analysis are described in Materials and Methods.

[b] Average number of transductants per PFU. The standard deviations were between 2 and 30% of the transduction frequencies.

ing mutation *HT105-1* and the integration mutation, *int-201*, that are present in the standard transducing phage.) Transduction experiments were performed with the *pyrE*::MudA donor strain. Results are shown in Table 8.

When the transducing phage were Abc$^+$, the recipient *recD* mutation had a significant effect on MudA inheritance only in a lysogenic recipient. This is the same result described above for the *HT105-1 int-201* phage. However, with *abc* mutant transducing phage, a recipient *recD* mutation had a significant stimulatory effect in both lysogenic and nonlysogenic recipient strains. These results suggest that the P22 Abc protein (produced in a nonlysogenic recipient) can mask recombination stimulation by a *recD* mutation, presumably by inhibiting the RecBCD exonuclease in *recD*$^+$ strains and mimicking the effect of the *recD* mutation. It should be noted that even with the *abc* deletion mutant as donor phage, a *recD* mutation still has a greater effect in the lysogenic recipients than in the nonlysogenic recipients; this finding suggests that additional phage functions may modulate RecBCD activity.

For His$^+$ selections, the stimulatory effect of the *recD* mutation is similar for Abc$^+$ and Abc$^-$ donor phage (lysogen and nonlysogens alike). This is expected because His$^+$ crosses are performed with a low MOI so the transduced recipient cells are unlikely to be coinfected by a functional phage genome. More importantly, long sequences flank the selected *his*$^+$ information, so many Chi sites may be present and DNA degradation by the RecBCD exonuclease should not limit inheritance of this marker.

(v) Transductional repair of an insertion mutation and a large deletion in the same region. The effects of a *recD* mutation noted above reflect difficulties presented by a long sequence element whose inheritance requires that exchanges occur in the short regions at the two ends of the transduced fragment. We were concerned that this effect was generated by Mu functions that are expressed when MudA enters the recipient cell (which lacks Mu repressor). To eliminate this possibility, we tested repair of a large chromosomal deletion. This cross (Fig. 4) is similar to MudA inheritance because it requires inheritance of a large intact sequence element (the deleted material) which has no homology to the recipient chromosome. We tested the transductional repair of a deletion that extends from *cysA* across the nearby *eut* operon, Δ763 (*eut cys*) (Fig. 4B). (The recipient deletion mutation has a MudA element at the deletion site.) For a comparison, we tested repair of a MudA element inserted in the *cysA* gene (Fig. 4A). To repair the recipient *cysA1585*::MudA mutation, the donor fragment needs only to provide a short sequence to bridge the insertion site; the exchanges can occur anywhere within approximately 22 kb of DNA sequence that flank the insertion site in the *cysA* gene.

The deletion Δ763 was constructed by recombination between the MudA sequences of the *cysA1585*::MudA insertion and the MudA sequences of a nearby *eut-171*::MudA insertion (28, 44). As judged by the low genetic linkage of the *cysA1585*::MudA and *eut-171*::MudA markers (less than 1% cotransduction) and the reduced frequency of Δ763 transductional repair, this deletion removes a large block of DNA sequence (estimated from the linkage results to be greater than 33 kb), but the exact size is unknown (44). The deletion strain is isogenic with the *cysA1585*::MudA insertion mutant, and both strains have a copy of the MudA element at the mutant site.

Prototrophy (Cys$^+$) was selected in both crosses, and the results are shown in Table 9. In a nonlysogenic recipient, a *recD* mutation increases the frequency of Cys$^+$ transductants two- to threefold both for the *cysA1585*::MudA insertion and for the large deletion (Δ763). In lysogens, repair of Δ763 is increased approximately 10-fold by a recipient *recD* mutation. Repair of the *cysA1585*::MudA insertion is stimulated only threefold. Each recipient also carries the 28-bp *hisG* deletion (*hisG618*), and repair of this mutation is increased only twofold in a *recD* mutant recipient, with or without a prophage. These data are consistent with that from the MudA experiments: a *recD* mutation has a greater effect on inheritance of a large block of DNA sequence. This effect is not dependent on inheritance of a MudA prophage and is apparent only when the Abc functions of the P22 phage are eliminated so that the host RecBCD activity is maximal.

DISCUSSION

We describe here the isolation and characterization of insertion mutations in the *recD* gene of *S. typhimurium*. The new *Salmonella* mutations are assigned to the *recD* gene by the

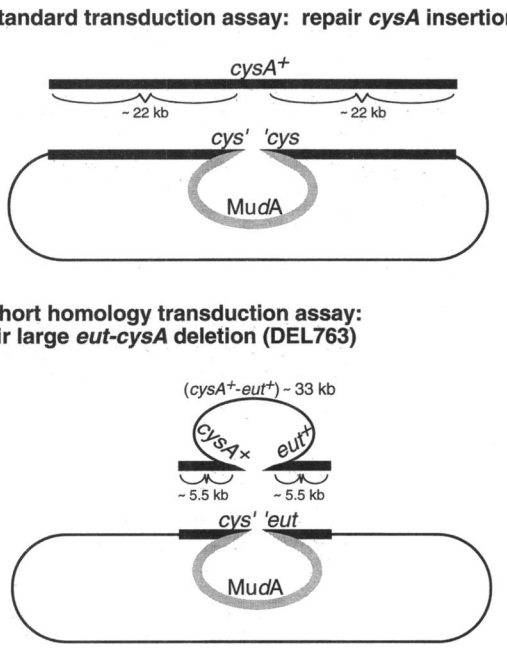

FIG. 4. Standard and short transduction assays that select for inheritance of a cys^+ gene. Heavy black lines denote sequences of identity between the transduced fragment and recipient chromosome. (A) Standard assay: repair of a *cysA* insertion mutation (*cysA1585*::MudA). The transduced fragment provides cys^+ sequences that bridge the MudA insertion site (gray loop); exchanges can occur within about 22 kb of flanking sequence that is homologous to the recipient chromosome. (B) Short homology assay: repair of a large *eut-cys* deletion (Δ763). The recipient chromosome has a large deletion that removes DNA from the *cysA* locus to the *eut* operon (43) (about 33 kb); a MudA element is located at the deletion site (gray loop). The transduced fragment contains all the deleted DNA; recombination must occur within about 5.5 kb of flanking sequence.

following criteria. (i) They allow growth of phage P22 *abc* mutants which grow poorly in $recBCD^+$ *Salmonella* strains. (ii) They do not cause UV sensitivity. (iii) They are complemented by plasmids carrying the *recD* gene of *E. coli*; plasmids carrying a mutant *recD* gene fail to complement. (iv) They are closely linked to the *argA* gene and are located between the *argA* and *recB* genes. (v) When tested in a *recBCD E. coli* mutant, the *Salmonella* mutations allow growth of a phage T4 gene *2* mutant. (vi) The mutations eliminate the ATP-dependent exonuclease activity (assayed in *E. coli*). The absence of RecBCD exonuclease in our new *recD* mutants should greatly facilitate genetic manipulations involving transformation with linear plasmid DNA (46).

In a transduction recipient, the new *recD* mutations stimulate transductional inheritance of his^+ sequences (28 bp) only slightly (about twofold; the standard assay in Fig. 3A), in agreement with the behavior of *E. coli recD* mutants (34). The *Salmonella recD* mutations cause a greater increase in ability of recipient cells to inherit a long block of DNA sequence. We observed this effect by testing recombinational inheritance of transposition-defective MudA elements (38 kb; Fig. 3B) and the repair of a large recipient deletion (about 33 kb; Fig. 4A). Inheritance of long sequence blocks differs from the standard assay in two respects. First, the recombination substrate is short: exchanges must occur within approximately 3 kb of sequence that flank the long sequence block. Second, these crosses require more transducing phage (a higher MOI) because the long sequence blocks are infrequently included within a single transduced fragment. This means that recipient cells are frequently infected with phage genomes which express RecBCD-modifying activities (the Abc system).

Current models for RecBCD activity propose that the enzyme enters the DNA duplex at a double-stranded end (54), degrading until it encounters a Chi site. At Chi, degradation ceases (perhaps as a result of expulsion of the RecD subunit) and the RecBC enzyme proceeds as a helicase to stimulate recombination (15, 16, 45, 51). In the absence of the RecD subunit, the RecBC enzyme does not degrade DNA but enters the double-stranded end and immediately acts like a Chi-stimulated recombinase (51, 56).

Transductional recombination is mediated by the RecBCD enzyme (11, 21), which presumably enters the ends of the transduced DNA and degrades much of the fragment prior to stimulating exchange at Chi (Fig. 1). (This view is supported by the earlier observations of Ebel-Tsipis et al. [19].) The amount of a transduced DNA fragment incorporated into the recipient chromosome would depend on the position of properly oriented Chi sequences within the transduced fragment. In a *recD* mutant, degradation would be minimized so that exchanges would occur near the ends of the transduced fragment: most of the transduced fragment would be incorporated into the recipient chromosome (even if the fragment lacked a Chi

TABLE 9. RecD transduction stimulation: repair of a small *his* deletion, a large *eut-cysA* deletion (Δ763), and a *cysA* insertion[a]

Recipient strain	Relevant recipient genotype	Inheritance of his^+ gene		Inheritance of cys^+ gene	
		No. His^+ (10^4)[b]	Fold stimulation by *recD* mutation	No. Cys^+ (10^6)[b]	Fold stimulation by *recD* mutation
TT17499	*cysA*::MudA $recD^+$	0.37		3.9	
TT17500	*cysA*::MudA *recD*	0.88	2.4	8.4	2.2
TT17501	Δ763[*eut**MudA**cysA*] $recD^+$	0.46		0.22	
TT17502	Δ763[*eut**MudA**cysA*] *recD*	0.74	1.6	0.65	3.0
TT17503	*cysA*::MudA $recD^+$ (P22-19)	1.7		2.1	
TT17504	*cysA*::MudA *recD* (P22-19)	4.0	2.4	6.2	3.0
TT17505	Δ763[*eut**MudA**cysA*] $recD^+$ (P22-19)	1.8		0.70	
TT17506	Δ763[*eut**MudA**cysA*] *recD* (P22-19)	4.2	2.3	6.4	9.6

[a] The transduction assays are described in Results and Fig. 4. The recipient strains are isogenic and carry the 28-bp *hisG618* deletion; the strain genotypes are listed in Table 1. The donor lysate used in these crosses was TT9521. The MOIs used were as follows: Δ763 repair (Cys^+), 1; *cysA1585* repair (Cys^+), 0.1; His^+, 0.01.

[b] Number of transductants per PFU, determined as described in Materials and Methods. The standard deviations were less than 30%.

sequence; Fig. 1B). Thus, *recD* mutations should stimulate inheritance of large sequence blocks (e.g., the 38-kb MudA elements) which require exchange near the ends of the transduced fragment; this effect should be greatest when the short flanking sequences lack a Chi sequence (Fig. 3). We would predict that the particular donor MudA insertions that show the largest effects of a recipient *recD* mutation are located at sites without properly oriented Chi sites nearby; at these sites, RecBCD degradation would be extensive. The donor MudA insertions that show a small stimulation by a recipient *recD* mutation may have flanking sequences with properly oriented Chi sites which prevent RecBCD degradation and stimulate the needed exchanges. This prediction is consistent with the behavior MudA elements located in the *his* operon.

The greatest effect of a *recD* mutation on inheritance of large sequence blocks was seen when expression of the Abc protein of transducing phage P22 was prevented. This was achieved by repressing *abc* expression in a lysogenic recipient or by using phage *abc* mutants as transducing phage. The effect of a *recD* mutation was modest when the nonlysogenic recipient cells are infected with Abc^+ phage. By inhibiting the RecBCD nuclease, Abc protein might allow efficient incorporation of MudA elements, regardless of RecD function.

Our results support the idea that P22 transduction crosses involve exchanges that occur in the absence of the RecBCD exonuclease (in agreement with the models proposed by Stahl et al. [51] and by Rosenberg and Hastings [45]). The exonuclease could be inactivated by Chi sites or inhibited by the phage P22 Abc protein (for crosses performed at high phage multiplicity). These factors may both contribute to the efficiency of P22-mediated transduction.

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REFERENCES

1. **Amundsen, S. K., A. F. Taylor, A. M. Chaudhury, and G. R. Smith.** 1986. *recD*: the gene for an essential third subunit of exonuclease V. Proc. Natl. Acad. Sci. USA **83**:5558–5562.
2. **Barbour, S. D., and A. J. Clark.** 1970. Biochemical and genetic studies of recombination proficiency in *Escherichia coli*. I. Enzymatic activity associated with $recB^+$ and $recC^+$ genes. Proc. Natl. Acad. Sci. USA **65**:955–961.
3. **Bender, J., and N. Kleckner.** 1986. Genetic evidence that Tn*10* transposes by a nonreplicative mechanism. Cell **45**:801–815.
4. **Biek, D. P., and S. N. Cohen.** 1986. Identification and characterization of *recD*, a gene affecting plasmid maintenance and recombination in *Escherichia coli*. J. Bacteriol. **167**:594–603.
5. **Burland, V., G. Plunkett, D. L. Daniels, and F. R. Blattner.** 1993. DNA sequence and analysis of 136 kilobases of the *Escherichia coli* genome: organizational symmetry around the origin of replication. Genomics **16**:551–561.
6. **Casadaban, M. J., and S. N. Cohen.** 1979. Lactose genes fused to exogenous promoters in one step using a Mu-*lac* bacteriophage: *in vivo* probe for transcriptional control sequences. Proc. Natl. Acad. Sci. USA **76**:4530–4533.
7. **Chan, R. K., D. Botstein, T. Watanabe, and Y. Ogata.** 1972. Specialized transduction of tetracycline by phage P22 in *Salmonella typhimurium*. II. Properties of a high frequency transducing lysate. Virology **50**:883–898.
8. **Chaudhury, A. M., and G. R. Smith.** 1984. Escherichia coli *recBC* deletion mutants. J. Bacteriol. **160**:788–791.
9. **Chaudhury, A. M., and G. R. Smith.** 1984. A new class of *Escherichia coli recBC* mutants: implications for the role of RecBC enzyme in homologous recombination. Proc. Natl. Acad. Sci. USA **81**:7850–7854.
10. **Ciampi, M. S., and J. R. Roth.** 1988. Polarity effects in the *hisG* gene of Salmonella require a site within the coding sequence. Genetics **118**:193–202.
11. **Clark, A. J.** 1973. Recombination deficient mutants of *E. coli* and other bacteria. Annu. Rev. Genet. **7**:67–86.
12. **Clark, A. J., and A. Margulies.** 1965. Isolation and characterization of recombination-deficient mutants of *Escherichia coli* K12. Proc. Natl. Acad. Sci. USA **53**:451–459.
13. **Dabert, P., S. D. Ehrlich, and A. Gruss.** 1993. Chi sequence protects against RecBCD degradation of DNA *in vivo*. Proc. Natl. Acad. Sci. USA **89**:12073–12077.
14. **Davis, R. W., D. Botstein, and J. R. Roth.** 1979. Advanced bacterial genetics. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
15. **Dixon, D. A., and S. C. Kowalczykowski.** 1991. Homologous pairing *in vitro* stimulated by the recombination hotspot, Chi. Cell **66**:361–371.
16. **Dixon, D. A., and S. C. Kowalczykowski.** 1993. The recombination hotspot χ is a regulatory sequence that acts by attenuating the nuclease activity of the *E. coli* RecBCD enzyme. Cell **73**:87–96.
17. **Dower, N. A., and F. W. Stahl.** 1981. Chi activity during transduction-associated recombination. Proc. Natl. Acad. Aci. USA **78**: 7033–7037.
18. **Dykstra, C. C., D. Prasher, and S. R. Kushner.** 1984. Physical and biochemical analysis of the cloned *recB* and *recC* genes of *Escherichia coli* K12. J. Bacteriol. **157**:21–27.
19. **Ebel-Tsipis, J., M. S. Fox, and D. Botstein.** 1972. Generalized transduction by bacteriophage P22 in *Salmonella typhimurium*. II. Mechanism of integration of transducing DNA. J. Mol. Biol. **71**:449–469.
20. **Eichler, D. C., and I. R. Lehman.** 1977. On the role of ATP in phosphodiester bond hydrolysis catalyzed by the recBC deoxyribonuclease of *Escherichia coli*. J. Biol. Chem. **252**:499–503.
21. **Eisenstark, A., R. Eisenstark, J. van Dillewijn, and A. Rorsch.** 1969. Radiation-sensitive and recombinationless mutants of *Salmonella typhimurium*. Mutat. Res. **8**:497–504.
22. **Elliott, T., and J. R. Roth.** 1988. Characterization of Tn*10*d-Cam: a transposition-defective Tn*10* specifying chloramphenicol resistance. Mol. Gen. Genet. **213**:332–338.
23. **Emmerson, P. T., and P. Howard-Flanders.** 1967. Cotransduction with *thy* of a gene required for genetic recombination in *Escherichia coli*. J. Bacteriol. **93**:1729–1731.
24. **Fenton, A. C., and A. R. Poteete.** 1984. Genetic analysis of the *erf* region of the bacteriophage P22 chromosome. Virology **134**:148–160.
25. **Goldmark, J., and S. Linn.** 1970. An endonuclease activity from *Escherichia coli* absent from certain rec^- strains. Proc. Natl. Acad. Sci. USA **67**:434–444.
26. **Goldmark, P. J., and S. Linn.** 1972. Purification and properties of the *recBC* DNase of *Escherichia coli* K-12. J. Biol. Chem. **247**: 1849–1860.
27. **Hughes, K. T., and J. R. Roth.** 1984. Conditionally transposition-defective derivative of Mu d1 (Amp *lac*). J. Bacteriol. **159**:130–137.
28. **Hughes, K. T., and J. R. Roth.** 1985. Directed formation of deletions and duplications using Mu d (Ap, *lac*). Genetics **109**: 263–282.
29. **Kowalczykowski, S. C., D. A. Dixon, A. K. Eggleston, S. D. Lauder, and W. M. Rehrauer.** 1994. Biochemistry of homologous recombination in *Escherichia coli*. Submitted for publication.
30. **Kushner, S. R.** 1974. Differential thermolability of exonuclease and endonuclease activities of the RecBC nuclease isolated from thermosensitive *recB* and *recC* mutants. J. Bacteriol. **120**:1219–1222.
31. **Kushner, S. R.** 1974. In vivo studies of temperature-sensitive *recB* and *recC* mutants. J. Bacteriol. **120**:1213–1218.
32. **Lam, S. T., M. M. Stahl, K. D. McMilin, and F. W. Stahl.** 1974.

Rec-mediated recombinational hot spot activity in bacteriophage lambda. II. A mutation which causes hot spot activity. Genetics **77**:425–433.
33. **Lieberman, R. P., and M. Oishi.** 1974. The *recBC* deoxyribonuclease of *Escherichia coli*: isolation and characterization of the subunit proteins and reconstitution of the enzyme. Proc. Natl. Acad. Sci. USA **71**:4816–4820.
34. **Lovett, S. T., C. Luisi-DeLuca, and R. D. Kolodner.** 1988. The genetic dependence of recombination in *recD* mutants of *Escherichia coli*. Genetics **120**:37–45.
35. **Mahan, M. J., and J. R. Roth.** 1989. *recB* and *recC* genes of *Salmonella typhimurium*. J. Bacteriol. **171**:612–615.
36. **Maniatis, T., E. F. Fritsch, and J. Sambrook.** 1982. Molecular cloning: a laboratory manual. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
37. **McKittrick, N. H., and G. R. Smith.** 1989. Activation of Chi recombinational hotspots by RecBCD-like enzymes from enteric bacteria. J. Mol. Biol. **210**:485–495.
38. **Murphy, K. C., A. C. Fenton, and A. R. Poteete.** 1987. Sequence of the bacteriophage P22 anti-RecBCD (*abc*) genes and properties of P22 *abc* region deletion mutants. Virology **160**:456–464.
39. **Oishi, M.** 1969. An ATP-dependent deoxyribonuclease from *Escherichia coli* with possible role in genetic recombination. Proc. Natl. Acad. Sci. USA **64**:1292–1299.
40. **Oliver, D. B., and E. B. Goldberg.** 1977. Protection of parental T4 DNA from a restriction exonuclease by the product of gene *2*. J. Mol. Biol. **116**:877–881.
41. **Palas, K. M., and S. R. Kushner.** 1990. Biochemical and physical characterization of exonuclease V from *Escherichia coli*. J. Biol. Chem. **265**:3447–3454.
42. **Poteete, A. R., A. C. Fenton, and K. C. Murphy.** 1988. Modulation of *Escherichia coli* RecBCD activity by the bacteriophage λ Gam and P22 Abc functions. J. Bacteriol. **170**:2012–2021.
43. **Rinken, R., B. Thomas, and W. Wackernagel.** 1992. Evidence that *recBC*-dependent degradation of duplex DNA in *Escherichia coli recD* mutants involves DNA unwinding. J. Bacteriol. **174**:5424–5429.
44. **Roof, D. M., and J. R. Roth.** 1988. Ethanolamine utilization in *Salmonella typhimurium*. J. Bacteriol. **170**:3855–3863.
45. **Rosenberg, S. M., and P. J. Hastings.** 1991. The split-end model for homologous recombination at double-strand breaks and at Chi. Biochimie **73**:385–397.
46. **Russell, C. B., D. S. Thaler, and F. W. Dahlquist.** 1989. Chromosomal transformation of *Escherichia coli recD* strains with linearized plasmids. J. Bacteriol. **171**:2609–2613.
47. **Schmieger, H.** 1971. A method for detection of phage mutants with altered transducing ability. Mol. Gen. Genet. **110**:378–381.
48. **Shyamala, V., E. Schneider, and G. F.-L. Ames.** 1990. Tandem chromosomal duplications: role of REP sequences in the recombination event at the join-point. EMBO J. **9**:939–946.
49. **Smith, G. R.** 1990. RecBCD enzyme, p. 78–98. *In* F. Eckstein and D. M. J. Lilley (ed.), Nucleic acids and molecular biology, vol. 4. Springer-Verlag, Berlin.
50. **Smith, G. R., S. K. Amundsen, A. M. Chadbury, K. C. Cheng, A. S. Ponticelli, C. M. Roberts, D. W. Shultz, and A. F. Taylor.** 1984. Roles of RecBC enzyme and Chi sites in homologous recombination. Cold Spring Harbor Symp. Quant. Biol. **49**:485–495.
51. **Stahl, F. W., L. C. Thomason, I. Siddiqi, and M. M. Stahl.** 1990. Further tests of a recombination model in which χ removes the RecD subunit from the RecBCD enzyme of *Escherichia coli*. Genetics **126**:519–533.
52. **Stahl, M. M., I. Kobayashi, F. W. Stahl, and S. K. Huntington.** 1983. Activation of Chi, a recombinator, by the action of an endonuclease at a distant site. Proc. Natl. Acad. Sci. USA **80**:2310–2313.
53. **Taylor, A. F.** 1988. The RecBCD enzyme of *Escherichia coli*, p. 231–263. *In* R. Kucherlapati and G. R. Smith (ed.), Genetic recombination. American Society for Microbiology, Washington, D.C.
54. **Taylor, A. F., and G. R. Smith.** 1985. Substrate specificity of the DNA unwinding activity of the RecBC enzyme of *Escherichia coli*. J. Mol. Biol. **185**:431–443.
55. **Thaler, D. S., E. Sampson, I. Siddiqi, S. M. Rosenberg, F. W. Stahl, and M. Stahl.** 1988. A hypothesis: Chi-activation of RecBCD enzyme involves removal of the RecD subunit, p. 413–422. *In* E. Freidberg and P. Hanawalt (ed.), Mechanisms and consequences of DNA damage processing. Alan R. Liss, Inc., New York.
56. **Thaler, D. S., E. Sampson, I. Siddiqi, S. M. Rosenberg, L. C. Thomas, F. W. Stahl, and M. M. Stahl.** 1989. Recombination of bacteriophage λ in *recD* mutants of *Escherichia coli*. Genome **31**:53–67.
57. **Tomizawa, J., and H. Ogawa.** 1972. Structural genes of an ATP-dependent deoxyribonuclease of *Escherichia coli*. Nature (London) New Biol. **239**:14–16.
58. **Triman, K. L., D. K. Chattoraj, and G. R. Smith.** 1982. Identity of a Chi site of *Escherichia coli* and Chi recombinational hotspots of bacteriophage λ. J. Mol. Biol. **154**:393–398.
59. **Vogel, H. J., and D. M. Bonner.** 1956. Acetylornithase of *Escherichia coli*: partial purification and some properties. J. Biol. Chem. **218**:97–106.
60. **Way, J. C., M. A. Davis, D. Morisato, D. E. Roberts, and N. Kleckner.** 1984. New Tn*10* derivatives for transposon mutagenesis and for construction of *lacZ* operon fusions by transposition. Gene **32**:369–379.
61. **Willetts, N. S., and D. W. Mount.** 1969. Genetic analysis of recombination-deficient mutants of *Escherichia coli* K-12 carrying *rec* mutations cotransducible with *thyA*. J. Bacteriol. **100**:923–934.