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Evidence that SbcB and RecF Pathway Functions Contribute to RecBCD-Dependent Transductional Recombination

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A role for the RecF, RecJ, and SbcB proteins in the RecBCD-dependent recombination pathway is suggested on the basis of the effect of null *recF*, *recJ*, and *sbcB* mutations in *Salmonella typhimurium* on a "short-homology" P22 transduction assay. The assay requires recombination within short (~3-kb) sequences that flank the selected marker and lie at the ends of the transduced fragment. Since these ends are subject to exonucleolytic degradation, the assay may demand rapid recombination by requiring that the exchange be completed before the essential recombining sequences are degraded. In this assay, *recF*, *recJ*, and *sbcB* null mutations, tested individually, cause a small decrease in recombinant recovery but all pairwise combinations of these mutations cause a 10- to 30-fold reduction. In a *recD* mutant recipient, which shows increased recombination, these pairwise mutation combinations cause a 100-fold reduction in recombinant recovery. In a standard transduction assay (about 20 kb of flanking sequence), *recF*, *recJ*, and *sbcB* mutations have a very small effect on recombinant frequency. We suggest that these three proteins promote a rate-limiting step in the RecBC-dependent recombination process. The above results were obtained with a lysogenic recipient strain which represses expression of superinfecting phage genomes and minimizes the contribution of phage recombination functions. When a nonlysogenic recipient strain is used, coinfecting phage genomes express functions that alter the genetic requirements for recombination in the short-homology assay.

The genetic analysis of recombination in *Escherichia coli* was initiated by A. J. Clark and coworkers using an Hfr conjugational assay (for a review of early work, see reference 8). In this assay, a linear donor molecule is introduced into a recipient strain as a single strand that is replicated after transfer. Recombinants that have incorporated a selected donor marker into the recipient chromosome in place of the corresponding recipient allele are selected (for reviews, see references 46 and 58). In this assay, the exchange events can occur at any point within the extensive sequences that flank the selected marker. For a typical 15-min mating, the recombination substrate is about 700 kb. The assay scores the number of recombinant clones that appear after several days and puts no time constraints on completion of recombination. That is, the same number of recombinants might be scored if the exchanges were completed in 10 s as if their completion required 10 h.

By this conjugation assay, mutants of the recipient which are impaired in formation of recombinants were detected (for a review, see reference 8). This led initially to discovery of the *recA*, *recB*, and *recC* genes, indicating that a rather short list of enzymes was required for recombination (7, 10, 15, 16, 22, 23, 36). The initially identified functions included only two enzymes, since the RecB and RecC proteins are subunits (with RecD) of a single functional complex (1, 5, 20, 66).

Later, the list of proteins involved in recombination was greatly extended by isolating mutants of a more complicated parental reference strain. In strains lacking *recBC* function, two suppressor mutations (*sbcB* plus *sbcCD*) restore recombination (2, 19, 31, 59). New mutants were characterized as defective for conjugational recombination in this triply mutant but recombination-proficient strain. This revealed an extensive list of proteins contributing to recombination (including RecF, RecJ, RecN, RecO, RecQ, RecR, UvrD, and Ruv) (2, 7, 21, 27, 30, 32, 34, 37, 45).

It appeared that the three mutations in the parental reference strain, *recBC*, *sbcB*, and *sbcC*, activated a complex recombination pathway involving many proteins. This is often designated the RecF pathway (8). Since the second set of mutations (*recF* and *recJ*, etc.) had little or no effect on conjugational recombination in an otherwise wild-type strain, it was inferred that the RecF pathway is not normally active in conjugational recombination but can be activated by the combination of *recBC*, *sbcB*, and *sbcC* mutations. It seems surprising that the majority of the known recombination functions contribute to a pathway that is inactive in wild-type cells; therefore, other explanations of the phenomenon have been sought.

We are interested in the possibility that the unusual pattern of *rec* mutant phenotypes, outlined above, reflects an inadequacy of the conjugational recombination assay which was used to identify and/or characterize the known mutations. Perhaps long sequences provide many opportunities for repeated attempts at exchange. Recombinants may form at a normal frequency even if a mutation makes the recombination process inefficient. Some recombination functions may act to accelerate the recombination process, and the conjugation assay may be insensitive to even substantial extensions of the time required to complete an exchange.

To approach these possibilities, we developed a "short-homology" transductional assay (42). In this assay, the donor marker, whose inheritance is selected, is a transposition-defective MudA element inserted in the donor chromosome (Fig. 1). This element is a 38-kb sequence including a gene for ampicillin resistance (Ap^r) (6, 25). The recipient cell includes no sequences homologous to the MudA element. Since the transducing phage P22 packages only 44 kb, single transduced fragments that include the entire donor MudA element have an average of only 3 kb of chromosomal sequence flanking each side of the element (42). (In fact, each flanking sequence can

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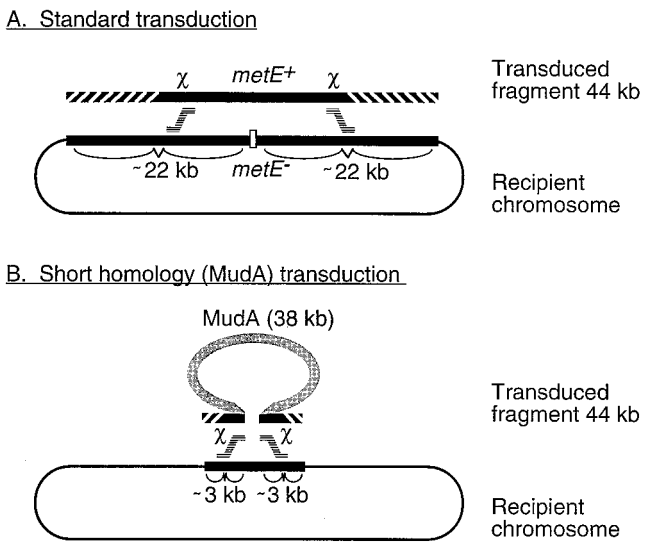

FIG. 1. The standard and short-homology assays. Thick black bars denote sequences of identity between the transduced fragment and the recipient chromosome. Degradation of the transduced fragment is indicated by white slashes. (A) Standard P22 transduction assay. Repair of a *metE* point mutation is selected. The entire transduced fragment (44 kb) is homologous to the recipient chromosome, and recombination events can occur at any site flanking the point of the *met*$^+$ information (average, about 22 kb). Degradation by the RecBCD enzyme does not limit incorporation of a *met*$^+$ 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, and so the flanking sequences homologous to the recipient chromosome are approximately 3 kb (at the ends of the transduced fragment). The RecBCD exonuclease can rapidly degrade the homologous sequences and prevent incorporation of these elements.

be anywhere between 0 and 6 kb because of random DNA packaging by the P22 high-frequency transducing mutant HT105-1.) When fragments including the MudA element are injected into a recipient cell, formation of an Ap^r transductant requires that a recombination event occur within each of these short flanking sequences (average of 3 kb). Previous work has demonstrated that P22-mediated transduction involves incorporation of a double-stranded fragment of donor material into the recipient chromosome and thus must require an exchange at each end of the transduced fragment (12). These recombination events must be completed before the short flanking sequences are degraded.

Inheritance of the MudA element in this assay is sensitive to the RecBCD exonuclease activity which presumably degrades the ends of the transduced fragment (42). For some flanking sequences (presumed to lack *chi* sites), a recipient *recD* mutation (which eliminates the RecBCD exonuclease activity) stimulates inheritance of Ap^r as much as 60-fold. Thus, we might expect other recipient mutations that minimize degradation of fragment ends to cause a similar increase in the recombinant frequency. Conversely, the frequency of recombination is likely to be reduced by a mutation that increases end degradation or extends the time required to complete the recombination event. In essence, delaying completion of the exchange should allow more time for degradation of the short sequences essential for recombinational inheritance. Once either flanking sequence is destroyed, recombinant formation is impossible. Such timing considerations are less likely to affect standard conjugation and transduction crosses, in which much longer recombining sequences are provided and which allow multiple attempts at recombination.

We have used this short-homology assay to determine the effects of null mutations in the *recF*, *recJ*, and *sbcB* genes. The *recJ* and *sbcB* genes are known to encode exonuclease activities (29, 35, 59). These activities are major contributors to degradation of ends (53), and so we expected the mutations to stimulate recombinant formation in this assay. We report here that on the contrary, the short-homology assay reveals a slight recombination defect for *recJ*, *sbcB*, and *recF* mutations tested individually, while any pairwise combination of these mutations causes a severe reduction in recombination frequency. Since the loss of known nucleases causes a decrease in recombination frequency, and since recombinant formation also depends on RecBC function, we suggest that these nucleases act in conjunction with the RecBCD enzyme and serve to accelerate the recombination process. We conclude that the RecF, RecJ, and SbcB proteins act to stimulate exchanges made by the RecBCD pathway of recombination.

MATERIALS AND METHODS

Bacterial strains. Most of the *Salmonella typhimurium* strains used in this study are derivatives of strain TR6583, which is our reference wild-type strain and carries the *metE205* point mutation (Table 1). For some experiments, a P22-19 lysogenic derivative of this strain was used (strain TT17663). Phage P22-19 (*sieA44 Ap68tpfr49*) was a gift from M. Susskind. All *rec* mutations tested in this study are insertion mutations (Tn*10*d*Tc*, Tn*10*d*Cm*, Mud-Cm, and Tn*5*) which have an associated antibiotic resistance. The Tn*10*d*Tc* element refers to the Tn*10* Del16 Del17 Tc^r element described by Way et al. (64), and Tn*10*d*Cm* refers to a transposition-defective Tn*10* derivative constructed by Elliott and Roth (14). The Mud-Cm element is a Mu derivative that has only the ends of phage Mu (13). The *recF* insertion mutant was obtained from Ken Rudd (55). The *recB*, *recD*, *recJ*, and *sbcB* mutations were identified and characterized in this laboratory (4, 38, 39, 42). The *rec* insertion mutations were introduced into tested strains by standard P22-mediated transduction (11). Phage MudA, used in transduction tests, is a transposition-defective derivative of the Mu*d*1(Ap *lac*) phage which is about 38 kb in length and is not homologous to any sequence in the *S. typhimurium* chromosome (6, 25).

Media and chemicals. Methods for preparing media have been described previously (11). Minimal medium was E medium (11, 63) supplemented with 0.2% glucose. The rich medium was Luria broth (LB) (40). Solid media contained agar from Baltimore Biological Laboratories (BBL) (1.5% [wt/vol]). Nutritional supplements to minimal media and antibiotics used in LB medium were added at the final concentrations described previously (11). All antibiotics were purchased from Sigma Chemical Co. unless otherwise noted.

Determination of cell viability. Since some *rec* mutants show reduced viability and a significant fraction of cells are nonviable, we determined viability (the fraction of recipient cells that are viable) and corrected recombinant numbers to take this into account. The method for determination of cell viability has been described previously (4). A fresh overnight culture was diluted 100-fold in LB and grown to an optical density of 100 Klett units. The cells were then diluted and plated on LB. Viability is expressed as the average number of CFU per Klett unit of culture formed by a particular strain divided by the average number formed by the isogenic *rec*$^+$ strain (strain TR6583 or TT17663).

Killing of recipients by phages was not significant for lysogenic recipients, regardless of their *rec* genotype (multiplicities of 3 and 10 were tested). With one exception, killing of nonlysogenic recipients (at a multiplicity of 3) was between 30 and 50%, regardless of *rec* genotype. The exception was recipient strain TT17675 (*recJ sbcB recD*), which showed 12% survival. Data presented do not correct for these slight effects, and even in the exceptional case, phage killing does not detract from the conclusions drawn here.

Assays for transduction proficiency. Transduction crosses were mediated by a P22 *int-201* mutant derived from the high-frequency generalized transducing phage P22 HT105/1 (57). Methods for preparing P22 transducing lysates were described previously (11).

Two assays were used to determine a strain's ability to serve as a transductional recipient. The standard assay involves selection for repair of the *metE205* point mutation (present in all recipients). In the short-homology assay, Ap^r transductants which had inherited the *pyrE2768*::MudA insertion were selected. For both assays, strain TT9521 (*metE*$^+$ *pyrE2768*::MudA) was the transduction donor. In some crosses, the recipient strains were lysogenic for a P22 phage (P22-19) which is defective in superinfection exclusion (*sieA*) and host surface antigen conversion (*a1*). Cells with the P22-19 prophage can be infected with P22 and transduced, but expression of superinfecting phage genes and host killing during transduction are prevented by repression of the superinfecting phage genomes.

The standard transduction assay was performed with a low phage multiplicity (multiplicity of infection [MOI] = 0.1 for the lysogenic recipients and MOI = 1.0 for the nonlysogenic recipients). These multiplicities differed because the standard assay generated much larger numbers of recombinants with the lysogenic

TABLE 1. Bacterial strains[a]

Strain	Genotype
TR6583	*metE205 ara-9*
TT9521	*pyrE2678*::MudA
TT17606	*metE205 ara-9 recB546*::Tn*10*d*Cm*
TT17607	*metE205 ara-9 recD543*::Tn*10*d*Tc*
TT17608	*metE205 ara-9 recJ504*::Mu*d*J
TT17654	*metE205 ara-9 sbcB2*::Tn*10*d*Cm*
TT17655	*metE205 ara-9 recF522*::Tn*5*
TT17656	*metE205 ara-9 sbcB2*::Tn*10*d*Cm recJ504*::Mu*d*J
TT17657	*metE205 ara-9 sbcB2*::Tn*10*d*Cm recF522*::Tn*5*
TT17658	*metE205 ara-9 recJ504*::Mu*d*J *recD543*::Tn*10*d*Tc*
TT17659	*metE205 ara-9 sbcB2*::Tn*10*d*Cm recD543*::Tn*10*d*Tc*
TT17660	*metE205 ara-9 recF522*::Tn*5 recD543*::Tn*10*d*Tc*
TT17661	*metE205 ara-9 sbcB2*::Tn*10*d*Cm recJ504*::Mu*d*J *recD543*::Tn*10*d*Tc*
TT17662	*metE205 ara-9 sbcB2*::Tn*10*d*Cm recF522*::Tn*5 recD543*::Tn*10*d*Tc*
TT17663	*metE205 ara-9* (P22-19; *sieA44 Ap68tpfr49*)
TT17664	*metE205 ara-9 recB546*::Tn*10*d*Cm* (P22-19; *sieA44 Ap68tpfr49*)
TT17665	*metE205 ara-9 recD543*::Tn*10*d*Tc* (P22-19; *sieA44 Ap68tpfr49*)
TT17666	*metE205 ara-9 recJ504*::Mu*d*J (P22-19; *sieA44 Ap68tpfr49*)
TT17667	*metE205 ara-9 sbcB2*::Tn*10*d*Cm* (P22-19; *sieA44 Ap68tpfr49*)
TT17668	*metE205 ara-9 recF522*::Tn*5* (P22-19; *sieA44 Ap68tpfr49*)
TT17669	*metE205 ara-9 sbcB2*::Tn*10*d*Cm recJ504*::Mu*d*J (P22-19; *sieA44 Ap68tpfr49*)
TT17670	*metE205 ara-9 sbcB2*::Tn*10*d*Cm recF522*::Tn*5* (P22-19; *sieA44 Ap68tpfr49*)
TT17671	*metE205 ara-9 recF522*::Tn*5 recJ504*::Mu*d*Cm(P22-19; *sieA44 Ap68tpfr49*)
TT17672	*metE205 ara-9 recJ504*::Mu*d*J *recD543*::Tn*10*d*Tc* (P22-19; *sieA44 Ap68tpfr49*)
TT17673	*metE205 ara-9 sbcB2*::Tn*10*d*Cm recD543*::Tn*10*d*Tc* (P22-19; *sieA44 Ap68tpfr49*)
TT17674	*metE205 ara-9 recF522*::Tn*5 recD543*::Tn*10*d*Tc* (P22-19; *sieA44 Ap68tpfr49*)
TT17675	*metE205 ara-9 sbcB2*::Tn*10*d*Cm recJ504*::Mu*d*J *recD543*::Tn*10*d*Tc* (P22-19; *sieA44 Ap68tpfr49*)
TT17676	*metE205 ara-9 sbcB2*::Tn*10*d*Cm recF522*::Tn*5 recD543*::Tn*10*d*Tc* (P22-19; *sieA44 Ap68tpfr49*)

[a] All strains were constructed in this laboratory and are derivatives of strain LT2. The *recF522*::Tn*5* mutation was identified by Ken Rudd (55); the *recJ*, *sbcB*, *recD*, and *recB* mutations were identified in this laboratory (4, 40, 44). Phage P22-19 was a gift from M. Susskind.

recipients than with the nonlysogenic recipients. The short-homology assay was performed at high (MOI = 10) and at lower (MOI = 3) phage multiplicities in lysogenic and nonlysogenic recipients alike. Despite the high-multiplicity requirement, the MudA element is transduced predominantly by a single fragment (in lysogenic recipients) since the recombinant frequency shows a first-order dependence on phage multiplicity (discussed below).

To measure recombination defects of the *rec* mutants, cultures of each mutant were grown to a density of 4×10^8 cells per ml (100 Klett units) in LB and placed on ice for 30 min to 1 h. For Ap^r selections performed with an MOI of 3, a single aliquot of culture (1.5 ml) was combined with 1.5 ml of lysate dilution and phage adsorption was allowed to proceed at 37°C for 30 min. For lysogenic recipients, the transduction mixture was divided into 10 0.2-ml aliquots and each aliquot was spread onto a selective plate. For nonlysogenic recipients (which form much larger numbers of recombinants), four 0.2-ml aliquots of the transduction mixture were spread onto selective plates. At an MOI of 3, the lysogenic rec^+ recipient (TT17663) formed about 50 recombinants per transduction mixture after incubation at 37°C for 20 h; the nonlysogenic rec^+ recipient (TR6583) formed about 150 recombinants per transduction mixture. These crosses were performed at least three times with different recipient cultures. Larger numbers of Ap^r recombinants were observed by performing crosses with an MOI of 10. For these crosses, five 0.5-ml aliquots from a single culture were each combined with 0.5 ml of lysate dilution. Four aliquots (0.2 ml) of a transduction mixture were spread onto each of four plates of selective medium. Under these conditions, the lysogenic recipient strain (TT17663) formed an average of 110 Ap^r recombinants per transduction mixture. For selection of Met^+ prototrophs, three 0.5-ml aliquots of a single culture were combined with 0.5 ml of lysate dilution and transductants were counted following a 60-h incubation at 37°C. Transduction frequencies are expressed as the average number of transductants per PFU of donor lysate; the frequency measured is corrected for any defect in recipient strain viability.

To assay the effects of phage multiplicity on Ap^r recombinant frequencies, recipient strains were grown to 4×10^8 cells per ml (100 Klett units) in LB and cultures were placed on ice for 30 min to 1 h. Twofold serial dilutions of the donor lysate were prepared in T2 buffer (11). An aliquot (0.5 ml) of this culture was combined with 0.5 ml of phage lysate dilution. After phage adsorption at 37°C for 30 min, four 0.2-ml aliquots of the transduction mixture were spread onto selective medium (ampicillin). Datum points in the figures are based on averages for at least three independent crosses done with different recipient cultures. Numbers of total transductants counted range from zero (for low multiplicities) to several thousand (for high multiplicities). The standard deviation for these replicas with more than 30 transductants was less than 30% of the mean. Two exceptional datum points had deviations that were 36% of the mean: TT17665 (MOI = 25) and TT17607 (MOI = 100 with LT2 helper phage).

Phage P22 plaquing efficiency. A fresh overnight culture was diluted 100-fold in LB and grown to an optical density of 100 Klett units. A 0.1-ml aliquot of culture was combined with 0.1 ml of phage suspension, and the cell-phage mixture was incubated at room temperature for 20 min prior to plating. Phage P22 plating efficiency on any host is expressed relative to the plating ability of strain TR6583. The phage used for determining plating efficiency was the virulent P22 phage mutant MS58-vir3, obtained from Miriam Susskind. Plaquing efficiencies are reported with and without correction for viability of the host strains. Viability correction makes the phage appear to plaque more efficiently on particular *rec* mutant strains than on the wild-type strain. We suspect that in some cases, the phage may be able to initiate a plaque after infecting a cell that is dead by the colony-forming viability assay.

RESULTS

The *rec* mutations. The *Salmonella rec* mutations used here are analogous to those defined for *E. coli* on the basis of mutant phenotype and map location (4, 38, 39, 42, 55). All *rec* mutations used are insertion mutations and thus are probably null alleles. The identities of the *recB*, *recD*, *recF*, and *recJ* mutations have been verified by complementation with the corresponding *E. coli* clones (38, 39, 42, 55). The *sbcB* insertion mutation results in the same phenotype as an *sbcB* deletion and (unlike many *sbcB* alleles) is recessive in complementation tests. On the basis of results of complementation and sequence analysis, we know that the *sbcB* insertion mutation is a null allele (3a, 4). Phenotypes of *S. typhimurium* strains containing the combination of a *recB* mutation and both the *sbcB1* and *sbcCD* suppressor mutations suggest that the recombination pathways in *Salmonella* spp. are analogous to those in *E. coli* (4).

Establishing the short-homology recombination assay. The MudA element (~38 kb) is rarely inherited on a single P22-transduced fragment which is about 44 kb in length. Previous

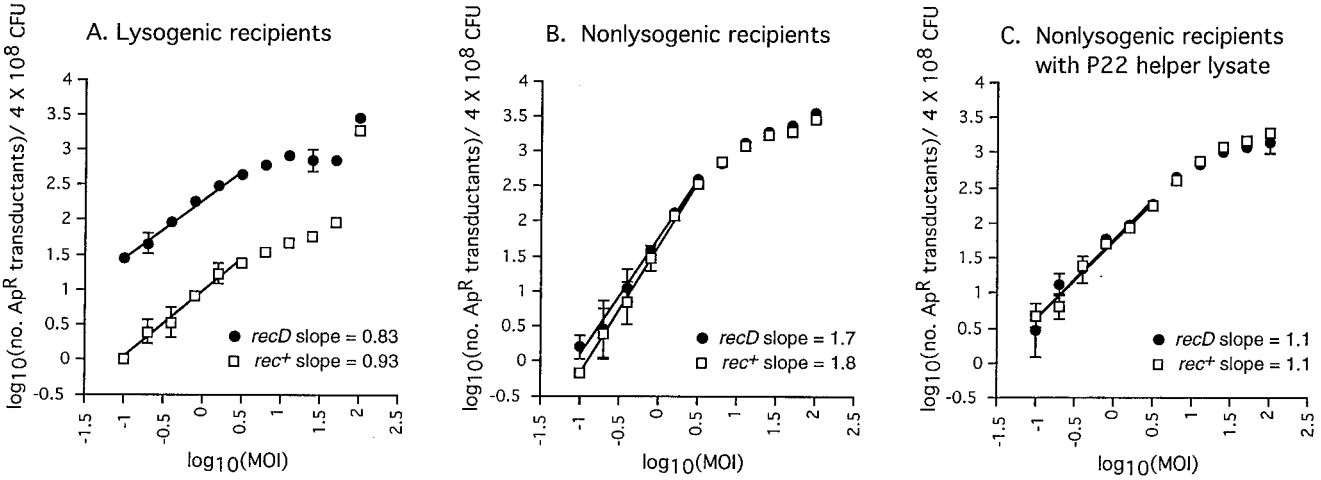

FIG. 2. Multiplicity dependence of the short-homology assay. A plot of the average of the logarithm of Ap^r transductant number as a function of the logarithm of the donor phage multiplicity of infection is shown. Crosses were performed by using 0.5 ml of lysate dilution with 0.5 ml of a constant number of recipient cells (4×10^8 CFU/ml) as described in Materials and Methods. The donor strain is TT9521, which carries the *pyrE2678*::MudA element. (A) Results of crosses done with P22-19 lysogenic recipient strains TT17663 (*recD*$^+$) and TT17665 (*recD*). (B) Results of crosses done with nonlysogenic recipient strains TR6583 (*rec*$^+$) and TT17607 (*recD*). (C) Results of crosses with the same nonlysogenic recipient strains as described for panel B; in these crosses, a P22 helper lysate (prepared on strain LT2) is provided at a constant MOI of 3.0. The helper lysate cannot transduce the recipient cells to Ap^r. Error bars represent the standard deviations of the $\log_{10}$ of transductant number determined for three independent repeats of each cross. Error bars are presented for all datum points, but the bar is obscured by the symbol for many datum points because the errors are small.

studies of MudA transduction (using nonlysogenic recipients and very high phage multiplicities) showed that these elements can be transductionally inherited by incorporation of two independently transduced fragments (24, 26); this was demonstrated by observing deletions and duplications formed by recombination between the two transduced fragments. For the studies described here, we wished to establish conditions under which inheritance of the MudA element was frequently due to a single transduced fragment. Therefore, we chose a particular donor MudA insertion (*pyrE*::MudA) that was transduced with a rather high frequency. We hoped to identify a MudA insertion site that placed the element in proper register to a chromosomal analog of the P22 *pac* site so that the element would frequently be transduced by one fragment.

To demonstrate the single-fragment inheritance of the *pyrE*::MudA element, we tested the effect of infection multiplicity on the number of transductants. A two-fragment event should show a second-order dependence on donor phage concentration, whereas single-fragment transmission should show first-order dependence (24). We performed crosses in which the number of recipient cells was held constant and the MOI of donor phage was varied. Transductants inheriting the Ap^r phenotype of the MudA element were selected. Transductant number (T) is expected to be a function of the phage multiplicity (P) raised to the power of the number of particles required for inheritance (n); that is $T = kP^n$, with k representing a proportionality constant. Thus, if one plots the logarithm of T versus the logarithm of the number of phage per cell, one expects a straight line ($\log T = \log k + n\log P$) whose slope is the number of particles required for a transductant. We measured the frequency of recombination as a function of phage MOI using isogenic *recD*$^+$ and *recD* mutant recipient strains. Crosses were performed with twofold serial dilutions of the donor lysate; multiplicity was varied from MOI = 0.01 to MOI = 100.

Effect of donor multiplicity on recombinant frequency in a lysogenic recipient. To minimize the contribution of phage functions to recombination, the first set of crosses was performed with a recipient strain lysogenic for prophage P22-19. Use of a lysogenic recipient serves to repress the expression of phage recombination functions that might otherwise contribute to the recombination process. In addition, use of a lysogenic recipient minimizes killing of recipient cells by phage. The P22-19 prophage is defective for superinfection exclusion (*sieA*) and cell surface antigen conversion (*a1*), but it provides repressor protein to prevent expression of superinfecting phage genomes.

The plot in Fig. 2A shows the logarithm of the frequency of recombinants as a function of the logarithm of the donor MOI for the P22-19 lysogenic recipients. The linear portion of the curve shows slopes of 0.9 for the *recD*$^+$ strain and 0.8 for the *recD* mutant (MOI of 0.1 to 3.0). We infer that inheritance of the *pyrE*::MudA element by the lysogenic recipient occurs primarily by incorporation of a single transduced fragment.

In the lysogenic recipient, a *recD* mutation causes elevated recombinants frequencies at all donor multiplicities. The recipient *recD* mutation is presumed to stimulate transduction by reducing RecBCD-mediated digestion of the short sequences that flank the MudA element. This finding indicates that the half-life of these short recombining sequences limits the recovery of Ap^r recombinants.

At multiplicities greater than 3, recombination frequency no longer increased linearly with donor multiplicity. We suggest that early-injected phages (*sie*$^+$) establish superinfection exclusion that limits later injection of transducing phages. (Killing of the lysogenic recipient by infecting phage genomes is unlikely to reduce recombinant recovery because there is no significant killing, even at an MOI of 10.) Superinfection exclusion by the phage in the transducing lysate may also explain why the slopes of the plots are slightly less than 1. The same effect was observed when inheritance of a *met*$^+$ allele was selected (Fig. 3); in this case, recombinants also form by incorporation of a single transduced fragment.

Crosses performed at an MOI of 100 generated much larger numbers of recombinants than expected (Fig. 2A). The *rec*$^+$ recipient formed 17-fold more recombinants at an MOI of 100

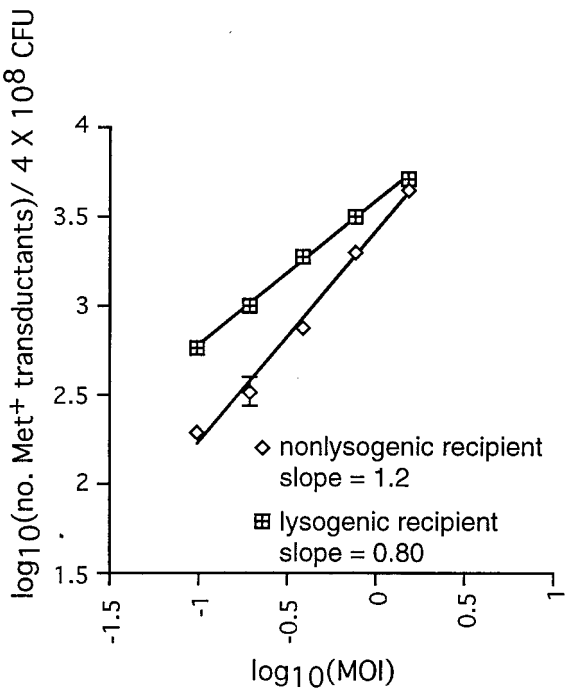

FIG. 3. Multiplicity dependence of the standard assay. Shown is a plot of the average of the logarithm of the number of Met^+ transductants as a function of the logarithm of the donor phage multiplicity of infection. Crosses were performed by using 0.5 ml of lysate dilution with 0.5 ml of a constant number of recipient cells (4×10^8 CFU/ml) as described in Materials and Methods. The donor strain is TT9521, which carries the *metE*$^+$ allele, as was used for the crosses described in the legend for Fig. 2. Diamonds represent the nonlysogenic *met* mutant recipient, strain TR6583; hatched squares denote the P22-19 lysogenic *met* mutant recipient, strain TT17663.

than it did at an MOI of 50. At high donor multiplicities, the prophage repressor becomes ineffective because of titration by the large number of superinfecting phage genomes. This may allow expression of phage recombination functions that stimulate recombination (discussed below). In addition, this high multiplicity may allow multiple transduced fragments to contribute toward inheritance of the MudA element.

Effect of donor multiplicity in a nonlysogenic recipient. When recombinant frequency is measured with a nonlysogenic recipient (permitting expression of phage functions), MudA transduction shows a second-order dependence on multiplicity (Fig. 2B). The slopes of the plots are 1.8 for the *rec*$^+$ strain and 1.7 for the *recD* recipient (over the range of MOI = 0.1 to MOI = 3). Thus, the nonlysogenic recipients appear to require two phage particles to inherit a MudA element. This requirement may be due to a need to be injected with a coinfecting (helper) phage genome and a single transduced fragment. The coinfecting phage genome could promote the rapid recombination events needed for MudA inheritance by the short-homology assay. Another explanation is that the recipient cells may need to be injected with two overlapping transduced fragments to inherit the MudA element.

To test for the requirement for a coinfecting helper phage, we performed the crosses as described above but, in addition, infected each cell with P22 helper particles (MOI = 3) prepared from a strain (LT2) that lacks the donor MudA element. This helper phage can promote recombination but cannot transduce the cells to Ap^r. If the second-order dependence of transduction on phage concentration described above is due to participation of a phage genome, the provided helper phage should cause MudA transduction to show a first-order dependence on the *pyrE*::MudA donor lysate. The plot in Fig. 2C shows a slope of 1.1 for both the *rec*$^+$ strain and the *recD* mutant. We infer from these data that coinfecting phage P22 genomes express functions that promote MudA transduction; this effect is seen only with nonlysogenic recipient cells which allow expression of phage genes.

The *recD* mutation has only a slight stimulatory effect on MudA transduction in the nonlysogenic recipient strain (Fig. 2B). We suggest that phage coinfection causes all recipient cells to show a phenotype caused by expression of the phage Abc protein, which has been shown to inhibit many activities of the RecBCD enzyme (44, 49). (Abc is a lytic function of P22 that is analogous to the Gam function of lambda.) Thus, it appears that transductional crosses with nonlysogenic recipients proceed in the absence of the RecBCD exonuclease activity, regardless of the nature of the recipient *recD* allele (42). It should be noted that the recombinant frequency for the nonlysogenic *recD* mutant recipients is lower than the frequency observed for the lysogenic *recD* mutant recipients. (Compare results for the *recD* mutant in Fig. 2A and C.) Below, we suggest that the phage Abc function inhibits the RecBC recombinase as well as the exonuclease.

Effect of *rec* mutations on transduction in a lysogenic recipient. The standard (long-homology) assay involves transductional repair of a *met* point mutation. The donated fragments provide an average of 22 kb of sequence flanking each side of the *metE*$^+$ site in the donor fragment whose inheritance is required to repair the recipient point mutation. In the short-homology assay, about 3 kb of flanking sequence is available for recombination. In comparing the results of the standard and short-homology assays, one should keep in mind that the standard assay is, of necessity, done at a substantially lower phage multiplicity than is the short-homology assay. In these crosses, all recipients are lysogenic for the P22-19 prophage as described above. While we believe that the prophage blocks expression of superinfecting phage genes, we cannot eliminate the possibility that some phage gene expression occurs at high multiplicities.

(i) Standard transduction assay. A *recB* mutation greatly reduces the frequency of Met^+ transductants, and a *recD* mutation has a slight stimulatory effect (Table 2). A mutation in any one of the *recJ*, *sbcB*, and *recF* genes does not significantly reduce the frequency of Met^+ transductants, but multiply mutant strains have a modest recombination defect (recombination is 30 to 60% reduced) relative to the Rec^+ strain. Single mutations in the *recJ*, *sbcB*, or *recF* genes slightly reduce the stimulatory effect conferred by a *recD* mutation, and combinations of these mutations have a more dramatic effect.

(ii) Short-homology assay. As shown in Table 2, recombination in the short-homology assay requires the *recB* gene product and is stimulated at least 11-fold by a *recD* mutation (42). Single mutations in either the *recJ* or the *sbcB* gene caused a 2-fold reduction in transduction frequency. A *recF* mutation reduces transduction fivefold. Strains carrying pairwise combinations of *sbcB*, *recJ*, and *recF* mutations are severely defective. The *sbcB recJ* or *sbcB recF* double mutants are as defective as a *recB* mutant. (The transduction frequency is reduced by a factor of 33.) The *recJ recF* double mutant has a 10-fold defect. All of these mutant phenotypes are observed in *recBCD*$^+$ recipient strains.

Single mutations in the *recF*, *recJ*, or *sbcB* genes also reduce the stimulatory effect conferred by a *recD* mutation. As in the *recD*$^+$ strains, the mutation pairs, *sbcB recJ* and *sbcB recF*, have a severe defect in a *recD* mutant (the transduction frequency is

TABLE 2. Transduction proficiencies of recipients carrying the P22-19 prophage

Recipient strain no.	*rec* mutation(s) carried[a]	Viability[b]	Standard assay (inheritance of *met*$^+$ gene) (MOI = 0.1)		Short-homology assay (inheritance of *pyrE*::MudA element)			
					MOI = 10		MOI = 3	
			Transduction frequency[c] (no. Ap^r [10^8])	Relative transduction proficiency[d]	Transduction frequency[c] (no. Ap^r [10^8])	Relative transduction proficiency[d]	Transduction frequency[c] (no. Ap^r [10^8])	Relative transduction proficiency[d]
TT17663	*rec*$^+$	1.00 (0.04)	13.0 (1.01)	1.0	5.3 (0.5)	1.0	3.9 (0.6)	1.0
TT17664	*recB*	0.26 (0.05)	0.2 (0.05)	0.015	0.18 (0.1)	**0.034**	0.5 (0.4)	**0.1**
TT17665	*recD*	0.99 (0.05)	61.9 (5.6)	4.8	57.4 (5.2)	10.8	65.0 (3.9)	16.6
TT17666	*recJ*	0.84 (0.09)	15.3 (1.9)	1.2	2.7 (0.5)	0.51	1.0 (0.1)	0.3
TT17667	*sbcB*	0.87 (0.12)	9.5 (1.4)	0.73	2.2 (0.4)	0.42	1.1 (0.3)	0.3
TT17668	*recF*	0.84 (0.10)	10.8 (1.3)	0.83	1.1 (0.3)	0.21	0.7 (0.3)	0.2
TT17669	*sbcB recJ*	0.89 (0.12)	3.9 (1.1)	0.30	0.17 (0.10)	**0.032**	0.08 (0.09)	**0.02**
TT17670	*sbcB recF*	0.74 (0.16)	4.7 (1.2)	0.36	0.17 (0.10)	**0.032**	0.1 (0.1)	**0.03**
TT17671	*recF recJ*	0.98 (0.14)	7.0 (0.6)	0.54	0.55 (0.21)	**0.10**	0.3 (0.25)	**0.08**
TT17665	*recD*	0.99 (0.05)	61.9 (5.5)	4.8	57.4 (5.2)	10.8	65.0 (3.9)	16.6
TT17672	*recD recJ*	0.70 (0.10)	16.1 (3.4)	1.2	19.6 (3.5)	3.7	19.0 (3.1)	4.9
TT17673	*recD sbcB*	1.02 (0.11)	26.1 (4.2)	2.0	7.1 (1.1)	1.3	7.5 (2.9)	1.9
TT17674	*recD recF*	0.87 (0.11)	31.7 (5.1)	2.4	18.3 (0.3)	3.4	5.3 (1.6)	1.3
TT17675	*recD sbcB recJ*	0.12 (0.01)	8.3 (1.6)	0.64	0.77 (0.74)	0.15	1.7 (1)	0.4
TT17676	*recD sbcB recF*	0.50 (0.08)	7.0 (1.2)	0.54	0.51 (0.21)	0.096	0.4 (0.3)	0.1

[a] All strains carry the phage P22-19 prophage and are isogenic derivatives of strain TR6583, which has the *metE205* mutation; the complete genotypes are listed in Table 1. The donor strain for all crosses is TT9521, which was used at MOIs of 10 and 3 for Ap^r selections and an MOI of 0.1 for Met$^+$ selections.

[b] The viability of a strain is the CFU of a culture at an optical density of 100 Klett units relative to CFU of the isogenic *rec*$^+$ strain, as described in Materials and Methods. The standard deviations are in parentheses.

[c] Transduction frequency is the number of transductants formed per PFU divided by the viability of that strain, as described in Materials and Methods. The standard deviations are indicated in parentheses.

[d] Transduction proficiency is the frequency of transductants relative to values for the wild-type strain; these values are adjusted for any viability defects. Boldface indicates critical entries supporting a role of RecF, RecJ, and SbcD function in RecBC-dependent recombination.

reduced by at least a factor of 40 in comparison with the level for the *recD* strain [Table 2]).

Effect of *rec* mutations with nonlysogenic recipient strains. We described above how phage functions promote inheritance of the *pyrE*::Mud element by a mechanism that is insensitive to the recipient strain's *recD* phenotype. Thus, we expected that the requirement for host *rec* functions might be altered when phage proteins are expressed from coinfecting phage genomes. To test the effects of host *rec* mutations in the presence of phage functions, we performed the transduction crosses with nonlysogenic recipient strains.

(i) Standard transduction assay. For the standard assay (nonlysogenic recipient), crosses were performed at an MOI of 1, so that most transduced cells are coinfected by infective phage. One can assess the contribution of phage functions by comparing the results of crosses done with nonlysogenic recipients (Table 3) with those seen previously for lysogenic recipients (Table 2). In this comparison, it should be noted that crosses with the lysogenic parent were done at an MOI of 0.1, since these crosses show a much higher recombination frequency (Fig. 3).

The infecting phage genomes alter the effect of host *rec* mutations. The *recD* mutation has only a slight stimulatory effect in the nonlysogenic recipients (described above). Strains with a *recJ* mutation have a recombination defect that is more significant than that observed for the same mutation in a P22-19 lysogenic recipient. Strains carrying mutations in both the *recD* and *recJ* genes are as defective as a *recB* mutant. This defect was not observed with the lysogenic recipients. Thus, it appears that phage gene expression makes recombination more dependent on the RecJ protein. The frequency of Met$^+$ recombinants is greatly reduced by a *recB* mutation, which indicates that phage gene expression does not alter the requirement for the RecBC recombinase (Table 3).

(ii) Short-homology assay. As described above, these crosses require two particles: a single transduced fragment and a helper phage genome. Not surprisingly, host *rec* mutations have effects different from those seen for a lysogenic recipient. (The absolute recombination frequencies are shown in Table 3).

The *recJ* mutation caused a more significant defect than observed with the lysogenic recipients. The *recJ* mutation, in a *recD* mutant background, caused a 250-fold reduction in recombination frequency. The single *sbcB* null mutation slightly increased the recombination frequency, whereas it decreased the recombination frequency in the lysogenic recipient. The *recB* mutation caused about a 15-fold defect in recombinant formation, which is similar to the defect observed with the lysogenic recipient. Together, these data indicate that phage functions alter host recombination, making the process more dependent on the RecJ activity. This recombination retains the requirement for the RecBC activity.

DISCUSSION

The role of the RecF, RecJ, and SbcB functions in recombination has been tested by a short-homology transduction assay which requires that recombination events occur in short (about 3-kb) sequences at the ends of the transduced fragment. The effect of host recombination mutations on this assay was compared with the effect of these mutations in a standard (long-homology) transduction assay which provides about 20 kb of flanking sequence for recombination. Since transduction frequencies are strongly reduced by a *recB* mutation in both assays, all the scored events occur by the RecBCD pathway of homologous recombination. The tests were done with both a lysogenic recipient (in which genes of superinfecting phage are

TABLE 3. Transduction proficiencies of nonlysogenic recipients

Recipient strain no.	*rec* mutation(s) carried[a]	Viability[b]	Phage P22 plating efficiency[c]		Standard assay (inheritance of *met*$^+$ gene) (MOI = 1)		Short-homology assay (inheritance of *pyrE*::MudA element)			
							MOI = 10		MOI = 3	
			Unadjusted	Adjusted for viability defects	Transduction frequency[b] (no. Met$^+$ [10^6])	Relative transduction proficiency[b]	Transduction frequency[b] (no. Ap^r [10^8])	Relative transduction proficiency[b]	Transduction frequence[b] (no. Ap^r [10^7])	Relative transduction proficiency[b]
TR6583	*rec*$^+$	1.0 (0.11)	1.0 (0.21)	1.0 (0.22)	1.8 (0.04)	1.0	27.9 (2.9)	1.0	8.6 (2.1)	1.0
TT17606	*recB*	0.23 (0.03)	0.70 (0.15)	3.0 (0.72)	0.04 (0.04)	0.02	1.9 (0.8)	0.07	0.4 (0.3)	0.05
TT17607	*recD*	0.97 (0.18)	0.94 (0.16)	0.97 (0.24)	3.1 (0.56)	1.7	50.0 (9.6)	1.8	12.9 (2.6)	1.5
TT17608	*recJ*	0.94 (0.18)	0.96 (0.19)	1.0 (0.27)	0.69 (0.12)	0.38	5.0 (0.9)	**0.2**	0.4 (0.08)	**0.04**
TT17654	*sbcB*	0.95 (0.14)	1.00 (0.19)	1.1 (0.23)	1.6 (0.20)	0.88	50.8 (10.1)	**1.8**	12.7 (1.8)	**1.5**
TT17655	*recF*	0.86 (0.07)	0.78 (0.12)	0.91 (0.14)	0.60 (0.13)	0.33	4.6 (0.5)	**0.2**	1.0 (0.2)	**0.1**
TT17656	*sbcB recJ*	1.1 (0.12)	0.78 (0.13)	0.72 (0.14)	0.36 (0.05)	0.20	2.8 (0.31)	0.1	0.4 (0.07)	0.05
TT17657	*sbcB recF*	1.0 (0.12)	0.77 (0.12)	0.77 (0.14)	0.48 (0.07)	0.27	2.9 (0.57)	0.1	0.7 (0.2)	0.08
TT17607	*recD*	0.97 (0.18)	0.94 (0.16)	0.97 (0.24)	3.1 (0.56)	1.7	50.0 (9.6)	1.8	12.9 (2.6)	1.5
TT17658	*recD recJ*	0.72 (0.07)	0.68 (0.17)	0.94 (0.25)	0.04 (0.02)	0.02	0.2 (0.1)	0.01	0.1 (0.05)	0.01
TT17659	*recD sbcB*	1.0 (0.13)	0.77 (0.13)	0.74 (0.15)	2.4 (0.29)	1.3	55.7 (9.5)	2.0	12.5 (1.9)	1.5
TT17660	*recD recF*	1.1 (0.12)	0.84 (0.14)	0.78 (0.14)	1.2 (0.12)	0.67	10.0 (1.4)	0.4	2.7 (0.3)	0.3
TT17661	*recD sbcB recJ*	0.11 (0.02)	0.49 (0.11)	4.3 (1.06)	0.02 (0.03)	**0.01**	0.1 (0.2)	**0.004**	0	**0**
TT17662	*recD sbcB recF*	0.69 (0.07)	0.65 (0.11)	0.94 (0.17)	0.83 (0.15)	0.46	9.1 (4.2)	0.33	1.2 (0.1)	0.1

[a] All strains are isogenic derivatives of strain TR6583, which carries the *metE205* mutation; the complete genotypes are listed in Table 1. The donor strain for all crosses is TT9521, which was used at MOIs of 10 and 3 for Ap^r selections and an MOI of 1 for Met$^+$ selections.

[b] The viability, transduction frequencies, and transduction proficiencies are as described in Table 2. Standard deviations are in parentheses. Boldface indicates critical entries supporting a role of RecF, RecJ, and SbcD function in RecBC-dependent recombination.

[c] The P22 plating efficiency is the average number of PFU observed on that strain relative to values for the isogenic *rec*$^+$ strain. The viability defects of the *rec* mutants are factored into the P22 plating efficiencies in the fifth column. The standard deviations are indicated in parentheses.

repressed) and nonlysogenic cells (in which recombination events depend on genes expressed by superinfecting phage).

Recombination without phage functions. With a lysogenic recipient to minimize the contribution of phage functions, the short-homology assay detects modest recombination defects (2- to 5-fold) caused by single mutations in the *recF*, *recJ*, or *sbcB* genes; a smaller defect is seen with the standard transduction crosses (20 to 30%). Pairs of mutations in the *recF*, *recJ*, and *sbcB* genes cause a major decrease in recombination in the short-homology assay (10- to 33-fold). These double mutants show a slight defect in the standard transduction assay (40 to 60%). The phenotypes associated with these mutations are observed in both *recD*$^+$ and *recD* mutant strains.

Results of the short-homology assay demonstrate that the *recF*, *recJ*, and *sbcB* gene products participate in RecBCD-mediated recombination events, supporting the idea that the RecF and RecBCD pathways are not independent. This possibility has been suggested previously (9, 28, 33, 65). A similar conclusion has recently been drawn from studies of Feng and Hays, who found that *uvrD*, *recF*, *recJ*, *sbcB*, and *recBC* mutations reduce recombination induced by mismatch repair of UV-irradiated phage lambda DNA (17). We suggest that other RecF pathway proteins (identified to be present in *E. coli recBC sbcBC* mutants) may also prove to participate in RecBCD-mediated exchanges and that their role will be more easily detected by using the short-homology transduction assay.

The transduction deficits reflect recombination defects. The transduction defects observed reflect impaired recombination ability and cannot be explained by effects of *rec* mutations on viability, phage penetration, or ability of recipient strains to survive infection. Most of the *rec* strains used in this study have only modest viability defects, and the data have been corrected for these defects (Tables 2 and 3). We have tested all the recipient strains for phage P22 plating efficiencies, and none are substantially defective (Table 3). Lysogenic strains are not killed by phage infection, regardless of their *rec* genotype, and while nonlysogenic strains are sensitive to killing, this sensitivity is not significantly altered by *rec* mutations (described in Materials and Methods). We conclude that the transduction defects noted here are caused by reduced abilities to perform recombination.

Roles for the RecF, RecJ, and SbcB proteins in RecBCD-mediated recombination. A hypothetical role for the RecF, RecJ, and SbcB proteins in recombination is diagrammed in Fig. 4 and discussed below. The RecJ and SbcB proteins are single-strand exonucleases of opposite polarity (29, 35, 59); these exonucleases might contribute to recombination by helping to produce a single-strand substrate for the RecA strand exchange protein. The single DNA strand required by RecA protein can be produced by the highly processive RecBCD helicase activity (reviewed in reference 28). We propose that DNA strands unwound by RecBCD enzyme tend to reanneal; exonuclease degradation of either strand would help stabilize the other single-stranded region (Fig. 4). Degradation of a 5′ end by RecJ protein would stabilize an unwound 3′-ended strand as diagrammed at the left of Fig. 4. Degradation of a 3′-ended strand by SbcB protein would stabilize a 5′-ended single strand (right side of Fig. 4). Figure 4 portrays the RecA protein as able to promote invasion of either a 3′- or a 5′-ended strand as suggested earlier (52, 54).

By degrading a displaced strand, these single-strand exonucleases may also stimulate strand exchange and branch migration reactions (3). The synergistic effect of the paired *sbcB recJ* mutations is consistent with the idea that the encoded proteins have functionally alternative activities in promoting recombination, as diagrammed in Fig. 4. Other studies have found that paired *sbcB recJ* mutations cause a synergistic defect for recombination activation by a Chi site (a RecBCD-dependent process) (52). If RecBCD-mediated recombination can proceed by either a 3′- or a 5′-end invasion mechanism, as diagrammed in Fig. 4, the severe defect of *sbcB recJ* double mutants would be explained. It should be noted that while the exonucleases may drive strand invasion and branch migration, the process of recombination might proceed without them, albeit at a slower pace and with reduced efficiency.

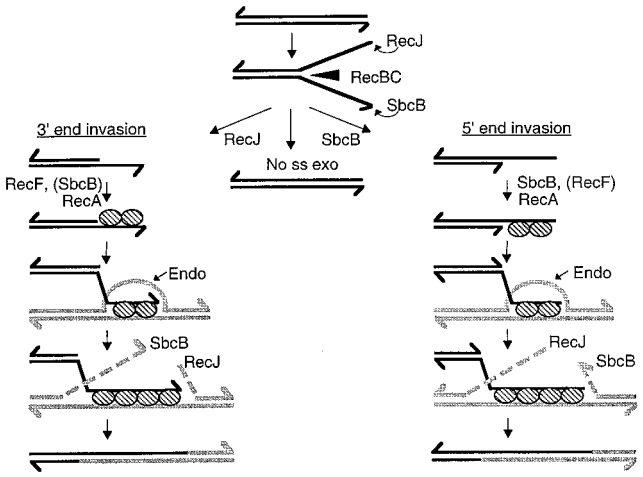

FIG. 4. Model for the role of RecF, RecJ, and SbcB proteins in recombination. The RecBCD enzyme after Chi stimulation is presumed to have no nuclease activity (RecBC [filled arrowhead]). It unwinds duplex DNA (black lines) to generate single strands that are substrates for RecA-mediated strand exchange. If the strands reanneal, the substrate becomes inert to strand exchange (center). The RecJ and SbcB proteins have single-stranded exonuclease activities which could degrade one of the complementary single strands and leave a recombinogenic single-stranded overhang. The RecJ protein is a 5′-to-3′ single-stranded (ss) exonuclease that would leave a 3′-ended strand; SbcB is a 3′-to-5′ ss exonuclease that would leave a 5′-ended strand. RecA (oval) can bind to the single-stranded DNA; we propose that this process is facilitated by the RecF protein (mainly for 3′-end invasion) and by a second activity of the SbcB protein (for 5′-end invasion). RecA promotes pairing with the homologous duplex (gray lines) and strand exchange. Endonucleolytic cleavage (Endo) of the displaced strand provides ended substrates for ss exonuclease. The exonucleases could enhance the rate of strand exchange by degrading the displaced strand (gray dashes).

The RecF protein has no known exonuclease activity. Current genetic and biochemical data suggest that the RecF protein (with RecO and RecR) displaces the Ssb protein from single-stranded DNA and thereby promotes RecA binding (28, 60). In Fig. 4, we suggest that RecF contributes to the loading of RecA protein to stimulate both 5′- and 3′-end invasion. We suggest that the SbcB protein may also facilitate RecA binding. This would explain the severe recombination defect of *sbcB recF* double mutants. This proposed interaction of SbcB and RecA proteins could explain the observation that SbcB protein copurifies with both the RecA and Ssb proteins through many chromatographic steps (28).

Short recombination substrates increase the sensitivity of a recombination assay. We suggest that an assay of recombination using short (3-kb) sequences at the ends of the transduced fragment is more sensitive to the speed of the recombination process because recombination competes with substrate degradation. Results obtained with the short-homology assay suggest a new interpretation of the conjugation crosses, which first suggested multiple recombination pathways. In wild-type strains, conjugational recombination would employ the highly processive RecBCD helicase which produces long single-stranded DNA substrates (after stimulation by Chi). Without RecBCD, single-stranded substrates would be generated by alternative helicases which unwind DNA at a slower rate with less processivity. The RecQ, helicase II, helicase IV, and Rep helicases are all potential substitutes for the RecBC helicase; these enzymes unwind only short tracks of DNA (41, 61, 62, 67, 68). Thus, recombination in *recBC sbcB sbcC* triple mutants would involve single-stranded DNA regions that are effectively much shorter than those used in *recBCD*$^+$ strains. Continuous degradation of these short regions may (like the short-homology assay) demand rapid recombination. This sort of recombination may be sensitive to mutational defects that slow the process and may therefore have allowed detection of the RecF pathway mutations.

Phage P22 gene expression alters host cell recombination. In the crosses performed with nonlysogenic recipients, expression of P22 genes stimulated MudA transduction, resulting in a second-order dependence of MudA (Ap^r) transductants on donor lysate concentration (Fig. 2B). This dependence becomes first order when helper phage (no MudA) is provided in excess (Fig. 2C). Lysogenic recipients show a first-order dependence in the short-homology assay (Fig. 2A). The standard transduction assay (long homology) showed first-order dependence on transducing phage in both lysogenic and nonlysogenic recipients (Fig. 3); however, in nonlysogenic recipients, phage gene expression reduced transductant recovery to extents which varied with donor MOI. Thus, phage functions seem to promote recombination in the short-homology assay but impair recombinant recovery in the standard assay.

Several phage recombination proteins which could stimulate the short-homology assay are known. The phage recombination functions promote phage genome circularization by using the short (1.7-kb) terminal repeats present at the ends of the injected molecule (reviewed in reference 47). Phage Abc function (an analog of the lambda Gam function) inhibits the exonuclease activity of RecBCD enzyme and the ability to promote homologous recombination (assayed by conjugation and phage lambda crosses) (44, 49). By inhibiting the RecBCD exonuclease, Abc protects the short terminally redundant ends of the phage genome prior to circularization and protects the end of the concatemeric genome required for packaging (43). The phage Erf protein (an analog of lambda β protein) promotes pairing of homologous single strands (48), which contributes to the ability of P22 to transduce plasmids (18) and promotes recombination in phage lambda and phage P22 crosses (50). Erf can act with the phage P22 Arf protein (accessory recombination function) to stimulate recombination in the presence or absence of the host *recA* function (50). Phage P22 also appears to have a functional analog of the host RecF protein (3a); this is similar to the situation for lambda, which has a functional analog of the host RecF, RecO, and RecR proteins (56).

The increase in MudA transduction caused by phage gene expression is probably due to combined effects of multiple P22 recombination functions that modify host cell recombination. When phage genes are expressed, recombination in the short-homology assay requires the *recJ* gene product (Table 3). Perhaps the P22-promoted recombination is driven primarily by long 3′-ended single strands and requires RecJ to destroy the competing 5′ complementary strand. The phage Abc function may stimulate recombination in the short-homology assay by inhibiting the RecBCD exonuclease and thereby stabilizing the short recombining sequences that flank the MudA element. This may explain why a *recD* mutation has almost no effect on MudA transduction in nonlysogenic recipients (Fig. 2B) (42), while the same *recD* mutation significantly increases MudA transduction in a lysogenic recipient (Fig. 2A).

Phage gene expression reduces MudA transduction in a *recD* mutant (Fig. 2) and also reduces recombinant recovery in the standard transduction assay (Fig. 3). We suggest that phage functions (including Abc) inhibit the exonuclease activity and slightly inhibit the recombinase activity of the RecBCD enzyme. In the short-homology assay, the half-life of the short recombining sequences limits recombinant recovery such that the phage functions increase recombinant recovery. However,

these phage functions have an inhibitory effect when recombinant recovery is limited by the RecBC recombinase activity (in the *recD* mutant and in the standard transduction assay). Previous studies have shown that the P22 *abc1* and *abc2* gene products inhibit the RecBC recombinase in *E. coli*, when assayed by conjugation and phage lambda crosses (44, 49, 51). This fits with the above interpretation of our results. However, the phage functions do not completely inhibit the RecBC recombinase because all the recombination in our assays requires RecBC.

The question of two-fragment transduction. In previous work with P22 transduction of MudA elements, we reported second-order dependence of recombinational inheritance of MudA on donor lysate concentration (24). Those results were interpreted as reflecting the cooperation of two transduced fragments, each carrying a portion of the large MudA element. We suggested that these two fragments recombine with each other to generate a complete MudA element on a hybrid fragment which, in turn, recombined with the chromosome to generate the detected MudA-bearing recombinant. The results presented here suggest that this interpretation is likely to be incorrect and that (at low phage multiplicities) the second-order dependence on donor lysate reflects involvement of a helper phage genome that provides stimulation of recombination.

Despite this reinterpretation, it is clear that, under some conditions, multiple transduced fragments can cooperate to carry the large MudA element. This has been seen in crosses performed at a very high transducing-phage multiplicity (higher than those used here). This cooperation between two overlapping transduced fragments was demonstrated by showing that two transduced fragments derived from different donor MudA elements (even from different donor strains) can recombine in the recipient cell and can form chromosomal deletions and duplications when the hybrid fragment recombines with the chromosome (26). Fragment cooperation is not occurring in the crosses reported here, and the exact multiplicity dependence of cooperative crosses that lead to rearrangements has not been tested. In view of the results reported here, we suspect that rearrangements may require three phage particles, two carrying transduced fragments and one infective (helper) genome.

In conclusion, the short-homology transduction assay provides evidence that recombination via the major RecBCD-dependent pathway is stimulated by the products of the *recF*, *recJ*, and *sbcB* genes and that phage P22-encoded functions contribute to the process of transductional recombination.

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