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Suppression of RecA Deficiency in Plasmid Recombination by Bacteriophage λ β Protein in RecBCD⁻ ExoI⁻ *Escherichia coli* Cells

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Plasmid recombination, like other homologous recombination in *Escherichia coli*, requires RecA protein in most conditions. We have found that the plasmid recombination defect in a *recA* mutant can be efficiently suppressed by the β protein of bacteriophage λ. β protein is required for homologous recombination of λ chromosomes during lytic phage growth in a *recA* host and is known to have a strand-annealing activity resembling that of RecA protein. The bioluminescence recombination assay was used for genetic analysis of β-protein-mediated plasmid recombination. Efficient suppression of the *recA* mutation by β protein required the absence of the *E. coli* nucleases exonuclease I and RecBCD nuclease. These nucleases inhibit a RecA-mediated plasmid recombination pathway that is more efficient than the pathway functioning in wild-type cells. Like RecA-mediated plasmid recombination in RecBCD⁻ ExoI⁻ cells, β-protein-mediated plasmid recombination depended on concurrent DNA replication and on the activity of the *recQ* gene. However, unlike RecA-mediated plasmid recombination, β-protein-mediated recombination in RecBCD⁻ ExoI⁻ cells was independent of *recF* and *recJ* activities. We propose that inactivation of exonuclease I and RecBCD nuclease stabilizes a recombination intermediate that is involved in RecA- and β-protein-catalyzed homologous pairing reactions. We suggest that the intermediate may be linear plasmid DNA with a protruding 3′ end, since these nucleases are known to interfere with the synthesis of such linear forms. The different *recF* and *recJ* requirements for β-protein-dependent and RecA-dependent recombinations imply that the mechanisms of formation or processing of the putative intermediate differ in the two cases.

The low frequency of recombinants in *recA* mutants of *Escherichia coli* indicates that the role of RecA in recombination is indispensable (9, 18). However, some RecA-independent recombination systems have been described. These include Red- and RecE-mediated recombination of bacteriophage λ and plasmid DNAs (12, 14, 22, 33, 39). One class of proteins whose activity may facilitate RecA-independent recombination is the helix-destabilizing proteins (see reference 35 for a review). Members of this class, *Escherichia coli* single-stranded-DNA-binding protein (SSB), T4 gene 32 product, and λ β protein, can play an accessory role in RecA-mediated strand invasion and strand exchange reactions (29, 31, 38). In addition, these proteins catalyze a RecA-independent strand-annealing reaction that may be involved in recombination (1, 8, 17, 30).

β protein is one component of the phage λ Red recombination system. The other component, *red* exonuclease, converts a double-stranded DNA end to a 3′ single-stranded end by exonucleolytic digestion in a 5′-to-3′ direction (34). The activity of *red* enzymes and the demonstrated role of double-stranded ends in recombination have led to the proposal that the Red recombination pathway functions by a manner similar to that of the double-strand-break repair pathway of recombination in yeast cells (41, 42, 45–47). According to this proposal, a double-stranded end, which is formed in the process of λ DNA rolling-circle replication or by λ terminase activity at *cos* sites, is converted by *red* exonuclease to a 3′ single-stranded end. This end serves as a substrate in a β-protein-mediated homologous pairing reaction.

Genetic analysis of plasmid recombination has demonstrated that in wild-type *E. coli* cells, plasmids recombine by a pathway that differs from that of conjugational recombination. *recB* and *recC* mutations, which lower recombinant frequency in conjugation (9), do not affect plasmid recombination. On the other hand, plasmid recombination depends on *recF*, *recJ*, and *recO* gene activity (11, 16, 19). These genes are involved in the RecF conjugational pathway, which is functional in *recB recC sbcB sbcC* mutants (9, 14, 19). Plasmid recombination is difficult to test experimentally in these mutants, since plasmid maintenance depends on the activity of exonuclease I and RecBCD nuclease (36, 48). To investigate plasmid recombination in RecBCD⁻ ExoI⁻ cells, the bioluminescence recombination assay and conditional expression of λ *gam*, which inhibits RecBCD nuclease, were used. The results indicate that RecBCD nuclease and exonuclease I inhibit a plasmid recombination pathway that is more efficient than the recombination pathway in wild-type cells and that recombination by this pathway depends on conditions that divert the plasmid mode of replication from a Θ mechanism to the synthesis of linear multimers. Similar requirements were reported for plasmid recombination by the λ Red and RecE pathways (33).

The dependence of Red-mediated and Gam-dependent plasmid recombination in *sbcB* mutants on conditions that facilitate plasmid linear-multimer synthesis and the demonstration that ends of linearized plasmid dimers are recombinogenic in *recB recC sbcA* and in *recB recC sbcB* mutants (44; C. Luisi-DeLuca and R. Kolodner, unpublished data), in which the RecE and RecF recombination pathways, respectively, are functional, implied that double-stranded ends play a role in plasmid recombination. A hypothesis that relates plasmid mode of replication to recombination by the RecE, RecF, and Red recombination pathways has been presented (33). According to this hypothesis, DNA ends that serve as recombinogenic elements in these pathways are produced in the process of plasmid linear-multimer synthesis. In the Red and RecE pathways, double-stranded ends of linear multim-

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TABLE 1. *E. coli* strains

Strain	Genotype						Reference or source
	recA	*recB*	*recC*	*sbcB*	*sbcC*[a]	Other[b]	
AB1157	+	+	+	+	+		2
JC2926	*13*	+	+	+	+		A. J. Clark
JC5495	*13*	*21*	*22*	+	+		A. J. Clark
JC7623	+	*21*	*22*	*15*	*201*		15
JC11451	+	+	+	*15*	(*201*)		A. J. Clark
JC12123	+	+	+	+	+	*recJ284*::Tn*10*	A. J. Clark
JC12334	+	+	+	+	+	*recF143 tna300*::Tn*10*	A. J. Clark
JC15329	Δ*306*::Tn*10*	*21*	*22*	*15*	(*201*)		A. J. Clark
JC15390	+	+	+	*15*	(*201*)	*ilv2168*::Tn*5 recQ1801*	A. J. Clark
N2364	+	+	+	+	*201*	*phoR*::Tn*10*	24
N2375	+	+	+	*15*	+		24
AC146	+	+	+	*15*	(*201*)	*recJ284*::Tn*10*	40
AC116	+	+	+	*15*	(*201*)	*recF143 tna300*::Tn*10*	40
AC127	+	*21*	*22*	*15*	(*201*)	*dnaB558*::Tn*10*Δ*16*Δ*17* Kan^r	This work[c]
AC135	Δ*306*::Tn*10*	+	+	*15*	(*201*)		33
AC145	Δ*306*::Tn*10*	+	+	*15*	(*201*)	*recF143*	This work[d]
AC148	+	+	+	*15*	(*201*)	*eda*::Tn*10 ruvA4*	This work[e]
AC151	Δ*306*::Tn*10*	*21*	*22*	*15*	(*201*)	*dnaB558*::Tn*10* Δ*16*Δ*17* Kan^r	This work[f]

[a] All *sbcB15* mutants except N2375 are derivatives of JC7623 and therefore are likely to also have an *sbcC201* mutation (24). The *sbcC201* mutation was not verified in these strains and is given in parentheses.

[b] All strains listed are *thr-1 ara-14 leuB6 (gpt-proA)622 lacY1 tsx-33 supE44 galK2 hisG4 rpsL31 kdoK51 xyl-5 mtl-1 argE3 thi-1*.

[c] AC127 is a temperature-sensitive Kan^r derivative of JC7623 constructed by transduction with P1 · AC122. AC122 is a *dnaB558*::Tn*10*Δ*16*Δ*17* Kan^r strain (40).

[d] AC145 is Tc^r UV^s derivative of AC116 constructed by transduction of AC140 with PI · JC15329. AC140 is a Tc^s derivative of AC116. JC15329 has a (*recA-srlR*)*306*::Tn*10* mutation (10).

[e] AC148 is a Tc^r MC^s derivative of JC11451 constructed by transduction with PI · CS40. CS40 has a *ruvA4 eda*::Tn*10* mutation (23).

[f] AC151 is a Tc^r UV^s derivative of AC127 constructed by transduction with P1 · JC15329.

ers are converted by *red* exonuclease and exonuclease VIII, respectively, to 3′ single-stranded ends. In the RecF pathway, 3′ single-stranded ends are by-products of DNA synthesis and are stabilized by the absence of RecBCD nuclease and exonuclease I (10). It follows from this hypothesis that similar substrates are available for the RecA-mediated pairing reaction in the RecF pathway and for the β-protein-mediated pairing reaction in the Red pathway. In such a case, β-protein activity should suppress *recA* mutations in plasmid recombination in RecBCD$^-$ ExoI$^-$ cells. To test this prediction, the bioluminescence plasmid recombination assay (33) and controllable *gam* and *bet* gene expression from *E. coli* plasmids were used. The genetic requirements of RecA- and β-protein-mediated plasmid recombination in RecBCD$^-$ ExoI$^-$ cells are compared.

MATERIALS AND METHODS

Bacterial strains and growth conditions. The *E. coli* K-12 strains used in this study are listed in Table 1 . All strains are isogenic derivatives of AB1157 (2). Plasmid-carrying strains were derived by transformation, and temporary cultures were kept on LB (26) agar plates. Cells were routinely grown on L broth at the indicated temperatures. When appropriate, media were supplemented with ampicillin (100 μg/ml), chloramphenicol (10 μg/ml), or both. Tetracycline-sensitive derivatives of tetracycline-resistant strains were selected by the method of Bochner et al. (4).

Plasmids. Plasmids used are listed in Table 2. pIB507 is a pSF117 (13) derivative, expressing λ *gamS*$^+$ and *bet* genes from a λ p_L promoter under *c*I857 repressor control. It was constructed by ligating the *Sal*I-*Sma*I fragment of pSJS6, which includes the 3′ end of the *bet* gene, to the *Sal*I-*Nru*I fragment of pSF117, which includes the replication origin, the *gamS*$^+$ gene, and the 5′ end of the *bet* gene. pIB509 is isogenic to pIB507 but carries a mutation in the *gamS*$^+$ gene. It was constructed by ligating the same *Sal*I-*Sma*I fragment of pSJS6 to a *Sal*I-*Nru*I fragment of pSF119 (13). All plasmids used except pAC604 are derivatives of pBR322 (5).

Bioluminescence plasmid recombination assay. The bioluminescence recombination assay uses mutated *Vibrio fisheri* luciferase genes cloned in *E. coli* plasmids. As in the enzymatic recombination assay (3), activity is determined at the gene expression level by assaying a functional gene product rather than by scoring viable recombinants. pAC604 is a pACYC184 (7) derivative carrying a duplication of the *V. fisheri luxA* gene. Each copy of the gene has a mutation at a different restriction endonuclease site. This plasmid is a substrate for the bioluminescence recombination assay (33). Assay conditions and determination of bioluminescence activity were essentially as described by Nussbaum and Cohen (33). Bioluminescence was measured at 28°C in a liquid scintillation spectrometer, and activity is expressed as counts per minute.

RESULTS

Suppression of *recA* mutations by λ *bet* activity. To determine whether λ β protein can suppress *recA* in plasmid

TABLE 2. Plasmids

Plasmid	Relevant genes	Source or reference
pAC604	*lacPO luxA*$^-$ *luxB*$^+$	33
pSF117	*c*I857 λ p_L *gamS*$^+$	13
pSF119	*c*I857 λ p_L *gamS*$^-$	13
pSJS6	*c*I857 λ p_R p_L *bet*$^+$ *exo*$^+$	S. Sandler and A. J. Clark
pSM701	*c*I857 λ p_R p_L *bet*$^+$ *exo*$^-$	S. Maor
pSM704	*c*I857 λ p_R p_L *bet*$^-$ *exo*$^-$	S. Maor
pIB507	*c*I857 λ p_R p_L *gamS*$^+$ *bet*$^+$	This work
pIB509	*c*I857 λ p_R p_L *gamS*$^-$ *bet*$^+$	This work

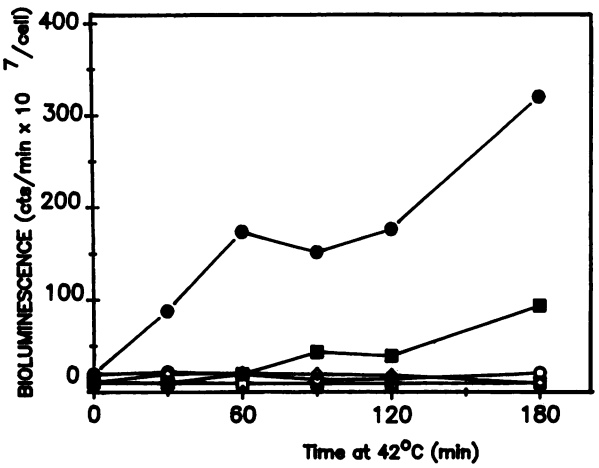

FIG. 1. Suppression of *recA* mutations by λ β-protein activity. *recA* mutants, harboring pAC604 and either pSM701 or pSM704, were grown at 28°C. After incubation at 42°C for the indicated times, the cultures were returned to 28°C for 1 h to allow luciferase synthesis (33), and bioluminescence activity was determined. Cultures expressing *bet* from pSM701 were of the following genotypes: *recA* (JC2926) (□), *recA recB recC* (JC5495) (◇), *recA sbcB* (AC135) (■), and *recA recB recC sbcB* (JC15329) (●). One culture contained *recA recB recC sbcB* (JC15329) cells harboring pSM704 and pAC604 (○).

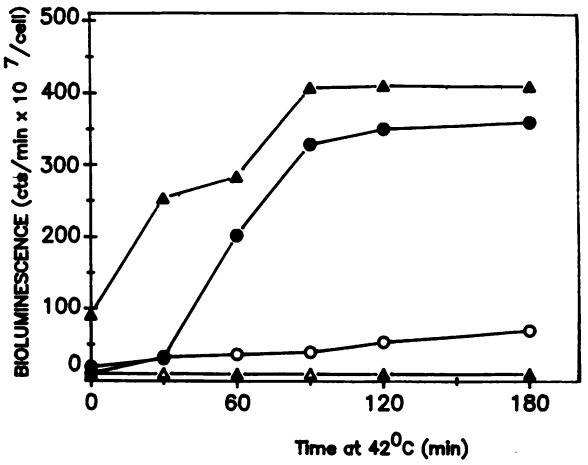

FIG. 2. Effects of $gamS^+$ and *bet* expression on plasmid recombination in *recA sbcB* mutants. Cultures of *recA sbcB* (AC135) cells harboring pAC604 and pIB507 (●), pIB509 (○), or pSF117 (△) were transferred from 28 to 42°C for the indicated times, and bioluminescence activity was determined as described in the legend to Fig. 1. The effect of $gamS^+$ expression from pSF117 on intramolecular recombination of pAC604 in *sbcB* (JC11451) cells (▲) is shown as a reference.

recombination, *recA* mutants were transformed by a plasmid substrate of the bioluminescence recombination assay (pAC604) and by pSM701, which expresses *bet* under λ *c*I857 control, and the effect of *bet* expression on plasmid recombination was determined by measuring bioluminescence activity (Fig. 1). Bioluminescence activity was not detectable after a temperature shift in *recA* mutants harboring pAC604 and pSM701. Therefore, *bet* activity did not facilitate RecA-independent plasmid recombination in $RecBCD^+$ $ExoI^+$ cells. Elimination of exonuclease I by an *sbcB* mutation allowed a relatively low level of β-protein-mediated recombination. In *recA recB recC sbcB* cells, in which both exonuclease I and RecBCD nuclease are nonfunctional, the level of β-protein-mediated recombination activity was substantially higher than in *recA sbcB* cells. Bioluminescence was not detectable in these mutants if pSM704, which is isogenic to pSM701 but carries a mutated *bet* gene, was substituted for pSM701. Little or no recombination activity was observed in *recA recB recC* cells after *bet* expression. The inhibitory effect of RecBCD nuclease and exonuclease I on β-protein-mediated recombination was apparent also in experiments in which RecBCD nuclease was inactivated by λ $gamS^+$ protein (Fig. 2). Recombination activity was not detectable after thermal derepression of $gamS^+$ expression in *recA sbcB* mutants harboring pAC604 and pSF117. However, expression of both *bet* and $gamS^+$ by pIB507 in these cells facilitated plasmid recombination. A lower level of recombination was measured in *recA sbcB* cells when pIB509, which expresses *bet* but has a *gam* mutation, was substituted for pIB507. The observation that recombination kinetics after *bet* and $gamS^+$ expression by pIB507 in *recA sbcB* cells was similar to that after $gamS^+$ expression by pSF117 in *sbcB* cells indicated that β-protein-mediated recombination in $RecBCD^-$ $ExoI^-$ cells is as efficient as RecA-mediated recombination in the same background.

Genetic analysis of RecA-mediated plasmid recombination in $RecBCD^-$ $ExoI^-$ cells. Conjugational recombination by the RecF pathway depends on two *recB recC* suppressor mutations, *sbcB* and *sbcC* (20, 24). To determine the effect of each of these mutations on plasmid recombination, *sbcB*, *sbcC*, or *sbcB sbcC* mutants were transformed by pAC604 and pSF117, and recombination activity was determined after thermal derepression of $gamS^+$ gene expression. pSF119 was substituted for pSF117 in control cultures (Fig. 3). Gam-dependent recombination was observed in *sbcB* and *sbcB sbcC* cells but not in *sbcC* cells. The rate of increase in bioluminescence activity in *sbcB* cells after $gamS^+$ expression was similar to that in *sbcB sbcC* cells, which indicated that unlike conjugational recombination by the RecF pathway, which depends on both *sbcB* and *sbcC* mutations (24),

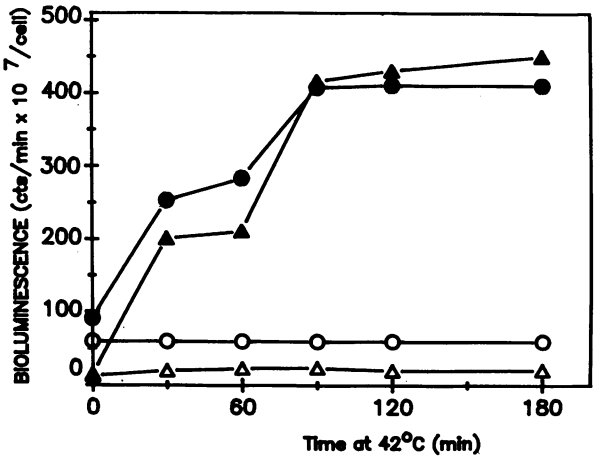

FIG. 3. Dependence of $gamS^+$-dependent plasmid recombination on *sbcB* and *sbcC* mutations. Cultures of *sbcB* (N2375) (▲), *sbcC* (N2364) (△), and *sbcBsbcC* (JC11451) (●) cells harboring pSF117 and pAC604 and a culture of *sbcB sbcC* cells harboring pSF119 and pAC604 (○) were transferred from 28 to 42°C for the indicated times, and bioluminescence activity was determined as described in the legend to Fig. 1.

TABLE 3. Effects of *recF*, *recJ*, and *recQ* mutations on plasmid recombination in wild-type cells and in *sbcB* mutants

Mutation tested			Relative recombination frequency[a]	
recF	*recJ*	*recQ*	*sbcB*$^+$	*sbcB15*
+	+	+	1.0	0.55
143	+	+	0.01	0.15
+	*284*::Tn*10*	+	<0.005	0.02
+	+	*1801*	1.0	0.6

[a] Recombination frequency is defined as the bioluminescence activity of cells harboring pAC604 divided by the activity of cells harboring isogenic plasmids with functional *luxA* genes (30). Relative recombination frequency is recombination frequency of the tested strain divided by recombination frequency of a wild-type (AB1157) strain.

plasmid recombination in RecBCD$^-$ ExoI$^-$ cells is not affected by the *sbcC* mutation.

Conjugational recombination in *recB recC sbcB sbcC* cells depends on the activity of at least seven genes: *recA*, *recF*, *recJ*, *recO*, *recQ*, *recN*, and *ruvA* (9, 15, 19, 25, 32). The effect of four RecF pathway mutations, *recF*, *recJ*, *ruvA*, and *recQ*, on Gam-dependent plasmid recombination in *sbcB* mutants and in *sbcB*$^+$ cells was investigated.

Bioluminescence activity was not detectable in *recF* mutants (JC12334) harboring pAC604 (Table 3) or pAC604 and pSF119 (Fig. 4), but expression of *gamS*$^+$ from pSF117 in the same cells led to an increase in bioluminescence activity (Fig. 4). These observations are consistent with previous results indicating dependence of plasmid recombination in wild-type cells on *rec*F activity (11, 16) and *recF*-independent plasmid recombination in *recB recC* mutants (11). A partial suppression of the *recF* mutation by the *sbcB* mutation in plasmid recombination was suggested by the low level of bioluminescence activity in *recF sbcB* mutants harboring pAC604 (Table 3). This level was about one-third of that in *sbcB* cells harboring the same plasmid. A Gam-dependent increase in bioluminescence activity was apparent in *recF*

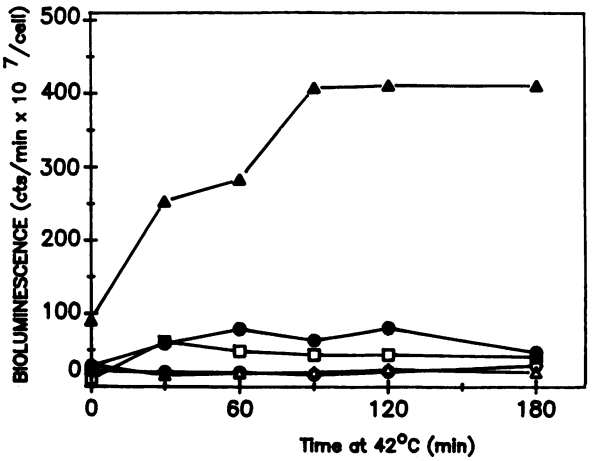

FIG. 4. Effects of *recF* and *recJ* mutations on Gam-dependent plasmid recombination. Cultures of cells of the indicated genotypes harboring pAC604 and either pSF117 or pSF119 were transferred from 28 to 42°C for the indicated times, and bioluminescence activity was determined as described in the legend to Fig. 1. Cultures harboring pSF117 and pAC604 were of the following genotypes: *recF* (JC12334) (□), *sbcB* (JC11451) (▲), *recF sbcB* (AC116) (●), and *recJ sbcB* (AC146) (○). A culture of *recF sbcB* cells (AC116) was cotransformed by pSF119 and pAC604 (△).

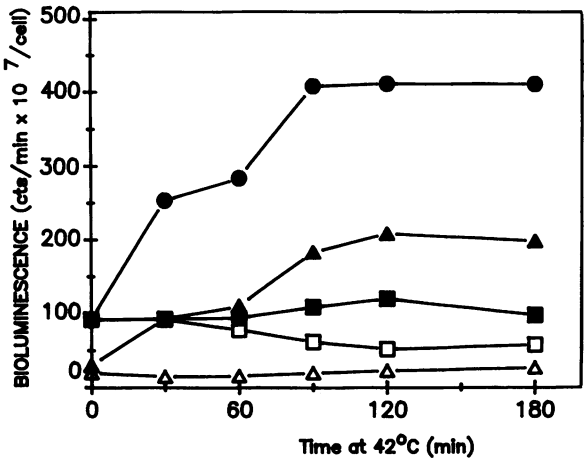

FIG. 5. Effects of *ruvA* and *recQ* mutations on Gam-dependent plasmid recombination in *sbcB* mutants. Cultures of *sbcB* (JC11451) (●), *ruvA sbcB* (AC148) (▲), and *recQ sbcB* (JC15390) (■) cells harboring pAC604 and either pSF117 (filled symbols) or pSF119 (open symbols) were transferred from 28 to 42°C for the indicated times, and bioluminescence activity was determined as described in the legend to Fig. 1.

sbcB cells harboring pSF117 and pAC604 after a temperature shift-up. However, the rate of increase was lower than that observed in *sbcB* cells harboring the same plasmids. Whereas a *recF* mutation only partially inhibited Gam-dependent plasmid recombination in *sbcB* cells, a *recJ* mutation inhibited it completely (Fig. 4). Bioluminescence activity was not detectable after *gamS*$^+$ expression in *recJ sbcB* cells harboring pAC604 and pSF117.

ruvA and *recQ* gene activities are not involved in plasmid recombination in wild-type cells (19; Table 3) but are required for conjugational recombination by the RecF pathway. The effect of mutations in these genes on plasmid recombination in RecBCD$^-$ ExoI$^-$ cells was investigated (Fig. 5). The *ruvA* mutation partially inhibited Gam-dependent recombination in *sbcB* mutants. An increase in the bioluminescence activity is observed after *gam* expression in *ruvA sbcB* cells. The rate of increase, however, was lower than that in *sbcB* cells. On the other hand, expression of *gamS*$^+$ from pSF117 in *recQ sbcB* mutants did not lead to an increase in plasmid recombination activity, which indicated the dependence of plasmid recombination in RecBCD$^-$ ExoI$^-$ cells on *recQ* activity.

Genetic analysis of β-protein-mediated recombination in RecBCD$^-$ ExoI$^-$ cells. Mutations in the *recF*, *recJ*, and *recQ* genes inhibit RecA-mediated plasmid recombination in RecBCD$^-$ ExoI$^-$ cells. To determine the role of these genes in β-protein-mediated recombination, the effect of *bet* expression on Gam-dependent plasmid recombination in *sbcB* cells, mutated in each one of these genes, was determined. A low level of β-protein-mediated, RecBCD nuclease-resistant plasmid recombination activity was apparent in *recF sbcB* cells harboring pIB509 and pAC604 (Fig. 6). Expression of *bet* and *gamS*$^+$ from pIB507 increased the level of β-protein-mediated plasmid recombination activity in *recF* mutants to the level found in *recA sbcB* cells expressing *gamS*$^+$ and *bet*. Similar kinetics of Gam-dependent and β-protein-mediated recombination was observed in *sbcB* cells, mutated in both the *recA* and the *recF* genes. As with *recF*, *recJ* mutation did not affect β-protein-mediated

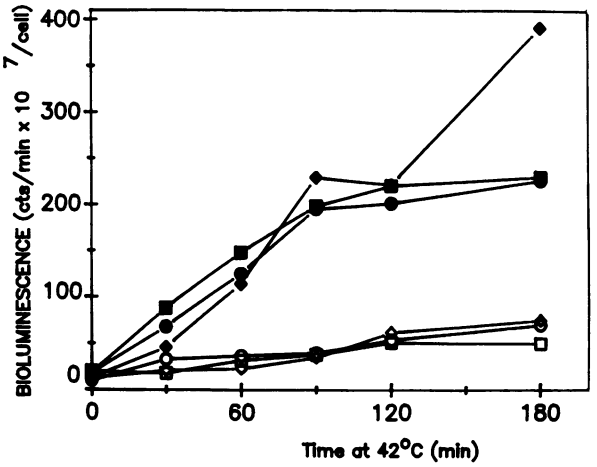

FIG. 6. Effects of *recF* mutation on β-protein-mediated plasmid recombination in RecBCD⁻ ExoI⁻ cells. Cultures of *recA sbcB* (AC135) (●), *recF sbcB* (AC116) (■), and *recA recF sbcB* (AC145) (◆) cells harboring pAC604 and either pIB507 (filled symbols) or pIB509 (open symbols) were transferred from 28 to 42°C, and bioluminescence was determined as described in the legend to Fig. 1.

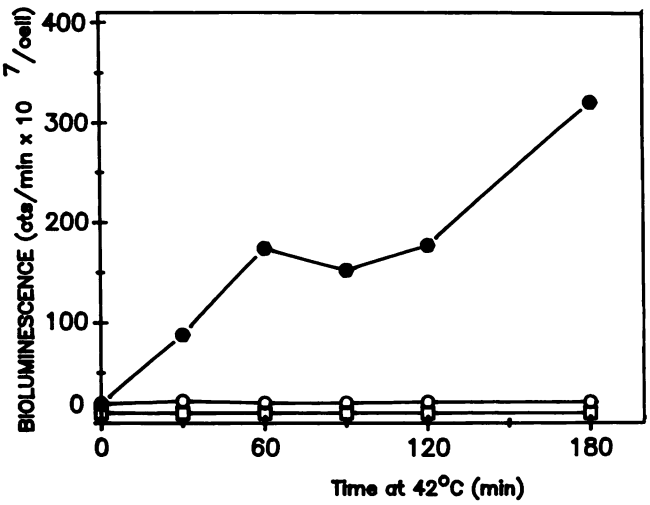

FIG. 8. Dependence of β-protein-mediated recombination on *dnaB* activity. Cultures of *recA recB recC sbcB* (JC15329) (●) and *recA recB recC dnaB558 sbcB* (AC151) (□) cells harboring pAC604 and pSM701 and a culture of *recA recB recC sbcB* cells harboring pAC604 and pSM704 (○) were transferred from 28 to 42°C, and bioluminescence was measured as described in the legend to Fig. 1.

plasmid recombination in *sbcB* mutants (Fig. 7). The kinetics of bioluminescence activity in *recJ sbcB* cells after expression of *bet* from pIB509 or expression of *bet* and *gamS*⁺ from pIB507 was similar to the kinetics after expression of these λ functions in *recA sbcB* cells. *recQ* mutation partially inhibited β-protein-mediated plasmid recombination in RecBCD⁻ ExoI⁻ cells. An increase in bioluminescence activity after *gamS*⁺ and *bet* expression in *recQ sbcB* cells harboring pIB507 and pAC604 was observed, but the rate of increase was lower than that in *recA sbcB* cells harboring the same plasmids.

Dependence of β-protein-mediated recombination on *dnaB* activity. Red-mediated and Gam-dependent plasmid recombination in *sbcB* mutants depends on concurrent DNA replication and on conditions that facilitate plasmid linear-multimer synthesis (33). Presumably, linear plasmid multimers serve as intermediates in plasmid recombination under these conditions. To test whether β-protein-mediated plasmid recombination in RecBCD⁻ ExoI⁻ cells is also replication dependent, *recA recB recC sbcB dnaB558*(Ts) cells harboring pAC604 and pSM701 were transferred from 28 to 42°C, and the effect of the temperature shift on bioluminescence was determined (Fig. 8). In this system, temperature shift-up has a dual effect: it derepresses *c*I857-controlled *gamS*⁺ and *bet* expression from pIB507 and stops DNA replication by inactivating the thermolabile *dnaB* product. No increase in plasmid recombination activity was measured in *recA recB recC sbcB dnaB558* mutants after the temperature shift, which indicated that β-protein-mediated, like RecA-mediated, plasmid recombination in RecBCD⁻ ExoI⁻ cells depends on concurrent DNA replication.

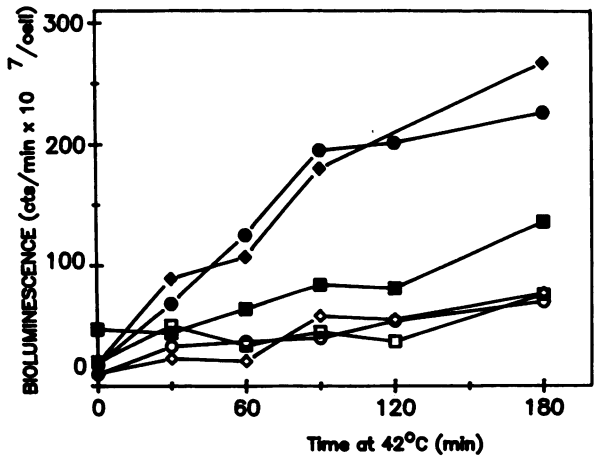

FIG. 7. Effects of *recJ* and *recQ* mutations on β-protein-mediated recombination in RecBCD⁻ ExoI⁻ cells. Cultures of *recA sbcB* (AC135) (●), *recJ sbcB* (AC146) (◆), and *recQ sbcB* (JC15390) (■) cells harboring pAC604 and either pIB507 (filled symbols) or pIB509 (open symbols) were transferred from 28 to 42°C, and bioluminescence was measured as described in the legend to Fig. 1.

DISCUSSION

RecBCD enzyme and exonuclease I inhibit a RecA-mediated plasmid recombination pathway that is more efficient than the pathway functioning in wild-type cells (33). In addition to its difference in sensitivity to these two nucleases, the recombination pathway in RecBCD⁻ ExoI⁻ cells differs from that in wild-type cells by its dependence on DNA replication (33) and on *recQ* activity and by its partial dependence on *ruvA* activity (Table 4). An additional property that distinguishes the two pathways is the ability of β protein to suppress RecA deficiency in recombination in RecBCD⁻ ExoI⁻ but not in RecBCD⁺ ExoI⁺ cells. This distinction may reflect differences between the two backgrounds in the availability of substrates for the homologous pairing reactions catalyzed by RecA and by β protein. RecA protein catalyzes two types of homologous pairing reaction: annealing of complementary single strands (6, 50) and invasion of duplex DNA by homologous single-stranded DNA (28, 37). On the other hand, β protein has been found to catalyze only a strand-annealing reaction (17, 31). The sensitivity of β-protein-mediated recombination to RecBCD enzyme and to exonuclease I suggests that the β-protein-

TABLE 4. Dependence of RecA- and β-protein-mediated recombination in RecBCD⁻ ExoI⁻ cells on RecF pathway gene activities[a]

Recombination system	Dependence on RecF pathway gene			
	recF	*recJ*	*recQ*	*ruvA*
Conjugational	+	+	+	+
Plasmid				
RecA mediated	+	+	+	±
β protein mediated	−	−	+	ND
In RecBCD⁺ ExoI⁺ cells	+	+	−	−

[a] Summary of data in Fig. 4 to 7. +, ±, and −, Dependence, partial dependence, and independence, respectively, of the tested recombination system with respect to the activity of the indicated gene; ND, not determined. Dependencies of conjugational recombination in RecBCD⁻ ExoI⁻ cells (9, 15, 19, 23, 32) and plasmid recombination in RecBCD⁺ ExoI⁺ cells (11, 16, 19) are shown as references.

catalyzed annealing reaction utilizes a substrate (or substrates), such as a 3′ single-stranded end, that is degradable by these two nucleases and is therefore not available in wild-type cells. Stahl and Stahl previously found that Red-mediated recombination in phage λ DNA is inhibited by RecBCD enzyme (43).

The fact that RecA- and β-protein-mediated plasmid recombinations are both sensitive to RecBCD nuclease and exonuclease I suggests that both pathways use a common intermediate that the two nucleases attack.

Genetic and enzymatic analyses of *recF* suppressor mutations have led to the proposal that one difference between the RecBCD and RecF recombination pathways lies in the nature of the presynaptic single-stranded DNA intermediates (27, 49). One interpretation of these findings is that in RecBCD⁻ ExoI⁻ cells, RecA catalyzes a homologous pairing reaction that is distinguishable from the reaction catalyzed in wild-type cells (49). This interpretation and the indication that in RecBCD⁻ ExoI⁻ cells, RecA- and β-protein-catalyzed reactions use a common intermediate raise the intriguing possibility that in this background, RecA- and β-protein-mediated recombination may involve a strand-annealing reaction. An alternative interpretation of these observations is that both RecA and β protein utilize a common substrate for different homologous pairing reactions. These alternative explanations may also be applied to the differences between RecA- and β-protein-mediated plasmid recombination in their dependence on RecF pathway activities (Table 4). These differences may indicate that β protein and RecA are involved in two separate recombination pathways. On the other hand, they could imply that the two enzymes play a similar role in one pathway, which is sensitive to RecBCD nuclease and exonuclease I, with RecA activity in this pathway depending on *recF* and *recJ* functions.

A direct role for *recF* activity in a RecA-catalyzed reaction is suggested by the finding that the *recA* mutation, *recA803*, suppresses the *recF143* mutation in promoting recombination in *recB recC sbcB* mutants (27). It is plausible that the homologous pairing reaction is RecF dependent when catalyzed by RecA and is RecF independent when catalyzed by β protein or RecA803. The RecA803-catalyzed homologous pairing reaction is less susceptible to increased SSB concentration than is the reaction catalyzed by RecA (27). This observation and the suppression of *recF143* by *recA803* have led to the proposal that RecF assists RecA in removing SSB from SSB–single-stranded DNA or in the formation of a RecA–single-stranded DNA complex that resists competition with SSB (27). Since β protein, like SSB, is a helix-destabilizing protein, a β protein–single-stranded DNA complex may resist SSB competition independently of RecF activity. Understanding of the mechanism by which β-protein activity suppresses *recJ* mutations in plasmid recombination must await further characterization of the role of *recJ* activity in RecA-mediated recombination.

Functions of at least four genes, *recA*, *recF*, *recJ*, and *recQ*, which are required for conjugational recombination by the RecF pathway, are also involved in plasmid recombination in RecBCD⁻ ExoI⁻ cells (Table 4). Differences between the genetic requirements for plasmid versus conjugational recombination have been noticed. Conjugational recombination in RecBCD⁻ ExoI⁻ cells depends on *sbcC* mutations, but plasmid recombination in the same background is not affected by the *sbcC* genotype. Mutations in the *ruvA* gene decrease the recombination frequency in both systems, but the decrease affects plasmid recombination less than conjugational recombination. These differences between the two recombination systems may stem from differences in substrate structure or molecular mechanism. However, since the genetic requirements of conjugational recombination were determined by scoring viable recombinants and those for plasmid recombination were determined by assaying transcribable products, one cannot rule out the possibility that these differences are due to the levels at which recombination activities are assayed in the two systems.

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