Properties of *Escherichia coli* Expressing Bacteriophage P22 Abc (Anti-RecBCD) Proteins, Including Inhibition of Chi Activity

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Escherichia coli strains bearing plasmids expressing phage P22 anti-RecBCD functions *abc1* and *abc2* were tested for the presence of *recBC*-like phenotypes. *Abc2* induces moderate sensitivity to UV light in wild-type and *recD* mutant strains but severely sensitizes both *recF* and *recJ* mutants. *Abc1* has little effect on UV sensitivity in wild-type or *recF* or *recJ* mutant hosts but increases the sensitivity of *recD* mutants to a UV dose of 20 J/m² about 10-fold. *Abc2* induces *E. coli* to segregate inviable cells during growth, interferes with the growth of λ *red gam* χ^+ and χ^0 phage (the effect is greater with χ^+ phage), inhibits Chi and Chi-like activity as measured by λ *red gam* crosses, and prevents SOS induction in response to nalidixic acid; *Abc1* has no effect in these tests. *Abc2*, alone or with *Abc1*, does not allow the growth of λ *red gam* in the presence of a P2 prophage but does not kill the P2 lysogenic host (as λ Gam does). Finally, *Abc2* inhibits conjugational recombination in wild-type cells to the level seen in *recBC* mutants. These data suggest that *Abc2* inhibits the recombination-promoting ability of RecBCD but leaves the exonuclease functions intact.

The RecBCD enzyme is a multifunctional protein capable of promoting homologous recombination in *Escherichia coli*. *recB* or *recC* mutants are deficient for recombination, sensitive to DNA-damaging agents, and unable to restrict foreign DNA in vivo (e.g., T4 gene 2 mutant phage DNA) and produce inviable cells in culture (for reviews, see references 44 and 54). These deficiencies are the result of loss of one or more of the enzymatic activities of the RecBCD enzyme. These activities include highly processive ATP-dependent double-stranded DNA (dsDNA) exonuclease and helicase activities, an ATP-dependent single-stranded DNA (ssDNA) exonuclease, an ATP-stimulated ssDNA endonuclease, and a DNA-dependent ATPase (for reviews, see references 54 and 59). RecBCD can also promote nicking on the 3' side of Chi sites (5'GCTGGTGG3') in vitro (32, 58). Chi sites are known to be hotspots for recombination in the RecBCD pathway (49, 52).

E. coli recD mutants are recombinationally proficient (in some instances, hyper-Rec), resistant to DNA damage, and as viable as wild-type cells (2, 6, 20). However, like *recBC* mutants, *recD* mutant strains allow the growth of T4 gene 2 mutant phage (2). In vitro examination of the RecBC(D⁻) enzyme shows that this species retains significant amounts of the ssDNA endonuclease and helicase activities but lacks both ssDNA and dsDNA exonuclease activities (31). Thus, the lack of exonuclease functions prevents restriction of foreign DNA yet has little effect on the levels of RecBC-promoted recombination and repair. However, RecBC(D⁻)-promoted recombination is qualitatively modified; unlike the RecBCD pathway and like the λ *red* pathway, exchanges among unreplicated λ phages are concentrated near double-stranded ends and are not influenced by Chi (6, 51, 53, 60). Additionally, conjugational recombination and UV resistance in *recD* mutant strains become dependent on *recJ*

function (20, 26); this is not true for RecBC(D⁻)-promoted λ *red gam* mutant crosses (60).

Current models for the mechanism of RecBCD-promoted recombination suggest that the enzyme binds to a flush or nearly flush dsDNA end and travels along the DNA by either unwinding or degrading one or both strands (44, 53–56). The energy for this process is provided by RecBCD's ATPase activity. At some point, RecBCD encounters a correctly oriented Chi site and is modified so that the enzyme becomes recombinogenic, capable of promoting and/or resolving Holliday junction-type intermediates (47, 53, 57, 60). This modification occurs at high frequency at Chi sites (for details, see references 44, 45, 50, and 54).

The role of dsDNA ends in RecBCD enzymology places dsDNA-containing phages at high risk following infection. As a result, the bacteriophages of *E. coli* (and *Salmonella typhimurium*) have developed defense mechanisms to counteract the deleterious effects of one or more of the activities of RecBCD (3, 30, 39). Phages T4 and Mu encode proteins (gene 2 product and Gam, respectively) which bind to the ends of the chromosomes and prohibit access of RecBCD to the DNA ends (29, 61). Presumably, this mode of defense affords protection from all host cell exonucleases. In other instances, RecBCD is targeted directly. For example, the λ Gam protein binds to RecBCD and inhibits all the known activities of the enzyme (17, 26). Hosts containing Gam-producing plasmids display most (but not all) of the phenotypes associated with *recBC* mutations (12, 26).

Bacteriophage P22's defense mechanism against RecBCD is provided by two genes, *abc1* and *abc2* (anti-RecBCD). These genes are necessary for prolific growth of P22 in wild-type hosts but not in *recBC* mutants (28). While both functions are required independently to restore wild-type plaque size to *abc* region deletion mutants, *abc2* has the greater effect. Previous studies (33) and unpublished observations have shown that *abc2* encodes a protein that binds directly to RecBCD, similar to the λ Gam protein. However, unlike Gam, *Abc2* (with or without *Abc1*) cannot comple-

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ment T4 gene 2 mutant phage, promote plasmid multimerization in *sbcB* strains, or support the growth of λ *red gam* phage in *recA* hosts (33, 34). On the other hand, Abc2 is more effective than Gam at inhibiting both RecBCD-promoted λ *red gam* recombination in a Chi-containing interval (33) and Hfr-mediated conjugational recombination (37).

Clearly, the modifications of RecBCD by P22 Abc and the λ Gam protein are quite different. In this study, we have further investigated the properties of *E. coli* strains expressing plasmid-encoded Abc. We find that Abc sensitizes *E. coli* to UV light, induces inviable-cell formation, does not kill P2 lysogens as λ Gam does, inhibits the growth and recombination of λ *red gam* phage, silences Chi, and prevents induction of the SOS response by nalidixic acid. We also find that Abc, despite its incomplete inactivation of RecBCD, is capable of reducing conjugational recombination to the levels seen in *recBC* mutants.

MATERIALS AND METHODS

Bacteria and phages. *E. coli* W3110 *lacI^r* L8 (5) was used for propagation of plasmids. *E. coli* AB1157 (*argE3 his-4 leuB6 proA2 thr-1 ara-14 galK2 lacY1 mtl-1 xyl-5 thi-1 rpsL31 tsx-33 supE44*) and isogenic strains JC5519 (*recB21 recC22*) and JC9239 (*recF*) were obtained from A. J. Clark. KM353 (*recD*) and KM354 (*recJ*) are otherwise isogenic to AB1157 and have been described previously (26). FS2152 (*recD* *Su⁻ hflA*) was obtained from F. Stahl. TP337 (Hfr PO3 *metB1 relA1 spoT1 thr-35::Tn9 fuc-3072::Tn10*) has been described previously (26). QD5003 (*mel-1 supF58*) was obtained from M. Susskind; QD5003 lysogenized with P2 was obtained from S. Adhya. DM4000 [*sulA::Mu-d(bla lac)::Tn9*] Δ (*lac-pro*)X111 *hisG4 argE3 thr-1 ara-14 xyl-5 mtl-1 rpsL31 tsx-33 ilv*(Ts) *Su⁻* was obtained from M. Volkert. λ MMS556 (*Rsus5 b1453 χ D*), λ MMS558 (*Rsus5 b1453 χ 76*), λ JMC307 (*Jsus6 b1453 cI857 χ^0*), λ REM267 (*Rsus5 b1453 cI857 χ^0*), λ MMS659 (*b1453 cI857 χ^0*), and λ MMS885 (*b1453 cI857 χ D*) were obtained from F. Stahl. λ KM1 (*b1453 χ^0*) was obtained by crossing λ MMS556 with λ JMC307 and selecting turbid plaques that grew well on FS2152 but poorly on AB1157. λ KM2 (*Rsus5 b1453 χ^0*) was obtained by crossing λ KM1 with λ REM267 and selecting turbid plaques that grew well on KM353 but not on FS2152.

Plasmids. Plasmids were constructed and propagated by standard techniques (23). Plasmids pTP30, pTP550, pKM331, pKM446, pKM452, and pKM590 have been described previously (4, 26, 28, 41). To construct pKM579, the 95-bp *PvuII-EcoRI* *P_{lac}*-containing fragment from pTP30 was filled in with the Klenow fragment and ligated to the *EcoRI*-cut, filled-in, dephosphorylated backbone of pTP550. *P_{lac}* reads clockwise in the conventional sense of the pBR322 map. For pKM581, the 460-bp *RsaI-XmnI abc1*-containing fragment from pKM331 was ligated to *EcoRI* linkers, cut with *EcoRI*, isolated on low-melting-point agarose, and ligated to *EcoRI*-digested pKM579 so as to create a *P_{lac}-abc1* operon fusion. To construct pKM583, the 397-bp *P_{lac}-abc2*-containing *EcoRI* fragment from pKM446 was ligated into the *EcoRI* site of pTP550; *P_{lac}* is transcribed clockwise. For pKM584, the 885-bp *P_{lac}-abc1,abc2*-containing *EcoRI* fragment from pKM452 was ligated into the *EcoRI* site of pTP550; *P_{lac}* is transcribed clockwise. These plasmids are similar to Abc-producing plasmids described earlier (28) except that the P22 *t_{ant}* transcription terminator is situated between the *P_{lac}-abc* fusions and the pBR322 gene conferring tetracycline resistance. These plasmids also contain the *lacI^r* promoter and gene sequence from pMC7 (41). In the

experiments described in this article, the *abc* functions are induced in vivo by the addition of 1 mM isopropylthiogalactopyranoside (IPTG) to plasmid-bearing host cultures.

General. LB, TB, and M9 media have been described previously (23); M9CAA is M9 supplemented with 0.3% casamino acids, 0.4% glucose, and 10 mM MgSO_4 . Phage platings, UV sensitivity measurements, and Chi assays were done as described previously (26, 28). Chi assays were performed several times. Although recombination levels varied in experiments done on different days with different phage stocks, the relative recombination rates among the strains tested remained the same. A representative experiment is shown in Table 4.

Viability measurements. Host cells containing control, Abc-, or Gam-producing plasmids were grown overnight in LB containing 12 μg of tetracycline per ml. Fresh cultures were started up by dilution in the same medium and rolled at 37°C, with IPTG added to a final concentration of 1 mM. The cultures were grown to densities of 2×10^8 cells per ml, diluted 100-fold in the same medium, and regrown to a final concentration of 2×10^8 /ml. The cell density was determined by counting the cells with a microscope in a Petroff-Hausser bacterial counting chamber. The cells were diluted in LB and plated immediately on LB plates containing 1 mM IPTG and 12 μg of tetracycline per ml. Viability is determined by the number of visible cells that are capable of forming colonies after a 24-h incubation at 37°C. The viability values shown in Table 2 are averages from three experiments (with standard deviations) performed on different days.

Burst size determination. Host cells were grown overnight at 37°C in TB containing 0.4% maltose and 10 mM MgSO_4 . Fresh cultures (5 ml) were started in LB broth containing 10 mM MgSO_4 . For plasmid-bearing strains, tetracycline was present at 12 $\mu\text{g}/\text{ml}$. When the cells reached a density of $10^7/\text{ml}$, IPTG was added to a final concentration of 1 mM. At a density of $2 \times 10^8/\text{ml}$, the culture was divided in half and infected with either λ MMS659 (*b1453 cI857 χ^0*) or λ MMS885 (*b1453 cI857 χ D*) at a multiplicity of infection of 0.5, and the phage were allowed to adsorb for 15 min at 37°C. Portions of the cultures were removed for counting unadsorbed phage on strain KM353 (*recD*). The number of infected centers was corrected by subtracting the number of unadsorbed phage. The cultures were then diluted 10^4 -fold with warm LB containing 12 μg of tetracycline per ml and 0.1 mM IPTG. After aeration for 2 h at 37°C, a few drops of chloroform were added to the infected cultures, and the phage in the lysates were titered on KM353 (*recD*). Burst sizes are reported as the number of phage produced per infected cell. Experiments were done several times; a representative set is shown in Table 3.

Conjugational recombination. Recombination following conjugation was done essentially as described earlier (26). Donor cells (TP337) were grown overnight in LB medium containing 25 μg of chloramphenicol per ml. Recipient strains (AB1157 or derivatives thereof), bearing control or Abc-producing plasmids, were grown overnight in LB containing 100 μg of streptomycin per ml and 12 μg of tetracycline per ml. Fresh cultures were started in LB medium (for the donors) and LB medium containing 12 μg of tetracycline per ml (for the recipients). When the cells reached a density of $10^7/\text{ml}$, IPTG was added to the recipient cultures to a final concentration of 1 mM. Recipient cultures were rolled at 37°C until they reached a concentration of 3×10^8 to $4 \times 10^8/\text{ml}$; the donor culture was swirled slowly until it reached a concentration of $10^8/\text{ml}$. Both donor and recipient cultures were diluted with warm LB containing 1 mM IPTG to final

concentrations of 4×10^7 and 2×10^8 cells per ml, respectively, just before mixing. Mating mixtures, containing a 2:1 (vol/vol) ratio of recipient to donor cultures were kept at 37°C for 45 min, diluted 10-fold with M9CAA medium, and vortexed. Exconjugants were grown and counted on LB plates containing both streptomycin (100 µg/ml) and chloramphenicol (25 µg/ml) with and without 1 mM IPTG. Donor and recipient cells by themselves showed no growth on exconjugant selection plates.

SOS induction experiments. Host cells (DM4000) containing control, Abc-, or Gam-producing plasmids were grown overnight in LB broth containing 12 µg of tetracycline per ml. Fresh cultures (15 ml) were started with a 1:200 dilution of the overnight culture into M9CAA medium containing 12 µg of tetracycline per ml and swirled at 37°C. When the cultures reached density of 10^7 cells per ml, IPTG was added to a final concentration of 1 mM. When the cultures reached a cell density of 3×10^8 to 5×10^8 per ml (A_{600} ~0.6), various concentrations of nalidixic acid were added to 2-ml portions of the cultures. The cultures were further incubated for 1 h, and β -galactosidase assays were performed as described by Miller (25).

Nomenclature. The term Abc is used throughout this article to describe the effects of Abc2, with or without the presence of Abc1. When Abc1 displays a significant effect or contributes to the effects of Abc2, the two functions are described separately. *E. coli* strains bearing a plasmid that produces Abc under the control of P_{lac} are designated Abc⁺ hosts. The term Chi is used to describe the hotspot sequence 5'-GCTGGTGG3' in general. Chi activity refers to the geometric mean of the factor by which Chi alters the ratio of turbid to clear plaques among the J^+R^+ recombinants from the crosses diagrammed in Fig. 2 (see reference 52 and footnote *d* in Table 4 for calculation of Chi activity). As described previously (22), the terms χ^+ and χ^0 are used to designate the active orientation and the inactive orientation (or absence) of Chi, respectively, in phage λ .

RESULTS

UV sensitivity of Abc⁺ hosts. Plasmids that express the anti-RecBCD functions (Abc) from phage P22 were tested for their ability to sensitize *E. coli* to UV radiation. The results are shown in Fig. 1. Abc1 increased the sensitivity of wild-type *E. coli* to UV light about two- to fourfold, while Abc2 increased the UV sensitivity about 25-fold. When expressed together, Abc1 and Abc2 sensitized wild-type cells to the level seen in a *recBC* mutant strain (50- to 100-fold increase in UV sensitivity). These results parallel the effects of Abc1 and Abc2 on restoring wild-type plaque sizes to P22 Δabc deletion mutants (28): a small effect with Abc1, a much greater effect with Abc2, and full complementation with both Abc proteins. When P22 Abc and λ Gam were compared in the same experiment, Abc was always more effective than Gam at increasing the level of UV sensitivity of a wild-type strain to that of a *recBC* mutant strain (data not shown).

The sensitivities of *E. coli* mutant strains containing Abc-producing plasmids to UV light at a dose of 20 J/m² were also examined; the results are shown in Table 1. Abc1 and Abc2, together or singly, did not further increase the UV sensitivity of a *recBC* mutant, arguing against the possibility that modification of other potential targets of Abc has a role in UV sensitization. Abc2 alone or in combination with Abc1 increased the UV sensitivity of a *recD* mutant strain to that observed for a *recBC* mutant; Abc1 alone increased the

FIG. 1. UV sensitivity of *E. coli* containing Abc-producing plasmids. Cells were grown by aeration in LB; tetracycline (12 µg/ml) was added to cultures of plasmid-bearing hosts. IPTG was added to 1 mM. After continued aeration, the cells were spun down and resuspended in 0.5 volume of M9CAA medium. The cells were then diluted in M9CAA and spread on LB plates containing tetracycline and IPTG. Plates were exposed to the indicated doses of UV and incubated overnight at 37°C in the dark. The cells on plates containing between 100 and 200 surviving colonies were counted and compared with the number on unirradiated plates to determine percent survival. A *recBC* mutant (△) and wild-type strains carrying plasmids expressing phage gene *abc1* (■), *abc2* (○), *abc1* plus *abc2* (●), or no phage gene (control plasmid) (□) were tested.

sensitivity of a *recD* strain about 10-fold. Furthermore, Abc2 dramatically increased the sensitivity of both *recF* and *recJ* mutant strains to UV radiation; Abc1 had no effect in these mutants.

These experiments show that hosts carrying an Abc-producing plasmid (Abc⁺ hosts) display a phenotype similar to that of *recBC* mutants with respect to UV sensitivity: they are moderately UV sensitive and become extremely UV sensitive in the presence of mutations in *recF* or *recJ* (14, 19). This is in contrast to Gam⁺ hosts, which are extremely sensitive only in the presence of a *recJ* mutation (26). Abc1⁺ *recD* mutants are more sensitive to UV radiation than Abc1⁺ wild-type hosts. It is possible that Abc1 binds directly to RecBCD but its effects are masked by the exonuclease activities or that RecBC(D⁻) is a more susceptible target for Abc1. However, there is no biochemical evidence that the effects of Abc1 are dependent on a direct interaction with RecBCD.

Viability of Abc⁺ hosts. *E. coli recBC* mutant strains segregate inviable cells during growth (13); the nature of this inviability is not well understood. It has been shown before that Gam can cause the same effect (12). The viability of Abc⁺ hosts was tested and compared with that of Gam⁺ hosts and *recBC* mutant strains. Cells were grown in the presence of IPTG for several generations, counted with a microscope, and then plated in the presence of IPTG to determine the number of CFU. Viability is measured as the percentage of visible cells that go on to form colonies. Table 2 shows that Abc⁺ hosts exhibited lower viability (44%) than control cells. This value is a bit higher than that for either Gam⁺ hosts (27%) or *recBC* strains (20%). Further dilutions in medium containing IPTG to increase the number of generations prior to plating did not further decrease the viability of Abc⁺ hosts (data not shown).

TABLE 1. UV sensitivity of *E. coli* strains containing Abc-producing plasmids^a

Host strain	Plasmid	abc gene(s) on plasmid	% Surviving cells after UV irradiation
AB1157 (wild type)	pKM579	None	6.1
	pKM581	<i>abc1</i>	1.5
	pKM583	<i>abc2</i>	0.36
	pKM584	<i>abc1,2</i>	0.12
JC5519 (<i>recBC</i>)	pKM579	None	0.15
	pKM581	<i>abc1</i>	0.15
	pKM583	<i>abc2</i>	0.16
	pKM584	<i>abc1,2</i>	0.14
KM353 (<i>recD</i>)	pKM579	None	5.0
	pKM581	<i>abc1</i>	0.58
	pKM583	<i>abc2</i>	0.12
	pKM584	<i>abc1,2</i>	0.11
JC9239 (<i>recF</i>)	pKM579	None	0.60
	pKM581	<i>abc1</i>	1.5
	pKM583	<i>abc2</i>	<0.0001
	pKM584	<i>abc1,2</i>	<0.0001
KM354 (<i>recJ</i>)	pKM579	None	6.6
	pKM581	<i>abc1</i>	5.8
	pKM583	<i>abc2</i>	<0.0001
	pKM584	<i>abc1,2</i>	<0.0001

^a Cells were grown in LB containing tetracycline and IPTG, collected by centrifugation, resuspended in M9 medium, and placed on ice. Dilutions of the resuspended cultures were spread on LB plates containing tetracycline and IPTG and irradiated with a UV lamp (254-nm wavelength) at a dose of 20 J/m². The plates were incubated overnight in the dark at 37°C, and colonies were counted on the following day; unirradiated plates were used to determine cell counts at the time of UV exposure.

Consistent with the generation of inviable progeny, Abc⁺, Gam⁺, and *recBC* mutant strains all displayed moderate filamentation (about 1% of the culture). Despite these similarities, differences were noted in the growth properties of the colonies in these experiments. For instance, *recBC* mutant cells but not Abc⁺ or Gam⁺ host cells were generally small and tended to form clumps after a few minutes in the counting chamber. Furthermore, colonies of Gam⁺ hosts but not of Abc⁺ hosts or *recBC* strains varied in size and displayed slightly serrated edges. These results are consistent with earlier reports (33) and the results described below that the properties of Abc⁺ hosts are different from those of Gam⁺ hosts and that neither case truly resembles the properties of null mutations in *recBC*.

Abc inhibits the burst size of χ^+ λ phage. λ red gam phage

TABLE 2. Viability of *E. coli* strains containing Abc- and Gam-producing plasmids^a

Host strain	Plasmid (phage gene)	Viability
AB1157 (wild type)	pKM579 (none; control)	0.96 ± 0.07
	pKM583 (<i>abc2</i>)	0.44 ± 0.02
	pKM584 (<i>abc1, abc2</i>)	0.43 ± 0.01
	pKM590 (<i>gam</i>)	0.27 ± 0.05
JC5519 (<i>recBC</i>)	pKM579 (none; control)	0.20 ± 0.03

^a Cells were grown in LB containing tetracycline and IPTG as described in Materials and Methods. Cells were counted with a microscope, and the cultures were diluted and plated on LB plates containing tetracycline and IPTG. Viability was calculated as CFU/number of cells. Values listed are the averages of three experiments done on different days ± standard deviation.

grow poorly on a wild-type host. The defect lies in the phage's inability to produce concatemeric forms of its chromosome, the immediate precursor to packaging (11). This deficiency is alleviated if either RecBCD exonuclease is deactivated (by mutation or the presence of λ Gam), which permits rolling-circle replication to ensue, or the phage contains a Chi site, with which phage DNA monomers can recombine via the RecBCD pathway to produce a dimeric substrate for packaging. We tested the effects of Abc and Gam on the burst size of χ^+ and χ^0 λ red gam phage; the results are shown in Table 3.

In wild-type cells containing control plasmid pKM579, χ^+ phage showed a fivefold increase in burst size relative to χ^0 phage, as expected (21, 52). When Abc was present (Table 3, experiments A and B), Chi did not confer a growth advantage on λ red gam phage. This effect was also evident from the identical plaque sizes of χ^+ and χ^0 phages on wild-type Abc⁺ hosts (see below). In wild-type hosts expressing the λ Gam protein (Gam⁺ hosts), an increase in burst size relative to that in the Abc⁺ host was detected and was independent of the presence of Chi. This is consistent with the fact that Gam promotes rolling-circle replication by inhibiting RecBCD (11, 17) but, in so doing, inhibits Chi-activated recombination (26, 52). The burst sizes in *recD* mutants were the highest. In this host, the phage may take advantage of both rolling-circle replication and an active RecBC pathway to promote concatemerization. Alternatively, the smaller burst size in the Gam⁺ host relative to the *recD* mutant strain may be due to the absence of the plasmid in the latter (see below). Finally, Abc1 had no effect on the growth of χ^+ or χ^- phage.

Growth of λ red gam was also tested in a *recD* mutant host bearing Abc-producing plasmids (Table 3, experiment C). The presence of the control plasmid in *recD* hosts decreased λ red gam burst size twofold; this was not true for wild-type hosts (data not shown). This inhibition of phage growth is interpreted to be a consequence of the fact that *recD* hosts generate linear multimers of plasmid DNA, resulting in competition between the resident plasmid and incoming phage for host DNA replication functions. In any case, the Abc-producing plasmid (pKM583), relative to the control plasmid (pKM579), inhibited λ red gam phage growth in *recD* mutant strains, suggesting that RecBC(D⁻) is a target for Abc. This inhibition of λ red gam growth was independent of the presence of Chi (as expected, since *recD* mutants are blind to Chi).

Gam-producing plasmids also inhibited the growth of λ red gam in *recD* strains (Table 3, experiment C). This result is counterintuitive, because one normally thinks of Gam as promoting the growth of λ red gam phage. However, the RecBCD exonuclease-inhibiting function of Gam will not promote phage growth in a host in which exonuclease is already inactivated (*recD*). It may be that competition between the linear plasmid multimers and the phage chromosome for rolling-circle replication functions makes the phage more dependent on the RecBC(D⁻) recombination pathway for growth. This inhibition (which was also observed in plating experiments; data not shown) suggests that Gam can modify some activity of the RecBC(D⁻) enzyme, consistent with earlier results (26).

Abc inhibits RecBCD Chi activity. It has previously been shown that, in vivo, production of Abc inhibits RecBCD-promoted λ red gam recombination within a Chi-containing interval and less so or not at all in a non-Chi-containing interval (33). Here, a formal measurement of Chi activity was performed with the phages described by Stahl and Stahl

TABLE 3. Burst sizes of χ^+ and χ^0 λ *red gam* phages^a in the presence of Abc- and Gam-producing plasmids

Expt	Strain	Plasmid (phage gene)	Burst size ^b		Ratio, χ^+/χ^0
			χ^+	χ^0	
A	AB1157 (wild type)	pKM579 (none; control)	120.2	20.2	5.9
		pKM583 (<i>abc2</i>)	23.8	26.0	0.92
		pKM590 (<i>gam</i>)	70.6	68.6	1.0
	KM353 (<i>recD</i>)	None	159.0	143.5	1.1
B	AB1157 (wild type)	pKM579 (none; control)	108.6	20.2	5.4
		pKM581 (<i>abc1</i>)	111.3	18.3	6.1
		pKM583 (<i>abc2</i>)	25.2	32.3	0.78
		pKM584 (<i>abc1</i> + <i>abc2</i>)	24.2	24.1	1.0
C	KM353 (<i>recD</i>)	None	159	138	1.1
		pKM579 (none; control)	60	61	1.0
		pKM583 (<i>abc2</i>)	32	26	1.2
		pKM590 (<i>gam</i>)	30	23	1.3
	JC5519 (<i>recBC</i>)	pKM579 (none; control)	30	28	1.1

^a λ MMS885 (*b1453 cI857* χ^D) and λ MMS659 (*b1453 cI857* χ^0).^b Number of phage produced per infected cell.

(52), as shown in Fig. 2. In these experiments, both Chi activity (see legend to Fig. 2) and recombination were measured for each cross. The results are shown in Table 4. Abc inhibited Chi activity to the level seen in *recD* mutants (and *recBC* mutants; data not shown). However, unlike the *recD* mutant, which exhibited wild-type levels of recombination, Abc⁺ hosts displayed a 10-fold decrease in the rate of recombination. This phenotype is similar to that seen in *E. coli* expressing the λ Gam protein (26) and suggests that Abc, like Gam, inhibits Chi-stimulated recombination. Abc⁺ hosts, however, consistently showed a more complete inhibition of Chi activity than Gam⁺ hosts (1.0 versus 1.5, respectively). Abc1 had little effect on Chi activity and no effect on the rate of recombination.

A third cross was performed in addition to the two crosses used to calculate Chi activity. In this cross, neither parent had a Chi site (χ^0 cross). This cross was performed to examine the effects of Abc on the distribution of exchanges and the rate of recombination in the absence of a known Chi site. In such a scenario, the combined effects of all the Chi-like sites would be expected to contribute to the overall

rate of recombination. (Chi-like sites are defined by sequences that differ from Chi by 1 bp. There are 44 such sites in the λ chromosome which are correctly oriented to be active in these crosses. Some of these sites are known to promote low-level Chi activity; see references 8 and 9.) These results for these χ^0 crosses are shown in Table 4. In the wild-type host containing control plasmid pKM579, recombination rates in the χ^0 crosses were only twofold lower than those in isogenic χ^+ crosses. This modest decrease in recombination is presumably due to the presence of Chi-like sites in these phages. When Abc was present during the χ^0 cross, recombination rates fell an additional fivefold, to the level seen in the χ^+ crosses in the presence of Abc.

TABLE 4. Chi assays

Host/plasmid (phage gene)	Cross ^a	% Recombination ^b	Relative burst size ^c	Chi activity ^d
AB1157/pKM579 (none; control)	1	13.0	1.0	10.2
	2	10.3	0.79	
	χ^0	5.0	0.19	
AB1157/pKM581 (<i>abc1</i>)	1	10.5	0.86	7.7
	2	12.8	0.55	
	χ^0	5.1	0.17	
AB1157/pKM583 (<i>abc2</i>)	1	1.0	0.34	0.9
	2	1.1	0.22	
	χ^0	1.3	0.26	
AB1157/pKM584 (<i>abc1</i> + <i>abc2</i>)	1	0.93	0.31	1.0
	2	0.74	0.18	
	χ^0	0.92	0.12	
KM353 (<i>recD</i>)/none	1	7.8	1.4	1.1
	2	12.7	1.2	
	χ^0	9.1	1.1	

^a Cross 1, λ MMS556 $\times$ λ JMC307; cross 2, λ MMS558 $\times$ λ JMC307; χ^0 cross, λ KM2 $\times$ λ JMC307.^b (No. of phage on a *recD* Su⁻ host/no. of phage on a *recD* Su⁺ host) $\times$ 100.^c Relative to that of the control; 1.0 = 55 phage per infected cell.^d Chi activity = [(no. of turbid plaques/no. of clear plaques in cross 1) $\times$ (no. of clear plaques/no. of turbid plaques in cross 2)]^{1/2}.

FIG. 2. Chi activity determination. Diagrams of the phages used to determine Chi activity as described in the text and by Stahl and Stahl (52). Cross 1 is λ MMS556 $\times$ λ JMC307; cross 2 is λ MMS558 $\times$ λ JMC307. The *b1453* deletion (boxed area) removes λ 's *red* and *int* recombination systems; therefore, these phage recombine via the RecBCD pathway. Chi activity was calculated as described in Table 4, footnote d. This value is equal to 1 if Chi is inactive.

TABLE 5. Exchanges in the *cI-R* interval and in the *J-cI* interval^a

Strain/plasmid (phage gene)	Ratio, turbid plaques/clear plaques		
	Cross 1	Cross 2	χ^0 cross
AB1157/pKM579 (none; control)	4.0	0.037	0.33 ^b
AB1157/pKM581 (<i>abc1</i>)	3.6	0.060	0.33
AB1157/pKM583 (<i>abc2</i>)	0.58	0.62	0.71
AB1157/pKM584 (<i>abc1</i> + <i>abc2</i>)	0.74	0.65	0.74
KM353 (<i>recD</i>)/none	0.68	0.69	0.74

^a Expressed as the ratio of turbid plaques to clear plaques in the crosses diagrammed in Fig. 2. See Table 4, footnote *a*, for crosses.

^b This value reflects the activity of the Chi-like sites in these intervals. Of the 44 correctly oriented Chi-like sites in λ , 8 are within the *J-cI* interval, 5 are within the *cI-R* interval, 6 are within the *J* gene, and 1 is within the *R* gene.

This result is interpreted to be due to Abc's inhibition of both Chi and Chi-like activity.

One could argue that the drop in recombination caused by Abc in the χ^0 crosses is due to inhibition of some Chi-like independent mechanism (that is, a mechanism that does not involve activation of RecBCD by a Chi-like site but simply relies on one or more of the Chi-independent biochemical activities of the enzyme). One way to answer this question is to examine the distribution of exchanges between the defined intervals in these crosses. If all the Chi-like sites within the *J-cI* and *cI-R* intervals were of equal densities and strengths, one would not expect any change in the distribution of exchanges upon their inactivation by Abc in these freely replicating crosses. However, given the probability that most Chi-like sites are weak or inactive, the distribution of exchanges in the χ^0 crosses is probably dependent on a few very active Chi-like sites. If so, then Abc's inhibition of Chi-like activity would alter (albeit to a small degree) the distribution of exchanges. Furthermore, if Abc does inactivate Chi-like sites, the distribution of exchanges in Abc⁺ χ^0 (and χ^+) crosses should be the same as that seen in hosts in which Chi is inactive (*recD*).

Both of these predictions were fulfilled (Table 5). In both χ^0 crosses without Abc, the ratio of exchanges in the *cI-R* to those in the *J-cI* intervals was 0.33. This number is presumably a reflection of the combined effects of all the Chi-like sites distributed throughout the λ chromosome within these intervals (see footnote *b* in Table 5). With Abc present, this ratio increased twofold in the χ^0 crosses. Furthermore, the distribution-of-exchange ratio for all crosses (both χ^0 and

χ^+) containing Abc was the same as in *recD* strains (0.68 ± 0.05 [standard deviation]). Thus, these data support the contention that Abc makes RecBCD blind to both Chi and Chi-like sequences.

Conjugation recombination in Abc⁺ hosts. The comparison of the relative rates of recombination of λ *red gam* crosses in wild-type and Abc⁺ hosts is complicated by the fact that λ *red gam* phage display different burst sizes in the two hosts. One might argue that the drop in apparent recombination rates in Abc⁺ hosts might be due to the lower number of phage replicating in these hosts. This suggestion seems unlikely, given that Abc inhibits recombination in χ^0 crosses without altering the burst sizes (for further details, see the Discussion). In any case, a different RecBCD-dependent recombination assay was performed.

In an earlier study, Abc was shown to inhibit recombination after conjugation about fivefold relative to that in a wild-type host containing a control plasmid (33), whereas mutations in *recB* and *recC* reduced recombination 300-fold. In those experiments, Abc⁺ recipient cells were grown in the presence of IPTG and mated with Hfr cells. Recombinants were selected in the absence of IPTG. We added IPTG to the exconjugant selection plates to determine whether continued production of Abc after the mating period increased the recombination-inhibiting activity of Abc. The results are shown in Table 6.

When recipient cells were grown in IPTG, mated with donor cells, and then plated in the absence of IPTG in exconjugant selection medium, Abc inhibited conjugational recombination fivefold, as seen previously (37). When Abc production continued after the mating period (that is, when 1 mM IPTG was included in the exconjugant selection plates), the recombination rate fell to the level exhibited by *recBC* mutants (100-fold decrease). This result is interpreted to mean that most of the recombination events inhibited by Abc occur after the mating period and during the selection process. It may be that induction of Abc during the mating period (but not on the selection plate) delays both recombination and digestion of the transferred DNA fragment. After plating in the absence of IPTG, Abc production is turned off, resulting in turnover of the Abc-modified RecBCD and production of the native species. In any case, full expression of Abc during the entire period inhibits conjugational recombination to the level seen in *recBC* mutant strains. Abc1 by itself caused only a twofold decrease in the recombination rate.

It has been reported that Gam has no effect on the level of conjugational recombination (12, 26, 37). Table 6 shows that

TABLE 6. Recombination in Hfr crosses with strains expressing Abc and Gam^a

Host	Plasmid	Phage gene on plasmid	Without IPTG		With IPTG	
			No. of exconjugants/ 100 donor cells	Relative recombination rate	No. of exconjugants/ 100 donor cells	Relative recombination rate
AB1157	pKM579	None	2.5	1.0	2.7	1.0
	pKM581	<i>abc1</i>	3.3	1.3	1.3	0.48
	pKM583	<i>abc2</i>	0.44	0.18	0.041	0.015
	pKM584	<i>abc1</i> + <i>abc2</i>	0.46	0.18	0.070	0.026
	pKM590	<i>gam</i>	4.0	1.6	0.33	0.12
JC5519 (<i>recBC</i>)	pKM579	None	0.037	0.015	0.032	0.012

^a Recipient cells were grown in the presence of 1 mM IPTG and mixed with donor cells (TP337) at a ratio of 10:1 (as described in Materials and Methods). The mating mixture was incubated at 37°C for 45 min. The cells were then vortexed, diluted with M9CAA and plated on exconjugant selection plates with and without IPTG. Positions of relevant markers: *thr-35::Tn9* in TP337, 0 min; *rpsL31* in AB1157 and JC5519, 73 min; *recBC*, 61 min. The direction of transfer is from *pro* (7 min) to *thr* (0 min).

TABLE 7. Growth of λ phages on strains containing Abc- and Gam-producing plasmids in the absence and presence of a P2 prophage

Host strain	Phage gene on plasmid	Growth ^a		
		Wild-type λ	λ MMS659 (<i>red gam</i> χ^0)	λ MMS885 (<i>red gam</i> χ^+)
QD5003	None	++	+	++
	<i>abc1</i>	++	+	++
	<i>abc2</i>	++	±	±
	<i>abc1, abc2 gam</i>	++	±	±
P2 lysogen of QD5003	<i>gam</i>	++	++	++
	None	—	±	++
	<i>abc1</i>	—	±	++
	<i>abc2</i>	—	—	—
	<i>abc1, abc2 gam</i>	—	—	—
		NT	NT	NT

^a ++, large plaques; +, small plaques; ±, pinpoint plaques; —, no growth; NT, not tested due to inviability of host.

continued expression of Gam during the exconjugant selection process induces a 10-fold drop in the rate of recombination. This drop in the recombination rate is still far short of the 100-fold drop seen in *recBC* strains and Abc⁺ hosts and supports the previous contention that Abc targets the recombinational machinery of RecBCD more precisely than does Gam (33).

Growth of λ in P2 lysogens containing Abc. P2 lysogens do not survive in *recBC* backgrounds (42). The mechanism of this phenomenon involves P2 prophage expression of the *old* function, which kills cells with mutations in *recBC* (18). P2 lysogens do not support the growth of wild-type λ (the Spi⁺ phenotype [sensitive to P2 interference]) because λ produces the Gam protein, which inhibits the host RecBCD enzyme (thus mimicking a *recBC* mutant genotype; see reference 43 for a review). This is a general P2 phage exclusion system, as most of the phages of *E. coli* produce functions which inactivate RecBCD following infection. The Abc functions were therefore tested for their ability to prevent the growth of an *E. coli* P2 lysogen.

Abc- and Gam-producing plasmids were successfully transformed into a P2 lysogenic strain. These plasmid constructs also express *lacI* (see Materials and Methods). P2 lysogens carrying the Gam producer pKM590 did not grow in the presence of IPTG, as expected. This inviability of P2 lysogens in the presence of Gam has been seen previously (12). In the presence of Abc, the growth of P2 lysogens was unaffected by the addition of IPTG (data not shown; results discussed below reveal that Abc is being made in these hosts). Thus, whatever modifications Abc inflicts upon RecBCD, it does not include inactivation of the function responsible for inhibiting the growth of P2 lysogens.

The effects of Abc on the growth of λ *red gam* in P2 lysogens were also examined; the results are shown in Table 7. Since Abc was shown above to inhibit Chi activity, both χ^+ and χ^0 phages were tested in these experiments. In the nonlysogenic strain, Abc diminished the plaque sizes of both χ^+ and χ^0 λ *red gam* phages, making them indistinguishable (consistent with the results described above). Abc did not diminish the plaque size of wild-type lambda phage in the nonlysogenic host, presumably because of the dominant effect of Gam produced from the infecting phage as well as the presence of λ 's *red* recombination system. In P2 lysogens

carrying a control plasmid, the λ *red gam* χ^0 phage (but not the χ^+ phage) displayed a smaller plaque size than that seen on the nonlysogen. Furthermore, Abc prevented the growth of both χ^+ and χ^0 λ *red gam* phages in the P2 lysogen. This result contrasts with the effect seen with Gam expression, which kills the host carrying a P2 lysogen. These observations suggest a greater dependency on Chi-stimulated recombination for growth on λ *red gam* in a P2 lysogen than on a nonlysogen.

Abc inhibits SOS induction in response to nalidixic acid. RecBCD is not required for induction of the SOS response when *E. coli* is exposed to UV light (24). However, RecBCD is required to generate the SOS response in the presence of nalidixic acid, a gyrase inhibitor (24). Although the mechanism of this dependency is not known, it has been suggested that inhibition of gyrase creates stalled replication forks, resulting in the formation of dsDNA breaks (10, 48). Chaudhury and Smith (7) have shown that *recBC* mutants, which lack RecBCD exonuclease activity, are proficient for SOS induction in response to nalidixic acid. They concluded that these dsDNA breaks could act as an entry site for RecBCD helicase activity (or some unknown activity), which is responsible for generation of ssDNA, the putative signal for SOS induction.

The *abc* functions were tested for their ability to inhibit SOS induction by nalidixic acid. Induction of SOS was followed by an increase in the β -galactosidase level in strain DM4000, which contains a Mu-*d(lac bla)* fusion to the SOS-inducible *sulA* promoter. *sulA* encodes a cell division inhibitor which is induced soon after exposure of *E. coli* to damaging agents (15). As shown in Fig. 3A, Abc1 had no effect on the induction of β -galactosidase in DM4000 in response to nalidixic acid. However, no increase in β -galactosidase activity was observed in the presence of Abc2 or Gam; this effect of Gam has been observed previously (12). The inhibition of SOS by Abc2 was IPTG dependent (Fig. 3B). Furthermore, Abc2 had no effect on the *recBC*-independent induction of SOS by UV light (Fig. 3C), showing that Abc2 is not simply a general inhibitor of SOS.

Since the RecBCD ssDNA endonuclease activity is not sufficient for SOS induction (16) and the dsDNA and ssDNA exonucleases are not necessary (7), this leaves the helicase activity of RecBCD (or some unknown activity) as the *recBC*-dependent function responsible for the nalidixic acid-induced SOS response. However, recent results (unpublished data) have revealed that a purified Abc2-RecBCD complex retains wild-type levels of helicase activity. Thus, RecBCD helicase activity is not sufficient for induction of SOS in response to nalidixic acid. These results suggest that in wild-type cells, Chi activation is also required, perhaps to suppress the nonspecific nuclease activity of RecBCD. This model is consistent with the nalidixic acid-induced SOS response in *recD* mutants (7), which lack RecBCD exonuclease functions. According to this line of reasoning, unattenuated Abc-modified RecBCD exonuclease activity would not generate adequate levels of ssDNA to activate the SOS response.

DISCUSSION

The Abc proteins of bacteriophage P22 are produced soon after infection to counteract the deleterious effects of the host cell RecBCD enzyme. In an effort to understand the nature of RecBCD modification by these phage proteins, the properties of *E. coli* strains bearing Abc-producing plasmids have been examined. We found that wild-type cells contain-

FIG. 3. Abc inhibits SOS response induced by nalidixic acid. Expression of SOS is monitored by β -galactosidase activity in a strain harboring a fusion of the *sulA* promoter to *lacZ* (DM4000). Cells were grown by rolling in M9CAA medium; plasmid-bearing strains were grown with tetracycline. IPTG was added to a final concentration of 1 mM. Nalidixic acid was added to the cultures at the concentrations indicated (A and B), or the cells were exposed to UV irradiation in uncovered petri dishes for the times indicated (C). After 1 h of continued rolling, β -galactosidase activities were determined as described by Miller (25). (A) Symbols: $\square$, no plasmid; $\diamond$, pKM579 (control); $\blacklozenge$, pKM581 (Abc1); $\circ$, pKM590 (Gam); $\square$, pKM583 (Abc2).

ing Abc possess some but not all of the properties of *recBC* mutants. Table 8 summarizes the similarities and differences in the phenotypes of *Abc*⁺, *Gam*⁺, and *recBC* mutant strains. Overall, Abc inhibits the recombination proficiency of the RecBCD enzyme more than Gam does. While both Abc and Gam inhibit Chi-stimulated λ recombination, Abc is generally more proficient at inhibiting both Chi activity and conjugational recombination. Furthermore, Abc induces extreme UV sensitivity in *recF* hosts; Gam does not. Given Abc's inhibition of Chi activity, it may be that the greater UV sensitivity of *Abc*⁺ *recF* hosts relative to *Gam*⁺ *recF* hosts is a reflection of the greater recombination-inhibitory ability of Abc. On the other hand, Gam is clearly more

proficient than Abc at inhibiting RecBCD nuclease activity both in vivo and in vitro (26, 33; unpublished observations).

It has been noted previously that extracts of cells containing Abc-producing plasmids retain about 25% of the ATP-dependent ds DNA nuclease activity found in wild-type extracts (33). The facts that *Abc*⁺ hosts retain ATP-dependent dsDNA nuclease activity and that *Gam*⁺ and *recBC* mutant cells do not may explain the differences shown in Table 8 with regard to Abc's inability to support the growth of T4 gene 2 mutant phage (and λ *red gam* in *recA* strains) and to promote plasmid multimerization in combination with *sbcB*.

The differential effects of Abc and Gam are likely due to

TABLE 8. Properties of *E. coli recBC* strains and wild-type hosts expressing λ Gam and P22 Abc proteins

Property	<i>E. coli</i> host			Reference
	<i>recBC</i>	<i>Gam</i> ⁺	<i>Abc</i> ⁺	
Viability ^a	20%	27%	44%	This report
ATP-dependent dsDNA exonuclease activity in extracts	ND ^b	ND	25% of wild-type activity	33
Supports growth of T4 gene 2 mutant phage	+	+	—	33
Promotes plasmid multimerization in combination with <i>sbcB</i>	+	+	—	33
Supports growth of λ <i>red gam</i> in <i>recA</i> host	+	+	—	34
Viability within P2 lysogen	—	—	+	This report
UV sensitivity				
In otherwise wild-type hosts	Moderate	Moderate	Moderate	12, 26; this report
In <i>recF</i> hosts	High	Moderate	High	12, 14; this report
In <i>recJ</i> hosts	High	High	High	19, 26; this report
Relative rate of conjugation recombination in otherwise wild-type host (= 1.0)	0.012	0.12	0.015	This report
Chi activity ^c	1.0	1.5	1.0	26, 52; this report
SOS response induced by nalidixic acid	—	—	—	12; this report

^a See Table 2, footnote a.

^b ND, none detected.

^c See Table 4, footnote d.

selective inactivations of one or more of the functions of the enzyme. In the case of Gam, all the known functions of RecBCD are inhibited in vitro (17, 26). The inability of Gam⁺ cells to display all the properties of *recBC* mutant strains has been suggested to be the result of an unknown function of RecBCD or the presence of a second (Gam-resistant) function encoded by either *recB* or *recC* (26). [The *sbcC* protein(s), a proposed secondary target by λ Gam, is not thought to play a role in Gam's inability to mimic mutations in *recBC*. If modification of the *sbcC* protein(s) by Gam stimulated conjugational recombination (offsetting the inhibitory effect of Gam on RecBCD-promoted recombination), one would expect to see this effect in *recBC sbcC*⁺ hosts. However, Gam does not stimulate conjugational recombination in *recBC sbcC*⁺ strains (26, 37).] The situation with Abc is less complicated. Abc does in fact modify the multiple activities of RecBCD differentially; it leaves the helicase, ssDNA exonuclease, and ssDNA endonuclease functions intact and inhibits dsDNA exonuclease activity to 25% of wild-type levels (unpublished data).

Abc inhibits Chi activation. Chi sites are hotspots for recombination promoted by the RecBCD pathway. Current models of the mechanisms of Chi-stimulated recombination suggest that Chi promotes a change in the structure and/or conformation, and hence the enzymology, of RecBCD. The modification results in a RecBCD-promoted nick in the dsDNA on the 3' side of a correctly oriented Chi site as the enzyme unwinds DNA (32, 58) and may suppress the exonuclease function of the enzyme as RecBCD digests dsDNA, as suggested previously (53, 60). In any case, Abc probably prevents RecBCD from either recognizing or responding to the signal, as opposed to preventing access to Chi, since Abc-modulated RecBCD retains full helicase activity (unpublished observations).

Gam has previously been shown to inhibit Chi-stimulated λ recombination close to the level seen in *recBC* mutants (26). This interpretation is clouded by the fact that the absence of RecBCD exonuclease activity (in both *recBC* mutants and Gam⁺ hosts) favors the growth of the phage by allowing rolling-circle DNA replication. In such a healthy environment, recombinants may be underrepresented. This argument cannot be used in the case of Abc⁺ hosts. Abc-modulated RecBCD retains significant levels of exonuclease activity in vivo (33) and in vitro (unpublished observations), does not support the formation of linear plasmid multimers in an *sbcB* mutant background (33), and is unable to support the growth of λ *red gam* in *recA* mutant hosts (34). These observations suggest that Abc does not allow the phage to enter rolling-circle replication. Thus, the inhibition of Chi-stimulated λ recombination in Abc⁺ hosts is clearly indicative of inhibition of RecBCD's recombination machinery by Abc and not the result of an alteration in λ physiology.

One additional observation in these Chi activity experiments is worth noting. Removal of Chi (χ^0 control crosses) caused a typical fivefold drop in burst size relative to that in χ^+ control crosses but only a twofold drop in recombination, while inhibition of Chi and Chi-like activity in the χ^+ crosses by Abc resulted in an additional fivefold drop in the rate of recombination, with little or no further effect on the burst size. These observations suggest that Chi-like sites contribute significantly to recombination rates in λ *red gam* crosses, at least in the *R-J* interval. It is likely that within smaller intervals, where Chi-like sites are few or absent, Abc would have little or no effect on recombination (as was observed in λ *R* $\times$ *S* crosses [33]). Furthermore, it was surprising that the fivefold decrease in burst size of the χ^0 control crosses

(relative to the χ^+ control crosses) was accompanied by only a modest decrease in recombination. Perhaps the presence of χ , besides stimulating recombination, also stabilizes the rolling-circle mode of replication by attenuating the dsDNA exonuclease activity of RecBCD, as recently suggested by Rosenberg and Hastings (38). However, inhibition of the RecBCD exonuclease activity cannot be χ 's only role, as λ *red gam* χ^+ phage do not grow in *recA* mutant hosts.

Why is it that Abc inhibits recombination fivefold in the χ^0 crosses but has no effect on burst size? [In fact, Abc mildly inhibits the growth of λ (χ^0) as monitored by plaque size; see Table 7. Presumably, the effect is too small to be observed in single burst experiments.] Since Chi-stimulated recombination is inactive (Abc⁺ hosts) and rolling-circle replication is not allowed (Gam⁻ infection), there should be no route for packaging λ *red gam* in Abc⁺ hosts. The host RecF pathway probably does not contribute significantly to packaging via recombination in Abc⁺ hosts. The recombination rates of λ *R* $\times$ *J* χ^0 crosses in Abc⁺ hosts were unaffected by the presence of a mutation in *recJ* (unpublished observation). Sawitzke and Stahl (40) recently described a λ analog of *E. coli*'s *recO*, *recR*, and *recF* functions in the λ *nin* region called *orf*, which may be responsible for the residual recombination of λ *red gam* in Abc⁺ hosts. Alternatively, it may be that Abc-modified RecBCD, as one component of the P22 phage recombination system, stimulates low levels of Chi-independent recombination or rolling-circle DNA replication, allowing phage packaging at some low frequency. Whatever the mechanism, it is inhibited by some function produced by the P2 prophage (perhaps *old*), since P2 lysogenic Abc⁺ hosts do not allow the growth of λ *red gam*.

Finally, while an Abc-modified RecBCD may be incapable of recognizing Chi, it may still retain the capability of being activated in a random, Chi-independent fashion. In this case, Abc may prove a valuable tool with which to assess the Chi dependency of RecBCD-promoted events (i.e., from the results reported here, one could conclude that RecBCD-promoted conjugational recombination is totally dependent on Chi). Alternatively, Abc may simply prevent the Chi-induced conformational change under any circumstances, inhibiting all RecBCD-stimulated recombination. To distinguish between these possibilities, the biochemical properties of the Abc-RecBCD complex need to be examined. Such studies are currently in progress.

Is Abc-modified RecBCD a class II mutation? Amundsen et al. (1) have isolated and characterized 10 different *recBC* mutations and placed them into three classes based on a variety of genetic tests and biochemical analyses. Abc⁺ hosts are strikingly similar to their class II mutants (in particular, those carrying *recB2154* and *recB2155*). Both Abc⁺ hosts and class II mutants are Chi inactive and recombinationally deficient, inhibit the growth of λ *red gam* phage, do not allow T4 gene 2 mutant phage to grow, and retain unwinding and exonuclease activities. Furthermore, extracts of *E. coli* containing the *recB2154* and *recB2155* alleles encode enzymes with depressed dsDNA exonuclease activity (43 and 34% of wild-type activity, respectively), a property shared by Abc-modified RecBCD (25% of wild-type activity; unpublished observations), although they show higher levels of ssDNA exonuclease than the wild-type enzyme a phenotype not found with Abc-modified RecBCD (unpublished observations). This last finding may be a consequence of the fact that Amundsen et al. examined extracts of mutant strains containing these *rec* alleles, whereas Abc-modified RecBCD exonuclease functions were determined with purified enzyme (unpublished data). Whether the mod-

ification of RecBCD by Abc is truly the same as the alteration of RecBCD in class II mutants will have to await characterization of these enzymes in vitro.

Abc in *S. typhimurium*. We used P22 Abc protein as a tool for modifying the function of *E. coli* RecBCD in vivo. Normally, Abc is produced in *S. typhimurium* after phage P22 infection. Do the modifications described here for the *E. coli* RecBCD apply to the *Salmonella* RecBCD enzyme? It has been shown that Abc modifies the *E. coli* RecBCD in vivo in a manner that supports the growth of a P22 Δ abc prophage after induction (33). Given this result and the demonstration that *Salmonella* RecBCD possesses Chi activity (46), Abc2 probably modifies the two RecBCDs in an identical fashion, although this has not been tested directly.

The role of Abc1 in the P22 recombination system is not clear. The designation of Abc1 as an anti-RecBCD function was based on the observation that it was required (along with Abc2) for full complementation of P22 Δ abc phage. However, in most of the tests described here, Abc2 is fully active in the presence or absence of Abc1. Abc1 may be involved in coordinating the activities of other P22 recombination functions. Sequence comparisons between phage P22 and HK97 revealed that HK97 has sequences identical to P22's abc2 as well as the region of the *erf* gene that encodes the N-terminal ssDNA-binding domain of the Erf protein (13a). No sequence identity was found for abc1 or the C-terminus-encoding region of *erf*, which encodes a domain of unknown function (27, 36). Given this observation and the fact that the C-terminus-encoding region of *erf* overlaps abc1, Abc1 protein may interact with the C-terminal region of Erf, possibly in a manner that aligns Erf with Abc2-modified RecBCD in vivo.

Finally, can the alterations of *Salmonella* RecBCD by Abc be explained in terms of how such modifications help P22 grow in *Salmonella* cells? This question can be answered only when we understand the biochemical nature of Abc-modified RecBCD. Abc clearly does not simply shut down the enzyme, as λ Gam does. It seems likely that Abc-modified RecBCD exonuclease functions qualitatively differently than the native species. A recent comparison of the recombination systems of phages λ and P22 suggests a role for Abc (35). Phage λ 's recombination system *red* consists of *bet* (encoding the ssDNA-binding β -protein) and *exo* (encoding λ exonuclease). While P22 *erf* is functionally analogous to λ *bet*, no λ -like exonuclease function has yet been found in P22. It is possible that Abc2 modifies RecBCD in a manner that makes it isofunctional to λ exonuclease. If so, one could imagine that Abc serves two purposes, curtailing the destructive activity of RecBCD and generating a phage-like exonuclease function. This hypothesis is currently being tested. In support of this notion, a plasmid that expresses λ exonucleases alone partially supports the growth of a P22 Δ abc mutant (unpublished observation).

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