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Degradation of Bacteriophage Lambda Deoxyribonucleic Acid After Restriction by *Escherichia coli* K-12[1]

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Wild-type bacteria which restrict the deoxyribonucleic acid (DNA) of infecting phage when the phage do not carry the proper host modification rapidly degrade that restricted DNA to acid-soluble products. The purified restriction enzyme acts as an endonuclease in vitro to cleave restrictable DNA and does not further degrade the DNA fragments produced. We have examined mutants of *Escherichia coli* K-12 which lack various nucleases in order to determine which nucleases are involved in the rapid acid solubilization in vivo of unmodified λ DNA following restriction. Bacteria which are wild type, *recA*$^-$, or *polA1*$^-$ degrade about 50% of the unmodified phage DNA within 10 min of infection, with little subsequent degradation. Mutants which are *recB*$^-$ or *recC*$^-$ degrade unmodified DNA very slowly, solubilizing about 15% of the DNA by 10 min after infection. Two classes of phenotypic revertants of *recB*$^-$/*C*$^-$ mutants were also tested. Bacteria which are *sbcA*$^-$ restrict poorly and do not degrade much of the restricted DNA. Bacteria which are *sbcB*$^-$ restrict normally. This mutation does not appear to affect degradation of restricted phage DNA in *recB*$^-$/*C*$^-$ mutants, but such degradation is decreased in *recB*$^+$/*C*$^+$ bacteria. The presence of a functional λ exonuclease gene is not required for degradation after restriction.

Bacteriophage lacking the appropriate host-controlled modification of their deoxyribonucleic acid (DNA) are aborted or restricted in their infection of certain host bacteria (23). Restriction by *Escherichia coli* strains in vivo is accompanied by rapid degradation of the phage genome to acid-soluble pieces (7, 18, 20).

In contrast, restriction of phage λ by *E. coli* strain K-12 has been shown by Meselson and Yuan to be due to an endonuclease which in vitro cleaves only unmodified regions of the λ DNA to fragments of approximately one-quarter of the original length (24). No exonuclease activity has been found associated with the restriction enzyme in vitro.

Several exonucleases in *E. coli* which might carry out the observed extensive degradation of restricted DNA are reviewed by Lehman (22): (i) exonuclease I, specific for 3'-hydroxyl-terminated single-stranded DNA; (ii) exonuclease II of the Kornberg polymerase enzyme, active on either terminus of double-stranded DNA; (iii) exonuclease III, specific for 3'-termini of double-stranded DNA. At least two other nucleases may be considered also: (iv) an adenosine triphosphate (ATP)-dependent exonuclease activity present in *rec*$^+$ strains but absent or at reduced activity in *recB*$^-$ or *recC*$^-$ strains (1, 3, 25); (v) an ATP-independent exonuclease found at elevated levels in one class (*sbcA*$^-$) of recombination-proficient phenotypic revertants of *recB*$^-$*C*$^-$ strains (2).

Another exonuclease is derived from the infecting bacteriophage. After infection or induction of phage λ in *E. coli*, a phage-directed exonuclease is synthesized (16). This λ exonuclease which acts on 5'-termini of DNA also warrants consideration for a role in the further degradation of restricted λ DNA.

The present work is on the degradation of restricted λ DNA in strains mutant for exonuclease activity in order to estimate the role of these exonucleases in restriction in vivo.

MATERIALS AND METHODS

Media. TB medium contained 10 g of tryptone

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(Difco), 5 g of NaCl, and 5 mg of thiamine per liter. TBY medium was TB supplemented with 1 g of yeast extract (Difco) per liter. TA medium was TBY containing 1.2% Difco agar; STA medium was TBY containing 0.6% Difco agar. Medium LN was a synthetic medium containing: K_2HPO_4, 0.697 g; KH_2PO_4, 1.157 g; Na_2HPO_4, 5.325 g; NH_4Cl, 1.07 g; $CaCl_2$, 11.1 mg; $ZnCl_2$, 0.27 mg; $FeSO_4$, 1.4 mg; $MgSO_4$, 120.4 mg; and glucose, 5 g per liter. The following supplements were added per liter as required: thiamine, 5 mg; adenine, 20 mg; thymidine, 2 mg; required 1-amino acids, 20 mg; and charcoal-filtered, vitamin-free Casamino Acids (Difco), 100 mg. Medium LNCAA was LN supplemented with charcoal-filtered, vitamin-free Casamino Acids, 100 mg per liter. TM medium contained 0.01 M tris(hydroxymethyl)aminomethane (Tris) (*p*H 7.4) and 0.1 M $MgSO_4$; TM/10 contained 0.01 M Tris (*p*H 7.4) and 0.001 M $MgSO_4$; and TM gel was TM containing 0.01% gelatin.

Bacterial strains. The bacterial strains are listed in Table 1.

Bacteriophage strains. The phage strains are listed in Table 2.

Preparation of tritium-labeled phage for degradation experiments. Strain 342-1h lysogenic for λ*cI857* (or λ*cI857red3*) was grown at 32 C to about 5 × 10^8 cells per ml in LNCAA medium supplemented with thymidine-*methyl*-^3H at 1.8 mCi/mg and 2 mg/liter. The lysogens were induced by the addition of an equal volume of the same medium preheated to 54 C, and aerated for 10 min at 43 C and then at 38 C until lysis was complete. Bacterial cell debris was removed by centrifugation at 12,000 × *g* for 10 min. The phage were sedimented at 30,000 rev/min for 90 min at 15 C in a Beckman Spinco 30 rotor, and the phage were resuspended in TM by gentle shaking overnight at 4 C. 7.76 g of CsCl was added to 10 ml of the resuspended phage which were then centrifuged for 22 hr at 30,000

TABLE 1. *Strains of E. coli K-12*

Strain no.	Genotype						Phenotype		Source	Reference
	rec		*sbc*			Other[a]	Rec	r_Km_K[b]		
	A	B	C	A	B					
C600	+	+	+	+	+		+	++	M. Meselson	
C600.4	+	+	+	+	+		+	−−	M. Meselson	19
342	+	+	+	+	+	*tdr⁻met⁻ade⁻*	+	++	This lab from C600	
342-1	+	+	+	+	+	*tdr⁻met⁻ade⁻*	+	−−	This lab from C600	
p3478	+	+	+	+	+	*polA1⁻*	+	++	E. Signer	6
JC2926	13	+	+	+	+		−	++	A. J. Clark	
JC4583	+	+	+	+	+	*endI⁻*	+	++	S. Barbour	4
JC4584	+	21	22	+	+	*endI⁻*	−	++	S. Barbour	4
JC4588	56	+	+	+	+	*endI⁻*	−	++	S. Barbour	4
JC4681	+	21	+	+	7		+	++	A. J. Clark	17
JC5170	+	+	22	4	+	*endI⁻*	+	±+	S. Barbour	2
JC5172	+	21	+	2	+	*endI⁻*	+	±+	S. Barbour	2
JC5174	+	21	+	1	+	*endI⁻*	+	±+	S. Barbour	2
JC5175	+	+	22	3	+	*endI⁻*	+	±+	S. Barbour	2
JC5176	+	21	22	6	+	*endI⁻*	+	±+	S. Barbour	2
JC5188	+	21	22	5	+	*endI⁻*	+	±+	S. Barbour	2
JC5495	13	21	+	+	+		−	++	S. Barbour	32
JC5519	+	21	22	+	+		−	++	A. J. Clark	32
JC6721	+	+	22	+	+	*endI⁻*	−	++	S. Barbour	2
JC6722	+	21	+	+	+	*endI⁻*	−	++	S. Barbour	2
JC7507	56	21	+	+	+	*endI⁻*	−	++	S. Barbour	
JC7617	+	21	22	+	9		+	++	A. J. Clark	17
JC7619	+	21	22	+	11		+	++	A. J. Clark	17
JC7622	+	21	22	+	14		+	++	A. J. Clark	17
JC7623	+	21	22	+	15		+	++	A. J. Clark	17
JC7689	+		+	+	15		+	++	A. J. Clark	17
JC7694	+		+	+	15		+	++	A. J. Clark	17
AB1157	+		+	+	+		+	++	A. J. Clark	

[a] Only relevant genetic markers are included. Abbreviations: *tdr⁻*, *met⁻*, *ade⁻*: requires thymidine (or thymine), methionine, and adenine, respectively; *endI⁻*, defective in endonuclease I activity; *rec*, recombination proficiency; *sbc*, suppression of *recB* or *recC* recombination deficiency.

[b] The restriction and modification phenotype, respectively; subscript denotes strain K-12.

TABLE 2. *Phage strains*

Phage genotype[a]	Source	Reference
λ*c26*	M. Meselson	14
λ*cI857*	M. Meselson	30
λ*cI857red3*	E. Signer	29
λ*cI857S7*	M. Howe	9
λ*cI857pbio256*	I. Herskowitz	28

[a] Abbreviations: *c26*, a clear plaque mutant; *c1857*, a heat-inducible mutant in *cI*; *red3*, a recombination-deficient mutant defective in λ exonuclease; *S7*, a lysis-defective mutant useful for obtaining large burst sizes in one-step lysates; *pbio256*, a plaque-forming *bio*-transducing phage mutant with a deletion of the λ exonuclease region.

rev/min in a Beckman SW39 rotor at 15 C to form an isopycnic band. The phage were collected in drops from the bottom of the centrifuge tube. The phage-containing fractions were pooled and dialyzed against TM. The specific activity of the phage was estimated to be about 0.5 mCi/10^{14} phage. Modified λ*cI857* were prepared similarly after induction of a lysogen of strain 342.

For preparation of ^{3}H-labeled λ*cI857pbio256* phage, strain 342-1 was grown to about 5×10^8 cells/ml in the tritiated medium described above. The cells were collected by centrifugation, resuspended in one-twentieth volume of TM, and infected at a multiplicity of infection (MOI) of about 3. After 15 min at room temperature, the bacterial cells were collected by centrifugation and resuspended in the tritiated thymidine medium from which they had been collected. After lysis, phage were purified as before.

Efficiency of plating. Bacteria grown to 5×10^8 to 8×10^8 cells/ml in TBY containing 0.1% maltose were resuspended in an equal volume of TM and aerated at 37 C for 20 to 30 min. An appropriate dilution of modified or unmodified phage was added to 0.1 ml of bacteria. After 15 min at 22 C, 2.5 ml of STA was added, and the mixture was plated on TA. Nonspecific plating differences of strains are corrected by comparison of the assay of modified phage on the test strain as compared to modified phage on nonrestricting strain C600.4.

Degradation assays. Bacteria were grown to about 8×10^8 cells/ml in TBY containing 0.1% maltose and resuspended in one-fifth volume of TM at 37 C. Phage were then added (t = 0) at an MOI of 1 (between 20,000 and 30,000 counts/min of phage were added to each bacterial sample). At intervals thereafter, 0.2 ml of the infection mixture was removed to an equal volume of 4% perchloric acid (PCA) at 0 C (each sample removed contained 3,000 to 4,000 counts/min). After at least 30 min, the acidified sample was centrifuged at $12,000 \times g$ for 10 min. The supernatant fluid containing acid-soluble products (the cold acid-soluble fraction) was added to 5 ml of Aquasol scintillation fluid (New England Nuclear). The acid-insoluble precipitate was resuspended in 0.4 ml of 2% PCA and heated at 100 C for 60 min. It then was centrifuged as above, and the supernatant fluid (the cold acid-insoluble, hot acid-soluble fraction) was added to 5 ml of Aquasol. The radioactivity of label soluble in cold PCA and solubilized by boiling PCA was determined for each sample removed. More than 90% of the label was recovered. Samples were counted twice on a Packard liquid scintillation counter for at least 5 min. The total counts per minute of the two fractions from each sample were determined and divided into the counts per minute of cold acid-soluble fraction to determine the percent cold acid-soluble label. The addition of 0.4 ml of 2% PCA to 5 ml of Aquasol containing tritium reduced the efficiency of counting by 30%. Variations of 25% in the amount of PCA added did not significantly change the efficiency of counting tritium. The absolute efficiency of counting of tritium in Aquasol containing PCA was estimated to be about 20%.

RESULTS

Most of the mutant strains tested plated modified λ well and restricted unmodified λ as efficiently as wild-type strains (Table 3). However, the *sbcA*$^-$ mutant restricted poorly.

The concomitant loss of restriction and increase in ATP-independent exonuclease might be attributed to a mutation in the *hsp* locus for restriction. To examine this possibility, *recB*$^-$ *sbcA*$^-$ or *recB*$^-$*C*$^-$ *sbcA*$^-$ cells were mated with HfrH which carried a wild-type restriction system and which was resistant to phages T1 and T5 (*tonA*$^-$). The male strain was lysogenic for λ*cI857S7*. This lysogen neither forms colonies at 42 C nor lyses. The mating was carried out at 32 C and was interrupted after 15 min to allow ample time for the transfer of the genes for *hsp* (expected before 10 min of mating at 32 C). After four generations of growth at 32 C, presumptive recombinants were selected by plating dilutions of the culture at 42 C in the presence of phage T1. Recombinants were obtained as clones which were resistant to phages T1 and T5 and to the male-specific phage fd, and which could form colonies at 42 C. Sixteen such recombinants from two independent matings all retained their original phenotype of low level of restriction, although previous work (19) made it likely that most of these recombinants had received and integrated the donor *hsp* locus for restriction. Therefore, the *sbcA* mutation is probably not at *hsp*.

Restriction by and degradation in bacterial mutants. It may be seen (Fig. 1) that after the infection of a restricting host by unmodified phage, about half of the phage DNA is rapidly degraded to acid-soluble size. Only 10% of the unmodified λ DNA is de-

TABLE 3. *Efficiency of plating of modified and unmodified λc26 on various hosts*

Host	λc26·342[a]	λc26·342-1[b]
C600	1	2×10^{-4}
C600.4	1	1
p3478	0.9	3×10^{-4}
JC4583	0.75	2.5×10^{-4}
JC4584	0.8	2×10^{-4}
JC4588	0.7	2.5×10^{-4}
JC4681	0.7	1×10^{-4}
JC5170	0.8	0.25
JC5172	0.7	0.18
JC5174	0.85	0.19
JC5175	0.8	0.20
JC5176	0.75	0.05
JC5188	0.8	0.06
JC5495	0.7	8×10^{-4}
JC5519	0.6	1×10^{-4}
JC6721	0.8	2.5×10^{-4}
JC6722	0.8	3.5×10^{-4}
JC7689	0.8	2×10^{-4}
JC7694	0.85	2×10^{-4}
JC7507	0.8	2.5×10^{-4}
JC7617	0.75	3×10^{-4}
JC7619	0.6	4×10^{-4}
JC7622	0.8	5×10^{-4}
JC7623	0.7	5×10^{-4}
JC7689	0.8	2×10^{-4}
JC7694	0.8	2×10^{-4}
AB1157	1	2×10^{-4}

[a] λc26 was grown on strain 342, which gives the phage the K-12 host modification. The efficiency of plating is normalized to a value of 1 for λc26·342 on C600.

[b] λc26 was grown in strain 342-1, which does not confer any known host modification. The efficiency of plating is normalized to a value of 1 for λc26·342 on the host strain.

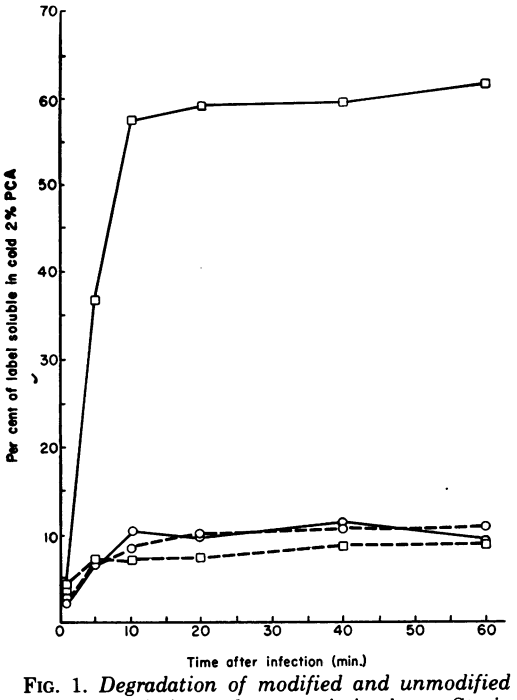

FIG. 1. *Degradation of modified and unmodified λcI857 by restricting and nonrestricting hosts. Strain C600 is $r_k^+m_k^+$ and C600.4 is $r_k^-m_k^-$. Symbols: ——, unmodified λ; ----, modified λ; □, C600; ○, C600.4.*

graded when the host is restriction-deficient (strain C600.4). Similarly, λ DNA carrying the K-12 host modification is only slightly solubilized by r_K^+ m_K^+ or r_K^- m_K^- hosts. This degradation in the absence of restriction may be due to defective phage in the population or to normal events of phage infection.

The function of the *recA*$^+$ gene is not known, although strains which are *recA*$^-$ *recB*$^+$*C*$^+$ degrade much more of their own DNA after ultraviolet (UV) irradiation than do strains which are *recA*$^+$ (5). However, the degradation of restricted DNA (Fig. 2) was not altered significantly from wild type in two independently derived *recA*$^-$ mutants.

Strain p3478 has an amber mutation in the Kornberg polymerase gene (12). This mutant has less than 1% of the wild-type polymerase activity (6). This mutant solubilizes unmodified DNA as efficiently as wild-type bacteria (Fig. 2).

In contrast to *recA*$^-$ or *polA1*$^-$ strains, bacteria with mutations in the *recB* or the *recC* gene, or both, show a reduction in the rate of acid solubilization of restricted DNA (Fig. 3). The decreased degradation by these strains is probably not due to a mutation in the restriction gene since some of these strains were derived by P1-mediated transduction from primary mutants (4). Furthermore, the *recB*$^-$ and *recC*$^-$ strains restrict almost as efficiently as the strains from which they were derived (Table 3). The pattern of degradation is essentially the same in the *recB*$^-$*C*$^+$, *recB*$^+$*C*$^-$, and *recB*$^-$*C*$^-$ strains.

The degradation of restricted DNA in *recB*$^-$ and *recC*$^-$ strains is not affected by the presence of a mutation in the *recA* gene (strains JC5495, JC7507).

Two classes of phenotypically Rec$^+$ revertants of *recB*$^-$ or *recC*$^-$ (or both) mutants are the result of indirect suppression of the *rec*$^-$ mutation (2, 17). The *sbcA* suppression is associated with elevated levels of an ATP-independent exonuclease (2). Therefore, it might be anticipated that *sbcA*$^-$ strains would de-

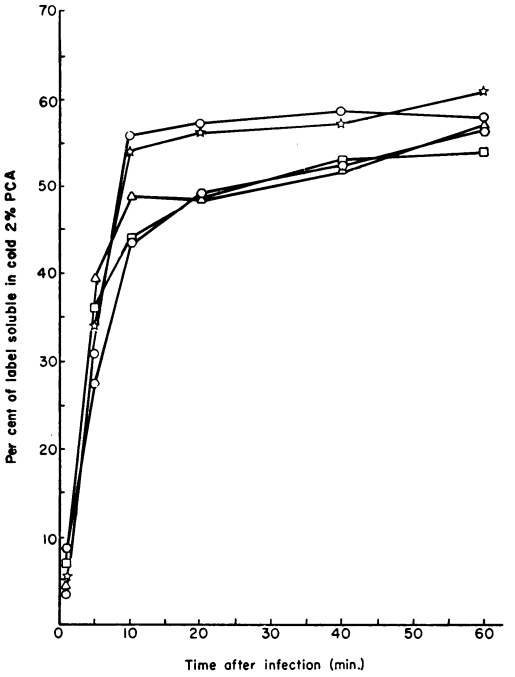

FIG. 2. *Degradation of unmodified λcI857 by rec⁺, recA⁻, and polA⁻ strains. Symbols:* □, *AB1157;* ☆, *JC4583 (rec⁺);* ⬭, *JC2926 (recA⁻);* △, *JC4588 (recA⁻); and* ○, *p3478 (polA1⁻).*

grade more restricted DNA than the *rec⁻* strains from which they were derived. On the contrary, these strains degrade less DNA than their *rec⁻* parents (Fig. 4). The reduced degradation may be due to the reduced restriction in these strains. Nevertheless, the amount of degradation which occurs by 60 min is less than might be expected for the residual number of phage restricted, on the assumption that the increased ATP-independent exonuclease could substitute for the missing *recB* exonuclease in degrading restricted DNA. Therefore, the ATP-independent exonuclease may not be able efficiently to degrade all of the DNA termini produced by the restriction enzyme.

The increased levels of the ATP-independent exonuclease in *sbcA* mutants may interfere with restriction by interacting with some component required for restriction, by altering the substrate of the restriction endonuclease, by facilitating repair, or by repressing the synthesis of restriction endonuclease.

The second class of phenotypically Rec⁺ revertants, *sbcB⁻*, results from a mutation which decreases the level of exonuclease I (17). When transferred to a *recB⁺C⁺* host, the *sbcB⁻* mutation results in a decrease in the amount of degradation of unmodified DNA (Fig. 5). The early, rapid degradation of DNA is the same as in *sbcB⁺*. However, within 10 min degradation has stopped, with about 40% of the DNA degraded. It therefore appears that exonuclease I is required for some but not all of the acid solubilization of restricted DNA. Presumably some of the DNA has been made single-stranded by the activity of the *recB⁺C⁺* ATP-dependent exonuclease. In contrast, the amount of DNA degraded in *recB⁻* or *recB⁻C⁻* strains is about the same even when the exonuclease I activity is decreased (Fig. 5). Therefore, the degradation which occurs in *recB⁻* or *recB⁻C⁻* strains does not produce a large amount of substrate for exonuclease I.

Restriction and degradation of λ exonuclease-deficient phage mutants. For several reasons, it would seem that λ exonuclease could not be responsible for degradation of restricted DNA. First, the rate of degradation of restricted DNA is the same in immune and sensitive hosts (20; Simmon, *unpublished data*). Furthermore, biochemical and genetic

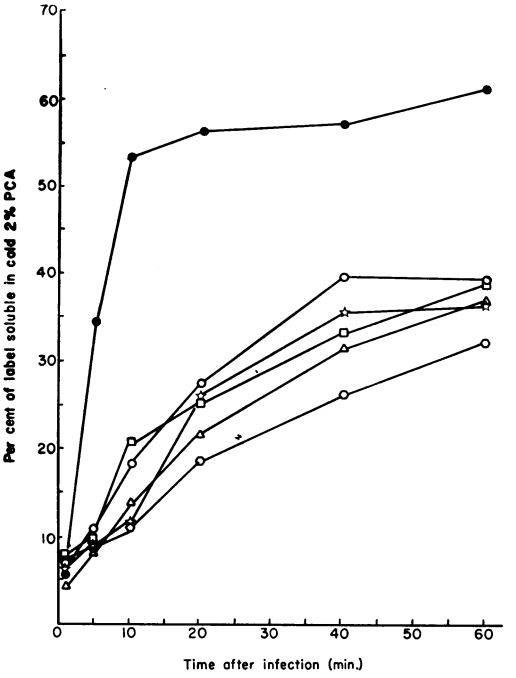

FIG. 3. *Degradation of unmodified λcI857 by rec⁺ and various rec⁻ strains. Symbols:* ●, *JC4583 (rec⁺);* △, *JC4584 (recB⁻C⁻);* ⬭, *JC5495 (recA⁻B⁻);* ○, *JC6721 (recC⁻);* ☆, *JC6722 (recB⁻); and* □, *JC7507 (recA⁻B⁻).*

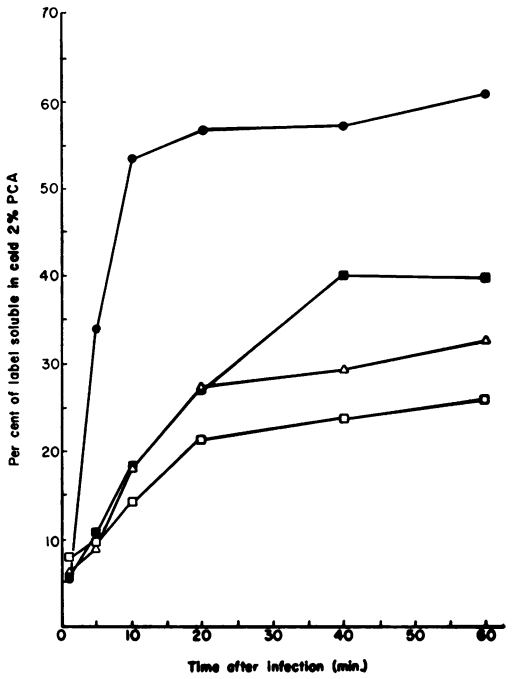

FIG. 4. *Degradation of unmodified λcI857 by rec⁺, rec⁻, and sbcA⁻ strains. Symbols:* ●, *JC4583 (rec⁺);* ■, *JC6721 (recC⁻);* □, *JC5170 (recC⁻sbcA⁻); and* △, *JC5176 (recB⁻C⁻sbcA).*

evidence suggests that one of the restriction sites is in the *N* gene of λ (8, 31). For the synthesis of λ exonuclease to occur, the protein product of the *N* gene is required, after which transcription of the exonuclease can occur (26). Thus an early scission in the *N* gene could prevent synthesis of exonuclease.

However, messenger ribonucleic acid synthesis in the λ exonuclease region is known to occur within 1 min after induction of thermolabile lysogens (15). Therefore, the possibility was considered that this region may be transcribed and translated prior to repression or restriction of infecting λ in an immune, nonaccepting host. To determine the effect of λ exonuclease on degradation of restricted DNA, phage mutants were studied. The phage *λcI857red3* makes neither λ exonuclease nor B protein (27). A biotin transducing phage (28) which has lost the exonuclease gene was also tested. We found that these phage are as restricted in their efficiency of plating as are wild-type phage. Also, the DNA of these λ mutants was rendered acid-soluble as rapidly as was the DNA of λ phage which carry the exonuclease gene (Fig. 6). The lack of effect of the λ exonuclease gene can best be seen in the *recB⁻* strain, JC4584, in which the rapid degradation by the bacterial ATP-dependent exonuclease is absent, thereby making more visible any hypothetical λ exonuclease activity.

DISCUSSION

Degradation of unmodified λ DNA. Restriction of unmodified DNA leads to production of exposed DNA termini. Exposure of these termini results in rapid degradation of the DNA in hosts which are *recB⁺C⁺*. If there are three double-stranded restriction scissions per λ DNA molecule (producing the approximately one-quarter-length molecules observed by Meselson and Yuan [24]), then there may be from six to eight exposed termini depending upon whether or not the two cohesive ends join. If it is assumed that the acid-solubilized fraction represents all of the degraded DNA, then the average degradation per terminus can be estimated. It will be an underestimate due to possible reincorporation of released radioactivity into host DNA and possibly due to incomplete extraction of the acid-soluble fraction. The maximum amount of DNA degraded in the experiments reported

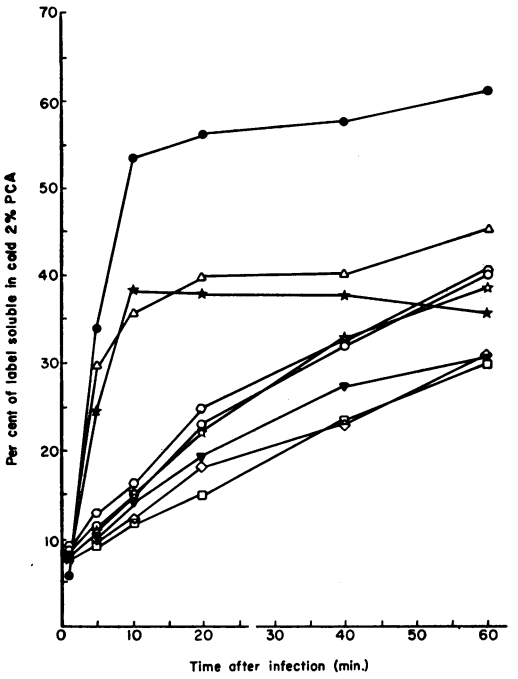

FIG. 5. *Degradation of unmodified λcI857 by rec⁺, rec⁻, and sbcB⁻ strains. Symbols:* ●, *JC4583 (rec⁺);* ○, *JC4681 (recB⁻ sbcB⁻);* ⬭, *JC5519 (recB⁻C⁻);* □, *JC7617 (recB⁻C⁻sbcB⁻);* ☆, *JC7619 (recB⁻C⁻, sbcB⁻);* ▼, *JC6722 (recB⁻C⁻sbcB⁻);* ◇, *JC7623 (recB⁻C⁻sbcB⁻);* △, *JC7689 (rec⁺sbcB⁻); and* ★, *JC7694 (rec⁺sbcB⁻).*

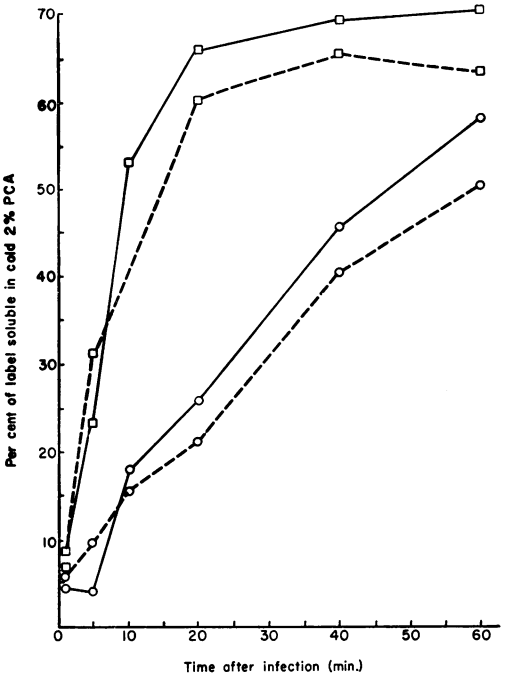

FIG. 6. *Degradation of unmodified λcI857 red3 and λcI857pbio256 after infection of rec⁺ and rec⁻ strains. Symbols: ----, λcI857red3; ——, λcI857pbio256; □, JC4583 (rec⁺); and ○, JC4584 (recB⁻C⁻).*

here was about 50%, after correction for soluble radioactivity in the phage preparation. If it is assumed that all termini produced by the restriction endonuclease are equivalent, then each terminus is degraded an average of 6 to 8% of the total molecule, or 6×10^3 to 8×10^3 bases. About two-thirds of this degradation occurs in strains with reduced levels of exonuclease I (Fig. 5, strains JC7689, JC7694). These strains have about one-quarter of the single-strand-specific exonuclease activity of the wild-type strain (17). Exonuclease I is single-strand-specific (21), and the restriction enzyme produces termini with little, if any, single-stranded DNA (24). Therefore, the following models are proposed to account for the degradation observed in these experiments. Soon after the restriction enzyme has produced a double-stranded scission, the ATP-dependent exonuclease begins to degrade the DNA (on the average 3×10^3 to 5×10^3 bases per terminus in these experiments).

Model 1: the ATP-dependent exonuclease is 5′-specific. Thus, degradation by it leaves a 3′-terminated single-stranded region. This single-stranded terminus is sensitive to exonuclease I, which degrades it.

Model 2: the ATP-dependent exonuclease is 3′-specific. Thus, it leaves a 5′-terminated single-stranded terminus. A single-strand-specific endonuclease is associated with the ATP-dependent exonuclease (10). The single-stranded region is clipped off by the associated endonuclease to yield a single-stranded piece whose 3′-terminus is sensitive to exonuclease I.

Model 3. The ATP-dependent exonuclease activity may start on either the 3′- or 5′-terminus, i.e., it is specific only for double-stranded DNA. The exonuclease I degradation would then follow by the mechanisms described in models 1 and 2.

The cold acid-insoluble DNA fragments remaining after extensive degradation in vivo have the same buoyant density in neutral CsCl as double-stranded DNA (S. Lederberg, *unpublished data*). Therefore, if they contain single-stranded termini, these termini cannot be very long.

Mutations in the *recB* or the *recC* gene, or both, reduce the amount of host cell DNA degraded after UV irradiation and after phage restriction. It is possible that the reduced degradation in these mutants is due to the inviable cells present in these cultures (4, 13). However, the *recB⁻* and *recC⁻* mutants appear to be as capable as *rec⁺* cells in yielding phage after infection (Table 3) and in restricting unmodified λ. It therefore seems reasonable to attribute the reduced degradation of *recB⁻* and *recC⁻* strains to the loss of exonuclease activity rather than to the absence of restriction or degradation, or both, in some of the infected cells.

The absence of a functional *recA* gene results in a large increase in the amount of DNA which is degraded after UV irradiation (5). In contrast, the amount of degradation after restriction does not appear to be influenced by mutations in the *recA* gene. It may be that in these experiments the amount of DNA degraded is limited by the exhaustion of a component required for degradation, for example, ATP. If *recB⁺C⁺* cells are aerated in TM at 37 C before the addition of unmodified phage, the amount of DNA degradation is decreased (*unpublished data*).

In the absence of the ATP-dependent nuclease activity, the restricted DNA is degraded, albeit much more slowly. The degradation may be carried out by exonuclease III, a low level of the *sbcA⁻* exonuclease and/or other exonucleases. The *recB⁻* and *recC⁻* strains exhibit a reduced, but measurable level of recombination, which may be mediated by the exonuclease(s) responsible for the slow degradation observed in these strains.

The activity of a functional exonuclease I gene (*sbcB*) does not significantly alter the amount of DNA degraded in *recB*$^-$ and/or *recC*$^-$ strains (Fig. 5). Thus the nuclease activity observed in *recB*$^-$ and/or *recC*$^-$ strains produces little single-stranded DNA which is sensitive to exonuclease I. Exonuclease III is specific for 3′-termini of double-stranded DNA. It would be expected to produce a single-stranded region with a 5′-terminus which should be insensitive to exonuclease I. The resolution of this question awaits the discovery of mutants lacking exonuclease III.

The presence of *endonuclease* I appears to be without effect upon the degradation of phage DNA after restriction. (Compare strains JC2926 and JC4588, JC4584 and JC5519 in Fig. 2, 3, and 5.)

Several mechanisms can explain the poor restriction observed in *sbcA* mutants. (i) They may result from secondary restriction-deficient mutations; (ii) some component required for restriction interacts with the exonuclease; (iii) the ATP-independent exonuclease alters the sensitivity of the substrate of the restriction enzyme.

The first possibility would require that the *sbcA* mutation be at the *hsp* locus or select for a secondary type of *hsp* mutation. Recombinants selected from crosses in which the *E. coli* genome between the Hayes origin and *tonA* is transferred to a recipient *sbcA* cell still have a *sbcA*$^-$ phenotype of DNA restriction and degradation, although most of the recombinants would be expected to have the donor *hsp*$^+$ locus from this transferred region. Therefore, allelism between the *sbcA* and the *hsp* loci is unlikely. A secondary mutation in *hsp* is also ruled out unless high levels of ATP-independent exonuclease are lethal for cells with a wild-type *hsp* locus and thereby select for a special recombinant class. Further analysis by transduction of *hsp* and *sbcA* loci into different background genomes will be of help here.

Apropos the second possibility, the ATP-independent exonuclease of *sbcA* is partially inhibited by ATP, a component required for in vitro activity of the restriction enzyme. Thus the *sbcA* nuclease may interfere with restriction by interacting with ATP. It would be necessary for this nuclease to reduce the ATP level in vivo, for which activity there is no evidence as yet. Alternatively, in vivo products of *sbcA* activity may regulate the level of *hsp* endonuclease.

Another possibility is that of substrate alteration. It has been shown in vitro that the restriction endonuclease cleaves first one strand and then the other of restricted DNA and that the restriction enzyme is inactive on single-stranded DNA (24). The *sbcA* exonuclease could alter the unmodified DNA substrate in two ways: (i) after the first DNA strand is cut, the exonuclease might bind to nicked DNA and thereby prevent the second scission from occurring; or (ii) the *sbcA* nuclease might partially degrade the nicked DNA, leaving a single-stranded gap opposite the site where the second restriction scission would normally occur. This might prevent the second scission from occurring since the restriction endonuclease is inactive on single-stranded DNA. A study of the levels of restriction endonuclease and of the physical properties of λ DNA in *sbcA* cells should clarify these possibilities.

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