Copyright © 1995 by the Genetics Society of America

DNA Structures Generated During Recombination Initiated by Mismatch Repair of UV-Irradiated Nonreplicating Phage DNA in *Escherichia coli:* Requirements for Helicase, Exonucleases, and RecF and RecBCD Functions

Wen-Yang Feng and John B. Hays

Department of Agricultural Chemistry, Oregon State University, Corvallis, Oregon 97331

Manuscript received January 31, 1995
Accepted for publication April 21, 1995

ABSTRACT

During infection of homoimmune *Escherichia coli* lysogens ("repressed infections"), undamaged nonreplicating λ phage DNA circles undergo very little recombination. Prior UV irradiation of phages dramatically elevates recombinant frequencies, even in bacteria deficient in UvrABC-mediated excision repair. We previously reported that 80–90% of this UvrABC-independent recombination required MutHLS function and unmethylated d(GATC) sites, two hallmarks of methyl-directed mismatch repair. We now find that deficiencies in other mismatch-repair activities—UvrD helicase, exonuclease I, exonuclease VII, RecJ exonuclease—drastically reduce recombination. These effects of exonuclease deficiencies on recombination are greater than previously observed effects on mispair-provoked excision *in vitro.* This suggests that the exonucleases also play other roles in generation and processing of recombinagenic DNA structures. Even though dsDNA breaks are thought to be highly recombinagenic, 60% of intracellular UV-irradiated phage DNA extracted from bacteria in which recombination is low—UvrD$^-$, ExoI$^-$, ExoVII$^-$, or RecJ$^-$—displays (near-)blunt-ended dsDNA ends (RecBCD-sensitive when deproteinized). In contrast, only bacteria showing high recombination (Mut$^+$ UvrD$^+$ Exo$^+$) generate single-stranded regions in nonreplicating UV-irradiated DNA. Both *recF* and *recB recC* mutations strikingly reduce recombination (almost as much as a *recF recB recC* triple mutation), suggesting critical requirements for both RecF and RecBCD activity. The mismatch repair system may thus process UV-irradiated DNA so as to initiate more than one recombination pathway.

ESCHERICHIA COLI contains versatile and efficient recombination systems; recombination-deficient mutants (reviewed by CLARK and SANDLER 1994) are sensitive to UV light and other genotoxic agents, suggesting that recombination mitigates the consequences of unrepaired DNA damage. Phage-λ nonreplicating-DNA systems have been successfully used to analyze damage-stimulated recombination *in vivo* (LIN and HOWARD-FLANDERS 1976; IKEDA and KOBAYASHI 1978; HAYS and BOEHMER 1978; PORTER *et al.* 1979; FENG *et al.* 1991). The intracellular substrates in these experiments are well-defined 50-kb circles, in which DNA replication and most transcription are blocked by (homoimmune) phage repressor. Normally recombination is negligible, but UV irradiation of phages before infection (of unirradiated bacteria) increases recombinant frequencies to ≥30% (HAYS and BOEHMER 1978); stimulation is not due to induction of the *E. coli* SOS system (HAYS and LEE 1985). Irradiated phage DNA can give rise to recombinagenic structures via excision repair, but phages irradiated at moderate doses show substantial recombination even in UvrABC$^-$ bacteria (LIN and HOWARD-FLANDERS 1976; PORTER *et al.* 1978; SMITH and HAYS 1985). FENG *et al.* (1991) showed UvrA-independent recombination to depend mostly on the *E. coli* mismatch-repair activities MutH, MutL, and MutS, and on the presence of unmethylated d(GATC) sequences in the phage DNA. This suggested that methyl-directed mismatch repair sometimes converted photoproduct-containing DNA into recombinagenic structures.

MutH, MutL and MutS proteins mediate incision at unmethylated d(GATC) sequences near base-mispairs (AU *et al.* 1992). Subsequent excision requires the UvrD helicase (helicase II) and, depending on the relative orientation of the mismatch and the unmethylated d(GATC) site, single-stranded-DNA exonucleases with 5′ → 3′ (RecJ exonuclease, exonuclease VII) or 3′ → 5′ (exonuclease I) specificity (COOPER *et al.* 1993). Here we have asked whether MutHLS-initiated recombination of nonreplicating UV-irradiated phage DNA in UvrA$^-$ bacteria also requires these helicase and exonuclease activities.

By analyzing phage DNA extracted from infected cells, we have further sought to determine whether UV-irradiated DNA is physically broken down by mismatch repair, whether any breakdown products resemble structures thought to be recombinagenic, and whether mutations that affect recombination affect DNA breakdown. Repressed infections afford significant advantages for study of recombination substrates and inter-

Corresponding author: John B. Hays, Department of Agricultural Chemistry, Oregon State University, ALS 1007, Corvallis, Oregon 97331-7301. E-mail: haysj@bcc.orst.edu

mediates *in vivo*; in the bacterial chromosome such structures are typically infrequent, and concomitant DNA replication complicates analysis.

Finally, we have asked whether this recombination of UV-irradiated DNA conforms to models for separate RecF-dependent and RecBC(D)-dependent pathways. Separate recombination pathways were originally proposed by CLARK and coworkers (HORII and CLARK 1973), to account for effects of certain mutations on conjugal and transductional recombination. Because *recB(C)* mutants and *recF* mutants are each moderately UV sensitive, but *recB(C) recF* double mutants highly sensitive, the separate-pathway hypothesis has been extended to recombinational repair of UV-damaged chromosomes. RecF is thought to assist recombinational filling of daughter-strand gaps arising from photoproduct-blocked DNA replication, and the RecBCD nuclease/helicase to carry out recombinational rejoining of double-strand breaks (GANESAN and SEAWELL 1975; SMITH and SHARMA 1987; SMITH and WANG 1989).

Here we find recombination to depend heavily on the same helicase and exonuclease activities that mediate repair of base-pair mismatches. The appearance of single-stranded DNA (ssDNA), but not blunt-ended linear double-stranded (dsDNA), is positively correlated with recombination. Both RecF and RecBC(D) function are required.

MATERIALS AND METHODS

Bacterial strains, bacteriophages, and plasmids: Bacterial strains, all *E. coli* K-12 derivatives, are described in Table 1. Lambda bacteriophages Y1722, λ*Aam32 Bam1 plac5 red3 cI857*, and Y1730, λ *plac5 lacZ118(oc) red3 cI857*, have been described (FENG *et al.* 1991). Plasmid pCSR606 encodes the *E. coli* phr^+ gene and some adjacent bacterial DNA (SANCAR *et al.* 1984); in pMS312, the *E. coli* $mutS^+$ gene is inserted into the *Eco*RI-nuclease-encoding gene of pSCC31 (SU and MODRICH 1986); pRC202 encodes the *E. coli* $xseA^+$gene (CHASE *et al.* 1986); we constructed pWF1 by inserting the phage λ *cI* (ind^-) gene (*Bgl*II fragment) into the *Bam*HI site of the pACYC-derived vector pAClac19 (SCHAEFER and HAYS 1990).

Preparation and irradiation of bacteriophages: Stocks of λ bacteriophages, radiolabeled with [^{3}H]thymidine, were prepared and UV-irradiated essentially as described by FENG *et al.* (1991). UV-induced cyclobutane pyrimidine dimers (CPDs) were estimated on three different occasions, by extraction of phage DNA and treatment with *Micrococcus luteus* or phage T4 UV endonuclease and sedimentation in alkaline sucrose, as described by HAYS *et al.* (1985). Exposure to 20 J/m^2 induced ~20 CPDs per λ duplex; (6-4) photoproducts would be expected to be ~15–30% as frequent as CPDs (FRANKLIN *et al.* 1982; MITCHELL 1988).

Repressed infections and recombination assays: Infection of *E. coli* bacteria lysogenic for λ *xisl red3* $c^+(ind^-)$, or bacteria carrying plasmid pWF1, with UV-irradiated homoimmune phages (λY1722 + λY1730 or λY1728 + λY1729; five of each parent per cell), incubation (with shaking) in broth at 38°, harvesting of samples at various times, and extraction of DNA for recombination and sedimentation assays, were essentially as described (FENG *et al.* 1991).

Sucrose gradient analyses: Zone sedimentation of DNA in sucrose gradients at neutral pH was essentially as described by BODE (1965). Phages were radiolabeled, UV irradiated, and infected into lysogenic bacteria. Samples of unlabeled bacterial DNA plus [^{3}H]thymidine-labeled phage DNA, extracted from repressed-infection bacteria, were mixed with [^{14}C]thymidine-labeled phage DNA that had been extracted from purified phage particles. Mixtures were layered directly onto the top of 5-ml 5–20% sucrose gradients and centrifuged at 45,000 rpm in an SW50.1 rotor (Beckman) at 20° for 1.5 hr. Fractions (170 μl) were collected from the bottom. Aliquots of 40 μL were mixed with 3.5 ml of liquid-scintillation fluid (New England Nuclear "formula 989") and counted in a Beckman scintillation counter for 10 min. Spillover of ^{3}H in the ^{14}C channel was negligible. A set of ^{14}C samples spanning a range of 40–10,000 cpm was counted, and the rate of spillover of ^{14}C cpm into the ^{3}H channel determined to be 16%. This correction was applied to ^{3}H-cpm values. The DNA remaining in each fraction was precipitated with EtOH, resuspended in TE Buffer (10 mM Tris-HCl, 10 mM Na_2 EDTA, pH 8), and used for other assays.

Analysis of DNA with RecBCD enzyme: DNA in neutral-sucrose-sedimentation fractions, or pools of several adjoining fractions (each sample contained ≥300 cpm) was precipitated by EtOH. Samples usually contained 2.5–7.5 ng phage DNA, plus a large excess of bacterial DNA. RecBCD enzyme was kindly provided by Dr. ANDREW TAYLOR (TAYLOR and SMITH 1985). He assayed the specific activity to be ~127,000 units per mg protein. Units are double-stranded exonuclease units of EICHLER and LEHMAN (1977): 1 nmole of nucleotides solubilized in 20 min at 37°; estimated purity was greater than 95% (by gel electrophoresis). Digestions were carried out as described by TAYLOR and SMITH (1985), in a 20 μl solution containing 50 mM Tris-HCl (pH 7.0), 1 mM dithiothreitol, 10 mM $MgCl_2$, 200 mM ATP, 1 mg/ml bovine serum albumin, 2.5–7.5 ng of sample DNA plus ~200 ng bacterial DNA, and two (or less, if indicated) units of RecBCD enzyme. After incubation at 37° for 30 minutes, reactions were terminated by addition of 0.2 volume of solution containing 200 mM EDTA, 0.1% sodium dodecyl sulfate, 50% sucrose, 0.2% bromophenol blue, and 0.2% xylene cyanol. Samples were mixed with 0.1 volume of 3 M Na-Acetate, two volumes of chilled 95% ethyl alcohol, and kept at −20° for 20 min. After centrifugation for 15 min at 13,000 rpm in a microfuge in a 4° room, pellets were dried and resuspended in 20 μl TE. The amount of nonalcohol-precipitable radioactivity was determined both directly, as the fraction of DNA radioactivity remaining soluble, and as [1-(fraction precipitated)]. The average of these two values was taken as the RecBCD sensitivity.

The resistance to RecBCD digestion of circular DNA duplexes containing gaps was verified using model substrates. Plasmid pBR322 was digested with restriction endonucleases *Eco*RI plus *Sal*I (i), or *Bam*HI plus *Sal*I (ii), and the long fragments electrophoretically purified. These were annealed to pBR322 linearized by *Pst*I endonuclease, to yield hydrogen-bonded circular heteroduplexes with (i) 0.65-kb or (ii) 0.28-kb gaps, separated from nicks at the *Pst*I site by 0.75 or 1.1 kb, respectively, plus linear homoduplexes. Gel electrophoresis revealed putative gapped-circle bands migrating more slowly than the doublet corresponding to linear duplexes, but ahead of presumed hydrogen-bonded concatamers trapped in the wells. Under the digestion conditions used, all of the presumed linear species were removed after 15 min, but the putative gapped-circle bands showed no loss in intensity after 60 min RecBCD treatment (data not shown). These experiments also rule out significant dsDNA endonuclease contamination of the RecBCD preparations.

Analysis of DNA with mung-bean endonuclease: Mung-bean endonuclease specifically cleaves single-stranded DNA or RNA. Reactions were carried out as recommended by the

TABLE 1

Bacterial strains

Strain	Pertinent genotype	Other markers	Source (reference)
BW1091	Δ*xonA* = Δ*(attP24-dcd)*	*rcsA*::(mini)Tn*10*	B. WEISS (NEUHARD and THOMASSEN 1976)
C600	*rec*$^+$	*supE thi-1 thr leu lacY*	(BACHMANN 1972)
DP302	*recA::cat*	F$^-$ *supE42*	(BIEK and COHEN 1986)
ES1574	*mutS201*::Tn*5*	As AB1157[a]	R. FISHEL (FISHEL *et al.* 1986)
FD2563	Δ*(uvrB-chlA)*	As C600	(HAYS, ACKERMAN and PANG 1990)
GM2159	*dam13*::Tn*9*	F$^-$ *thr-1 ara-14 leuBN6* Δ*(gpt-proA)*	M. MARINUS (CARRAWAY *et al.* 1983)
JC12123	*recJ284*::Tn*10*	similar to AB1157	(LOVETT and CLARK 1984)
JH158	*rec*$^+$ [λ*cI(ind*$^-$*) red3 xis1*]	*galK2 rpsL*	(HAYS and BOEHMER 1978)
JH303	*recF143*	As N99	This laboratory (SMITH and HAYS 1985)
JH310	*recB21 recC22 recF143*	As N99 but *zga921*::Tn*10 guaB*	This laboratory (SMITH and HAYS 1985)
KLC381	Δ*(xseA-guaB)*	*HfrH* Δ*(gal-bio)*	J. CHASE via S. LOVETT
MM2838	*rec*$^+$	*supE thi* Δ(lac-*pro*A13)	This laboratory [F$^-$ derivative of JM101 (YANISH-PERRON, VIERRA and MESSING 1985)]
MPh30	*rec*$^+$	F$^-$Δ*(argF-lacZ)U169 phoA*::Tn*5*	R. WOLF (MICHAELIS, GUARANTE and BECKWITH 1983)
N99	*rec*$^+$	*galK2 rpsL200*	Derived from W3102 (BACHMANN 1972)
RM1031	*dnaE52*::Tn*10*	*thi thy leu thr lacY str*^{r}	R. E. MOSES
SM2265	*uvrA6* (λ*cI* ind$^-$)	As JH158, but Δ*(lac-pro) X111 pyrA*::Tn*10*	Hfr(EG333) × TS9 → Tc^r[Lac$^-$][b]
SM2503	*uvrD*::Tn5 (λ*cI*ind$^-$)	As JH158, but Δ*(lac-pro) X111 pyrA*::Tn*10*	S. KUSHNER (unpublished)
STL114	*recJ 2003*::miniTn*10*km	As AB1157[a]	S. LOVETT (unpublished)
TS9	*uvrA6*	As N99	(SMITH and HAYS 1985)
WF2860	*uvrA6*	As SM2265, but *pyrA*$^+$	P1(C600) × SM2265 → Pyr$^+$[Tc^s]
WF2863	*uvrA6 mutS201*::Tn5	As WF2860	P1(ES1574) × WF2860 → Nm^r[Mut$^-$]
WF2871	*recA::cat*	As MPh30	P1(DPB302) × MPh30 → Cm^r[UV^{s}Rec$^-$]
WF2872	*recA::cat* pCSR606)[c]	As WF2871	This laboratory
WF2873	*dam13*::Tn*9*	*supF trp*::Tn*10*	P1(GM2159) × WF2869 → Cm^r[Dam$^-$]
WF2878	*(pGG503)*[d]	As MM2838	This laboratory
WF2902	Δ*(uvrB-chlA) recF*	As N99	P1(FD2563) × JH303 → Gal$^+$[UV^s]
WF2905	Δ*(uvrB-chlA) rec*$^+$	As N99	P1(FD2563) × N99 → Gal$^+$[UV^s]
WF2908	Δ*(uvrB-chlA) recJ*::Tn*10*	As N99	P1(JC12123) × WF2905 → Tc^r
WF2911	Δ*(uvrB-chlA) recJB21 recC22 revF143*	As N99, but *zga921*::Tn*10*	P1(JH210) × WF2902 → Tc^r[UV^s]
WF2916	Δ*(uvrB-chlA) recB21 recC22*	As N99	P1(FD2563) × JH314 → Gal$^+$[UV^s]
WF2961	(pMS312)[e] *rec*$^+$ Δ*(uvrB-cheA)*	*ara* Δ*(lac-proB)XIII F' (lacI*$^-$ *lacZ461-6 proB*$^+$*)*	This laboratory
WF3119	Δ*xonA*	As WF2860 but Δ*(attP2H-dcd) rcsA*::(mini)Tn*10*	P1(BW1091) × WF2860 → Tc^r[His$^-$]
WF3120	Δ*(xseA-guaB)*	As WF2860 but *zff-208*::Tn*10*	P1[KLC381*(zff-208*::Tn*10)*] × *WF2860* → Tc^{r}Gua$^-$
WF3121	Δ*(xseA-guaB)*	As WF2860 but *zff-3139*::Tn*10*km	P1(KLC3139 [*zff-3139*::Tn*10*km)] × WF2860 → Km^r[Gua$^-$]
WF3122	*recJ2003*::miniTn*10*km	As WF2860	P1(STL114) × WF2860 → Km^r
WF3123	Δ*(xseA-guaB)*Δ*xon*	As WF2860 but *zff-3139*::Tn*10*km Δ*(attP2H-dcd) rcsA*::Tn*10*	P1(KLC281 (*zff-3139*::Tn*10*km)) × WF3119 → Km^r[Gua$^-$]
WF3124	Δ*(xseA-guaB)*Δ*xon recJ2003*::minTn*10*km	As WF2860 but *zff-208*::Tn*10*	P1[KLC381 (*zff-208*::Tn*10*)] × WF3122 → Tc^r[Gua$^-$]
WF3125	Δ*xon recJ2003*::miniTn*10*km	*rcsA*::(mini)Tn*10*	P1(BW1091) × WF3122 → Tc^r[His$^-$]
WF3131	*dnaE511*::Tn*10*	As WF2860	P1(RM1031) × WF2860 → Tc^r[Ts]

Tc, tetracycline; Cm, chloramphenicol; Km, kanamycin; Nm, neomycin; UV, ultraviolet light. *s* and *r* superscripts indicate sensitivity and resistance respectively.

[a] HOWARD-FLANDERS and THERIOT (1962).

[b] P1 (donor strain) × recipient → selected marker [screened marker].

[c] *phr*$^+$, encoding plasmid.

[d] *dam*$^+$, encoding plasmid.

[e] *mutS*$^+$, encoding plasmid.

manufacturer (Gibco-BRL). Mixtures contained, in 0.5 ml, 10 mM Na-acetate (pH 5.0), 50 mM NaCl, 0.1 mM Zn-acetate, 1 mM cysteine, 5% (v/v) glycerol, 5–10 ng sample DNA plus ~200 ng bacterial DNA, and 0.1 unit mung-bean endonuclease (1 unit of enzyme hydrolyzes 1 μg of denatured DNA to acid-soluble material in 1 min at 37°). The apparent requirement for excess endonuclease presumably reflects the presence of excess bacterial DNA and RNA in the reaction mixture. Linear λ [^{14}C]dsDNA included in reaction mixtures was not affected under these conditions. After 20 min incubation at 37°, reactions were terminated by addition of 0.2 ml TE buffer, and the DNA precipitated in EtOH.

Preparation of partially single-stranded DNA by exonuclease III digestion: Digestion mixtures (1 ml) contained 0.4 mmol λ DNA, 70 mmol Tris-HCl, pH 8, 1.7 mmol $MgCl_2$, 1 mmol EDTA and 1 mmol dithiothreitol. Reactions were carried out at 37° for 10 min, using 0.25–2.0 units of exonuclease III per micromole of DNA (1 unit releases 10 nmol of acid-soluble nucleotide in 10 min at 37°).

Analysis of DNA by BND-cellulose chromatography: Benzoylated-napthoylated-DEAE-cellulose (BND-cellulose) was obtained from Sigma. A 25-g amount of resin was washed successively with 500 ml 2 M NaCl and 2 liters H_20, and resuspended in 100 ml NET (0.3 M) buffer [0.001 M EDTA and 0.01 M Tris-HCl (pH 7.5), containing 0.3 M NaCl]. Columns were formed by pouring 3 ml of the resuspended slurry substance into a 3-ml syringe plugged with glass wool, and then washed with 10 ml of NET (0.3 M) buffer. DNA was loaded by slow passage (1 ml/min) of solution through the column. Columns were eluted with three 2-ml aliquots of NET (0.3 M) buffer, followed by six 2-ml aliquots NET (1 M) buffer. Elution was continued with an 0–2% gradient of caffeine dissolved in NET (1M) buffer, and then with 6 ml of 50% formamide [in 2% caffeine and NET (1 M) buffer]. Twenty-one fractions were collected; each was divided into several 0.5-ml aliquots and mixed with 5 ml of liquid scintillation fluid. The radioactivity of each fraction was calculated as the sum of the aliquot radioactivities.

RESULTS

Recombination of UV-irradiated nonreplicating phage DNA in UvrA⁻ bacteria: We sought to determine whether activities that act in mismatch repair after MutHLS-mediated incision are also needed for recombination of nonreplicating UV-irradiated DNA. Mutant derivatives of *uvrA*(λ) bacteria, lacking helicase II (*uvrD*), exonuclease I (*xonA*), exonuclease VII (*xseA*), or RecJ exonuclease (*recJ*), were infected with UV-irradiated λ "Dam⁻" phages [grown on bacteria unable to methylate d(GATC) sequences], and intracellular DNA extracted and assayed for recombination at various times. In *uvrD* bacteria recombination was only 1/10 to 1/4 of wild-type (*exo*$^+$) values (Figure 1). We previously observed very similar reductions in *mutH*, *mutL*, and *mutS* bacteria (Feng *et al.* 1991). Recombination in exonuclease-deficient mutants—*recJ*, *xseA* (ExoVII⁻), or *xonA* (ExoI⁻)—was reduced substantially, to 1/5 to 2/5 of *exo*$^+$ frequencies (Figure 1: ●, ▲, ■, respectively). In previous studies of base-mismatch repair *in vitro*, the repair defect in ExoVII⁻ RecJ⁻ extracts could not be remedied by addition of purified *E. coli* exonuclease VII (ExoVII) (Cooper *et al.* 1993). Here however, an *xseA*$^+$

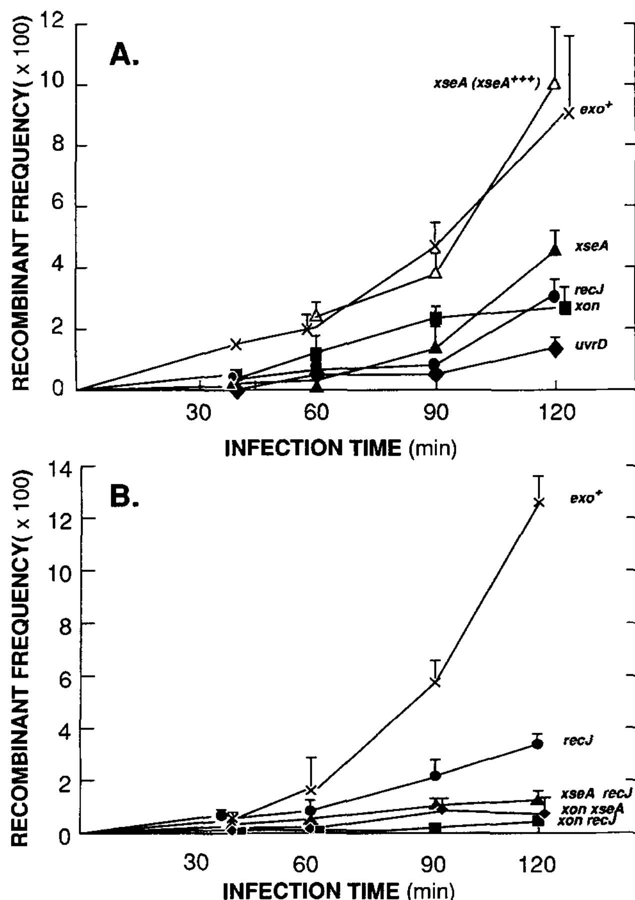

FIGURE 1.—Recombination in exonuclease-deficient and helicase-deficient mutants. Growth of λY1722 (*A*am *B*am *lac*$^+$) and Y1730 (A^+B^+ *lacZ118*) phages to 20 J/m^2, infections of indicated *uvrA*(λ) lysogens under room lighting, extraction of intracellular DNA at indicated times, electroporative transfection of WF2872 (Δ*lac recA*) bacteria, and measurement of the recombinant frequency (fraction of λ A^+B^+ *lac*$^+$ among total A^+B^+ infective centers) was as described under MATERIALS AND METHODS. Data correspond to average values for two or three repressed infections, with half standard deviations indicated. (A) Single mutants. Strains infected: *exo*$^+$(×), WF2860; *xseA*(▲), WF3121; *xseA*(*xseA*$^{+++}$)(△), WF3121-(pRC101); *recJ*(●), WF3122; *xon*(■), WF3119; *uvrD*(♦), SM2503. (B) Double mutants. Strains infected: *exo*$^+$(×), WF2860; *recJ*(●), WF3122; *xseA recJ* (▲), WF3124; *xon xseA* (♦), WF3123; *xon recJ* (■), WF3125.

plasmid fully corrected the recombination deficiency in Δ*xseA* bacteria (Figure 1A, △). This appears to be the first genetic evidence that ExoVII and *E. coli* exonuclease I (ExoI) can play positive roles in recombination; previously, *sbcB* alleles of *xonA*, and *xseA* mutations, were found to actually increase bacterial conjugal and intrachromosomal recombination (Kushner *et al.* 1972; Chase and Richardson 1977). In mutants lacking any two of the exonucleases, recombination was severely reduced, and in Δ*xonA recJ*::Tn*10* bacteria it was nearly abolished (Figure 1B).

Physical analysis of nonreplicating UV-irradiated DNA processed by the mismatch-repair system: There

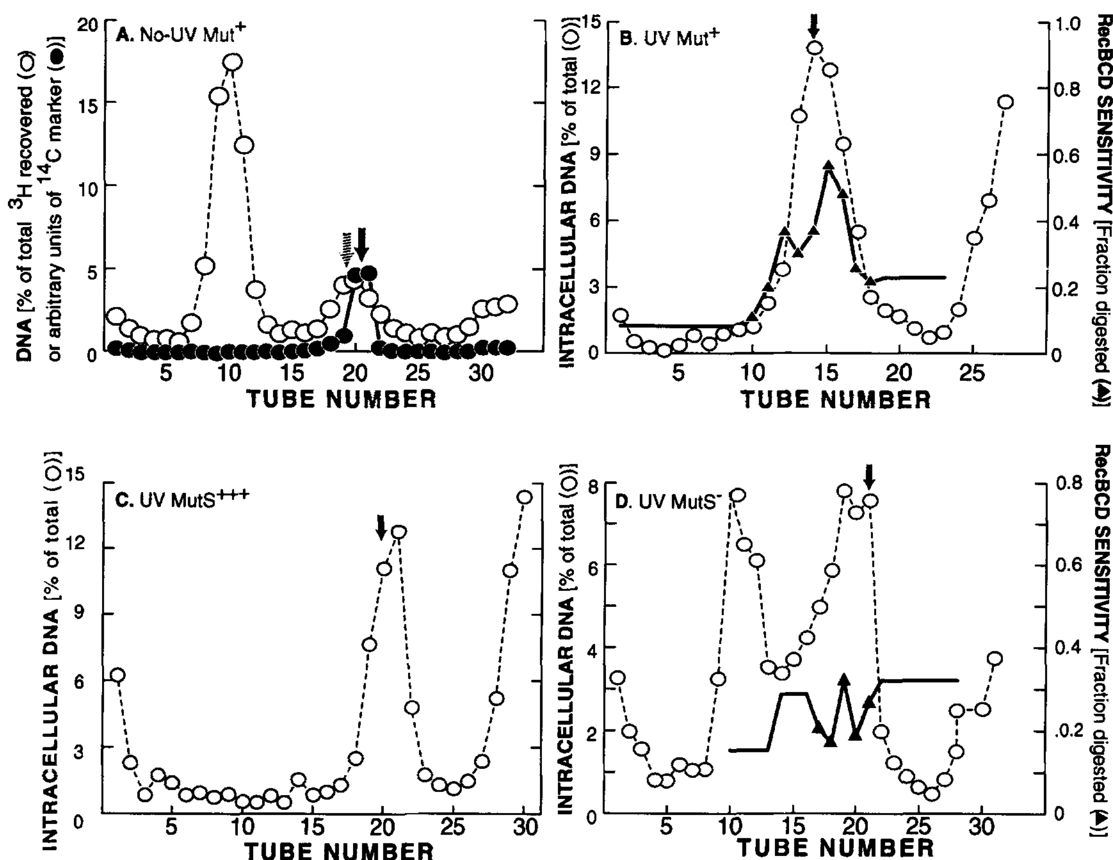

FIGURE 2.—Sedimentation and RecBCD-sensitivity of intracellular phage DNA. Growth of λ Y1730 phages on Dam$^-$ bacteria (strain WF2873) with ^{3}H- or ^{14}C-thymidine, irradiation to 100 J/m^2, infection of *uvrA*(λ) lysogens with indicated additional phenotypes, extraction of intracellular DNA after 60 min, sedimentation in neutral sucrose, analyses of fractions for radioactivity, and of fractions (▲) or pooled fractions (-) for sensitivity to RecBCD nuclease, were as described under MATERIALS AND METHODS. Sedimentation was right to left as shown. Radioactivity in individual fractions is expressed as percent of total [^{3}H]DNA recovered from the gradient (○). Linear [^{14}C]DNA from λ virions, added as a marker to all gradients is shown for each fraction in panel A (●), and the peak center (↓) indicated in other panels. Some of the gradients shown here or in subsequent figures reflect small differences in sedimentation time and/or fraction size that cause the linear marker to appear in different tubes. Representative gradients from two or more experiments are presented. (A) Unirradiated phage in Mut$^+$ bacteria (strain WF2860), (↓) apparent center at nicked-circle peak. (B) UV-irradiated phage in Mut$^+$ bacteria (strain WF2860). (C) UV-irradiated phage in MutS-overproducing (MutS^{+++}) bacteria (strain WF2860 (pMS312)). (D) UV-irradiated phage in MutS$^-$ bacteria (strain WF2863). Total [^{3}H]DNA recovered from gradients was 25,000–30,000 CPM; 600–6000 CPM were used for RecBCD analyses.

have been relatively few studies of DNA structures formed during recombination *in vivo*; much of this work has focused on detection of joint molecules (see for examples ANRAKU *et al.* 1969; IKEDA and KOBAYASHI 1978). Especially lacking have been analyses of potential recombinagenic substrates. In this initial physical study of the fate of nonreplicating UV-irradiated phage λ DNA in UvrA$^-$ Mut$^+$ bacteria, we employed neutral-sucrose sedimentation and treatment with various enzymes, to semiquantitatively address three questions: Does "mismatch repair" cause physical breakdown of nonreplicating UV-irradiated DNA *in vivo*? Do any breakdown products have properties associated with recombinagenicity, *e.g.*, double-strand ends or single-stranded regions? Do mutations that reduce recombination also alter the nature of the breakdown products?

Phage λ [^{3}H]DNA, extracted from bacteria after a 60-min repressed infection, was sedimented in neutral sucrose, along with exogenous linear λ [^{14}C]DNA. Unirradiated DNA (Figure 2A) was mostly (here 55%) in a fast-sedimenting supercoiled form, as previously observed (BODE 1965). A lesser portion (here 16%) sedimented at intermediate velocities, characteristic of nicked-circular and linear DNA. Although these latter two species are not well resolved, the differences in ^{3}H:^{14}C ratios for tubes 18 and 19 *vs.* 20 and 21 suggest that for unirradiated DNA most of the central peak consists of nicked-circular DNA (cross-hatched arrow), which sediments 1.14 times faster than linear λ DNA (filled arrow) (BODE 1965). Only ~9% of the radioactivity appeared in the last three fractions, the position of very small DNA or nucleotides. The remaining 20% was spread throughout the gradient. The amount of linear λ [^{14}C]DNA in each fraction is shown here, so as to illustrate typical peak shape, but in subsequent profiles only the peak position is shown.

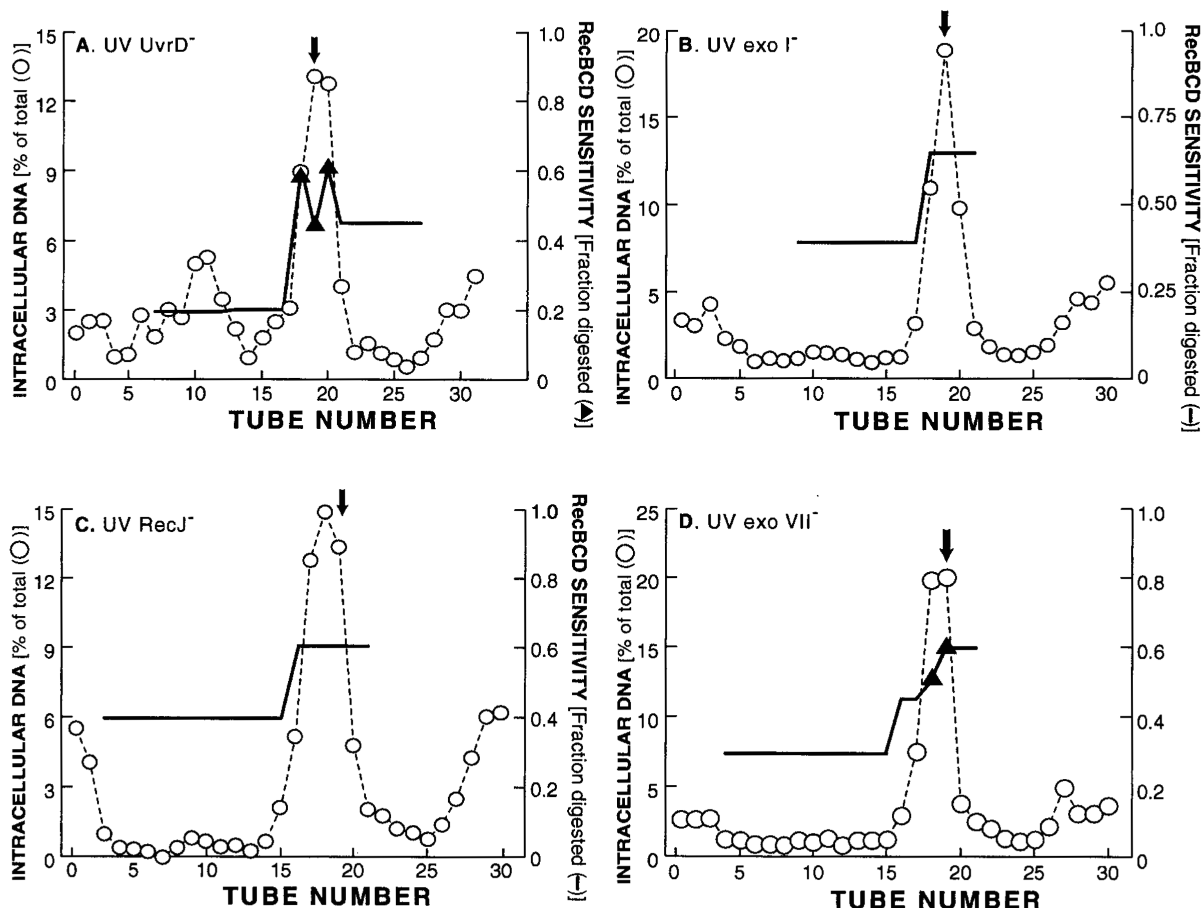

FIGURE 3.—Sedimentation and RecBCD analyses of UV-irradiated phage DNA from exonuclease-or helicase-deficient bacteria. Growth and ^{3}H-thymidine labeling of Y1730 phages on Dam$^-$ bacteria (strain WF2873), irradiation of phages to 100 J/m^2, infection of *uvrA*(λ) lysogens with indicated mutant phenotypes, extraction of intracellular DNA after 60 min, sedimentation in neutral sucrose together with linear ^{14}C-labeled λ DNA (peak position, ↓), determination of percent of recovered ^{3}H CPM in individual fractions (▲), and of proportion of RecBCD-digestible ^{3}H DNA in individual fractions (▲) or pools of fractions (-), were as described under MATERIALS AND METHODS. RecBCD sensitivity corresponds to average between acid-soluble CPM and 1 − (fraction CPM precipitated). Representative gradients from three experiments are shown; RecBCD sensitivities varied by less than ±35% among experiments. Total ^{3}H CPM recovered from gradients was 25,000–30,000; 1600–12,000 CPM were used to analyze RecBCD sensitivity. (A) UV-irradiated phage in UvrD$^-$ bacteria (strain SM2503). (B) UV-irradiated phage in ExoI$^-$ bacteria (strain WF3119). (C) UV-irradiated phage in RecJ$^-$ bacteria (strain WF3122). (D) UV-irradiated phage in ExoVII$^-$ bacteria (strain WF3121).

"Dam$^-$" phages irradiated to 100 J/m^2 lose ~80% of their biological activity within 40–60 min after (repressed) infection of Mut$^+$ UvrA$^-$ bacteria (FENG *et al.* 1991). Figure 2B shows that during this time all supercoiled DNA is eliminated, and ~25% degraded to very small fragments (last three tubes). Breakdown was more pronounced in cells overproducing MutS protein (Figure 2C): most of the products sedimented at the positions of full-length or slightly smaller linear λ DNA, or very small DNA (now 33% of total). In MutS$^-$ bacteria (Figure 2D), most DNA appeared to sediment as supercoiled or nicked-circular DNA, but the resolution was not good enough to exclude linear products.

Linear dsDNA with blunt or near-blunt ends, a good substrate for RecBCD helicase/nuclease (TAYLOR and SMITH 1985), might be expected to initiate recombination by the RecBCD pathway. Also, linearized chromosomes would be expected to transfect poorly, accounting for the previously observed loss in biological activity (FENG *et al.* 1991). We deproteinized aliquots from tubes (▲) or pools of tubes (——) and determined the fraction of DNA in each tube that was digestible by RecBCD nuclease. Nearly 60% of the DNA from Mut$^+$ Exo$^+$ cells that sedimented at the velocity of linear λ DNA appeared RecBCD-sensitive (Figure 2B). The central peak therefore appears to be a mixture of RecBCD-sensitive (near-) blunt-ended dsDNA, and RecBCD-resistant nicked-circular DNA and/or linear molecules with overhanging ssDNA tails. Linear DNA with single-stranded tails longer than 50 nt, or circular DNA with large gaps, is known to be resistant to RecBCD digestion (PRELL and WACKERNAGEL 1980), although excess enzyme is reported to slowly digest circular DNA with gaps >650 nt (TAYLOR and SMITH 1985). We verified that

under our reaction conditions, model substrates containing 275- or 650-nt gaps were fully RecBCD-resistant (see MATERIALS AND METHODS). DNA from a MutS$^-$ infection showed no distinctive RecBCD-sensitive peak (Figure 2D). We cannot explain the significant background of RecBCD sensitivity across the gradient in this case; it might partially reflect breakage during manipulation of the fractions. The RecBCD nuclease preparation did not show any endonuclease activity in control reactions (see MATERIALS AND METHODS).

DNA from bacteria lacking post-MutHLS mismatch-repair activities—UvrD helicase, exonuclease I, exonuclease III, RecJ nuclease—was similarly analyzed by sedimentation and RecBCD treatment. In UvrD$^-$ Mut$^+$ bacteria (Figure 3A), loss of supercoiled DNA was pronounced but incomplete. Perhaps in the absence of UvrD helicase, DNA ligase sometimes reseals nicks introduced by MutHLS, and gyrase reintroduces supercoils. Very small DNA accounted for only 13% of the total. DNA from exonuclease-deficient cells (Figure 3, B–D) showed central peaks, at the positions of linear DNA, that were narrower than those in Exo$^+$ gradients (Figure 2B), and there was less very small DNA. About 60% of the (deproteinized) DNA from UvrD$^-$ or Exo$^-$ bacteria (Figure 3) was RecBCD-sensitive, *i.e.*, appeared to consist of blunt-ended linear dsDNA, even though recombination was severely reduced in these cases (Figure 1). This suggests that intracellular linear dsDNA is not itself a sufficient substrate for recombination, because it lacks long ssDNA tails, or because its ends are protected by proteins, or for other reasons. Although the apparent background RecBCD sensitivity of the fastest-sedimenting DNA appeared as high as 30–40% in the Exo$^-$ gradients, there was very little of this DNA (Figure 3, A–C).

We assayed for another structure expected to initiate recombination, namely ssDNA, in two ways. First, we analyzed fractions from neutral sucrose gradients by chromatography on BND-cellulose. Second, we exhaustively treated aliquots of the fractions whose RecBCD digestibility is shown in Figures 2, B and D, and 3, A-D with mung-bean endonuclease to remove all ssDNA, both in tails and at interior gaps, and then digested any newly formed blunt-ended linear DNA with RecBCD nuclease. The amount of DNA previously resistant to RecBCD nuclease, but now made susceptible, would correspond to the amount of DNA attached to ssDNA regions. The actual amount of ssDNA might be much less.

For preliminary calibration experiments with BND-cellulose, we digested λ DNA (from phage particles) to 10% ethanol solubility with exonuclease III, which selectively digests 5′ → 3′ at dsDNA ends (RICHARDSON *et al.* 1964). A mixture of this partially single-stranded [^{3}H]DNA and untreated linear λ [^{14}C]DNA was applied to a BND-cellulose column, and eluted with a series of solutions (Figure 4A). Elution with 1 M NaCl displaced most of the fully dsDNA (□), but virtually no partially

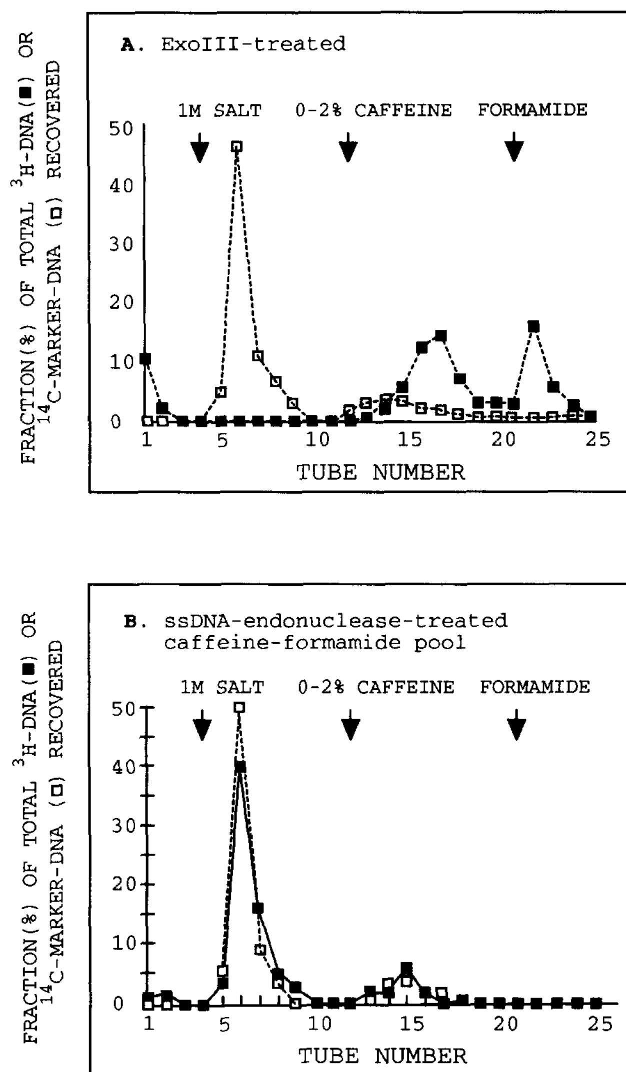

FIGURE 4.—BND-cellulose analysis of DNA standards. (A) Partially single-stranded λ DNA. Growth of λ Y1730 phages with [^{3}H]thymidine or [^{14}C]thymidine radiolabeling, preparation of phage DNA, treatment of [^{3}H]DNA with exonuclease III to 10% ethanol solubility, and analysis by BND-cellulose chromatography, were as described under MATERIALS AND METHODS. A mixture of 3000 CPM (~300 ng) ExoIII-treated [^{3}H]DNA (■) plus untreated [^{14}C]DNA (□) was loaded onto a BND-cellulose column and eluted successively with 0.3 M NaCl, 1 M NaCl, a 0–2% gradient of caffeine in 1 M NaCl, and a solution of 50% formamide in 1 M NaCl plus 2% caffeine. Typically 80–90% of loaded CPM was recovered in the tubes. Data represent fraction of total recovered. (B) Analysis of caffeine-formamide DNA with mung-bean endonuclease and BND-cellulose. DNA from fractions 14–25 from gradient shown in A, mostly ^{3}H-labeled (■) was precipitated in EtOH, mixed with fresh untreated λ [^{14}C]DNA (□), and treated with mung-bean endonuclease and rechromatographed on BND-cellulose, as described under MATERIALS AND METHODS.

ssDNA (■). A gradient of 0–2% caffeine in 1 M NaCl eluted about half of the ssDNA, and a mixture of 50% formamide, 1 M NaCl and 2%-caffeine eluted another fourth. About 1/10 of the dsDNA was eluted by caffeine but not by 1 M salt. This was the case even with rechro-

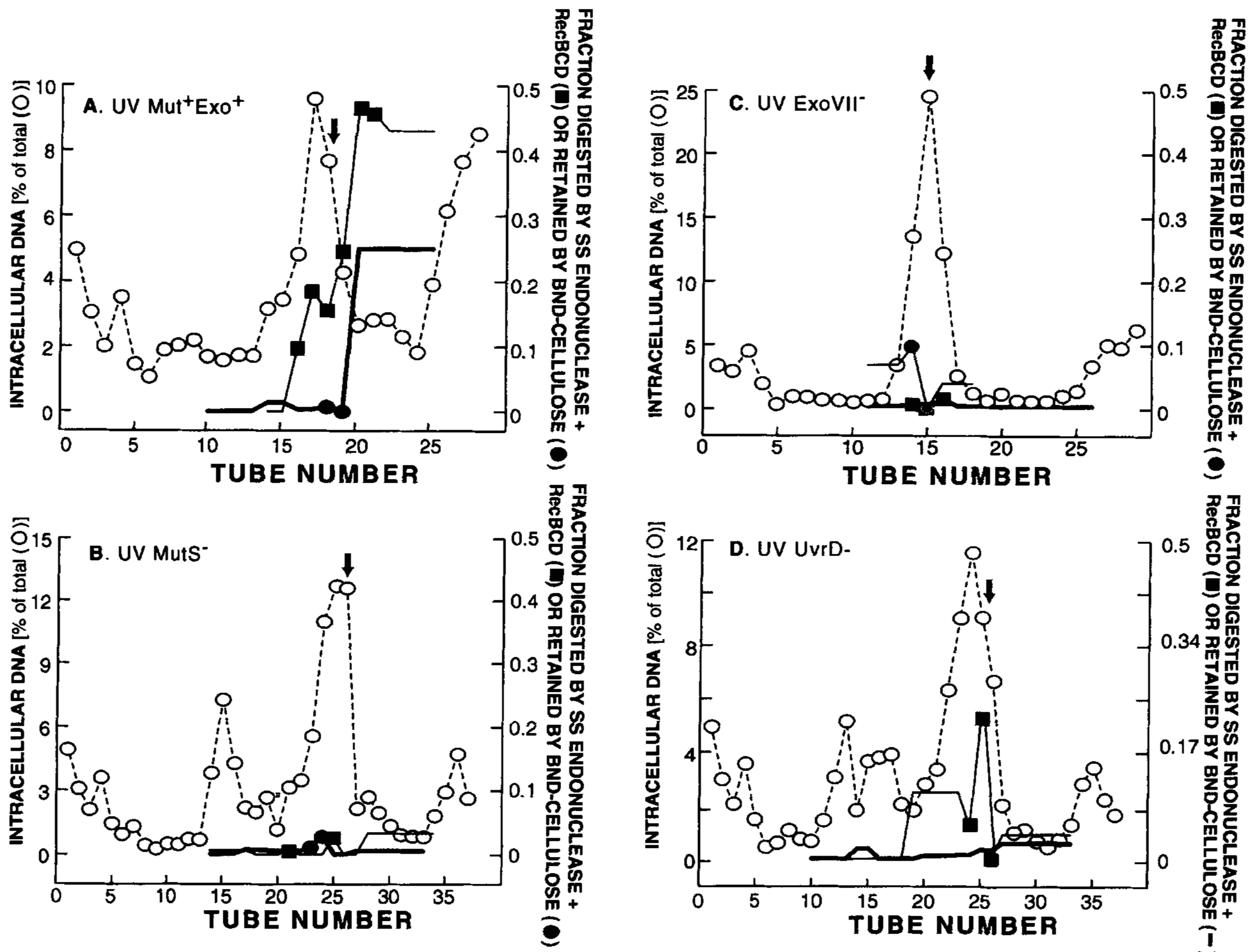

FIGURE 5.—Single-stranded character of intracellular phage DNA. Growth and [^{3}H]thymidine labeling of Y1730 phages on Dam$^-$ bacteria (strain WF2873), irradiation of phages to 100 J/M^2, infection of uvrA(λ) lysogene with indicated additional phenotypes, extraction of intracellular DNA after 60 min, sedimentation in neutral sucrose together with [^{14}C]thymidine-labeled λ DNA (peak position, ↓), determinations of percent of recovered ^{3}H CPM in individual tubes (○), of fractions of DNA in individual tubes (■) or pools of tubes (-) digestible by RecBCD nuclease after treatment with mung-bean endonuclease, and of fraction of DNA in individual tubes (●) or pools of tubes (■) retained by BND-cellulose in 1 M salt, were as described under MATERIALS AND METHODS. The data for treatment with mung-bean endonuclease plus RecBCD nuclease correspond to analyses of fractions from the gradients shown in Figure 2, B and D, and 3, A and D, respectively. These have been replotted at the positions of the corresponding fractions in A–D above, respectively, to facilitate comparison of the two measurements of single-stranded character.

matographed 1-M-salt-eluted material. DNA eluted by the caffeine-gradient and formamide steps, when treated with ssDNA/RNA specific mung-bean endonuclease (Figure 4B, ■), chromatographed identically with untreated dsDNA (□). The fraction of "caffeine-formamide DNA" therefore reflects the fraction with some single-stranded character. The BND-cellulose technique could not be used to estimate the actual degree of single-strandedness: samples digested with exonuclease III to varying extent showed similar chromatographic properties (data not shown).

We fractionated DNA from repressed infections by neutral sucrose sedimentation and analyzed individual fractions or pools of fractions by BND-cellulose chromatography (Figure 5). Comparison of the gradient profiles in Figure 5 with corresponding profiles in Figures 2 and 3 indicates the reproducibility of the sedimentation analyses. We also measured, in aliquots of the fractions shown in Figures 2, B (Mut$^+$UvrD$^+$Exo$^+$) and D (Mut$^-$UvrD$^-$Exo$^+$), and 3, D (Mut$^+$UvrD$^+$ Exo-VII$^-$) and A (Mut$^+$UvrD$^-$Exo$^+$), the extent to which RecBCD sensitivity was increased by digestion with mung-bean ssDNA-endonuclease. To facilitate comparison of the two measures of ssDNA character, we replotted the fraction-by-fraction mung-bean-endonuclease/RecBCD-nuclease data obtained from the gradients shown in Figures 2, B and D, and 3, D and A, at the positions of the corresponding fractions of the gradients shown, respectively, in Figure 5, A–D, along with the BND-cellulose data for these latter gradients.

In Mut$^+$UvrD$^+$Exo$^+$ bacteria, a significant fraction of material sedimenting more slowly than linear λ DNA (fractions 20–26) contained single-stranded regions, by both criteria. DNA corresponding to fractions 20–26

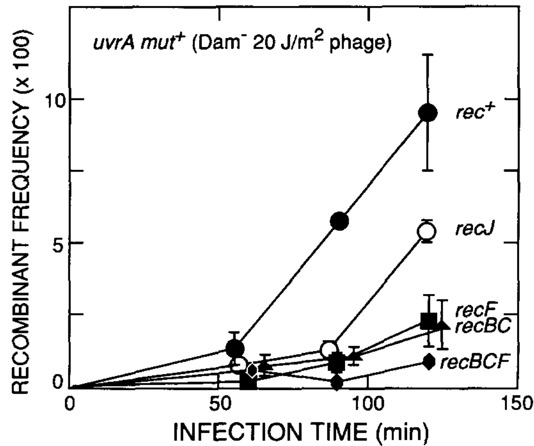

FIGURE 6.—Effects of *recF, recBC,* and *recJ* mutations on recombination. Growth of Dam⁻ λ Y1722 (*Aam Bam lac*⁺) and λ Y1730 (*A*⁺*B*⁺ *lacZ118*) phages, irradiation to 20 J/m², infections of indicated *uvrA*(λ) repressor-expressing bacteria, extraction of intracellular DNA at indicated times, electroporative transfection of Δ *lac recA* bacteria (strain WF2872), and measurement of *A*⁺*B*⁺ *lac*⁺ among *A*⁺*B*⁺ infective centers, were as described under MATERIALS AND METHODS. Data represent averages from two independent experiments, with ranges indicated. *uvrA* (λ) derivatives employed: *rec*⁺(●), WF2905-(pWF1); *recJ*(○), WF2908(pWF1); *recF*(■), WF2902(pWF1); *recBC*(▲), WF2916(pWF1); *recF recBC* (◆), WF2911(pWF1).

from a similar Mut⁺ UvrD⁺ Exo⁺ gradient, after purification by a second round of neutral-sucrose sedimentation, again chromatographed on BND-cellulose as if partially single stranded (data not shown). In contrast, MutS⁻, UvrD⁻, and ExoVII⁻ bacteria yielded little or no ssDNA, by either criterion (Figure 5, B–D). RecJ⁻ and ExoI⁻ bacteria showed patterns very similar to ExoVII⁻ patterns (data not shown). Thus, only in Mut⁺ UvrD⁺ Exo⁺ bacteria did irradiated phages yield high recombinant frequencies (Figure 1), and high ssDNA levels (Figure 5A).

Dependence of recombination on RecF and RecBCD functions: HORII and CLARK (1973) hypothesized separate RecF- and RecBCD-dependent recombination pathways. If the recombination initiated here by mismatch repair of UV-irradiated DNA proceeds only via a RecF-dependent recombination pathway, or only via a RecBCD-dependent pathway, then recombination should be reduced by a *recF* mutation, but not a *recB(C)* mutation, or vice versa. If however, significant recombination takes place via each of two separate pathways, *recF* or *recB*(*C*) mutations should have only partial effects and *recF recB*(*C*) mutations should completely eliminate recombination. In fact, neither prediction was fulfilled: recombination appeared heavily dependent on both RecF and RecBCD functions (Figure 6). Perhaps structures processed respectively by RecF and RecBCD are both involved in the same recombination pathway, or distinct substrates initiate separate pathways that each require both RecF and RecBCD function.

DISCUSSION

Experiments described here, together with our earlier work (FENG *et al.* 1991), implicate seven mismatch-repair activities—MutH, MutL, and MutS, UvrD helicase, exonuclease I, exonuclease VII, and RecJ nuclease—in recombination of UV-irradiated nonreplicating d(GATC)-undermethylated phage λ DNA in UvrA⁻ bacteria, and in physical breakdown of irradiated DNA. This breakdown thus seems the likely cause of progressive loss of biological activity of the DNA *in vivo* (FENG *et al.* 1991). Breakdown products appear to include linear dsDNA, even in UvrD⁻ or Exo⁻ bacteria, in which recombination is very low; in contrast, significant amounts of ssDNA appear only in recombination-proficient Mut⁺ UvrD⁺ Exo⁺ cells. The strong dependence of this recombination on both RecF and RecBCD function suggests that the operative pathway or pathways differ from those postulated for conjugal and transductional recombination.

The severe effects of *uvrD, recJ, xonA* or *xseA* mutations on recombination (Figure 1) point to more than one function for the respective gene products, perhaps involving more than one recombinagenic substrate. Possible routes to recombinagenic DNA structures via mismatch "repair" of UV-irradiated d(GATC)-unmethylated DNA are depicted in Figure 7. MutHLS function is presumed to nick one or both strands at fully unmethylated d(GATC) sites (AU *et al.* 1992) [Figure 7, Step (1)]. UvrD helicase might be required not only to generate excision gaps [Figure 7 (2A)], but also to prevent religation of near-blunt dsDNA ends [Figure 7 (2B)], and perhaps facilitate resection of such ends by ssDNA-specific exonucleases [Figure 7 (4)].

Mispair-protected excision *in vitro* requires only ExoI if the d(GATC) is 5′ to the mismatch [Figure 7 (2A)], or either ExoVII or RecJ exonuclease, if the d(GATC) is 3′ to the mismatch (not shown, for simplicity) (COOPER *et al.* 1993; GRILLEY *et al.* 1993). If excision were the only role for these exonucleases here, and d(GATC) sites 5′ to photoproducts were as likely to be incised as 3′ sites, absence of ExoI, or of both ExoVII and RecJ, should therefore have reduced recombination only by one-half; absence of only one 5′ → 3′ exonuclease (ExoVII or RecJ) should have had even less effect. The strong mutation effects (Figure 1) thus suggest multiple exonuclease roles. One or more exonucleases may help prevent ligation of near-blunt dsDNA ends [Figure 7 (2B)] and/or resect these ends to produce ssDNA overhangs [Figure 7 (4)]. Such overhangs might also be formed by endonucleolytic scission of ssDNA gaps where repair synthesis was delayed [Figure 7 (3B and 5)]. The gaps might themselves be recombinagenic [Figure 7 (6A)]. A gap resulting from blockage by a template photoproduct of repair synthesis [Figure 7 (3A)] would be formally identical to a daughter-strand

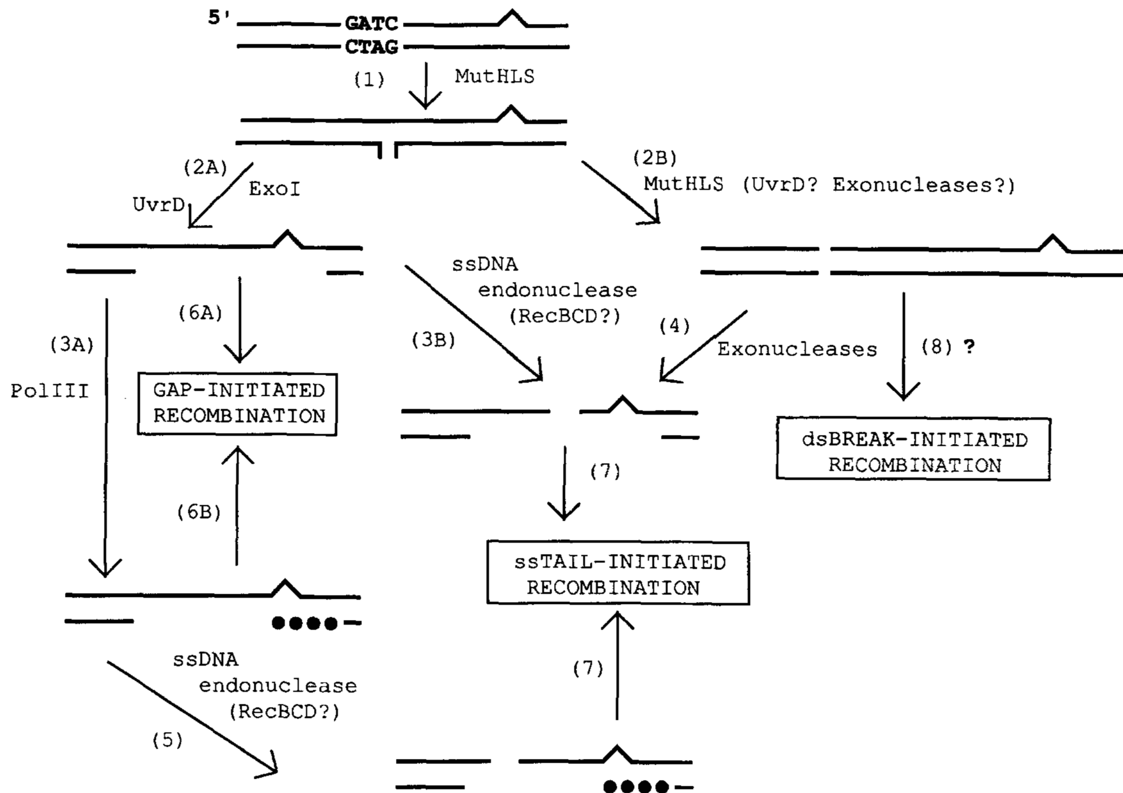

FIGURE 7.—Pathways for recombination initiated by UV photoproducts and MutHLS activity (see text for discussion). MutHLS recognizes DNA in which a UV photoproduct is near a fully unmethylated d(GATC) site (arbitrarily shown 5′ to the photoproduct) and produces a nicked d(GATC) site (arbitrarily shown on the nonphotoproduct-containing strand) (1), which is converted by MutHLS into a dsDNA break (2B) or expanded into an excision gap (2A) by UvrD helicase + ExoI. dsDNA breaks might initiate recombination directly (8) or be resected by end-specific dsDNA exonucleases or helicase plus ssDNA exonucleases (4) to structures with ssDNA tails. Alternatively, PolIII might fill excision gaps by DNA synthesis (3A), until encounter with a photoproduct in the template strand, producing a partial-gap structure analogous to postulated daughter-strand gaps in semiconservatively replicating UV-irradiated DNA. Gapped structures might themselves initiate recombination (6A) or (6B), or be converted by endonucleases to ssDNA-tailed structures (3B, 5), which might then initiate recombination (7).

gap in semiconservatively replicating UV-irradiated DNA. Such blocked-resynthesis gaps might be more recombinagenic than delayed-resynthesis gaps [Figure 7 (6A) *vs.* (6B)] if, for example, RecA protein were already bound to photoproduct sites (LU *et al.* 1986). Recombination might also be initiated by 5′- or 3′-ended ssDNA tails [Figure 7 (7)]. In model "three-strand" recombination reactions between circular ssDNA and linear dsDNA, reactions initiated by 3′-ssDNA ends, such as RecJ or ExoVII might produce by resection of linear dsDNA, appear favored (reviewed by KONFORTI and DAVIS 1992). However, initiation by a 5′-ssDNA end, as ExoI might produce by resection, can also be significant in certain circumstances (DUTREIX *et al.* 1991; KONFORTI and DAVIS 1991). Furthermore, 5′-ssDNA-initiated three-strand reactions go efficiently to completion when the 3′-ended ssDNA displaced during branch migration is continuously digested by ExoI (KONFORTI and DAVIS 1992; BEDALE *et al.* 1993). Exonucleases might play analogous roles here. In summary, the severe reductions in recombination seen in Figure 1 suggest that UvrD helicase and/or one or more exonucleases may act at one or more points downstream in the recombination pathway, as well as in initial MutHLS-coupled excision.

The positive correlation of recombination with the production of ssDNA fits well with the requirement for an ssDNA loading site for RecA in model reactions (reviewed by KOWALCZYKOWSKI *et al.* 1994), and with the postulated gap-filling role of recombination during replication of UV-damaged DNA (RUPP and HOWARD-FLANDERS 1968). Surprisingly, (near-) blunt-ended dsDNA, the substrate for RecBCD helicase/nuclease, appears to be stable in $UvrD^-$ or Exo^- mutants [note the paucity of smaller fragments in Figure 3, A–D) and nonrecombinagenic (Figure 1)]. Linear dsDNA in Exo^- cells cannot have been protected against RecBCD simply by ssDNA tails (PRELL and WACKENAGEL 1980): mung-bean nuclease would have removed the tails and increased RecBCD-sensitivity, but this was not observed in $UvrD^-$ or Exo^- experiments (Figure 4, C and D). Perhaps the dsDNA ends are protected *in vivo* by an unknown protein.

The recombination seen here does not proceed by a single pathway dependent on RecF but not RecBCD function, or vice versa. This contrasts with evidence for separate RecF- and RecBCD-dependent pathways for

conjugal recombination and transduction (HORII and CLARK 1973) and for recombinational toleration of unrepaired DNA damage (SMITH and SHARMA 1987; SMITH and WANG 1989). The fact that *recF* and *recB recC* are here each nearly as recombination-deficient as *recF recB recC* mutants argues also against roughly equal recombinant production by two independent pathways. Perhaps both activities act in the same pathway. For example, RecBCD helicase might open up (blunt-ended) dsDNA to produce ssDNA ends, and RecF facilitate recombination of ssDNA gaps with these ends. This dual-substrate model would explain as well the apparent failure of the linear dsDNA produced in Exo$^-$ repressed infections to initiate recombination in the absence of ssDNA (Figures 1, 4, and 5).

The semiquantitative correlation of recombination with the presence of ssDNA among the breakdown products of UV-irradiated DNA, seen in this initial analysis, points the way to more refined studies of recombining DNA *in vivo*. By adjusting the ratios of irradiated to unirradiated DNA and the levels of rate-limiting activities, it should be possible to simultaneously maximize measurable recombination and observable DNA intermediates. Analyses of precursor-product relationships should then yield new insights into the structures of key recombination substrates.

We thank ANDREW TAYLOR and GERRY SMITH for generous gifts of RecBCD enzyme, JACK CHASE, SUSAN LOVETT, MARTIN MARINUS, PAUL MODRICH, GRAHAM WALKER, and BERNARD WEISS for providing bacterial strains and plasmids, TOM ELLEN for assisting with some experiments, and JUDITH HAYS for figures and manuscript preparation. MICHAEL COX, ALVIN J. CLARK, STEPHEN KOWALCZYKOWSKI and PAUL MODRICH made helpful comments on the manuscript. This work was supported in part by pilot project grants from the Oregon Unit, American Cancer Society, and the OSU Environmental Health Sciences Center (center grant ES00210).

LITERATURE CITED

ANRAKU, N., Y. ANRAKU and I. R. LEHMAN, 1969 Enzymatic joining of polynucleotides VIII. Structure of hybrids of parental T4 DNA molecules. J. Mol. Biol. **46:** 481–497.

AU, K. G., K. WELSH and P. MODRICH, 1992 Initiation of methyl-directed mismatch repair. J. Biol. Chem. **267:** 12142–12148.

BACHMANN, M. B., 1972 Pedigrees of some mutant strains of *Escherichia coli* K-12. Bacteriol. Rev. **36:** 525–557.

BEDALE, W. A., R. B. INMAN and M. M. COX, 1993 A reverse DNA strand exchange mediated by recA protein and exonuclease I. J. Biol. Chem. **36:** 525–557.

BIEK, D. P., and S. N. COHEN, 1986 Identification and characterization of *recD*, a gene affecting plasmid maintenance and recombination in *Escherichia coli*. J. Bacteriol. **167:** 597–603.

BODE, V. C., 1965 Changes in the structure and activity of DNA in a superinfected immune bacterium. J. Mol. Biol. **14:** 399–417.

CARRAWAY, M., A. Z. FREY, L. BROWN and J. A. ARRAJ, 1983 Insertion mutations in the *dam* gene of *Escherichia coli* K12. Mol. Gen. Genet. **192:** 288–289.

CHASE, J. W., and C. C. RICHARDSON, 1977 *Escherichia coli* mutants deficient in exonuclease VII. J. Bacteriol. **129:** 934–947.

CHASE, J. W., B. A. RABIN, J. B. MURPHY, K. L. STONE and K. R. WILLIAMS, 1986 *Escherichia coli* endonuclease VII. Cloning and sequencing of the gene encoding the large subunit. J. Biol. Chem. **261:** 14929–14935.

CLARK, A. J., and S. J. SANDLER, 1994 Homologous genetic recombination: the pieces begin to fall into place. Crit. Rev. Microbiol. **20:** 125–142.

COOPER, D. L., R. S. LAHUE and P. MODRICH, 1993 Methyl-directed mismatch repair is bidirectional. J. Biol. Chem. **268:** 11823–11829.

DUTREIX, M., B. J. RAO and C. M. RADDING, 1991 The effects on strand exchange of 5′ *versus* 3′ ends of single-stranded DNA in RecA nucleoprotein filaments. J. Mol. Biol. **219:** 645–654.

EICHLER, D. C., and I. R. LEHMAN, 1977 On the role of ATP in phosphodiester bond hydrolysis catalyzed by the *rec*BC deoxyribonuclease of *Escherichia coli*. J. Biol. Chem. **252:** 499–503.

FENG, W.-Y., E. LEE and J. B. HAYS, 1991 Recombinagenic processing of UV-light photoproducts in nonreplicating phage DNA by the *Escherichia coli* methyl-directed mismatch repair system. Genetics **129:** 1007–1020.

FISHEL, R. A., E. C. SIEGEL and R. KOLODNER, 1986 Gene conversion in *Escherichia coli*. Resolution of hereroallelic mismatched nucleotides by co-repair. J. Mol. Biol. **188:** 147–157.

FRANKLIN, W. A., K. M. LO and W. A. HASELTINE, 1982 Alkaline lability of fluorescent photoproducts produced in ultraviolet light-irradiated DNA. J. Biol. Chem. **257:** 13535–13543.

GANESAN, A. K., and P. C. SEAWELL, 1975 The effect of *lexA* and *recF* mutations on post-replication repair and DNA synthesis in *E. coli* K12. Mol. Gen. Genet. **141:** 189–205.

GRILLEY, M., J. GRIFFITH and P. MODRICH, 1993 Bidirectional excision in methyl-directed mismatch repair. J. Biol. Chem. **268:** 11830–11837.

HAYS, J. B., and S. BOEHMER, 1978 Antagonists of DNA gyrase inhibit repair and recombination of UV-irradiated phage λ. Proc. Natl. Acad. Sci. USA **75:** 4125–4129.

HAYS, J. B., and E. LEE, 1985 Repair and recombination of nonreplicating UV-irradiated DNA in *E. coli*. III. Enhancement of excision repair in UV-treated bacteria. Mol. Gen. Genet. **201:** 402–408.

HAYS, J. B., S. J. MARTIN and K. BHATIA, 1985 Repair of nonreplicating UV-irradiated DNA: Cooperative dark repair by *Escherichia coli* Uvr and Phr functions. J. Bacteriol. **161:** 602–608.

HAYS, J. B., E. J. ACKERMAN and Q. PANG, 1990 Rapid and apparently error-prone excision repair of nonreplicating UV-irradiated plasmids in *Xenopus laevis* oocytes. Mol. Cell. Biol. **10:** 3505–3511.

HORII, Z.-I., and A. J. CLARK, 1973 Genetic analysis of the RecF pathway to genetic recombination in *Escherichia coli* K-12: isolation and characterization of mutants. J. Mol. Biol. **80:** 327–344.

IKEDA, H., and I. KOBAYASHI, 1978 *recA*-mediated recombination of bacteriophage λ: structure of recombinant and intermediate DNA molecules and their packaging in vitro. Cold Spring Harbor Symp. Quant. Biol. **43:** 999–1008.

KONFORTI, B. B., and R. W. DAVIS, 1991 DNA substrate requirements for stable joint molecule formation by the RecA and SSB proteins of *Escherichia coli*. J. Biol. Chem. **265:** 6916–6920.

KONFORTI, B. B., and R. W. DAVIS, 1992 ATP hydrolysis and the displaced strand are two factors that determine the polarity of RecA-promoted DNA strand exchange. J. Mol. Biol. **227:** 38–53.

KOWALCZYKOWSKI, S. C., D. A. DIXON, A. K. EGGLESTON, S. D. LAUDER and W. M. REHRAUER, 1994 Biochemistry of homologous recombination in *Escherichia coli*. Microbiol. Rev. **58:** 401–465.

KUSHNER, S., H. NAGAISHI and A. J. CLARK, 1972 Indirect suppression of *recB* and *recC* mutations by exonuclease I proficiency. Proc. Natl. Acad. Sci. USA **69:** 1366–1370.

LIN, P.-F., and P. HOWARD-FLANDERS, 1976 Genetic exchanges caused by ultraviolet photoproducts in phage λDNA molecules: the role of DNA replication. Mol. Gen. Genet. **146:** 107–115.

LOVETT, S. T., and A. J. CLARK, 1984 Genetic analysis of the *recJ* gene of *Escherichia coli* K12. J. Bacteriol. **157:** 190–196.

LU, C., R. H. SCHEUERMAN and H. ECHOLS, 1986 Capacity of RecA protein to bind preferentially to UV lesions and inhibit the editing subunit ε of DNA polymerase III: a possible mechanism for SOS-induced targeted mutagenesis. Proc. Natl. Acad. Sci. USA **83:** 619–623.

MICHAELIS, S., L. GUARANTE and J. BECKWITH, 1983 *In vitro* construction and characterization of *phoA-lacZ* gene fusions in *Escherichia coli*. J. Bacteriol. **154:** 356–365.

MITCHELL, D., 1988 The relative cytotoxicity of (6–4) photoproducts and cyclobutane dimers in mammalian cells. Photochem. Photobiol. **48:** 51–57.

NEUHARD, J., and E. THOMASSEN, 1976 Altered deoxyribonucleotide pools in P2 eductants of *Escherichia coli* K12 due to deletion of the *dcd* gene. J. Bacteriol. **126:** 999–1001.

PORTER, R. D., T. MCLAUGHLIN and B. LOW, 1978 Transduction versus conjduction: evidence for multiple roles for exonuclease V in genetic recombination in *Escherichia coli.* Cold Spring Harbor Symp. Quant. Biol. **43:** 1043–1047.

PRELL, A., and W. WACKERNAGEL, 1980 Degradation of linear and circular DNA with gaps by the *recBC* enzyme of *Escherichia coli* K-12. Eur. J. Biochem. **105:** 109–116.

RICHARDSON, C. C., I. R. LEHMAN and A. KORNBERG, 1964 A deoxyribonucleic acid phosphatase-exonuclease from *Escherichia coli* II characterization of the exonuclease activity. J. Biol. Chem. **239:** 251–258.

RUPP, W. D., and P. HOWARD-FLANDERS, 1968 Discontinuities in the DNA synthesized in an excision-defective strain of *Escherichia coli* following ultraviolet irradiation. J. Mol. Biol. **31:** 291–304.

SANCAR, G. B., F. W. SMITH, M. S. LORENCE, C. S. RUPERT and A. SANCAR, 1984 Sequences of the *Escherichia coli* photolyase gene and protein. J. Biol. Chem. **259:** 6033–6088.

SCHAEFER, T. S., and J. B. HAYS, 1990 The *bof* gene of bacteriophage P1: DNA sequence and evidence for roles in regulation of phage *cl* and *ref* genes. J. Bacteriol. **172:** 3269–3277.

SMITH, K. C., and R. C. SHARMA, 1987 A model for *recA*-dependent repair of excision gaps in UV-irradiated *Escherichia coli* K-12. Mutat. Res. **183:** 1–9.

SMITH, K. C., and T.-C. V. WANG, 1989 *recA*-dependent DNA repair processes. Bioessays **10:** 12–16.

SMITH, T. A. G., and J. B. HAYS, 1985 Repair and recombination of nonreplicating UV-irradiated DNA in *E. coli.* II. Stimulation of RecF-dependent recombination by excision repair of cyclobutane pyrimidine dimers and of nondimer photoproducts. Mol. Gen. Genet. **201:** 393–401.

SU, S.-S., and P. MODRICH, 1986 *Escherichia coli mutS*-encoded protein binds to mismatched DNA base pairs. Proc. Natl. Acad. Sci. USA **83:** 5057–5061.

TAYLOR, A. F., and G. R. SMITH, 1985 Substrate specificity of the DNA unwinding activity of the RecBC enzymes of *E. coli.* J. Mol. Biol. **185:** 431–443.

WEST, S., 1992 Enzymes and molecular mechanisms of genetic recombination. Annu. Rev. Biochem. **61:** 603–640.

YANISCH-PERRON, C., J. VIERA and J. MESSING, 1985 Improved M13 cloning vectors and host strains: nucleotide sequence of M13mp18 and pUC vectors. Gene **33:** 103–119.

Communicating editor: P. L. FOSTER