Antagonists of DNA gyrase inhibit repair and recombination of UV-irradiated phage λ

(supertwisted DNA/UV repair/coumermycin/oxolinic acid)

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Communicated by Bruno H. Zimm, May 26, 1978

ABSTRACT Intracellular λ DNA (from EDTA-sensitive tandem duplication phages) was extracted from infected *recA*⁺ bacteria and scored for infectivity and recombination (loss of duplication) by transfection of *recA recB* spheroplasts and subsequent assay for EDTA resistance. When phage development was blocked by repressor or by antibiotics (chloramphenicol and/or rifampin), the apparent recombination frequency was about 0.1% above the background value for *recA* infections. Prior irradiation of the phage greatly stimulated recombination; the frequency was 20% when UV fluence was 140 J/m². Repair (recovery of infectivity) and recombination of irradiated phage DNA proceeded readily in the presence of chloramphenicol and rifampin. Inhibitors of DNA gyrase (coumermycin and oxolinic acid) blocked repair and reduced recombination. UV-stimulated recombination was very low in *recA* but nearly normal in *recB* cells; repair was reduced in both mutant strains. The recombination remained high at phage/cell ratios less than unity.

In *Escherichia coli*, as in other organisms, replication, repair, and recombination of DNA are accomplished by a complex, interconnected set of metabolic pathways (1). Thus, general (homology-dependent) recombination is strongly affected by the nature and extent of DNA replication and repair. Although genetic analyses of mutants deficient in recombination (2, 3) have led to the identification of some recombination enzymes, two basic questions about the physiology of general recombination remain incompletely answered: what kinds of DNA structures are recombinogenic, and what cellular functions are needed for recombination?

We have studied general recombination under conditions where the effects of DNA replication are minimized, using the DNA of bacteriophage λ as the substrate for cell-mediated recombination *in vivo*. When phage development is blocked, λ DNA molecules remain covalently closed circles, ideal probes for the action of cellular enzymes. The recombination assay is indirect: intracellular DNA is extracted from phage-infected cells and then used to transfect recombination-deficient spheroplasts. The indirect assay permits the selective inhibition of cellular functions during the recombination process; inhibitors of cellular RNA and DNA synthesis and antagonists of DNA gyrase were tested. The latter enzyme, which introduces negative supertwists into intracellular λ DNA (4, 5), is necessary for integrative recombination of λ (6). Unlike assays that depend on the packaging of phage DNA into virions (7, 8), the transfection assay does not discriminate against monomer circles, which comprise the bulk of intracellular DNA under blockage conditions.

Detection of recombined DNA is simplified by the use of λ tandem duplication phages whose chromosomes are sufficiently long for the phages to be highly sensitive to EDTA treatment

(9). Recombination (intramolecular or intermolecular) between homologous regions within the duplications yields "single-copy" DNA. The single-copy phages contain 18% less DNA and are EDTA resistant. Since the phages lack all of the λ recombination systems and the spheroplasts lack the primary cellular ones, transfection of a mixture of unrecombined (duplication) and recombined (single-copy) DNA yields the corresponding mixture of EDTA-sensitive and EDTA-resistant phages. Transfection techniques have previously been used to study the λ site-specific (10, 11) and general (12) recombination systems.

We find that unreplicated λ DNA molecules are poor substrates for recombination *in vivo*, unless the phages are UV-irradiated prior to infection. In the latter case, the measurement of infectivity by transfection becomes an assay for repair as well. Neither repair nor UV-stimulated recombination prove to require concomitant RNA or protein synthesis. Both processes, however, are inhibited by antagonists of DNA gyrase. The recombination requires RecA, but not RecB function, and does not depend on phage multiplicity.

MATERIALS AND METHODS

Media. TBM broth was described by Nash (13). TBMMT broth is TBM broth plus maltose (0.2%) plus Tris-HCl (pH 7.4, 50 mM). TM buffer is 20 mM Tris-HCl/10 mM MgSO₄, pH 7.4. TE buffer is 10 mM Tris-HCl/10 mM Na₃EDTA, pH 7.4. BTE buffer is 50 mM Tris-HCl/1 mM Na₃EDTA, pH 8. TS buffer is 50 mM Tris-HCl, pH 7.4, plus 20% sucrose.

Antibiotics. Chloramphenicol and rifampin, obtained from Sigma, were dissolved in 95% ethanol and in dimethyl sulfoxide, respectively. Coumermycin A₁, a gift of I. J. Pachter, Bristol Laboratories, was dissolved in dimethyl sulfoxide. Oxolinic acid, a gift of J. D. Stein, Warner-Lambert Research Institute, was dissolved in 0.1 M KOH.

Bacteria and Bacteriophages. Cells used were N99, *recA*⁺ *cou*^s (Meselson 28); N100, N99 *recA3* (Meselson 152); HN356, N99 *recB21*; N4164, N99 *cou*^R (from M. Gellert); KL176, *recA1recB21*; and lysogens of these strains (with *lcI857r-ed3xis1*). The tandem duplication of the *la106-19* phages (14) is 18% of λ length (genes A–V). The *b538* phages are deleted for 16% of λ length, including the *att-int-xis* region involved in site-specific recombination; the *red3* mutation inactivates the phage general recombination system. In the text, "duplication" refers to *a106-19b538* and "single-copy" to *b538* phages and DNA. Stocks of duplication phages, purified by two rounds of CsCl equilibrium sedimentation, contained less than 0.05% EDTA-resistant plaque-forming units (PFU). Specific activities of radioactive phages (labeled with [³H]thymidine) were typically 0.5–2.5 $\times 10^{-6}$ cpm per λ equivalent.

Irradiation with Ultraviolet Light. Phage stocks were ir-

Abbreviation: PFU, plaque-forming units.

radiated with a 15-W germicidal lamp at a distance of 50 cm. The lamp output was measured with a calibrated Blak-Ray ultraviolet meter (Ultraviolet Products, San Gabriel, CA). Phage survival after irradiation with 140 J/m² was 17–22%. All multiplicities of infection with irradiated phage were calculated from the titer *prior* to irradiation. When irradiated phage were used for infections, all containers were wrapped in foil and all manipulations performed in partial darkness.

Growth and Infection. Cells were grown overnight in TBMMT broth at 30° from a low inoculum to late exponential phase (4–9 × 10⁸ cells per ml), harvested by centrifugation, and infected according to procedure A (for accurate time courses) or procedure B (when antibiotics were used). *Procedure A:* Cells were resuspended in cold TM buffer (final density, 1 × 10⁹ cells per ml) and mixed with radioactive phage. After 20–30 min of adsorption at 5°, the mixture was diluted into 10 vol of 31° broth (time zero) and shaken at the appropriate temperature. *Procedure B:* Cells were resuspended in TBMMT broth (final density, 1 × 10⁹ cells per ml) and shaken for 5 min at 31°. After addition of 0.01 vol of antibiotics (or pure solvents) and 10 min of additional shaking, the culture was mixed with phage (time zero), shaken for 15 min, and diluted with 10 vol of 31° TBMMT broth (containing the same concentration of antibiotics or pure solvents); phage adsorption was more than 90% complete in 15 min.

Lysis and DNA Extraction. Samples (usually 5 ml) were moved from the infected cultures at designated times, diluted with 10 ml of ice-cold TS buffer, harvested by centrifugation, and washed twice with 20 ml of cold TS buffer. The final washed pellet was resuspended in 0.6 ml of lysis buffer (20% sucrose/50 mM potassium phosphate, pH 7.5/0.1 M NaCl/10 mM NaN₃). The final pellets usually contained 45–50% of the original phage DNA radioactivity, and the final supernatants less than 3%. Pellets were lysed by treatment with lysozyme (CalBiochem), sodium lauroyl sarcosinate, and Proteinase K (E. Merck) (5). The lysates were diluted 1:1 with BTE buffer, and extracted by gently rocking with buffered (pH 7.4) phenol. The DNA solutions were dialyzed overnight against BTE buffer (twice with 200 vol), and stored at 5°. Lysis and extraction of dilute resuspended pellets (*A*₆₅₀ ≤ 0.5) usually resulted in recovery of 80–100% of DNA radioactivity. Yields from more concentrated pellets were sometimes 60–80%. Neither the total yield of infective DNA nor that of recombined DNA was increased by variations in the lysozyme or Proteinase K or phenol treatments.

Transfection. Spheroplasts prepared from strain KL176/λ *recArecB* were transfected with DNA samples essentially as described by Nash (10), except that the chloroformed lysates were thoroughly aerated before assay of volumes exceeding 0.05 ml. Most transfection mixtures contained 1–10 ng of λ DNA per ml. Transfection efficiencies (for unirradiated DNA) were usually 0.2–1 × 10⁻³ PFU per λ DNA equivalent. Single-burst experiments indicate that each infected spheroplast yields 10–40 phage. The apparent transfection efficiencies of duplication and single-copy DNA, both from λ*I*26Sam7 and λ*I*857 phages, were the same. Sometimes duplication DNA from λ*I*857Sam7 phages was 0.5–0.8 as efficient as the corresponding single-copy DNA.

Assay of Recombined (EDTA-Resistant) Phage. Method I: Purified phage stocks or chloroform-lysed cultures were diluted with 50 vol of TE buffer, incubated for 15 min at 41°, chilled on ice, and made 50 mM in MgSO₄. Lysates from single lytic cycles with duplication phages under recombination-negative conditions yielded about 0.1% EDTA-resistant PFU. **Method II:** Transfection lysates were diluted with 1 vol of 0.1 M Na₃EDTA (pH 7.4), heated at 43° for 20 min, chilled, and

mixed with 0.4 vol of 1 M MgSO₄. Lysates from control transfections of KL176/λ spheroplasts with duplication DNA yielded 0.1–0.3% EDTA-resistant PFU; lysates from transfections with single-copy DNA were 80–100% EDTA resistant.

RESULTS

General Recombination Is Low in Absence of Phage DNA Replication and Repair. When *rec*⁺ cells were infected in the presence of λ repressor and the intracellular DNA was extracted and assayed by the transfection-EDTA procedure (method II), the apparent recombination frequency was less than 0.1% above the background level for *recA* infections (Fig. 1 B). When the same procedure was used to assay cultures infected with Sam7 duplication phage under lytic conditions (Fig. 1 A), the values (10–15% recombination at 150 min) are comparable to those obtained by direct assay of the whole phage (method I). Recombined DNA can be detected, by the transfection assay, before new virions are assembled (*inset*). In an infection with S⁺ duplication phage at 37° (not shown), recombination at-

FIG. 1. Recombination of unirradiated phage. (A) Lytic infection. Infection and incubation were according to procedure A: cells, *rec*⁺ and *recA*; phage, unirradiated λ*I*06-19b538*I*26*red*3Sam7 (15 per cell); incubation at 30° or 37°. Chloroform lysis and EDTA treatment (method I) or sampling (at indicated times), harvest, lysis, DNA extraction, transfection, and EDTA treatment (method II) were as in *Materials and Methods*. Typical burst sizes: 100 min, 50 PFU/cell; 200 min, 100 PFU/cell. *rec*⁺ infections: (Δ) 37°, whole phage assay (method I); (▲) transfection assay (method II); (○) 30°, method II. *recA* infection: (□) 37° and 30°, method II. (*Inset*) Data at early times, expanded scale. (B) Infection with repression. Procedure was as in A, except that phage were unirradiated λ*I*06-19b538*I*857-*red*3Sam7, cells were *rec*⁺(λ) or *recA*(λ), and all incubations were at 31°. (○) *rec*⁺(λ) infections, method II; (□) *recA*(λ) infections, method II.

tained a limiting value of about 2% at approximately 60 min after infection, although phage production continued for another 30 min.

If phage development was blocked by chloramphenicol (200 $\mu\text{g/ml}$), rifampin (150 $\mu\text{g/ml}$), or by both drugs, instead of by repressor, recombination was similarly low (0.3–0.5% in *rec⁺* infections, 0.2–0.4% in *recA*). The results were essentially the same at multiplicities of 15–40 phage per cell and for infection times of 15–100 min.

Recombination of UV-Irradiated Phage. Recombination of UV-irradiated phage was highly efficient, even in the presence of repressor (the *cI857* repressor is *ind⁻*). The frequency depended on the UV fluence to the phage, but was not changed by irradiation of the cells just before infection (Table 1). When the *recArecB* spheroplasts were transfected with DNA extracted directly from irradiated phage, the infectivity was extremely low (Table 2, line 10). Thus the increases in the infectivity of UV-irradiated DNA observed for the infection-extraction-transfection experiments reflect repair activity during the first infection (Table 2, lines 5–8). Repair, by the infectivity criterion, was usually depressed 50% or less in *recA* and 50–67% in *recB* infections. The recombination frequency was nearly normal in *recB* cells, but very low in *recA*. There were no significant differences in the recovery of DNA from these strains (Table 2, lines 1–4). Recombination and repair of UV-irradiated repressed phage require only preexisting cellular enzymes, although both the yield of infective DNA and the recombination frequency were somewhat reduced in the presence of chloramphenicol and rifampin (Table 3). Recombination of UV-irradiated phage in the presence of these drugs was comparable in the absence of repressor (infections of strain N99 with irradiated *cI26* duplication phage; not shown). Experiments reported elsewhere (15) demonstrate that the EDTA-resistance assay measures recombination between the duplicated DNA segments, not UV-stimulated production of deletions. The data of Table 4, for infections at low multiplicity, indicate that there was substantial intramolecular recombination of UV-irradiated DNA.

UV-Stimulated Recombination in Presence of DNA Gyrase Inhibitors. Supertwisted DNA is the obligatory substrate for integrative recombination of λ phage; coumermycin and novobiocin inhibit this process *in vitro* (6) and *in vivo* (Y. Kikuchi and H. Nash, unpublished data). The target for these drugs is a subunit of DNA gyrase (5), an enzyme that introduces

Table 2. Repair and recombination of UV-irradiated phage in "Rec" mutants (repressor present)

Cells	UV, J/m ²	Relative infectivity	Recombination, %
<i>rec⁺</i>	None	(1.00)	0.5
<i>recA⁺</i>	None	1.5	0.5
<i>recB⁺</i>	None	0.8	0.3
<i>recArecB⁺</i>	None	1.0	0.4
<i>rec⁺</i>	120	0.18	21
<i>recA⁺</i>	120	0.12	0.1
		(0.05–0.17) [†]	(0.1–0.5) [†]
<i>recB⁺</i>	120	0.07	19
		(0.06–0.11) [†]	(10–23) [†]
<i>recArecB⁺</i>	120	0.03	0.2
		(0.02–0.03) [†]	(0.1–0.2) [†]
None [§]	None	(1.00)	0.5
None [§]	140	0.005	<0.1

Infection and incubation were according to procedure B: cells, as indicated; phage, UV-irradiated (120 J/m²) λ a106-19b538cI857-red3Sam7 (20 per cell); incubation, 31°. Sampling (60 min), harvest, lysis, DNA extraction, transfection, and EDTA treatment (method II) were as in *Materials and Methods*. Recoveries of total DNA from infections with irradiated phage were depressed by less than 10%, relative to corresponding infections with unirradiated phage. Recoveries from mutant strains were decreased (relative to *rec⁺*) by 12% (*recA*), 6% (*recB*), and 20% (*recA recB*). For lines 9 and 10, DNA was extracted with phenol from irradiated and unirradiated phage and used to transfect spheroplasts by the standard procedure. Transfections with DNA alone or in the presence of a dummy extract prepared from uninfected cells gave similar results. A relative infectivity of 1.00 corresponds to 36,000 (lines 1–8) or 50,000 (lines 9 and 10) PFU/ml. Data are averages from two transfections.

* Isogenic series.

† Lysogen of transfection strain.

‡ Range in other experiments.

§ Control transfections of DNA extracted directly from irradiated or unirradiated phage.

supertwists into circular λ DNA (4). Nalidixic acid and oxolinic acid inhibit gyrase by acting on a different subunit (16, 17). The effects of gyrase inhibitors on repair and general recombination of UV-irradiated λ DNA are seen in Table 5 and Fig. 2. Repair (formation of infective DNA) was nearly abolished by low concentrations of the inhibitors. Recombination was depressed by gyrase inhibitors, but was apparently less sensitive than repair. In a coumermycin-resistant strain, both repair and recombination were unaffected by the drug (Fig. 2).

Table 1. Stimulation of recombination of repressed phage DNA by UV irradiation

UV, J/m ²		Infectivity, PFU/ml	EDTA ^R infectivity, PFU/ml	Recombination frequency, %
Phage	Cells			
None	None	35,000	130	0.4
None	36	32,000	120	0.4
48	None	18,000	2100	12
48	36	7,500	660	9
120	None	6,000	1900	30
120	36	1,980	660	33
190	None	2,800	1620	58
190	36	610	320	52

Infection and incubation were according to procedure A: cells, *rec⁺*(λ), irradiated just before phage adsorption as indicated; phage, UV-irradiated (as indicated) λ a106-19b538cI857red3 (17 per cell); incubation, 31°. Sampling (at 120 min), harvest, lysis, DNA extraction, transfection, and EDTA treatment (method II) were as in *Materials and Methods*. Data are averages from two transfections.

Table 3. Repair and recombination of UV-irradiated phage in presence of chloramphenicol and rifampin

Cells	Drugs	35-min sample		70-min sample	
		Relative infecti- vity	Recombi- nation, %	Relative infecti- vity	Recombi- nation, %
<i>rec⁺</i>	None	1.14	17	(1.00)	25
<i>rec⁺</i>	Both	0.63	11	1.14	16
<i>recA</i>	None	0.41	0.3	0.68	0.4
<i>recA</i>	Both	0.54	0.2	1.09	0.2

Infection and incubation were according to procedure B: cells, *rec⁺*(λ) or *recA*(λ); antibiotics, chloramphenicol (150 $\mu\text{g/ml}$) and rifampin (200 $\mu\text{g/ml}$); phage, UV-irradiated (140 J/m²) λ a106-19b-538cI857red3, 15 per cell; incubation, 31°. Sampling (at indicated times), harvest, lysis, DNA extraction, transfection, and EDTA treatment (method II) were as in *Materials and Methods*. DNA recoveries were slightly depressed (10–20%) in the drug and *recA* experiments. Relative infectivity of 1.00 corresponds to 14,000 PFU/ml. Data are the averages from two transfections.

Table 4. Effect of multiplicity on recombination of UV-irradiated phage (repressor present)

Multiplicity*, phage/ cell	Time, min	Recombination, %	Time, min	Recombination, %
0.2			70	57
0.8			70	17
3.2			70	23
12.0			70	26
0.5	30	20	60	14
4.0	30	20	60	22

Infection and incubation were according to procedure A: cells, *rec*⁺(λ); phage, UV-irradiated (140 J/m²) λ a106-19b538cI857red3 (multiplicity indicated); incubation, 31°. Sampling (at indicated times), harvest, lysis, DNA extraction, transfection, and EDTA treatment (method II) were as in *Materials and Methods*. Absolute infectivities were 25–6000 PFU/ml (lines 1–4) and 250–2000 PFU/ml (lines 5 and 6).

* Multiplicity was calculated from titer of unirradiated phage.

DISCUSSION

General recombination between metabolically inert λ DNA molecules proves to be relatively inefficient when assayed by a method that does not depend on phage DNA replication and gene expression. These results, obtained with λ repressor (Fig. 1) or with chloramphenicol and/or rifampin, are similar to those from previous studies of prophage-prophage and phage-prophage recombination (18, 19). Other investigators, using assays

Table 5. Repair and recombination of UV-irradiated phage in the presence of DNA gyrase inhibitors

Exp.	Drug, μ g/ml	Relative infectivity	Recombination frequency, %
1	Coumermycin		
	None	(1.00)	20
	0.6	1.65	21
	2.0	0.50	17
	6.0	0.21	5
2	None	(1.00)	9
	12	0.08	3
	25	0.05	3
	50	0.05	3
3	Oxolinic acid		
	None	(1.00)	23
	0.5	1.55	27
	5.0	0.17	6
	50.0	0.11	<1
4	None	(1.00)	28
	0.8	0.65	23
	2.5	0.88	21
	7.5	0.50	10

Infection and incubation were according to procedure B: cells, *rec*⁺(λ); antibiotics as indicated; phage, UV-irradiated (140 J/m² in Exps. 1, 3, and 4; 60 J/m² in Exp. 2) λ a106-19b538cI857red3 (Exps. 1 and 4) or λ a106-19b538cI857red3Sam7 (Exps. 2 and 3), 20–25 phage per cell. Sampling (at 70 min), harvest, lysis, DNA extraction, transfection, and EDTA treatment (method II) were as in *Materials and Methods*. Relative infectivities are not corrected for effects of drugs on recovery of infective DNA observed in control infections with unirradiated phage (30–50% loss at 5 μ g of coumermycin per ml, 20% loss at 5 μ g of oxolinic acid per ml). Relative infectivity of 1.00 corresponds to 4600, 46,000, 1800, or 3400 PFU/ml in Exps. 1–4, respectively. Data correspond to the averages from five, one, three, or one transfections, respectively.

FIG. 2. Repair and recombination of UV-irradiated phage in coumermycin-resistant cells. Infection and incubation were according to procedure B: cells, *rec*⁺*cou*^S(λ) or *rec*⁺*cou*^R(λ); phage, UV-irradiated (140 J/m²) λ a106-19b538cI857red3Sam7 (16 per cell). Sampling (at indicated times), harvest, lysis, DNA extraction, transfection, and EDTA treatment (method II) were as in *Materials and Methods*. Relative infectivity of 1.0 corresponds to 5000 PFU/ml. (O) *cou*^S cells, no coumermycin; (●) *cou*^S, 5 μ g of coumermycin per ml; (▲) *cou*^R, 5 μ g of coumermycin per ml.

that select against unrecombined DNA, have reported substantial recombination frequencies for inert λ DNA (7, 8, 20). The selectivity of these assays stems from the inability of the λ phage maturation system to use the monomer circles that prevail under blockage conditions; some recombination products, presumably multimeric DNA, are readily packaged into infective virions.

The DNA gyrase inhibitors, coumermycin and oxolinic acid, block the repair of UV-irradiated DNA (Table 5 and Fig. 2) at concentrations well below those required to detectably alter the degree of supertwisting of intracellular λ DNA (6, 16), but comparable to those which inhibit cell growth (21, 22). The inhibition of repair is in apparent conflict with reports that gyrase inhibitors efficiently block semiconservative, but not repair, replication of DNA (23, 24). Our criterion for repair is restoration of DNA infectivity. It seems likely that some step in the pathway to this biological endpoint is more sensitive to gyrase inhibitors than is the incorporation of precursors into bulk DNA under the conditions that define repair replication. Alternatively, the observed loss of DNA infectivity could be the direct result of double-strand breaks, analogous to those engendered *in vitro* by treatment of a mixture of gyrase, DNA, and oxolinic acid with detergent (16, 17). However, the breaks do not occur *in vitro* when coumermycin replaces oxolinic acid, so simple drug-induced cutting *in vivo* cannot explain the

coumermycin effects seen in Table 5 and Fig 2. It is possible that excision repair of pyrimidine dimers (25) does not require gyrase action, but that repair of some unidentified 254-nm photoproduct does.

Reliable measurements of recombination frequency require appreciable levels of infectivity, so it is difficult to determine whether or not recombination is less sensitive to gyrase inhibitors than repair. It appears, however, that recombination, although reduced to $\frac{1}{3}$ – $\frac{1}{6}$ that of untreated DNA, is not abolished by these drugs. It may be that different cellular processes depend on gyrase action to different degrees, i.e., some step(s) in UV repair and in processes critical for growth are highly gyrase dependent, integrative recombination of λ and UV-stimulated general recombination are less dependent, and the degree of supertwisting of bulk intracellular DNA even less so (6, 16). On the other hand, recombination could proceed by two pathways, only one of which requires gyrase action. Supertwisted DNA has been postulated as a recipient in single-strand transfer during general recombination (26). The requirement suggested here may be only for a localized negative supertwisting stress; it is unlikely that many molecules remain covalently closed during excision repair of the pyrimidine dimers. Furthermore, there is substantial intramolecular recombination of the irradiated DNA (Table 3), and the same molecule cannot be fully supertwisted and a source of single-stranded ends at the same time. The replication of the linear phage T7 chromosome appears to require DNA gyrase (27), a result consistent with a localized role for supertwisting.

The recombinogenic photoproduct has not been identified. We have shown elsewhere (14) that for phages irradiated as described under Table 2 [about 50 dimers per λ genome (19)], *in vivo* photoreactivation and subsequent infection of *rec*⁺ cells yields DNA with normal infectivity, but the absolute number of recombined molecules is not changed by the photoreactivation. This result, similar to that obtained for phage-prophage recombination by Lin and Howard-Flanders (19), suggests that the primary recombinogenic lesions are not pyrimidine dimers, but rather some unidentified 254-nm photoproducts.

Although the mechanism of the repair-recombination process remains to be determined, preliminary experiments suggest that the repair process observed is a complete one. No covalently closed λ DNA can be detected by ethidium bromide/CsCl density gradient analysis of DNA extracted from *recA* lysogens superinfected with UV-irradiated phage. However, about 10% of the DNA from *rec*⁺ infections is covalently closed, and this fraction is enriched more than 5-fold for infectivity and 10-fold for recombined DNA. It may be that some of the branched structures observed by electron microscopy after infections of *polArecA* cells by irradiated phage (28) represent intermediates on the pathway leading to these recombined circles.

We thank Dr. Howard Nash for many helpful suggestions and discussions, Drs. Bruce Duncan and Martin Gellert for comments on the manuscript, and Dr. Lawrence Grossman for the calibrated UV meter. The technical assistance of Mr. Richard Gough is gratefully acknowledged. This work was supported by U.S. Public Health Service Grant GM22586 from the National Institute of General Medical Sciences.

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