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Host-Mediated Repair of Discontinuities in DNA From T4 Bacteriophage

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Discontinuities of T4 DNA which are caused by excision of UV-damaged areas, by decay of ^{32}P atoms, or which are present in DNA from $rII^{-}lig_{am}^{-}$ phage produced in a host nonpermissive for amber mutants are all repaired by bacterial enzymes after infection in the presence of chloramphenicol. *Escherichia coli* DNA polymerase I participates in the host-mediated repair, but an approximately 20-fold variation in the levels of host polynucleotide ligase does not affect either the kinetics or the extent of repair observed. Upon removal of chloramphenicol, host-repaired DNA from UV-irradiated phage undergoes a secondary cycle of breakage, which ultimately results in solubilization of most of the phage DNA. If the cells are co-infected with nonirradiated helper phage, the secondary breaks are repaired and the continuity of the polynucleotide chain is restored. The close coincidence in the extent of primary and secondary breakage suggests that phage-coded enzymes recognize and excise areas improperly repaired by the host. In contrast to host-mediated repair, repair mediated by rescuing phage probably restored functionality to the damaged DNA.

Although bacteriophage T4, after infection of bacterial cells, codes for a number of enzymes involved in its DNA metabolism (review in reference 19), it is quite possible that some of the enzymes involved in this process may be injected into the cell together with the phage DNA upon infection or be of bacterial origin. This possibility was explored by Kozinski and Lorkiewicz in 1967 in a study of intracellular breakage and repair of UV-damaged DNA (15). ("Break" and "breakage" refer to single-stranded discontinuities in DNA with no implications to the nature and size of this discontinuity. "Repair" refers to the restoration of integrity of broken DNA.) The authors found that when the synthesis of new proteins was inhibited by the addition of chloramphenicol (CM) shortly before infection, DNA from UV-irradiated phage acquired single-standed interruptions shortly after infection. (DNA of the radiation-sensitive mutant V_1 [11] did not acquire such breaks.) The broken strands were subsequently repaired to full integrity, still in the presence of CM. The results were interpreted to indicate that bacterial enzymes performed both breakage and repair.

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This work provided the first indication that DNA from a virulent phage can be repaired by host enzymes. More recent experiments in this laboratory have shown that a phage enzyme injected with the DNA is responsible for the intracellular breakage of UV-damaged DNA (R. Shames, Z. K. Lorkiewicz, and A. W. Kozinski, J. Virol., in press). This enzyme is the product of the v gene in T4, is found in the mature phage, and has endonucleolytic activity specific for UV-damages when assayed in vitro (C. J. Castillo, Ph.D. thesis, University of Pennsylvania, in preparation).

The objective of the present communication is to characterize the repair event following breakage. Evidence will be presented showing that several different types of damage in phage DNA can be repaired, and that the enzymes responsible for the restoration of integrity are of bacterial origin. *Escherichia coli* DNA polymerase I participates in this function, but a 20-fold variation in the levels of bacterial polynucleotide ligase does not affect either kinetics or extent of repair. The host-repaired DNA from UV-damaged phage is genetically incompetent, and only a second round of phage-directed excision repair restores functionality to the damaged DNA.

MATERIALS AND METHODS

Strains. The bacterial strains *E. coli* W3110 su^- thy^- and its derivatives P3478 $polA^-$ and a spontaneous revertant of this mutant (6) were obtained from J. Cairns. *E. coli* strains N953 lac^- $galE^-$ $galK_{am}^-$ trp_{am}^- ara_{am}^- $T6^R$ su^- and its derivatives N1323 lop8 and N1325 lop8 lig2 correspond to the strains N1071, N1072 lop8, and N1252 lop8 lig2 of Gellert and Bullock (9) which have been cured of their lambda prophage. These strains were obtained through H. Krisch. *E. coli* B23 is the normal host for T4 used in this laboratory.

The bacteriophages used were $T4BO_1^r$, an osmotic shock-resistant mutant, and T4D r59 H39X, an $rII^-lig_{am}^-$ double mutant (2).

Phage preparation. Methods for preparing radioactive phage have been described (15, 16). (i) UV irradiation of bacteriophage was performed with a germicidal lamp (GE) without a filter, with a dose corresponding to 6 to 9 lethal hits. The phage was suspended in 0.15 M NaCl-0.01 M Tris-hydrochloride, pH 7.4, during irradiation.

(ii) To obtain phages with DNA nicks due to ^{32}P decay, ^{32}P-labeled phage of a specific activity of 10 to 20 mCi of phosphorus per mg were stored at a concentration of 10^{10} particles per ml in $3 \times D$ medium (8) at +4 C until about 6 to 8 single-stranded scissions due to ^{32}P decay had accumulated, as determined by alkaline sucrose gradient sedimentation.

(iii) $rII^-lig_{am}^- \cdot B$ denotes phage which were prepared by a single growth cycle in *E. coli* B23 (2). This strain is nonpermissive for the amber ligase mutation, but partial suppression of the amber phenotype by the rII mutation results in productive infection. About 10% of the resulting phages are viable, and the phages contain DNA with numerous single-stranded interruptions (2).

Determination of intracellular strand integrity of phage DNA. All experiments were performed at 37 C in TCG medium 16) at a cell concentration of 3×10^8/ml. The medium was supplemented with thymidine (TdR) at 5 μg/ml in experiments with thy^- strains and L-tryptophan at 20 μg/ml in experiments with trp^- strains. CM was added to the cultures at a final concentration of 150 μg/ml immediately before the cells were infected with ^{32}P-labeled phage at a multiplicity of 3 to 5 particles per cell. At desired intervals after infection the samples were lysed (together with ^3H-labeled mature T4 phage as reference) with lysozyme, Triton X-100, and NaOH to a final concentration of 0.2 M (20). The lysates were sedimented through alkaline sucrose gradients (5 to 20% in 1 M NaCl-0.2 M NaOH; 26,700 rpm for 3 h in a Spinco SW50 rotor). The distance sedimented by the intracellular DNA molecules relative to the distance sedimented by the marker DNA (D_2D_1) (4) serves as a measure of strand integrity.

Enzymological techniques. DNA extracted from phage with phenol was dialyzed against 1 mM EDTA, pH 7.4, and incubated with T4 polynucleotide ligase by the method of Weiss and Richardson (25). Incubation with T4 DNA polymerase was by the method of Aposhian (1). After conclusion of the enzymatic reactions the mixtures were again dialyzed against 1 mM EDTA and analyzed on alkaline sucrose gradients.

RESULTS

Intracellular repair of different types of single-stranded discontinuities in phage DNA. It was demonstrated previously that, shortly after infection in the presence of CM, UV-damaged T4 DNA acquires a number of single-stranded nicks (15) corresponding to the number of lethal hits (17). These nicks are repaired upon prolonged incubation (15). To test the generality of such repair, we prepared phages with different types of interruptions in their DNA.

(i) Breaks in DNA due to ^{32}P-Decay. Litwin et al. (17) showed that there is a one-to-one relationship between the number of decayed ^{32}P atoms and the number of resulting single-stranded interruptions, which indicates that there is a chain break adjacent to the decayed ^{32}P.

Decayed phage were prepared by allowing ^{32}P-labeled phage (specific activity 20 mCi of phosphorus per mg to age in $3 \times D$ medium. Such phage acquired a number of single-stranded breaks (Fig. 1A). Bacteria were infected (MOI 1) with the decayed phage in the presence of CM. Thirty minutes later the integrity of the phage DNA was analyzed (Fig. 1B). Most of the radioactivity now coincided fairly well with the integral reference. This indicates that discontinuities due to ^{32}P decay can be repaired.

(ii) Discontinuities in DNA from $rII^-lig_{am}^-$ B phage. In a bacterial host nonpermissive for amber mutants, partial suppression of the amber ligase mutation by rII mutations allows the production of mature phages (2, 7, 13). Such phages, however, contain discontinuous DNA (2). The nature of these discontinuities is not known. To obtain information about this, we tested the repair of such discontinuous DNA in vitro by subjecting the DNA to repairing enzymes. DNA extracted from $rII^-lig_{am}^- \cdot B$ phage (Fig. 2A, insert) was incubated with T4 polynucleotide ligase or a combination of both ligase and T4 DNA polymerase. It was expected that a simple break not involving loss of nucleotides should be repaired by ligase alone, whereas a combination of the two enzymes would be required if the break consisted of gaps in the polynucleotide chain. Samples were then subjected to alkaline sucrose gradient analysis. Ligase alone (Fig. 2A) could not effect any repair of the DNA. A combination of the two enzymes, however, achieved significant extent

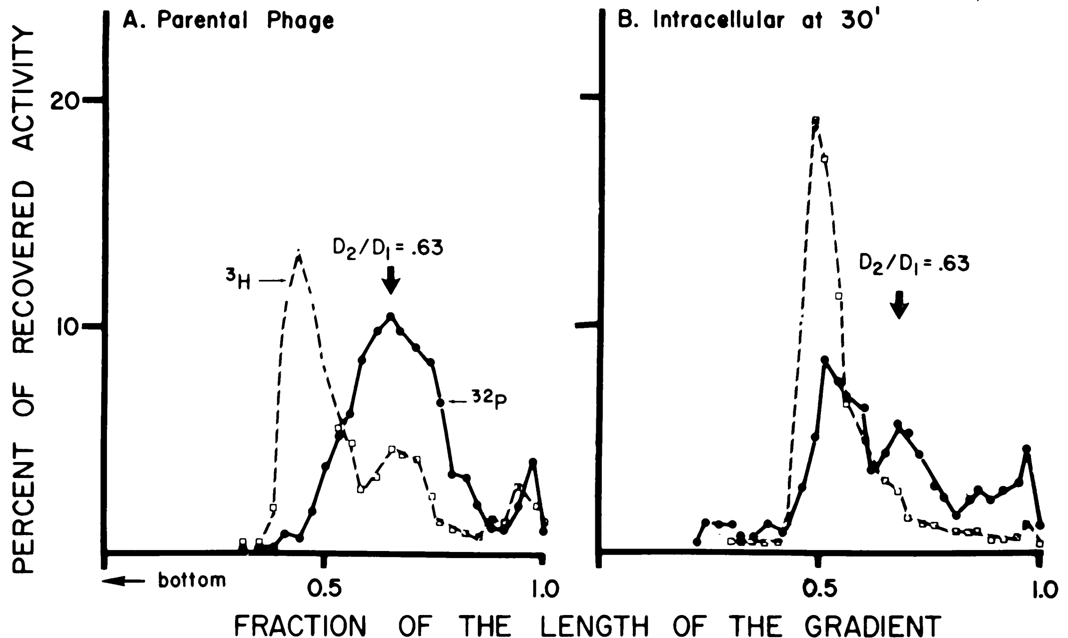

FIG. 1. *Repair of breaks inflicted by ^{32}P decay in T4 DNA. ^{32}P-labeled phage (specific activity 20 mCi of phosphorus per mg) was stored in 3 × D medium allowing limited decay to occur. The decayed phage was used to infect E. coli B in the presence of CM (150 μg/ml). Thirty minutes after infection part of the infected cells were lysed and analyzed on alkaline sucrose gradients. A, Analysis of the parental phage used for the experiment; B, intracellular DNA from the same phage. Note significant repair. ^{3}H represents reference DNA from mature T4 phage.*

of repair (Fig. 2B). This result indicates that the discontinuities in $rII^{-}lig_{am}^{-}\cdot B$ DNA are gaps, not nicks, requiring both repair synthesis and sealing.

The extent of in vivo repair of this same phage DNA was tested upon infection of *E. coli* B (MOI 3) in the presence of CM. Intracellular DNA was analyzed on alkaline sucrose gradients (Fig. 2C and D). The intensity of repair is quite similar to what was observed in vitro with a combination of ligase and polymerase (cf. Fig. 2B and C). Note that extensive repair occurred already at 5 min. There is no further repair if incubation is prolonged beyond 5 min. In both cases about 35% of the DNA mass is integral. A sizable fraction of the DNA is unrepaired in vivo as well as in vitro. This fraction may correspond to a fragmented moiety of DNA, i.e., where double-stranded scissions have occurred in addition to the single-stranded gaps. Invariably, when DNA from $rII^{-}lig^{-}$ phage is extracted with phenol and sedimented through neutral sucrose gradients, a sizable fraction sediments more slowly then integral DNA. It is uncertain whether fragments are found in the phage, or whether the gapped DNA is unusually susceptible to fragmentation during extraction. The extent of gapping varies considerably from experiment to experiment. In other experiments where the starting material was less broken, full restoration of integrity was observed upon infection in the presence of CM.

Role of bacterial enzymes in repair of discontinuous phage DNA. Although the occurrence of repair after infection in the absence of new protein synthesis suggested that preexisting (bacterial) enzymes were responsible (15), the host origin of the repair enzymes was not proven. The repair may be effected by a phage protein injected from the infecting particle or by a protein the synthesis of which is unusually resistant to CM. The notion that repairing enzymes are indeed of bacterial origin is supported by the experiments described as follows.

Role of host polynucleotide ligase and DNA polymerase I in repair. Polynucleotide ligase seals a single-stranded nick in bihelical DNA in vitro (26), joining a 3'OH end to a 5'P end. This enzyme, therefore, is an obvious candidate for performing the last step in repair.

E. coli mutants deficient in DNA polymerase I (6) are fully viable, but display increased sensitivity to UV (6). The increased sensitivity

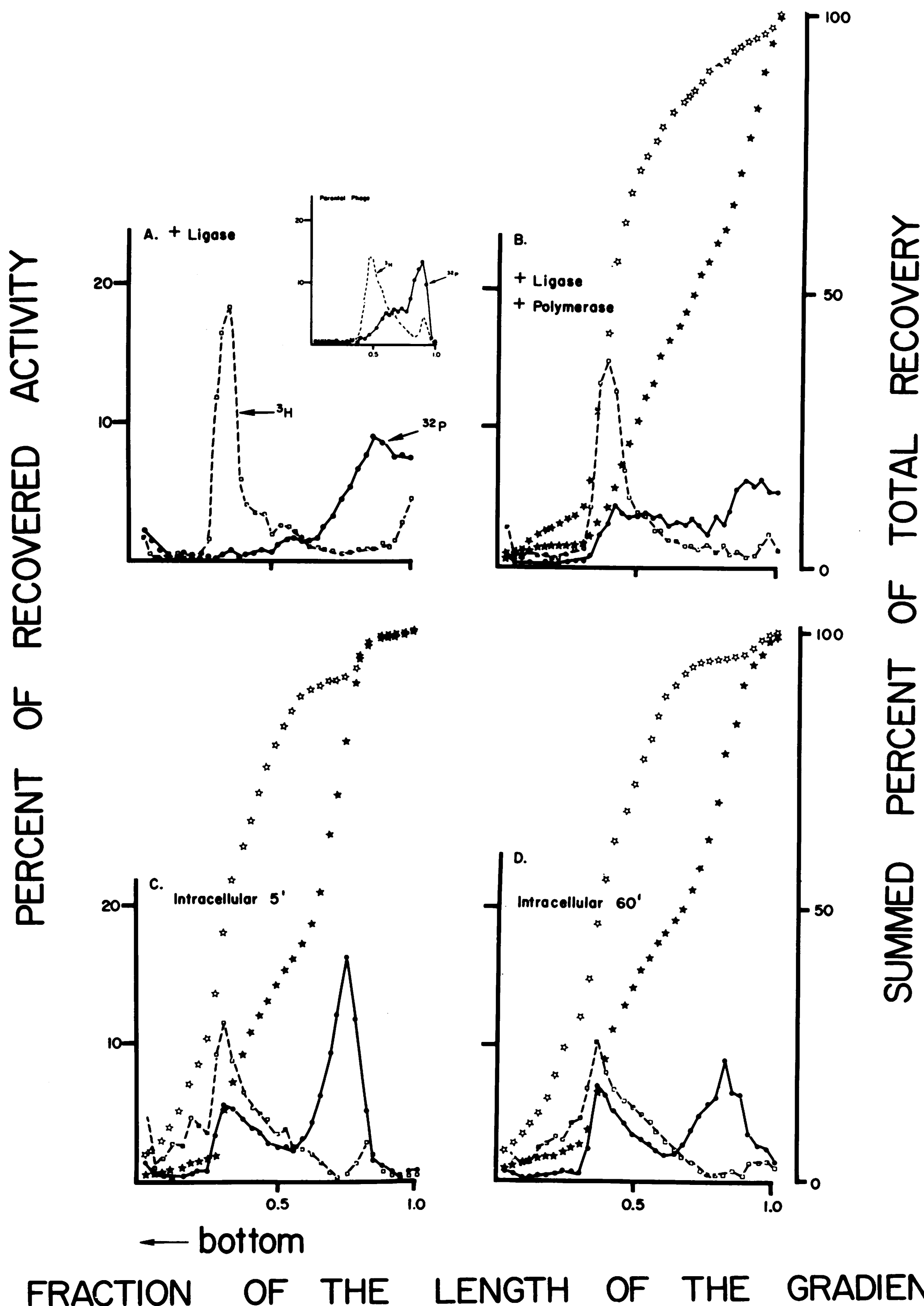

FIG. 2. *Repair of discontinuities in DNA from rII⁻lig_{am}⁻·B phage produced in "nonpermissive" host in vitro and in vivo. Phages were obtained as described in Materials and Methods and analyzed in an alkaline sucrose gradient (insert in panel A). Note that all DNA is discontinuous. DNA was extracted from this phage and subjected to repair by ligase alone (A) or by a combination of ligase and polymerase (B). Note partial repair in B, as compared to lack of repair in A. C and D, Intracellular fate of DNA from the same phage upon infection of E. coli B in the presence of CM. The infected cells were sampled at 5 and 60 min after infection and analyzed in alkaline sucrose gradients. Note significant repair reaching maximum already 5 min after infection. A comparison of panels B and C reveals a comparable extent of repair of the discontinuous DNA in vitro and in vivo. The stars represent summed percent recovered activity. Symbols: open stars, ^{3}H; solid stars, ^{32}P. ^{3}H represents reference DNA from mature T4 phage.*

is probably due to diminished repair after excsion of UV-damaged DNA (12). Both a 5′ to 3′ and a 3′ to 5′ exonuclease are firmly associated with *polI*; the combination of exonuclease and polymerase activities in the purified enzyme will excise and repair UV-damaged DNA in vitro (14). These findings all suggest a role of *polI* in repair synthesis and not in replication per se. The involvement of *polI* and ligase in the repair of damaged phage DNA was tested in experiments analogous to those described above.

Ligase mutants. Two types of ligase mutants in *E. coli* characterized by Gellert (9) and their parental strain were scrutinized: N1323, which overproduces ligase, N953 producing normal amounts, and N1325 which is deficient in polynucleotide ligase. The ligase levels differ at least 20-fold between the overproducer and the deficient strain (9). Intracellular repair of DNA from UV-irradiated phage is illustrated in Fig. 3. Neither deficiency (Fig. 3E, F, G, and H) nor overproduction (Fig. 3I, J, K, l) affected either the extent or the kinetics of repair of UV-damaged DNA. The same was true for repair of breaks due to ^{32}P decay and for breaks in $rII^{-}lig_{am}^{-}\cdot B$ DNA (data not shown).

DNA polymerase I mutant. Similar experiments were performed with bacteria deficient in *polI* (Fig. 4). Deficiency in *polI* impaired the repair process. While similar extent of initial breakage of UV-damaged DNA was found in both the *polI*-deficient strain and in a spontaneous *polI*$^{+}$ revertant thereof (Fig. 4A and D), subsequent restoration of strand integrity was less efficient in *polA*$^{-}$ cells (Fig. 4B, C, E, and F). Even 60 min of incubation in the presence of CM did not restore the integrity in *polA*$^{-}$ cells (cf. Fig. 4C and F).

The fact that deficiency in *polI* reduced the extent of observed repair further strengthens the argument that bacterial enzymes perform this repair of phage DNA and strongly suggests that *polI* is partially responsible for the repair.

While there are no doubts that ligase is capable of repairing single-stranded breaks in DNA (22, 25), we feel that its physiological role is still uncertain. It is interesting to observe that ligase deficiency does not impair the repair of discontinuities in phage DNA. This observation leaves room for speculations about the role of ligase in the cell.

Host-repaired areas may be recognized by phage-coded repairing enzymes. Kozinski and Lorkiewicz (15) showed that, despite a nearly perfect restoration of integrity of UV-damaged DNA upon removal of CM, there was no accompanying restoration of phage viability, as if repair was incompetent. UV excisions in *E. coli* DNA produce quite large gaps (3, 24). If the same is true for UV excisions in T4 DNA (23), the filling of such gaps would lead to insertion of cytosine instead of hydroxymethylcytosine (HMC) (since phage enzymes required to synthesize HMC and glucosylate it are not produced in the presence of CM). Phage-coded excision (and repair) enzymes may be able to recognize such inadequately repaired areas of the DNA. If this were the case, expression of phage functions after removal of CM from the culture may result in a second round of excision and possibly repair. This was tested in the following experiment. A culture of B23 was divided into two parts. One part was infected with ^{32}P-labeled, UV-irradiated T4 phage (MOI 0.1), and the second part was mixedly infected with the same UV-irradiated phage (MOI 0.1) and nonirradiated helper phage (MOI 3). CM was added to both parts at the moment of infection. Sixty minutes later the infected bacteria were sedimented and resuspended in fresh medium without CM, and incubation was continued. Figure 5 shows alkaline sucrose gradient analysis of samples from the mixedly infected culture at different times after infection. Prompt nicking occurs at 3 min after infection (Fig. 5A), and repair in the polynucleotide chain has occurred by 60 min (Fig. 5B). Twenty minutes after the removal of CM, the DNA repaired by the host has undergone a second cycle of nicking (compare Fig. 5C and A and note that the average D_2/D_1 of secondarily nicked DNA is virtually identical). The patterns of distribution depicted in Fig. 5A, B, and C are, for all practical purposes, identical also for bacteria which were singly infected with UV-irradiated phage without any unirradiated helper phage (not documented here). Figure 5D represents the fate of DNA from UV-irradiated phage 100 min after infection together with helper phage and shows that there is secondary repair. This is a typical pattern only for the suspensions co-infected with rescuing cold, viable phage. In the case of infection with UV-irradiated phage alone, there is no secondary repair, but gradual solubilization of DNA (up to 80% of the parental label, not documented here). In a typical experiment the secondary nicking is first detectable at 10 min after removal of CM, reaching its maximum at approximately 20 min after removal of CM. The close coincidence in the extents of primary and secondary nicking indicates that the phage enzymes may recognize excise and repair those areas that were previously subjected to host-mediated repair. This experiment also indicates

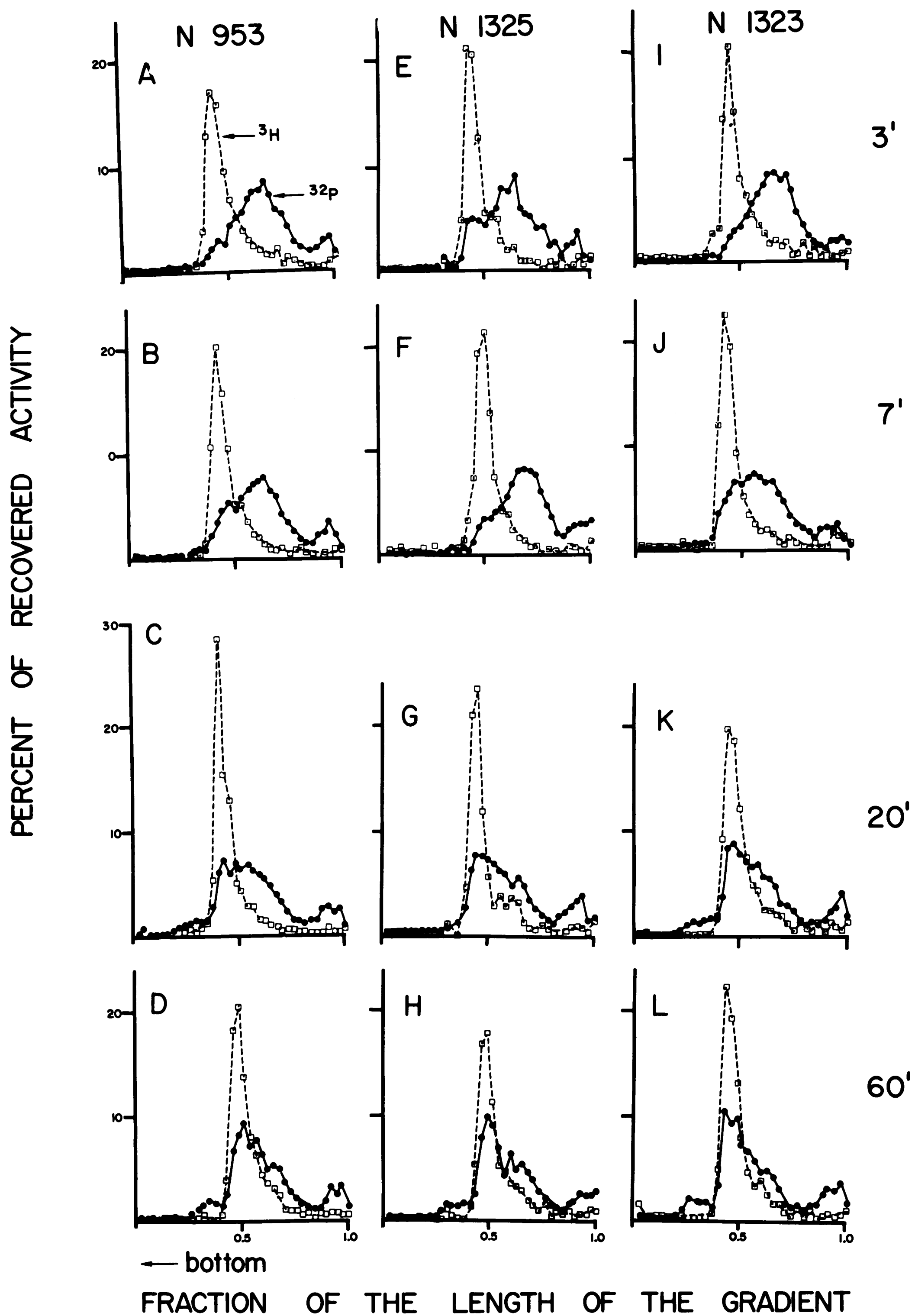

FIG. 3. *Repair of UV-damaged DNA in ligase mutants of E. coli. E. coli N953 (wild-type ligase levels), N1325 (deficient in ligase), or N1323 overproducing ligase) was infected with UV-irradiated phage in the presence of CM. At intervals samples were withdrawn and analyzed as described in preceding legends. Note the striking similarity in the pattern of occurring repair as a function of time and the seeming lack of effect of ligase levels on the kinetics of the repair.* ^{3}H *represents reference DNA from mature T4 phage.*

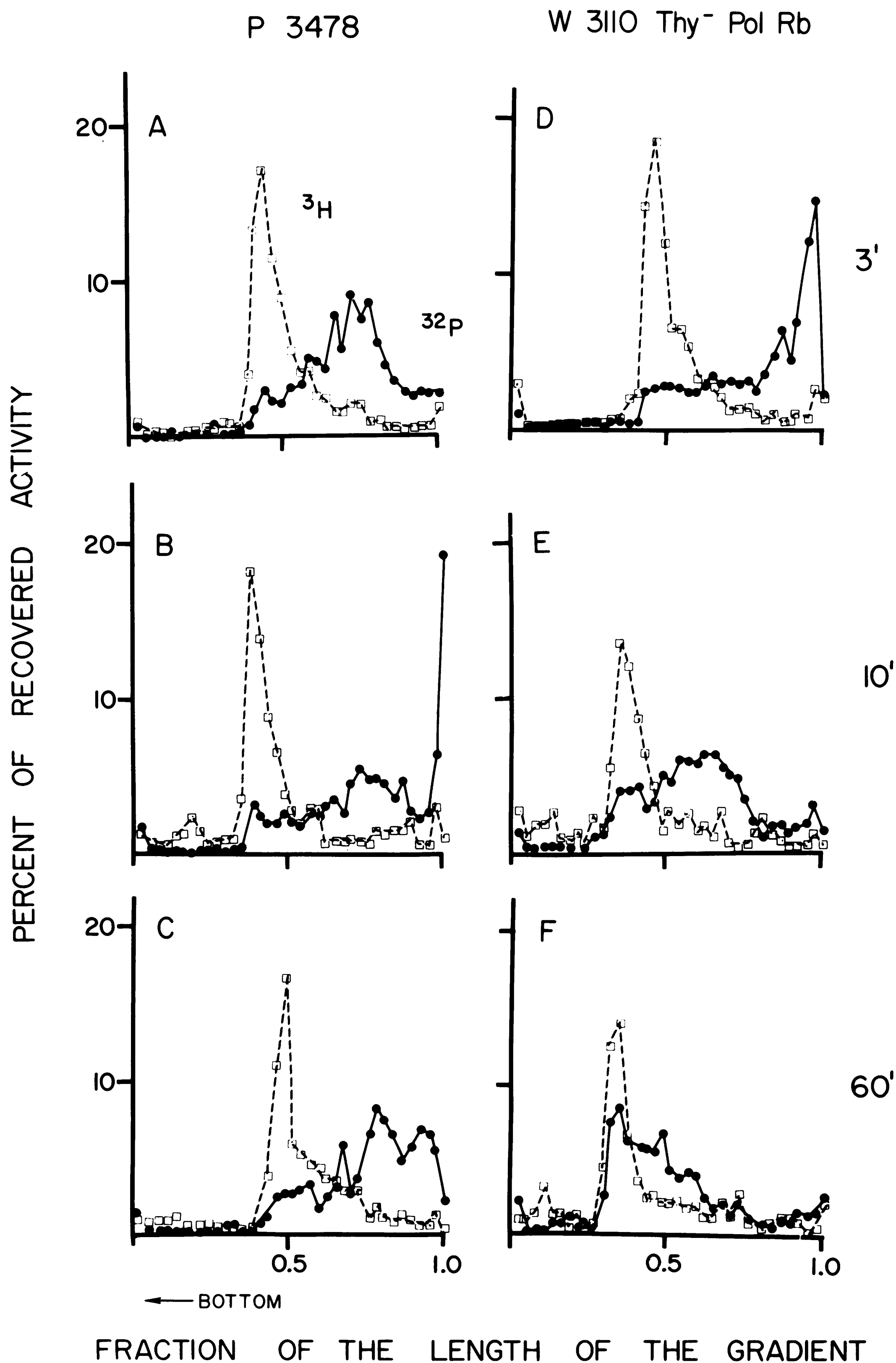

FIG. 4. *Repair of UV-damaged DNA in a DNA polymerase mutant of E. coli. E. coli P3478 polA⁻ or a polA⁺ revertant of this strain were infected with UV-irradiated phage as described in legend to Fig. 3, and the infected cells were sampled at intervals. Both strains allow the infliction of nicks at 3 min; however, a drastic difference is observed in the extent of subsequent repair in pol⁺ and pol⁻ mutants. A, B, and C, polA⁻; D, E, and F, polA⁺ revertant.*

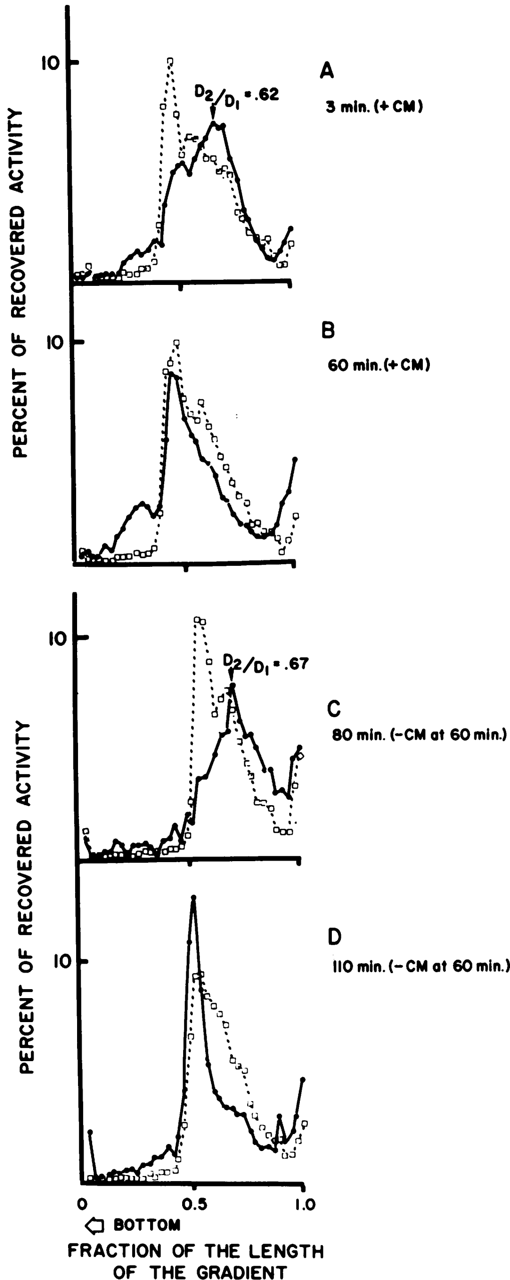

FIG. 5. *Primary and secondary breakdown and repair of UV-damaged phage DNA. E. coli B23 was infected in the presence of CM with an MOI of 0.1 of ^{32}P-labeled, UV-inactivated (6 lethal hits) T4 phage and an MOI of 3.0 of cold, nonirradiated T4 phage. A, Pattern obtained at 3 min after infection in the presence of CM; note the peaking of the breakdown product at D_2/D_1 = 0.62. This is the product of primary nicking. B, Fate of injfected DNA at 60 min after infection; note the extensive repair (primary repair). The reference ^{3}H DNA (broken line) is partially degraded, therefore after repair the distribution of ^{32}P tends to be more homogeneous than that of the reference. C, Secondary nicking occurring at 20 min after the removal of CM; note that the D_2/D_1 of the fragmented moiety = 0.67; this is very similar to the product of primary nicking shown in A. D, Secondary repair which occurs at 40 min after the removal of CM; note the repair in the continuity of polynucleotide chain and compare with the repair in B.*

that host repair of UV damages in phage DNA does not affect the coding for excision enzyme(s) recognizing host-repaired areas, but that production of enzyme(s) restoring strand integrity are prevented.

DISCUSSION

Experiments documented here support our earlier conclusion that there is host-mediated repair of discontinuous phage DNA under conditions when the synthesis of phage-induced proteins is prevented. Physiological ($rII^-lig_{am}^- \cdot B$) and physical (^{32}P decay) discontinuities in phage DNA, as well as discontinuities inflicted upon the DNA after it has entered the cell (UV damage), are promptly repaired upon infection of *E. coli* B in the presence of CM. The three types of damage are quite different: $rII^-lig_{am}^- \cdot B$ phage DNA has single-stranded gaps, ^{32}P decay causes a single-stranded nick where the newly exposed end may have an S terminus, which would be a rather unyielding candidate for repair, and UV-irradiated phage have damaged DNA which is not discontinuous to begin with but becomes broken after infection of the cells.

It is possible that the repair itself it quite similar in all three cases. It has been shown that excision of UV-irradiated DNA both in vivo in *E. coli* (3, 24) and in vitro using purified *E. coli* polymerase I (14) or a mixture of T4 endonuclease I and T4 "excision enzyme" (23) results in the release of oligonucleotides in size exceeding a thymine-thymine dimer by 10 to 30 residues. The terminus at the nick arising due to ^{32}P decay provides a very unusual substrate for repairing enzymes. It appears simpler to postulate that in this case repair is preceded by a preparatory, "editing" enzyme which recognizes that the DNA termini are somehow unusual, and excises adjacent nucleotides in "anticipation" of repair of the damage. Possibly, this excision of nucleotides may proceed by a similar mechanism as the UV excision, maybe utilizing the same enzyme(s). The discontinuous DNA of $rII^-lig_{am}^- \cdot B$ phage was shown here to contain single-stranded gaps of undefined

length, since the discontinuities are repairable only by a combination of DNA polymerase and ligase. In all three cases, then, repair may involve filling of a single-stranded gap.

Among several candidates for the role of bacterial repairing enzyme(s), DNA polymerase I and polynucleotide ligase attract special attention, being well characterized enzymes which have been shown to repair DNA discontinuities in vitro (18, 22, 25). Our finding that deficiency in *polI* interferes with the repair of T4 DNA is well in line with the diminished repair activities in this strain reported from another laboratory (12). Deficiency in *polI* has also been reported to interfere with replication and recombination of T4 DNA (21). In some repetitions of our *polA* experiments (not documented here), some residual repair was observed, though this repair was significantly less than what was found in the pol^+ revertant. The origin of the residual repair activity in the $polI^-$ mutant is not known. $recA^-$ bacteria lack ability to repair discontinuities in phage DNA (15) and are also UV sensitive (5). This suggests that the *recA* protein may be involved in the residual repair observed in $polA^-$ cells. It is of interest here to note that it is not possible to obtain a double mutant deficient in both *recA* and *polA*, suggesting that the two enzymes perform similar complementary functions (10).

More surprising was the discovery that neither deficiency nor overproduction of ligase affected the rate or extent of repair. The ligase-deficient mutant employed in this study shows increased UV sensitivity, suggesting impaired repair activity, although there is no effect on DNA replication or on the viability of the cells (9). It is possible that the small amounts of ligase found in the ligase-deficient mutant are sufficient to "seal" the gaps in phage DNA once they are filled. Another possibility is that the "sealing" may be performed by a highly specialized, as yet undescribed ligating enzyme, or by the gap-filling enzyme itself.

It was shown by Kozinski and Lorkiewicz (15) that functionality can not be restored by the host-mediated repair of phage DNA. Experiments described in this paper provide an explanation for this observation. Upon removal of CM there is a secondary breakdown of UV-irradiated, host-repaired phage DNA. The extent of this secondary breakdown coincides closely with that of the primary breakdown, and proceeds with an identical pattern both in singly and in mixedly (UV-damaged and non-UV-damaged phage) infected bacteria. After completion of the secondary round of nicking, secondary repair takes place, but only if the cells have been co-infected with non-UV-damaged "rescuer". In the absence of unirradiated helper phage the irradiated DNA is gradually solubilized. Thus, host-mediated repair not only did not improve the DNA, but actually proved very detrimental.

This suggests that secondary nicking and repair are caused by newly synthesized phage-coded enzymes which at first are able to recognize areas improperly repaired by the host enzyme(s) and then restore the damaged area to its integrity. The former, but not the latter, may be coded from UV-irradiated DNA. There are reasons to believe that viability is also restored, since it was demonstrated that the parental label of such rescued phage is being transferred to progeny phage as segments of parental strands which underwent semiconservative replication (E. Shahn, Ph.D. thesis, 1965, University of Pennsylvania, Philadelphia).

Stringent proof that the host-repaired areas are recognized, excised, and repaired by phage enzymes could be obtained if, for instance, ^{3}H-TdR was incorporated during the host-mediated repair in CM. Upon removal of CM one should then observe solubilization of this ^{3}H label coincidentally with the appearance of secondary breaks. However, after infection in the presence of CM most of the ^{3}H-TdR uptake will go to bacterial DNA. This bacterial DNA synthesis, and the phage-mediated breakdown of labeled host DNA after removal of CM, would overshadow any label entering and being released from the repaired areas of the phage DNA.

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LITERATURE CITED

1. Aposhian, H. V. 1966. DNA polymerase from T2-infected Escherichia coli, p. 277-283. *In* G. L. Cantoni and D. R. Davies (ed.), Procedures in nucleic acid research. Harper and Row, New York.
2. Berger, H., and A. W. Kozinski. 1969. Suppression of T4D ligase mutations by rIIA and rIIB mutations. Proc. Nat. Acad. Sci., U.S.A. **64**:897-904.
3. Boyce, R. P., and P. Howard-Flanders. 1964. Release of ultraviolet light-induced thymine dimers from DNA in Escherichia coli K-12. Proc. Nat. Acad. Sci. U.S.A. **51**:293-300.
4. Burgi, E., and A. D. Hershey. 1963. Sedimentation rate as a measure of molecular weight of DNA. Biophys. J. **3**:309-321.
5. Clark, A. J., and A. D. Margulies. 1965. Isolation and

characterization of recombination-deficient mutants in Escherichia coli K12. Proc. Nat. Acad. Sci. U.S.A. **53**:451-459.

6. DeLucia, P., and J. Cairns. 1969. Isolation of an Escherichia coli strain with a mutation affecting DNA polymerase. Nature (London) **224**:1164-1166.
7. Ebizuzaki, K., and L. Campbell. 1969. On the role of ligase in genetic recombination in bacteriophage T4. Virology **38**:701-703.
8. Fraser, D., and E. A. Jerrel. 1953. The amino acid composition of T3 bacteriophage. J. Biol. Chem. **205**:291-295.
9. Gellert, M., and M. L. Bullock. 1970. DNA ligase mutants of Escherichia coli. Proc. Nat. Acad. Sci. U.S.A. **67**:1580-1587.
10. Gross, J. D., J. Grunstein, and E. M. Witkin. 1971. Inviability of recA$^-$ derivatives of the DNA polymerase mutant of DeLucia and Cairns. J. Mol. Biol. **58**:631-634.
11. Harm, W. 1963. Mutants of phage T4 with increased sensitivity to ultraviolet. Virology **19**:66-71.
12. Kanner, L., and P. C. Hanawalt. 1970. Repair deficiency in a bacterial mutant defective in DNA polymerase. Biochem. Biophys. Res. Commun. **39**:149-155.
13. Karam, J. D. 1969. DNA replication by phage T4rII mutants without polynucleotide ligase (gene 30). Biochem. Biophys. Res. Commun. **37**:416-422.
14. Kelly, R. B., M. R. Atkinson, S. A. Huberman, and A. Kornberg. 1969. Excision of thymine dimers and other mismatched sequences by DNA polymerase of E. coli. Nature (London) **224**:495-501.
15. Kozinski, A. W., and Z. K. Lorkiewicz. 1967. Early intracellular events in the replication of T4 phage DNA. IV. Host-mediated single-stranded breaks and repair in ultraviolet-damaged DNA. Proc. Nat. Acad. Sci. U.S.A. **58**:2109-2116.
16. Kozinski, A. W., and W. Szybalski. 1959. Dispersive transfer of the parental DNA molecule to the progeny of phage ϕX 174. Virology **9**:260-274.
17. Litwin, S., E. Shahn, and A. W. Kozinski. 1969. Interpretation of sucrose gradient sedimentation pattern of deoxyribonucleic acid fragments resulting from random breaks. J. Virol. **4**:24-30.
18. Masamune, Y., and C. C. Richardson. 1971. Strand displacement during deoxyribonucleic acid synthesis at single strand breaks. J. Biol. Chem. **246**:2692-2761.
19. Mathews, C. K. 1971. Bacteriophage biochemistry. Van Nostrand Reinhold Company, New York.
20. Miller, R. C., and A. W. Kozinski. 1970. Early intracellular events in the replication of bacteriophage T4 deoxyribonucleic acid. Further studies on the T4 protein-deoxyribonucleic acid complex. J. Virol. **5**:490-501.
21. Mosig, G., D. W. Bowden, and S. Bock. 1972. E. coli DNA polymerase I and other host functions participate in T4 DNA replication and recombination. Nature N. Biol. **240**:12-16.
22. Olivera, B. M., and I. R. Lehman. 1967. Linkage of polynucleotides through phosphodiester bonds by an enzyme from Escherichia coli. Proc. Nat. Acad. Sci. **57**:1426-1433.
23. Sekiguchi, M., S. Yasuda, S. Okubo, H. Nakayama, K. Shimada, and Y. Takagi. 1970. Mechanisms of repair of DNA in bactriophage. I. Excision of pyrimidine dimers from ultraviolet DNA by an extract of T4-infected cells. J. Mol. Biol. **47**:231-242.
24. Setlow, P. B., and W. L. Carrier. 1964. The disappearance of thymine dimers from DNA: an error-correcting mechanism. Proc. Nat. Acad. Sci. U.S.A. **51**:226-231.
25. Weiss, B., and C. C. Richardson. 1967. Enzymatic breakage and joining of deoxyribonucleic acid. I. Repair of single-stranded breaks in DNA by an enzyme system from Escherichia coli infected with T4 bacteriophage. Proc. Nat. Acad. Sci. U.S.A. **57**:1021-1028.