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Role of DNA Polymerase I and the *rec* System in Excision-Repair in *Escherichia coli*

(UV irradiation/CsCl centrifugation/5-bromouracil)

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ABSTRACT **The DNA polymerase I-deficient mutant *polA1* is shown to perform an *increased* amount of UV-stimulated repair synthesis relative to its *pol*$^+$ parent. In contrast, a *recA recB* double mutant is found to perform little detectable repair synthesis. Analysis of the density distribution of sheared DNA of the *recA recB* mutant reveals that none of the repair synthesis in this strain is in the large repair patches previously demonstrated by us in wild-type strains. These results are interpreted in terms of a model involving both DNA polymerase I and the *rec* system in the excision-repair process, with polymerase I performing an efficient short patch repair and *rec* system enzymes producing predominantly large patches of repair synthesis.**

An excision-repair mechanism for the correction of structural defects in DNA has been demonstrated in several living systems (1–7). The process involves enzymatic recognition and excision of an oligonucleotide containing the lesion, repair synthesis in the deleted region using the undamaged complementary strand as template, and a final rejoining of the repaired segment to the contiguous parental strand. The process was first discovered in UV-irradiated *Escherichia coli*, and it has been most extensively studied in that system. One class of UV-sensitive mutants (*uvr*$^-$) is deficient in excision of UV-induced pyrimidine dimers from DNA (1, 2). A mutant of this class, carrying *uvrA6*, has been tested and found, as predicted, to be defective in repair synthesis after UV irradiation (8).

A second general class of UV sensitive mutants (*rec*$^-$) has been shown to be deficient in genetic recombination (9, 10). These mutants appear to have defects in a repair pathway other than that initiated by the removal of dimers (11). Because *uvr rec* double mutants are more sensitive to UV irradiation than either *rec* or *uvr* single mutants, it has been generally assumed that at least two separate and distinct repair systems exist in *E. coli*, the excision-repair scheme and a recombination-dependent (*rec*) system. The latter performs post-replication repair of gaps left in *daughter* strands as the replicase bypasses dimers in the normal sequential progression of the growing point (12).

The *in vitro* properties of the DNA polymerase I from *E. coli* render this enzyme a very likely candidate for *both* the repair replication step and the dimer excision step in excision-repair (13). However, a mutant, *polA1*, deficient in this enzyme (14) is not as UV sensitive as the *uvrA6* strain, suggesting that some repair does occur in this mutant (15). The UV sensitivity of a *polA uvrA* double mutant is similar to that of the *uvrA* mutant, indicating that the defect in *polA1* is in the excision-repair pathway (16). Dimers are excised to a nearly normal extent in the *polA1* strain (17), but the analysis of the rejoining of parental strands shows that this final step is delayed relative to that in the parent strain (15). Monk, Peacey, and Gross have proposed that the *recA* system may substitute for DNA polymerase I in repairing the gaps produced by dimer excision in the *polA1* mutant (16). The fact that *polA*$^-$ *recA*$^-$ is a lethal genotype is also consistent with the suggestion that polymerase I and the *rec* system can overlap in carrying out some *essential* function (*18*).

We have previously demonstrated that the size of repair patches in *E. coli* DNA varies from short stretches of nucleotides to several thousand nucleotides in extent, and we have suggested that different enzymes may be responsible for the small and large patches (19). In the present study we have examined repair replication in the *polA1* mutant and in a *recA recB* double mutant. Our results support the following model: (*i*) the DNA polymerase I performs short patch repair of damaged DNA *in vivo;* (*ii*) the *rec* system also participates in excision-repair, but it produces predominantly large patches; (*iii*) either of these systems is capable of completing the excision-repair process in the absence of the other.

MATERIALS AND METHODS

The following bacterial strains were used: *E. coli* W3110 *thy*$^-$ and its DNA polymerase I-deficient derivative, P3478 *polA1*, obtained through the generosity of Dr. John Cairns; *E. coli* K12 strains AB2497, AB2500 *uvrA6*, SR111 *recA13 recB21* (a *thy*$^-$ derivative of *JC5495*), and SR78 *recB21* (a *thy*$^-$ derivative of JC5743). The latter four strains, kindly supplied by Dr. Ann K. Ganesan, are all F$^-$ *thy*$^-$ *thr*$^-$ *leu*$^-$ *thi*$^-$ *arg*$^-$ *pro*$^-$ *his*$^-$.

Cells were cultured aerobically at 37° in glucose–salts medium as described (20), supplemented with 2 μg/ml thymine, 1 mg/ml vitamin-free casamino acids, and 0.1 μg/ml thiamin where required. The DNA was uniformly labeled by addition to the medium of 1 μCi/ml of carrier-free [^{32}P]-phosphate (giving a final specific activity of 0.2 Ci/mol). Density labeling was accomplished either by substitution of 10 μg/ml 5-bromouracil (BrUra) for thymine in the medium or by preparation of the buffered salts solution with $^{15}NH_4Cl$ and supplying 0.1% [^{13}C]glucose as energy source. Radio-

Abbreviation: BrUra, 5-bromouracil.

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active density labeling was usually performed with 10 μCi/ml of [^{3}H]BrUra (8.8 Ci/mmol).

Cultures were irradiated in Tris buffer at 2 × 10^8 cells/ml with a Westinghouse low-pressure germicidal lamp (254 nm, dose rate 200 ergs/mm^2 per min). After UV irradiation [^{3}H]BrUra was incorporated into the cells. The cell lysate was then centrifuged in an equilibrium CsCl density gradient to separate parental-density DNA from normally replicated, hybrid-density DNA. Fractions from the parental-density region were selected and centrifuged a second time in CsCl for further isolation of the parental DNA. The [^{3}H]BrUra that was incorporated into the parental-density DNA was taken to be a measure of repair replication. Details of the assay for repair replication are given elsewhere (20).

The method of Couch and Hanawalt was used to correct for the effect of endogenous nucleotide pools on the measure of repair replication (21). Cultures were prelabeled for several generations with 0.5 μCi/ml [^{3}H]thymine (23.2 Ci/mmol); the cells were harvested by filtration and then resuspended in Tris buffer at 5% of the usual phosphate concentration. After UV irradiation, supplements required for growth were added, with thymine replaced by 10 μg/ml BrUra, along with 20 μCi/ml [^{3}H]BrUra (8.8 Ci/mmol) and 20 μCi/ml [^{32}P]-phosphate (carrier-free) as repair labels. After incubation the repaired DNA was isolated pycnographically from the cell lysate as described (20). The parental density fractions from the second CsCl centrifugation were subjected to gentle alkaline hydrolysis for removal of contaminating RNA, and spleen phosphodiesterase digests were then prepared. The resulting 3′-mononucleotides were separated by two-dimensional thin-layer chromatography on PEI-cellulose and the ^{3}H and ^{32}P radioactivity in each cut-out spot was determined by liquid scintillation spectrometry. The base composition of the repaired DNA was then derived from the ^{32}P radioactivity associated with each of the 3′-deoxymononucleotides (21, 22). The apparent amount of repair replication was then corrected as follows to take into account the dilution of the repair label. The number of moles of BrUra incorporated by repair and the total moles of thymine were calculated from the ^{3}H radioactivity associated with the respective spots and from the known specific activities of [^{3}H]BrUra and [^{3}H]thymine supplied. Assuming that the ^{32}P specific activities were the same for dTMP and BrdbUMP, the moles of thymine incorporated by repair were then calculated from the T:BrUra ratio in repaired DNA and the computed moles of BrUra in repair. If one assumes that the base composition of the repaired regions is similar to that of the DNA as a whole, then the true fraction of the parental density DNA synthesized during repair is equal to (number of moles of [^{32}P]dTMP plus total number of moles of BrdUMP) divided by (number of moles of [^{3}H]dTMP).

RESULTS

If DNA polymerase I is responsible for the synthesis step in the excision-repair of UV-damaged DNA, then the DNA polymerase I-deficient mutant of *E. coli*, *polA1* (14), should be unable to perform repair replication. However, when we compared P3478 *polA1* and its *pol*$^+$ parent W3110, we were surprised to find that the mutant performed an apparently similar amount of UV-stimulated repair replication. The results of a typical experiment with BrUra as the density label are illustrated in Fig. 1. In 20 min of incubation after

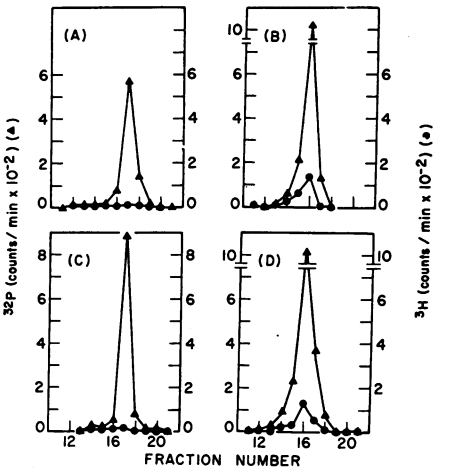

FIG. 1. Recentrifugation in neutral CsCl of parental-density DNA from (*A*) unirradiated W3110, (*B*) W3110 exposed to 200 ergs/mm^2 UV, (*C*) unirradiated P3478 (*polA1*), and (*D*) P3478 (*polA1*) exposed to 200 ergs/mm^2 of UV irradiation. Cells were uniformly labeled with ^{32}P, and half of each culture was irradiated in buffer. During 20 min of incubation after UV irradiation thymine was replaced by [^{3}H]BrUra in the growth medium (*see Methods*). The culture lysate was sedimented to equilibrium in a neutral CsCl density gradient, and parental-density DNA fractions were selected for recentrifugation as shown.

a 1-min exposure to UV the amount of repair synthesis (as indicated by the ratio of ^{3}H repair label to ^{32}P prelabel in DNA of parental density) was stimulated about 5-fold in the parent (Figs. 1, *A* and *B*) and about 4-fold in the mutant (Fig. 1, *C* and *D*). In many other similar experiments involving UV exposures ranging from 15 sec to 2 min and incubation periods after UV irradiation of from 10 min to 90 min the results were similar, with the *polA1* strain always incorporating about as much BrUra repair label as the parent strain. This result was also obtained when the heavy isotopes ^{13}C and ^{15}N were used as density labels instead of BrUra, thus ruling out possible artifacts due to the thymine analogue.

In interpreting the results of repair experiments with the *polA1* strain, however, it is necessary to take into account the fact that the DNA of this strain undergoes extensive UV-induced degradation (17, 23). The resulting large quantity of thymidine nucleotides released into the internal pools competes with the radioactive density label in DNA synthesis after UV irradiation. To correct for this we determined the actual base composition of the repaired DNA, as described in *Methods*. While there is an appreciable amount of reincorporated thymine in repaired DNA of the *pol*$^+$ strain W3110, the T:BrUra ratio in P3478 *polA1* repair is about 8-fold greater (Table 1). When the apparent amount of repair replication is corrected for this dilution of the BrUra repair label, it is seen that about a 4-fold *greater* extent of repair synthesis has occurred in the *polA1* strain than in its *pol*$^+$ parent.

The same UV dose should generate the same number of dimers in both strains. Since dimer excision has been shown to proceed at a slightly slower initial rate in the mutant compared to the parent but to about the same final extent after 40 min of incubation (17), it is reasonable to assume that there is an equal number of repairable sites in the two strains. Then, on the average, the lengths of the repair patches must

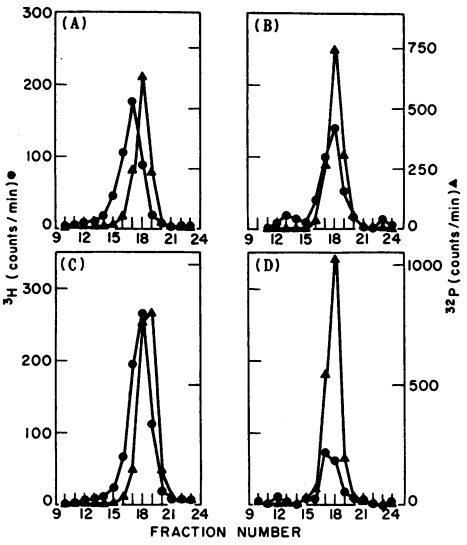

FIG. 2. Recentrifugation in neutral CsCl of parental-density DNA of (*A*) unirradiated *rec*$^+$ strain, (*B*) *rec*$^+$ strain after 100 ergs/mm^2 UV, (*C*) unirradiated *recA*$^-$ *recB*$^-$ strain, and (*D*) *recA*$^-$ *recB*$^-$ strain after 100 ergs/mm^2 of UV irradiation. The experiment was performed as described in the legend to Fig. 1, except that incubation after UV irradiation was for 30 min.

be longer in the *polA1* mutant than in the parent strain to give the result we observe. We have recently demonstrated that some very long stretches of nonconservative DNA replication occur in the course of repair (19). Hence, it seems likely that the defect in P3478 somehow causes more of the excision sites to become involved in this long patch synthesis. This interpretation is consistent with other known facts about repair in the *polA1* strain, such as the extensive DNA degradation (17, 23) and the delay in the rejoining step (15). Unfortunately, this explanation is not amenable to direct proof by shearing the DNA and observing the density shift of large repair patches containing BrUra. The T:BrUra ratio of 4:1 found in repaired DNA of P3478 (Table 1) is too high to allow resolution of long patches of repair synthesis as DNA fragments of intermediate density, even if the entire fragment consists of a repair patch. Shearing experiments have been performed and yield the result predicted on the basis of the base composition analysis: after extensive shearing, density label in repaired DNA of P3478 is found in fragments that band in alkaline CsCl at the density position expected for DNA that is 20% substituted with BrUra (23). This is in contrast to the result obtained with W3110 and other strains with less UV-induced DNA degradation, where much of the repair label is found in fragments banding at higher densities, up to that of a strand fully substituted with BrUra (19, 23).

There are several possible mechanisms by which a mutant with a defective repair polymerase might produce large patches of repair synthesis. One possibility is that the large patches represent the aberrant behavior of a partially functional enzyme. However, it has been shown that the residual DNA polymerizing activity in extracts from the *polA1* strain can be attributed to other polymerases, such as DNA polymerase II (24) and DNA polymerase III (25). Alternatively, there might be two polymerases normally competing for excision gaps and one of these, but not the other, produces long regions of degradation and resynthesis. We examined the possibility that the *rec* enzymes may function in long patch repair synthesis by applying our shearing analysis of repaired DNA (19) to repair in a *recA recB* double mutant. In this procedure, extensive sonic shearing of the isolated parental-density DNA permits the resolution of fragments containing BrUra patches of different lengths in a subsequent alkaline CsCl gradient. Since the DNA of *recA* mutants undergoes very extensive UV-induced degradation, but *recA recB* double mutants show the reduced level of degradation characteristic of *recB* or *recC* mutants carrying *recA*$^+$ (26), we used an *E. coli* strain carrying the double mutation to minimize dilution of the repair label by degradation products.

When *E. coli* strain SR111 *recA13 recB21* and the related *rec*$^+$ strain AB2497 were exposed to a UV dose of 100 ergs/mm^2 and then incubated for 30 min with [^{3}H]BrUra, DNA synthesis was strongly depressed in the mutant, but not in the *rec*$^+$ strain. In both strains a sizable amount of the newly-synthesized DNA appeared at a density intermediate between parental and hybrid, whether or not the cells were irradiated. The proportion of new DNA having this intermediate density seemed to depend upon the extent of normal semiconservative replication. While it is apparently unrelated to any effects of UV damage, its equilibrium position in CsCl gradients is so close to the normal parental density that it interferes with the quantitation of repair synthesis. This problem has been referred to previously in connection with an analysis of repair occurring in the excision-defective *uvrA6* mutant derived from AB2497 (8).

Recentrifugation in neutral CsCl of fractions containing parental-density DNA selected from the initial CsCl bandings of unirradiated and irradiated samples of both strains reveals that there is a much larger amount of nonconservative synthesis after UV in the *rec*$^+$ strain than in the *recA recB* double mutant (Fig. 2). The central fractions of the parental-density peaks of the rebanded DNA of irradiated AB2497 and SR111 (Fig. 2, *B* and *D*) were selected, sheared by sonication as described (19) to a single-strand molecular weight of about 0.5×10^6, denatured, and analyzed in alkaline CsCl gradients, as shown in Fig. 3. As with other strains previously examined (19), repaired DNA of strain AB2497 contains large patches of repair synthesis, evidenced by the large proportion of the repair label that appears in fragments of intermediate density after shearing (Fig. 3*A*). This large patch component is strikingly absent in the *recA recB* double mutant (Fig. 3*B*).

Since nonconservative DNA synthesis after exposure to UV irradiation in the *recA*$^-$ *recB*$^-$ strain is strongly depressed

TABLE 1. *Use of endogenous thymine in repair patches in E. coli strain W3110 and its polymerase I-deficient derivative, P3478 polA1*

	W3110	P3478
% T	7.85	20.20
% BrUra	16.59	4.97
T:BrUra	1:2	4:1
% Corrected repair	0.2	0.8

The base composition of repair patches in DNA labeled with ^{32}P during a 45-min period after 200 ergs/mm^2 UV.

% T = % of all bases incorporated into parental DNA during repair that are thymine; % BrUra = % of all bases incorporated into parental DNA during repair that are BrUra; % Corrected repair = % of parental DNA resynthesized by repair.

relative to that in the rec^+ strain and does not contain a large patch component, it is of interest to compare the extent of such synthesis in the $recA^-$ $recB^-$ strain to that in a related strain carrying the *uvrA6* mutation. Strains AB2497 (uvr^+ rec^+), AB2500 (*uvrA6*), and SR111 (*recA13 recB21*) were grown in a ^{32}P-containing "light" medium, exposed to an incident UV dose of 100 ergs/mm^2, and incubated for 60 min with [^{3}H]BrUra. The extent of repair synthesis determined from the ratio of ^3H to ^{32}P in the second CsCl banding of the parental-density DNA is given in Table 2. Although the second bandings were performed in alkaline CsCl in an attempt to increase the resolution of parental-density DNA from the replicated DNA of intermediate density, the $^3H/^{32}P$ ratios for control cultures of the two mutant strains and for the irradiated culture of AB2500 still reflect significant amounts of contamination by this normally replicated DNA. Unfortunately it is therefore impossible to assess with certainty the extent of stimulation of nonconservative synthesis by exposure to UV in SR111. It is clear that any repair synthesis that occurs in the $recA^-$ $recB^-$ strain is insignificant in amount relative to that in the rec^+ strain, and in fact may be undetectably different from that in the *uvrA6* strain.

The resemblance between the absence of measurable repair synthesis in a $recA^-$ $recB^-$ strain and a *uvrA6* strain (Table 2) could be due to an inability of the *rec* double mutant to perform the excision step of repair. Although it had previously been concluded that a *recA* mutant is capable of dimer excision (10), the additional effect of the deficiency in the *recB*, *recC*-determined ATP-dependent nuclease is not known. This possibility is especially worth considering since *polA1* excises dimers normally although the 5′ → 3′ exonuclease activity of DNA polymerase I thought to be responsible for excision is reportedly also absent in the mutant (28). Accordingly, a preliminary experiment was performed to test for excision of dimers in the $recA^-$ $recB^-$ strain. The rec^+ and $recA^-$ $recB^-$ strains were exposed to a UV dose of 400 ergs/mm^2 and samples were taken after 0 and 30 min of incubation after UV irradiation. The acid-soluble and acid-precipitable fractions were assayed for [^{3}H]thymine and [^{3}H]thymine-containing dimers by the column chromatographic method of Dellweg and Wacker (29), as modified by Nakayama (personal communication). A comparison of the amount of thymine and dimers released into the acid-soluble fraction with the estimated total amount of each indicated that dimers are preferentially released in *both* strains. That the excision of dimers in the $recA^-$ $recB^-$ strain is less efficient than in the rec^+ strain remains a possibility, however. Obviously a quantitative study of excision in a *recA recB* mutant would be of interest.

TABLE 2. *Ratio of* [^{3}H]*BrUra label to parental* [^{32}P] *DNA label as an indication of repair replication in several E. coli strains*

Strain	Unirradiated control	100 ergs/mm^2 of UV irradiation
AB2497 (uvr^+ rec^+)	0.045	0.09
AB2500 (*uvrA6*)	0.02	0.02
SR111 (*recA13 recB21*)	0.06	0.035

The experiment was performed as in the legend to Fig. 1, except that incubation after UV irradiation was for 60 min. Fractions containing parental-density DNA were selected from first bandings in neutral CsCl gradients and recentrifuged in alkaline CsCl. The ratio of [^{3}H]BrUra repair label to parental [^{32}P] DNA label was determined for the peak of parental-density DNA.

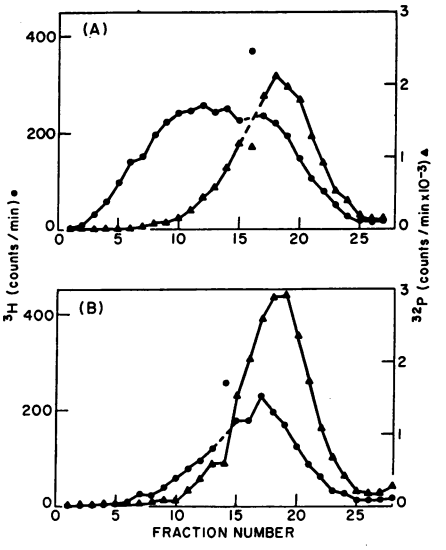

FIG. 3. Density analysis in alkaline CsCl of extensively sheared repaired DNA of (*A*) rec^+ strain and (*B*) $recA^-recB^-$ strain. The central fraction from the parental-density regions of the CsCl gradients of Fig. 2*B* and *D*, were selected and sonicated to reduce the single-strand molecular weight to 0.5–0.8 × 10^6 (19). The sheared DNA was then denatured at pH 12.5 and centrifuged in alkaline CsCl gradients.

DISCUSSION

The results of our experiments demonstrate that in the UV-sensitive *polA1* mutant a deficiency in DNA polymerase I results in a greatly *increased* extent of repair synthesis. On the other hand, a *recA recB* double mutant performs little detectable repair synthesis, none of which is found in the large repair patches that in rec^+ strains account for the major proportion of the label incorporated into repaired DNA. In contrast, studies on toluene-treated cells of the *polA1* strain have shown that repair, as defined by stimulation of nonconservative synthesis by nuclease action, is *absent* in this strain (27). However, repair synthesis after exposure to UV irradiation has not been examined in these cells after toluene treatment.

Assuming as is indicated by our preliminary experiments that dimer excision occurs in the $recA^-$ $recB^-$ mutant, one possible interpretation of the apparent absence of repair synthesis in the $recA^-$ $recB^-$ strain is that *all* the repair synthesis in the excision-repair process is mediated by *rec* enzymes. However, this interpretation does not account for the fact that the UV sensitivity of the *uvrA recA* double mutant is greater than that of a *uvrA* single mutant. It also does not explain the fact that a deficiency in DNA polymerase I confers UV sensitivity while increasing the extent of repair synthesis.

Our preferred explanation is the model involving both DNA polymerase I and *rec* enzymes in the resynthesis step of repair. Thus it could be supposed that both enzyme systems can perform repair synthesis and normally compete for exci-

sion sites. The system mediated by *rec* enzymes may be less efficient in repair, since it produces repaired regions of random length, some of which might be very large, corresponding to a single strand molecular weight of at least 0.5×10^6 (19). DNA polymerase I is involved in another, more efficient, system that excises only a few adjacent nucleotides along with each dimer in a manner analogous to that of the *M. luteus in vitro* system (30). The correspondingly small patch of resynthesis could make only a negligible contribution to the total label incorporated in repaired DNA. In the absence of DNA polymerase I, the *rec* system would then operate at all damaged sites, producing large patches at some of them. Conversely only small patches would be produced in *rec*$^-$ mutants. Operation of the two systems in excision-repair might be analogous to the *pol*-controlled fast repair and *rec*-mediated slow repair of x-ray damage reported by Town, Smith, and Kaplan (31). This model is attractive in that it explains the high level of repair synthesis in the *polA1* strain and also could account for the observed delay in the final rejoining step of excision-repair in this strain (15).

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