REPAIR OF NEAR-ULTRAVIOLET (365 NM)–INDUCED STRAND BREAKS IN *ESCHERICHIA COLI* DNA

The Role of the *polA* and *recA* Gene Products

ANN G. MIGUEL and REX M. TYRRELL
Instituto de Biofisica, Centro de Ciências da Saúde (Bloco G), Universidade Federal do Rio de Janeiro, Rio de Janeiro, Brazil; and Institut Suisse de Recherches Expérimentales sur le Cancer, CH-1066 Epalinges s/Lausanne, Suisse

ABSTRACT The action of near-ultraviolet (UV-365 nm) radiation in cellular inactivation (biological measurements) and induction and repair of DNA strand breaks (physical measurements) were studied in a repair-proficient strain and in *polA-*, *recA-*, *uvrA-*, and *polA uvrA*–deficient strains of *Escherichia coli* K-12. The induction of breaks in the *polA* and *polA uvrA* strains was linear with dose (4.0 and 3.7 $\times$ 10^{-5} breaks/2.5 $\times$ 10^{9} daltons/Jm^{-2}, respectively). However, in the *recA-*, *uvrA-*, and repair-proficient strains, there was an initial lag in break induction at low doses and then a linear induction of breaks at higher doses with rates of 4.6, 2.8, and 3.2 $\times$ 10^{-5} breaks/2.5 $\times$ 10^{9} daltons/Jm^{-2}, respectively. We interpret these strain differences as indicating simultaneous induction and repair of breaks in polymerase 1 (*polA*)–proficient strains under the 0°C, M9 buffer irradiation conditions that, for maximum efficiency, require both the *polA* and *recA* gene products. Strand-break rejoining also occurred at 30°C in complete growth medium. We propose that at least three (and possibly four) distinct types of pathways can act to reduce the levels of 365-nm radiation–induced strand breaks. A quantitative comparison of the number of breaks remaining with the number of lethal events remaining after repair in complete medium at 30°C showed that between one and three breaks remain per lethal event in the wild-type and *recA* strains, whereas in the *polA* strain one order of magnitude more breaks were induced.

INTRODUCTION

The increased sensitivity of *Escherichia coli* to near-UV radiation when irradiated under aerobic conditions (1) in conjunction with the oxygen dependence of single-strand break induction at 365 nm (2) has made the single-strand break a candidate for a critical lethal lesion at this near-UV wavelength. Irradiation at 365 nm has been shown to induce single-strand breaks (and alkali-labile bonds) in extracted T_4 phage DNA at a rate equal to the induction of pyrimidine dimers, and in bacterial DNA in vivo at a rate approximately one-half that of pyrimidine dimers (2, 3). While the pyrimidine dimer is the major lethal lesion induced in *E. coli* strains by far-UV radiation at 254 nm (4), pyrimidine dimers are not significant lethal lesions at 365 nm in either the *uvrA* excision deficient or wild-type strains (5).

Although two classes of strand-break repair have been characterized for x-ray–induced single-strand breaks, work on repair of 365-nm irradiation-induced strand breaks is less complete. Repair processes known to be effective in the repair of x-ray–induced single-strand breaks include a fast *polA*-dependent pathway that occurs within 2–5 min after irradiation and a slower *recA*-dependent pathway that takes 40–60 min to seal the breaks (6). A fast *polA*-dependent repair capacity has been found for 365-nm irradiation–induced breaks (7), but no evidence has been presented for additional pathways.

In this study we have (*a*) determined the initial 365-nm break induction rates for several strains of *E. coli* both deficient and proficient in repair, (*b*) partially defined several break repair systems, and (*c*) compared the number of single-strand breaks remaining after repair with calculated values for lethal event induction, using a quantitative physical method for break determination and biological viability for determination of lethal events.

MATERIALS AND METHODS

Bacterial Strains

The *E. coli* K-12 strains W3110 *thy* (repair-proficient), p3478 *polA1 thy* (8), TN207 *uvrA6 thy* (9), and JG136 *uvrA6 polA1 thy* (10) were obtained from Ronald Ley (Lovelace Medical Foundation, Albuquerque, NM). MM450 *recA56 thy lac rha str* (11) was originally obtained from Marilyn Monk (Medical Research Council Genetics Unit, London, UK). All five strains were derived from the W3110 parental strain.

Growth and Radioactive Labeling

Cells were grown overnight in a shaking incubator at 37°C in M9 buffer (1 g NH_4Cl, 5 g NaCl, 6 g Na_2HPO_4, 3 g KH_2PO_4, 0.8 g $MgSO_4 \cdot 7H_2O$,

H_2O to 1 liter) supplemented with 4 mg/ml glucose, 2.5 mg/ml Casamino acids, 10 μg/ml niacin, 10 μg/ml thiamine, and 10 μg/ml thymine. For labeling, a sample of an overnight culture was diluted 50-fold into prewarmed supplemented M9 medium containing 20 μCi/ml [^{3}H]methyl-thymidine (New England Nuclear [Boston, MA], 6.78 Ci/mmol) at a final total thymidine concentration of 2 μg/ml. After 2.5 h of growth under aeration at 37°C, the cells were harvested by centrifugation, washed twice with M9 buffer, and resuspended in M9 buffer at a cell concentration of 1 to 1.5 × 10^8 cells/ml.

Irradiation

The irradiation source was a 2.5-kW mercury-xenon lamp (929B; Canrad-Hanovia Inc., Newark, NJ) in combination with a predispersion prism (Schoeffel Instrument Division of Kratos, Westwood, NJ) and a high intensity grating monochromator (Schoeffel model GMA 252-10). The dose rate was measured using a radiometer (Kettering model 65A; Yellow Springs Instruments Co., Yellow Springs, OH). A Corning 0-52 filter (Corning Medical and Scientific, Corning Glass Works, Medfield, MA) with a sharp cut-off at 350 nm was used in conjunction with 6-mm entrance and exit slits, resulting in a half band width of 9.6 nm for 365-nm irradiations. The dose rate was in the range of 600–700 Wm^{-2}. Samples were irradiated with air stirring in a jacketed Pyrex cuvette at 0°C. In experiments in which samples were taken over a range of doses, all samples were held on ice until the end of the irradiation.

Postirradiation Incubation

Unirradiated control and irradiated samples in M9 buffer were diluted with an equal volume of two times tryptone broth (6 g nutrient broth, 10 g tryptone, 5 g NaCl, H_2O to 1 liter) before incubation at 30°C for various times to allow for repair before viability and break determinations. Shorter time points were held on ice until completion of the longest incubation time for each experiment.

Viability Determination

Either before or after postirradiation incubation, cells were diluted in M9 buffer on ice and plated in triplicate on tryptone plates (12), which were then incubated for 48 h at 37°C before counting. All procedures were conducted under yellow light to prevent photoreactivation. The number of lethal events (h) was calculated from the equation $N/N_o = e^{-h}$, where N/No is the fraction of the initial population surviving and e is the base of natural logarithms.

Break Determination

A modification of the McGrath and Williams (13) alkaline sucrose gradient technique was used to estimate the sizes of single-stranded DNA before and after postirradiation incubation. Samples (50 μl) were layered onto 4.8-ml alkaline sucrose gradients (5–20% sucrose, 0.7 M NaCl, 0.3 M NaOH, 0.01 M EDTA, pH 12.2) that were capped with 0.2 ml of lysing solution (0.5 M NaOH, 0.05% SDS, 0.1 ml mM EDTA). The gradients were allowed to stand at room temperature for 30 min before centrifugation in a SW 50.1 rotor (Beckman Instruments, Inc. Palo Alto, CA) at 25,000 rpm for 2 h at 20°C. The gradients were fractionated and analysed for radioactivity, and the number average molecular weights were calculated according to the method described by Ley (14). The number of single-strand breaks per *E. coli* genome (2.5 × 10^9 daltons) were calculated as described by Tyrrell et al. (2). All break results

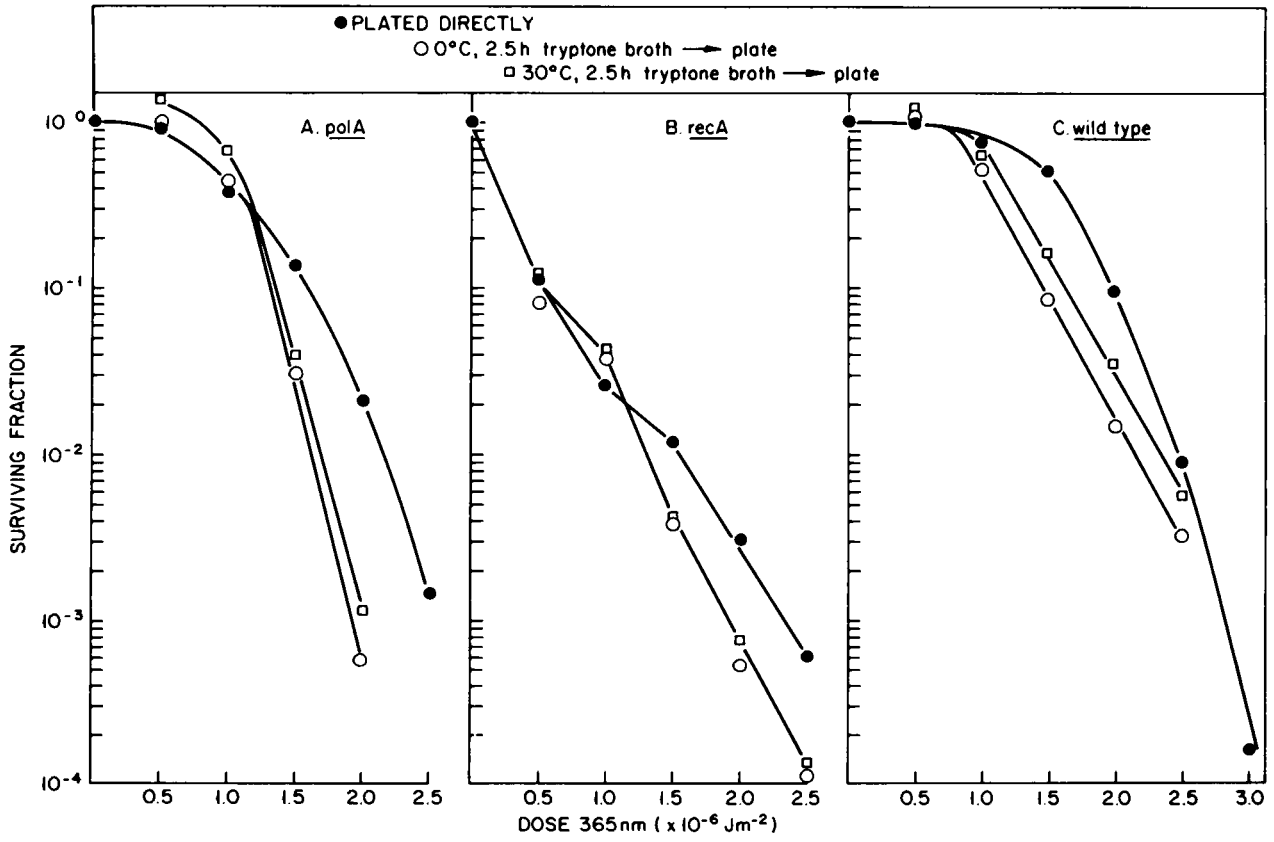

FIGURE 1 Viability of strains after 365-nm irradiation. (*A*) p3478, *polA;* (*B*) MM450, *recA;* and (*C*) W3110, wild type. Cells were irradiated at 0°C in M9 minimal buffer and given one of the following treatments: plated directly on tryptone plates (●); diluted with an equal volume ot two times tryptone broth, left on ice for 2.5 h and plated on tryptone plates (○); or diluted with an equal volume of two times tryptone broth, incubated at 30°C for 2.5 h and plated on tryptone plates (■).

presented are the average of at least three separate determinations. DNA base damage, generating alkali-labile bonds, as well as directly induced strand breaks, including both single-strand and double-strand breaks, are measured by alkaline sucrose gradients, but for simplicity we will refer to them collectively as breaks.

RESULTS

Viability Studies

The lethal action of 365-nm radiation on a repair-proficient strain and two mutants differing in their repair capacities are presented in Fig. 1 *A–C*. While the survival curve for the *recA* strain shows no lag or shoulder in inactivation and is the most sensitive, the wild-type and *polA* strains both contain a shoulder that most probably indicates an increased repair capacity at the lower doses.

One objective of this study was to compare the number of biological lethal events (viability) with the number of strand breaks (physical lesions) so that conditions had to be provided for biological measurements as close as possible to those used for physical measurements. Since the final biological measurement involves incubation for an extended period in a nutrient agar medium, we are limited to modifying the conditions in the immediate postirradiation period. Irradiated cells were either plated immediately or diluted with an equal volume of two times tryptone broth and divided into two parts. One of these parts was then incubated at 30°C to allow for break repair while the other was held on ice as a control before plating. The data illustrated in Fig. 1 show that all the strains held on ice for 2.5 h after irradiation at doses $>1 \times 10^6$ Jm^{-2} decreases in viability. Cells incubated at 30°C in tryptone broth before plating also showed decreased survival as compared to cells plated on tryptone plates directly after irradiation at doses $>1 \times 10^6$ Jm^{-2}, but the survival was slightly higher than when held on ice. The only increase in survival was observed for the *polA* strain, irradiated at low doses and incubated at 30°C in tryptone broth for 2.5 h before plating (Fig. 1 *A*). Although a near-UV radiation–induced growth delay would be expected (15) even at the lowest dose of 0.5×10^6 Jm^{-2}, this slight increase in survival could be due to a small amount of growth, equivalent to 0.62 of a doubling time at most.

Break Induction

The 365-nm radiation induction of breaks for the repair-proficient strain and four mutant strains is shown in Fig. 2. Breaks were induced linearly with dose in the *polA* and *polA uvrA* strains; however, the *recA*, *uvrA*, and wild-type strains all showed in a lag in break induction followed by a linear induction at higher doses. The introduction of a *uvrA* mutation into either the wild-type or *polA* strains did not affect the break yield. However, the large differences in the break induction curves for the other strains with different repair capacities (Fig. 2) could be an indication that induction and repair of breaks are occurring simultaneously in M9 buffer at 0°C during the irradiation treatment. Leaving the irradiated cells on ice in M9 buffer for up to 24 h did not lead to further repair of breaks in any of the strains.

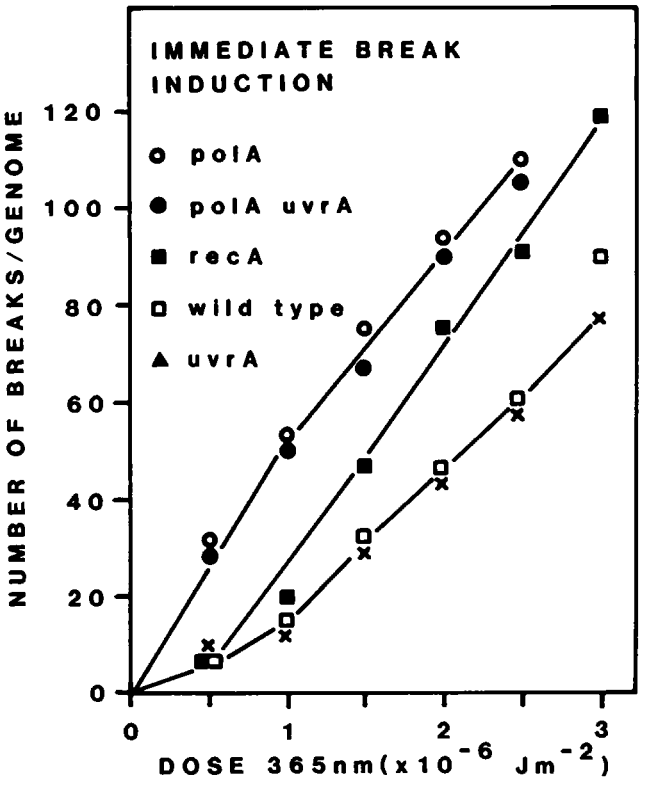

FIGURE 2 Immediate strand break induction. Cells were irradiated (365 nm) at 0°C in M9 minimal buffer to the stated dose and layered into alkaline sucrose gradients that were centrifuged at 25,000 rpm for 2 h at 20°C in order to determine the number of strand breaks per genome. The strains used were the following: p3478, *polA* (○); JG136, *polA uvrA* (●); MM450, *recA* (■); W3110, wild type (□); and TN207, *uvrA* (x).

Table I shows a comparison of the immediate break

TABLE I
COMPARISON OF 365-nm RADIATION STRAND-BREAK INDUCTION RATES FOR E. COLI K-12 STRAINS

Strain	Single-strand breaks/genome/Jm^{-2} $\times 10^{-5}$*
W3110 (wild type)	3.2 ± 0.8‡
	2.5§
	3.5‖
P3478 (*polA*)	4.0 ± 0.6‡
	7.5§
JG136 (*polA uvrA*)	3.7 ± 0.5‡
	4.7§
TN207 (*uvrA*)	2.8 ± 0.5‡
MM450 (*recA*)	4.6 ± 0.6‡

*Single-strand break per 2.5×10^9 daltons/Jm^{-2} ± 95% confidence interval.
‡Values determined in present work.
§Values as given in the work of Ley et al. (7).
‖Values as given in the work of Yoakum (16).

induction rates measured in this study with those determined by other workers. The value from our laboratory presented for each strain is the slope from the pooled data of three to four separate experiments (15–20 points) obtained by a least squares linear regression analysis. It should be noted that the value given for the *polA uvrA* strain from Ley et al. (7) is our estimate from their break yield at a single dose and assuming a linear induction with dose. Our results are in reasonable agreement with those obtained by other laboratories, except that we obtain a rather lower value for the *polA* strain.

Repair of 365-nm Radiation–induced Breaks

Ley et al. (7) have shown that postirradiation incubation in M9 buffer for 10 min at 30°C results in rejoining of breaks in the wild-type (81%) and *uvrA* (65%) strains but not in the *polA* strain. However, to compare the viability studies with the physical break studies, we wanted to find the total final repair levels for each strain. Thus, after irradiation the cells were diluted into complex tryptone broth medium and incubated at 30°C for 2.5 h. As demonstrated in Fig. 3, all strains repaired breaks with similar efficiency at doses $<1 \times 10^6$ Jm^{-2}, but the *polA* strain was markedly deficient in repair capacity at high doses. The *recA* and wild-type strains repair breaks with the same efficiency over the entire dose range, resulting in break yields after repair of 7.6 and 7.8 $\times$ 10^{-6} breaks/genome/Jm^{-2}, respectively (Fig. 3).

Also shown in Fig. 3 is the effect of holding irradiated cells on ice in the presence of tryptone broth for 2.5 h. At doses $>1 \times 10^6$ Jm^{-2}, there was a considerable increase in the number of breaks during the postirradiation treatment in the *polA* strain. Since repair experiments in which the *polA uvrA* strain was given initial doses of 1.5 or 2 $\times$ 10^6 Jm^{-2} gave results similar to those for the *polA* strain both at 0°C and 30°C (not shown), the *uvrAB* endonuclease appears not to be involved in this destructive process.

Comparison of the Number of 365-nm Radiation–induced Lethal Lesions and Strand Breaks Remaining after Repair

Several authors have suggested the single-strand break as a candidate for the critical lesion responsible for bacterial

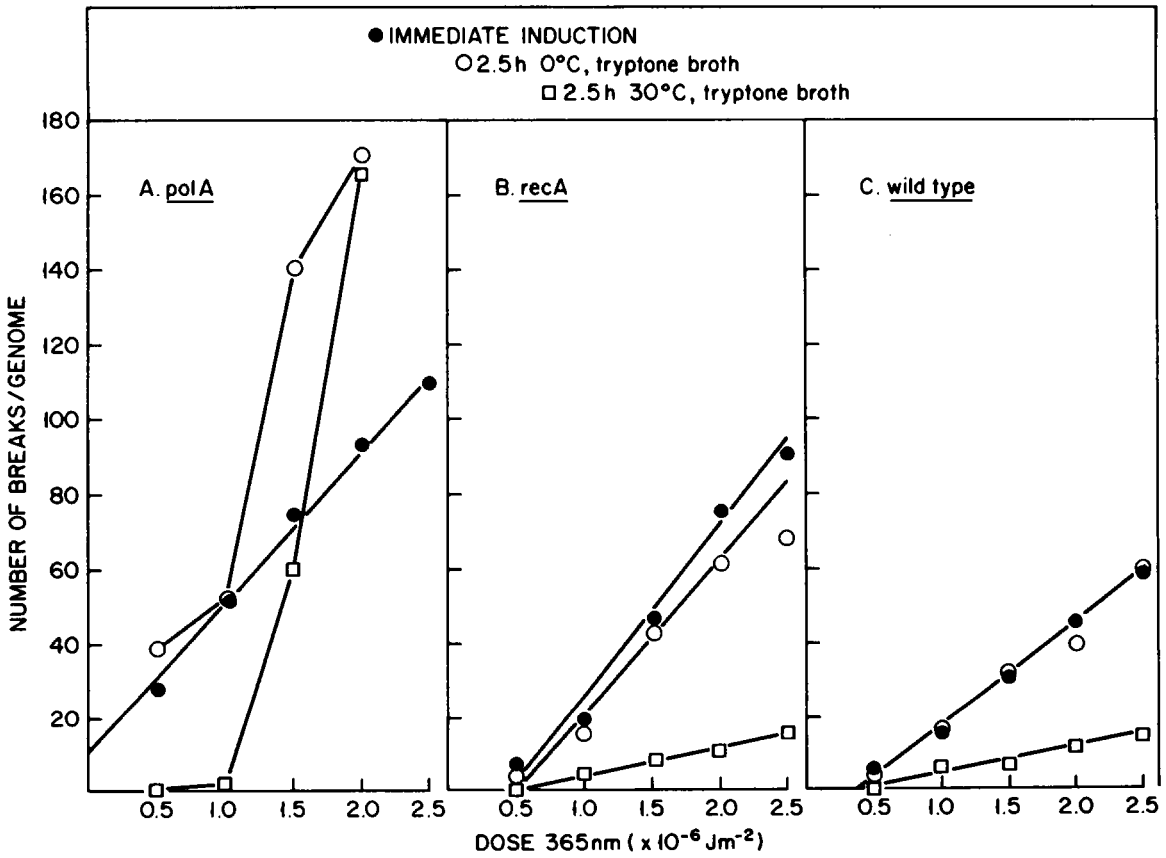

FIGURE 3 Effect of tryptone broth incubation on the number of strand breaks. (*A*) p3478, *polA;* (*B*) MM450, *recA;* and (*C*) W3110, wild type. Cells were irradiated (365 nm) at 0°C in M9 minimal buffer to the stated dose and given one of the following treatments: loaded directly onto alkaline sucrose gradients (●); diluted with an equal volume of two times tryptone broth and left on ice for 2.5 h before loading gradients (○); or diluted with an equal volume of two times tryptone broth and incubated for 2.5 h at 30°C before loading gradients (□).

inactivation by 365-nm radiation (2, 7, 17). A correlation between the number of single-strand breaks and the number of lethal events (viability) remaining after repair would support this idea.

So that a quantitative comparison of the number of single-strand breaks and lethal events could be made, experiments were carried out in which all physical measurements (gradients) and biological measurements (viability) were performed simultaneously on the same cell culture. Fig. 4 shows the data for both the number of strand breaks and number of lethal events remaining after repair. Although there was some correspondence between the threshold dose at which both strand breaks and lethal events began to appear in the *polA* and wild-type strains, there were many times more breaks remaining than lethal events remaining in the *polA* strain at equivalent doses. The discrepancy is much less in the wild-type strain and is only a factor of two or three at the highest doses employed. While the *recA* strain shows a threshold for strand break induction, there is no such threshold for the lethal event induction (Fig. 1 *B*). When net break repair did occur, the kinetics were rapid within the first 10 min in all strains, but the *polA* strain contained a second slower element out to 2.5 h (not shown). Thus, holding cells in complete medium for >2.5 h would probably not result in a further decrease in the number of breaks as visualized by alkaline sucrose gradients.

DISCUSSION

Radiation at 365 nm induces several different types of lesions (1), among which the predominant is the single strand break. We have studied repair of this lesion since it is dependent upon oxygen for its induction (2) and so may be responsible for the oxygen enhancement of lethality by near-UV radiations (1). We observed a marked difference in the levels of breaks induced in wild-type strains and in those mutant at the *polA* or *recA* loci (Fig. 2). Since these gene products are both known to be involved in strand break repair (6,18), we interpret this data to mean that repair of a fraction of these lesions (single-strand breaks, alkali-labile lesions, and double-strand breaks) was taking place during the 365-nm irradiation period. An alternative explanation is that additional breaks are introduced in the *polA* and *recA* strains at sites of lesions during the 0°C holding period and that this is prevented by the presence of the *polA* and *recA* proteins. However, we consider this unlikely, since even in the *polA* strains (Table I) the rate of break induction is 50% slower than the rate originally observed in extracted bacteriophage DNA (2). There is clearly no endonucleolytic incision (19) occurring at

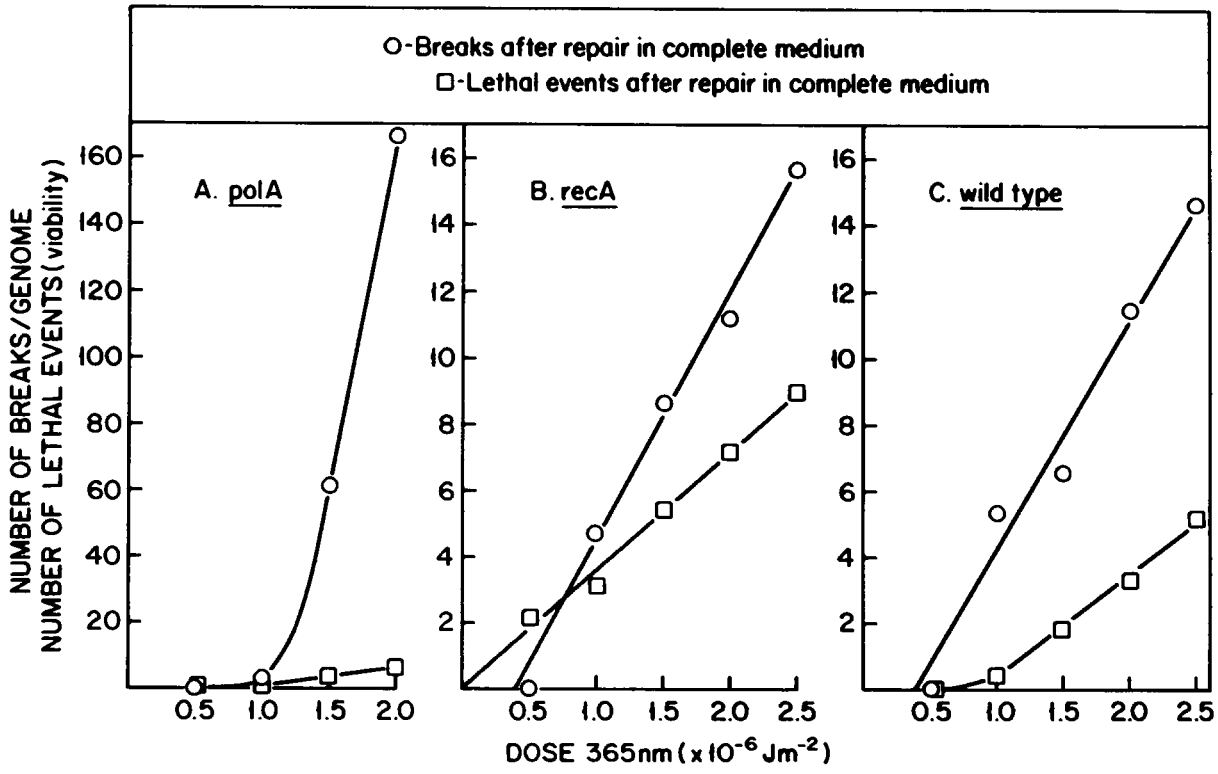

FIGURE 4 Comparison of number of strand breaks and number of lethal events remaining after postirradiation incubation (30°C) repair. (*A*) p3478, *polA*; (*B*) MM450, *recA*; and (*C*) W3110, wild type. Cells were irradiated (365 nm) at 0°C in M9 minimal buffer to the stated dose and diluted with an equal volume of two times tryptone broth. After allowing repair for 2.5 h at 30°C, the number of strand breaks (O) was determined by alkaline sucrose gradients and the number of lethal events (□) was calculated from the viability as described in the Methods.

pyrimidine dimer sites, since the *uvrA* and wild-type break induction curves are superimposable and the *polA uvrA* and *polA* curves are also superimposable with each other under the 0°C M9 buffer irradiation conditions. These results indicate that the *uvrA* coded endonuclease is neither involved in break repair nor inducing single strand breaks in the DNA as a result of nicking during repair of other lesions.

Assuming that the strain differences really do indicate repair, then the existence of two repair systems that operate in M9 buffer at 0°C may be inferred by comparison of the 365-nm irradiation break induction curves (Fig. 2). The difference between the wild-type and *recA* induction curves is attributed to the *recA*-dependent repair process, whereas the difference between the *recA* and *polA* strains is attributed to the additional repair capacity of the *polA*-dependent process.

The results in Fig. 3 are clear evidence that additional repair process(es) dependent upon complete growth medium can act on 365-nm radiation–induced strand breaks. Since such repair can occur in all strains at doses up to and including 1×10^6 Jm^{-2}, we suggest that either a single repair process is controlled by an additional unknown gene product or that both *polA* and *recA* gene products are involved in two independent medium-dependent repair pathways that can substitute for each other. The rapid loss in efficiency of repair in the *polA* mutant at doses $>1 \times 10^6$ Jm^{-2} could be due to saturation of repair or to inhibition of repair by the near-UV radiation itself (cf. reference 20).

The fast *polA* repair process we observed at 0°C (Fig. 2) appears to be analogous to the fast *polA* dependent repair of x-ray–induced breaks (6, 18) which even at 0°C in minimal medium removes half the breaks in 10 min (6). Since our total irradiation times are >60 min, this does not imply the operation of an ultra-fast strand-break repair process for which no evidence could be found using fast (<180 ms) alkaline lysis techniques (18). The *recA*-dependent repair process, which occurs at 0°C in minimal buffer and which we observed for the repair of 365-nm irradiation–induced breaks has no analogy with any other system seen in *E. coli*, and, under the conditions in which it occurs, is clearly constitutive and does not require DNA synthesis. The repair seen in the *polA* strain at 30°C (Fig. 3 *A*) would appear to represent a third repair pathway that could well be related to the *recA*-dependent repair of x-ray damage (8). Finally, the medium-dependent repair of breaks observed in the *recA* strain (Fig. 3 *B*) could represent an additional uncharacterized repair pathway.

An examination of the relationship between breaks and lethal events after repair as illustrated in Fig. 4 demonstrates that the threshold doses for both parameters are similar for the *polA* and wild-type strains. The discrepancy observed in the *recA* strain is consistent with the observation that a fraction of the lethal damage is photoreactivable in a *recA* mutant at low doses (5) so that in this dose range pyrimidine dimers must be involved in cell death. However, it is evident that at the upper end of the dose range employed, a lethal event corresponds to one to two (*recA* strain [Fig. 4 *B*]) or two to three (wild-type strain [Fig. 4C]) unrepaired strand breaks per genome. It is therefore noteworthy that after irradiation at 254 nm, in which the pyrimidine dimer has been clearly implicated as the major lethal lesion in DNA, a lethal hit corresponds to one to two pyrimidine dimers per genome in repair-deficient yeast, bacteria, and bacteriophage (see reference 21). By analogy, the data in Fig. 4 is at least consistent with a major role for the strand break in lethality after irradiation at 365 nm in $polA^+$ strains. The large discrepancy between breaks and lethal events observed in the *polA* strain at doses $>10^6$ Jm^{-2} (Fig. 4 *A*) could reflect the apparent inhibition of repair by higher doses of 365-nm radiation (20), which could then lead to the accumulation of strand breaks during the 2.5-h repair period in liquid medium (Fig. 3 *A*). This repair inhibition could be reversible during the longer incubation period on solid medium plates, which are required for the viability determination. This could then lead to a discrepancy between the measured levels of breaks and the lethal events estimated from the viability. An alternative possibility is that the *polA* strain may only be able to slowly repair the alkali-labile base damage that constitutes 20% of the initial damage detected as breaks in alkaline sucrose gradients (7, 22).

In summary, we have observed that in addition to the *polA*-dependent pathway for rejoining of 365-nm irradiation–induced strand breaks previously reported (7), several additional pathways also operate. The fast-acting *polA*-dependent pathway appears analogous to the fast repair of ionizing radiation-induced breaks that occurs in buffer even at 0°C, while a second pathway resembles the slower *recA*-controlled medium-dependent repair of ionizing radiation damage. An additional fast *recA*-dependent rejoining occurs in buffer at 0°C during the irradiation period and has no analogy with break repair systems previously observed. Finally, there is probably an additional slow medium-dependent pathway dependent upon the *polA* gene product. Between one and three strand breaks remain unrepaired per lethal event in the wild-type and *recA* strains, so that these lesions are a firm candidate for the major lethal lesion induced in pol^+ strains by radiation at 365 nm.

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