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Role of Deoxyribonucleic Acid Polymerase III in the Repair of Single-Strand Breaks Produced in *Escherichia coli* Deoxyribonucleic Acid by Gamma Radiation

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Cell survival, deoxyribonucleic acid (DNA) degradation, and the repair of DNA single-strand breaks were measured for *Escherichia coli* K-12 *pol*$^+$, *polA1*, *polC1026*(ts), and *polA1 polC1026*(ts) cells after ^{137}Cs γ irradiation. The results indicate that DNA polymerase III is required for growth medium-dependent (type III) repair in *polA*$^+$ or *polA* cells and is necessary for growth medium-independent (type II) repair in *polA* cells. In *pol*$^+$ or *polC* cells, DNA polymerase I performs type II repair efficiently. The relative deficiencies of each of these strains in DNA repair generally correlate with their relative sensitivities to cell killing and with the extent of DNA degradation observed.

Ionizing radiation introduces strand breaks and other radiochemical lesions into cellular deoxyribonucleic acid (DNA) (25). The ability of the cell to repair these radiation products is closely correlated with cell survival, since radiosensitive *Escherichia coli* strains such as the *recA*, *recB*, *lexA*, and *polA* mutants are partially deficient in DNA strand break repair (25).

Studies with the *polA* mutant, which has greatly reduced DNA polymerase I activity (5), have indicated that these cells retain some ability to repair radiation-induced DNA strand breaks (30). The residual DNA polymerase activity detectable in extracts of *polA* cells has been resolved into two additional enzymes: DNA polymerase II (9, 16) and DNA polymerase III (10, 11). It seemed likely that the residual repair of single-strand breaks that occurs in a strain lacking DNA polymerase I may be due to the activity of one or both of these polymerizing enzymes.

Youngs and Smith (32) have shown that a mutant deficient in DNA polymerase II has about the same X-ray sensitivity as the wild-type strain, suggesting that DNA polymerase II plays no major role in the repair of ionizing radiation damage. Thus, by elimination, DNA polymerase III seemed likely to be the enzyme responsible for residual repair activity in *polA* cells. The available data support this idea. Tomilin (22) has shown that the *polA polC* strain (the *polC* mutation results in the production of a thermolabile DNA polymerase III molecule [7, 27]) is more sensitive to γ rays than is the *polA* strain. It has also been demonstrated that DNA polymerase III is involved in the excision (15, 21, 31) and post-replicational repair processes (18, 21, 23) occurring after ultraviolet irradiation. The observation by Livingston and Richardson (14) that DNA polymerase III has a 5'-3' nuclease activity capable of excising dimers from incised, ultraviolet-irradiated DNA also supports involvement of this enzyme in excision repair. In addition, DNA polymerase III appears to be required for normal DNA replication (7, 17).

We have examined the role of DNA polymerase III in the repair of DNA single-strand breaks in relation to cell survival and DNA degradation after γ irradiation and find that DNA polymerase III plays an important role in the repair of DNA damage produced by ionizing radiation, particularly in cells lacking DNA polymerase I.

MATERIALS AND METHODS

Bacterial strains. The following closely related strains of *E. coli* K-12 were used: (BT1026) F$^+$ *polC1026*(ts) *polA1 thy str endA tsx* was initially obtained from K. Geider; (DY138) F$^+$ *polC1026*(ts) *thy str metE endA tsx*, (DY144) F$^+$ *polA1 tonA thy str endA tsx*, and (DY147) F$^+$ *tonA thy str metE endA tsx* were derived from BT1026 by P1-mediated transductions.

Growth conditions. Cells were grown at 30°C in minimal medium (MM, reference 6) supplemented with thiamine hydrochloride (0.5 μg/ml), thymine (10 μg/ml for overnight cultures or 2 μg/ml for exponentially growing cells), and L-methionine (10^{-3} M) when required.

Survival after γ irradiation. Overnight cultures grown in MM medium were diluted 1:50 into fresh

medium and grown for about three generations to midlog phase (1×10^8 to 2×10^8 cells/ml). Cells were collected on membrane filters (0.45-μm pore size; Millipore Corp.) and suspended in DTM buffer (MM without glucose or organic supplements) at a cell density of about 2×10^8 cells/ml. The cell suspensions were incubated at 30 or 42°C in a shaking water bath for 30 min and then equilibrated with air by bubbling for 5 min in a water-jacketed glass vessel before and during γ irradiation with an 8,000-Ci ^{137}Cs source (as previously described [2]). Samples were maintained at room temperature (~23°C) or 42°C during irradiation as desired. After irradiation, samples were added to an equal volume of DTM buffer or MM medium (containing twice the normal concentrations of glucose and supplements) and incubated for 80 min either at 30 or 42°C. For survival measurements the cells were diluted in phosphate buffer (11.7 g of Na_2HPO_4 and 7.1 g of KH_2PO_4 per liter, pH 7.0) and plated on MM agar (MM medium solidified with 1.6% Noble agar) supplemented with 10 μg of thymine per ml, 0.5 μg of thiamine hydrochloride per ml, and 10^{-3} M L-methionine. The plates were incubated 48 h at 30°C to allow for colony formation.

Measurement of DNA single-strand breaks. Gradient techniques described by D. A. Youngs and K. C. Smith (Photochem. Photobiol., in press) were adopted for these experiments. Cells were grown for about three generations in the presence of 100 μCi of [*methyl*-^{3}H]thymine (New England Nuclear Corp., 16 Ci/mmol) per ml to label their DNA. Deoxyguanosine was present at a concentration of 100 μg/ml during labeling since the thymine requirement does not seem to be absolute at 30°C (27). The labeled cells were preincubated, irradiated, and postincubated as described in the previous section. Approximately 10^6 cells (in 0.05 ml) were layered onto a 0.1-ml cap of 0.5% Sarkosyl (Geigy NL30), 0.01 M ethylenediaminetetraacetic acid, and 0.5 N NaOH that was layered on top of a 4.8-ml, 5 to 20% (wt/vol) linear alkaline sucrose gradient just before the addition of the cells. After at least 40 min at room temperature, the gradients were centrifuged at 20°C for 16 h at speeds ranging from 10,000 rpm (for samples from unirradiated cells) to 14,000 rpm (for samples of small DNA pieces from irradiated cells) in an SW50.1 rotor in a Beckman model L2 or L2-65B ultracentrifuge. This variation from the general technique described previously (26) was adopted so as to avoid the possibility of a speed-dependent decrease in the sedimentation rate of high-molecular-weight DNA samples (13).^{14}C-labeled DNA from bacteriophage T2 was used in most centrifugation runs as a molecular weight marker. After centrifugation, the bottom of each tube was pierced and the fractions were collected onto Whatman no. 17 filter paper strips that were dried, washed in 5% trichloroacetic acid-95% ethanol-acetone, dried, and counted in a liquid scintillation spectrometer. The number average molecular weight was calculated from the DNA sedimentation profiles as previously described (Youngs and Smith, in press).

DNA degradation. For the measurement of DNA degradation after γ irradiation, the strains were labeled with 25 μCi of [^{3}H]thymine per ml, irradiated at a density of 1×10^8 to 2×10^8 cells/ml, and incubated as described above. After postirradiation incubation, triplicate, 0.1-ml samples were pipetted onto filter paper disks that had been previously soaked in 10% trichloroacetic acid and dried. The technique for processing the disks has been described (24).

RESULTS

DNA strand breakage. The DNA strand breakage results are shown in Fig. 1, where the reciprocal of the number average molecular weight is plotted as a function of the γ-ray dose. The slopes of the regressed lines (expressed as the number of breaks per 10^8 daltons per krad) are listed in Table 1.

Figure 1A shows the results for the wild-type, *polC*, *polA*, and *polA polC* strains incubated in DTM buffer at 42°C before and after γ irradiation. The slopes of the regressed lines for the *polC* and *pol*$^+$ strains are not significantly different, but they are less than those obtained for the *polA* and *polA polC* strains by factors of about 10 and 13, respectively. These data also show that the *polA polC* double mutant is more deficient in the repair of DNA single-strand breaks at 42°C than is its isogenic *polA* partner. Approximately 1.3 times more strand breaks remained unrepaired in the *polA polC* strain than in the *polA* strain (Table 1).

When the irradiated cells were incubated in DTM buffer at 30°C instead of at 42°C (Fig. 1B), an increased amount of repair of breaks was observed in all the strains. The decrease in the DNA strand breakage values (Table 1) was the greatest for *polA* and *polA polC* strains (~four-fold), but was also noticeable with the *polC* and *pol*$^+$ strains (1.5-fold). The yield of unrepaired strand breaks in *polC* cells was again quite similar to that observed for wild-type cells incubated in DTM buffer under the same conditions.

However, the results shown in Fig. 1C indicate that when the irradiated cells were held at 42°C in MM medium instead of DTM buffer, the extent of repair was slightly increased in the wild-type strain and slightly reduced in the *polC* strain. The most significant effects were observed with the *polA* and *polA polC* strains. The *polA* strain showed about 5% fewer unrepaired single-strand breaks when incubated in MM medium than in DTM buffer at 42°C. The *polA polC* double mutant, however, showed about 2.4 times more unrepaired single-strand breaks after reincubation in MM medium than in DTM buffer.

The extent of DNA strand break repair for

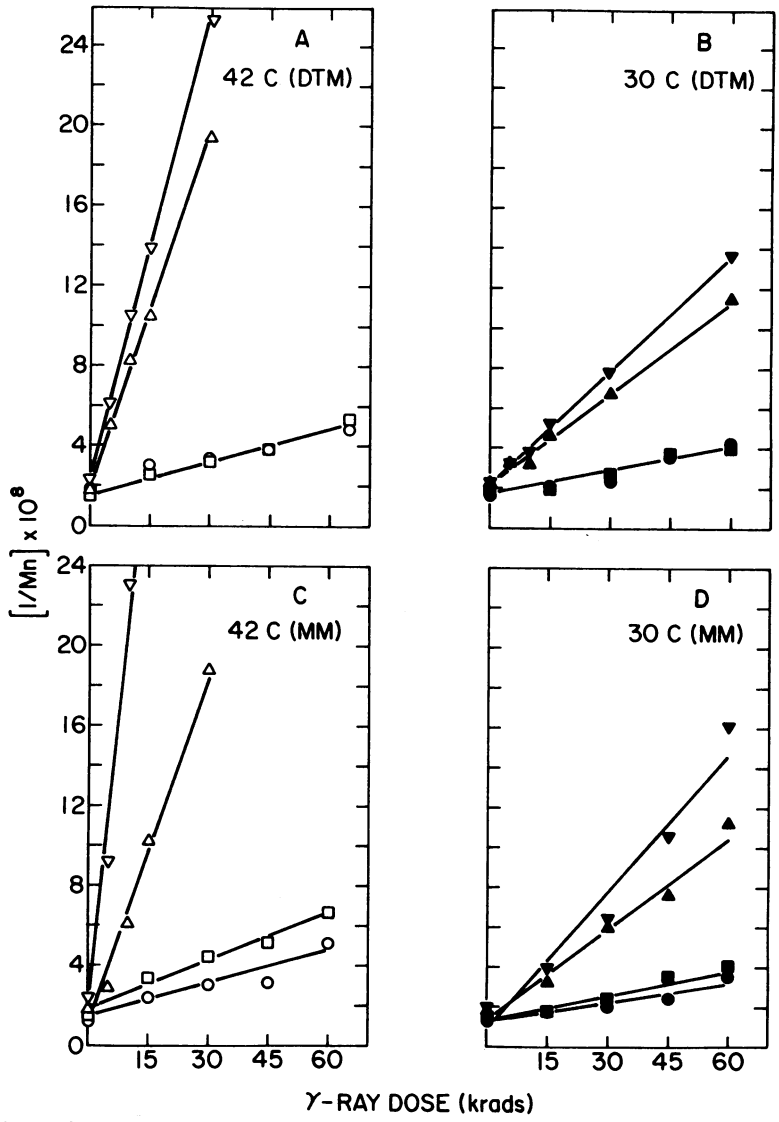

FIG. 1. *DNA single-strand breakage in E. coli K-12 cells after γ irradiation. The number average molecular weights were calculated from DNA profiles from alkaline sucrose gradients. The pol⁺ (○, ●), polC (□, ■), polA (△, ▲), and polA polC (▽, ▼) cells were preincubated at 42°C (open symbols) or 30°C (closed symbols) for 30 min, irradiated, and then incubated for an additional 80 min in DTM buffer at 42°C (A) or 30°C (B) or in MM medium at 42°C (C) or 30°C (D). The samples were aerated vigorously for 5 min before and during γ irradiation, which was carried out either at 42°C or room temperature (~23°C). The lines were fitted to the data by linear regression analysis. Each point represents the average of at least two independent experiments. The strain numbers are given in Material and Methods. One data point for the polA polC strain is not shown in Fig. 1C: that value is 29.7 at 15 krads.*

each of the strains at 30°C was essentially the same whether the cells were incubated in MM medium or DTM buffer (Table 1). However, the wild-type strain did show slightly more repair than the *polC* strain at 30°C in MM medium, but not in DTM buffer (Fig. 1D).

Survival curves. To assess the possible contributions of unrepaired DNA single-strand breaks toward the sensitivity of the strains to γ rays, data were obtained for the survival of the four isogenic strains irradiated in equilibrium with air under the same conditions used for the sedimentation experiments (Fig. 2). The D_0 values (dose required to reduce survival by a factor

TABLE 1. *Cell survival and DNA single-strand break repair after γ irradiation of E. coli K-12 cells*

Strain	Single-strand breaks[a] (per 10^8 daltons per krad)				D_0 values[b] (krads)			
	DTM		MM		DTM		MM	
	42°C	30°C	42°C	30°C	42°C	30°C	42°C	30°C
polA polC	0.78 ± 0.05[c]	0.19 ± 0.02	1.91 ± 0.05	0.23 ± 0.01	1.06 ± 0.01	1.32 ± 0.01	0.90 ± 0.03	1.36 ± 0.03
polA	0.60 ± 0.04	0.15 ± 0.02	0.57 ± 0.04	0.15 ± 0.02	1.32 ± 0.01	1.55 ± 0.01	1.24 ± 0.03	1.77 ± 0.02
polC	0.06 ± 0.01	0.04 ± 0.00	0.08 ± 0.01	0.04 ± 0.01	3.95 ± 0.00	3.62 ± 0.01	2.29 ± 0.02	3.95 ± 0.00
pol$^+$	0.06 ± 0.01	0.04 ± 0.01	0.05 ± 0.01	0.03 ± 0.01	3.62 ± 0.01	3.34 ± 0.01	3.95 ± 0.00	3.62 ± 0.00

[a] Calculated from the data in Fig. 1.
[b] Calculated from the data in Fig. 2: the D_0 value is the dose required to reduce survival by a factor of *e* on the exponential part of the survival curve.
[c] Standard deviation of the slopes.

of *e* on the exponential part of the survival curve) obtained from these survival curves are listed in Table 1.

The *polA* and *polA polC* strains were ~1.2 times more sensitive to killing by γ rays after incubation in DTM buffer at 42°C than at 30°C. However, incubation at 42°C in DTM buffer increased slightly the survival of the *polC* and *pol*$^+$ strains relative to the survival observed at 30°C (Table 1).

When the cells were incubated in MM medium, instead of buffer, the *polA* and *polA polC* strains were, respectively, about 1.3 and 1.5 times more sensitive to cell killing by γ rays at 42°C than at 30°C. The *polC* strain was also more sensitive to killing by γ rays after incubation in MM medium at 42°C than at 30°C. This was unlike the wild-type strain, which was more resistant after treatment at the higher temperature. Thus, under all of the incubation conditions used, the *polA* strain was more sensitive to killing by γ rays than either *polC* or *pol*$^+$, but not as sensitive as the *polA polC* strain. The *polC* strain was more sensitive than *pol*$^+$ when incubated in MM medium at 42°C, but the two strains showed similar survival under the other conditions (Fig. 2).

DNA degradation. DNA degradation during post-irradiation incubation in either DTM buffer or MM medium, at both permissive and nonpermissive temperatures, was examined in these strains. The *polA* strain generally degraded more of its DNA than did the *polC* and wild-type strains, although less DNA was degraded than in the *polA polC* strains under the same conditions (Fig. 3).

When irradiated cells were held for 80 min in DTM buffer at 30°C (Fig. 3B), DNA degradation increased with dose, but to a smaller extent than at 42°C (Fig. 3A); up to 20% more labeled material remained precipitable after incubation at 30°C than at 42°C. At either temperature, the *polA polC* strain degraded its DNA more extensively than did the *polA* strain, and both strains exhibited more degradation than did the *polC* and wild-type strains.

Similar results were obtained when the cells were incubated at both temperatures in MM medium instead of buffer. The only differences were the greater extent of DNA degradation in growth medium and the fact that the *polC* strain degraded slightly more of its DNA than did the wild-type strain. In fact, when *polC* cells were incubated at 30°C in MM medium, they degraded their DNA as extensively as did *polA* cells under the same conditions (Fig. 3D).

To complete these experiments, the extent of DNA degradation was followed as a function of time (up to 3 h) as the irradiated (30 krads) cells were incubated in DTM buffer or MM medium at 42 and 30°C (data not shown). The extent of DNA degradation in γ-irradiated cells increased with incubation time, was both slower and less extensive at 30°C than at 42°C, and was also less extensive in DTM buffer than in MM medium. The relative rates of DNA degradation in each of the strains were analogous to the relative differences in the final extent of degradation described above for Fig. 3.

DISCUSSION

The number of DNA single-strand breaks remaining after completion of growth medium-independent (type II) repair at 42°C was approximately the same for the *polC* and wild-type strains (Fig. 1A and Table 1). Thus, DNA polymerase III, which is inactivated in *polC* strains under these conditions (7, 27), is not required for type II repair in wild-type cells. The much greater yield of unrepaired DNA single-strand breaks in the *polA* strain indicates that DNA polymerase I is a key enzyme in the growth medium-independent process. However, the limited ability of the DNA polymerase I-deficient mutant to repair DNA single-strand breaks after γ irradiation is further decreased when *polC* is placed in combination with this mutation, indicating that the residual growth medium-independent repair present in *polA* cells is dependent on DNA polymerase III activ-

ity. On the basis of these results, it appears that DNA polymerase III is needed for growth medium-independent repair when DNA polymerase I is absent.

However, the higher yield of unrepaired DNA single-strand breaks in *polA* cells, as compared to *polC* and *pol*$^+$ cells, indicates that DNA polymerase III is not as efficient as DNA polymerase I in completing type II repair of strand breaks. This could be related to the inability of the former enzyme to repair single-strand nicks in duplex DNA (12). For a nick to be repaired by DNA polymerase III, exonucleolytic action must first occur to create a small gap. This could result in the increased DNA degradation (Fig. 3), increased double-strand breakage (2), and decreased efficiency of strand break repair (Fig. 1) characteristic of *polA* cells.

The *polC* gene product appears to be essential for the growth medium-dependent (type III) repair of DNA single-strand breaks produced after γ irradiation. This is indicated by the observation that there appeared to be some additional repair of strand breaks in the DNA of *polA* and *pol*$^+$ strains, but not in *polC* and *polA polC* cells after post-irradiation incubation in MM medium rather than DTM buffer at 42°C (Fig. 1 and Table 1). In fact, the *polA polC* and *polC* strains showed increased DNA strand breakage in MM medium at 42°C as compared to DTM buffer. Thus, DNA polymerase III appears to be needed for growth medium-dependent repair whether DNA polymerase I is pres-

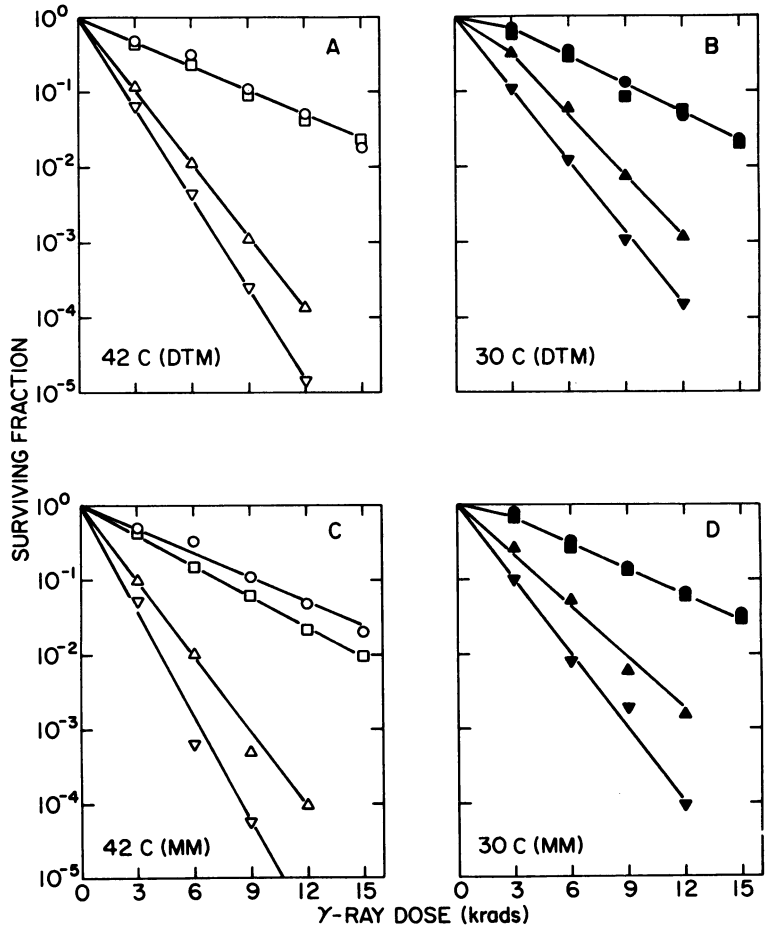

FIG. 2. *Survival of E. coli K-12 cells after γ irradiation. The experimental conditions and symbols are the same as shown in Fig. 1. Following post-irradiation incubation, the cells were diluted in phosphate buffer, plated on MM agar, and incubated for 48 h at 30°C. The lines were fitted to the data by linear regression analysis. Each survival curve represents the average of two independent experiments. One data point for the polA polC strain is not shown in Fig. 2C: that value is 1.4 × 10^{-6} at 12 krads.*

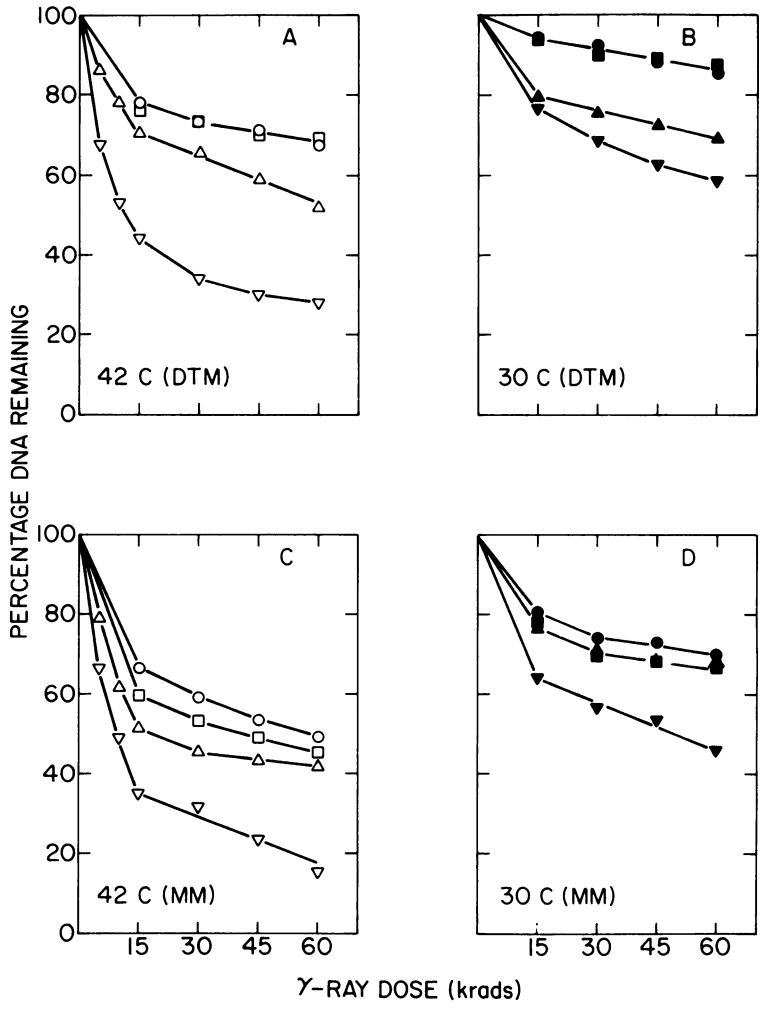

FIG. 3. *Kinetics of DNA degradation in E. coli K-12 cells after γ irradiation. The extent of DNA degradation was measured as the fraction of labeled material remaining trichloroacetic acid insoluble after various doses of γ rays and an 80 min post-irradiation incubation period. Each data point represents the average of three independent experiments. The experimental conditions and symbols are the same as in Fig. 1.*

ent or not. The requirement for DNA polymerase III in the growth medium-dependent repair process could be related to the known adenosine 5'-triphosphate requirement of DNA replication by certain DNA polymerase III-dependent systems (see reference 8).

We also noted a slight, but persistent, increase in the level of unrepaired DNA single-strand breaks in the *polA polC* strain, compared to the *polA* strain, after incubation at 30°C in either MM medium or DTM buffer (Fig. 1B and D). This observation correlates with the survival data shown in Fig. 2B and D and indicates that the *polC* gene product is partially deficient in repair capacity even at the permissive temperature.

A noteworthy correlation was found between the relative number of single-strand breaks remaining unrepaired after post-irradiation incubation at 42°C and the sensitivity of a strain to killing by γ irradiation. The survival data show that the *polA* strain is more sensitive to killing by γ rays than are the *polC* and *pol*$^+$ strains, which showed similar survival after incubation in buffer (Fig. 2A). The *polA polC* strain was even more sensitive than the *polA* strain, suggesting that the function of the *polC* gene product in the type II repair system, in the absence of DNA polymerase I, contributes to cell recovery. Support for the involvement of DNA polymerase III-dependent type III repair in cell recovery comes from the survival data shown in

Fig. 2C, where the cells were incubated in growth medium at 42°C after irradiation. In this case, the presence of the *polC* mutation in either the *polA* or wild-type background resulted in an increased sensitivity to γ rays. The reduced viability found with the *polA polC* double mutant after post-irradiation incubation at the restrictive temperature (references 22 and 25; Fig. 2) thus appears to correlate with the reduced type II (growth medium-independent) and type III (growth medium-dependent) repair of DNA single-strand breaks characteristic of this strain.

In addition, extensive DNA degradation has often been found to correlate with sensitivity to irradiation and the lack of repair of DNA single-strand breaks (29). In agreement with this idea, our results (Fig. 3) indicate that the extent of γ ray-induced DNA degradation generally correlates inversely with the radioresistance of the strain and its capacity to repair DNA single-strand breaks. For example, DNA degradation in the most sensitive and repair-deficient strain, *polA polC*, was always faster and more extensive than in any of the other strains studied, regardless of the post-irradiation incubation conditions. Thus, cell recovery, DNA strand break repair, and DNA degradation all appear to be interrelated under the present experimental conditions.

An interesting observation concerning the correlation of DNA strand breakage with cell survival is that more DNA strand breaks appear to remain after the completion of repair than would be required to account for cell killing, if each unrepaired single-strand break constituted a lethal event. The DNA lesion that has been most clearly correlated with cell killing after irradiation is the DNA double-strand break (1–3). It now appears that double-strand breaks may arise by at least two general modes: (i) initial radiochemical events and (ii) post-irradiation enzymatic processes, perhaps associated with excision repair (1, 2). It seems likely that the increase in unrepaired single-strand breakage and decreased cell survival, which we observed for the *polA* and *polA polC* strains, is at least partly due to an increase in the number of double-strand breaks present in the DNA. Bonura et al. (2) have, in fact, observed a greater yield of double-strand breaks in *polA* cells than in wild-type cells after γ irradiation under conditions that allowed enzymatic processes to occur. We have not yet determined the extent of double-strand breakage in *polA polC* cells after ionizing irradiation.

Although the *polA* strain is not known to contain any temperature-sensitive mutation, more radiation-induced DNA single-strand breakage was observed at 42°C than at 30°C. DNA single-strand breaks are produced in *E. coli* after heating at 52°C (4, 18, 28), and it has been suggested that such breaks may occur at lower physiological temperatures, but they are probably repaired with a high efficiency (28). Consequently, their presence would become apparent only if the repair system were inhibited or saturated, as is probably the case for γ-irradiated *polA* and *polA polC* cells. Thus, at 42°C the recovery systems presumably have to cope with both thermal and radiation damage. This may explain why the *polA* strain showed more DNA strand breakage at 42°C than at 30°C. The wild-type strain also showed slightly more DNA strand breakage and degradation at the higher temperature, suggesting that thermal lesions may be of some importance even in γ-irradiated wild-type cells incubated at 42°C.

The importance of DNA polymerase III in both replicative and repair processes seems well established. The present data indicate the involvement of this enzyme in both the growth medium-dependent (type III) and also the growth medium-independent (type II) repair processes that act after ionizing irradiation. In agreement with the suggestion that DNA polymerases I and III may substitute for each other in performing certain repair processes (19–21), we find that DNA polymerase III is required for type II repair only if DNA polymerase I is absent. However, the requirement of DNA polymerase III for type III repair seems to be independent of the status of DNA polymerase I. This involvement of DNA polymerase III in the repair of ionizing radiation-induced DNA single-strand breaks, as well as the two analogous branches of the excision repair process acting after ultraviolet irradiation (20, 31), supports the idea that the repair of DNA single-strand breaks produced after either type of radiation requires similar incubation conditions and gene products (20).

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