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Photoreactivation, Excision, and Strand-Rejoining Repair in R Factor-Containing Minicells of *Escherchia coli* K-12

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Chromosomeless "minicells" are formed by misplaced cell fissions near the polar extremities of an *Escherichia coli* K-12 mutant strain. Resistance (R)-factor deoxyribonucleic acid (DNA) can be introduced into minicells by segregation from an R^+ (R64-11) derivative of the original mutant. We have assessed the ability of R^+ minicells to correct defects produced in their plasmid DNA by ultraviolet (UV) and gamma radiations. Minicells harboring plasmid DNA, in comparison with their repair-proficient minicell-producing parents, possess (i) an equal competence to rejoin single-strand breaks induced in DNA by gamma rays, (ii) a reduced capacity for the photoenzymatic repair of UV-induced pyrimidine dimers, and (iii) a total inability to excise dimers, apparently owing to a deficiency in UV-specific endonuclease activity responsible for mediating the initial incision step in excision repair. Assuming that the DNA repair properties of R^+ minicells reflect the concentration of repair enzymes located in the plasmid-containing polar caps of entire cells, these findings suggest that: (i) the enzymes responsible for rejoining single-strand breaks are distributed throughout the cell; (ii) photoreactivating enzyme molecules tend to be concentrated near bacterial DNA and to a lesser extent near plasmid DNA; and (iii) UV-specific endonuclease molecules are primarily confined to the central region of the *E. coli* cell and, thus, seldom segregate with R-factor DNA into minicells.

Although exposure of living organisms to ultraviolet (UV) and ionizing radiations produces potentially lethal defects in deoxyribonucleic acid (DNA), many species possess enzymatic repair mechanisms for the removal of such damage. For example, the bacterium *Escherichia coli* is known to possess several repair processes responsible for the elimination of such UV-induced lesions, as cyclobutyl pyrimidine dimers from DNA (for reviews, see 15, 16, 33). One mechanism, enzymatic photoreactivation, involves repair by conversion of dimers to monomers in situ by a photoreactivating enzyme in the presence of visible light (360 to 450 nm). A second mechanism, referred to as excision repair, results in the physical removal of dimers from the DNA of UV-irradiated *E. coli* cells. The most widely accepted model (16) for this process consists of three sequential light-independent steps: (i) a single-strand incision near the lesion by an endonuclease that recognizes structural distortions in the DNA duplex; (ii) exonucleolytic excision of the dimer as part of an oligonucleotide, followed by the widening of the ensuing gap, with concomitant repolymerization in the region of the gap, by use of the intact complementary strand as a template [the Kornberg DNA polymerase (EC 2.7.7.7) has been reported to perform both the exonucleolytic and repolymerization functions associated with excision repair in vitro (20)]; and (iii) formation of a phosphodiester bond between newly synthesized and preexisting DNA by the strand-rejoining enzyme, polynucleotide ligase, to restore the continuity of the repaired DNA strand.

Unlike the well-characterized repair of UV photoproducts, the sequence of events leading to the rejoining of single-strand breaks produced in DNA by ionizing radiation is largely unknown at the present time. There appear to be at least two mechanisms, rapid and slow strand-rejoining repair, responsible for over-

coming X ray-induced strand breaks (31). The rapid process, which is completed in a few minutes, requires a functional Kornberg DNA polymerase, whereas the slow process, which requires a longer postirradiation incubation period (10 to 45 min), is controlled by *recA*, *recB*, and *recC*—three genes that code for proteins involved in genetic recombination.

Although specific enzymes have been implicated in the correction of UV damage in DNA, there is very little insight into the distribution of these DNA repair enzymes in the *E. coli* cell. Indirect evidence suggests that certain repair enzymes tend to be concentrated near bacterial DNA. For example, integration into the recipient genome of *Haemophilus influenzae* cells is a necessary prerequisite for excision repair of UV-irradiated transforming DNA (28). *E. coli* K-12 singly infected with phage λ preferentially excises dimers from host-cell rather than λ DNA (3). In phage T1-infected *E. coli* B, the number of photoreactivating enzyme molecules available for the monomerization of dimers in bacterial DNA exceeds that correcting UV photoproducts in phage DNA by fourfold (23).

The opportunity to measure directly the relative activities of DNA repair enzymes in the polar regions and in the entire cell was provided by the isolation of an *E. coli* mutant possessing an aberrant cytokinetic mechanism (1). Under normal growth conditions, cells of the mutant strain may undergo, in addition to normal binary fissions, abnormal fissions at the polar extremities, leading to the production of small, anucleate, spherical bodies. These bodies, minicells, can be isolated in a highly purified state by sucrose density gradient centrifugation and do not normally contain detectable amounts of chromosomal DNA.

Several investigators (18, 19, 21, 26) have demonstrated that, when a minicell-producing strain harbors a fertility (F), F′, resistance (R), or colicinogenic (Col) factor, plasmid DNA may segregate into the otherwise DNA-deficient minicells. Recent studies have also indicated that minicells containing plasmid DNA are capable of performing certain indispensable cellular functions, including DNA (18, 26) ribonucleic acid, and protein syntheses (27). In the experiments reported here, we have made use of the presence of R-factor DNA in minicells to examine the relative abilities of minicells and their parents to perform strand-rejoining repair after gamma irradiation, as well as enzymatic photoreactivation and excision repair after UV irradiation. By inference, it is reasonable to suppose that differences in DNA repair capabilities between minicells and parental cells may reflect an unequal distribution of specific repair enzymes in the *E. coli* cell. These studies have permitted us to assess the potential of the minicell system for elucidating processes involved in DNA repair and genetic recombination. A preliminary account of our findings has been reported elsewhere (Abstr., Genetics **68**:s48–s49, 1971).

MATERIALS AND METHODS

Bacterial strains. Strain χ1009 is a minicell-producing strain of *E. coli* K-12 that harbors the drug-resistance transfer factor R64-11. The genotype and the construction of this strain have been described (26). R64-11 has been isolated from the cell as a covalently closed circular molecule with a molecular weight of about 76×10^6 daltons (32).

P678 is the K-12 strain from which the original minicell producer, P678-54, was derived (1) and is, therefore, an ancestor of χ1009. The response of P678 to UV and gamma radiations, as measured by colony-forming ability, is that of a wild-type *E. coli* strain (H. I. Adler, *personal communication*).

Media. Growth medium was a mineral salts solution (7) enriched with 0.5% Casamino Acids (Difco), 2 μg of thiamine HCl per ml, and 0.5% glucose (w/v). Plating medium was growth medium supplemented with 12 g of nutrient agar (Difco) per liter. As radiation suspension media, either phage buffer (24) or mineral salts solution was used. Washing and lysis buffers were phosphate-containing (*p*H 7.0) and tris-(hydroxymethyl)aminomethane-containing (*p*H 9.1) buffers, respectively, as prepared by Freifelder et al. (12). The lysing solution was 1% sodium dodecyl sulfate (SDS) in 0.8 N NaOH.

Growth conditions. Logarithmic-phase bacteria were cultivated in growth medium to a concentration of approximately 4×10^8 cells/ml by incubation at 37 C for 4.5 hr with vigorous shaking on a New Brunswick rotary shaker. When required, DNA was labeled by incubation of the cells in growth medium supplemented with 100 μg of adenosine/ml, 1 μg of unlabeled thymidine/ml, and 10 μCi of thymidine-*methyl*-^3H (18.1 Ci/mmole) per ml, purchased from Schwarz BioResearch Inc.

Biological survival assay. Nonradioactive cells in the late exponential phase of growth were harvested by centrifugation, resuspended in phage buffer at a concentration of about 2×10^7 cells/ml, and exposed to either UV or gamma radiation at 4 C. After appropriate serial dilutions in phage buffer, the cells were plated out on plating medium to give 100 to 250 surviving colonies per plate. All plates were incubated at 37 C for 24 hr.

General experimental design. To compare the DNA repair capabilities of minicells and cells, the two were separated before irradiation. However, for reasons outlined in the Discussion, this procedure proved unsatisfactory. Therefore, the following protocol was adopted: (i) irradiation of a freshly harvested culture resuspended in a mineral salts solution (7), (ii) postirradiation incubation of culture

samples in growth medium, (iii) separation of treated samples into minicell and cell fractions by sedimentation in neutral sucrose, and (iv) analysis of the purified minicell and cell suspensions for the extent of radiation-induced damage remaining in the DNA.

Purification of minicells and parental cells. High yields of purified minicells (1 cell per 10^6 minicells) and parental cells were isolated from a culture of χ1009 by two successive sedimentations through 35-ml linear gradients of 5 to 20% sucrose (w/v) in buffered saline with gelatin, as described earlier (27).

Method of irradiation. In preparation for gamma and UV radiation treatments, samples were washed and diluted in the appropriate medium to an absorbancy of 0.2 units measured at 620 nm, which corresponds to about 10^8 cells/ml or 10^9 minicells/ml. Samples to be UV-irradiated were diluted a further 10-fold.

The general procedure for UV radiation, including dosimetry, was as reported elsewhere (24). Stirred samples at 4 C were irradiated with far UV light, chiefly at 254 nm, emitted from two 15-w low-pressure mercury germicidal lamps; the incident UV exposure rate was 10 ergs per mm^2 per sec. For exposure to gamma rays, chilled samples were irradiated with ^{60}Co gamma rays in a Gammacell 200 unit (Atomic Energy of Canada, Ltd., Ottawa) at a dose rate ranging from 4.17 to 4.10 krad/min, as measured by ferrous sulfate dosimetry (25).

Postirradiation incubation conditions. Irradiated samples were incubated in growth medium at 37 C on a rotary shaker for suitable time intervals to permit strand rejoining and dimer excision. To prevent photoreactivation, UV-treated suspensions were incubated under dim yellow light emitted from GE "gold" fluorescent lamps.

For photoreactivation of UV-irradiated bacteria, samples were maintained at 37 C on a rotary shaker and illuminated with two GE blacklight lamps (F15T-BLB) emitting light in the 300- to 400-nm region with an incident intensity of approximately 15,000 ergs per mm^2 per min. The photoreactivating light was filtered with plate glass 13 mm thick to remove any radiation below 320 nm. To avoid excision repair, samples were illuminated in growth medium containing 10^{-2} M KCN (29).

Determination of the percentage of R factor DNA present as covalently closed circles. (i) Cell lysis. The technique was similar to that developed by Freifelder et al. (12) for the isolation of plasmid DNA in the form of covalently closed circles. After appropriate periods of postirradiation incubation, 1-ml samples at 4 C were centrifuged, washed in washing buffer, and resuspended gently by manual agitation in 300 μliters of lysis buffer. The suspensions were transferred at 23 C to a cuvette, in which cell lysis was achieved by introducing 60 μliters of lysis solution into the stirred suspension over a 2-min period. Lysates of cells were agitated with a Vortex mixer in a manner similar to that reported elsewhere (12), a procedure that shears chromosomal DNA but leaves plasmid DNA intact (12), so that a clear sedimentation profile of plasmid DNA is obtained. Profiles of sheared and unsheared lysates of minicell suspensions were indistinguishable from each other.

(ii) Velocity centrifugation in alkaline sucrose. Samples (100 μliters) of alkali-SDS lysates derived from about 10^7 cells (15×10^3 to 30×10^3 counts/min) or about 10^8 minicells (5×10^3 to 15×10^3 counts/min) were layered on 3.6-ml linear gradients of 5 to 20% sucrose (w/v) in 0.5 M NaCl, 0.2 M NaOH, and 0.01 M ethylenediaminetetraacetic acid. Gradients were centrifuged at 30,000 rev/min (21 C) for 40 min in an SW 56 swinging-bucket rotor driven by a Spinco model L ultracentrifuge. Approximately 28 fractions of eight drops each were collected on paper strips by gravity drainage from the bottom of the gradient tube, according to the method of Carrier and Setlow (5). The gradient fractions were treated with 5% trichloroacetic acid, washed twice in 95% ethanol, and dried, and the radioactivity was counted in a toluene-2-5-bis[2-(5-*tert*-butylbenzoxazolyl)]-thiophene scintillator by use of a Packard Tri-Carb liquid scintillation spectrometer. The radioactivity recovered from each gradient was within ±20% of the input activity.

Measurement of single-strand breaks in chromosomal DNA. The procedure used for measuring single-strand scissions in chromosomal DNA of gamma-irradiated parental cells was that of McGrath and Williams (22). All manipulations after removal of cell samples from post-gamma-ray incubation medium were carried out in the presence of 10^{-2} M KCN, so as to prevent further strand-rejoining repair. About 8×10^6 protoplasts (10^4 to 2×10^4 counts/min), formed according to the procedure of Fraser et al. (10), were layered onto 5 to 20% alkaline sucrose gradients. Thereafter, the procedure was as described above for sedimentation of R-factor DNA, except that the gradients were centrifuged at 30,000 rev/min for 90 min instead of 40 min, a modification that sediments covalently closed circular plasmid DNA to the bottom of the gradients while distributing untreated chromosomal DNA near the middle of the gradients. The small amounts of remaining plasmid DNA, as compared to chromosomal DNA, were not detected in the sedimentation profiles. The radioactivity recovered from each gradient was within ±10% of the input activity.

Dimer analysis. Thymine-containing pyrimidine dimers in UV-irradiated DNA were measured by the radiochromatographic method of Carrier and Setlow (4). Briefly, 0.5-ml samples of post-UV-incubated minicell and parental cell suspensions were treated with ice-cold 5% trichloroacetic acid. DNA in the acid-precipitable fraction containing 2×10^4 to 4×10^4 counts per min per minicell sample and 4×10^5 to 8×10^5 counts per min per cell sample was hydrolyzed by formic acid to individual bases and pyrimidine dimers. The two ^3H-thymidine-labeled components in the acid hydrolysate were separated by two-dimensional paper chromatography. The radioactivity in the resulting monomer and dimer regions was eluted from the paper with water and counted in a dioxane-naphthalene scintillation medium.

RESULTS

Only data derived from experiments in which a culture was first irradiated and incubated prior to purification of minicells and cells are used to measure strand-rejoining repair, photoreactivation, and excision repair in minicells and in their progenitors. Minicells and cells handled in this fashion are referred to as sucrose-untreated. Later, results obtained from experiments in which minicells and cells were purified prior to irradiation and incubation (sucrose-treated) will be compared with results obtained for sucrose-untreated minicells and cells, to determine the degree to which sucrose gradient centrifugation prior to UV or gamma radiation retards DNA repair.

Biological survival. A prerequisite to measuring the ability of minicells per se to repair radiation-induced defects in their R-factor DNA is to establish that χ1009, the strain from which R^+ minicells are derived, exhibits the capacity to overcome both UV and gamma-ray inactivation. In fact, as clearly illustrated by the survival curves in Fig. 1, χ1009 is more resistant than P678, a wild-type strain in terms of colony-forming ability following radiation treatment, toward the lethal effects produced by UV and gamma rays. Table 1 shows χ1009 to be about 4.0 times more UV-resistant and approximately 2.7 times more resistant to gamma rays than P678. Survival of the R^- minicell-producing strain P678-54 after UV irradiation is similar to that of P678 but after gamma-ray treatment is indistinguishable from χ1009 (Paterson and Roozen, *unpublished data*). Thus, the enhanced gamma-ray resistance of χ1009, like that reported earlier for P678-54 (1), can be attributed to mutations in chromosomal DNA responsible for minicell formation; however, the increased UV resistance of χ1009 appears to be conferred primarily by the presence of R-factor DNA. This latter finding is in agreement with an observation first reported by Howarth (17) for the Col I plasmid and later shown for R-factor (8, 9) and F-factor (2) DNA; namely, *E. coli* strains containing plasmids exhibit increased resistance to UV inactivation.

Fate of gamma ray-induced single-strand breaks in plasmid and bacterial DNA. (i) Yield of single-strand breaks. As previously demonstrated by Freifelder (11) for various species of covalently closed circular plasmid DNA (F, F′, and λ), sedimentation in alkaline sucrose permits an accurate quantitative estimation of the number of single-strand breaks acquired during exposure to ionizing radiation.

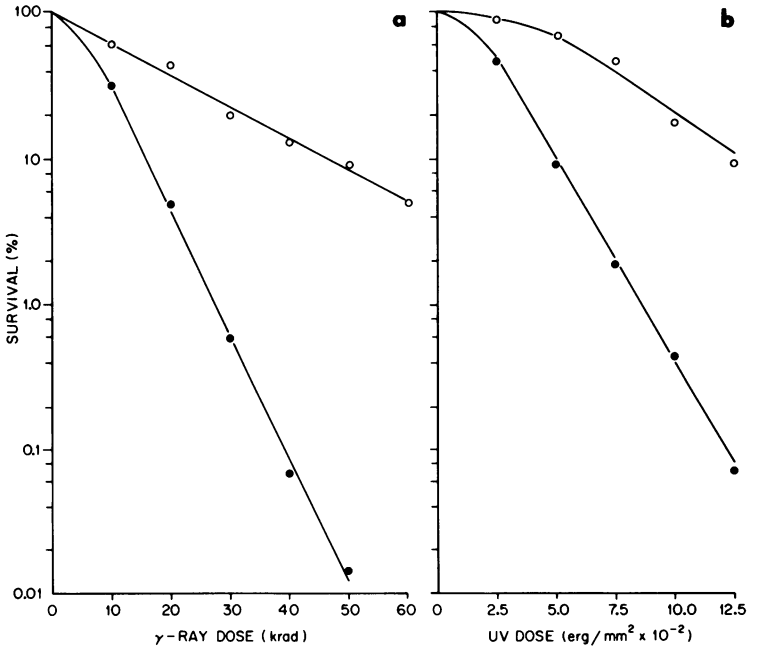

FIG. 1. *Gamma-ray and UV survival curves for colony-forming ability of E. coli strains χ1009 and P678. Stirred samples of chilled cells at 2 × 10⁷ per ml in phage buffer were irradiated with gamma rays (a) and germicidal light (b). Symbols:* ○, *χ1009;* ●, *P678.*

The introduction of one single-strand break in R-factor DNA is sufficient to convert a covalently closed double-stranded circle to a "nicked" or open circle, the single-stranded products of which sediment three to four times more slowly in alkaline sucrose than does the closed circular structure. Figure 2 shows typical sedimentation profiles for an unirradiated and for a gamma-irradiated minicell suspension. The fast-sedimenting component contains only double-stranded covalently closed circular DNA, whereas the more slowly sedimenting material at the top of each gradient is a mixture of circular and linear single-strand DNA molecules. In a series of eight independent lysate preparations of unirradiated control samples, the values for the fraction of the total radioactivity recovered from the gradients in covalently closed circular DNA range from 24 to 37% with an average of about 30% for minicells and from 4.4 to 6.1% with an average of approximately 5% for parental cells. The kinetics of gamma ray-induced single-strand breaks in R-factor DNA from either cells or minicells is first-order, as indicated by an exponential decline in the surviving fractions of covalently closed circular DNA as a function of gamma-ray dose (Fig. 3). It can be seen in Fig. 3 that loss of covalently closed circular DNA is independent of the host; R-factor DNA is nicked by gamma rays with the same efficiency in minicells as in normal-sized cells. If the breaks are randomly distributed, a gamma ray dose of approximately 3.5 krad produces, on the average, one single-strand break per R64-11 DNA molecule.

The appearance of single-strand breaks induced in bacterial DNA by gamma radiation is manifested as reduced rates of sedimentation in alkaline sucrose gradients. As shown in Fig. 4a and 4b, 10 krad of gamma rays reduces the

TABLE 1. *Summary of biological survival*

Determination	Strain	Resistance to radiation	
		UV (ergs/mm²)	Gamma rays (krad)
D_0 value[a]	χ1009	400	20
	P678	150	5
Ratio of resistances[b]	χ1009/P678	2.7	4.0

[a] The increment of dose necessary to reduce survival by 63% in the exponential region of the curve.
[b] Proportional to the ratio of the D_0 values.

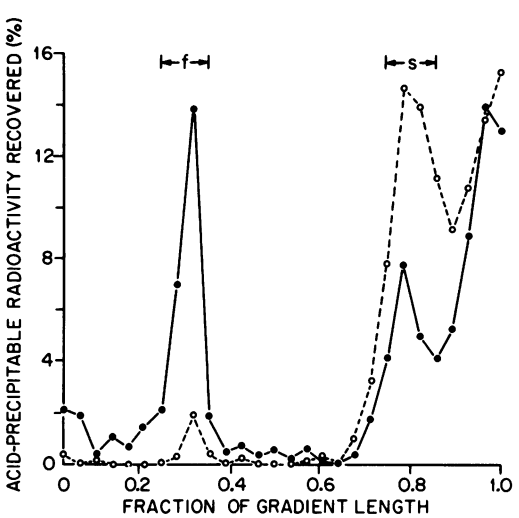

FIG. 2. *Alkaline sucrose gradient profiles of unirradiated and gamma-irradiated minicells containing R-factor DNA. Radioactivity profiles are plotted as a fraction of the gradient, with sedimentation from right to left. The rapidly sedimenting component containing covalently closed circular (CCC) DNA is indicated by (| ← f → |), while (| ← s → |) identifies the more slowly sedimenting component. Symbols: ●, unirradiated; ○, irradiated with 10 krad of gamma rays. Gamma irradiation converts about 90% of CCC DNA from the fast to the slow fraction.*

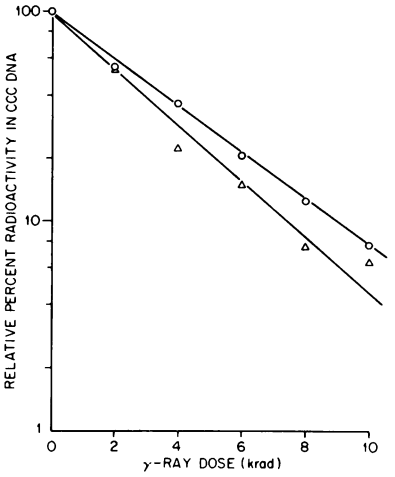

FIG. 3. *Dose-dependence curves for the survival of R-factor DNA as covalently closed circular (CCC) DNA in minicells and cells after gamma irradiation. Data for these curves are derived from an appropriate family of sedimentation profiles of the type illustrated in Fig. 2. These data are normalized by expressing the percentage of the total radioactivity in CCC DNA in profiles of irradiated samples as a percentage of the value (i.e., approximately 30 and 5% of the total radioactive counts in unirradiated minicells and cells, respectively, is in CCC DNA) that is representative of unirradiated control samples. Symbols: ○, minicells; △, cells.*

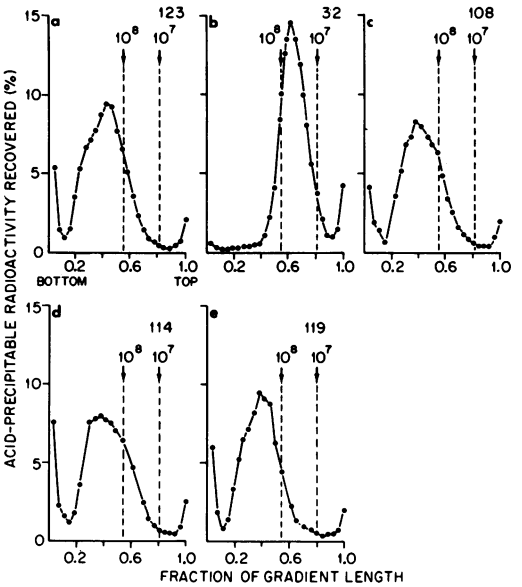

FIG. 4. *Sedimentation patterns in alkaline sucrose gradients of chromosomal DNA from parental cells irradiated with 10 krad of gamma rays and incubated for various periods of time. Radioactivity profiles are plotted as a fraction of the gradient, with sedimentation from right to left in each panel. Panels refer to unirradiated (a) and to irradiated cells incubated for 0, 10, 20, and 30 min (b-e). Reference arrows indicating the expected sedimentation positions of single-stranded molecules with molecular weights of 10^7 and 10^8 daltons, respectively, were obtained from an independent calibration of the gradients by using the observed sedimentation of ^{3}H-thymidine-labeled phage T4 DNA and the data of Studier (30). The number average molecular weight (M_n) $\times$ 10^6 daltons of the chromosomal DNA profile is indicated in each panel. The first two fractions on the top as well as the last three fractions on the bottom of each gradient were excluded from the M_n calculations.*

number average molecular weight (M_n) from 123×10^6 to 32×10^6 daltons. Therefore, if the breaks are randomly distributed, the gamma-radiation treatment has produced on the average approximately three breaks in each *E. coli* DNA molecule with an M_n equal to 123×10^6 daltons.

(ii) Strand-rejoining repair. At the outset, it must be pointed out that no attempt has been made in this study to distinguish between fast and slow strand-rejoining processes. Although it may be assumed that both processes occur in cells, the data presented below indicate only that minicells possess at least one of the two.

Data in Fig. 5 demonstrate that minicells are as competent as their progenitors at mending strand scissions produced in R-factor DNA by 10 krad of gamma radiation; after 10 min of incubation, the amount of covalently closed circular DNA increases from a low of 10% immediately after irradiation to 70 to 80% of that found in the unirradiated control samples. As shown in Fig. 4c, 4d, and 4e, the sedimentation patterns of chromosomal DNA obtained from gamma-irradiated parental cell samples incubated for 10 min or more have returned to that representative of unirradiated cells. These data suggest that the strand restitution processes quickly repair gamma ray-induced strand breaks not only in chromosomal DNA but also in R-factor DNA. We conclude, then, that minicells and cells are equally proficient at rejoining strand scissions produced by 10 krad of gamma rays.

Fate of UV-induced pyrimidine dimers. (i) Yield of dimers. Since minicells contain only R-factor DNA, and all but 5% of the total trichloroacetic acid-insoluble radioactivity in

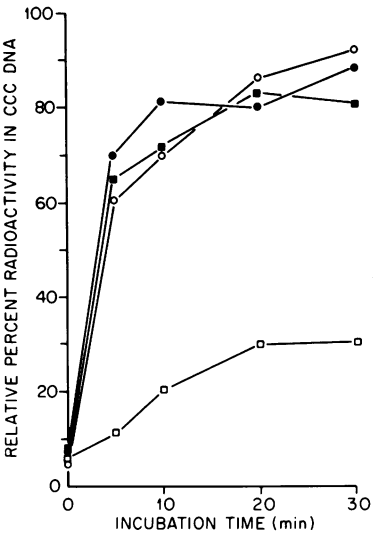

FIG. 5. *Kinetics of strand-rejoining repair of R-factor DNA contained in minicells and cells exposed to 10 krad of gamma irradiation. At suitable intervals during post-gamma-ray incubation, samples were subjected to alkaline sucrose centrifugation, and the per cent radioactivity in covalently closed circular (CCC) DNA was calculated from a sedimentation profile of the type shown in Fig. 2. For both minicells and cells, the percentage of the total radioactivity in CCC DNA from unirradiated control samples incubated for 15 and 30 min did not vary by more than ±10% from that found for nonincubated control samples. DNA was obtained from sucrose-treated (○) and sucrose-untreated (●) minicells, and from sucrose-treated (□) and sucrose-untreated (■) cells.*

parental cells is found in chromosomal DNA, cells and R+ minicells can be used to measure the production of dimers in chromosomal and R-factor DNA, respectively. Radiochromatographic analysis of cell and minicell samples exposed to 250 ergs/mm² of 254-nm light reveals that 0.105 to 0.124% of the radioactivity in both bacterial and R-factor DNA is associated with thymine-containing dimers.

(ii) Enzymatic photoreactivation. The criterion for the presence of photoenzymatic activity in cells and minicells is a reduction in the relative number of dimers present in UV-irradiated DNA as a function of time of exposure to a photoreactivating light source. Monomerization of dimers in both minicell and parental cell suspensions exhibits exponential kinetics, as shown in Fig. 6. However, photoreactivating illumination reduces dimers twice as quickly in cells as in minicells. For example, 60 min of exposure eliminates 60 to 65% of the total number of dimers induced by UV in cells but only 30% of the dimers initially produced in minicells.

(iii) Excision repair. (a) Dimer excision. The relative amounts of UV-induced pyrimidine dimers selectively excised from DNA in cell and minicell preparations during post-UV incubation are shown in Fig. 7. Parental cells remove dimers in a manner comparable to that previously reported for excision-proficient strains of *E. coli* (29). Within 60 min, these cells remove up to 75% of the dimers produced in their DNA by exposure to 250 ergs/mm² of germicidal light. In sharp contrast to the efficient excision mechanism operative in their parents, minicells appear defective in the excision process, since no net dimer removal was detected even after an incubation period of 60 min.

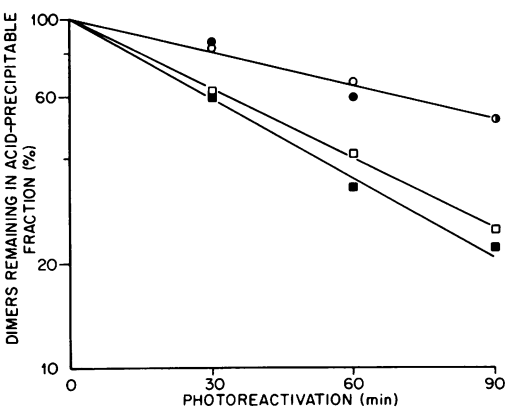

FIG. 6. *Kinetics for the photorepair of pyrimidine dimers produced in the DNA of minicells and cells by exposure to 250 ergs/mm² of germicidal light at 254 nm. At appropriate intervals, samples from sucrose-treated* (○) *and sucrose-untreated* (●) *minicells, and from sucrose-treated* (□) *and sucrose-untreated* (■) *cells, were assayed for dimer content.*

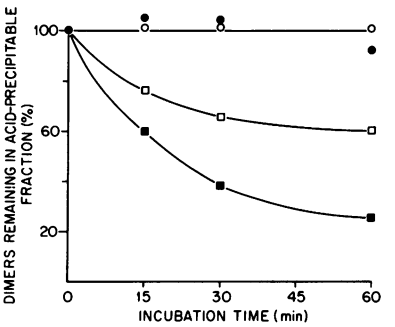

FIG. 7. *Rate of dimer excision in DNA obtained from minicells and cells irradiated with 250 ergs/mm² of germicidal light. At suitable intervals, samples from sucrose-treated* (○) *and sucrose untreated* (●) *minicells, and from sucrose-treated* (□) *and sucrose-untreated* (■) *cells, were assayed for dimer content.*

(b) Appearance of single-strand breaks in R-factor DNA during post-UV incubation. In contrast to gamma irradiation, UV light does not directly introduce single-strand breaks into DNA. Instead, single-strand breaks appear in DNA of *E. coli* during post-UV incubation as a result of metabolic events involved in the excision-repair process. The number of strand breaks observed in UV-irradiated DNA at any given instance is interpreted to be a measure of the instantaneous rate of excision repair in the organism. That is, the number of single-strand breaks in the DNA is representative of the number of excision-repair events which at that instance have proceeded to the initial incision step but have not yet completed the final repair step, which involves the restitution of the polynucleotide chain. As expected in an excision-proficient strain of *E. coli*, single-strand breaks in R-factor DNA, manifested in sucrose sedimentation profiles as a decrease in the relative percentage of total radioactivity in covalently closed circular DNA, appear and subsequently disappear in the parental cell fraction during normal excision repair of UV-induced lesions (see Fig. 8). After 60 min of incubation, the amount of plasmid DNA in the form of covalently closed circles returns from a low of 5% at 20 min to 70% of that present immediately after UV treatment. In UV-irradiated minicells, however, relatively few single-strand scissions appear in R-factor DNA during the incubation

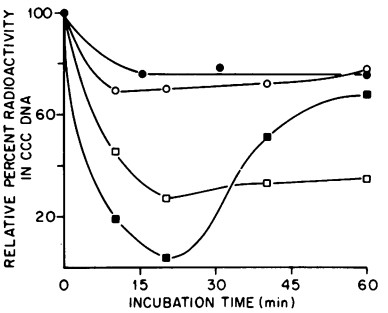

FIG. 8. *Occurrence of single-strand breaks in R-factor DNA contained in minicells and cells irradiated with 250 ergs/mm² of 254-nm light. At appropriate intervals, samples were treated as in Fig. 5 so as to obtain the per cent radioactivity in covalently closed circular DNA. DNA was obtained from sucrose-treated* (○) *and sucrose-untreated* (●) *minicells, and from sucrose-treated* (□) *and sucrose-untreated* (■) *cells.*

period. A simple interpretation of this observation is that minicells are defective in performing the initial incision step in excision repair, an enzymatic deficiency that explains the lack of dimer excision in minicells, as illustrated in Fig. 7. We conclude, then, that minicells do not possess a functional UV endonuclease, the enzyme responsible for making a single-strand break in UV-irradiated DNA adjacent to a pyrimidine dimer (13, 14). In addition, our data suggest that parental cells are capable not only of excising pyrimidine dimers in R-factor DNA but also of filling in the resulting gaps.

Effect of centrifugation in neutral sucrose on the DNA repair properties of cells and minicells. Although they are not relevant to the general conclusions, we feel that the following points are worth making in regard to the relative DNA repair capabilities of sucrose-treated and untreated cells and minicells. (i) As compared with that found for sucrose-untreated cells, the rates of strand-rejoining repair (Fig. 5) and dimer excision (Fig. 7) but not photoenzymatic repair (Fig. 6) are much reduced in sucrose-treated cells. (ii) Sucrose-treated minicell suspensions possess the same capacity for strand-rejoining repair (Fig. 5) and photoreactivation (Fig. 6) as sucrose-untreated suspensions. Consequently, sedimentation through 5 to 20% neutral sucrose gradients may retard the activity of certain DNA repair enzymes in cells, but this treatment has no measurable effect in minicells.

DISCUSSION

Certain repair enzymes may be concentrated near DNA to detect and repair rapidly any harmful physicochemical defects in the normal double helical structure of DNA. The comparison of the DNA repair properties of minicells and parental cells enables us to test this viewpoint in a more direct manner than was possible in earlier studies (3, 23, 28). The cellular distribution of photoreactivating enzyme molecules in *E. coli* K-12 appears to be consistent with the above notion. Extracts of R^- parental cells clearly possess photoreactivating enzyme activity; in comparison, extracts of minicells lacking DNA do not exhibit a detectable level of enzyme activity, as determined by an in vitro transformation assay (J. Setlow and A. Cohen, cited in reference 6). In contrast, our data from in vivo studies indicate that minicells which possess DNA can eliminate pyrimidine dimers during post-UV incubation in the presence of photoreactivating illumination. Thus, it can be inferred that the enzyme is concentrated in the vicinity of R-factor DNA and, therefore, tends to segregate with the plasmid into minicells. On the average, there is approximately one R-factor plasmid per minicell (R. Curtiss III, *personal communication*). Assuming two chromosomes per parental cell, there is approximately eight times more chromosomal DNA per unit mass of cells than plasmid DNA per unit mass of minicells. It may then be estimated from the data of Boyle and Setlow (3) that 250 ergs/mm² of UV makes about 2,800 dimers per cell and about 35 dimers per minicell. Nevertheless, after 60 min of photoreactivating illumination, about 1,700 (i.e., 60% of the initial number) dimers are monomerized in cells, but only about 10 (i.e., 30% of the initial number) are eliminated in minicells. If the relative rates at which photoenzymatic repair occurs in chromosomal DNA of cells and in plasmid DNA of minicells reflect, at least partially, the concentration of enzyme, and hence the relative affinity of the photoreactivating enzyme for the two DNA species, then the enzyme molecules in *E. coli* cells have a greater affinity for bacterial DNA than for R-factor DNA.

In contrast to the photoreactivating enzyme, at least one of the enzymes involved in the excision-repair process appears to segregate rarely with plasmid DNA into minicells. R^+ minicells formed from terminal regions of excision-proficient cells are appreciably deficient in UV-specific endonuclease activity in vivo. A simple explanation for this observation is that endonuclease molecules tend to be confined to the central region of cells and, thus, seldom segregate into R^+ minicells. Two alternative explanations are as follows. (i) Minicells pos-

sess UV-specific endonuclease molecules, but for some unknown reason the endonuclease is catalytically inactive toward UV-irradiated R-factor DNA. (ii) The molecular turnover of the endonuclease is rapid in both cells and minicells; in cells, but not in minicells, the degraded endonuclease molecules are being replaced continuously.

R^+ minicells are as competent as parental cells at rejoining single-strand breaks in DNA induced by 10 krad of gamma rays (Fig. 4 and 5). Although these data, coupled with an inability to assay for strand-rejoining repair in R^- minicells, preclude an unequivocal estimation of the distribution of enzymes mediating strand-rejoining repair, the finding does suggest that the enzymes involved are distributed throughout the *E. coli* cell.

An objection to our basic experimental design involving the use of sucrose-untreated minicells and cells might be that a significant fraction of the minicell population eventually assayed arises during the postirradiation incubation period preceding minicell and cell separation. In that case, radiation-induced defects in R-factor DNA of these minicells might have been corrected by repair processes operative in parental cells prior to minicell formation. In fact, such repair is a negligible fraction of that observed in minicells for the following reasons. (i) In experiments assaying the strand-rejoining (Fig. 5) and photoreactivation (Fig. 6) capabilities of R^+ minicells, it was demonstrated that minicells separated from parental cells after irradiation and incubation have repair properties identical to those of minicells purified prior to irradiation and incubation. (ii) As pointed out in the Results, data presented in Fig. 8 suggest that parental cells, as expected (3), perform excision repair on the extrachromosomal element, R-factor DNA. If a significant fraction of the final minicell population arises during the 60-min incubation period, then the R-factor-containing minicells should, but clearly do not (Fig. 7), exhibit dimer excision. These considerations demonstrate that the repair or lack of repair observed in minicells is a property of the minicells themselves and is not due to events that occur in parental cells prior to minicell formation.

Experimental data presented above (Fig. 5–8) indicate that sedimentation through sucrose gradients impairs the subsequent functioning of certain DNA repair processes in cells [i.e., strand rejoining (Fig. 5) and excision repair (Fig. 7 and 8) but not enzymatic photoreactivation (Fig. 6)] but not in minicells. At present, there is no satisfactory explanation for the detrimental effects of this treatment on cells but not on minicells. However, one possible reason, based on the fact that during centrifugation minicells are exposed to at most 8% sucrose whereas cells experience 20% sucrose, can be rejected on the basis of the following observation. Minicells exposed to 20% sucrose for the same period as cells perform strand-rejoining repair with kinetics indistinguishable from those of minicells isolated in the normal manner.

In summary, a comparison of the DNA repair properties operative in minicells and in cells suggests the compartmentalization of certain repair enzymes (i.e., photoreactivating and UV-specific endonuclease) but not of others (i.e., enzymes involved in strand-rejoining repair) in the *E. coli* cell. The demonstration that R^+ minicells are capable of strand-rejoining repair makes them a potentially useful tool for investigating rapid and slow strand-rejoining processes.

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LITERATURE CITED

1. Adler, H. I., W. D. Fisher, A. Cohen, and A. A. Hardigree. 1967. Miniature *Escherichia coli* cells deficient in DNA. Proc. Nat. Acad. Sci. U.S.A. **57**:321–326.
2. Axelrod, D. E., and H. I. Adler. 1969. Influence of the fertility episome on the survival of X-irradiated *Escherichia coli*. J. Bacteriol. **98**:329–330.
3. Boyle, J. M., and R. B. Setlow. 1970. Correlations between host-cell reactivation, ultraviolet reactivation and pyrimidine dimer excision in the DNA of bacteriophage λ. J. Mol. Biol. **51**:131–144.
4. Carrier, W. L., and R. B. Setlow. 1971. The excision of pyrimidine dimers (the detection of dimers in small amounts), p. 230–237. *In* L. Grossman and K. Moldave (ed.), Methods in enzymology, vol. 21, part D. Academic Press Inc., New York.
5. Carrier, W. L., and R. B. Setlow. 1971. A paper strip method for assaying gradient fractions containing radioactive macromolecules. Anal. Biochem. **43**:427–432.
6. Cohen, A., W. D. Fisher, R. Curtiss III, and H. I. Adler. 1968. The properties of DNA transferred to minicells during conjugation. Cold Spring Harbor Symp. Quant. Biol. **33**:635–641.
7. Curtiss, R., III. 1965. Chromosomal aberrations associated with mutations to bacteriophage resistance in *Escherichia coli*. J. Bacteriol. **89**:28–40.
8. Drabble, W. T., and B. A. D. Stocker. 1966. Transducibility and other properties of some R (transmissible drug-resistance) factors. Heredity **21**:166–167.
9. Drabble, W. T., and B. A. D. Stocker. 1968. R (transmissible drug-resistance) factors in *Salmonella typhimurium*: Pattern of transduction by phage P22 and ultra-

violet-protection effect. J. Gen. Microbiol. **53**:109–123.
10. Fraser, D., H. R. Mahler, A. L. Shug, and C. A. Thomas. 1951. The infection of subcellular *Escherichia coli*, strain B, with a DNA preparation from T2 bacteriophage. Proc. Nat. Acad. Sci. U.S.A. **43**:939–947.
11. Freifelder, D. 1968. Rate of production of single-strand breaks in DNA by X-irradiation *in situ*. J. Mol. Biol. **35**:303–309.
12. Freifelder, D., A. Folkmanis, and I. Kirschner. 1971. Studies on *Escherichia coli* sex factors: evidence that covalent circles exist within cells and the general problem of isolation of covalent circles. J. Bacteriol. **105**:722–727.
13. Grossman, L., J. C. Kaplan, S. R. Kushner, and I. Mahler. 1968. Enzymes involved in the early stages of repair of ultraviolet-irradiated DNA. Cold Spring Harbor Symp. Quant. Biol. **33**:229–234.
14. Grossman, L., J. Kaplan, S. Kushner, and I. Mahler. 1969. Enzymatic mechanisms for the repair of UV irradiated DNA, p. 318–333. *In* A. Castellani (ed.), Proceedings of the photobiology meetings on factors affecting cellular photosensitivity. Special Suppl. 1 to Ann. Ist. Super. Sanita, vol. 5.
15. Hanawalt, P. C. 1968. Cellular recovery from photochemical damage, p. 203–251. *In* A. C. Giese (ed.), Photophysiology IV. Academic Press Inc., New York.
16. Howard-Flanders, P. 1968. DNA repair. Annu. Rev. Biochem. **37**:175–200.
17. Howarth, S. 1965. Resistance to the bactericidal effects of ultraviolet radiation conferred on Enterobacteria by the colicine factor *col* I. J. Gen. Microbiol. **40**:43–55.
18. Inselburg, J. 1970. Segregation into and replication of plasmid deoxyribonucleic acid into chromosomeless segregants of *Escherichia coli*. J. Bacteriol. **102**:642–647.
19. Kass, L. R., and M. B. Yarmolinsky. 1970. Segregation of functional sex factor into minicells. Proc. Nat. Acad. Sci. U.S.A. **66**:815–822.
20. Kelly, R. B., M. R. Atkinson, J. A. Huberman, and A. Kornberg. 1969. Excision of thymine dimers and other mismatched sequences by DNA polymerase of *Escherichia coli*. Nature (London) **224**:495–501.
21. Levy, S. B., and P. Norman. 1970. Segregation of transferable R factors into *Escherichia coli* minicells. Nature (London) **227**:606–607.
22. McGrath, R. A., and R. W. Williams. 1966. Reconstruction *in vivo* of irradiated *Escherichia coli* deoxyribonucleic acid; the rejoining of broken pieces. Nature (London) **212**:534–535.
23. Muraoka, N., and S. Kondo. 1969. Kinetics of enzymatic photoreactivation studied with phage T1 and *Escherichia coli* B_{s-1}. Photochem. Photobiol. **10**:295–308.
24. Paterson, M. C., J. M. Boyle, and R. B. Setlow. 1971. Ultraviolet- and X-ray-induced responses of a DNA polymerase-deficient mutant of *Escherichia coli*. J. Bacteriol. **107**:61–67.
25. Randolph, M. L. 1969. Measurement and properties of ionizing radiation, p. 1–115. *In* D. H. Moore (ed.), Physical techniques in biological research, II B. Academic Press Inc., New York.
26. Roozen, K. J., R. G. Fenwick, Jr., and R. Curtiss III. 1971. Isolation of plasmids and specific chromosomal segments from *Escherichia coli* K-12, p. 249–266. *In* L. Ledoux (ed.), Informative molecules in biological systems. North Holland, Amsterdam.
27. Roozen, K. J., R. G. Fenwick, Jr., and R. Curtiss, III. 1971. Synthesis of ribonucleic acid and protein in plasmid-containing minicells of *Escherichia coli* K-12. J. Bacteriol. **107**:21–33.
28. Setlow, J. K., M. E. Boling, and K. L. Beattie. 1970. Repair of DNA in *Haemophilus influenzae*. III. Excision and recombination defects and the site of repair of ultraviolet-irradiated transforming DNA. M. D. Anderson Symp. Genetic Concepts and Neoplasia. Collect. Pap. Annu. Symp. Fundam. Cancer Res. **23**: 555–568.
29. Setlow, R. B., and W. L. Carrier. 1968. The excision of pyrimidine dimers in vivo and in vitro, p. 134–141. In W. J. Peacock and R. D. Brock (ed.), Replication and recombination of genetic material. Australian Academy of Science, Canberra.
30. Studier, F. W. 1965. Sedimentation studies on the size and shape of DNA. J. Mol. Biol. **11**:373–390.
31. Town, C. D., K. C. Smith, and H. S. Kaplan, 1971. DNA polymerase required for rapid repair of X-ray-induced DNA strand breaks *in vivo*. Science **172**:851–854.
32. Vapnek, D., M. B. Lipman, and W. D. Rupp. 1971. Physical properties and mechanism of transfer of R factors in *Escherichia coli*. J. Bacteriol. **108**:508–514.
33. Witkin, E. M. 1969. Ultraviolet-induced mutation and DNA repair. Annu. Rev. Genet. **3**:525–552.