Proc. Natl. Acad. Sci. USA
Vol. 78, No. 6, pp. 3450–3453, June 1981
Biochemistry

Repair of DNA double-strand breaks in *Escherichia coli* cells requires synthesis of proteins that can be induced by UV light

(induced DNA repair)

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Communicated by Richard B. Setlow, March 3, 1981

ABSTRACT **The repair of DNA double-strand breaks in *Escherichia coli* cells irradiated with γ rays occurs only after new proteins are synthesized in response to damage introduced in the genome DNA. One protein whose synthesis is thus induced is the *recA* protein, and previous work has shown that *recA*⁻ cells do not repair double-strand breaks. However, inducing *recA* protein by treating cells with nalidixic acid does not induce repair of double-strand breaks, so this repair requires more than the presence of the *recA* protein. When repair of double-strand breaks is blocked, the genome DNA is degraded by an endonuclease-like action. Evidence is presented to show that the inducible inhibition of DNA degradation after x-irradiation [Pollard, E. C. & Randall, E. P. (1973) *Radiat. Res.* 55, 265] is probably caused by the inducible repair of DNA double-strand breaks.**

Double-strand breaks in intracellular DNA are created by the action of various physical and chemical agents, of which ionizing radiation in its various forms is the most prevalent and important. Studies of the consequences of such double-strand breaks are made difficult by the small numbers found in cells treated so that some reasonable fraction (say 10%) can still replicate. Nevertheless, the repair of double-strand breaks has been demonstrated in bacteria (for review see ref. 1) and in yeasts (for review see ref. 2); the direct evidence for such repair in cultured mammalian cells is less convincing, but the information available supports the view that DNA double-strand breaks are resealed in such cells (1).

In *Escherichia coli* cells, repair of DNA double-strand breaks requires an active *recA* gene and the presence of another DNA duplex that has the same base sequence as the broken double helix (3); normally growing K-12 strains can repair double-strand breaks because they have four or five genomes per cell.

In this paper, we show that such repair is not carried out by enzymes present in normal cells but requires the synthesis of proteins induced by damage to intracellular DNA.

Our experiments were suggested by those of Pollard and his associates (4, 5). They found that the colony-forming ability of *E. coli* cells was sensitized to x-rays by treatment with rifampicin just before irradiation; however, cells exposed to UV light and incubated for 45 min before addition of rifampicin had regained their resistance to x-rays. Similar results have been reported by Smith and Martignoni (6). This suggests that the repair of some lesion, such as a DNA double-strand break, could be one of those processes that Radman (7) and Witkin (8) have classified as coordinate responses to DNA damage in *E. coli recA*⁺ *lexA*⁺ cells: mutagenesis, Weigle mutagenesis and Weigle reactivation (the increase in mutagenesis and plaque-forming ability of UV-irradiated λ phage when the host cells are also irradiated), filamentation, induction of prophage, synthesis of *recA* protein, and others.

Kenyon and Walker (9) have evidence that damage to the DNA in *E. coli* cells induces increased transcription at several specific loci on the chromosome; one site is known to be close to the *uvrA* locus coding for one component of an endonuclease that incises DNA next to a pyrimidine cyclobutane dimer. The increased transcription occurs only in cells that have *recA* and *lexA* functions.

Little *et al.* (10) have isolated the *lexA* protein and shown that it both represses the synthesis of *recA* protein and is specifically cleaved by the *recA* protease. Presumably, damage to intracellular DNA activates the *recA* protease and causes it to cleave the *lexA* protein in the same way that *recA* protease cleaves the λ repressor (11–14). A reduced concentration of *lexA* protein leads in turn to increased synthesis of *recA* protein. It is not known whether the *lexA* protein is also a repressor for any of the other sites of transcription activated by DNA damage. Kenyon and Walker (9) state that the various loci differ widely in their basal levels, lag times for expression, and final induced levels, which suggests they are not all controlled in the same way.

The other loci are assumed to code for, among others, proteins that aid in DNA repair. Sedgwick (15) has found a small but reproducible increase in *recA*-dependent postreplication repair of UV-irradiated DNA in *E. coli* cells permitted to synthesize proteins, compared with cells blocked in protein synthesis. Cooper and Hunt (16) concluded that long patch repair, with 1000–2000 nucleotides inserted at a damage site, is inducible as well as *recA* dependent (17).

MATERIALS AND METHODS

Bacteria. *E. coli* AB2497 *thyA⁻rec⁺arg⁻his⁻pro⁻thi⁻thr⁻* cells were originally obtained from P. Howard-Flanders.

Media. Cells were grown in K medium [M9 buffer (19 mM NH_4Cl/42 mM Na_2HPO_4/22 mM KH_2PO_4/1 mM $MgSO_4$/0.1 mM $CaCl_2$, thiamine at 0.1 μg/ml); 1% (wt/vol) glucose; 1% (wt/vol) Casamino acids (decolorized, vitamin-free); and [*methyl*-^{14}C]thymine at 49 mCi/mmol (1 Ci = 3.7×10^{10} becquerels) and 5 μg/ml, [*methyl*-^{3}H]thymine at 2.2 Ci/mmol and 5 μg/ml, or nonradioactive thymine at 5 μg/ml]. Aspartate medium was M9 buffer, amino acids required by AB2497 at 50 μg/ml each, 0.4% L-aspartic acid neutralized with NH_4OH, and [^{3}H]thymine as above.

Irradiation. Cells were irradiated with 254-nm UV light from a germicidal lamp at 1.0 J m^{-2} sec^{-1}. The cells were in a layer less than 3 mm thick and stirred continuously. They were irradiated under aerobic conditions with ^{60}Co γ rays at 300 rads/min (1 rad = 1.0×10^{-2} gray) at ice temperature.

Cell Lysis and Neutral Sedimentation. The methods have been described (3). Briefly, cells at ≈10^8/ml in nonradioactive

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growth medium were diluted 10-fold with 50 mM Tris base/2 mM EDTA, pH 7.6 and incubated with egg white lysozyme at 200 μg/ml for 10 min at 0°C. About 0.1 ml (10^6 spheroplasts) was layered on linear 5–20% (wt/vol) sucrose gradients in 0.5% $NaDodSO_4$/0.01 M NaCl/1 mM EDTA/1 mM sodium citrate/5 mM Tris base, pH 7.4, saturated at 20°C with chloroform. After 90 min at room temperature, the gradients were centrifuged at 20°C in an SW50.1 rotor at speeds low enough so that anomalous sedimentation of large DNA (3) did not complicate interpretation of the data. About 32 fractions of equal volume were obtained per gradient through a hole pierced in the bottom of the tube. In all experiments except one (see below), each fraction was collected directly in a vial, scintillant was added, and the radioactivity determined in a scintillation counter. Better than 90% of the activity added was recovered.

Acid-Soluble DNA Fragments in Gradient. Two samples of one cell preparation were sedimented on neutral sucrose gradients. One gradient was processed as described above. A second gradient was fractionated on Whatman 17 filter paper discs, which were soaked in 5% trichloroacetic acid, washed in ethanol, dried, and assayed for radioactivity. Multiplication of the observed radioactivity by a constant (of the magnitude expected to take into account the altered counting efficiency) gave a sedimentation profile in good agreement with that of the first gradient for the rapidly sedimenting fractions 1–25; this is as expected, as both profiles should show the same distribution of large DNA. The first gradient showed, however, more radioactivity in the fractions from the top of the gradient, representing the acid-soluble activity removed when the filter papers in the second gradient were washed.

Colony-Forming Ability. Cells were diluted with M9 buffer, spread on Luria agar plates (1), and incubated 24 hr at 37°C, and the colonies were counted.

RESULTS

Fig. 1 shows that *E. coli* cells treated with rifampicin have little ability to repair DNA double-strand breaks caused by γ rays. Incubation for 45 min after irradiation gives a small increase in fast-sedimenting DNA (fractions 1–14) compared with the pattern for unincubated cells. However, cells irradiated with UV light at a time 45 min before rifampicin is added can readily repair double-strand breaks, as shown by sedimentation of much of the DNA to near the bottom of the gradient.

If chloramphenicol at 100 μg/ml is added rather than rifampicin, the results (not shown) are similar to those in Fig. 1 with one exception. For cells not previously irradiated with UV light, there is no detectable increase in the fast-sedimenting DNA (fractions 1–14) on incubation after γ-ray irradiation. DNA from cells irradiated with UV light and treated immediately with chloramphenicol (rather than 45 min later) sediments as does DNA from cells not previously irradiated with UV light, and shows no detectable repair during incubation after γ-ray irradiation.

Our interpretation is that the repair of DNA double-strand breaks requires proteins whose synthesis is induced by DNA damage. Cells treated with rifampicin to block mRNA synthesis or chloramphenicol to block protein synthesis cannot make these proteins. Irradiation of cells with UV light (which does not produce double-strand breaks) induces synthesis of the needed proteins during the following incubation; these enzymes are then available if protein synthesis is subsequently blocked and double-strand breaks are introduced with γ rays. The low level of repair in rifampicin-treated cells could be from any of a number of causes (e.g., nonoptimal drug concentration) and its presence does not affect the conclusion.

It is known that an active *recA* gene is required for repair of

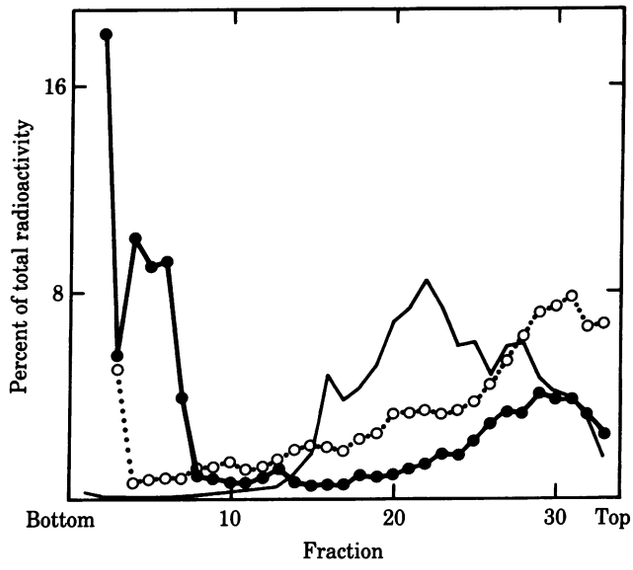

FIG. 1. Repair of DNA double-strand breaks in cells that cannot synthesize protein. *E. coli* AB2497 cells in exponential growth in K medium containing [^{14}C]thymine were chilled, washed, and suspended in fresh K medium containing nonradioactive thymine. One fraction of the culture was exposed to 10 J/m^2 of UV light, and the other fraction was not. After 45 min of incubation at 37°C, each fraction received rifampicin at 75 μg/ml, 10 min of additional incubation, and 12 kilorads of γ rays. ——, Sedimentation of DNA from cells given UV light and lysed immediately after γ-ray irradiation [the sedimentation of DNA from cells not receiving UV light and lysed immediately after γ-ray irradiation was not significantly different (data not shown)]; ●, sedimentation of DNA from cells exposed to UV light and incubated in K medium containing nonradioactive thymine for 45 min after γ-ray irradiation; ○, sedimentation of DNA from cells not exposed to UV light but incubated 45 min after γ-ray irradiation. These sedimentations were for 38.5 hr at 3760 rpm. Linear DNA sedimenting to fraction 15 has a M_r larger than 10^9. DNA sedimenting beyond this is in a more compact structure characteristic of DNA in unirradiated cells (ref. 3).

double-strand breaks and that the synthesis of *recA* protein is induced by UV light. Another agent that also induces synthesis of *recA* protein is nalidixic acid (18, 19). Fig. 2 shows that, when nalidixic acid is used as the inducing agent, there is only a low level of repair of double-strand breaks, very much less than when the inducing agent is UV light (Fig. 1). Thus, the induction of *recA* protein is not, by itself, sufficient to cause repair of double-strand breaks.

Incubation of cells treated with rifampicin but not UV light (Fig. 1) or chloramphenicol without UV light (data not shown) actually causes some degradation, as shown by the appearance of more slowly sedimenting DNA. Fig. 2 also shows the extensive degradation of DNA in cells that do not repair double-strand breaks. It may therefore be asked whether UV light induces the synthesis of proteins essential for repair of the breaks or of an inducible inhibitor of nucleases that reduces degradation of broken DNA before the repair enzymes can act.

To help answer this question, AB2497 cells were grown in aspartate medium (doubling time, 3 hr), so that there was only a little more than one genome per cell on average. Such cells repair only a small fraction of DNA double-strand breaks (3). We have hypothesized that repair needs a DNA template having the same base sequence as at the break; repair would occur only in those asparate-grown cells that have partially duplicated DNA. Fig. 3 shows the sedimentation of DNA from aspartate-grown cells after treatment with rifampicin and γ rays. Compared with cells not previously irradiated with UV light, previously irradiated cells show a low level of repair; there was a small DNA decrease in fractions 15–17 and a corresponding in-

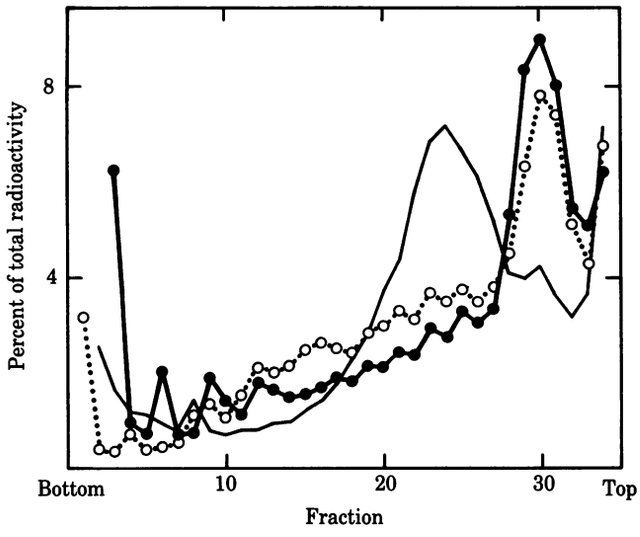

FIG. 2. Repair of DNA double-strand breaks in cells that cannot synthesize protein and have been pretreated with nalidixic acid. Exponentially growing AB2497 cells with [^{3}H]thymine were washed and suspended in K medium containing nonradioactive thymine. One fraction of the culture was treated with nalidixic acid at 40 μg/ml, and the other was not. The treatment with nalidixic acid stopped DNA synthesis and caused cell filamentation. Both fractions were incubated for 45 min; then, the cells were washed and suspended in fresh K medium containing nonradioactive thymine and rifampicin at 75 μg/ml. After 10 min of incubation, the cultures were given 12 kilorads of γ rays. ——, Sedimentation of DNA from cells treated with nalidixic acid and lysed directly after γ-ray irradiation [the sedimentation did not differ from that of DNA cells not treated with nalidixic acid (data not shown)]; ●, sedimentation of DNA from cells pretreated with nalidixic acid and incubated for 45 min at 37°C after γ-ray irradiation; ○, sedimentation of DNA from cells not pretreated with nalidixic acid but incubated for 45 min at 37°C after γ-ray irradiation. Sedimentation was for 45.25 hr at 3440 rpm.

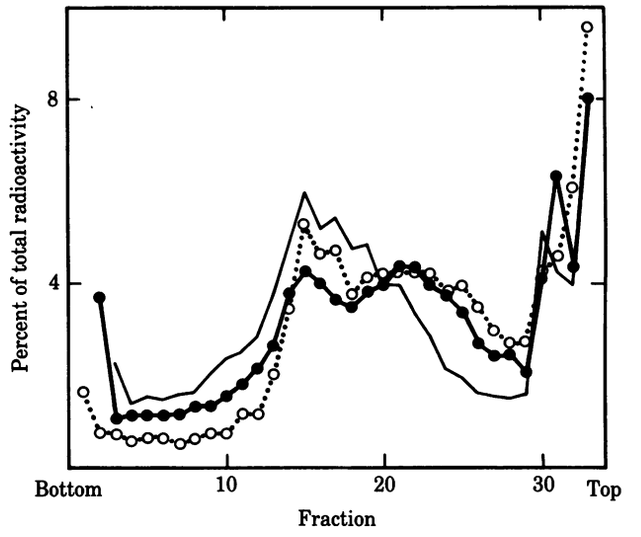

FIG. 3. Degradation of DNA after γ-ray irradiation of cells that cannot repair double-strand breaks. AB2497 cells were grown in aspartate medium containing [^{3}H]thymine for several generations; doubling time was 3 hr. After suspension in fresh aspartate medium containing nonradioactive thymine, one fraction of the culture was exposed to 10 J/m^2 of UV light and the other was not. Both fractions were incubated, treated with rifampicin, and irradiated with γ rays exactly as described in the legend to Fig. 1. ——, Sedimentation of DNA from cells exposed to UV light that were lysed immediately after γ-ray irradiation [sedimentation of DNA from cells not exposed to UV light was essentially the same (data not shown)]; ●, sedimentation of DNA from cells irradiated with UV light and incubated for 45 min at 37°C after γ-ray irradiation; ○, sedimentation of DNA from cells not exposed to UV light and incubated for 45 min at 37°C after γ-ray irradiation. Sedimentation was for 18 hr at 7400 rpm. Here the value of (rpm)^2 hr was almost twice that for the experiments described in Figs. 1 and 2, which accounts for the greater distance sedimented by the DNA from cells that had not been incubated.

crease in fractions 1–13. The important point is this: the similar amount of degraded DNA in fractions 20–29 for cells with and without pretreatment shows that an inhibitor of DNA degradation is not induced by UV irradiation of aspartate-grown cells. This cannot be ascribed to a general loss of *recA*-dependent functions in such cells because UV light induces Weigle mutagenesis in λ phage adsorbed to aspartate-grown AB2497 cells (unpublished results). We cannot exclude the possibility that specific *recA*-dependent functions, such as the synthesis of a nuclease inhibitor, are lost in the process of reducing DNA content per cell by growth in aspartate medium.

The mean sedimentation coefficient of the large DNA molecules in Figs. 1 and 2 decreases by a factor of almost 2 in a 45-min incubation, which corresponds to a decrease in molecular length of roughly a factor of 5. If this decrease were brought about by exonuclease attack of the broken ends, the enzymes would have to digest 80% of the DNA to small fragments. The sucrose gradient patterns in Figs. 1 and 2 show both the large DNA molecules and the small fragments produced by degradative processes. Incubation produces only modest increases in fragments in the top gradient fractions; thus, the degradation observed must be primarily due to endonuclease action.

For the cells in Fig. 3 that were not previously irradiated with UV light but were incubated for 45 min after γ-ray irradiation, a direct measurement was made of the acid-soluble fraction as described in *Materials and Methods*. Only 7% of the radioactivity was soluble, which supports the hypothesis that degradation is mostly due to endonucleases.

Colony-forming ability of cells treated in various ways was measured by suitably diluting the cells directly after various γ-ray exposures and plating (Table 1). Cells treated with agents that block protein synthesis are more sensitive to γ rays than untreated cells. They are not quite as sensitive as cells grown in aspartate medium. This may be in part because diluting out the drug for plating could allow some repair to take place; note that rifampicin seems slightly more effective than chloramphenicol (20). The small number (<2) of genomes in the cells grown in aspartate medium may also decrease survival in this case.

The sensitization of colony-forming ability by the drugs is

Table 1. Sensitivity to γ rays of colony-forming ability of *E. coli* AB2497 cells in exponential growth given various treatments and then irradiated

Treatment	Drug added just before γ irradiation	D_{37}*
None	None	25–28
None	Rifampicin at 75 μg/ml	4–6
UV at 10 J/m^2, 45 min of incubation	Rifampicin at 75 μg/ml	25–28
None	Chloramphenicol at 100 μg/ml	6–9
UV at 10 J/m^2, 45 min of incubation	Chloramphenicol at 100 μg/ml	25–28
Exponential growth in asparate medium	None	3†

* D_{37} is the number of kilorads required to reduce colony-forming ability of irradiated cells to 37% of that for cells not exposed to γ rays. The values given here are somewhat higher than those previously reported (3) because irradiation here is in K medium rather than buffer.

† Irradiation in buffer (from ref. 3).

removed effectively by previous irradiation with UV light and incubation, as has been reported (4–6).

DISCUSSION

Our results show that the repair of DNA double-strand breaks in *E. coli* cells is blocked by inhibitors of protein synthesis. Efficient repair of the breaks requires proteins that are synthesized in response to DNA damage.

One protein whose synthesis is greatly increased as a consequence of DNA damage is the *recA* protein (7, 8, 19, 21). The current picture (10, 14, 22, 23) is that DNA damage activates protease activity of the low levels of *recA* protein in normal cells, which cleaves the *lexA* protein, as shown by Little *et al.* (10). As the *lexA* protein behaves as a repressor for the *recA* locus (10), there is a consequent increase in synthesis of *recA* protein, which leads to even more rapid cleavage of *lexA* protein.

The need for *recA* protein in the repair of DNA double-strand breaks (3) could relate to the apparent need for a DNA duplex having the same base sequence as the DNA at the double-strand break (3). The *recA* protein could partially unwind the duplex DNA (24) and also catalyze base pairing of the broken ends with homologous double-helical DNA (24–30).

More than the *recA* protein seems to be needed for repair, because cells induced to synthesize *recA* protein by nalidixic acid show little repair of double-strand breaks (Fig. 2). Witkin (8) has pointed out that *recA-lexA*-dependent functions fall in two classes. Some functions, such as synthesis of *recA* protein, induction of λ prophage, and cell filamentation, are induced by DNA damage. They are also efficiently induced by treatments that stop DNA replication forks, such as nalidixic acid, thymine starvation, and heating *dnaB(ts)* mutants to the nonpermissive temperature. Stopped replication forks, however, seem far less effective than DNA damage in inducing other functions. For example, *E. coli* cells starved for thymine show little mutagenesis (8). This could reflect a requirement for both induced repair enzymes and damage in the gene in which mutation is being observed. In Weigle mutagenesis (enhanced mutation in UV-irradiated λ phage when the host cells are treated), there are lesions in the phage gene being mutated. However, Weigle mutagenesis is not found when the host cells are treated with nalidixic acid although it is strongly induced when the host cells are irradiated with UV light (31). The induction of repair of DNA double-strand breaks falls in the same class as mutagenesis and Weigle mutagenesis.

One possibility is that DNA damage produces at least one extra inducing signal in addition to the signal from stopped replication forks; indeed, the latter signal could conceivably come from replication forks stopped at damage sites. As discussed above, Kenyon and Walker (9) found that the various sites of transcription activated by DNA damage differ in such aspects as lag time for expression, which suggests different triggers for activation. It would be interesting to know which of these sites are induced when DNA replication forks are stopped by nalidixic acid, for example.

It is also possible that stopping DNA replication forks produces changes in metabolic patterns that, in some other way, inhibit the induction of certain *recA-lexA*-dependent functions.

Another recognized *recA-lexA*-dependent function induced by DNA damage in *E. coli* cells is an inhibition of DNA degradation (8, 32). From Figs. 1–3, the simplest explanation is that DNA degradation is inhibited when the repair of double-strand breaks is induced—i.e., the repair enzymes are, in effect, the inducible inhibitors.

Our results show that the initial degradation is largely due to endonucleases. It is easy to understand how exonucleases could degrade DNA that has double-strand breaks by attacking the free ends, but it is less obvious how an endonuclease would identify the DNA to be degraded. One possible identifying characteristic of DNA that has double-strand breaks is the lack of supercoiling. The gyrase responsible for supercoiling *E. coli* DNA can, under certain conditions, introduce DNA double-strand breaks (33, 34). Perhaps this enzyme plays some role in forming the double-strand breaks during incubation.

We are deeply grateful to Judith Aronson for her expert help. This research was supported by Contract AC02-76EV03571 with the U.S. Department of Energy and by National Institutes of Health Grant GM 25260.

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