Proc. Nat. Acad. Sci. USA
Vol. 70, No. 4, pp. 1064–1068, April 1973

Repair of DNA Containing Interstrand Crosslinks in *Escherichia coli*: Sequential Excision and Recombination

(genetic recombination/psoralen)

RONALD S. COLE*

Yale University, Department of Radiology, Department of Molecular Biophysics and Biochemistry, New Haven, Connecticut 06520

Communicated by Norman H. Giles, January 26, 1973

ABSTRACT **The repair of DNA containing interstrand crosslinks induced by psoralen-plus-light in *E. coli* cells has been investigated. During a 30-minute incubation after psoralen-plus-light treatment, crosslinks were excised and the cellular DNA was cut into discrete pieces. The molecular weight of these pieces corresponds to about twice the single-strand distance between crosslinks, as measured by sedimentation velocity in alkaline sucrose. During further incubation, these DNA fragments were covalently joined into high molecular weight DNA. This joining did not occur in cells carrying a mutation at *recA*; in these strains the DNA was further degraded to smaller polynucleotides and acid-soluble material. The possibility that repair of crosslinked DNA involves strand exchanges between homologous duplexes was investigated. Cells were grown in ^{13}C,^{15}N-containing medium for several generations, then switched to medium of normal density that also contained [^{3}H]thymidine for about 0.5 generation. After the crosslinking treatment, the cells were incubated in medium of normal density in order for repair to occur. The DNA was extracted and centrifuged in alkaline CsCl density gradients, where the light and heavy strands were separated. Molecules of intermediate density that contained ^{3}H accumulated during repair in wild-type cells, but not in control cells or treated *recA*$^-$ cells. After molecular weight reduction of the intermediate-density DNA, the ^{3}H could be separated from the heavy strands, demonstrating that covalent joining between heavy and light strands of homologous duplexes accompanies repair. A mechanism involving sequential excision and genetic recombination is proposed for the repair of DNA containing interstrand crosslinks.**

Covalent linkages between the complementary strands of DNA *in vitro*, or in cells, are detected after treatment with several agents, including nitrous acid (1), nitrogen or sulfur mustards (2), mitomycin C (3), or psoralen-plus-light (4–6). Crosslinks are also formed in low yields after exposure to ultraviolet light (7), and ionizing radiation (8). Such interstrand crosslinks prevent the complementary strands from diffusing apart after exposure to denaturation conditions, and a crosslinked DNA duplex is therefore reversibly bihelical (1).

Crosslinks that remain in cellular DNA should block the separation of complementary strands during semiconservative replication and, if left unrepaired, might prevent cells from dividing normally. Cells, however, survive treatments producing many crosslinks in their DNA, suggesting that DNA that contains crosslinks may be successfully repaired. Animal cells growing in tissue culture maintain their ability to grow and divide after treatments with bifunctional alkylating agents that produce thousands of interstrand crosslinks per cell (9). Bacteria also retain their colony-forming ability after exposure to crosslinking agents. Wild-type *Escherichia coli* cells survive treatments producing about 55–70 crosslinks per genome, and strains defective in genetic recombination (*recA*$^-$), and in excision repair (*uvr*$^-$), survive after the introduction of 5–20 crosslinks per genome (10, 11). However, a double mutant, *uvr*$^-$ *recA*$^-$, is killed by treatments producing not more than 1 crosslink per genome (10). Evidently, excision and genetic recombination contribute to recovery from damages induced by crosslinking agents in bacterial cells.

A major discovery by Lawley and Brookes (11, 12) and by Kohn, Steigbigel, and Spears (13) was the finding that crosslinks appear to be removed from the DNA of *E. coli* cells, and that this process is controlled by the *uvrB* gene. This removal was demonstrated by several techniques, which include loss of the reversibly bihelical property, release of the crosslinking residue into acid-soluble material, and changes in the alkaline sucrose sedimentation properties (14). The excision of crosslinks from the DNA of mammalian cells in tissue culture has also been demonstrated by Reed and Walker (15). In these experiments, the rate of crosslink removal was considerably faster than the release of the crosslinking residue.

It is also known that crosslinking agents induce extensive genetic exchanges in eukaryotic cells. Morpurgo (16) and Holliday (17) have shown that nitrogen mustard and mitomycin C induce crossing-over in fungi and yeast. More recently, mitomycin C has been shown to induce genetic exchanges in Drosophila (18, 19). Most striking, however, are the nonsister homologue exchanges reported by Shaw and Cohen (20), German and La Rock (21), and Evans and Scott (22). These exchanges are detected at metaphase in the cell division cycle after treatment with mitomycin C or nitrogen mustard of human leukocytes or fibroblasts.

A covalent link between the complementary DNA strands necessarily damages bases in both strands at nearly the same point along the helical axis. It has been difficult to visualize how such a defect might be corrected by cellular repair systems responsible for maintaining the integrity of the DNA. Several possible mechanisms for the repair of crosslinked DNA have been suggested, but none are satisfactory when examined in detail. For example, repair might involve deletion of a duplex segment containing the crosslink (19). This process would permit separation of the strands during the next round of replication, but would appear to cause the loss of at least

Abbreviation: psoralen, 4,5′,8-trimethylpsoralen.

* Present address: Department of Biochemistry and Department of Microbiology, University of Georgia, Athens, Ga. 30601.

one base pair for each crosslink repaired. Kohn, Steigbigel, and Spears (13) and Reed and Walker (15) have suggested another mechanism, in which one arm of the crosslink is first excised from one strand and the resultant gap is filled by repair synthesis. A difficulty with this mechanism is the proposed use of a single-stranded region still containing a damaged base (one arm of the partially excised crosslink) as a template for synthesis of an intact complementary strand. Rupp and Howard-Flanders (23) have shown that damaged bases remaining in template DNA prevent synthesis of intact daughter strands. During replication, a gap is thought to be left in the daughter strand at the position of each dimer in the template strand. A partially excised crosslink may be structurally analogous to the dimer and daughter-strand gap, which is relatively stable (23, 24) in cells, and is evidently corrected by recombinational events (25).

All known crosslinking agents produce several different structural damages in DNA (5, 11, 12, 26). An additional complication is the known instability to heat, or alkali, of crosslinks formed by alkylating agents, which are spontaneously released by depurination events (12). The psoralen-plus-light reaction is highly specific for native DNA, and the induced crosslinks are stable to heat and alkali (4, 5, 27). This reaction also induces monofunctional adducts to pyrimidine bases in DNA at about a three-times higher yield than of crosslinks obtained (5). The biological significance of these monoadducts remains to be demonstrated, although some experiments on the inactivations of selected biological functions in *E. coli* suggest that crosslinks, rather than monofunctional adducts, are primarily responsible for the sensitizing action of psoralen-plus-light (10).

Taking advantage of the desirable properties of the psoralen-plus-light reaction, the repair of crosslinked DNA in *E. coli* cells has been investigated further. In these studies, it was found that crosslinked DNA undergoing repair is first cut into discrete pieces by excision enzymes. Subsequent joining of these pieces into high molecular weight DNA is accompanied by joining of strands from homologous duplexes, and is controlled by the *recA* gene.

MATERIALS AND METHODS

Bacterial Strains. *E. coli* K12 derivatives used are: W3110 *thyA tlr su* Str^S/F^- (obtained from Dr. John Cairns), and KL163 *recA1 thyA drm nalA* Hfr KL16 (obtained from Dr. K. Brooks Low).

Media. For the sedimentation studies, cells were grown in K medium (28) (salts, casamino acids, glucose) with thymidine added to 2 μg/ml. Bacterial DNA was labeled with heavy isotopes by growth in a medium containing: 0.1 M Tris·HCl (pH 7.4); 10 mM NaCl; 10 mM KCl; 2 mM $MgSO_4$; 0.2 mM $CaCl_2$; 0.01 μg of thiamine per ml; 1.0 mg of $^{15}NH_4Cl$ per ml (96% atom purity); 0.5 mg of [^{13}C]glucose (55% atom purity) per ml; 10 mM KH_2PO_4; and 2 μg of thymidine per ml.

Psoralen-Plus-Light Treatment. This treatment and procedures used in the sucrose gradient sedimentations are described in Fig. 1. The number-average molecular weight of the DNA in these distributions was estimated as described by Rupp and Howard-Flanders (23). The experimental format used to search for strand exchanges by the use of density isotopes is described in Fig. 2.

RESULTS

The DNA in *E. coli* strains W3110 (wild type) and KL163 (*recA1*) was labeled uniformly by growth in the presence of [^{3}H]thymidine, then treated with psoralen-plus-light. In earlier experiments done under similar conditions, a 10-min exposure to 360-nm light produced an average of one crosslink per 11×10^6 (single-strand) molecular weight, or about 114 crosslinks and 350 monoadducts per *E. coli* genome (4, 5, 10). Fig. 1 shows alkaline sucrose sedimentation profiles of the labeled *E. coli* DNA before and after the crosslinking treatment, and after 30 and 90 min of incubation after irradiation. Unirradiated control DNA sedimented as expected, with the position of the radioactivity peak corresponding to a molecular weight of about 150×10^6. After irradiation, the DNA sedimented even further. This increased sedimentation velocity is apparently due to the higher molecular weight of the covalently linked strands released during lysis (14), and to the unusual sedimentation behavior of DNA duplexes containing many crosslinks (27). After the 30-min incubation,

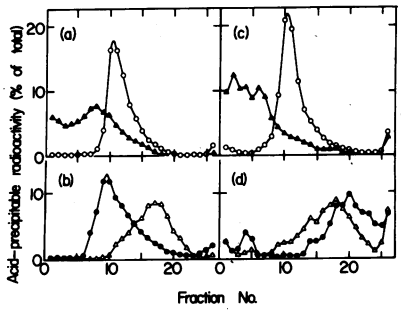

FIG. 1. Alkaline sucrose sedimentation profiles of DNA released from wild-type and *recA*$^-$ cells exposed to psoralen-plus-light, and incubated in growth medium after the crosslinking treatment. Data shown in this figure demonstrate that cellular DNA containing psoralen photoproducts and crosslinks is cut into discrete pieces, and that the subsequent joining of these pieces into high molecular weight DNA occurs in *recA*$^+$, but not in *recA*$^-$, cells. (*a*) and (*b*), sedimentation profiles obtained from wild-type cells; (*c*) and (*d*) comparable data for *recA*$^-$ cells. Sedimentation is from *right* to *left* and the *bar* over fraction 15 shows the position to which ^{14}C-labeled DNA (added as an internal molecular weight marker) from T2 phage sedimented. The symbols denote: O——O, unirradiated; ▲——▲, 10 min of psoralen-plus-light; △——△, irradiation followed by 30 min of incubation; ●——●, irradiation followed by 90 min of incubation. The total number of acid-precipitable counts in each gradient in the above order for W3110 is: 8×10^3, 7.5×10^3, 6.8×10^3, and 4.7×10^3, and for KL163 *recA* is: 55×10^3, 55×10^3, 31×10^3, and 4.3×10^3. Wild-type and *recA*$^-$ strains of *E. coli* were grown in K medium containing [^{3}H]thymidine (2 μg/ml). 4,5′,8-trimethylpsoralen (17 μg in 10 μl of ethanol per ml of medium) was added; the cells were chilled and kept at 0° for 30 min, then irradiated 10 min with 360-nm light (5, 6). Excess [^{3}H]thymidine and psoralen were removed by several washings in chilled saline buffer. After the desired incubation times, cells were chilled to 0° and converted to spheroplasts with lysozyme–EDTA (23); about 10^7 spheroplasts were placed in a 0.1-ml overlayer of 0.5 N NaOH on a 5-ml 5–20% alkaline sucrose gradient (pH 12.4). Gradients were then centrifuged at 40,000 rpm for 45 min at 20° in a Spinco SW50.1 rotor; tube bottoms were pierced and 25.5 10-drop fractions were collected on filter discs. Discs were washed with cold 5% trichloroacetic acid, ethanol, and acetone, dried, and counted in vials containing scintillation fluid.

the DNA of both strains sediments more slowly than does control DNA. The number-average molecular weight of these

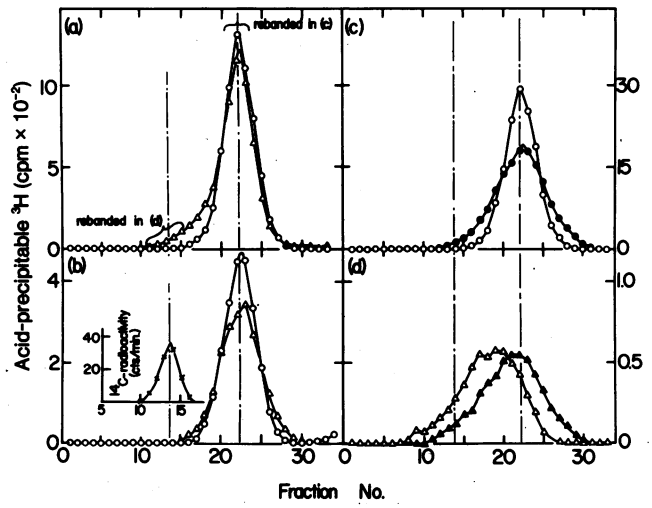

FIG. 2. Density profiles in alkaline CsCl of DNA from wild-type and *recA*⁻ cells labeled with density isotopes, treated with psoralen-plus-light, and incubated in growth medium. Data shown in this figure demonstrate that the joining of strands carrying different density labels occurs in *recA*⁺, but not *recA*⁻, cells exposed to psoralen-plus-light. Cells were grown in ^{13}C,^{15}N-containing medium and then transferred to light medium (see Fig. 1) for 5 min to exhaust the intracellular pools. [^{3}H]-Thymidine was added; after 20 min at 37°, the cells were washed and resuspended in K medium containing 20 μg/ml of thymidine for an additional 5 min of incubation. Psoralen was added; the cells were chilled, irradiated for 10 min with 360-nm light, and washed as described in Fig. 1. After a 60-min incubation at 37°, the cell suspensions were chilled, converted to spheroplasts with lysozyme–EDTA, and lysed by the addition of Sarkosyl 97 (Geigy Industrial Chemicals) to 0.5% final concentration. Cell lysates were added to alkaline CsCl solutions to give final volumes of 5.0 ml having pH = 13, EDTA = 0.03 M, and η = 1.4032. These solutions were centrifuged for 60 hr at 35,000 rpm in a Ti50 rotor at 20° in a Beckman L2-65B ultracentrifuge. 35 Fractions of equal volume were collected from the pierced tube bottoms into plastic vials, and a 25-μl aliquot of each fraction was placed on a filter disc, which was subsequently acid-washed and counted 50 min for radioactivity, as described in Fig. 1. Density alignment of the gradients was confirmed by the position at which ^{13}C,^{15}N,^{14}C-DNA from *E. coli* (added as a density marker to each gradient) banded, as shown in the inset in (*b*). The *broken lines* through fractions 13–14 and 22, respectively, show the positions at which fully heavy and normal-density DNA strands band when centrifuged to equilibrium. The average single-strand molecular weight of molecules in such preparations is about 1 to 2 × 10^7, as measured by alkaline sucrose sedimentation velocity. (*a*) Density profiles of DNA from unirradiated wild-type cells (O——O) and from cells exposed for 10 min to psoralen-plus-light and incubated 60 min before lysis (Δ——Δ). The density distribution of DNA from control cells is not appreciably changed by the 60-min incubation (not shown). (*b*) Corresponding data for *recA*⁻ cells, except that both control (O——O) and irradiated (Δ——Δ) cells were incubated for 90 min before lysis and centrifugation in CsCl. (*c*) DNA in fractions 21–23 from unirradiated wild-type cells [(O——O in (*a*)] was centrifuged (●——●) or reduced to about 10^6 molecular weight (by boiling for 70 min) and centrifuged (O——O). (*d*) DNA from fractions 11–15 from irradiated and incubated wild-type cells [Δ——Δ in (*a*)] was centrifuged (Δ——Δ) or reduced in molecular weight and centrifuged (▲——▲).

distributions is 20 to 24 × 10^6, or about twice the average distance between crosslinks. Experiments with other incubation times and light exposures indicate that the molecular weight is at a minimum at about this time, and that the length of the fragments is nearly equal to twice the average single-strand spacing of crosslinks. Strand cutting was not detected in strains carrying mutations at *uvrA* or *B* (data not shown).

As shown in Fig. 1, further incubation results in the joining of these discrete pieces into fast-sedimenting DNA strands of high molecular weight in wild-type, but not *recA*⁻, cells. In the *recA* strain, these DNA fragments are further degraded into smaller pieces and into acid-soluble material. Evidently, the joining of these fragments is dependent upon the *recA*⁺ function. This surprising result, and the genetic and cytological evidence described in the *Introduction*, suggested that strand exchanges between homologous duplexes may play an important role in the repair of crosslinks. The covalent joining of strands from DNA duplexes undergoing genetic recombination has been successfully demonstrated by the use of density labels and radioactive isotopes (25, 28–30). In order to test whether strand exchanges occur in cells treated with psoralen-plus-light, an experiment of a second type was conducted.

Wild-type and *recA*⁻ strains were grown in medium containing heavy isotopes, transferred to light medium, and incubated 5 min to exhaust the intracellular pools. [^{3}H]-Thymidine was added and the cells were incubated for 20 min and then transferred to nonradioactive light medium for 5 min. Replication of a fully heavy duplex in the ^{3}H–light medium should yield two sister duplexes of hybrid density (31). A heavy strand in one duplex will thus have the same 3′- to 5′-direction as the light strand in its sister duplex (as shown in the top diagram in Fig. 3), although this generalization may not apply to all homologs in a cell. Psoralen was then added and the cell suspensions were chilled and irradiated for 10 min with 360-nm light. Irradiated cells were then incubated in light, nonradioactive medium for 60 or 90 min to permit strand cutting, removal of crosslinks, and subsequent strand joining to be completed. The lysate from these cells was centrifuged to equilibrium in alkaline CsCl density gradients, where the complementary heavy and light strands separate and band at positions of their respective densities, as shown in Fig. 2. As expected, the ^{3}H radioactivity, corresponding to light strands from untreated cells, bands at the light-density positions (see Fig. 2*a*). However, after crosslinking and incubation of the wild-type cells for 60 min, the ^{3}H-radioactivity peak develops a tail extending into the fully heavy region of the gradient. This heavy tail is not seen after incubation for 30 min, when excision appears to be completed, as judged by the decrease in molecular weight (Fig. 1*b*). The heavy tail begins to appear during a 30- to 45-min incubation after psoralen-plus-light treatment (data not shown). Radioactivity did not shift into the intermediate density region after treatment and incubation of cells carrying *recA*⁻. [^{3}H]-DNA from the treated *recA*⁻ strain bands in a slightly broader peak, possibly because of the lower molecular weight of the unjoined fragments produced by excision enzymes and degradation.

The heavy tail in Fig. 2*a* could be the result of: (*a*) covalent joining between heavy and light strands of sister duplexes, (*b*) isotope mixing through breakdown, followed by rein-

corporation, or (*c*) repair synthesis after the excision of damaged bases, or (*d*) a combination of these processes. In order to distinguish among these possibilities, the material in fractions 21–23 (light-density region) of the unirradiated control of Fig. 2*a* was directly centrifuged in alkaline CsCl, or was reduced from 10^7 to about 10^6 molecular weight by boiling for 70 min, then centrifuged. As shown in Fig. 2*c*, reduction in molecular weight broadens the density profile, but does not shift the peak position. The heavy-density tail of DNA from irradiated and incubated wild-type cells (pooled fractions 11–15) was also centrifuged as described above. This material banded at intermediate densities, but after molecular weight reduction, the peak position shifted toward that of light-density DNA, as shown in Fig. 2*d*. Evidently, part of the ^3H radioactivity recovered from intermediate- and heavy-density regions consists of discrete segments of light, ^3H-labeled polynucleotide strands covalently attached to heavy DNA strands. In a similar experiment, radioactivity from fractions 16–17 remained at intermediate densities when centrifuged, but the peak position was not substantially shifted after the reduction in molecular weight (not shown).

These results demonstrate that the radioactive material having an intermediate density, formed in wild-type cells treated with psoralen-plus-light, is of two types: (*i*) heavy molecules covalently linked to segments that contain both ^3H and light isotopes in lengths greater than 3000 bases (about 10^6 molecular weight), and (*ii*) intermediate-density molecules in which the ^3H was not readily detached from the heavy isotopes. Molecules in the second class could be composed of many short segments (less than 1000 bases each) of heavy and light strands, or might be produced by DNA breakdown followed by reincorporation of isotopes mixed in the intracellular pools. Excision of monoadducts, followed by repair synthesis, might also contribute to this class of molecules. The contribution from this source should be quite small compared to the relatively large amount of intermediate-density DNA detected. By comparison with the repair of pyrimidine dimers induced by 254-nm light, about 20 DNA bases are thought to be removed for each dimer excised, and the resultant gap is then filled by repair synthesis (32, 33). With this estimate, excision–repair of the 400 psoralen adducts to single strands should replace only about 0.2% of the original DNA bases by repair synthesis. This small amount would not be expected to appreciably change the density of the originally labeled strands.

If repair of each of the 114 crosslinks per genome involves an obligatory strand exchange, and the length of the exchanged region is between 3,000 and 15,000 bases (as estimated from the molecular weight reduction and recentrifugation results), 5–25% of the ^3H radioactivity should become associated with heavy DNA strands. In apparent agreement with this estimate, 9% of the total radioactivity was shifted into the intermediate-density region and 5% was shifted into the fully heavy region after psoralen-plus-light treatment and repair in wild-type cells.

DISCUSSION

The DNA of wild-type *E. coli* cells containing monoadducts and interstrand crosslinks induced by psoralen-plus-light is evidently repaired by reactions that involve a partial excision of the crosslink followed by strand exchanges between homologous duplexes, controlled by the *recA* gene. Results reported in this paper extend the findings of Kohn, Steibigel, and Spears (13) and Venitt (14), who suggested that crosslinks formed by bifunctional alkylating agents are excited from cellular DNA, and that this removal is controlled by the *uvr*B gene. After psoralen-plus-light exposure, it was found that, in addition to crosslink excision, cellular DNA undergoing repair is cut into discrete segments having a single-strand molecular weight about equal to twice the single-strand spacing of the induced crosslinks. These DNA fragments may be the product of excision repair enzymes making two incisions at each crosslink, as shown in the first step in Fig. 3. If the resulting gap at each crosslink remains open after this partial excision, the DNA released in alkaline sucrose should sediment as segments having an average single-strand length of about twice the crosslink spacing, as was observed in these studies. After partial excision, the resulting gap would differ from that thought to be formed by excision of a pyrimidine dimer in one DNA strand, as the partially excised crosslinking residue would be still attached to one side in a single-stranded region. This intermediate, however, may be structurally analogous to the dimer and daughter-strand gap produced after replication of DNA containing unexcised pyrimidine dimers, as proposed by Rupp and Howard-Flanders (23). A partially excised crosslink remaining in a single-stranded region might thus be expected to block filling of the gap by repair synthesis, and this excision-produced gap would be stable in cells, as was observed. During extended incubation,

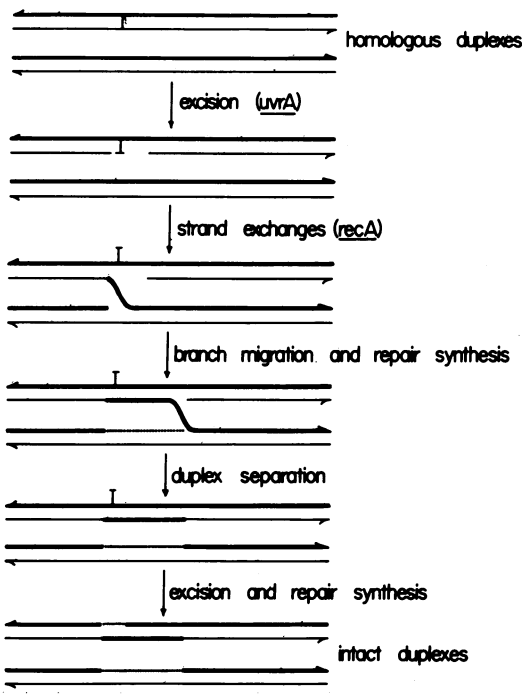

FIG. 3. One possible mechanism for the repair of crosslinked DNA is shown here to illustrate the types of products and intermediates involved, and to indicate steps controlled by the *uvr* and *recA* genes. The sequence proceeds as follows: Two incisions are first made near each crosslink. A nuclease then widens the gap, exposing a single-stranded region. Ensuing strand exchanges between homologous duplexes insert an intact base sequence complementary to the strand still carrying the partially excised crosslinking residue. When the twin helical DNA structure is restored, the remaining arm of the crosslink is excised. *Dashed lines* indicate regions of repair synthesis.

these fragments became joined into high molecular weight DNA strands, and this joining is apparently controlled by the *recA* gene. Recombinational events, detected as strand exchanges between sister duplexes of hybrid density, occur during the joining of the polynucleotide fragments as described above, and these strand exchanges are also controlled by the *recA* locus.

One possible mechanism for the repair of crosslinks is shown in Fig. 3, and proceeds as follows. Incisions are first made in one strand of a crosslinked duplex, on both sides of one arm of the crosslink. As described, this process takes place so that the complementary strands are no longer linked together. A nuclease then widens the gap, exposing a single-stranded region still containing one arm of the crosslinking residue. Such an intermediate may then enter genetic recombination pathways controlled by the *recA* gene. Ensuing strand exchanges between homologous duplexes insert an intact base sequence complementary to the strand containing the crosslinking residue still attached on one side. Now that the twin helical structure is restored, excision enzymes presumably can remove the remaining arm of the crosslink. Repair synthesis and strand joining complete reconstruction of an intact duplex.

Apparently, wild-type *E. coli* cells correct crosslinking defects in their DNA by a repair sequence involving partial excision followed by strand exchanges between homologous duplexes. As bacterial cells normally contain more than one copy of their haploid genome, strand exchanges between these duplexes could restore the intact base sequence to a duplex region containing damages in both strands at the same point along the helical axis. This type of mechanism may also be operative in eukaryotic cells. Other crosslinking agents are known to induce genetic exchanges in fungi, yeast, Drosophila, and human cells. These types of genetic and cytological evidence are consistent with a repair sequence involving nonsister homologue exchanges in eukaryotic cells treated with crosslinking agents.

I am indebted to Prof. Paul Howard-Flanders for his intensely stimulating discussions and advice, and thank Deborah Levitan for excellent technical assistance. This work was supported by United States Public Health Service Grants CA 06519 and GM 11014.

1. Geiduschek, E. P. (1961) *Proc. Nat. Acad. Sci. USA* **47**, 950–955.
2. Brookes, P. & Lawley, P. D. (1961) *Biochem. J.* **80**, 496–503.
3. Iyer, V. N. & Szybalski, W. (1963) *Proc. Nat. Acad. Sci. USA* **50**, 355–362.
4. Cole, R. S. (1970) *Biochim. Biophys. Acta* **217**, 30–39.
5. Cole, R. S. (1971) *Biochim. Biophys. Acta* **254**, 30–39.
6. Dall'acqua, F., Marciani, S. & Rodighiero, G. (1970) *FEBS Lett.* **9**, 121–123
7. Marmur, J. & Grossman, L. (1961) *Proc. Nat. Acad. Sci. USA* **47**, 778–787.
8. Lett, J. T., Stacey, K. A. & Alexander, P. (1961) *Rad. Res.* **14**, 349–362.
9. Crawthorn, A. R. & Roberts, J. J. (1966) *Nature* **211**, 150–153.
10. Cole, R. S. (1971) *J. Bacteriol.* **107**, 846–852.
11. Lawley, P. D. & Brookes, P. (1965) *Nature* **206**, 480–483.
12. Lawley, P. D. & Brookes, P. (1968) *Biochem. J.* **109**, 433–447.
13. Kohn, K., Steigbigel, N. & Spears, C. (1965) *Proc. Nat. Acad. Sci. USA* **53**, 1154–1159.
14. Venitt, S. (1968) *Biochem. Biophys. Res. Commun.* **31**, 355–360.
15. Reid, B. D. & Walker, I. G. (1969) *Biochim. Biophys. Acta* **179**, 179–188.
16. Morpurgo, G. (1963) *Genetics* **48**, 1259–1263.
17. Holliday, R. (1964) *Genetics* **50**, 323–335.
18. Schewe, M., Suzuki, D. & Erasmus, U. (1971) *Mutat. Res.* **12**, 255–267.
19. Schewe, M., Suzuki, D. & Erasmus, U. (1971) *Mutat. Res.* **12**, 269–279.
20. Shaw, M. & Cohen, M. (1965) *Genetics* **51**, 181–190.
21. German, J. & La Rock, J. (1969) *Tex. Rep. Biol. Med.* **27**, 409–418.
22. Evans, H. J. & Scott, D. (1969) *Proc. Roy. Soc. Ser. B.* **173**, 491–512.
23. Rupp, W. D. & Howard-Flanders, P. (1968) *J. Mol. Biol.* **31**, 291–304.
24. Cole, R. S. (1971) *J. Bacteriol.* **106**, 143–149.
25. Rupp, W. D., Wilde, C. E., Reno, D. & Howard-Flanders, P. (1971) *J. Mol. Biol.* **61**, 25–44.
26. Fishbein, L., Flamm, W. G. & Falk, H. L. (1970) in *Chemical Mutagens* (Academic Press, New York), pp. 25–26.
27. Cole, R. S. & Zusman, D. (1970) *Biochim. Biophys. Acta* **224**, 660–662.
28. Weigle, J., Meselson, M. & Paigen, K. (1959) *J. Mol. Biol.* **1**, 379–386.
29. Oppenheim, A. B. & Riley, M. (1967) *J. Mol. Biol.* **28**, 503–511.
30. Meselson, M. (1964) *J. Mol. Biol.* **9**, 734–745.
31. Meselson, M. & Stahl, F. W. (1958) *Proc. Nat. Acad. Sci. USA* **44**, 671–679.
32. Pettijohn, D. E. & Hanawalt, P. C. (1964) *J. Mol. Biol.* **9**, 395–410.
33. Ley, R. D. & Setlow, R. B. (1972) *Biophys. J.* **12**, 420–431.