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Effect of Photoreactivation on the Filling of Gaps in Deoxyribonucleic Acid Synthesized After Exposure of *Escherichia coli* to Ultraviolet Light

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Daughter-strand gaps in deoxyribonucleic acid (DNA) synthesized after exposure of excision-deficient *Escherichia coli* to ultraviolet light are filled during subsequent incubation in buffer, and the rate of filling is increased when the incubation in buffer is carried out in the presence of 360-nm light. It is concluded that daughter-strand discontinuities are prevented from being rapidly sealed in the dark not because of some structural feature of the daughter-strand but because of the presence of a pyrimidine dimer on the opposite (parental) strand. "Photoreactivation-stimulated gap filling" is dependent on the *polA*$^+$ and *recA*$^+$ but not the *exrA*$^+$ genes. It is suggested that the removal of the dimer allows gap-filling by DNA polymerase I and polynucleotide ligase. The *recA*$^+$ gene may be needed at a very early stage, possibly for gap stabilization.

When bacteria unable to excise pyrimidine dimers from their deoxyribonucleic acid (DNA) are exposed to doses of ultraviolet (UV) light, DNA synthesis is slowed down and the newly synthesized DNA contains gaps (10) of around 1,000 nucleotides in length (7). There is roughly one such gap for each pyrimidine dimer passing through the replication complex (10), and there is genetic evidence that the gaps are opposite the dimers (3). Under normal growth conditions these daughter-strand gaps are filled with both new DNA (R. D. Ley and R. B. Setlow, Biophys. Soc. Abstr., p. 151a, 1972) and pieces of parental DNA as a result of recombination exchanges (11). This process is often referred to as "recombinational repair" and it requires the *recA*$^+$ gene.

We became interested in the nature of these daughter-strand gaps; in particular we asked whether the gap is a simple one, possibly with 5'P and 3'OH ends, which could be filled in if the dimer were not there by repair replication using DNA polymerase I and polynucleotide ligase, or a more complex one with some unknown structural feature which commits it to a requirement for a recombinational exchange. These two possibilities should be distinguishable by following gap filling after removal of the dimer by photoreactivation. By using repair-deficient strains we have attempted to define and compare the genetic requirements for post-replication repair in the dark and in the light.

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MATERIALS AND METHODS

Bacteria. The following near isogenic bacterial strains were used (all deficient in excision repair): *Escherichia coli* WP2 *uvrA* (Hill) (6), CM611 (*uvrA exrA*) (2), WP100 (*uvrA recA*), and WP12 (*uvrA exrA polA1*) (the latter two kindly supplied by E. M. Witkin).

Bacteria were grown to about 2×10^8/ml in M9 minimal medium plus 1% casein hydrolysate and 20 μg of tryptophan per ml except for WP12 which required similarly supplemented Davis and Mingioli minimal medium (4). The bacteria were exposed to UV light and grown for a further period in the same medium supplemented with 50 μg of deoxyadenosine per ml, 0.5 μg of thymine per ml and 20 μCi of [*methyl*-^{3}H]thymidine. The bacteria were then membrane filtered, washed with 10 volumes of prewarmed buffer, and suspended (except where otherwise stated) in phage buffer (1), in which the postirradiation degradation of DNA typical of *exrA* and *recA* strains was drastically reduced. The subsequent change in size of the DNA which had been synthesized during the 10-min pulse was observed by alkaline sucrose centrifugation, after subsequent incubation either in the dark or in the presence of light from two Philips Black Light tubes, 10 cm away. Maximum photoreversal of survival occurred by about 20 min under these conditions. Pulse-label periods were adjusted so that equal amounts of label uptake occurred in samples synthesizing DNA at different rates. In addition, the labeling period extended over at least 1/6th of a replication

cycle. These two precautions were to avoid "end error artefact" (8).

RESULTS

With WP2 *uvrA* incubation in growth medium, repair of daughter-strand gaps was almost complete within 15 min in the dark; it was in fact too fast to be able to show a photoreactivation effect with any confidence. In buffer at room temperature more repair had occurred after 10 min in the light than in the dark. After 20 min, repair was complete in both light and dark incubated cultures (data not shown). These results suggest that removal of pyrimidine dimers by photoreversal permits a more rapid repair of gaps with no requirement for growth medium. Even in the dark, however, gap filling occurred in buffer, presumably by recombination repair.

A clearer distinction between gap filling in the dark and in the light was obtained with CM611 (*uvrA exrA*). This strain possesses a limited capacity in buffer for filling in of daughter-strand gaps formed after low doses of UV. Above about 40 ergs/mm^2, however, little or no gap filling is seen. In the experiment shown in Fig. 1 there was little increase in molecular weight during the 30 min in buffer in the dark after 50 ergs/mm^2 (Fig. 1B) but in the presence of light, repair was complete within 30 min (Fig. 1C). Photoreversibility of survival was also observable under these conditions (Table 1).

Repair of most parental strand breaks induced by ionizing radiation is rapid and occurs in buffer at room temperature. Town et al. (12, 13) have shown that it is brought about by DNA polymerase I and, probably, polynucleotide ligase. Similar physiological characteristics are observed by us for gap filling after photoreversal of dimers, suggesting that this process might also be carried out by these enzymes. If this were the case, it should be dependent on the *polA*$^+$ gene. The DNA polymerase I-deficient strain WP12 (*uvrA exrA polA*) was like CM611 in being able to join up in 30-min daughter-strand gaps produced after UV doses of less than about 40 ergs/mm^2 but not after higher doses, whether incubation was in buffer or in growth medium. In contrast to the behavior of CM611, however, exposure to light after the formation of daughter-strand gaps did not stimulate the joining process (Fig. 2). We conclude that the *polA*$^+$ gene is required for light-accelerated joining of daughter-strand gaps which occurs when cells are incubated in buffer. The low-molecular-weight, newly synthesized DNA formed in unirradiated WP12 bacteria is a typical feature of *polA1* mutants (9). This DNA is joined to form pieces of normal control size (i.e., as found in a *pol*$^+$ strain) DNA during 15 min of incubation in buffer (Fig. 2a).

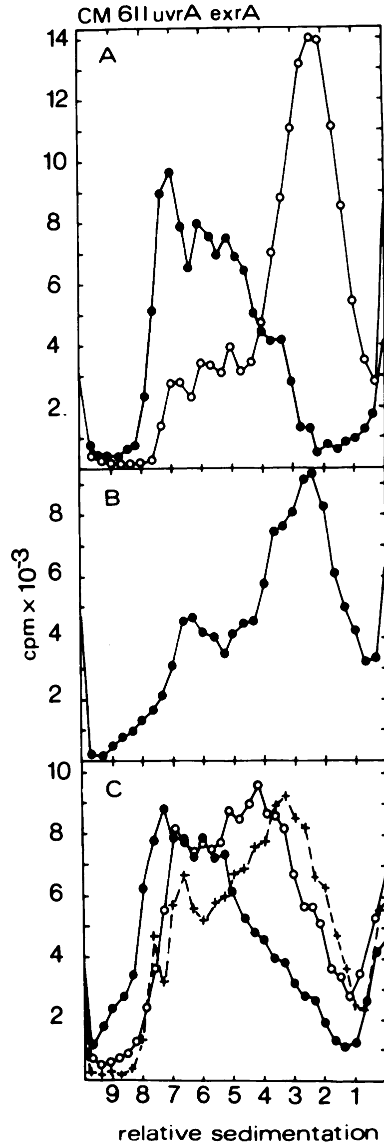

FIG. 1. *Alkaline sucrose gradient sedimentation profiles of newly synthesized DNA from E. coli CM611. Symbols: (A)* ●, *no UV, 5 min in [^{3}H]thymidine;* O, *50 ergs/mm^2 UV, 10 min in [^{3}H]thymidine. (B)* ●, *50 ergs/mm^2 UV 10 min in [^{3}H]thymidine followed by 30 min in buffer in the dark. (C) 50 ergs/mm^2 followed by 10 min in [^{3}H]thymidine followed by 10 min (+), 20 min (O) and 30 min (●) in buffer in the light.*

We have also looked at gap filling in a *uvrA recA* strain (WP100). As in WP12 there was no daughter-strand gap filling whether incubation

TABLE 1. *Photoreversal of CM611*[a]

Time of photoreversal (min)	Surviving fraction[b]
0	1.37×10^{-5}
1	4.45×10^{-5}
3	5.75×10^{-4}
7	3.06×10^{-3}
11	1.02×10^{-2}
15	1.72×10^{-2}
22	4.82×10^{-2}
30	4.08×10^{-2}

[a] The surviving fraction after 50 ergs/mm² UV was 1.39×10^{-5}.

[b] After 50 ergs/mm² UV, 10 min in growth medium, and resuspension in buffer. Over the same period in buffer in the dark there was no significant increase or decrease in survival.

was in the presence or absence of light, and there was some loss of labeled DNA (Fig. 3).

DISCUSSION

The light-stimulated, daughter-strand gap filling demonstrated in CM611 and WP2 *uvrA* is a *recA*$^+$-dependent process like that occurring in the dark. One trivial explanation of this might be that removal of the dimers accelerates recombinational repair. There is no theoretical reason to expect this, and a strong argument against it is that we have shown that the light-dependent gap filling requires *polA*$^+$ and there is no evidence that recombination repair is *polA*$^+$ dependent. WP12 (*uvrA exrA polA*) can perform dark recombination repair in buffer as well as CM611 (*uvrA exrA*) after lower UV doses, and *polA uvrA* bacteria as well as *uvrA* bacteria, after both high and low doses (unpublished data). We suggest the most likely explanation for the *recA*$^+$ dependence of daughter-strand gap filling in the light is that the *recA*$^+$ gene product acts before the light and dark pathways diverge. It might conceivably interact with the newly formed gap in some way, such that degradation is inhibited and repair (by any pathway) is enhanced. Another instance in which early action of the *recA*$^+$ gene product has been inferred is for DNA synthesis immediately after exposure to low doses of ionizing radiation where an effect can be detected in *recA* strains within 1 or 2 min (4). An immediate interaction of *recA*$^+$ gene product with a single-strand gap resulting in some form of stabilization was suggested (5). If one were to assume that the *recA*$^+$ gene product acted at a later stage in repair, one would need to postulate a new *recA*$^+$-dependent pathway that required the *polA*$^+$ gene product.

Another trivial explanation that might explain light-stimulated gap filling is that removal of dimers permits a larger amount of normal semiconservative replication and that the normal-molecular-weight DNA seen after incubation in the light represents new daughter DNA synthesized on dimer-free template. There are at least three arguments against this proposition. First, normal DNA synthesis in buffer at room temperature is minimal even in undamaged bacteria. Secondly, normal semiconservative DNA replication is not inhibited in *polA1* bacteria. Thirdly, any semiconservative

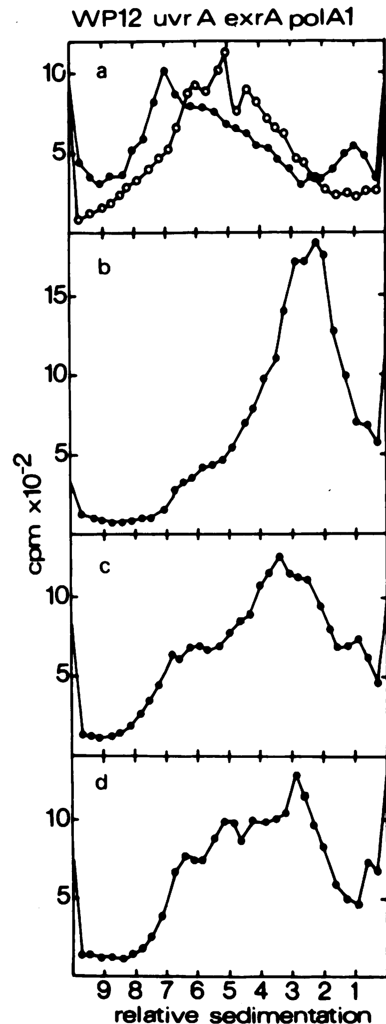

FIG. 2. *Alkaline sucrose gradient sedimentation profile of newly synthesized DNA from E. coli WP12. (a) no UV, 5 min in [³H]thymidine (O) followed by 30 min in buffer in the dark (●). 80 ergs/mm² UV, 10 min in [³H]thymidine (b), followed by 30 min in buffer in the dark (c) or in the light (d).*

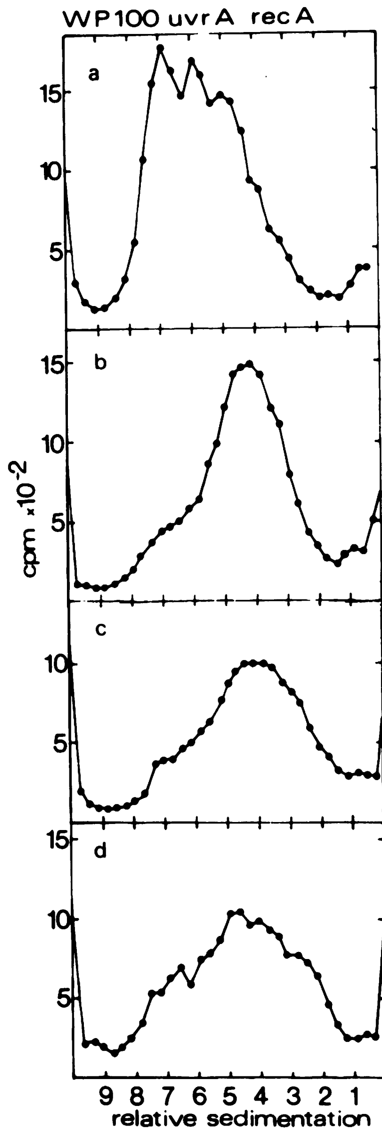

FIG. 3. *Alkaline sucrose gradient sedimentation profiles of newly synthesized DNA from E. coli WP100. No UV, 5 min in [³H]thymidine (a); 40 ergs/mm² UV, 10 min in [³H]thymidine (b), followed by 30 min in buffer in the dark (c) or in the light (d).*

DNA synthesis that occurs would be expected to use largely unlabeled precursors synthesized de novo. If DNA synthesis made use of a large pool of [³H]thymidine, an increase in acid-insoluble activity should have been found during incubation. If products of massive DNA degradation were used, one would have expected to detect such degradation in the dark incubated cultures. In our experiments, the total number of counts per gradient was similar in CM611 bacteria incubated in either the dark or the light.

We conclude that when the pyrimidine dimers presumed to be opposite daughter-strand gaps are removed by exposure to light, the resulting joining process (which might be termed photoreactivation-stimulated gap filling) has characteristics which differ from recombination repair and all other hitherto-reported mechanisms for repair of DNA breaks or gaps. It occurs rapidly in buffer at room temperature and requires the *recA*$^+$ and *polA*$^+$ genes but not the *exrA*$^+$ gene. We further conclude that daughter-strand discontinuities are not committed to recombination repair by virtue of some structural feature of the daughter strand but are prevented from being sealed simply by the presence of a pyrimidine dimer on the opposite (parental) strand. We propose that removal of the dimer allows simple closure of the gap to occur by the action of DNA polymerase I and polynucleotide ligase and that the *recA*$^+$ gene product is most likely needed at a very early stage, perhaps to stabilize the gap. In contrast, the need for the *exrA*$^+$ gene may be relieved by photoreactivation and presumably occurs at a later stage.

Finally, observation of photoreactivation-stimulated gap filling may be taken as further circumstantial evidence that a large proportion of daughter-strand gaps are opposite pyrimidine dimers and not opposite other non-photoreversible lesions.

ACKNOWLEDGMENTS

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LITERATURE CITED

1. Boyle, J. M., and N. Symonds. 1969. Radiation-sensitive mutants of T4D. I. T4y; a new radiation-sensitive mutant; effect of the mutation on radiation survival, growth and recombination. Mutat. Res. **8**:431-439.
2. Bridges, B. A., R. P. Mottershead, M. A. Rothwell, and M. H. L. Green. 1972. Repair-deficient strains suitable for mutagenicity screening: tests with fungicide captan. Chem.-Biol. Interactions **5**:77-84.
3. Cole, R. S. 1971. Properties of F' factor deoxyribonucleic acid transferred from ultraviolet-irradiated donors: photoreactivation in the recipient and the influence of *recA*, *recB*, *recC*, and *uvr* genes. J. Bacteriol. **106**:143-149.
4. Davis, B. D., and E. S. Mingioli. 1950. Mutants of *Escherichia coli* requiring methionine or vitamin B12. J. Bacteriol. **60**:17-28.
5. Gray, W. J. H., M. H. L. Green and B. A. Bridges. 1972. DNA synthesis in gamma-irradiated recombination-deficient strains of *Escherichia coli*. J. Gen. Microbiol. **71**:359-366.
6. Hill, R. F. 1965. Ultraviolet induced lethality and reversion to prototrophy in *Escherichia coli* strains with normal and reduced dark repair ability. Photochem. Photobiol. **4**:563-568.

7. Iyer, V. N., and W. D. Rupp. 1971. Usefulness of benzoylated napthoylated DEAE-cellulose to distinguish and fractionate double-stranded DNA bearing different extents of single-stranded regions. Biochim. Biophys. Acta **228**:117-126.
8. Lehmann, A. R., and M. G. Ormerod. 1969. Artefact in the measurement of the molecular weight of pulse-labelled DNA. Nature (London) **221**:1053-1056.
9. Okazaki, R., M. Arisawa and A. Sugino. 1971. Slow joining of newly-replicated DNA chains in DNA polymerase I-deficient *Escherichia coli* mutants. Proc. Nat. Acad. Sci. U.S.A. **68**:2954-2957.
10. Rupp, W. D., and P. Howard-Flanders. 1968. Discontinuities in the DNA synthesized in an excision defective strain of *Escherichia coli* following ultraviolet irradiation. J. Mol. Biol. **31**:291-304.
11. Rupp, W. D., C. E. Wilde, D. L. Reno, and P. Howard-Flanders. 1971. Exchanges between DNA strands in ultraviolet-irradiated *Escherichia coli*. J. Mol. Biol. **61**:25-44.
12. Town, C. D., K. C. Smith, and H. S. Kaplan. 1971. DNA polymerase required for the rapid rejoining of X-ray-induced DNA strand breaks *in vivo*. Science **172**:851-853.
13. Town, C. D., K. C. Smith, and H. S. Kaplan. 1972. Influence of ultrafast repair processes (independent of DNA polymerase I) on the yield of DNA single-strand breaks in *Escherichia coli* K-12 X-irradiated in the presence or absence of oxygen. Radiat. Res. **52**:99-114.