Loss of Photoreversibility of Damage to Deoxyribonucleic Acid Replication in Ultraviolet-Irradiated *Escherichia coli B*r thy trp

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Loss of photoreversibility (LOP) of the ultraviolet (UV) damage which prevents reinitiation of deoxyribonucleic acid (DNA) replication occurred with incubation of *Escherichia coli B*r thy trp cultures after UV doses of 240, 320, and 400 ergs/mm². LOP occurred at the time of reinitiation of DNA replication in the cultures (i.e., after postirradiation lag periods of 45 min or more). Neither the absence of thymine nor the absence of tryptophan prevented LOP of the damage to DNA replication, suggesting that neither DNA replication nor protein synthesis is necessary for the process. These findings suggest that attempted initiation of DNA replication results in transformation of pyrimidine damage into permanent damage to chromosome structure at the reinitiation site.

Deoxyribonucleic acid (DNA) replication initiation requires protein synthesis in ultraviolet (UV)-damaged *Escherichia coli* cultures (2, 4, 9, 11). With bacteria which have been allowed to complete their replication cycle in the absence of a required amino acid, initiation of DNA replication also requires protein synthesis (10). A reasonable model (1, 4) explaining the need for protein synthesis before DNA replication in UV-damaged bacteria is that UV damages the active DNA replication site. Protein synthesis would be thus required at the chromosomal origin for initiation of DNA replication. Billen (1) has reported some results which support this model. He showed that, after damage by relatively high doses of UV (600 ergs/mm²), semi-conservation DNA replication requires protein synthesis and replication begins at the chromosomal fixed origin. We (8) studied the UV dose kinetics of induction of the need for protein synthesis, as evidenced by inhibition of DNA replication with high concentrations of chloramphenicol (an antibiotic which specifically blocks protein synthesis). These studies indicated that the above model holds after lower UV doses also. The initiation proteins appeared to be formed during the recovery period after the UV exposure. When chloramphenicol was added at the time that DNA replication began, it did not limit the DNA replication.

Since initiation of DNA replication after UV damage is at the chromosomal origin (7), then the rapid initiation of DNA replication usually seen could result in synchronous synthesis of all the chromosomes in the culture (8). Synchrony is supported by results which show that cell division begins in the UV-damaged culture immediately after the doubling of amount in DNA, suggesting that completion of chromosomes at that time triggers a subsequent initiation of cell division. Further results (8) have shown a precise limitation of DNA replication by rifampin and a very rapid acquisition of capacity for DNA replication in the presence of this antibiotic. These findings support the possibility of replication synchrony.

In previous studies, Nishioka and Doudney (13, 14) investigated the process of loss of photoreversibility (LOP) of lethal UV damage and UV damage causing mutation. In this study we explored the process of LOP of UV damage which prevents reinitiation of DNA replication. We found that, after doses of 240, 320, and 400 ergs/mm², LOP occurred at the time of reinitiation of DNA replication in the culture after the UV-induced lag period. This finding supports synchronous initiation of DNA replication after UV exposure and suggests the hypothesis that attempted reinitiation of DNA replication after UV damage results in the transformation of pyrimidine damage into permanent damage to the chromosome structure at a site close enough to the reinitiation site to inhibit reinitiation.

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MATERIALS AND METHODS

Bacterial strain. E. coli strain B/r WP2 thy trp (101) was kindly supplied by Daniel Billen.

Growth media. The minimal medium was Davis (12) minimal salts-glucose (1%) medium containing thymine (2 μg/ml) and L-tryptophan (40 μg/ml). Solid agar plates were made up of the same liquid medium solidified with 1.5% agar (Difco).

Culture conditions. Stock cultures for inoculum harvested in log phase (4 × 10⁶ cells/ml) were maintained at 6 C in minimal medium with thymine and tryptophan and were regrown periodically (usually the day before an experiment). Subsequently, the logarithmic growth-phase bacteria needed for the experiment were obtained by inoculating 0.5 ml of stock culture into 10 ml of fresh minimal medium containing 2 μg of thymine per ml and 40 μg of L-tryptophan per ml. The [2-¹⁴C] thymine was at a radioactivity of 0.1 μCi/μl in all growth media including the inoculum culture. The inoculum was added in 0.1-ml quantities every 5 min during the first 45 min of incubation to promote random growth. The bacteria were grown at 37 C with rapid rotary agitation from this inoculation to an absorbance of 0.2 measured at 660 nm with a Coleman spectrophotometer (model 6D). The bacteria were separated from the medium in preparation for UV exposure by pouring through a membrane filter, washed twice with the filter with 6 C minimal medium lacking thymine and tryptophan, and then resuspended in 2 volumes of such minimal medium at 6 C and adjusted by dilution to an absorbance of 0.09 (ca. 1.8 × 10⁸ bacteria per ml).

UV irradiation and postirradiation incubation. The bacterial suspension was irradiated with vigorous magnetic stirring at a standard distance (about 53 cm) from a Gates “Raymaster” UV lamp (model MR-r) containing a 15-W mercury vapor bulb (General Electric). The output of the lamp at the position of the cells (as determined by an Ultraviolet Products, Inc., long-wavelength UV meter, model J221) was 53 ergs per mm² per s, and the exposure was 2 min. It has been shown that this exposure gives maximum photoreactivation (7; Doudney, unpublished data).

Samples of 2.5 ml were taken from the 3-ml portions (both photoreactivated and not photoreactivated) and were added to 30-ml incubation tubes. These 2.5-ml cultures were put in a 37 C water bath and incubated with vigorous rotary agitation. For studies of DNA replication, samples of 0.2 ml were taken periodically and added to tubes containing 0.1 ml of 8% formaldehyde solution.

Radioactive assay of DNA increase. The procedure used for measuring DNA replication by labeled-thymine incorporation has been described (7). Since we used radioactive medium of the same consistency both before and after UV exposure, the relative increase in counts per minute after exposure could be used to determine relative increase in DNA and such is indicated on the left vertical scale of the DNA replication figures. The data represent the averaged results of three identical experiments.

RESULTS AND DISCUSSION

Lower doses of UV (up to about 200 ergs/mm²) produce a delay in DNA replication during which restoration processes reinstitute full capacity for DNA replication (6). This delay appears to be due to the inactivation of active DNA replication sites by UV (4, 7). Reactivation of DNA replication then involves normal formation of capacity to initiate DNA synthesis at the chromosomal fixed origin. Higher doses of UV (above 200 ergs/mm²) progressively decrease the rate of DNA replication in the culture. Presumably such damage causes inactivation of capacity for institution of DNA replication at the chromosomal origin (4, 7). This damage (unlike the damage after UV doses near 200 ergs/mm² which produces only delay) is partially photoreversible, indicating that at least part of the damage to initiation at the chromosomal origin is caused by a pyrimidine dimer.

Studies of LOP are able to follow the fate of pyrimidine dimers causing a given effect during restoration processes (13, 14). For example, it has been shown that in Hcr+ strains certain mutation induction processes lose photoreversibility during the first 20 min of postirradiation incubation and such LOP is unaffected by the absence of required thymine or the presence of nalidixic acid, an antibiotic which blocks DNA replication specifically. This was not so in the otherwise isogenic Hcr- strain; no such immedi-
ate LOP occurred with this strain. This indicated that immediate LOP, which can occur even under conditions where DNA cannot be formed, shows dimer excision and suggested that the gap left after excision is involved in the mutation process. It should be understood that this finding does not necessarily mean that prereplication repair is involved in this type of mutagenesis. Nishioka and Doudney (13) suggested that the gap could persist until the time of postreplication repair to cause mutation. Since the dimers cannot be excised in the Hcr strain, they persist until the time of DNA replication. The mutagenic damage then loses photoreversibility with progression of DNA replication, suggesting that gaps formed around the pyrimidine dimers lead to mutation with subsequent postreplication recombination-repair processes (13, 14). Both the absence of thymine and the presence of nalidixic acid prevented this LOP. Thus it is possible to differentiate experimentally between LOP due to excision and LOP due to subsequent repair during DNA replication both on the basis of timing and requirement for DNA replication.

In the present study we wanted to know whether UV damage affecting DNA replication itself would show LOP with dimer excision or with subsequent DNA replication. To determine this we followed LOP after several doses producing varying degrees of both photoreversible and non-photoreversible damage to DNA replication. With 400 ergs/mm² of UV, the rate of DNA formation in the culture after initiation was severely depressed (Fig. 1). Photoreversing light caused a considerable reversal of this damage when applied during the lag period, so that the rate of DNA formation in the culture was increased relative to the non-photoreactivated rate. This is presumably because those chromosomal origins suffering photoreversible damage regained the ability to initiate DNA replication. No loss of such capacity for photoreversal response was seen up to 55 min of incubation or just 5 min before initiation of DNA replication in the culture occurs. At 5 min after such initiation, very little photoreactivation was seen. Thus, within a 10-min period bracketing the time of initiation of DNA replication in the culture, pyrimidine damage was converted into permanent damage to the DNA replication system. Results with lower UV doses showed the same response. LOP occurred after 240 ergs/mm² or 320 ergs/mm² only at the time of DNA replication initiation (Fig. 2 and 3). It is interesting that with the two lower doses greater photoreversal response occurred when the light was applied after 20 min of incubation [broken line] rather than immediately after UV exposure. This could be due to excision of

![Fig. 1. LOP of UV (400 ergs/mm²) damage to DNA replication. DNA replication was measured by radioactive thymine incorporation (right vertical scale) and this was used to calculate the relative increase in amount of DNA (left vertical scale). Portions (3 ml) of the UV-exposed culture were exposed to photoreversing light after different periods of postirradiation incubation and then reincubated. The numbers following the symbol PR indicate elapsed time (minutes) of postirradiation incubation at the time of photoreactivation treatment. All cultures were sampled every 15 min, and thymine incorporation per 0.1 ml of the suspension was determined. The upper solid line with the UV-exposed bacteria demonstrates the response with photoreversing light when applied at the start of incubation. The lower solid line shows response to UV when no photoreversing light was applied. The bacteria (1.8 × 10⁷/ml) were reduced by UV exposure (400 ergs/mm²) to 1.4 × 10⁴.

![Fig. 2. LOP of UV (240 ergs/mm²) damage to DNA replication. Except for UV dose, the experiment was carried out exactly as described in Fig. 1. The bacteria (1.8 × 10⁷/ml) were reduced by UV exposure to 2.1 × 10⁴.]
dimers around the damage during the first 20 min of incubation rendering the photoreactivation more efficient.

The findings described above suggest that attempted initiation of DNA replication transforms pyrimidine damage into permanent damage to the chromosome structure presumably at the reinitiation site at the chromosomal origin. Since the evidence (5, 7, 15) suggests that active replication sites are eliminated by nonphotoreversible damage inflicted by UV doses about 100 ergs/mm², then reinitiation of DNA replication after higher doses given here must be from the chromosomal origin.

Absence of thymine after 50 min of incubation did not prevent the occurrence of LOP (as indicated by change in slope) (Fig. 4). This supports the suggestion of the data above that the initiation event does not involve a significant amount of DNA replication. It cannot be ruled out that a very small amount of DNA was made utilizing residual intracellular thymine. Almost identical data (Fig. 5) were obtained in the absence of tryptophan. With lack of tryptophan, protein synthesis cannot occur and initiation of DNA replication in UV-damaged bacteria requires protein synthesis (7). The data suggest that the LOP process in the case of DNA synthesis damage is unlike that in the case of UV-induced mutation in that neither immediate LOP is seen nor LOP dependent on DNA replication. (It should be noted that both thymine and tryptophan deprivation for the above periods causes a delay of another 15 min. The reason for this is unknown, but it is probable that such induced delay is not significant to the LOP results described here).

It has been shown by biophysical studies (3; Doudney, unpublished data) that the postirradiation decrease in rate of DNA replication with dose in cultures of UV-damaged bacteria is due to inhibition of replicating units rather than a slowed rate of replication in each bacterium, and that postirradiation recovery of capacity to synthesize DNA in the presence of chloramphenicol involves the contribution to DNA replication of increasing numbers of such units rather than an increased rate of synthesis in each bacterium. Since the undamaged bacteria in each case are synthesizing DNA at maximum rate, it is evident that the increase in rate of DNA replication in the culture after exposure to 366-nm light could not be due to an increase in rate of DNA replication in each active bacterium but must represent an increase in replicating units. It appears unlikely that light treatment can increase the rate of replication in the undamaged bacteria.

Since reinitiation of DNA replication after UV exposure is probably at the chromosomal origin (1, 7), the rapid initiation of DNA replication usually seen (6) could result in the
Thus, photoreversible damage could have the effect of the reflection of 10 light. Only with initiation event, replication occurs within a synchronous replication of all the chromosomes in the culture (8). This is supported by the correlation of LOP with initiation of DNA replication reported here. This shows that the initiation event involved in LOP occurs within a period of 10 min for almost all bacteria so damaged.

It is evident that we are dealing in this paper with only those bacteria which respond to photoreversing light. However, the results could reflect the effect of the non-photoreversible (and possibly nondimer) damage in the culture. Thus, photoreversible and non-photoreversible damage could have essentially the same effect except in the photoreversal response. We now have no evidence bearing on this question.

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**LITERATURE CITED**


