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Postreplicational formation and repair of DNA double-strand breaks in UV-irradiated *Escherichia coli uvrB* cells

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Summary

The number of DNA double-strand breaks formed in UV-irradiated *uvrB recF recB* cells correlates with the number of unrepaired DNA daughter-strand gaps, and is dependent on DNA synthesis after UV-irradiation. These results are consistent with the model that the DNA double-strand breaks that are produced in UV-irradiated excision-deficient cells occur as the result of breaks in the parental DNA opposite unrepaired DNA daughter-strand gaps. By employing a temperature-sensitive *recA200* mutation, we have devised an improved assay for studying the formation and repair of these DNA double-strand breaks. Possible mechanisms for the postreplication repair of DNA double-strand breaks are discussed.

Few, if any, DNA-strand interruptions are produced directly by the action of UV radiation (see Rahn and Patrick, 1976). However, DNA-strand interruptions may be enzymatically produced during the course of the excision repair of lesions such as pyrimidine dimers. These DNA-strand interruptions are predominantly single-strand breaks, although occasionally some DNA double-strand breaks are also produced. Bonura and Smith (1975) attributed the production of DNA double-strand breaks in excision-repair-proficient cells of *E. coli*, following high fluences of UV radiation, to the converging excision of two pyrimidine dimers that occur in close proximity but on opposite strands of a DNA duplex, and suggested that these enzymatically-induced DNA double-strand breaks may be responsible for a significant fraction of the lethal events in UV-irradiated excision repair-proficient cells.

DNA double-strand breaks can also be produced via an excision-repair-independent process (Wang and Smith, 1982, 1983). For cells that are grossly deficient in the repair of DNA daughter-strand gaps (i.e., *recA* or *recF* cells), the production of DNA double-strand breaks can be easily detected following low fluences of UV radiation (i.e., 0.25 J/m^2 or less). On the other hand, to detect DNA double-strand breaks in cells that are proficient in the repair of DNA daughter-strand gaps requires that higher UV radiation fluences (e.g., ~ 3 J/m^2 for *uvrB recB* cells) be used so that unrepaired DNA daughter-strand gaps begin to accumulate (unpublished data).

In the present work, we have investigated the nature of the DNA double-strand breaks formed in UV-irradiated *E. coli uvrB* cells, and their repair. Models for the postreplicational formation and repair of DNA double-strand breaks are discussed.

Materials and methods

Bacterial strains

The following *E. coli* K-12 strains were used:

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SR1203 (*uvrB5 recB21 recF143*), SR1347 [Δ(*uvrB-chlA*) *recA200*] and SR1376 [Δ(*uvrB-chlA*) *recA200 recB21*]. All strains are derivatives of KH21, and are F$^-$ and λ^-, and carry *leuB19 thyA deo(C2?) lacZ53 rha-5 rpsL151*. SR1347 and SR1376 also carry *malB45* and *srlA300*::Tn*10*. The transduction technique used in strain construction was similar to that described by Miller (1972).

Media

Supplemented minimal medium and DTM buffer were as described (Wang and Smith, 1982).

Irradiation

The source (254 nm) and measurement of fluence rate for UV irradiation have been described (Wang and Smith, 1981).

Alkaline and neutral sucrose gradient sedimentation

Unless otherwise stated, the cells were UV-irradiated and pulse-labeled with [^{3}H]thymidine as previously described (Wang and Smith, 1983). Sedimentation of DNA on alkaline and neutral sucrose gradients (5–20%, wt./vol.), calculation of DNA molecular weight, and the determination of UV radiation-induced DNA-strand breaks were as described (Wang and Smith, 1983).

Results and discussion

One explanation for the formation of DNA double-strand breaks in UV-irradiated *uvrB* cells is that breaks occur in the parental DNA strand opposite unrepaired DNA daughter-strand gaps, possibly through the attack of an endonuclease specific for single-stranded DNA. Although definitive proof of such a model may require the identification of the enzyme(s) that are responsible for the nicking of single-stranded DNA, several predictions can be made according to this model: (1) In the absence of DNA replication, DNA double-strand breaks should not be produced, and (2) if every unrepaired DNA daughter-strand gap is converted to a DNA double-strand break and remains unrepaired, then the number of UV radiation-induced DNA double-strand breaks per genome should be equal to half the number of DNA single-strand breaks per genome.

When [^{3}H]thymidine-prelabeled *uvrB recF recB* (SR1203) cells were UV-irradiated and incubated in growth medium, DNA double-strand breaks were formed (Fig. 1), as previously reported (Wang and Smith, 1983, 1985). In contrast, if the irradiated cells were incubated in buffer, the formation of DNA double-strand breaks was inhibited during the entire course of incubation (Fig. 1). This is consistent with the first prediction that DNA synthesis is a necessary step in the formation of DNA double-strand breaks.

To test for a correlation in the relationship between unrepaired DNA daughter-strand gaps and the formation of DNA double-strand breaks, *uvrB recF recB* cells were pulse-labeled with [^{3}H]thymidine after UV-irradiation. Then, after 4 h of incubation, at which time the maximum production of DNA double-strand breaks was observed (data not shown), the DNA samples were

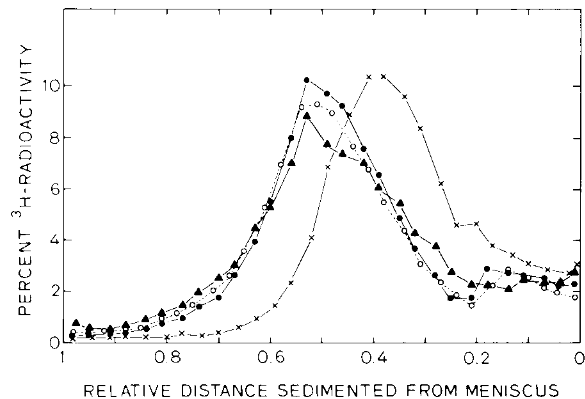

Fig. 1. Growth-medium dependent formation of DNA double-strand breaks in UV-irradiated *E. coli* K-12 *uvrB recF recB* (SR1203). Cultures were prelabeled with [^{3}H]thymidine (20 μCi/ml) for 2 generations at 37°C. They were filtered, washed, and resuspended in DTM buffer at 5×10^7 cells per ml. After incubation at 37°C for 30 min, the cells were UV-irradiated (0.4 J/m^2), and incubated either in buffer or in supplemented minimal medium at 37°C. The DNA was sedimented on a neutral sucrose gradient (5–20%). Symbols: unirradiated control (●); UV-irradiated with no repair incubation (○); UV-irradiated and incubated at 37°C for 4 h in supplemented minimal medium (×) or in DTM buffer (▲). Irradiated samples incubated in buffer for times up to 4 h at 37°C had sedimentation profiles (data not shown) similar to that of the unirradiated control (●), while irradiated samples incubated in supplemented minimal medium during this time showed a progressive shift (data not shown) towards the profile shown for that of the 4 h-incubated samples (×).

sedimented in both alkaline and neutral sucrose gradients. A representative sedimentation profile of DNA is shown in Fig. 2. The number-average molecular weight of the DNA was calculated relative to a ^{14}C-labeled bacteriophage T2 DNA marker, and was used to calculate the average number of DNA strand breaks in UV-irradiated cells (Wang and Smith, 1983). As shown in Table 1, the number of single-strand breaks (i.e., unrepaired DNA daughter-strand gaps plus any additional rapair-induced single-strand breaks) is approximately twice that of the number of DNA double-strand breaks in the irradiated cells. This suggests that most of the single-strand breaks measured after long times of repair incubation are the result of DNA double-strand breaks. Taken together, these data are consistent with the model that these DNA double-strand breaks occur as the result of the formation of single-strand breaks in the parental DNA opposite DNA daughter-strand gaps.

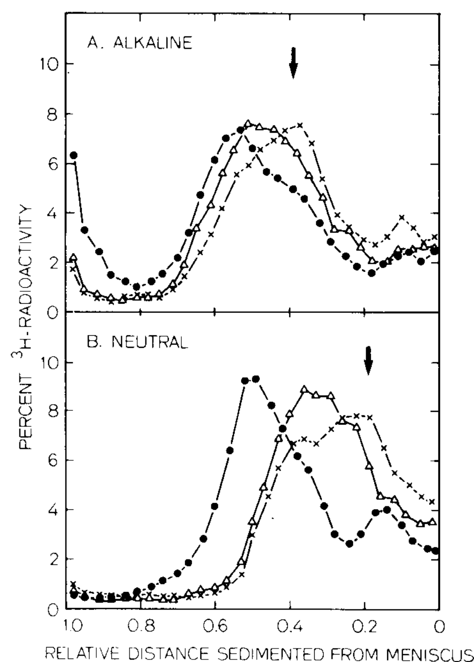

Fig. 2. Sedimentation on alkaline and neutral sucrose gradients of DNA synthesized after UV irradiation. Cells of strain SR1203 (*uvrB recB recF*) were UV-irradiated, pulse-labeled with [^{3}H]thymidine at 37°C for 5 min, and incubated in nonradioactive supplemented minimal medium for 4 h. The cells were converted to spheroplasts and sedimented on (A) alkaline and (B) neutral sucrose gradients as described in Materials and Methods. The arrow indicates the position of a bacteriophage T2 DNA marker. Symbols: unirradiated control (●); 0.4 J/m^2 (△); and 0.8 J/m^2 (×).

The conversion of DNA daughter-strand gaps to DNA double-strand breaks creates a new lesion that needs to be repaired. We have used a *ΔuvrB recA200*(ts) strain (SR1347) to further study the postreplicational formation and repair of DNA double-strand breaks. By growing these cells at a nonpermissive temperature (42°C), the irradiated cells will behave like *uvrB recA* cells and, therefore, won't be able to repair DNA daughter-strand gaps. These unrepaired daughter-strand gaps should then be converted to DNA double-strand breaks, which will remain unrepaired at 42°C. Then by shifting to the permissive temperature (30°C) the cells become RecA$^+$, and the repair of DNA double-strand breaks can proceed in the absence of the competing process of double-strand break formation.

When UV-irradiated *uvrB recA200* cells were pulse-labeled with [^{3}H]thymidine for 10 min at 42°C, the newly synthesized DNA appeared to consist of two components on a neutral sucrose gradient (Fig. 3). One component sedimented at high molecular weight like unirradiated control DNA, and the other component sedimented at low molecular weight, indicating that a good fraction of newly synthesized ^{3}H-DNA was rapidly con-

TABLE 1

CORRELATION BETWEEN THE NUMBER OF DNA SINGLE-STRAND BREAKS AND DNA DOUBLE-STRAND BREAKS PRODUCED IN UV-IRRADIATED *uvrB recF recB* CELLS OF *E. coli* K-12 [a]

UV radiation fluence (J/m^2)	Number of DNA single-strand breaks per genome	Number of DNA double-strand breaks per genome
0.4	7.9	3.5
0.8	14.4	7.4

[a] UV radiation-induced DNA strand breaks per genome was calculated by the formula $(Mc/Muv - 1) \times (2.8 \times 10^9/Mc)$, where Mc and Muv are the number-average molecular weights (Mn) of DNA from unirradiated control and UV-irradiated samples, respectively. The Mn values of bacterial DNA were calculated relative to a bacteriophage T2 DNA marker from sedimentation data obtained from alkaline and neutral sucrose gradients (Fig. 2). Data are the average of 2 Expts.

verted to slowly sedimenting molecules, i.e., double-strand breaks were produced. During the subsequent 100 min of incubation at 42°C, most of the ^{3}H-DNA from the irradiated cells was converted to slowly sedimenting DNA, as one would expect if unrepaired daughter-strand gaps were converted to DNA double-strand breaks. When the irradiated culture was then shifted to 30°C, the repair of DNA double-strand breaks was evident, beginning at about 60 min of incubation at 30°C (data not shown), and reached a maximum after about 2 h of incubation at 30°C (Fig. 3A). This repair of DNA double-strand breaks was not observed if the incubation of the irradiated *uvrB recA200* cells was continued at 42°C (data not shown). Similarly, when a *uvrB recA200 recB* (SR1376) strain was used, the postreplication repair of DNA double-strand breaks was inhibited (Fig. 3B). These results confirm our previous observation that the postreplication repair of DNA double-strand breaks is dependent both on functional *recA* and *recB* genes (Wang and Smith, 1983), and illustrate the usefulness of this assay system in further studies on the postreplicational formation and repair of DNA double-strand breaks produced after UV-irradiation.

The essential role of the *recB* gene product in the repair of UV radiation-induced DNA double-strand breaks suggests that this repair may utilize the *recB*-dependent pathway of genetic recombination. This is consistent with the recent findings that the substrates for *recB*-dependent recombination are linear duplex DNA molecules having nearly flush-ended termini (Taylor and Smith, 1985). However, the mechanism for the repair of DNA double-strand breaks remains open for speculation. At least two recombinational mechanisms may allow cells to produce a DNA duplex free of double-strand breaks. First, if the two free ends of DNA double-strand break initiate recombination simultaneously with a homologous sister DNA duplex, the DNA double-strand breaks can be repaired during the recombination process by DNA synthesis and ligation (Fig. 4, process I). Variations on such a model for the repair of DNA double-strand breaks have been proposed (Resnick, 1976; Szostak et al., 1983). Second, if the two free ends of a DNA double-strand break do not initiate recombination concurrently, then the DNA

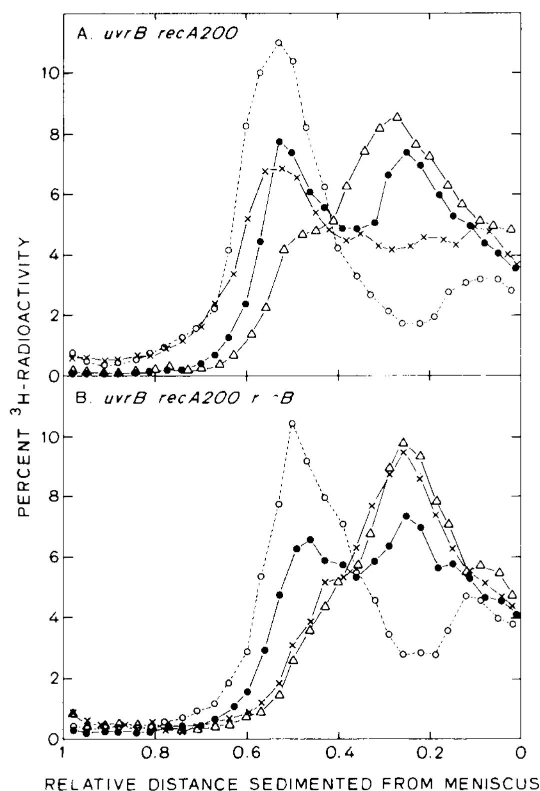

Fig. 3. Postreplicational formation and repair of DNA double-strand breaks in UV-irradiated *ΔuvrB recA200* (A) and *ΔuvrB recA200 recB21* (B) cells of *E. coli* K-12. Log-phase cells grown in supplemented minimal medium at 30°C were diluted to 7×10^7 cells per ml. After incubation at 42°C for 30 min, cells were UV irradiated (0.5 J/m^2) and pulse-labeled with [^{3}H]thymidine (20 μCi/ml) for 10 min at 42°C. The pulse-labeled cells were harvested by filtration, washed with warm buffer (42°C), and resuspended in prewarmed supplemented minimal medium. Samples were maintained at 42°C for an additional 100 min before shifting to 30°C. Symbols: unirradiated cells incubated at 42°C for 100 min (○), UV-irradiated cells incubated at 42°C for 0 min (●) or 100 min (△); UV-irradiated cells incubated at 42°C for 100 min, and then at 30°C for 120 min (×).

double-strand break cannot be repaired, but the cells can produce one DNA duplex free of double-strand breaks by segregating out the double-strand breaks into its homologous DNA duplex (Fig. 4, process II). This latter mechanism was proposed for the postreplication repair of DNA double-strand breaks in UV-irradiated *uvrB recF* cells (Wang and Smith, 1983). One major difference between these two mechanisms of repair is

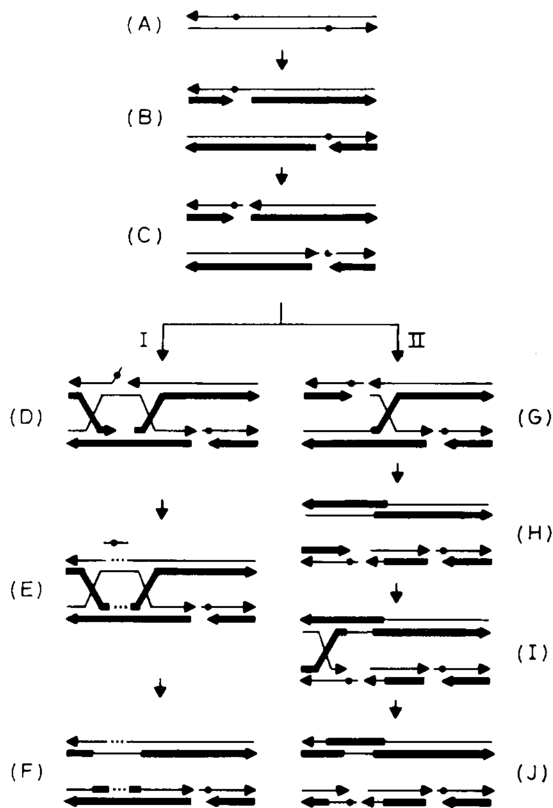

Fig. 4. Model for the postreplicational formation and repair of DNA double-strand breaks in UV-irradiated *uvrB* cells of *E. coli*. When DNA containing lesions (A), such as pyrimidine dimers (●), is replicated, the newly synthesized DNA (thick lines) is discontinuous opposite each dimer (B). When not repaired, these daughter-strand gaps can be converted to double-strand breaks by endonucleolytic attack on the single-stranded parental DNA opposite the gaps (shown here by a break at the 5′-end of the dimer in the irradiated parental DNA) (C). Two possible mechanisms are depicted, which may allow the cells to produce a DNA duplex free of DNA double-strand breaks. In Process I, the two ends of a DNA double-strand break initiate recombination simultaneously (D) and this can lead to the repair of the DNA double-strand break by DNA synthesis (dotted lines) and ligation (E, F). In Process II, the two ends of a DNA double-strand break do not initiate recombination at the same time. In this case the DNA double-strand break is not repaired, but two separate recombination events involving double-strand exchanges on the opposite sides of a double-strand break can segregate the double-strand break into the homologous DNA duplex (G–J). It is likely that the two ends of a DNA double-strand break may first be processed by exonucleases before they initiate recombination. In the present model, dimers were shown to be excised (step E) during these processes, but this is not a necessary event.

that one would expect that an increase in the number of high molecular weight DNA molecules would be accompanied by an increase in the number of smaller molecular weight DNA molecules by Process II, but not by Process I. In the present work, after the maximal repair of DNA double-strand breaks was completed in UV-irradiated *uvrB recA200* cells, a significant fraction of the ^{3}H-DNA still sedimented at low molecular weight (Fig. 3A). However, it is difficult to determine from these sedimentation profiles whether the formation of low molecular weight DNA molecules is causally related to the regeneration of high molecular weight DNA. Therefore, the mechanism for the repair of these DNA double-strand breaks remains to be elucidated.

The observation of the postreplicational formation and repair of DNA double-strand breaks in UV-irradiated *E. coli* cells supplements the current understanding of the major 'dark' repair system for the processing and repair of UV-radiation-damaged DNA in *E. coli* cells. Pyrimidine dimers produced in DNA by UV-irradiation can be repaired by an efficient *uvrABC*-dependent excision repair process (Setlow and Carrier, 1964; Boyce and Howard-Flanders, 1964). If these pyrimidine dimers are not removed from the DNA and replication proceeds past them, the dimers will then appear in single-stranded DNA that is opposite a gap in the newly-synthesized DNA. These daughter-strand gaps are the secondary lesions that the cells need to repair, and *E. coli* possess efficient recombinational processes for the repair of DNA daughter-strand gaps (Rupp and Howard-Flanders, 1968). Finally, a daughter-strand gap can be converted to a tertiary lesion, a DNA double-strand break. The repair of DNA double-strand breaks is dependent on the *recB*-pathway of postreplication repair (Wang and Smith, 1983).

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References

Bonura, T., and K.C. Smith (1975) Enzymatic production of deoxyribonucleic acid double-strand breaks after ultraviolet irradiation of *Escherichia coli* K-12, J. Bacteriol., 121, 511–517.

Boyce, R.P., and P. Howard-Flanders (1964) Release of ultraviolet-light induced thymine dimers from DNA in *E. coli* K-12, Proc. Natl. Acad. Sci. (U.S.A.), 51, 293–300.

Miller, J.H. (1972) Experiments in molecular genetics, Cold Spring Harbor Laboratory, Cold Spring Harbor, NY.

Rahn, R.O., and M.H. Patrick (1976) Photochemistry of DNA; secondary structure, photosensitization, base substitution, and exogenous molecules, in: S.Y. Wang (Ed.), Photochemistry and Photobiology of Nucleic Acids, Vol. 2, Academic Press, New York, pp. 97–145.

Resnick, M.A. (1976) The repair of double-strand breaks in DNA: a model involving recombination, J. Theoret. Biol., 59, 97–106.

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.

Setlow, R., and W. Carrier (1964) The disappearance of thymine dimers from DNA; an error-correcting mechanism, Proc. Natl. Acad. Sci. (U.S.A.), 51, 226–231.

Szostak, J.W., T.L. Orr-Weaver, R.J. Rothstein and F.W. Stahl (1983) The double-strand-break repair model for recombination, Cell, 33, 25–35.

Taylor, A.F., and G.R. Smith (1985) Substrate specificity of the DNA unwinding activity of the RecBC enzyme of *Escherichia coli*, J. Mol. Biol., in press.

Wang, T.V., and K.C. Smith (1981) Effect of *recB21*, *uvrD3*, *lexA101* and *recF143* mutations on ultraviolet radiation sensitivity and genetic recombination in *ΔuvrB* strains of *Escherichia coli* K-12, Mol. Gen. Genet., 183, 37–44.

Wang, T.V., and K.C. Smith (1982) Effects of the *ssb-1* and *ssb-113* mutations on survival and DNA repair in UV-irradiated *ΔuvrB* strains of *Escherichia coli* K-12, J. Bacteriol., 151, 186–192.

Wang, T.V., and K.C. Smith (1983) Mechanisms for *recF*-dependent and *recB*-dependent pathways of postreplication repair in UV-irradiated *Escherichia coli uvrB*, J. Bacteriol., 156, 1093–1098.

Wang, T.V., and K.C. Smith (1985) Role of the *umuC* gene in postreplication repair in UV-irradiated *Escherichia coli uvrB*, Mutation Res., 145, 107–112.