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NOTES

recF-Dependent and *recF recB*-Independent DNA Gap-Filling Repair Processes Transfer Dimer-Containing Parental Strands to Daughter Strands in *Escherichia coli* K-12 *uvrB*

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The processes for repairing DNA daughter-strand gaps were studied in UV-irradiated *uvrB*, *uvrB recB*, *uvrB recF*, and *uvrB recB recF* cells of *Escherichia coli* K-12. The dimer-containing parental DNA was found to be joined to daughter strands during postreplication repair in all four strains examined. Therefore, both the major (*recF*-dependent) and the minor (*recF recB*-independent) gap-filling processes repair DNA daughter-strand gaps by transferring parental strands into daughter strands.

In UV-irradiated cells of *Escherichia coli*, if the replication complex encounters unexcised pyrimidine dimers, gaps opposite the dimers are produced in the nascent DNA. These daughter-strand gaps are the substrates for postreplication repair (5).

Rupp et al. (10) suggested that the filling of daughter-strand gaps was achieved by using a single-stranded piece of the parental DNA to fill the gap. Using assays involving dimer-specific endonuclease V of bacteriophage T4, Ganesan (3) observed that the repair of daughter-strand gaps was accompanied by the transfer of parental DNA containing dimers into the daughter strands. Alternatively, daughter-strand gaps may be filled by a non-recombinational process such as the proposed transdimer type of DNA synthesis (1, 2), although there is little biochemical evidence to support this hypothesis.

Genetic studies have indicated the existence of two major, independent pathways for postreplication repair, i.e., the *recF*-dependent and *recB*-dependent pathways (9, 11). The *recF*-dependent pathway is largely responsible for the filling of daughter-strand gaps (4, 6, 8, 13). However, the *recB*-dependent pathway plays little role in the filling of daughter-strand gaps, but has recently been shown to be required for the repair of DNA double-strand breaks that arise from unrepaired daughter-strand gaps (13). In addition to the two major pathways mentioned above, a minor, *recF recB*-independent repair of DNA daughter-strand gaps has been observed (13).

In this communication, we have studied the genetic control of the covalent joining of parental and daughter DNA during the repair of DNA daughter-strand gaps in UV-irradiated *uvrB* cells of *E. coli* K-12. The strains used were SR596 Δ(*uvrB-chlA*), SR305 Δ(*uvrB-chlA*) *recF143*, SR1160 Δ(*uvrB-chlA*) *recB21*, and SR840 Δ(*uvrB-chlA*) *recF143 recB21*. All strains are F^- and λ^- and carry *leuB19 thyA deo(C2?) lacZ53 malB45 rha-5* and *rpsL151* mutations. The source or deviation of these strains has been described previously (13).

For *uvrB recB* cells, the [^{3}H]DNA, newly synthesized after UV (254 nm) irradiation (3 J/m^2), sedimented more slowly on alkaline sucrose gradients than the irradiated parental [^{14}C]DNA, which sedimented at the same rate as DNA from unirradiated cells. Upon treatment with endonuclease V, both [^{3}H]DNA and [^{14}C]DNA sedimented slowly on the alkaline sucrose gradients, with a number-average molecular weight (M_n) of 3×10^7 (Fig. 1A). After 2 h of repair incubation, the [^{3}H]DNA sedimented at high molecular weights, approaching those for parental [^{14}C]DNA. Upon treatment with endonuclease V, both [^{3}H]DNA and [^{14}C]DNA sedimented at lower molecular weights (Fig. 1D); the [^{3}H]DNA had an M_n of 6×10^7, and the [^{14}C]DNA had an M_n of 3.9×10^7, indicating that there were fewer endonuclease V-sensitive sites (i.e., dimers) in the high-molecular-weight [^{3}H]DNA than in the [^{14}C]DNA. A similar pattern of gap-filling repair was also observed in irradiated *uvrB* cells (Fig. 1B and E; see also reference 3). Taken together, these results suggest that (i) the *recB*-dependent recombination process is not required for the covalent joining of dimer-containing parental DNA segments to daughter-strand DNA, as deduced from the presence of dimers in the repaired [^{3}H]DNA in irradiated *uvrB recB* cells (Fig. 1D) and (ii) the gap-filling repair process, which is mostly *recF* dependent (4, 6, 8, 13), repairs DNA daughter-strand gaps, resulting in both single-strand exchanges and double-strand exchanges as proposed by Ganesan (3). This conclusion is based upon the observation that there are fewer dimers in the [^{3}H]DNA than in the [^{14}C]DNA (Fig. 1D and E) after repair incubation, suggesting that although a portion of gap-filling repair resulted in the covalent joining of dimer-containing parental-strand DNA to the daughter-strand DNA (i.e., double-strand exchanges or single-strand exchanges involving long pieces of parental DNA containing dimers, or both), a portion of gap-filling repair did not result in the transfer of dimers into the daughter-strand DNA (i.e., single-strand exchanges involving parental DNA segments containing no dimers).

In addition to the two major pathways for postreplication repair, a "transdimer DNA synthesis" (1, 2) mechanism has been proposed to fill daughter-strand gaps without joining daughter-strands to dimer-containing parental strands. Evidence supporting the existence of transdimer DNA synthesis came mainly from the observation that there was UV radiation-induced reactivation of UV-irradiated single-stranded DNA bacteriophages such as φX174 and S13. It has

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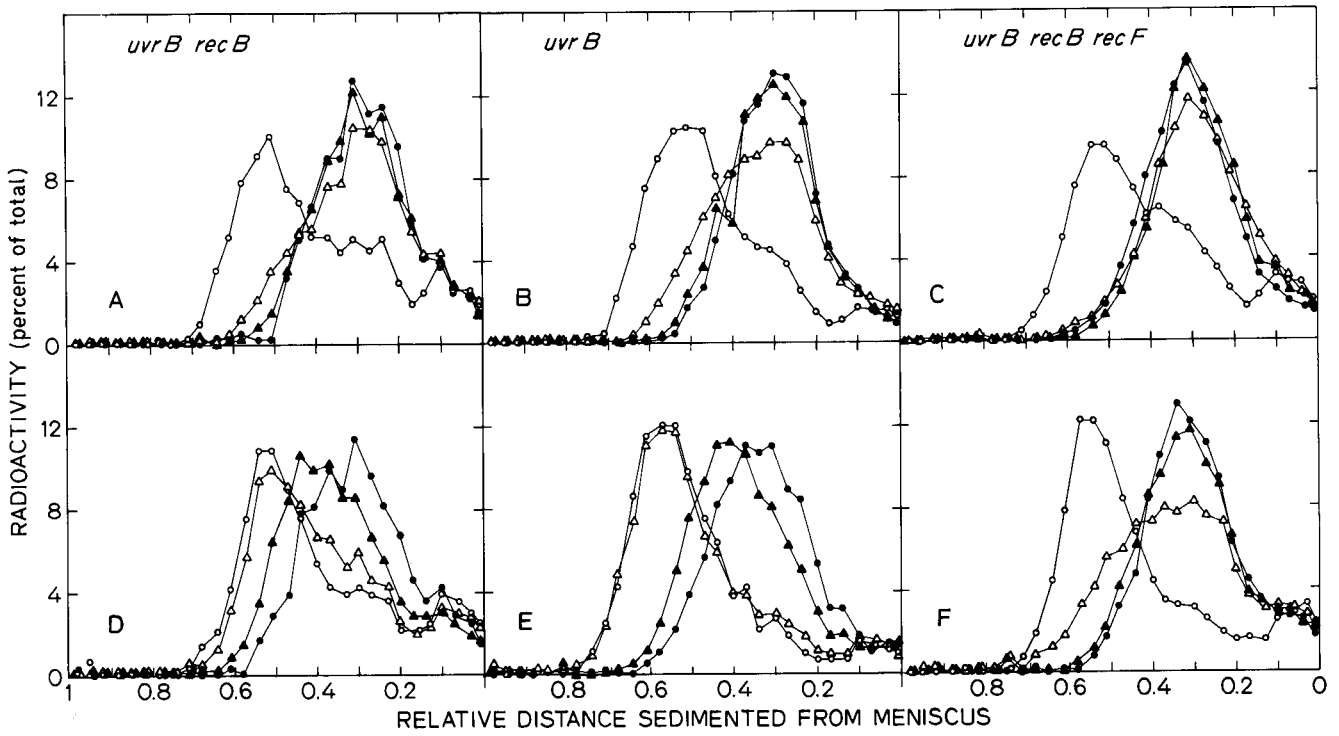

FIG. 1. Sensitivity to endonuclease V of DNA synthesized after UV irradiation (254 nm; 3 J/m^2). Parental [^{14}C]DNA without (○) and with (●) endonuclease treatment; daughter [^{3}H]DNA without (△) and with (▲) endonuclease treatment. The *E. coli* cells were grown for at least four generations in supplemented minimal medium (12) containing thymine at 2 μg/ml and [2-^{14}C]thymine (54.5 Ci/mol; New England Nuclear Corp.) at 2 μCi/ml. When the culture reached a cell density of 2 × 10^8 cells per ml, the cells were harvested by filtration, washed, and resuspended at 8 × 10^7 cells per ml in supplemented minimal medium containing thymine at 2 μg/ml. The resuspended cells were UV irradiated and pulse-labeled with [*methyl*-^{3}H]thymidine (41 Ci/mmol; Amersham Corp.) at 20 μCi/ml for 5 min as previously described (13). After 0 h (A, B, C) and 2 h (D, E, F) of repair incubation at 37°C, the cells were collected by centrifugation, made permeable, and treated with T4 endonuclease V as described by Ganesan (3). Samples, with or without endonuclease treatment, were layered on alkaline sucrose gradients (5 to 20% in 0.1 N NaOH). The gradients were centrifuged, fractionated, and processed for assaying radioactivity as described previously (13). The sedimentation of DNA from unirradiated cells was similar to irradiated parental [^{14}C]DNA without UV-endonuclease treatment and was not affected by treatment with the enzyme (data not shown). Data are from one of two experiments with similar results.

been suggested that transdimer DNA synthesis is *recF* dependent (2), since *recF* mutants showed no UV radiation-induced reactivation of UV-irradiated S13 phage. On the other hand, *recF* mutants showed normal levels of UV radiation mutagenesis (7). These two pieces of apparently conflicting data argue either that transdimer DNA synthesis does not operate on irradiated cellular DNA or that, if it does operate on cellular DNA, it plays little role in UV radiation mutagenesis. Alternatively, the process that is responsible for UV radiation-induced reactivation of UV-irradiated single-stranded DNA bacteriophages may be due to a mechanism other than transdimer DNA synthesis. In a recent study (13), we observed that *uvrB recB recF* cells, although deficient both in the *recF*-dependent gap-filling repair process and in the *recB*-dependent sister duplex recombination process, are able to perform a limited repair of daughter-strand gaps, as compared to "repairless" *uvrB recA* cells. Since a *uvrB recB recF* strain had been previously shown to be nearly normal in UV radiation mutagenesis (7), it is possible that this limited repair of daughter-strand gaps observed in *uvrB recB recF* cells may reflect a type of mutagenic repair, such as the proposed transdimer DNA synthesis. However, after repair incubation, the [^{3}H]DNA of irradiated *uvrB recB recF* cells was also sensitive to endonuclease V treatment (Fig. 1C and F), indicating the presence of dimers in the repaired [^{3}H]DNA. Therefore, the limited repair of daughter-strand gaps observed in irradiated *uvrB recB recF* cells cannot be due largely to transdimer DNA synthesis, which by inference should not result in the transfer of dimers into daughter strands. Similar results were also observed for *uvrB recF* cells (data not shown; see reference 4, Fig. 7A and B). If the limited repair of daughter-strand gaps observed in UV-irradiated *uvrB recF* and *uvrB recF recB* cells is not due to a leakiness in the mutations *recF143* and *recB21* employed, our results raise further questions about the existence of transdimer DNA synthesis in UV-irradiated *E. coli* cells and the validity of the transdimer DNA synthesis hypothesis as an explanation for UV radiation mutagenesis.

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LITERATURE CITED

1. **Caillet-Fauquet, P., M. Defais, and M. Radman.** 1977. Molecular mechanisms of induced mutagenesis. Replication *in vivo* of bacteriophage φX174 single-stranded, ultraviolet light-irradiated DNA in intact and irradiated host cells. J. Mol. Biol. **117**:95–112.
2. **Clark, A. J., and M. R. Volkert.** 1978. A new classification of pathways repairing pyrimidine dimer damage in DNA, p. 57–72. *In* P. C. Hanawalt, E. C. Friedberg, and C. F. Fox (ed.), DNA

repair mechanisms. Academic Press, Inc., New York.
3. **Ganesan, A. K.** 1974. Persistence of pyrimidine dimers during post-replication repair in ultraviolet light-irradiated *Escherichia coli* K-12. J. Mol. Biol. **87**:103–119.
4. **Ganesan, A. K., and P. C. Seawell.** 1975. The effect of *lexA* and *recF* mutations on post-replication repair and DNA synthesis in *Escherichia coli* K-12. Mol. Gen. Genet. **141**:189–205.
5. **Hanawalt, P. C., P. K. Cooper, A. K. Ganesan, and C. A. Smith.** 1979. DNA repair in bacteria and mammalian cells. Annu. Rev. Biochem. **48**:783–836.
6. **Kato, T.** 1977. Effects of chloramphenicol and caffeine on postreplication repair in *uvrA*$^-$ *umuC*$^-$ and *uvrA*$^-$ *recF*$^-$ strains of *Escherichia coli* K-12. Mol. Gen. Genet. **156**:115–120.
7. **Kato, T., R. H. Rothman, and A. J. Clark.** 1977. Analysis of the role of recombination and repair in mutagenesis of *Escherichia coli* by UV irradiation. Genetics **87**:1–18.
8. **Rothman, R. H., and A. J. Clark.** 1977. The dependence of postreplication repair on *uvrB* in a *recF* mutant of *Escherichia coli* K-12. Mol. Gen. Genet. **155**:279–286.
9. **Rothman, R. H., T. Kato, and A. J. Clark.** 1975. The beginning of an investigation of the role of *recF* in the pathways of metabolism of ultraviolet-irradiated DNA in *Escherichia coli*, p. 283–291. *In* P. C. Hanawalt and R. B. Setlow (ed.), Molecular mechanisms for repair of DNA. Plenum Publishing Corp., New York.
10. **Rupp, W. D., C. E. Wilde III, D. L. Reno, and P. Howard-Flanders.** 1971. Exchanges between DNA strands in ultraviolet-irradiated *Escherichia coli*. J. Mol. Biol. **61**:25–44.
11. **Wang, T. V., and K. C. Smith.** 1981. Effect of *recB21*, *uvrD3*, *lexA101* and *recF143* mutations on ultraviolet radiation sensitivity and genetic recombination in Δ*uvrB* strains of *Escherichia coli*. Mol. Gen. Genet. **183**:37–44.
12. **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 Δ*uvrB* strains of *Escherichia coli* K-12. J. Bacteriol. **151**:186–192.
13. **Wang, T. V., and K. C. Smith.** 1983. Mechanisms for the *recF*-dependent and *recB*-dependent pathways of postreplication repair in UV-irradiated *Escherichia coli uvrB*. J. Bacteriol. **156**:1093–1098.