A92

Effect of the *uvrD3* mutation on ultraviolet radiation-induced DNA-repair replication in *Escherichia coli* K12

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Summary

Ultraviolet-radation-induced DNA-repair replication was measured in wild-type, *polA1*, *uvrD3*, and *polA1 uvrD3* strains of *Escherichia coli* K12. A large stimulation of repair replication was observed in the *uvrD3* strain, compared to the wild-type and *polA1* strains. This enhanced repair replication was reduced in the *polA1 uvrD3* strain. Therefore, a *uvrD3* mutation appears to affect the amount of repair replication performed by DNA polymerase I. In the *polA1* strain, there also appears to be an effect of the *uvrD3* mutation on the amount of repair replication performed by DNA polymerase III (and/or II). The enhanced repair replication observed for the *uvrD3* strains appears to be in response to the enhanced DNA degradation observed for these strains.

The *uvrD3* strain of *Escherichia coli* is deficient in host-cell reactivation (Ogawa et al., 1968). This suggests that the *uvrD3* strain is deficient in some aspect of excision repair. Since this strain is proficient in the initial endonucleolytic incision steps of excision repair (Shimada et al., 1968), it must be deficient in the excision and/or resynthesis steps of excision repair.

To gain further information on the involvement of the *uvrD* gene product in the excision repair of UV-radiation-produced DNA damage, DNA-repair replication was measured in the wild-type, *polA1*, *uvrD3* and *polA1 uvrD3* strains of *E. coli* K12 after UV-irradiation. There was a large stimulation of repair replication in the *uvrD3* strain, compared to the wild-type and *polA1 uvrD3* strains, that was reduced in the *polA1 uvrD3* strain. Thus, it appears that the *uvrD* gene product affects the amount of repair replication performed by DNA polymerase I, and may also affect the amount of repair replication performed by DNA polymerase III (and/or II), at least in the *polA1* strain.

Bacterial strains. The *E. coli* K12 strains used in this study are wild-type (SR308), *polA1* (SR309), *uvrD3* (SR310) and *polA1 uvrD3* (SR311). These strains were constructed (Van der Schueren et al., 1977) as DY182, DY183, DY184 and DY185, respectively. All the strains are F$^-$ *lacZ53 rpsL151 thyA36 deo(C2?)* λ$^-$.

Culture conditions. Cells were grown in Tris-buffered salts (TBS) medium (Hanawalt and Cooper, 1971) supplemented with glucose (at 0.5%), Difco Casamino Acids (at 0.1%), and thymine (at 10 μg/ml for overnight cultures; 2 μg/ml for exponential phase cultures).

Overnight cultures were diluted 1:100 or 1:50 into fresh prewarmed growth medium (30 ml) supplemented with [2-^{14}C] thymine (New England Nuclear Corp.; 53 Ci/mole) at 0.20 μCi/ml, and grown in exponential phase at 37°C until a cell density of ∼2 × 10^8 cells/ml was reached. Cells were collected on a membrane filter (0.45 μm pore size; Millipore Corp.), washed several times with 30 ml of warm Tris buffer, pH 7.4 (Hanawalt and Cooper, 1971), and resuspended in warm TBS medium at ∼2 × 10^8 cells/ml.

UV-irradiation. Two 10-ml portions were taken from the resuspended culture, with one portion being kept as an unirradiated control. The second portion was placed in an uncovered glass petri dish on a rotary shaker, and irradiated at room temperature with an 8-W General Electric germicidal lamp. The UV-radiation fluence rate (∼0.8 Jm^{-2}sec^{-1}) was determined with an International Light, Inc. germicidal photometer (No. IL-254). The stated UV-radation fluences have been corrected for cell-masking effects (Sargentini and Smith, 1979). All manipulations were carried out under General Electric "gold" fluorescent lamps to prevent photoreactivation.

Repair replication. We have used a modification of the repair-replication method of Smith et al. (1981). Control and UV-irradiated cell suspensions in TBS medium were supplemented with glucose at 0.5% and Casamino Acids at 0.1%, and [6-^{3}H] 5-bromouracil (5-BrUra) (New England Nuclear Corp.; 23 Ci/mmole) as a repair label was added at 10 μCi/ml plus nonradioactive 5-BrUra to give a final concentration of 10 μg/ml. Both control and UV-irradiated cultures were incubated at 37°C with aeration for 30 min. The repair period was stopped by adding an equal volume of ice-cold NET buffer [0.1 M NaCl, 10 mM ethylenediaminetetraacetic acid (EDTA) and 10 mM Tris buffer,pH 8.0]. Cells were then harvested on membrane filters presoaked in sterile bovine serum albumin (BSA, 1 mg/ml) and washed with 5 ml of BSA to minimize cell sticking, which is a problem for cells carrying a *polA1* mutation (unpublished observation). The cells were then washed with several volumes of ice-cold NET buffer, and resuspended in 1 ml of 1/10 NET buffer. A 0.1-ml sample of a freshly made solution of lysozyme (Worthington Biochemical Corp.) (1 mg/ml in 1/10 NET buffer) was added, and the mixtures were held at 4°C for 30 min to allow for spheroplast formation. 2 drops of a 10% solution of Sarkosyl NL30 (Geigy) and 0.1 ml of pronase (Calbiochem) (1 mg/ml in H$_2$O) were added to each cell preparation; they were then incubated at 60°C for 30 min. The lysates were

"vortexed" rapidly for 4 min to fragment the DNA into pieces having an average single-strand molecular weight of $\sim 2 \times 10^7$, as checked by alkaline sucrose gradient centrifugation with a [^{14}C] thymine-labeled bacteriophage T2 DNA marker (data not shown).

Each "vortexed" lysate was adjusted to 6 ml with 1/10 NET buffer, and 8.2 g of optical grade CsCl (Beckman) was added. The solutions were placed in cellulose nitrate tubes, filled with mineral oil, capped and centrifuged (Beckman L5-50) for 60 h at 37000 rpm and 20°C in a type 40 rotor to band the DNA. After centrifugation, the bottom of each tube was pierced, and 25–28 thirty-drop fractions were collected into polystyrene tubes (Falcon, 12 × 15 mm) to prevent the loss of DNA that occurs in glass tubes (P.K. Cooper, personal communication). A 0.02-ml sample from each fraction was spotted on 1.25-cm wide Whatman No. 17 filter paper strips marked at 1.75-cm intervals. The strips were dried, washed twice in 5% trichloroacetic acid (TCA), once in 95% ethanol, dried, and the individual fractions were cut off, placed in 5 ml of scintillation solution [4 g Omnifluor (New England Nuclear Corp.) per liter of toluene], and assayed for ^{3}H and ^{14}C in a Tractor Delta 300 liquid scintillation spectrometer. The 3 peak fractions contianing the parental density DNA were then pooled, and rebanded in neutral CsCl gradients as described above. Repair replication was determined as the ratio of [^{3}H] 5-BrUra repair label to [^{14}C] thymine prelabel in the parental DNA peak.

Results and discussion

UV radiation-induced DNA degradation. E. coli strains carrying *polA1* (Cooper and Hanawalt, 1972) or *uvrD3* (Ogawa et al., 1968) mutations degrade their DNA extensively after UV-irradiation. Therefore, various irradiation conditions were tested (Fig. 1) in order to find one that might limit UV-radiation-induced DNA degradation, while maximizing the number of lesions introduced into the DNA and the time allowable for repair. Based on such experiments (Fig. 1), 10 Jm^{-2} of UV-radiation (254 nm), and a 30-min repair period were chosen for our standard conditions.

Repair replication. Repair replication in *E. coli* was assayed by the incorporation of a repair label into parental density DNA after UV-irradiation. The DNA from a cell lysate was banded in a neutral CsCl density gradient, and the 3 peak fractions of the parental density DNA were pooled and banded in a second neutral CsCl density gradient (Fig. 2).

During the 30-min labeling period, the unirradiated *polA1* and *polA1 uvrD3* strains showed about a 2-fold reduction in the amount of semi-conservative DNA synthesis compared to the wild-type and *uvrD3* strains, which is consistent with the 2-fold slower growth rate of the *polA* strains (data not shown). Therefore, the large amount of [^{3}H] 5-BrUra incorporation in the parental density region in the unirradiated *polA1* and *polA1 uvrD3* strains (Fig. 2c,g) is probably not due to contamination by semi-conservative DNA synthesis, but may be due to long-patch DNA turnover
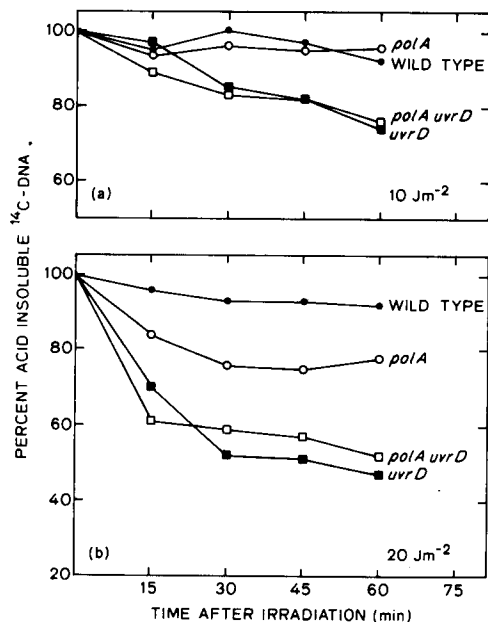
Fig. 1. DNA degradation during incubation after exposure to a UV-radiation fluence of (a) 10 Jm^{-2} or (b) 20 Jm^{-2}. Cultures were grown, labeled with [^{14}C] thymine, and irradiated as described in Methods. Irradiated cell suspensions were then supplemented with glucose (at 0.5%), Difco Casamino Acids (at 0.1%), and thymine (at 2 μg/ml), and incubated at 37°C with aeration. Immediately after irradiation and at intervals thereafter, 1.0 ml samples were removed and placed in tubes containing 1.0 ml of ice-cold 10% TCA. Precipitated samples were collected on Millipore filters (type EH; 0.5 μm pore size), washed, dried and counted as described in Methods. The acid-insoluble radioactivity remained contstant in the unirradiated cells held for 60 min (data not shown). 100% = 1167–4035 cpm. Symbols: ●, wild-type; O, polA1; ■, uvrD3; □, polA1 uvrD3.

performed by DNA polymerase III (and/or II).

The *uvrD3* cells showed more repair replication than did the wild-type or *polA1* strains after 10 Jm^{-2} (Fig. 2) or 20 Jm^{-2} of UV-radiation (data not shown). The enhanced repair replication observed in the *uvrD3* strain was reduced by the addition of a *polA1* mutation, but was still higher than that observed in the wild-type and *polA1* strains (Fig. 2).

While the *uvrD3* and *polA1 uvrD3* strains showed about the same amount of DNA degradation after UV-irradiation (Fig. 1), the amount of repair replication was much greater in the *polA*$^+$ strain (Fig. 2). The enhanced amount of repair replication observed in the *uvrD3* strain appears to be in response to the enhanced DNA degradation observed in this strain following UV-irradiation.

The *uvrD3*, *uvrE502* and *recL152* mutations in *E. coli* are different alleles of the same gene, but strains carrying these mutations often demonstrate quite dissimilar phenotypes (Kushner et al., 1978; Siegel and Race, 1981). Nevertheless, our results, which suggest that the *uvrD3* mutation affects the amount of repair replication after
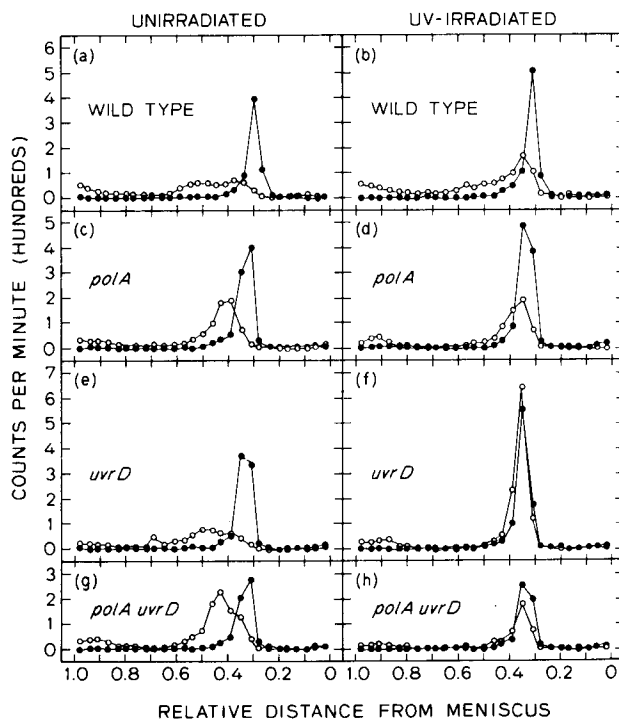
Fig. 2. Repair replication in strains of *E. coli* K12 after 10 Jm^{-2} of UV (254 nm) radiation and 30 min of incubation. The figure shows the rebanding in neutral CsCl density gradients of parental density DNA from unirradiated (a, c, e, and g, respectively) and UV-irradiated (b, d, f, and h, respectively) wild-type, *polA1*, *uvrD3* and *polA1 uvrD3* strains. Repair replication is assessed in the irradiated cells as the relative amount of [^{3}H] 5-BrUra repair label that is present in the [^{14}C] thymine-prelabeled parental density DNA peak (measured base line to base line). Symbols: ●, [^{14}C] thymine; ○, [^{3}H] 5-BrUra.

UV-irradiation, are consistent with the longer-than-average patch size observed in a *recL* strain after UV-irradiation, as measured by 5-BrUra photolysis (Rothman, 1978). Rothman (1978) and Kushner et al. (1978) have suggested that the *recL* and *uvrE* gene products are somehow involved in the regulation of DNA polymerase I and/or III, however, we favor the explanation that the enhanced activity of DNA polymerase I in the *uvrD3* strain after UV-irradiation is simply in response to the presence of longer single-strand templates on which repair replication can occur.

Just prior to submitting this paper, we became, aware of a paper by Kuemmerle and Masker (1980) describing similar studies on *E. coli* K-12 *uvrD101*. No difference was observed by these authors in the amount of repair replication or DNA degradation performed by the *uvrD101* strain after UV irradiation, compared to the wild-type strain. This is in marked contrast to the results obtained here for the *uvrD3* strain. These results further emphasize the conclusion that, although several strains have been isolated that carry different mutations in the *uvrD* gene (i.e., *uvrD3*, *uvrD101*, *recL152* and *uvrE502*), the phenotype of any one *uvrD* mutation

cannot be considered to be representative of all *uvrD* mutations (Siegel and Race, 1981).

Acknowledgments

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