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Involvement of DNA Polymerase III in Excision Repair after Ultraviolet Irradiation

THE dark repair of ultraviolet-induced damage in *Escherichia coli* K-12 involves at least two processes[1–3]: (1) post-replicational repair of gaps produced in daughter strands of the DNA by replication past ultraviolet-induced lesions, and (2) excision repair of these lesions. This report is concerned with only the latter repair process.

Mutants of *E. coli* with a *uvr* mutation are deficient in excision repair[4]; they are unable to produce incision breaks in the DNA at or near the ultraviolet-induced lesions. Production of incision breaks is followed by local DNA degradation, DNA resynthesis and presumably a ligase reaction to close the final gap[1–3].

The *polA*1 mutant of *E. coli* K-12 is deficient in DNA polymerase I activity[5]. In addition, the repair of incision breaks occurs at a slower rate in *polA*1 cells than *pol*$^+$ cells[6,7]. This is presumably due to a deficiency of the *polA*1 strain in the DNA resynthesis step of excision repair, thus implicating DNA polymerase I in this process. However, the *polA*1 strain is not completely deficient in the repair of incision breaks, suggesting two possibilities: (1) some residual DNA polymerase I activity accounts for this repair, and/or (2) another DNA polymerase is able to perform repair resynthesis. We have examined the latter possibility.

Strains of *E. coli* which lack DNA polymerase II activity are not ultraviolet-sensitive[8,9], suggesting that this enzyme is not involved to any great extent in the repair of ultraviolet damage. Thus, by elimination, DNA polymerase III seemed likely to be the enzyme responsible for the residual repair activity in *polA* cells.

The conditionally lethal *dnaE* mutants of *E. coli* produce a temperature sensitive DNA polymerase III[10]. We have examined the ultraviolet survival and repair characteristics of a *polA*1 *dnaE* strain (BT1026)[10–12] in permissive and restrictive conditions.

The *polA*1 *dnaE* strain was grown for several generations to log phase in minimal medium[13] supplemented with thymine at 2 μg ml^{-1} and thiamine at 0.5 μg ml^{-1}. At a density of ~10^8 cells ml^{-1} the cells were collected on a 0.45 μm 'Millipore' filter and resuspended in DTM buffer (minimal medium without glucose or supplements). Samples were incubated at 30° C or 42° C for 30 min before ultraviolet irradiation in DTM buffer and for 60 min after irradiation in minimal medium. The 42° C samples were irradiated while being stirred in a Petri dish

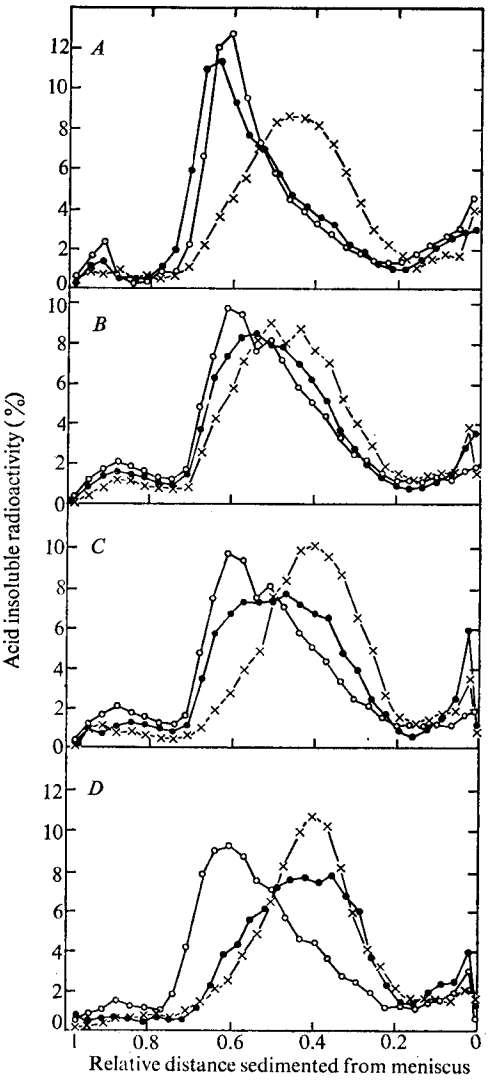

Fig. 1 Repair of ultraviolet-induced single strand breaks in a *polA1 dnaE* strain (BT1026). Log phase cells grown to ~10^8 cells ml^{-1} at 30° C in minimal medium supplemented with thiamine (0.5 μg ml^{-1}), thymine (2 μg ml^{-1} with 100 μCi ml^{-1} ^{3}H-thymine; New England Nuclear Corp.), and deoxyguanosine (100 μg ml^{-1}, added because the *thy* mutation of BT1026 is very leaky at 30° C[12]) were incubated at 30° C or 42° C for 30 min in DTM buffer before ultraviolet irradiation and then for 60 min in minimal medium after irradiation. The 42° C samples were irradiated at 42° C in a constant temperature holder; the 30° C samples were irradiated at room temperature. Samples (0.05 ml, ~10^6 cells) containing a minimum of 2,000 acid-insoluble c.p.m. were layered onto alkaline sucrose gradients and processed as described elsewhere[19]. A maximum of 37% degradation of DNA to acid soluble material was observed (ultraviolet treated for 60 min sample in *D*). ○, Control; +, 5 min after ultraviolet irradiation; ●, 60 min after ultraviolet irradiation. Exposures and incubation temperatures were (*A*) 0, 50 erg mm^{-2}, 30° C; (*B*) 0, 5 erg mm^{-2}, 42° C; (*C*) 0, 12 erg mm^{-2}, 42° C; (*D*) 0, 25 erg mm^{-2}. 42° C.

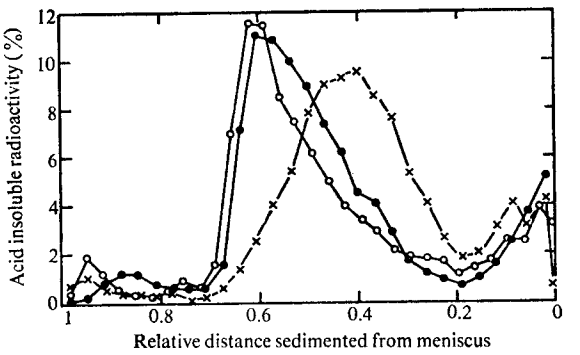

Fig. 2 Repair of ultraviolet-induced single strand breaks in a *polA*1 strain, DY144 (a *dnaE*$^+$ transductant of BT1026). The symbols and procedures are the same as for Fig. 1. Exposure was 50 erg mm^{-2} and temperature was 42° C.

maintained at 42° C. The cells incubated at 30° C were irradiated at room temperature.

At 30° C the *polA*1 *dnaE* strain repaired the incision breaks completely within 60 min after an exposure of 50 erg mm^{-2} (Fig. 1*A*) comparable with a *polA*1 *dnaE*$^+$ transductant at 42° C (Fig. 2). After incubation at 42° C the *polA*1 *dnaE* strain showed little or no repair after an ultraviolet exposure of 25 erg mm^{-2} (Fig. 1*D*). More extensive repair was observed after ultraviolet exposures of 5 or 12 erg mm^{-2} (Fig. 1*B* and *C*). The preincubation at 42° C was necessary for maximal inactivation as, if *polA*1 *dnaE* cells were raised to 42° C only after the ultraviolet exposure (25 erg mm^{-2}), a considerable shift in the DNA profile towards higher molecular weight was observed (unpublished results). The results are consistent with the hypothesis that DNA polymerase III is essential for most of the repair of incision breaks which occurs in a strain lacking DNA polymerase I.

The small amount of repair observed with the *polA*1 *dnaE* strain in the restrictive conditions at low ultraviolet doses may be due to: (1) residual DNA polymerase I activity[14], (2) incomplete inactivation of DNA polymerase III[12], or (3) the involvement of some other DNA polymerase in repair in these conditions. Masker and Hanawalt[15] have reported that ultraviolet-stimulated DNA synthesis occurred in toluene-treated *polA*1 *dnaE* (BT1026) cells held at the restrictive temperature. This repair was dependent on DNA polymerase II[16]. Thus the residual repair of incision breaks which we observe at the restrictive temperature in the *polA*1 *dnaE* strain may be due in part to DNA polymerase II.

The *polA*1 *dnaE* strain was sensitized to killing by ultraviolet irradiation by a combined pre- and post-irradiation incubation

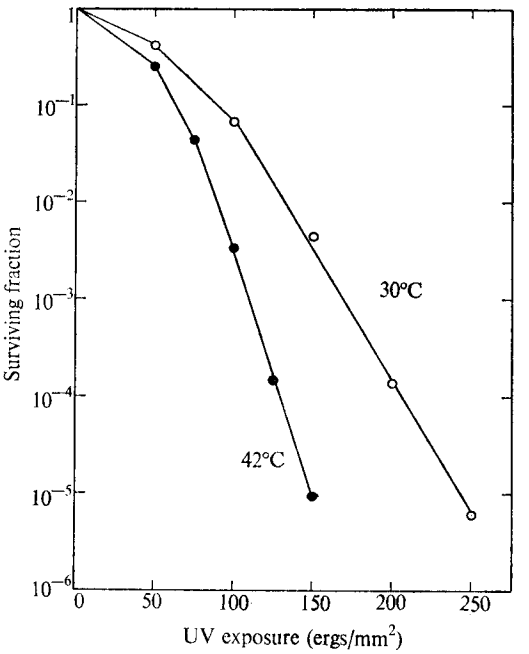

Fig. 3 Survival after ultraviolet irradiation of a *polA*1 *dnaE* strain (BT1026) in permissive and restrictive conditions. Cells were grown to log phase in supplemented minimal medium. Samples were treated as in Fig. 1 except that the post-irradiation incubation was for 80 min in DTM buffer at 30° C or 42° C. The cells were diluted in phosphate buffer (0.67 M, *p*H 7.0), spread on minimal medium solidified with 1.5% noble agar (Difco), and incubated at 30° C for 2–3 d to allow colony formation. The relative survival of control cells incubated at 42° C (●) compared with cells treated at 30° C (○) was 0.81.

in DTM buffer at 42° C, compared with cells treated in the same way at 30° C (Fig. 3). This sensitization correlates with the inhibitory effect of the 42° C treatment on the repair of incision breaks (Fig. 1). The fact that the 42° C treatment had little effect on survival after an ultraviolet exposure of 25 erg mm^{-2} suggests that the inhibitory effect of the 42° C treatment on the repair of incision breaks was partially reversed when the cells were subsequently incubated at 30° C to measure colony forming ability.

Cooper and Hanawalt[17,18] have found that excision repair results in the formation of large and small patches of DNA by repair resynthesis. The large patches were present in a *polA*1 strain but not in a *recA recB* strain, implying that the large patch repair is dependent on *recA* and/or *recB* gene products but not on DNA polymerase I. Our results with the *polA*1 *dnaE*

strain indicate that most of the repair resynthesis present in *polA*1 cells is dependent on DNA polymerase III activity, suggesting that the *recA* and/or *recB* gene dependent synthesis of large patches may require DNA polymerase III.

Thus the available evidence suggests that at least two pathways of excision repair may be present in a wild-type cell: (1) a DNA polymerase I dependent pathway, and (2) a DNA polymerase III dependent pathway.

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