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Host Cell Deoxyribonucleic Acid Polymerase I and the Support of T4 Bacteriophage Growth

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Ultraviolet irradiation of *Escherichia coli polA*$^-$ cells reduces their capacity to support the growth of T4 phage. There is no additional loss of capacity observed in *pol tsA*$^-$ *recA*$^-$ double mutants at the nonpermissive temperature. The reversion frequency of a T4 *rII* mutant after ultraviolet irradiation is not changed by the absence of host deoxyribonucleic acid polymerase I.

The in vivo function of deoxyribonucleic acid (DNA) polymerase I is not known; however, the in vitro properties of the enzyme indicate a role in repair processes (5). A mutant of *Escherichia coli* strain W3110, *polA1*, containing less than 1% DNA polymerase I activity in vitro, is about five times as sensitive to ultraviolet light (UV) as the parental strain (4), although it is able to excise dimers normally (3). The *polA1* mutation also affects the survival of some, but not all, UV-sensitive on *polA1* than on *polA*$^+$ cells (7), strain. Although ϕX174 and λ phages are more UV-sensitive on *polA1* than on *polA*$^+$ cells (7), the polymerase deficiency does not affect the survival of UV-irradiated T7 phage (4). We report here some observations on the effects of the *polA1* mutation of the host on the survival and mutagenesis of UV-irradiated bacteriophage T4.

Survival of phage after irradiation. T4 at a titer of 2×10^7/ml were suspended in tris(hydroxymethyl)aminomethane (Tris) diluent, placed on a rotating turntable to allow even exposure to all particles, and UV-irradiated with a 15-watt General Electric germicidal lamp (model no. G15T8) at a distance of 59 cm from the source. Under these conditions, an exposure of 1 sec corresponds to approximately 8 ergs/mm^2. Irradiated T4 phage were adsorbed to *polA1* and *polA*$^+$ cells at 37C at a multiplicity of infection of 0.1. Unadsorbed phage were removed with anti-T4 serum.

The survival of irradiated wild-type T4 is lower when assayed on *polA1* than on *polA*$^+$ cells (Fig. 1). After 40 sec of UV irradiation of the phage, the ratio of phage survival, *polA*/*polA*$^+$, is 1:4.5. These results confirm the findings of Smith, Symonds, and White (9). However, the UV-sensitive mutant T4v and wild-type phage T6 have the same UV sensitivity and plating efficiency on *polA1* and *polA*$^+$ cells (*unpublished observations*). T4v lacks an endonucleolytic activity (nicking enzyme) specific for UV-irradiated DNA (12) which apparently performs the first step in the excision repair of UV-induced lesions. Since the *polA1* mutation of the host does not further increase the UV sensitivity of T4v, it is probable that the effect of the *polA1* mutation on phage survival requires the presence of a single-stranded nick in the bacteriophage DNA.

Reversion frequency of rII mutants. To determine whether the reduced survival of UV-irradiated T4$^+$ on *polA1* cells is accompanied by an increased rate of mutagenesis, we assayed the reversion frequency of T4 *r375* (an *rII* nonsense mutant revertable by T4 gene 43 mutator polymerase [10]) infecting *polA1* and *polA*$^+$ cells. There were no significant differences in the reversion frequency of T4 *r375* infecting *polA1* or *polA*$^+$ cells, either before or after irradiation of the phage. The relative reversion frequency per surviving phage particle (on *polA1*/on *polA*$^+$) ranged from 0.8 to 1.2 for unirradiated phage and from 0.9 to 1.0 for irradiated phage.

Growth of T4$^+$ on UV-irradiated cells. The capacity of *E. coli* to support the growth of T-even phages is normally very resistant to UV irradiation (2, 11). We investigated the effect of the *polA1* mutation on the capacity of UV-irradiated cells to yield T4$^+$ phage. Bacterial cells grown to 2×10^8/ml in M9S medium (1) were resuspended in Tris diluent at 1×10^9/ml. Irradiated cell suspensions were less than 1.0 mm in depth. The cells were exposed to increasing doses of UV and then infected at a multiplicity of 0.1 with wild-type T4. The phage,

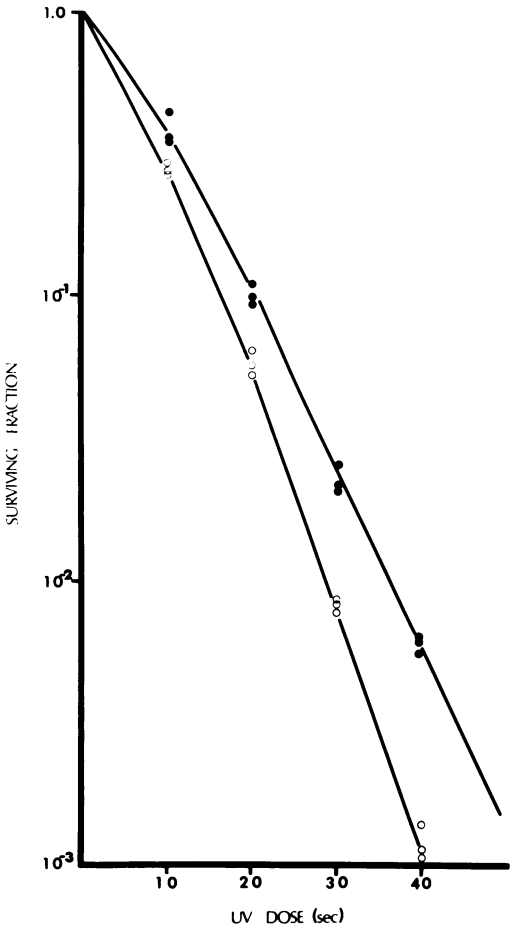

FIG. 1. *Survival of UV-irradiated T4v⁺ on polA⁺ (●) and polA1 (○). Unirradiated T4 has the same efficiency of plating on polA⁺ and polA1.*

either untreated or irradiated to about 1% survival, were assayed on *polA1* or *polA⁺* cells.

We found that the *polA1* cells showed a 50% decrease in their capacity to support phage growth at doses of UV irradiation to which the capacity of *polA⁺* cells was highly resistant (Fig. 2).

Attempts to construct doubly mutant bacteria (*polA recA*) deficient in DNA polymerase I and lacking the *recA⁺* gene product have been unsuccessful, leading to the conclusion that the double mutant is not viable (5, 8). Support for this conclusion was obtained by the isolation of *recA* derivatives with a temperature-sensitive *polA* mutation; these cells do not form colonies at the nonpermissive temperature (5, 8). The *recA* mutation of the host does not affect the survival of UV-irradiated T4 phage. We tested the survival of UV-irradiated T4 on *recA⁺* and

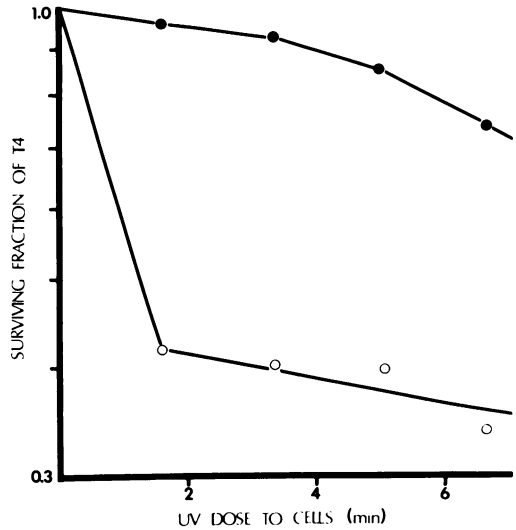

FIG. 2. *Capacity of UV-irradiated polA⁺ and polA1 cells to support the growth of unirradiated T4v⁺. Cells at 10^9/ml were subjected to increasing doses of UV irradiation and singly infected with unirradiated T4v⁺. T4v⁺ on polA⁺ (●) and polA1 (○). Each point represents the average of three experiments.*

recA56 cells that also contained the mutation, *polA12*, which results in the synthesis of a temperature-sensitive DNA polymerase I (8). After growth at 30 C, the cells were transferred to the nonpermissive temperature, 42 C, for 30 min before adsorption of irradiated T4. The infective centers were plated, with *polA⁺* cells as the indicator strain, and incubated overnight at 42 C. Under these conditions, there was no difference in the survival of UV-irradiated T4 on strains W3110 *polA12 recA56* and *polA12 recA⁺*. In both cases, the survival of the UV-irradiated T4 was lower than when assayed on *polA⁺* cells. At the nonpermissive temperature, the *polA12 recA56* double mutant displayed the same degree of decreased capacity after UV irradiation as previously demonstrated with *polA1*.

Therefore, although the presence of the Kornberg DNA polymerase of the host cell may be required for the efficient repair of UV-irradiated wild-type T4, the additional absence of *recA* product has no detectable further effect on the survival of the phage.

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LITERATURE CITED

1. Adams, M. H. 1959. Bacteriophages. John Wiley & Sons, New York.
2. Anderson, T. F. 1948. The growth of T2 virus on ultraviolet-killed host cells. J. Bacteriol. **56**:403-410.
3. Boyle, J. M., M. C. Paterson, and R. B. Setlow. 1970. Excision-repair properties of an *Escherichia coli* mutant deficient in DNA polymerase. Nature (London) **226**:708-710.
4. DeLucia, P., and J. Cairns. 1969. Isolation of an *E. coli* strain with a mutation affecting DNA polymerase. Nature (London) **224**:1164-1166.
5. Gross, J. D., J. Grunstein, and E. M. Witkin. 1971. Inviability of *recA* derivatives of the DNA polymerase mutant of DeLucia and Cairns. J. Mol. Biol. **58**:631-634.
6. Kelly, R. B., M. R. Atkinson, J. A. Huberman, and A. Kornberg. 1969. Excision of thymine dimers and other mismatched sequences by DNA polymerase of *Escherichia coli*. Nature (London) **224**:495-501.
7. Klein, A., and U. Niebch. 1971. Host cell reactivation in strains of *E. coli* lacking DNA polymerase in vitro. Nature (London) **229**:82-84.
8. Monk, M., and J. Kinross. 1972. Conditional lethality of *recA* and *recB* derivatives of a strain of *Escherichia coli* K-12 with a temperature-sensitive deoxyribonucleic acid polymerase I. J. Bacteriol. **109**:971-978.
9. Smith, S. M., N. Symonds, and P. White. 1970. The Kornberg polymerase and the repair of irradiated T4 DNA. J. Mol. Biol. **54**:391-393.
10. Speyer, J. F., and D. Rosenberg. 1968. The function of T4 DNA polymerase. Cold Spring Harbor Symp. Quant. Biol. **33**:345-349.
11. Tessman, E. S. 1956. Growth and mutation of phage T1 on ultraviolet-irradiated host cells. Virology **2**:679-688.
12. Yasuda, S., and M. Sekiguchi. 1970. T4 endonuclease involved in repair of DNA. Proc. Nat. Acad. Sci. U.S.A. **67**:1839-1845.