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

DONNA GEORGE AND DAVID ROSENBERG

Department of Biological Sciences, Douglass College, Rutgers-The State University, New Brunswick, New Jersey 08903

Received for publication 25 August 1972

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, polAl, 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 polAI mutation also affects the survival of some, but not all, UV-sensitive on polAI than on polA+ cells (7), strain. Although φX174 and λ phages are more UV-sensitive on polAI 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 polAI 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 × 10^10/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 polAI and polA+ cells at 37°C 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 polAI 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mutant T4v and wild-type phage T6 have the same UV sensitivity and plating efficiency on polAI and polA+ cells (unpublished observations).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 polAI mutation of the host does not further increase the UV sensitivity of T4v, it is probable that the effect of the polAI 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 polAI cells is accompanied by an increased rate of mutagenesis, we assayed the reversion frequency of T4 r375 (an rII nonsense mutant revertible by T4 gene 43 mutator polymerase [10]) infecting polAI and polA+ cells. There were no significant differences in the reversion frequency of T4 r375 infecting polAI or polA+ cells, either before or after irradiation of the phage. The relative reversion frequency per surviving phage particle (on polAI/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 polAI mutation on the capacity of UV-irradiated cells to yield T4+ phage. Bacterial cells grown to 2 × 10^9/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,
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 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.

We thank Marilyn Monk for E. coli strains W3110 polA12 recA\(^-\) and polA12 recA56.

Donna George is the recipient of a National Defense Education Act Title IV fellowship.
LITERATURE CITED