SHORT COMMUNICATION

Impossible Formation of Twisted Circular Form DNA after Infection with Ultraviolet Irradiated λ Phages

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ABSTRACT

There is no conversion of λ DNA into the twisted circular form DNA molecules when E. coli cells were infected with ultraviolet-irradiated λ phages.

It is known that the linear double stranded λ DNA injected into the host cells is converted to a circular form DNA as a first step of phage multiplication through the hydrogen-bonding the cohesive ends of both terminal single stranded parts of DNA, and then, to the twisted circular form DNA through the formation of covalent-bonds between the terminal nucleotides of both sites of DNA strands. The present communication will show the impossible formation of twisted circular DNA, when E. coli cells unable to repair the ultraviolet lesions to DNA were infected with ultraviolet-irradiated λ phages.

Escherichia coli K12 N3-5 (uvr B) in growth phase and λhC4 phages were used. The cells were grown in λ-broth to about 3×10⁸ cells/ml. ³²P-labeled phages were multiplied in the λ broth supplemented with P₇-deprived casamino acids in stead of polypeptone, 1 mM MgCl₂, 8.8 µg/ml KH₂PO₄ and 10 µC/ml ³²P-orthophosphate. λ phages (10⁹/ml) in λ-dilution buffer** and the host cells (3×10⁸/ml) in adsorption buffer*** were irradiated with ultraviolet light at a dose rate of 119 ergs and 2

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** λ-dilution buffer: 10 mM Tris, 1 mM MgCl₂ and 5 g NaCl in 1000 ml (pH 7.4)
*** Adsorption buffer: 10 mM Tris and 10 mM MgSO₄
ergs/mm²/sec, respectively. The cells (3 × 10⁹/ml) were infected with phages in the adsorption buffer at 0°C for 15 min at a moi 3. Two kinds of infection system were used. One, in which ultraviolet-irradiated cells were infected with ultraviolet-irradiated λ phages. The other, non-irradiated cells were infected with irradiated phages in the presence of 60 μg/ml chloramphenicol. The infected cells were incubated at 37°C and the DNA was extracted from the cells following to the procedures by Anraku et al.². The DNA preparation was heated for 10 min at 75°C and cooled rapidly before the sedimentation analysis of DNA patterns. The DNA samples were layered on the top of a linear gradient of sucrose (5—20%) prepared in 1×SSC* and centrifuged for 3 or 4 h at 30,000 rpm. The radioactivity of two-drops-fraction on a piece of filter paper from the bottom of the centrifuge tube was counted by a liquid scintillation counter (Mark I) after washing twice the paper pieces with 2% trichloroacetic acid, followed twice with alcohol.

The DNA synthetic activity of λ phages was measured by the incorporation of ³H-thymidine into acid insoluble materials. E. coli K12 N3-5 (T⁻) was used as host cells. To the suspension of ultraviolet-irradiated cells infected with λ phages, ³H-thymidine (3 μC/3 μg/ml) was added and 0.1 ml aliquot was withdrawn from the cell suspension at a regular time-interval into 5% trichloroacetic acid. The acid insoluble materials were collected on a glass filter (GB-100), washed, dried and counted. The DNA extracted from ³H-thymidine-labeled λ phages was used as marker DNA.

E. coli cells were irradiated with ultraviolet light at 20 ergs/mm² which dose is enough to abolish the cell DNA synthesis. (Data not shown) The synthesis of λ phage DNA, however, can proceed with normal rate in the irradiated cells³, although the rate of synthesis is decreased in proportion to the ultraviolet dose to the phages. (Fig. 1.)

The ultraviolet-irradiated cells were infected with ³²P-λ phages irradiated with ultraviolet light at 1190 ergs/mm². The change of molecular

* 1×SSC: 0.15 M NaCl+0.015 M Na-citrate

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Fig. 1. Inhibition of phage DNA synthesis after infection of ultraviolet-irradiated λ phages.
(a) non-irradiated phages, (b) irradiated with 50 ergs/mm², (c) 100 ergs/mm², (d) 200 ergs/mm², (e) 1000 ergs/mm².
form of parental phage DNA in the cells was shown in Fig. 2.

![Image of Fig. 2](image)

**Fig. 2.** Formation of twisted circular λ DNA in ultraviolet-irradiated host cells and impossible formation of the DNA form after infection of ultraviolet-irradiated λ phages.

(a) pattern of parental λ DNA at 0 time infection, (b) the pattern after incubation for 15 min, (c) pattern of non-irradiated λ DNA in the cells incubated for 15 min (centrifuged for 4 h), (d) pattern of DNA incubated for 15 min after infection with ultraviolet-irradiated λ phages at 1190 ergs/mm² (centrifuged for 4 h).

--- ○ --- ³²P-labeled parental λ DNA, ⋯⋯ ³H-marker DNA

Analogous experiments to Fig. 2 were done under an absence condition of progeny phage synthesis. Non-irradiated cells were infected with ultraviolet-irradiated ³²P-λ phages and incubated for 70 min in the presence of chloramphenicol. The results were shown in Fig. 3.

It is known from the both results in Figs. 2 and 3 that the λ DNA when irradiated with ultraviolet light loses the ability to convert the initial linear double stranded DNA molecules into the twisted circular form DNA in host cells. The process in the twisted circular DNA formation, however, is rather resistant to the ultraviolet irradiation as compared to the progeny formation, because the progeny DNA synthesis is highly inhibited by relatively low doses of ultraviolet light on λ phages. (Ref. Fig. 1) These facts indicate that the ultraviolet lesions to the λ DNA do not so much interfere in the formation of twisted circular DNA, although a high amount of lesions completely inhibits the structural conversion of DNA, but the
lesions affect more efficiently the λ DNA replication\(^{4,5}\).

The present experimental results suggest that the main cause for the inhibition of λ DNA synthesis by ultraviolet irradiation is not due to the impossible formation of twisted circular DNA molecules, but is probably due to the formation of pyrimidine dimers in DNA molecules\(^6\).
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REFERENCES