Nuclear Disruption After Infection of *Escherichia coli* with a Bacteriophage T4 Mutant Unable to Induce Endonuclease II

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Received for publication 12 April 1972

Nuclear disruption after infection of *Escherichia coli* with a bacteriophage T4 mutant deficient in the ability to induce endonuclease II indicates that either (i) the endonuclease II-catalyzed reaction is not the first step in host deoxyribonucleic acid (DNA) breakdown or (ii) nuclear disruption is independent of nucleolytic cleavage of the host chromosome. M-band analysis demonstrates that the host DNA remains membrane-bound after infection with either an endonuclease II-deficient mutant or T4 phage ghosts.

Within 2 or 3 min after infection of *Escherichia coli* cells with T-even bacteriophages, the nucleoids of the bacteria undergo nuclear disruption during which the host deoxyribonucleic acid (DNA) accumulates in numerous clumps along the cell membrane (2, 12, 14, 20, 21). Nuclear disruption is induced by ultraviolet light-inactivated T-even phages (20), but not by phage ghosts (2). Kellenberger et al. (14) have shown that nuclear disruption after T-even phage infection is blocked by the addition of chloramphenicol at the time of infection. If chloramphenicol is added 1 min after infection, nuclear disruption is delayed. The addition of chloramphenicol at 8 min after infection has no effect on nuclear disruption. These observations suggest that nuclear disruption results from the action of an “early” or “pre-early” phage function. Subsequent to nuclear disruption, all but about 20% of the host DNA is degraded to acid-soluble residues, and the nucleotides produced are reincorporated into progeny phage DNA (11, 16–18, 28). It is now clear, however, that extensive host chromosome breakdown is not essential for phage T4 replication (9, 27). It seems likely (and has been suggested, see reference 26) that nuclear disruption may be coincident with the first step in this process of degradation of host DNA. It is not clear, however, whether nuclear disruption is also not essential for phage T4 replication.

Bose and Warren (3) have reported that when chloramphenicol is added at the time of infection, the host DNA remains as material which sediments in glycerol gradients like intact bacterial DNA. When chloramphenicol is added at 5 min after infection, the host DNA sediments as material with a size comparable to intact T4 DNA. If chloramphenicol is added at later times, the host DNA undergoes much more extensive degradation. Their results suggest a very early, discrete endonucleolytic step in host DNA breakdown, yielding fragments of $10^8$ to $2 \times 10^8$ daltons in size. This initial step could account for the light and electron microscope observations of rapid nuclear disruption after T-even phage infection (2, 12, 14, 20, 21).

Warner et al. (27) and Hercules et al. (9) have recently isolated mutants of phage T4 which are unable to carry out an early step in the degradation of host DNA. These mutants are unable to induce endonuclease II (24), an enzyme which makes single-strand breaks in double-stranded cytosine-containing DNA (23). When *E. coli* cells are infected with endonuclease II-deficient T4 phage, the host DNA remains as material which sediments with an apparent molecular weight of about $2 \times 10^8$ daltons, or about one-tenth the size of the intact *E. coli* genophore. This observation, along with the results of Bose and Warren (3) and Kellenberger et al. (14), suggests that the initial nuclear disruption may be accompanied by endonucleolytic cleavage of about ten phosphodiester bonds per host genophore (for further dis-
cussion, see the review by Koerner, reference 15) and that endonuclease II, when present, then further degrades the host DNA fragments. Direct confirmation of this hypothesis by conventional density gradient ultracentrifugation studies of the DNA from cells infected with endonuclease II-deficient mutants is difficult since the reliability of this technique for analysis of DNA molecules larger than 10^6 daltons is still uncertain.

All of the conditional-lethal mutants of phage T4 examined so far have been found to induce normal nuclear disruption (8). Thus it was of interest to determine whether nuclear disruption occurs in cells infected with endonuclease II-deficient T4 phage. This report describes the results of thin-section electron microscopy and M-band analysis of the fate of host DNA after infection of E. coli with a T4 mutant deficient in the ability to induce endonuclease II.

MATERIALS AND METHODS

Phage and bacterial strains. The bacteriophage T4 endonuclease II-deficient mutant nd 28x6 has been described by Warner et al. (27). Amber mutants B22 (gene 43), N82 (gene 44), and H39 (gene 30) were generously provided by R. S. Edgar. Double mutants of nd 28x6 and these amber mutants were prepared as described earlier (27). Amber stocks were prepared on E. coli strain CR63; E. coli strain B/5 was used as the amber-restrictive host in the EM and M-band experiments.

Preparation of T4 “ghosts.” The procedure used to prepare T4 phage ghosts was adopted from Duckworth (6) and Herriott and Barlow (10). A wild-type T4 lysate was concentrated by membrane ultrafiltration (Dia-flo apparatus model 50 with XM-50 membrane, Amicon Corp., Cambridge, Mass.). Ghosts were prepared by adding 2 volumes of 3 M sodium acetate to 1 volume of concentrated T4, titr 2 X 10^9, equilibrating for 15 min at 0°C, and rapidly diluting into 100 volumes of cold distilled water (rapidly stirred). The shocked phage were treated with deoxyribonuclease I (5 µg/ml) after adjusting the suspension to 3 mM MgSO4. After incubation for 30 min at 36°C, the suspension was centrifuged at 3,000 X g for 15 min. The supernatant fluid was then centrifuged at 100,000 X g for 3 hr. The precipitate was suspended in a small volume of M-9 salt solution and used without further treatment.

Inactivation of plaque-forming ability was about 99%. Electron micrographs of the preparation (e.g., Fig. 6B) showed that most of the tail structures were indeed intact (sheaths not contracted) and that most of the heads were empty. Preliminary experiments demonstrated the killing ability of the ghosts as well as their ability to induce lysis of host cells at multiplicities above 10. The titer of the ghost preparation was estimated by assuming that one-half of the shocked phage were recovered as active ghosts (5).

Electron microscopy. Log-phase cells were prepared by inoculating fresh H-broth (25) with a 1,000-fold dilution of an E. coli B/5 overnight culture, aerating at 30°C for 2.5 hr, centrifuging, and resuspending the pellet in fresh H-broth. The log-phase cultures were adjusted to about 4.4 X 10^6 cells/ml by using a Petroff-Hauser counting chamber and infected immediately using a multiplicity of infection of 20. The infected cultures along with an uninfected control were aerated at 30°C. In two experiments, the infections were terminated by transferring 5-ml samples to an ice-water bath at 7.5 and 15 min after infection. In the third experiment, 5-ml samples were transferred to an ice-water bath at 10 min after infection. The infected and uninfected cells were then spun down and resuspended in 1% osmium tetroxide in Veronal-acetate buffer (13). After fixation for 16 hr, the cells were suspended in agar, dehydrated with ethanol, and embedded in Epon 812 (19). Thin sections were cut with an LKB Ultratome, mounted on carbon-coated Formvar, and doubly stained with uranyl acetate and Reynolds' lead citrate (22). Electron micrographs were taken with an RCA EMU-3G electron microscope.

M-band experiments. The M-band procedure used was adapted from that described by Earhart et al. (7). Cultures of E. coli B/5 were grown in M-9 medium (1) at 37°C to a density of about 10^9 cells/ml, at which time 0.5 mg of uridine per ml was added to suppress the induction of thymidine phosphorylase (4). Four minutes later 14C-thymidine was added (specific activity 33 µCi/µmole, final concentration 0.06 µCi/ml), and the culture was shaken for 80 min at 37°C. Cells were then sedimented by centrifugation for 5 min at 10,000 X g and suspended in warm (30°C), nonradioactive medium. After 2 min of incubation (30°C), phage were added at a multiplicity of infection (MOI) of about 5. At various times thereafter, 2.5-ml samples were removed from the 30°C water bath and quickly cooled, and the cells were pelleted by centrifugation. Cells were resuspended in 0.4 ml of 15% sucrose in 0.01 M Tris(hydroxymethyl)aminomethane (Tris)-chloride, pH 8.1. To this was added 0.1 ml of a 1:1 mixture of lysozyme (850 µg/ml in 0.25 M Tris-chloride, pH 8.1) and disodium ethylenediaminetetraacetate (2.7 mg/ml). After 10 min at 0°C, 0.1 ml of 0.1 M MgCl2 was added. The suspension (0.5 ml) was layered on a 9-ml gradient of 15 to 47% sucrose in TMK buffer (0.01 M Tris-chloride, pH 7.0; 0.01 M magnesium acetate; 0.1 M KCl, and 0.02% NaN3) over a 4 ml cushion of 47% sucrose in TMK buffer; 0.04 ml of 5% Sarkosyl was added and carefully mixed with the tip of a pipette. Gradients were prepared 4 to 6 hr before use and equilibrated at 4°C. Gradients were centrifuged in an SB269 rotor in an IEC ultracentrifuge (model B-35) at 25,000 X g (average) for 20 min. Fractions were collected by removing successive 0.5-ml samples from the meniscus with a syringe.

Fractions were analyzed by adding 0.1 ml of 25% trichloroacetic acid and 0.1 ml of bovine serum albumin (at 10 mg/ml), sedimenting precipitates by centrifugation for 10 min at 2,500 X g, washing twice with 1.0 ml of cold 5% trichloroacetic acid, dissolving in 0.5 ml of 1 M NaOH, plating on planchets, and counting on a Nuclear-Chicago planchet counting system (model 4338).
Fig. 1. Electron micrograph showing the typical nuclear morphology observed in uninfected E. coli B/5 control cells; magnification approximately $\times 51,000$. 
RESULTS AND DISCUSSION

When *E. coli* is infected with wild-type T4 phage, nuclear disruption is rapidly followed by the synthesis of a large pool of phage DNA. In studying the possible role of T4 endonuclease II in nuclear disruption, we have avoided the complicating effect of phage DNA synthesis by using double mutants carrying *nd 28x6* (endonuclease II−) and a DNA synthesis− amber mutation.

Figure 1 shows a thin section of an uninfected *E. coli* B/5 cell with the typical multilobed, but largely centrally located, nuclear regions. Figure 2 shows the occurrence of nuclear disruption (the accumulation of host DNA along the cell membrane) in B/5 cells infected with *am B22* (gene 43, DNA polymerase−). When *E. coli* B/5 cells are infected with an *nd 28x6* (gene *den A*)-*am B22* (gene 43) double mutant (endonuclease II−, DNA polymerase−), nuclear disruption occurs (Fig. 3) just as in *am B22*-infected cells, except that the peripheral DNA-containing regions generally are larger, presumably as a result of the block in host DNA breakdown imposed by the absence of endonuclease II. Nuclear disruption was also observed in cells infected with an *nd 28x6* (gene *den A*)-*am N82* (gene 44, DNA synthesis−) double mutant and an *nd 28x6* (gene *den A*)-*am N82* (gene 44)-*am H39* (gene 30, DNA ligase−) triple mutant. Essentially identical nuclear disruption was observed at 7.5, 10, and 15 min after infection.

Nuclear disruption is not merely a fortuitous event due to inhibition of host metabolism or killing following T-even phage infection since, as

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**Fig. 2.** Electron micrographs showing nuclear disruption in *am B22*-infected B/5 cells, MOI = 20; one showing about the minimum amount of membrane-associated DNA (A) and the other the maximum amount of membrane-associated DNA (B) observed at 15 min after infection. Magnification approximately ×58,000.
mentioned earlier, it does not occur in cells infected with T-even ghosts (2). Nuclear disruption also does not appear to occur in cells killed by lysis-from-without by high multiplicities of T4 phage "ghosts." Figure 4 shows the nuclear morphology observed in B/5 cells mixedly infected with viable nd 28x6-am B22 phage (MOI 7.5) and an excess (MOI approximately 100) of noninfectious T4 particles ("ghosts"). These cells showed varying degrees of "lysis-from-without" (cell wall breakdown) but no nuclear disruption. Rather, they exhibited a condensation of the nuclear region to the central part of the cell (much less lobed than in uninfected cells) typical of that observed in cells infected with T-even phage ghosts (2).

The M-band technique of Earhart et al. (7) was used to determine whether the host DNA remains membrane-bound or is released from the cell membrane in the absence of endonuclease II. Most—all but 10% and 30% of the radioactivity recovered in the gradient (some counts "leak" to the medium at late times after infection)—of the host DNA was released from the membrane by 30 min after infection with wild-type T4 and am B22, respectively. In contrast, most (77%) of the counts recovered in the gradient) of the host DNA remained in the M-band at 30 min after infection.

Fig. 3. Electron micrographs showing nuclear disruption in one longitudinal section and three cross sections of E. coli B/5 cells infected with the double mutant nd 28x6-am B22; MOI = 20; 10 min after infection. Magnification approximately X57,000 (A), X76,000 (B), and X37,000 (C).
Fig. 4. Electron micrograph showing the appearance of the nuclear region when E. coli B/5 cells were simultaneously infected with infectious T4 phage (nd 28x6-am B22; MOI = 7.5) and an excess (MOI approximately 100) of nonviable phage ("ghosts") sufficient to induce lysis-from-without. Magnification approximately ×57,000.
Fig. 5. M-band analysis of the DNA from E. coli B/5 cells infected with (A) T4 wild-type, (B) am B22, (C) nd 28x6, and (D) nd 28x6-am B22. Fraction 1 is the top of the gradient. The distinct peak corresponds to the M-band in each case. Progeny phage were not titered in these experiments; in earlier experiments, however, phage particles were almost all recovered in fractions 1 through 5. The latter undoubtedly account for most of the label found in fractions 1 through 5 at late times after infection with T4 am+. For details of the experiment, see Materials and Methods. Radioactivity is expressed as counts per minute per milliliter × 10^-3; (○) 7.5, (●) 10, (△) 15, and (▲) 30 min after infection.
Fig. 6. (A) M-band analysis of the DNA from E. coli B/5 cells infected with T4 phage ghosts, (○) 7.5, (△) 15, and (▲) 30 min after infection. Fraction 1 is the top of the gradient. (B) Electron micrograph of the ghost preparation used; magnification approximately ×135,000.
with nd 28x6-am B22 (Fig. 5). This indicates that the host DNA which accumulates as material with an apparent size of 10^6 to 2 × 10^6 daltons after infection with nd 28x6 (9, 27) is not released from the cell membrane.

In the light of Duckworth’s suggestion that many of the effects of phage T4 ghosts could be explained if infection with ghosts led to functional detachment of the host DNA from the cell membrane (5), it is of some interest that, at least under our conditions, infection with T4 ghosts did not release the host DNA from the M-band (Fig. 6A).

The evidence discussed earlier in this paper suggests that an “early” or, more likely, a “pre-early” phage gene is responsible for nuclear disruption after T-even phage infection. The results presented in this report indicate that the gene responsible for this phenomenon is not den A. When E. coli B/5 cells are infected with double mutants of phage T4 which are unable to induce the synthesis of endonuclease II and phage DNA, they undergo nuclear disruption, and most of the host DNA remains membrane-bound. The mechanism by which nuclear disruption occurs following infection of E. coli with phage T4 remains unknown; the process does not, however, appear to require endonuclease II activity. One rather attractive possibility is that nuclear disruption requires no phage-induced function(s) except the proposed pre-early endonuclease, and that the free ends produced by limited endonucleolytic hydrolysis of the host chromosome become attached to the cell membrane by one or more normal host components, perhaps by a membrane-bound enzyme or enzyme complex which functions in replication or repair of DNA in the uninfected cell.

 Majumdar, Dewey, and Frankel (personal communication) have recently shown that DNA polymerase I becomes transiently membrane-bound very soon after infection with phage T4, T7, or lambda. Polymerase I does not become membrane-bound following infection with T4 ghosts or a known deletion mutant of T7. Chloramphenicol inhibited the membrane association of DNA polymerase I when added at the time of infection and inhibited its further binding when added at 2.5 or 5 min after infection. These results strongly suggest that the DNA polymerase I-membrane association is mediated by phage-directed protein synthesis. One would expect the nicks or free ends, or both, produced by endonucleolytic cleavage of host DNA to bind to the membrane-associated DNA polymerase I. The 3'- or 5'- (or both) exonuclease activities of DNA polymerase I may, in fact, play a role in host DNA breakdown.

ACKNOWLEDGMENTS

This investigation was supported by Public Health Service research grants AI-07946 to D. Peter Snustad, AI-07988 to Huber R. Warner, and AI-08088 to Dwight L. Anderson (from the National Institute of Allergy and Infectious Diseases), and National Science Foundation research grant GB-29393 to Dwight L. Anderson.

D. Peter Snustad would like to thank Dwight L. Anderson and Donald D. Hickman for instruction on the techniques of thin-section electron microscopy and Susan Kay Harlander for technical assistance.

LITERATURE CITED


