NOTES

Analysis of Nuclear Disruption and Binding of Intermediates in Host DNA Breakdown to Membranes After Infection of *Escherichia coli* with Bacteriophages T4 and T7¹

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Received for publication 19 December 1972

Escherichia coli DNA polymerase I is implicated in the binding of intermediates in host DNA breakdown to membrane in T4-infected, but not T7-infected, cells. Nuclear disruption is observed in T4-infected *polA1* mutant cells.

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 DNA accumulates along the cell membrane (1, 11, 17, 19, 23). Several observations (for a discussion of these, see 14 and 23) suggest that nuclear disruption results from the action of a "pre-early" phage gene(s). Subsequent to nuclear disruption, all but about 20% of the host DNA is degraded to acid-soluble residues which are then reincorporated into progeny phage DNA (10, 13, 15, 16, 26). It has been suggested (24) that nuclear disruption may be coincident with the first step in the process of degradation of host DNA.

Recent studies with a T4 mutant unable to induce endonuclease II (22), an enzyme which produces single-strand breaks in double-stranded cytosine-containing DNA (20), indicate that either the endonuclease II-catalyzed reaction is not the first step in host DNA breakdown, or, if it is the first step, nuclear disruption is independent of nucleolytic cleavage of the host chromosome (23). When *E. coli* cells are infected with endonuclease II-deficient T4 phage, the host DNA remains in large molecules with a mol wt of at least 10⁸ (9, 25). The above observations and the results of Bose and Warren (2) have suggested the existence of

a pre-early endonuclease which makes about 10 breaks in the host chromosome.

Since the host DNA moves very rapidly from a largely central location within the cell into tight juxtaposition with the cell membrane during nuclear disruption, it is not surprising that the fragments produced during degradation of the host chromosome are found to be membrane bound (23).

Majumdar et al. (18) recently demonstrated that *E. coli* DNA polymerase I becomes transiently membrane bound shortly after infection with bacteriophages T4, T7, or lambda. One might expect that if nicks or free ends, or both, are produced by endonucleolytic cleavage of the host DNA, these would subsequently bind to the membrane-associated DNA polymerase I (8). If so, nuclear disruption and/or the binding of intermediates in host DNA breakdown to the cell membrane might be dependent on DNA polymerase I activity. We examined these possibilities by thin-section electron microscopy and M band analysis of the *E. coli* DNA polymerase I-deficient mutant *polA1* (6) and its *polA*⁺ parent infected with an endonuclease II-deficient mutant (*nd-28*) of phage T4 (22, 25).

Thin-section electron microscopy, performed by using procedures as previously described (23), showed that nuclear disruption does occur in T4-infected *polA*⁻ cells (Fig. 1), but in only 75 to 80% of the infected cells even when

¹This publication is Scientific Paper no. 8179 of the Minnesota Agricultural Experiment Station.

FIG. 1. Typical nucleoid morphology in an uninfected *polA1* cell (A) and nuclear disruption in *polA1* cells infected for 10 min at 30 C with a double mutant of phage T4 deficient in DNA synthesis (*am-N82*, gene 44) and endonuclease II (*nd-28x6*, gene *denA*) (B and C). The procedures used were as previously described (23); multiplicity of infection was about 20.

multiplicities as high as 20 phage per cell are used. Independent killing studies, again performed by using multiplicities of up to 20 phage per cell, showed that from 15 to 18% of the infected *polA1* cells survived T4 infection, even with wild-type T4. Killing of the parental *polA+* strain, on the other hand, approaches 100% under the same conditions. The reason for this survival of 15 to 20% of the population of wild-type T4-infected *polA-* cells is unknown.

M band analysis (7) of labeled host DNA as previously described (23) from cells infected with endonuclease II-deficient T4 phage indicated that considerably less of the host DNA is membrane bound in *polA-* than in *polA+* cells (Fig. 2A). At 30 min after infection about 51% of the host DNA is membrane bound in *polA-* cells compared to about 84% in *polA+* cells. When one takes into account the 15 to 20% of the *polA1* cells that survive infection and

FIG. 2. Percentage of host DNA found in the M band at various time after infection. Growth, infection of cells, and M band analysis were done as previously described (23). Multiplicity of infection was about 10. A T4nd-28x6-amB22-infected *polA*⁺ (○); *polA*⁻ (△); *polA*⁻ corrected for surviving cells by subtracting 20% of the DNA in the M band and the same amount from the total recovered in the gradient (▲). B T7am-29 (gene 3)-infected *polA*⁺ (○); *polA*⁻ (△).

thus contain essentially 100% membrane-bound DNA, it can be seen (Fig. 2A) that about three times as much DNA is released from the membrane in phage T4 nd-28-infected *polA1* cells as in similarly infected *polA*⁺ cells. These results suggest that membrane-bound DNA polymerase I in phage T4-infected cells plays a role in the binding of host DNA fragments to membranes.

Mutant *polA1* does possess the 5'-exonuclease activity associated with DNA polymerase I (I. R. Lehman, personal communication). It is possible, therefore, that the mutant polypeptide possessing this 5'-exonuclease activity still becomes membrane bound after T4 infection and that this activity is responsible for the nuclear disruption observed in T4-infected *polA1* cells.

Since the breakdown of host DNA after infection of *E. coli* with bacteriophage T7 appears to follow a pathway quite similar to that in T4-infected cells (3, 4, 12, 21) and since *E. coli* DNA polymerase I becomes membrane bound after T7 as well as T4 infection (18), we extended these studies to T7-infected cells. Gene 3 of phage T7 codes for an endonuclease which induces double- and single-strand breaks in native DNA, apparently about 600 breaks per strand of the host chromosome (4, 5, 21). M band analyses of host DNA from cells infected

with T7 am-29 (gene 3) showed that essentially all of the host DNA fragments produced remain membrane bound in *polA1* as well as *polA*⁺ cells (Fig. 2B). Moreover, T7⁺, unlike T4⁺, kills both *polA*⁺ and *polA*⁻ with an efficiency near 100%.

Luria and Human (17) first observed that T7 does not induce nuclear disruption as seen after T-even infection. Rather, T7 was found to induce the formation of condensed, central chromatin bodies. We used thin-section electron microscopy to verify the absence of nuclear disruption in cells infected with amber mutants in genes 1 through 6 as well as wild-type T7.

The above observations indicate that the binding of host DNA fragments to the cell membrane occurs by different mechanisms in T7-infected and T4-infected cells.

This investigation was supported by Public Health Service research grants AI-07946 to D.P.S., AI-07898 to H.R.W., and AI-08088 to D.L.A. (from the National Institute of Allergy and Infectious Diseases), and National Science Foundation research grant GB-29393 to D.L.A.

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