Polyamines and the Delay in Deoxyribonucleic Acid Synthesis in Some Bacteriophage T4 Infections

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Escherichia coli B infected by the DD mutant of T4, *am* N116, is stimulated to initiate deoxyribonucleic acid accumulation by 1 to 10 mM spermidine but not by 10 mM putrescine. The syntheses of putrescine and spermidine in cells infected by T4D and the mutant are similar, although slight differences are observed in the intracellular concentration of free spermidine. Unlike r-K12 (λ) systems, *am* N116-infected cells do not leak polyamine.

T4 amber mutants of genes 52, 60, and 39 (DD mutants) are expressed phenotypically as a delay in the onset of phage deoxyribonucleic acid (DNA) synthesis. It was suggested by Guttman and Begley (7) that T4 *am* N116 infection of a non-permissive host failed to induce an Mg^{2+} pump. These authors also cited the possible significance of the proximity of these DD genes to the *rII* locus, noting that abortive infections of *Escherichia coli* K-12 (λ) by *rII* mutants are reverted phenotypically by high Mg^{2+} concentrations (6).

The present study was designed to test further possible similarities between the DD and *rII* phenotypes under nonpermissive conditions by investigating the effect of polyamines in DNA synthesis. Ferroluzzi-Ames and Ames (5) have noted extensive polyamine leakage from *E. coli* K-12 (λ) infected with *rII* mutants. In addition, a number of workers (1, 2, 5) have reported phenotypic reversion of abortive infections of similarly infected cells by polyamines. We have tested for possible interrelations between the accumulation of DNA and of polyamines after infection of the restrictive host *E. coli* B with T4 *r⁺ am* N116.

Phage stocks of T4 *r⁺D* and T4 *r⁺ am* N116 (gene 39) were prepared by infecting *E. coli* B and *E. coli* CR63, respectively, at a multiplicity of infection (MOI) of 0.1. Purified stocks were obtained by two cycles of low-speed ($4,080 \times g$), and high-speed ($27,300 \times g$) centrifugation of overnight broth lysates. Methods for analyses of phage DNA accumulation and polyamine levels have been previously described. (4; A. S. Dion and S. S. Cohen, *manuscript in preparation*). Cultures in medium 52 (3) were grown to a cell density of approximately 2.0×10^8 /ml and infected at an MOI of 5.0 in the presence of L-tryptophan (25 μ g/ml) and glucose (3 mg/ml).

As shown in Fig. 1, infection of *E. coli* B with T4D resulted in the initiation of DNA accumulation at approximately 8 min after infection. Phage production by T4 *r⁺* strains has been reported to be unaffected by 40 mM spermidine (5). In contrast, the onset of DNA accumulation after

FIG. 1. Effect of spermidine on DNA synthesis in *E. coli* B infected with T4 *r⁺ am* N116 at 37°C (MOI = 5). Spermidine was added 5 min after infection. T4D infection of *E. coli* B included as control.

infection of the same host with T4 *am* N116 was typically delayed, beginning at approximately 20 min. In addition, the rate of DNA accumulation in the latter infection was slightly, but reproducibly, slower than in T4D-infected *E. coli* B. Addition of spermidine at the indicated concentrations, 5 min after infection, effected an earlier onset of DNA accumulation and slightly enhanced the rate of DNA synthesis to the control

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FIG. 2. Intracellular polyamine levels and DNA synthesis in *E. coli* B infected by T4D (●) and T4 am N116 (X) at 37 C (MOI = 5).

rate. Equimolar putrescine concentrations were generally without effect, i.e., there was no reduction in the time necessary for the inception of DNA accumulation; however, at times, putrescine addition slightly increased the rate of DNA synthesis.

Correlations between intracellular polyamine levels and phage DNA accumulation are demonstrated in Fig. 2. In T4D-infected *E. coli* B, the synthesis of DNA begins at approximately 7 to 8 min after infection; the onset of DNA synthesis in *E. coli* B infected with T4 am N116 begins at 18 to 19 min after infection. Increases in the intracellular level of putrescine in both types of infection were apparent from the first analysis after infection, i.e., at 10 min, resulting in approximately commensurate levels of intracellular putrescine throughout infection. The increase in intracellular spermidine was observed to proceed somewhat earlier and to be maintained at a slightly higher level in T4 am N116-infected cells than in T4D-infected cells. No additional increases in intracellular putrescine or spermidine were observed after hydrolysis in 6 N HCl at 110 C for 18 hr, a treatment which releases bound polyamines.

In the experiment presented in Fig. 2, samples were also removed for the analysis of polyamine content of acidified infected cultures, i.e., cells plus media. These results are shown in Fig. 3. Infection of *E. coli* B with either T4D or T4 am N116 resulted in similar levels of synthesis of free putrescine, free spermidine, and total (free plus bound) polyamines after infection. A higher level of total putrescine was always observed in cultures infected by T4 am N116 than in those infected by T4D. The bound forms of putrescine and

FIG. 3. Free and total polyamine levels in cells and media of T4D and T4 am N116-infected *E. coli* B. Cells were infected at 37 C at an MOI of 5, and samples were removed for analyses of free and total (free plus bound) polyamines. Solid line, putrescine; dashed line, spermidine; ● and ○, free; X and Δ, total.

spermidine have not been investigated; however, the above data indicate that these compounds are extracellular.

The results presented in Fig. 1 suggest that spermidine is effective in establishing an earlier initiation of phage DNA accumulation in T4 am N116-infected *E. coli* B. Putrescine was not observed to produce this effect. Although both putrescine and spermidine were shown to be effective in enhancing rII production in a restrictive

host (5), equimolar putrescine concentrations were at least one order of magnitude less effective than spermidine.

That the DD and rII phenotypes, under restrictive conditions, differ in one important respect, was observed in analyses of intracellular polyamines. We found no evidence for extensive polyamine leakage as observed for rII-infected cells (5).

In addition, we have found no delay in polyamine synthesis resulting from the delayed initiation of DNA synthesis. These observations extend our previous data demonstrating that, in strain B infected by ultraviolet-irradiated phage or by amber phage mutants, polyamine synthesis is not dependent on phage DNA synthesis (4). It may be noted that, although synthesis of phage DNA does not control the synthesis of the polyamines in infected cells, the synthesis of phage DNA has been found to be dependent upon exogenous polyamines in a polyamine-depleted, putrescine-requiring auxotroph (4; A. S. Dion and S. S. Cohen, *manuscript in preparation*).

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