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Partial Suppression of Bacteriophage T4 Ligase Mutations by T4 Endonuclease II Deficiency: Role of Host Ligase[1]

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Endonuclease II-deficient, ligase-deficient double mutants of phage T4 induce considerably more deoxyribonucleic acid (DNA) synthesis after infection of *Escherichia coli* B than does the ligase-deficient single mutant. Furthermore, the double mutant can replicate 10 to 15% as well as wild-type T4, whereas the single mutant fails to replicate. When the *E. coli* host is also deficient in ligase, the double mutant resembles the single mutant. The results indicate that host ligase can substitute for phage ligase when the host DNA is not attacked by the phage-induced endonuclease II.

Mutants of bacteriophage T4 which fail to induce ligase induce little net deoxyribonucleic acid (DNA) synthesis, and the DNA that is made has a very low molecular weight (10). Apparently the ligase activity already present in the *Escherichia coli* host cell cannot overcome the deficiency of the phage-induced enzyme even though the host enzyme is active with phage DNA in vitro (1). Anraku and Lehman (1) have recently suggested that this may be due to the binding of host ligase to the host DNA at nicks produced by the action of phage-induced nucleases, so that the ligase is unavailable to seal nicks in parental and newly synthesized phage DNA. (Nicks are defined as single phosphodiester-bond interruptions containing 5'-phosphoryl and 3'-hydroxyl terminii in one strand of double-stranded DNA. Gaps are nicks enlarged by the elimination of one or more nucleotides from the broken strand.) This hypothesis may be tested if conditions can be found whereby the nicking of host DNA and subsequent degradation do not occur after infection.

A T4 mutant, designated *nd*28, which lacks the ability to degrade the host DNA normally has recently been described (16). For the first 10 min after infection of *E. coli* B by this mutant, the host DNA sediments in neutral and alkaline sucrose gradients at rates corresponding to molecular weights of 2×10^8 and 10^8, respectively, and subsequently this DNA is only slowly converted to smaller pieces. These results suggested that *nd*28 fails to induce some nuclease essential for one of the first steps in degradation of the host DNA. The T4-induced endonuclease II described by Sadowski and Hurwitz (13) makes nicks only in cytosine-containing DNA and is a likely candidate for such an enzyme. Assays for T4 endonuclease II activity both in crude extracts and in partially purified fractions of *E. coli* infected by *nd*28 indicate that this phage induces very little, if any, of the normal endonuclease II activity (P. Sadowski, *personal communication*). This finding has been confirmed by E. Kutter (*personal communication*).

If the hypothesis of Anraku and Lehman (1) is correct, the failure to induce endonuclease II should prevent the production of many ligase-binding sites on the host DNA thus "sparing" ligase to bind to and seal nicks in both newly synthesized and parental phage DNA. Thus, an endonuclease II-deficient ligase-deficient double mutant should replicate better than a ligase-deficient mutant if such sealing is essential for phage replication. This paper describes experiments designed to test this hypothesis.

The T4 phages and *E. coli* B used in this work were grown and titered as described earlier (16). Phage *am*H39 was obtained from R. S. Edgar; this mutant contains a mutation in gene 30 and fails to induce the T4 ligase (4). [The *am*H39 mutant used also contains a defective α-glucosyl

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transferase gene (7); it is not known whether *nd*28-*am*H39 also carries this defective gene. However, this deficiency does not influence the results since we have obtained identical results using *am*H39X and *nd*28-H39X, mutants which have a normal α-glucosyl transferase gene (7).] Phage *nd*28 was isolated as described earlier (16); the double mutant *am*H39-*nd*28 was prepared and identified as described earlier (16). *E. coli* strains N1071 and N1254 were obtained from M. Gellert, and their isolation and characterization have been described (6). *E. coli* N1071 contains normal ligase activity at 30 and 42 C, whereas N1254 contains about 5% normal ligase activity at 42 C, but normal amounts at 30 C. These strains were grown in the media of Fraser and Jerrell (5) supplemented with 0.25% Yeast Extract (Difco).

The experiments were performed in a gyratory shaking water bath (New Brunswick Scientific Co., New Brunswick, N. J.) Cultures were used when they reached 4×10^8 to 5×10^8 cells/ml and were infected at a multiplicity of infection of about four. When titering cultures for phage, the first dilution tube always contained several drops of chloroform to lyse infected cells. *E. coli* CR63 was always used as the lawn.

The ^{14}C-thymidine was purchased from Schwarz BioResearch Inc., Orangeburg, N. Y. and had a specific activity of 45 μCi/μmole. Radioactivity was measured on a gas-flow planchet counting system (Nuclear-Chicago Corp., DesPlaines, Ill.). Incorporation of ^{14}C-thymidine into DNA was always carried out in the presence of 0.5 mg of uridine per ml to ensure linear incorporation for extended periods of time. Uridine not only prevents induction of thymidine phosphorylase but also inhibits the constitutive activity (11). To follow DNA synthesis in infected cells, 0.05 μCi of ^{14}C-thymidine was added per ml of culture. At various times, 0.5-ml samples were removed from the culture and added to 0.5 ml of cold 10% trichloroacetic acid. The precipitates were collected by centrifugation, washed two times with 2 ml of 5% cold trichloroacetic acid, dissolved in 0.5 ml of 1 N NH_4OH, plated, and counted.

The data shown in Fig. 1A indicate that cells infected with the double mutant *nd*28-*am*H39 accumulated 40 to 60% as much labeled DNA as cells infected with wild-type phage, whereas cells infected with *am*H39 accumulated only 5 to 10% as much. No progeny phage were made after infection with *am*H39, whereas 28 and 300 progeny phage were made per infected cell by 65 min after infection with *nd*28-*am*H39 and wild-type T4, respectively. Hence, the effects of ligase

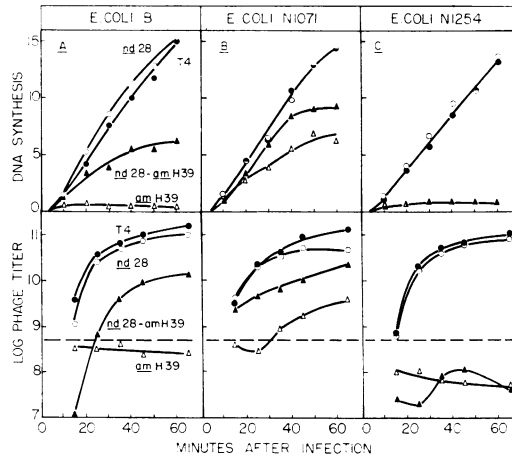

FIG. 1. *Deoxyribonucleic acid (DNA) and phage synthesis in various Escherichia coli hosts. Experiments with E. coli B, N1071, and N1254 were performed at 37, 42, and 42 C, respectively. DNA synthesis is expressed as counts per min* × 10^{-3} *from* ^{14}*C-thymidine incorporated into trichloroacetic acid-insoluble material per milliliter of culture. The phage production was measured by plating on E. coli CR63 and is expressed as log phage titer; the dashed, horizontal line represents one progeny phage per cell. The T4 phages used were wild-type* (●), *nd28* (○), *nd28-amH39* (▲), *and amH39* (△).

deficiency upon DNA and phage synthesis are both significantly suppressed by concomitant deficiency of endonuclease II. The burst size of *nd*28-*am*H39 on plates is not large enough to produce plaques on the nonpermissive host, *E. coli* B, however. Results with *nd*28 are similar to those with wild-type phage, indicating endonuclease II deficiency by itself does not seriously affect DNA and phage synthesis. Simultaneous infection by *am* H39 and *nd*28-*am*H39 gave results similar to *am*H39, indicating the suppression is recessive (*data not shown*).

The above results suggest that the hypothesis of Anraku and Lehman (1) is correct. Another way to confirm the validity of the above hypothesis is to perform a similar experiment in a ligase-deficient *E. coli* host. The data shown in Fig. 1B indicate that *E. coli* N1071, a K-12 strain with normal ligase activity at 42 C, is more permissive for *am*H39 and *nd*28-*am* H39 than is *E. coli* B. However, *E. coli* N1254, a ligase-deficient derivative of *E. coli* N1071, is completely restrictive to not only *am* H39 but also *nd*28-*am*H39, as shown in Fig. 1C. Again, results with *nd*28 are similar to those with wild-type phage. These results indicate that the suppression of the lethal effects of phage ligase deficiency by phage endonuclease

II deficiency is only possible when host ligase is functional.

The results indicate that when host DNA is not attacked by T4 endonuclease II, host ligase can apparently substitute for phage ligase and partially restore phage DNA synthesis. Nicks formed by endonuclease II are presumably widened into gaps by the action of an exonuclease (14), whereupon T4 endonuclease IV can then break the second strand to form short double-stranded fragments of host DNA. Ligase must be able to bind to nicks in DNA and in some cases to ends of DNA (15), and it presumably also binds to regions of DNA containing gaps. Thus, the failure to induce T4 endonuclease II, which prevents the formation of many new ligase binding sites on the host DNA, allows the host ligase to partially suppress the effects of phage-ligase deficiency. The amount of host ligase activity approximates one-third to one-half of that present in infected cells (9); when the amount of host ligase is increased four- to fivefold, the suppression is nearly complete (6) even when endonuclease II is induced. Host ligase has also been shown to substitute for the T7-induced ligase (6).

Recently it has been shown that rII mutations also suppress the effects of the phage-induced ligase deficiency (2, 8). Berger and Kozinski showed that rII mutations restored DNA and phage synthesis in cells infected with ligase-deficient phage to 80 to 90% and 20 to 40% of wild-type values, respectively (2), but this rescue cannot take place in ligase-deficient *E. coli* (6), indicating that host ligase is required for this suppression. Rutberg and Rutberg (12) reported that extracts of cells infected with rII mutants have less nuclease activity than extracts of cells infected with wild-type T4, suggesting that deficiency of some nuclease is responsible for this suppression. Chan et al. (3) have reported the isolation of revertants of ligase mutants which contain suppressor mutations in a gene other than rII, but these mutants have not been characterized further.

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