Degradation of *Escherichia coli* Chromosome After Infection by Bacteriophage T4: Role of Bacteriophage Gene D2a

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Received for publication 22 June 1972

Mutations in the D2a gene of bacteriophage T4 have recently been shown to result in the stabilization of cytosine-containing phage deoxyribonucleic acid (DNA) made after infection by phage gene 56 (deoxycytidine triphosphatase) mutants. In the experiments reported here, we investigate the role of the D2a gene in the degradation of the host chromosome. We find that if T4 endonuclease II, a product of the phage gene *denA*, is active, host chromosome degradation appears normal, regardless of the presence of the D2a gene product. However, if T4 endonuclease II is absent, a small amount of host chromosome degradation occurs, but only if the D2a product is present. These results are interpreted in terms of the hypothesis that D2a controls a nuclease which degrades cytosine-containing DNA. Neither D2a nor *denA* mutations affect the shut-off of host DNA synthesis.

Very quickly after infection of *Escherichia coli* by the T-even bacteriophages, synthesis of host deoxyribonucleic acid (DNA) ceases (3). Furthermore, infection results in the degradation of the host chromosome (7). This degradation of the host chromosome appears to be of some value to the phage, since a substantial fraction of the nucleotides in progeny phage DNA comes from the host chromosome (8, 16).

Some progress has been made in elucidating the mechanism of degradation of the host chromosome. Mutants in three genes of phage T4 are known to be defective in the process. From the behavior of mutants in either gene 46 or 47, it is inferred that these two genes control, directly or indirectly, an exonuclease activity which is responsible for the final degradation to nucleotides (13, 17). Mutants in the *denA* gene fail to induce T4 endonuclease II, an enzyme which can attack cytosine-containing DNA, such as the *E. coli* chromosome, but not hydroxymethylcytosine-containing DNA, such as the T4 chromosome (10, 11). Indeed, infection with the *denA* mutants leads to very little degradation of the host chromosome (6, 13). A result of this is that very few of the nucleotides of the host chromosome are transferred to DNA of progeny phage. This feature leads to a quick diagnostic test for *denA* mutants: their sensitiv-

ity to hydroxyurea (HU), which inhibits the alternative source of nucleotides for phage DNA synthesis (13). Based on analyses of these mutants, as well as the kinetics of degradation of the host chromosome after infection with wild-type phage, a reasonable model of host chromosome degradation has been presented (13). The model involves the more or less sequential function of a number of endo- and exonucleases, including those controlled by phage genes 46, 47, and *denA*. However, several steps of the model remain hypothetical.

We have recently shown that the D2a gene of T4 appears to control a nuclease activity which is involved in the degradation of the cytosine-containing phage DNA which is synthesized after infection by phages defective in the breakdown of deoxycytidine triphosphate (gene 56 mutants) (2). We have also shown that the cytosine-containing DNA synthesized in vitro in toluenized cells prepared from T4-infected *E. coli* is degraded if the D2a gene is active (J. Elliott, M.A. thesis, Rice University; Elliott, Bruner, and Souther, *manuscript in preparation*). We have therefore suggested that the D2a gene controls a nuclease activity which is specific for cytosine-containing DNA. One might suspect that such an enzyme would be involved in the degradation of the host chromosome. Therefore, in the experiments described below, we have investigated the role of the D2a gene of T4 in the degradation of the host chromosome.

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The mechanism of shut-off of host DNA synthesis is not understood. The problem of shut-off, and the possibility of its relationship to host chromosome degradation, will be considered.

MATERIALS AND METHODS

Phage strains. The amber mutation *amN82* (gene 44, function unknown) is used to prevent phage DNA synthesis; it is referred to as *am44*. The *rII* deletion mutations have been described earlier (2); those which extend into the nearby D2a region, *rII*PT8 and *rII*H23, are referred to as D2a mutations. The mutant *denA* S112 is defective in the gene for T4 endonuclease II (6, 11); it was provided by J. Wiberg.

Bacterial strains (all *Escherichia coli*). The following three host strains are nonpermissive for amber mutants: the standard strain BB; W3110, a thymine-requiring K-12 strain; and P3478, a derivative of W3110 carrying the mutation *polA1* in the gene for DNA polymerase I (4, 5). The strain 011 is an *E. coli* B strain which carries an amber suppressor (*suII*, which inserts glutamine). The strain 011/HU is a spontaneous mutant of 011, which we selected for resistance to HU. This strain was used in all plate tests for the *denA* mutation, since we found that the amount of HU necessary to clearly distinguish *denA* and *denA*⁺ phage severely retarded growth of wild-type indicator bacteria. In these tests, 0.2 ml of 2 M HU was added to the top agar along with about 10⁸ bacteria. Under these conditions, *denA* mutants do not form plaques (or clear spots, in spot tests), whereas *denA*⁺ phage plate at near normal efficiency. CR63, which also carries an amber suppressor (*suI*, serine), was used to prepare stocks of amber mutants, whereas BB was used to prepare stocks of *am*⁺ phage. A lambda lysogen of CR63 was used as the nonpermissive host to check for *rII* mutants.

Media. All media have been described previously (2).

Chemicals. HU was purchased from Sigma. Fresh 2 M stock solutions were prepared daily for use in quantitative experiments. Thymidine-*methyl*-³H and thymine-*methyl*-³H (both: 20 Ci/mmol, 1 mCi/ml) were purchased from New England Nuclear.

Measurement of DNA synthesis. The amount of DNA synthesized after infection was measured by the incorporation of ³H-thymidine in a 1.5-min pulse 15 min after infection at 37 C, as described previously (2).

Measurement of degradation of prelabeled host DNA. A thymine-requiring strain was grown in SMGCT plus ³H-thymine (1 μ Ci/ml, 5 μ g/ml) at 37 C for several generations, to about 2 $\times$ 10⁸/ml. Unincorporated ³H-thymine was removed by filtration. Ten minutes after suspending the culture in unlabeled medium, portions were infected with phage at a multiplicity of infection of 3 to 6 phages/bacterium. At intervals, 0.5-ml samples were taken for measurement of trichloroacetic acid-precipitable radioactivity. This was done by adding the samples to tubes containing 5 ml of iced 8% trichloroacetic

acid solution containing 200 μ g of thymine per ml. The tubes were allowed to sit on ice for 15 to 30 min, and then the precipitates were collected on 0.45- μ m membrane filters (Millipore Corp.) and were washed with the trichloroacetic acid solution, and finally with 0.01 M HCl. Dried filters were added to 5 ml of toluene-Liquifluor scintillation fluid (New England Nuclear) and counted in a Packard Tri-Carb scintillation counter. Within each figure, the curves show data from infections using portions of the same labeled culture.

Measurement of burst size. Bacteria were grown to about 2 $\times$ 10⁸/ml in SMGCT (plus thymine, if required). Portions (1 ml) were distributed to tubes standing at 37 C. At *t* = 0, KCN was added to 0.002 M. At *t* = 2 min, phage were added at a multiplicity of infection of about 0.4 phage/bacterium. At *t* = 7 min, anti-T4 serum was added to K = 0.8/min. At *t* = 10 min, the infected cultures were diluted 10⁴-fold into the "growth tubes," containing various concentrations of HU. The growth tubes were incubated, without shaking, for 1 hr at 37 C. Then CHCl₃ was added, and the phage yields were assayed. The numbers of infected cells and unadsorbed phage were measured early in the growth period. The latter number was always negligible. The burst size was calculated as the phage yield per infected cell.

For all burst-size experiments in the 011 strains, phage carrying the *am44* mutation were used. Since the 011 strains suppress the amber mutation, it is not relevant to the experiment and is not mentioned in the text below or in the tables. For burst-size experiments in the nonsuppressing strains, *am*⁺ phage were used.

RESULTS

Two kinds of experiments have been carried out to test the extent of host chromosome degradation after infection by various T4 mutants. In one kind of experiment, the host chromosomes are labeled with ³H-thymine prior to infection. The cells are then infected under conditions which minimize new DNA synthesis, so that any nucleotides released from host DNA cannot be reincorporated into high-molecular-weight DNA. The amount of label still acid-precipitable at various times after infection is a measure of the amount of host chromosome not yet degraded to small pieces.

In the second kind of experiment, the extent of phage production by various T4 mutants is determined in the presence of various concentrations of HU. The drug inhibits de novo synthesis of deoxyribonucleotides (12). Phage DNA synthesis is then dependent on the degradation of host DNA for a supply of precursors; phage which are defective in that degradation process will show a reduced yield of progeny in the presence of HU (6, 13).

These two kinds of experiments are comple-

mentary to each other. The former experiment will work only in the absence of new DNA synthesis. To prevent phage DNA synthesis, all phages used in these experiments carried a mutation in gene 44. To minimize repair replication of the host chromosome, most of the experiments were done in the *polA1* host, which lacks a major repair polymerase. The latter experiment requires phage production, and hence must be done with phage wild type for all essential genes. The former experiment makes use of a direct measurement of degradation, although by the rather ill-defined criterion of acid solubility. Since the latter experiment measures the reuse of liberated nucleotides by the phage, it clearly requires that the host DNA be degraded to free nucleotides. However, this experiment suffers from the problem that there is no assurance that the only effect of HU is on the de novo synthesis of deoxynucleotides. Fortunately, the results from both types of experiments have led to the same basic conclusions.

It is helpful to discuss separately the results obtained with *denA*⁺ and *denA* phage.

Effect of D2a mutations on degradation of host DNA by *denA*⁺ phages. The kinetics of degradation of prelabeled host DNA to acid-soluble material after infections by *am44* and by *am44-D2a* phages (both *denA*⁺) are shown in Fig. 1. It is seen that the kinetics are nearly the same. This experiment was carried out in the *polA1* strain, P3478; similar results were obtained in its *polA*⁺ parent, W3110. We therefore conclude that the D2a gene does not control an obligatory

step of the normal process of host DNA degradation.

The burst sizes of the wild-type and D2a phages (both *denA*⁺) in the presence of various concentrations of HU are shown in Table 1. Also shown are similar data for the *denA* phage, known to be very defective in host DNA degradation. It is seen that the two *denA*⁺ phages are about equally resistant to HU, compared to the *denA* mutant. Similar results have been obtained in 011/HU (Table 1), W3110, and P3478. These results are consistent with the idea that D2a mutants do not have any major defect in the process of degradation of the host DNA.

Effect of D2a mutations on degradation of host DNA by phages defective in T4 endonuclease II. The kinetics of degradation of prelabeled host DNA to acid-soluble material after infection by *am44-denA* and *am44-denA-D2a* phages are shown in Fig. 2. Comparison of these kinetics

TABLE 1. Effect of hydroxyurea on growth of *denA*⁺ phages^a

Hydroxyurea concn (molarity)	Burst size of phage		
	Wild type	<i>rII</i> Pt8	<i>denA</i>
0	156 ^b	156	100
0.01	19	17	3.2
0.1	8.3	7.2	0.01

^a Host: 011/HU.

^b Phage yield per infected cell.

FIG. 1. Degradation of prelabeled host DNA after infection by *denA*⁺ phages. Host: P3478 (*polA1*). Phages: *am44*, ○; *am44-rIIH23*, ●.

FIG. 2. Degradation of prelabeled host DNA after infection by *denA* phages defective in T4 endonuclease II. Host: P3478 (*polA1*). Phages: *am44-denA*, ○; *am44-denA-rIIH23*, ●; *am44-denA-rII1241*, ×. The scale on the ordinate has been expanded, compared to Fig. 1.

with those for the *denA*⁺ phages (Fig. 1), shows that the *denA* mutant phages cause much less breakdown of the host DNA than do the *denA*⁺ phages. However, it is seen that the *am44-denA* phage (D2a⁺) does cause a small amount of degradation of host DNA, but that much less degradation of host DNA occurs after infection with the *am44-denA*-D2a phage. That is, the small amount of host chromosome degradation which is observed after infection with the *denA* mutant is largely prevented by D2a mutations.

Both D2a mutations tested, *rIIH23* and *rIIPT8*, show a similar inhibition of host DNA degradation by *denA* mutants. The effect is ascribed to the D2a region by the same argument used in earlier work (2): a shorter *rII* deletion, *rII1241*, which does not extend into D2a, does not show the effect. That is, the kinetics of degradation of host DNA after infection by *am44-denA-rII1241* are like those for *am44-denA-rII*⁺ (Fig. 2).

(The results shown in Fig. 2, indicating some D2a⁺ product-dependent degradation of host DNA after infection by *denA* phages, are quite reproducible as long as the experiment is carried out in the *polA1* host, P3478. If the same experiment is carried out in the *polA*⁺ parental strain, W3110, the difference in host DNA degradation between *denA*-D2a⁺ and *denA*-D2a phages is markedly reduced: neither phage induces much breakdown. We suspect that in the *polA*⁺ host the D2a⁺ product-dependent degradation is largely obscured by repair replication of the host chromosome. This will be considered further in the Discussion.)

If indeed D2a mutations reduce host DNA degradation caused by *denA* mutants, it might be expected that *denA*-D2a mutants would be even more sensitive to HU than *denA* (*rII*⁺) phage. To test this prediction, the burst sizes of *denA* phages, with and without D2a mutations, were measured in the presence of various concentrations of HU. The results (Table 2) agree with

the prediction. The burst sizes of the *denA* phage and of both *denA*-D2a phages are about the same in the absence of HU. But in the presence of HU, either D2a mutation clearly leads to a small reduction in the burst size of the *denA* phage. The short *rII* deletion, *rII1241*, which does not extend into D2a, has no effect on the sensitivity to HU of *denA* phage (data not shown). Similar results have been obtained in strains 011/HU (Table 2), 011, W3110, and P3478.

Effect of D2a mutations on shut-off of host DNA synthesis. We have already presented some data indicating that D2a mutations do not prevent the normal shut-off of host DNA synthesis (2). However, in view of the fact that an effect of D2a mutations on host DNA degradation can be observed only under certain conditions, it seemed worthwhile to investigate more thoroughly the effect of D2a mutations on shut-off of host DNA synthesis. We have, therefore, examined the amount of DNA synthesis which occurs after infection by phages which carry (i) a mutation to prevent phage DNA synthesis and (ii) various combinations of wild-type and mutant alleles of the *denA* and D2a genes. These experiments have been carried out both in strain BB and in the K-12 strain P3478 (*polA1*). The general result is that none of the phage strains tested allow DNA synthesis to occur after infection. A sample of the results of such experiments is shown in Table 3. We thus conclude that D2a and *denA* mutations, even in combination, do not prevent the shut-off of host DNA synthesis.

(One exception to the general experimental result stated above is that strains of the type *am56*-D2a do allow DNA synthesis after infection. The DNA which is synthesized is phage DNA, not host DNA [2]. Therefore, this result does not affect the conclusion.)

TABLE 2. *Effect of hydroxyurea on growth of denA mutant phages*^a

Hydroxyurea concn (molarity)	Burst size of phage		
	<i>denA</i>	<i>denA-rIIPT8</i>	<i>denA-rIIH23</i>
0	119 ^b	122	133
0.002	40	33	34
0.01	5.5	2.5	3.6
0.04	0.27	0.02	0.04

^a Host: 011/HU.

^b Phage yield per infected cell.

TABLE 3. *Effect of D2a gene on shut-off of host DNA synthesis*^a

Phage strain	Surviving bacteria ^b	DNA synthesis ^b
Wild type.....	0.12	3.09
<i>am44</i>	0.08	0.03
<i>am44-denA</i>	0.04	0.03
<i>am44-rIIPT8</i>	0.13	0.08
<i>am44-denA-rIIPT8</i>	0.12	0.06

^a Host: P3478 (*polA1*).

^b As fraction of uninfected control. Typical values for uninfected control are 3×10^8 bacteria/ml, and 1,500 counts of ³H-thymidine incorporated per min.

DISCUSSION

Our previous work has implicated the D2a gene of T4 in the control of a deoxyribonuclease activity specific for cytosine-containing DNA (2). The purpose of the experiments reported here was to examine the effect of the D2a gene on the degradation of the host chromosome after T4 infection of *E. coli*. The experiments lead to one clear conclusion in that regard: the D2a gene plays no major obligatory role in the degradation process. This conclusion derives from the result (based on two types of experiments) that, if the phage are *denA*⁺, D2a phage and D2a⁺ phage show about the same host DNA degradation.

However, if host DNA degradation induced by *denA* phages is examined in a *polA1* host, a rather different result is found. Although the *denA* (*rII*⁺) phage are quite defective in the degradation process, we consistently find that a small amount, about 20%, of the prelabeled host DNA is degraded to acid-soluble material. The striking finding is that this degradation is observed only if the phage are D2a⁺, but not if they carry D2a mutations. This result indicates that the D2a-controlled nuclease can attack host DNA, as we expected, even though it does not take part in an essential step of the degradation process. In agreement with this, *denA*-D2a phage are even more sensitive to HU than *denA*-D2a⁺ phage, suggesting that the former phage are less effective at supplying nucleotides from host DNA for their own DNA synthesis.

The difference in host DNA degradation induced by *denA*-D2a⁺ and *denA*-D2a phages is markedly reduced in the *polA*⁺ host, as stated earlier. Two possible explanations for this result seem reasonable. Since the degradation experiments measure only net degradation, i.e., degradation less resynthesis, then repair replication mediated by the *polA*⁺ polymerase could reutilize liberated nucleotides and thus obscure the D2a product-dependent degradation. In fact, repair replication of the host chromosome after T4 infection has been observed (M. S. Scofield and C. K. Mathews, *personal communication*), in support of this explanation. The alternative is that the D2a⁺ and *polA*⁺ products interact directly in some way, so that the D2a⁺ product is actually functioning differently in the two hosts.

One other difference in results obtained in the *polA*⁺ and *polA*⁻ hosts is perhaps worth noting, although it is not relevant to our basic conclusions. In Fig. 1, the two curves, showing the kinetics of degradation of host DNA after infection of the *polA*⁻ host by *am44*-*denA*⁺ phages,

are probably within experimental error of each other. However, if the same experiment is carried out in the *polA*⁺ host, W3110, the *am44*-D2a mutant shows slightly faster and slightly more extensive degradation of the host DNA than does the *am44*-D2a⁺ phage. We have no explanation for this effect and have not investigated it further.

We do not understand why, in infections by *denA* phages, the D2a-controlled nuclease leads to degradation of only a limited amount of the host DNA. It is possible that this limited D2a-controlled degradation simply reflects the slow rate of degradation by a limited amount of enzyme, perhaps present only during part of the infection. Alternatively, the D2a-controlled nuclease might be specific for certain regions of the DNA.

As stated in the introduction, the mechanism by which T4 shuts off host DNA synthesis is not known. One conceivable possibility has been that some step of host chromosome degradation results in the shut-off of DNA synthesis. However, experiments to test this idea have failed to provide support (1, 9, 15). Our present experiments, likewise, provide no evidence for the model. The *denA* gene clearly is involved in a quite early step of degradation, but it does not affect shut-off of host DNA synthesis (Table 3; K. Hercules, *personal communication*). The D2a gene appears to play a nonobligatory role in degradation; however, all phage carrying D2a mutations, along with various other phage mutations, still cause shut-off of host DNA synthesis (Table 3; reference 2).

Throughout most of this paper we have referred to "D2a mutations." As in our earlier work (2), we must again point out that all available D2a mutations are *rII* deletions which extend through D1 into the D2a region. Thus the possibility remains that certain (or all) of the phenotypes which we ascribe to D2a mutations may in fact require, also, *rII* or D1 mutations, or both. However, it should be noted that the *rII* deletions used in these experiments do not extend into the portion of the genome containing the recently discovered *dexA* gene, which controls T4 exonuclease A (14).

ACKNOWLEDGMENTS

We thank C. K. Mathews and K. Hercules for sharing unpublished results; C. R. Stewart for helpful comments; and R. Edgar, S. Champe, J. Wiberg, and J. Cairns for sending strains.

This work was supported by Public Health Service Health Sciences Advancement Award 5-S04-FR-06136 from the Division of Research Facilities and Resources and by research grant 5-R01-AI-10025 from the National Institute of Allergy and Infectious Diseases.

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