Synthesis of Bacteriophage-Coded Gene Products During Infection of *Escherichia coli* with Amber Mutants of T3 and T7 Defective in Gene 1

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During nonpermissive infection by a T7 amber mutant in gene 1 (phage RNA polymerase-deficient), synthesis of the products of the phage genes 3 (endonuclease), 3,5 (lysozyme), 5 (DNA polymerase), and 17 (serum blocking power) was shown to occur at about half the rate as during wild-type infection. This relatively high rate of expression of "late" genes (transcribed normally by the phage RNA polymerase) seems to be a general feature of all T7 mutants in gene 1 from our collection. In contrast, T3 gene 1 mutants and a T7 gene 1 mutant from another collection showed late protein synthesis at very reduced rates. Synthesis of the gene 3 endonuclease by T7 gene 1 mutants was very sensitive to the addition of rifampin 2 min after infection, conditions under which there was very little inhibition during wild-type infection. This supports the notion that late gene expression during nonpermissive infection by gene 1 mutants is dependent on the transcription of the T7 genome by the host RNA polymerase. In contrast to T3 gene 1 mutants, the T7 gene 1 mutants of our collection directed the synthesis of phage DNA during nonpermissive infection. This DNA accumulated as a material sedimenting faster than mature T7 DNA.

The RNA polymerases (gene 1 products) coded by the related phages T3 and T7 have a central role regarding the pattern of gene expression of these viruses: the RNA polymerase of the host, *Escherichia coli*, is only able to efficiently transcribe a short segment at the "left" end of the phage genomes. In the case of T7 this segment has been shown to code for at least four proteins ("early" proteins), one of which is the phage DNA-specific RNA polymerase. The rest of the approximately 30 genes coded by these phages is efficiently transcribed by the phage RNA polymerase only. Thus, it was postulated that a terminator, i.e., a nucleotide sequence leading to the interruption of transcription by the host RNA polymerase, was located near the "right" end of the ligase gene (see reviews by Studier [20] and Summers [Annu. Rev. Genet. 6, in press]). Davis and Hyman (3) have found visual evidence for such a terminator, at about 20% of the length of the T7 genome, through electron microscopy of T7 DNA transcribed in vitro. And Studier (20) has located this terminator to the right of gene 1.3 (ligase). In addition to this terminator, *t1*, another terminator, *t2*, has been postulated to occur at about 30% of the genome, coming from the left (Summers, Annu. Rev.

Genet. 6, in press). This might correspond to a point between genes 4 and 5.

Reports vary regarding the efficiency of these termination sites (13, 19, 24). To better evaluate their in vivo roles, a series of gene 1 mutants of phages T3 and T7 have been tested here with respect to the expression of the genes 3 (endonuclease) and 3.5 (lysozyme), thought to be located between the terminators *t1* and *t2*, as well as genes 5 (DNA polymerase) and 17 (serum-blocking protein), located to the right of *t2*. In addition to this, we also tested the capacity of these gene 1 mutants to synthesize phage-directed DNA which involves the action of genes 1 through 6.

MATERIALS AND METHODS

Phage and bacteria. The strains of T3 and T7 were the same as described in previous work (7) except for the T7 mutants *am23* and *am345L5*, LG26. Both of these strains are from the collection of F. W. Studier. T7*am23* was obtained directly from F. W. Studier, and the double mutant *am345L5*, LG26 through M. Schweiger (Max Planck Institute, Berlin). This strain carries the amber mutation *am345L5* in gene 1 and a deletion, LG26, covering the terminator of transcription to the right of the ligase gene (20). T7*am23* also

carries a mutation which results in a defective DNA ligase (8, 17). Our T7 am^+ strain, characterized by Summers (23) as T7L, was originally obtained from Delbrück, but it has been reisolated from a single plaque at least twenty times. For changes in terminology, see Studier and Hausmann (21). Host bacteria were *E. coli* strains B/5 or B $_{s-1}$ which are nonpermissive for amber mutants, and *E. coli* BB/w which is permissive for amber mutants (7).

Media and growth conditions. Standard dilutions of phage as well as growth of bacteria and phages were in 1% nutrient broth. 3H -thymidine incorporation experiments were carried out in M9 medium supplemented with Casamino Acids (5 g/liter). To prevent host cell-directed thymidine incorporation, 10-ml samples of host cell cultures were ultraviolet irradiated (Sylvania germicidal lamp, at 15-cm distance) shortly before phage infection. The times of irradiation were from 50 to 180 S, as specified in the description of the experiments. All experiments were carried out at 37 C, as described in previous work (7, 8).

Chemicals and isotopes. Thymidine- 3H was purchased from Amersham-Buchler. Rifampin was a gift from Ciba-Geigy Pharmaceutical Co.

Isolation of 3H -labeled *E. coli* DNA. BBw/1 cells were grown in M9 supplemented with 3H -thymidine (10 μ Ci/ml) and adenosine (200 μ g/ml). Adenosine was added to allow optimal uptake of thymidine (1). The isolation procedure for DNA was that of Marmur (12).

Enzyme assays. Extracts of infected cells were obtained as previously described (8). Endonuclease was tested by the method of Weissbach and Korn (25), lysozyme was assayed by the method of Pryme and Berentsen (14), and DNA-polymerase was assayed by the method of Grippo and Richardson (6).

Sucrose gradients. Conditions for sucrose gradient centrifugation were as referred to by Hausmann and LaRue (9).

Serum-blocking protein. Tests for detecting anti-phage serum-blocking power were based on the method of DeMars (4). The data which led to the finding that gene 17 codes for the serum blocking protein of phages T3 and T7 will be published elsewhere (Issinger, Beier, and Hausmann; manuscript in preparation).

RESULTS

Patterns of enzyme synthesis, in the non-permissive host, by gene 1 amber mutants of T3 and T7. For evaluating the rate of synthesis of phage products during nonpermissive infection with a gene 1 mutant of T3 or of T7, an exponentially growing culture of B/5 cells was infected with phage and samples were taken at intervals after infection. The cells were then centrifuged and disrupted by sonic treatment. The cell-free extracts were used as enzyme sources or as a source of serum-blocking protein

(SBP). As shown in Fig. 1, the synthesis of all products tested was reduced much less upon infection with the T7 gene 1 mutant than with the T3 gene 1 mutant, when compared to infection with the respective wild types.

To establish whether this was a feature of the particular mutants tested or whether the differences could be traced back to the respective wild types, DNase synthesis by a series of gene 1 mutants of T3 as well as of T7 was examined (Fig. 2). In nonpermissive cells infected with T3 gene 1 mutants or with T7 $am23$ (from Studier's collection), DNase activity reached maximal values of only about 5% of those obtained during wild-type infection; all T7 gene 1 mutants from our collection (including a double mutant, T7 $amH13$ · $amH280$), however, correspondingly reached levels of 20 to 50%.

To see whether the factor determining the high levels of synthesis of DNase and other late phage proteins was also active in the *trans* position, nonpermissive cells were mixedly infected with T7 $am23$ and T7 $amH13$ · $amA50$ (a double mutant from our collection, defective in gene 1 and gene 3). The multiplicity of infection was 5 phages of each type per cell. Extracts from cells harvested at 10 and 20 min after infection were assayed for gene 3 DNase activity. The DNase activity in these extracts was slightly lower than in corresponding samples from cells infected with T7 $am23$ alone. Thus, it became clear that the factor determining high level of DNase synthesis by our T7 gene 1 mutants did not act *trans*.

Patterns of enzyme synthesis by a T7 mutant with a deletion covering the terminator *t1*. To evaluate the relative inefficiency of the *t1* terminator in our T7 gene 1 mutants (and by inference in our T7 am^+ reference stock), it was necessary to measure the rate of enzyme synthesis determined by an amber mutant in gene 1 with a deleted terminator such as characterized by Studier (20). The rate of synthesis in such a case was not expected to be necessarily the same as during wild-type infection. For instance, the specific activity of the host RNA polymerase on a T7 DNA template is known to be much lower than that of the T7 RNA polymerase (2). Thus, even with 100% read-through by the host RNA polymerase, less messenger would be made than in the case of transcription by the phage RNA polymerase. (It is implicitly assumed here that the availability of messenger is limiting the rate of enzyme synthesis). As shown in Fig. 2, DNase synthesis by the double mutant $am345L5$ ·LG26 (with an amber mutation in gene 1 and a deletion covering the terminator *t1*) was at a rate of not more than about 15% of the norm. It thus came as a surprise to

FIG. 1. Time course of synthesis of some phage T7- and T3-directed late proteins during infection with the wild types or with a gene 1 mutant (multiplicity of infection: 10 particles per cell). Infected host bacteria were harvested at the times indicated, and their cell-free extracts were used as enzyme source. The values obtained were normalized to the highest activity reached during wild-type infection. Residual synthesis of late proteins is shown to be much more pronounced in the gene 1 mutant of T7 (T7amH13) as compared to the T3 mutant (T3amH5). Symbols. ●, DNase; ▽, lysozyme; ○, DNA polymerase; ▲, SBP (serum blocking power).

observe that host RNA polymerase-dependent DNase synthesis by this double mutant with a deleted terminator was at a lower rate than that corresponding to some of our gene 1 mutants.

Rifampin sensitivity of DNase synthesis by gene 1 mutants. If the residual synthesis of DNase, lysozyme, DNA polymerase, and SBP in gene 1 amber mutants of T7 was indeed due to read-through of the host RNA polymerase past the terminator *t1* (rather than leakiness of the mutation in gene 1), then transcription of the corresponding genes in gene 1 mutants should be more sensitive to rifampin than in wild-type infection. (Rifampin is known to block the formation of initiation complexes by the host RNA polymerase but does not affect the gene 1 polymerase [2].) To test this, rifampin was added to samples of a B/5 culture 2 min after they had been infected with phage: one sample with T7am⁺, the other with T7amH13 (gene 1). The 2-min delay between infection and the addition of rifampin allowed a limited initiation of transcription by the host polymerase. In the case of gene 1 mutants, late gene expression was apparently directly limited by the limitation of transcription by the host polymerase. On the other hand, during wild-type infection, synthesis of rifampin-resistant phage polymerase led to a

pronounced enzyme synthesis, apparently as a result of recycling of phage polymerase and the consequently continued transcription of late genes (Fig. 3).

Phage-directed DNA synthesis during nonpermissive infection with gene 1 mutants. Since read-through by the host RNA polymerase in gene 1 mutants apparently allows the production of all the proteins required for phage DNA synthesis—albeit at a reduced rate—the question arose regarding the capacity of gene 1 mutants to synthesize DNA. Some residual DNA synthesis by some (but not all) gene 1 amber mutants of T7 had been observed by Hausmann and Gomez (7). This question has now been analyzed in more detail. Special attention was given to (i) the dependence of the residual DNA synthesis on the UV doses administered to the host cells prior to infection, (ii) the time delay in initiation of DNA synthesis in gene 1 mutants as compared to wild-type infection, and (iii) the sedimentation pattern of this DNA upon zone centrifugation.

B₈-1 cells were irradiated for 50, 100, and 180 s. They were then infected with either wild-type phage or with a T7 gene 1 mutant. The results are plotted in Fig. 4. It can be seen that, at the higher UV doses to the host, DNA synthesis

FIG. 2. Synthesis of gene 3 product (DNase) in various gene 1 amber mutants of T3 and T7, as compared to their respective wild types. The symbol LG26 refers to the double mutant from F. W. Studier, T7am345L5, LG26, which carries an amber mutation in gene 1 and a deletion covering the terminator to the right of the ligase gene. The experiments were executed and plotted as in Fig. 1.

directed by gene 1 amber mutants was at a rate of less than 5% that of DNA synthesis directed by the wild-type phage under the same conditions. At the lower UV doses to the host, the corresponding rates varied between 15 and 30%. The degree of DNA synthesis varied according to the mutants used. The reason for this variation is not clear but it is not correlated with the map position of the mutations.

As also shown in Fig. 4, phage DNA synthesis was always initiated with a 3-min time delay in the gene 1 mutants, compared to wild-type infection. The initial rate of synthesis in amH13 reached from 20 to 60% of the rate during wild-

type infection and proceeded often for more than 1 h, reaching levels of thymidine incorporation of up to 70% of the maximum level reached during wild-type infection. Zone centrifugation of such DNA samples revealed the presence of fast-sedimenting material (concatemers [18]), as shown in Fig. 5.

DISCUSSION

It seems likely that the late gene products synthesized in nonpermissive cells infected with gene 1 amber mutants of T7 originate as a consequence of read-through by the host polymerase rather than by virtue of a partial suppres-

FIG. 3. *T7*-directed DNase synthesis in the presence of rifampin. Experiments were carried out as those of Fig. 1, except that rifampin (200 μ g/ml) was added 2 min after infection.

sion of the amber mutation. This second alternative is made very unlikely by the observation that double mutants in gene 1 show the same degree of read-through as single mutants (20). This observation has been confirmed in the case

of our double mutant in gene 1, *T7amH13-amH280*. The first interpretation (read-through by the host polymerase) is supported by the rifampin sensitivity of phage DNase synthesis by gene 1 mutants and, to a certain degree, also by the high sensitivity of phage DNA synthesis by these mutants to ultraviolet irradiation of the host prior to infection. The ultraviolet doses administered seem to be within the range of inactivation of the host RNA polymerase, which is highly radiation sensitive (5, 22). Further evidence for a read-through by the host polymerase is the time delay of appearance of the late gene products, compared to wild-type infection: a 2-min delay for lysozyme and endonuclease, about a 3-min delay for DNA polymerase, and about an 8-min delay for SBP. These delays are consistent with the model of sequential transcription by the host polymerase of the gene 1 mutant genome, starting from only one promoter located at the left end of the DNA molecule (20). The agreement is qualitative, i.e., increasingly longer delays are observed for genes located further to the right; but quantitatively the time delays correspond to only about one-half the values arrived at by considering the physical length of the *T7* genome and the transcription speed (16) of the *E. coli* RNA polymer-

FIG. 4. Phage-directed DNA synthesized by *T7* gene 1 mutants infecting *B.*₋₁ cells after these cells had been UV irradiated for 50 s (A), 100 s (B), or 180 s (C). The rate of DNA synthesis in gene 1 mutants, as compared to wild-type, is much more sensitive to UV irradiation of the uninfected host.

FIG. 5. Patterns of sedimentation through a neutral sucrose gradient (5 to 20%) of phage DNA synthesized in the nonpermissive host infected by T7amH13 (gene 1). *E. coli* B₁ was UV irradiated to inhibit host DNA synthesis. ³H-thymidine (5 μCi/ml) and T7amH13 (multiplicity of infection: 10 particles per cell) was added. At 20 min after infection a sample was lysed with lysozyme, EDTA, and sodium dodecyl sulfate. ³²P-labeled T7 DNA was added as a marker (triangles). Centrifugation with a Spinco model L2 centrifuge (SM39 rotor) was for 150 min at 39,000 rpm. For comparison, two similar experiments were done using T7am⁺. One experiment (upper inset) corresponds to the normal course of phage growth. The sample was taken at 10 min after infection. In the second experiment (lower inset), chloramphenicol (50 μg/ml culture) was added at 5 min after infection. The sample was taken at 15 min after infection. Chloramphenicol is known to inhibit formation of mature phage DNA from fast-sedimenting precursor DNA (9). T7 wild type under these conditions is thus phenotypically similar to mutants defective in gene 1.

ase. Such a faster than expected transcription and translation of the genome of gene 1 mutants has also been observed by Studier (20) through gel-electrophoretic analysis of labeled T7-coded proteins. The nearly simultaneous appearance of lysozyme, DNase, DNA polymerase, and SBP during wild-type infection (8) could be due to the fast transcription by T7 polymerase (2) as well as to the possible existence of several phage polymerase-specific promoters on the T7 DNA.

As we have shown here, the rate of synthesis of gene 3 endonuclease by gene 1 mutants from our collection varies according to the mutant used and—as with mutant T7amH13—this rate may be higher than the corresponding rates of a mutant from Studier's collection, defective in gene 1 and with a deletion covering the terminator *t1*. The reasons for this phenomenon are not clear. One can assume that our T7 stocks also carry a deletion, which we were not aware of till now and which covers the terminator site for the host polymerase. But we have found no significant difference in heat stability between phage

particles of our and Studier's T7am⁺ reference types. This is noteworthy because an increased heat stability has been shown to be characteristic of deletion-carrying mutants (15).

An alternative to the hypothesis of a defective or absent terminator *t1* would be to postulate that our T7 strains carry an amber suppressor which partially suppresses the amber mutations in gene 1. This hypothesis seems unlikely for three reasons. First, our mutants are not more leaky than 18 representative mutants in different genes from Studier's collection. Second, no trace of phage RNA polymerase activity could be detected in extracts of nonpermissive cells infected with our T7 amber mutants in gene 1. Finally, upon mixed infection of nonpermissive cells by T7am23 (from Studier) and T7amH13-*amA50* (a double mutant for our collection, defective in gene 1 and gene 3) no increase in DNase synthesis was observed, as compared to single infection by T7am23, in otherwise identical conditions. This shows that the factor determining the high levels of synthesis of DNase and

other late phage proteins does not act in the *trans* situation. Such *trans* activity, however, would be expected from an amber suppressor.

Whatever the reasons for the differences between ours and Studier's gene 1 mutants, these differences are certainly one of the causes of some controversies raised by publications in which lysozyme and phage DNase were considered to be early phage proteins (10, 11, 17). In these publications, T7 gene 1 mutants from our collection were used, and the fact that in nonpermissive conditions lysozyme and phage DNase was synthesized was taken as an indication of the early nature of these proteins.

As shown here, the phage DNA which is synthesized after nonpermissive infection with T7 gene 1 mutants sediments faster than mature T7 DNA. Such fast sedimenting phage-directed DNA was also observed to occur after infection with amber mutants defective in either one of these genes: 8, 9, 18, or 19 (references 9, 21). Straetling and Knippers (Virology, in press) have also observed the accumulation of concatemers in gene 3 mutants of T7. Thus, the lack of transcription of one or all of these genes could explain the accumulation of concatemers in gene 1 mutants. But since it has here been shown that in these mutants a sizable amount of gene 3 endonuclease is synthesized (up to 50% that of wild-type), a lack of gene 3 product seems not to be an acceptable explanation for the accumulation of large pieces during infection with gene 1 mutants of T7. Thus, the reason for the accumulation of fast-sedimenting, phage-directed DNA during nonpermissive infection by gene 1 mutants remains unknown. Furthermore, the considerable synthesis of phage DNA polymerase (gene 5 product) and of SBP (gene 17 product) in such mutants of our collection is evidence against a terminator sequence to the right of gene 3, as proposed by Summers (Annu. Rev. Genet. 6, in press); however, since Summers has used T7 mutants from Studier's collection, it should be emphasized here again that there seem to be considerable differences between Studier's and our T7 strains.

Although, according to Studier (20), the read-through phenomenon allows synthesis of all late proteins, one should bear in mind that no phage production occurs in nonpermissive cells infected with gene 1 amber mutants. It could be that at least some late gene products have to be synthesized in a critical amount or at the right time, or both, i.e., that the mere presence of all phage gene products is not sufficient for a productive infection, but rather that these products have to be kept in a metabolic balance to each other.

Finally, the question of the physiological role

of the read-through phenomenon is to be discussed. As shown here, read-through in T3 gene 1 mutants as well as in T7am23 (from Studier's collection) is very low (late enzyme synthesis at the most is at a rate of about 5% of the normal rate, compared to up to 50% in our T7 gene 1 mutants). Since high levels of read-through have been found in all the tested T7 gene 1 mutants of our collection, it can be assumed that this common feature reveals a peculiarity of the common wild-type ancestor, our T7am⁺ reference stock. This stock does not have any growth disadvantages as compared to Studier's T7am⁺. Thus, the level of read-through by the RNA polymerase of the host does not seem to markedly influence the viability of the phage.

We would like to put forward the hypothesis that the read-through is a mere evolutionary atavism stemming from an ancestral phage which was possibly fully transcribed by the host polymerase. Evolutionary pressure would have led to the encoding by the phage of a new primitive RNA polymerase with the ancillary function of providing extra messenger of the genes whose products were needed in large amounts (like the major coat protein, for instance). The existence of a phage protein with RNA polymerase function then provided a system with excellent potentialities for further evolution. This new RNA polymerase and newly arising promoter sequence could adapt to each other in a way that high polymerase-promoter affinity was selected for in the case of mRNAs of which many copies were required, and low affinity was selected if only a small number of gene products was necessary. The role of the host polymerase in this evolving system would then have continuously declined.

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