

Rate of Translocation of Bacteriophage T7 DNA across the Membranes of *Escherichia coli*

L. RENÉ GARCÍA AND IAN J. MOLINEUX*

Department of Microbiology, University of Texas, Austin, Texas 78712-1095

Received 20 October 1994/Accepted 5 May 1995

Translocation of bacteriophage T7 DNA from the capsid into the cell has been assayed by measuring the time after infection that each GATC site on the phage genome is methylated by cells containing high levels of DNA adenine methylase. Methylation at GATC sites on T7 DNA renders both the infecting genome and any newly synthesized molecules sensitive to the restriction enzyme *DpnI*. In a normal infection at 30°C, translocation of the T7 genome into the cell takes between 9 and 12 min. In contrast, translocation of the entire phage λ genome or of a T7 genome ejected from a λ capsid can be detected within the first minute of infection. Entry of the leading end of the T7 genome occurs by a transcription-independent mechanism that brings both *Escherichia coli* and T7 promoters into the cell. Further translocation of the genome normally involves transcription by the RNA polymerases of both *E. coli* and T7; the rates of DNA translocation into the cell when catalyzed by each enzyme are comparable to the estimated rates of transcription of the respective enzymes. A GATC site located between the early *E. coli* promoters and the coding sequences of the first T7 protein made after infection is not methylated before the protein is synthesized, a result supporting the idea (B. A. Moffatt and F. W. Studier, *J. Bacteriol.* 170:2095–2105, 1988) that only certain proteins are permitted access to the entering T7 DNA. In the absence of transcription, the genomes of most T7 strains do not completely enter the cell. However, the entire genome of a mutant that lacks bp 393 to 808 of T7 DNA enters the cell in a transcription-independent process at an average overall rate of 50 bp per s.

Bacteriophage T7 contains a 39,937-bp linear genome enclosed within an icosahedral head attached to a stubby, non-contractile tail (25). Six fibers emanate from the tail, and these promote the initial attachment of the phage to the outer membrane lipopolysaccharide of a susceptible cell. The signal(s) that evokes ejection of the T7 genome into the cell is not known, although it has been established that the genetic left end is the first to penetrate the cytoplasmic membrane (27, 32).

About 2.5% of the T7 genome is thought to be ejected into the cell, while the remaining DNA enters as a result of transcription of the genome (24, 45). *Escherichia coli* RNA polymerase recognizes the three early promoters A1, A2, and A3, located within the first 2.5% of the genome. Transcription from these promoters causes the template DNA to be pulled from the phage head into the cell, simultaneously providing transcripts over the early region of the genome. Transcription halts at the terminator TE at about 19% from the left end of the genome, but T7 RNA polymerase, which is synthesized from these early transcripts, recognizes phage promoters and, also via transcription, pulls the remainder of the genome into the cell. From the temporal patterns of mRNA synthesis, complete entry of T7 DNA into the cell has been estimated to take 8 to 10 min, a time that corresponds to about one-third of the latent period of the phage (1, 21).

In the absence of T7 RNA polymerase, readthrough of the transcription terminator by *E. coli* RNA polymerase causes more than 19% of the phage genome to enter the cell. However, because of additional terminators downstream of TE, transcription by *E. coli* RNA polymerase allows only 60% of the genome to enter within 20 min of infection, at least in *E. coli* C (45). Conversely, entry of the genome is faster than normal if T7 RNA polymerase is present in the host cell prior

to infection (24), since the phage enzyme transcribes DNA much faster than the *E. coli* enzyme (6, 7).

It has been suggested that the slow entry of the T7 genome is important in the control of T7 gene expression (21). Early (class I) genes all lie within the leftmost 19% of the genome and are transcribed by *E. coli* RNA polymerase from about 2 to 6 min after infection at 30°C; their continued transcription is inhibited by the newly synthesized 0.7 protein (3, 4, 31). Class II and class III genes are both transcribed by T7 RNA polymerase; class II genes lie between 15.04 and 46.35% of the T7 genome and are transcribed between about 5 and 15 min, whereas class III genes lie between 46.58 and 99.00% and are transcribed from about 7 min onwards (21). By means of a slow mode of entry of the phage genome, transcription from the relatively weak class II promoters is thought to begin before the stronger promoters that direct class III gene expression enter the cell. As the transcriptional capacity of the infected cell declines late in infection because of the inhibition of T7 RNA polymerase by the gp3.5 lysozyme (23), the strong class III promoters are utilized preferentially (21).

Details of the mechanism of transfer of T7 DNA from its capsid into the cell were first studied by sonication of phage-bacterium complexes in the presence of transcriptional or translational inhibitors (45). These inhibitors are necessary because T7 gene expression destabilizes the cell wall, rendering the infected cell prone to sonic disruption. A second study used the susceptibility of T7 DNA (in the absence of gp0.3) to type I restriction enzymes as an assay and measured the time when infecting phage [³²P]DNA was degraded (24). Neither procedure is useful for studying the process of DNA entry in permissive infections. In order to understand the mechanics of the overall process of genome translocation from the phage head across the bacterial cell membranes, we have developed a noninvasive assay that measures the time of DNA adenine methylation of an infecting T7 DNA molecule. This assay has allowed measurements of the rates of T7 genome translocation

* Corresponding author. Phone: (512) 471-3143. Fax: (512) 471-7088. Electronic mail address: molineux@mail.utexas.edu.

from the phage head into the cell under various experimental conditions.

MATERIALS AND METHODS

Phages, plasmids, and bacteria. The T7 mutants 4101, D502, sRK836, and sRK836,1379 are described by Moffatt and Studier (24). T7 $\Delta 10$ -NB1 (35) is a gene 10 deletion mutant that can be propagated on T7 or T3 gene 10 plasmid-containing strains (11). T7 $\Delta 10$ -NB1 is routinely propagated on strains containing pSW5 (11), a plasmid containing T3 gene 10 under T7 promoter control, since recombinations yielding plasmid-independent phages are less frequent than when T7 gene 10 plasmids are employed. The T7 and T3 gene 10 major capsid proteins have 79% identity in predicted amino acid sequence, and the biological properties of the normal and chimeric T7 $\Delta 10$ -NB1 particles appear to be identical. In this work, both T7 and T3 gene 10 plasmids were used to propagate strains containing the $\Delta 10$ -NB1 mutation; for clarity, both T7 and T3 head-containing phages containing $\Delta 10$ -NB1 DNA are referred to simply as T7 $\Delta 10$ -NB1[T7] in order to distinguish them from T7 $\Delta 10$ -NB1[λ], in which the DNA is packaged in a λ particle.

Dam12, a T7 mutant containing six GATC sites in addition to those normally found in wild-type phage, was constructed in a multistep procedure involving several phages. Dam12 was constructed as follows. T7 3.5 *am13a* was found to contain two extra GATC sites near positions 15577 and 17477 of its genome, which are not associated with the amber mutation. The amber mutation in this phage was reverted to sense, and the DNA of one revertant was digested with *Bst*EII. The 20-kb left arm was ligated to a 19-kb *Bst*EII right arm of T7 $\Delta 10$ -NB1 (35) and transfected into cells containing the complementing gene 10 plasmid pAR5378 (kindly provided by A. Rosenberg and F. W. Studier). pAR5378 contains a 12-bp *Bgl*III linker near the beginning of gene 10 at position 23065 of the T7 genome. A plaque was resuspended in LB medium and titered on a noncomplementing host to select phages that had recombined the plasmid-encoded mutant gene 10 onto their chromosome. The DNA of one such phage was cut with *Pac*I and religated in the presence of a double-stranded oligonucleotide linker (5' GATCCACGGAATTCAT and 5' GAATTCCTGGATC AT) that preserves the normal termination codon of gene 12 and introduces a GATC site at position 27222. The DNA of this phage was digested with *Bcl*I, and the 31-kb right arm was ligated to the 7.7-kb *Bcl*I left arm of sRK836,1379. The resulting phage is called Dam12; it contains the deletion removing genes 0.4 to 0.7 associated with sRK836,1379 but grows well on most *E. coli* strains.

Figure 1 provides a *Dpn*I restriction map of all the T7 strains used in the methylation assays. Nucleotide positions are those from the complete T7 DNA sequence (13); they have been adjusted as necessary for deletion mutations, but for clarity, they have not been corrected to account for the linker oligonucleotides present in the sRK and Dam12 strains.

Phage stocks containing totally unmethylated DNA were grown in strains containing the *dam-13::Tn9* mutation from GM2163; both this strain and the *dam*-overexpressing plasmid pTP166 (Ap^r) were from M. G. Marinus (18). For growth of the gene 1 mutant 4101, a T7 gene 1 plasmid (both pAR1219 [12] and pPK41, a pACYC184 derivative containing the *Bam*HI gene 1 fragment from pAR1219, were used) was introduced into GM2163. Bacteriophage λ *cos* DNA was excised from plasmid pWP14 (36) with *Bam*HI and inserted into the unique *Bcl*I site of T7 $\Delta 10$ -NB1 to give T7 1.7:: λ *cos* $\Delta 10$ -NB1[T7]. Strain SMR10 (29), made *dam-13::Tn9* by transduction with P1 grown on GM2163, was used to package T7 1.7:: λ *cos* $\Delta 10$ -NB1[T7] into particles of λ . All phage stocks were purified by banding on density gradients of cesium chloride solutions; T7 was dialyzed into T7 buffer (10), and λ was dialyzed into SM buffer (34) lacking gelatin. Phage stocks were used within a few days of preparation; storage for longer periods often resulted in asynchronous adsorption and/or penetration of the phage genome. Assays of T7 DNA methylation during infection were performed in the *E. coli* K-12 strain RV (26) or derivatives of RV. The genotype of RV is $\Delta lacX74$ *thi*, that of the $r_K^- m_K^-$ strain IJ1133 is RV $\Delta lacX74$ *thi* $\Delta(mcrC-mrr)102::Tn10$, and that of IJ1134 is RV *lacUV5 lacZ::T7* gene 1-Kn^r *thi* $\Delta(mcrC-mrr)102::Tn10$. The source of the Tn10 replacement of the *hsd* genes was ER1648 [F⁻ *fluA2* $\Delta(lacZ)r1$ *supE44* *trp31* *mcrA1272::Tn10* *his-1* *rpsL104* *xyl-7* *mtl-2* *metB1* $\Delta(mcrC-mrr)102::Tn10$], from New England Biolabs, and that of the *lac*-T7 gene 1 insertion was KT842 (MG1655 *lacUV5 lacZ::T7* gene 1-Kn^r; kindly provided by K. Tedin, University of Vienna); both insertions were transduced into RV by using P1 vir.

Assays of phage genome entry into cells. The assay measures the time after infection when infecting phage DNA is methylated by Dam methylase, thereby becoming sensitive to cleavage by the restriction enzyme *Dpn*I. Cells containing plasmid pTP166 (overproducing Dam methylase) were grown with aeration in LB medium supplemented with 100 μ g of ampicillin per ml at 30°C to a density of 2×10^8 /ml and then infected with T7 phage. In some experiments, rifampin or chloramphenicol was added to the culture 10 min prior to phage infection; the concentrations of the antibiotics are provided in the appropriate figure legends. When specifically measured, more than 85% of added phage irreversibly adsorbed within 1 min; however, a variable level of adsorbed phage eject their DNA within the time course of an experiment. This methylation assay is designed to analyze only those genomes that initiate translocation into the cell; the genomes of adsorbed phages that do not eject their DNA never become accessible to Dam

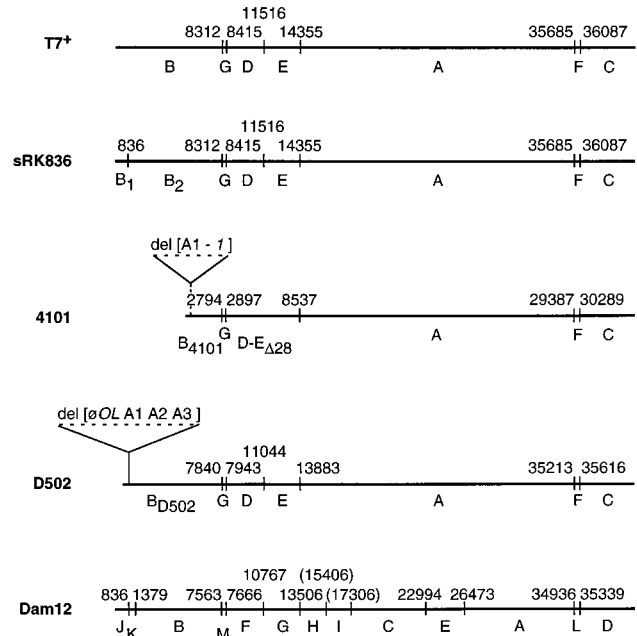


FIG. 1. Positions of GATC sites in the genomes of the T7 strains employed. Numbers are nucleotide positions of the complete T7 DNA sequence (13), ignoring insertions of linker oligonucleotides in phages sRK836 and Dam12. The positions of the two GATC sites of Dam12 that are in parentheses are based on restriction fragment sizes and are thus approximate. There are actually four GATC sites within the DNA inserted at position 836 and two GATC sites at position 1379; the second is accompanied by a 749-bp deletion. For more details of these insertions, the deletion in D502, and the two deletions in 4101, see reference 24.

methylase and remain as 40-kb molecules (as do genomes of unadsorbed phage) after restriction enzyme digestion. Infections by phage λ or by the transvoltage phage T7[λ], which contains a T7 genome packaged in a λ virion, were performed similarly to T7 infections except that (i) 10 mM MgSO₄ was added to the LB medium to improve adsorption (addition of maltose, which should increase the amount of the LamB receptor, had no effect on the intensity of *Dpn*I bands in a methylation assay) and (ii) the cells were centrifuged and resuspended in fresh medium at a cell density of 2×10^9 /ml prior to phage infection. The concentration step was necessary to increase both the efficiency and synchrony of infection. At 1 min after infection, the cells were diluted to a cell density of 2×10^8 /ml to reduce the rate of additional particle adsorption and to allow phage development.

At various intervals, 0.75 ml of the culture was mixed with 0.75 ml of ice-cold phenol-ethanol solution (2% [vol/vol] phenol, 75% [vol/vol] ethanol, 8 mM EDTA, 20 mM sodium acetate [pH 5.2]) and centrifuged, and the cell pellet was resuspended in 0.5 ml of 50 mM Tris-chloride [pH 7.5]-50 mM NaCl-5 mM EDTA-0.5% sodium dodecyl sulfate-100 μ g of proteinase K per ml and incubated at 37°C for 1 h. Samples were then extracted once with phenol and twice with phenol-chloroform-isoamyl alcohol (25:24:1, by volume), ethanol precipitated, dried, and dissolved in a buffer suitable for digestion by *Dpn*I. Each sample was digested with 20 U of *Dpn*I for 1 h at 37°C, and DNA fragments were separated by electrophoresis on 0.7 or 1% agarose gels in 40 mM Tris acetate-2 mM EDTA (pH 8.1) buffer. After capillary transfer to nylon membranes (MSI), Southern hybridizations were conducted following the procedures described in Sambrook et al. (34). T7 DNA was hybridized to probes of T7 DNA labeled by primer extension with [α -³²P]dCTP and primers of random-sequence hexamers or heptamers for the Klenow fragment of DNA polymerase I. After hybridization and washing, T7 DNA was visualized by autoradiography. For unknown reasons, certain T7 DNA sequences were routinely overrepresented in the labeled probe, since the band intensities of DNA extracted from phage particles were not proportional to *Dpn*I fragment sizes (see the accompanying paper [14] for more details). This lack of uniformity in labeling the probe prevents accurate estimates of the amount of DNA in different T7 DNA *Dpn*I fragments in phage-infected cells.

Analysis of protein synthesis during T7 infection. Cultures of IJ1133(pTP166) were grown at 30°C in B2 glucose medium (41) supplemented with 17 amino acids (lacking Cys, Met, and Trp) and ampicillin (100 μ g/ml) to a cell density of 2×10^8 /ml and irradiated with UV light to suppress host protein synthesis. Cells were collected by centrifugation, resuspended in fresh medium at a concentration of 2×10^9 /ml, and infected with T7 at a multiplicity of 3. One minute after

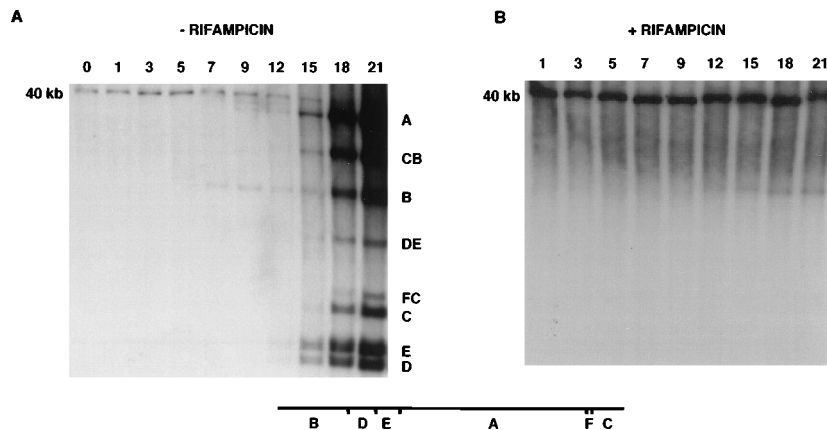


FIG. 2. Time course of methylation of wild-type T7 DNA during infection of RV(pTP166) in the absence (A) or presence (B) of 200 μg of rifampin per ml. The multiplicity of infection was 0.1. The time after infection that each sample was analyzed is shown above each lane (in minutes). *DpnI* fragments are indicated by letters on the right of each panel and on the phage genome map.

infection, the culture was diluted 10-fold to allow T7 development and to reduce further phage particle adsorption. The experiment involving T7[Δ] was performed similarly except that the medium contained 10 mM MgSO_4 and the multiplicity of infection was 10. Starting 1 min before and at various intervals after infection, 2×10^7 cells were pulse-labeled with 100 μCi of [^{35}S]methionine per ml for 1 min and chased for 30 s with 1 ml of LB. Labeled proteins were analyzed by electrophoresis through linear 12 to 20% gradient polyacrylamide gels in the presence of 0.1% sodium dodecyl sulfate and visualized by autoradiography.

RESULTS

Methylation of T7 DNA. When T7 is grown on *E. coli* strains containing normal levels of DNA adenine methylase (Dam), its DNA can be efficiently cut with *DpnII* but is largely resistant to *DpnI*. These restriction enzymes recognize the same GATC sequences, but their activities are affected by adenine methylation. *DpnII* cuts only unmethylated and *DpnI* cuts only fully methylated DNA. T7 is not known to directly inhibit DNA methylation, and it is assumed that phage DNA replication and packaging occur too fast for normal intracellular levels of Dam to efficiently methylate T7 DNA. However, the high intracellular levels of Dam methylase made from the high-copy-number *dam* plasmid pTP166 are sufficient to completely methylate the T7 genome, so that DNA isolated from phage particles grown on a host that contains this plasmid is efficiently cut by *DpnI*.

A high level of Dam in *E. coli* slows the rate of cell growth but does not grossly affect T7 infections. Plating efficiencies and plaque sizes of T7 on Dam-overproducing strains and their plasmid-free parents are identical. One-step growth experiments (not shown) demonstrated that the rates and efficiencies of phage adsorption and the lengths of the eclipse and latent periods are indistinguishable. However, the size of the T7 burst in a cell overproducing Dam is only half that obtained in a normal infection, a difference more likely due to the physiology of the Dam-overproducing cells than to any direct interference with T7 growth.

Kinetics of methylation of T7 DNA during infections of cells overproducing Dam. Previous studies showed that entry of T7 DNA into the cell is a gradual process dependent on both *E. coli* and T7 RNA polymerases (24, 45). In order to examine the transcription-coupled translocation of DNA from the phage head into the cell in more detail, the time of methylation at GATC sites on the T7 genome was measured. Since polarity of T7 DNA entry has been demonstrated (27), GATC sites near the genetic left end of an infecting unmethylated T7 genome

should be methylated by Dam before sites near the right end. The extent of methylation of the entering phage genome can be monitored by assaying the sensitivity of the DNA to the enzyme *DpnI*.

Figure 2A shows the time course of methylation of the wild-type T7 genome during an infection at low multiplicity of the *E. coli* strain RV. After 7 min, band B becomes visible, indicating that the GATC site 8,312 bp from the physical left end of T7 DNA has become accessible to Dam methylation. By 9 min, band A and a relatively broad band corresponding to the DNA fragments DEAFC, EAFC, and AFC can be detected; bands D and E (which are visible on the original autoradiogram) also can be inferred from the presence of band A at 9 min, since entry of T7 DNA is known to be polar (24, 32, 45). Between 12 and 15 min, the C band becomes visible, indicating that at least 90% of the phage genome has entered the cell. The relatively fast rate of entry for the last 80% of the genome is consistent with the idea that T7 RNA polymerase, which synthesizes RNA much faster than the *E. coli* enzyme (6, 7), catalyzes the entry of the majority of the T7 genome. Complete Dam methylation of an infecting T7 genome thus requires 9 to 12 min, a time that is in good agreement with estimates of the time of genome entry made from studies on the temporal patterns of RNA synthesis (1, 21). Therefore, the time of Dam methylation at the last GATC site to enter the cell likely reflects the time required for complete genome entry.

The band labeled CB in Fig. 2A becomes visible after 12 to 15 min; this band represents the junction fragment of a concatemer of *DpnI*-cut T7 DNA that forms during DNA replication. However, bands DE and FC arise when the GATC sites at positions 11516 and 36087 are not methylated but GATC sites at positions 8312 or 8415 (these are not distinguished here), 14355, and 35685 are methylated. The origin of these bands is unclear since their intensities relative to those of the D, E, and C bands (band F is not usually seen on these agarose gels) vary in different experiments. Bands DE and FC are not a result of incomplete digestion by *DpnI* since they are cut by *DpnII* (specific for unmethylated DNA), suggesting that certain GATC sites on T7 DNA may be less reactive to methylation *in vivo* even in cells containing the multicopy *dam* plasmid pTP166.

When cells are treated with rifampin, no significant methylation of wild-type T7 DNA occurs (Fig. 2B), indicating that in the absence of transcription, less than 8.3 kb of the wild-type genome enters the cell during 12 min of infection. This result

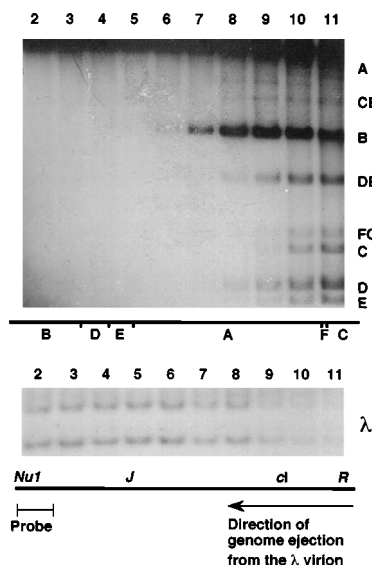


FIG. 3. Time course of phage DNA methylation during λ superinfection of T7-infected IJ1133(pTP166). Cells were infected with wild-type T7 at a multiplicity of 10. At 1-min intervals beginning at time zero, cells in a portion of the culture were superinfected with λ vir at a multiplicity of 0.1. At 2 min after infection by λ , DNA was isolated, digested with *DpnI*, and analyzed by Southern hybridizations. Membranes were probed with T7 [32 P]DNA (upper panel), stripped, and probed with a 5.1-kb *MluI* fragment of λ [32 P]DNA (lower panel). This *MluI* fragment (positions 458 to 5548 of the DNA sequence) contains 12 GATC sites. Only the two largest *DpnI* fragments are shown. The time after T7 infection that each sample was analyzed is shown above each lane (in minutes).

is consistent with previous studies that showed that entry of T7 DNA into the cell is coupled to transcription (24, 45). In part, the small amount of band B that is detected after 12 min of infection reflects incomplete inhibition of *E. coli* RNA polymerase by rifampin. When the experiment is continued for a longer time, the concatemer junction band (CB) eventually becomes visible, indicating that DNA replication is occurring in at least some infected cells (data not shown). T7 DNA replication requires, among other proteins, T7 RNA polymerase; incomplete inhibition of *E. coli* RNA polymerase by rifampin would allow synthesis of the T7 enzyme, and the latter would then transcribe the whole genome in a rifampin-insensitive reaction.

Methylation of DNA ejected from phage λ . The GATC site at position 8312 on T7 DNA becomes susceptible to cleavage by *DpnI* at 7 min after infection (Fig. 2A), about twice the time expected for transcription-mediated genome entry if *E. coli* RNA polymerase recognizes the A1, A2, and A3 promoters immediately after infection and synthesizes RNA at 40 base pairs per second (bp/s). In principle, the slow methylation of infecting T7 DNA could be due simply to an inability of Dam to methylate DNA rapidly. In order to test this idea, cells were infected at high multiplicity with unmethylated T7 and then, at various times, superinfected with unmethylated phage λ at low multiplicity. Thus, all λ -infected cells were previously infected with T7. After 2 min of infection by λ , DNA was extracted, and the transfer of T7 and λ DNA was analyzed by sequentially probing the separated membrane-bound *DpnI* fragments first with T7 DNA and then (after stripping) with λ DNA.

Methylation at T7 position 8312, leading to the appearance of the *DpnI* B band, occurs after 6 to 7 min of T7 infection, and that at position 36088, producing the C band, occurs after 10 min (Fig. 3). These bands appear at the same times in this experiment as they did in the experiment shown in Fig. 2, in

which a low multiplicity of infection was used. Therefore, the multiplicity of infection does not significantly affect the time course of methylation of the infecting T7 genome, and the failure to observe *DpnI* fragments immediately after phage infection is not a trivial consequence of a low sensitivity of the assay.

In contrast to the slow rate of methylation of T7 DNA, λ DNA is rapidly methylated by Dam and is therefore susceptible to *DpnI* digestion. When T7 and λ coinfect the same cells or when λ is added to cells either 2 or 4 min after infection by T7 and DNA is isolated after an additional 2 min of infection (lanes 2 through 6, Fig. 3), *DpnI* bands of λ DNA can be detected. The λ DNA probe used in this experiment is from the *NuI-Nu3* region of the phage genome and was chosen to have minimal hybridization to defective lambdoid prophages in the *E. coli* chromosome. This region is the last part of the chromosome to be ejected from the phage head into the cell (9, 33, 43), and thus the entire λ genome is ejected into the cell and methylated by Dam within 2 min of infection. In other experiments (not shown), λ DNA was shown to be methylated within the first minute of infection. Thus, in contrast to T7, the λ particle ejects the entire 48.5-kb genome into the cell extremely fast. After 6 to 8 min of T7 infection, the gene 3 and gene 6 nucleases are synthesized, and they degrade intracellular DNA, providing precursors for T7 DNA replication (40). As expected, therefore, when λ superinfects cells 5 min or more after T7 infection and the cells are incubated for a further 2 min (lanes 7 through 11, Fig. 3), the amount of methylated λ DNA fragments declines.

Dam methylation of λ DNA occurs within 2 min of infection, whereas methylation of T7 DNA, which is present in the same cells at a 100-fold-higher concentration than λ DNA, is delayed until at least 6 min after infection. Thus, the 3- to 4-min delay in T7 DNA methylation at position 8312 with respect to the time estimated for *E. coli* RNA polymerase to transcribe about 8 kb is not due to a slow rate of methylation by Dam. Furthermore, since the intensity of the two λ *DpnI* bands shown in Fig. 3 at early times is the same as in cells infected only with λ at the same low multiplicity (data not shown), no inhibitor of Dam is ejected from the T7 particle into the cell.

At the left end of the genome, methylation and gene expression occur at different times. In order to examine the time of methylation at a GATC site closer to the left end of the phage genome, IJ1133(pTP166) was infected by sRK836, a T7 mutant whose first GATC site to enter the cell lies at position 836 (ignoring linker sequences). This site is not methylated (and thus is not cleaved by *DpnI* to give band B₁) until 4 to 5 min after infection, whereas the site at position 8312, which gives rise to band B₂, is methylated at 7 min (Fig. 4A). The time that elapses between methylation at these sites corresponds to a rate of DNA translocation for 7.5 kb of the genome of 40 to 60 bp/s, a rate consistent with the idea that most of this region of the T7 genome enters the cell by *E. coli* RNA polymerase-catalyzed transcription. The apparent delay in methylation at position 836 is not due to interference with Dam activity by transcription from the upstream promoters for *E. coli* RNA polymerase, since pretreating cells with rifampin still results in methylation at position 836 after 5 min (Fig. 4B). The lack of Dam methylation of T7 DNA for several minutes after infection is a phenomenon similar to the delayed sensitivity of T7 DNA to various restriction enzymes (24).

In the presence of rifampin, the B₁ band is first detected at 5 min but increases in intensity with time (Fig. 4B). At least part of this increase seems to be due to genome entry in the absence of transcription. Any RNA polymerase that escaped inhibition by rifampin at the A1, A2, and A3 promoters not

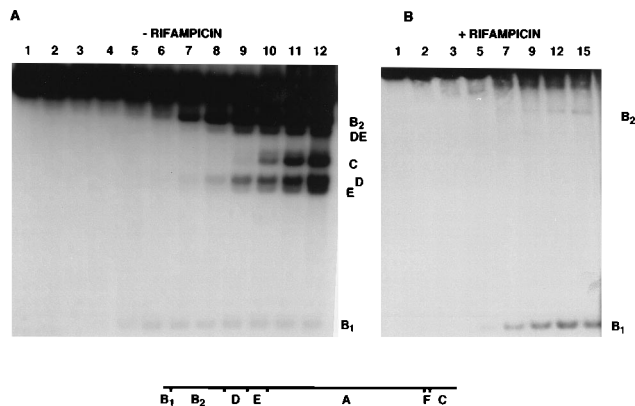


FIG. 4. Time course of methylation of sRK836 DNA during infection of IJ1133(pTP166) in the absence (A) or presence (B) of 200 µg of rifampin per ml. The multiplicity of infection was 3. The time after infection that each sample was analyzed is shown above each lane (in minutes).

only would allow Dam to methylate position 836 but would also transcribe gene 1. The gene 1 RNA polymerase should then draw the remainder of the genome in rapidly. Since only a trace of the B₂ band is visible and other downstream bands are undetectable, the rifampin treatment was effective. Thus, at least the first 836 bp of a nearly wild-type T7 genome enter the cell within 15 min of infection in the absence of transcription. Conversely, in the absence of transcription, most of the T7 genome cannot enter the cell and be methylated by Dam methylase.

The first Dam methylation site in sRK836 lies 338, 210, and 86 bp downstream of the start points of transcription from the A1, A2, and A3 *E. coli* promoters, respectively, and 89 bp upstream of the coding region of gene 0.3 (13). A priori, it would be expected that methylation at this site would occur prior to synthesis of the 0.3 RNAs and gp0.3. However, cells containing normal levels of Dam methylase are known (40) to express T7 gene 0.3 earlier after infection than the 4 to 5 min required for methylation at position 836. In order to determine if high levels of Dam methylase result in abnormal synthesis of T7 proteins, UV-irradiated IJ1133(pTP166) cells were infected with sRK836 at the same multiplicity used in the experiment shown in Fig. 4. The autoradiogram presented as Fig. 5 shows that gp0.3 is detectable within 2 min, gp0.7 within 3 min, and gp1 within 3 to 4 min of infection, i.e., at the same times seen

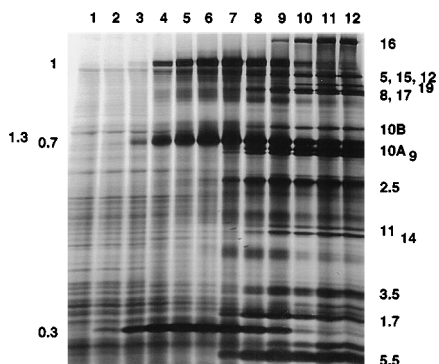


FIG. 5. Protein synthesis during sRK836 infection of UV-irradiated IJ1133(pTP166). Cells were infected at a multiplicity of 3. The time after infection when the 1-min pulse-labeling with [³⁵S]methionine was terminated is shown above each lane (in minutes). Gene numbers of prominent T7 proteins are indicated.

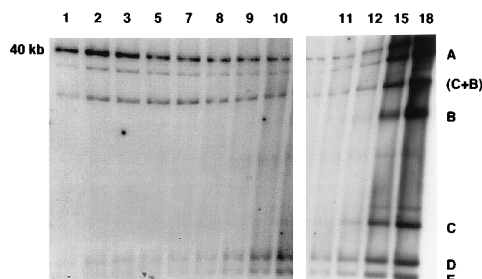
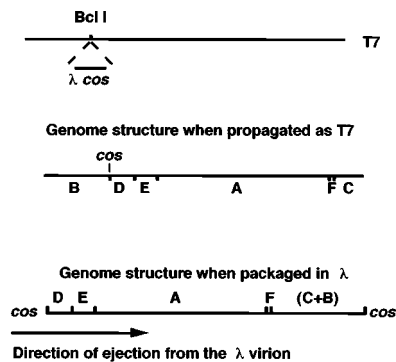


FIG. 6. Time course of methylation of T7 DNA when ejected from a λ particle. The construction of T7 I.7::λ cos Δ10-NB1 and genomic maps of the phage when packaged in λ and T7 virions are shown schematically. Cloning the *cos* BamHI fragment into the *Bcl*I site of T7 DNA leaves GATC sites flanking the insert. The hatch marks at both ends of the T7[λ] genome represent these GATC sites when the phage DNA is packaged in a T7 head. IJ1133(pTP166) was infected with T7 I.7::λ cos Δ10-NB1[λ] at a multiplicity of 0.1. The time after infection that each sample was analyzed is shown above each lane (in minutes). The two panels represent different exposures of the same experiment; parts of the 10- and 11-min lanes are visible in both panels.

after infection of wild-type cells. The genes for these three proteins must therefore have been transcribed and the RNAs translated before Dam can methylate a single upstream GATC site. Thus, *E. coli* RNA polymerase must be able to bind to the A1, A2, and A3 promoters and transcribe across the GATC sequence at position 836 before that site becomes accessible to Dam methylase.

Methylation of T7 DNA ejected from a λ particle. The difference in the times of methylation of T7 and λ DNAs in infected cells containing high levels of Dam should be due only to the different modes of genome ejection by the two phages. If this idea is correct, T7 DNA should be methylated rapidly if it is ejected from a λ particle. In order to package T7 DNA into λ heads, a 393-bp *Hinc*II fragment containing λ *cos* was cloned with *Bam*HI linkers into the unique *Bcl*I site of the DNA of T7 Δ10-NB1 (Fig. 6), a phage that contains a deletion of gene 10 sequences between the *Nhe*I and *Bss*HII sites. The resulting T7 I.7::λ cos Δ10-NB1[T7] can be propagated as a normal T7 phage, provided that gene 10 is provided in *trans*. However, the T7 genome can also be packaged, albeit rather inefficiently, into phage λ particles by infecting T7 I.7::λ cos Δ10-NB1 into an induced λ lysogen that cannot package its own DNA (30). Digestion of the DNA in the transvestite T7 particle (referred to as T7 I.7::λ cos Δ10-NB1[λ], or more simply as T7[λ]) with *Sau*3A I gives the fragments indicated in Fig. 6, showing that the C and B fragments, which are normally diagnostic of a T7 concatemer, are fused. Note that *Dpn*I will cut at GATC sites in and flanking λ *cos* DNA and thereby separate the C+B and D fragments that are fused when T7 DNA is circularized via the complementary ends of *cos* DNA. The orientation of the λ

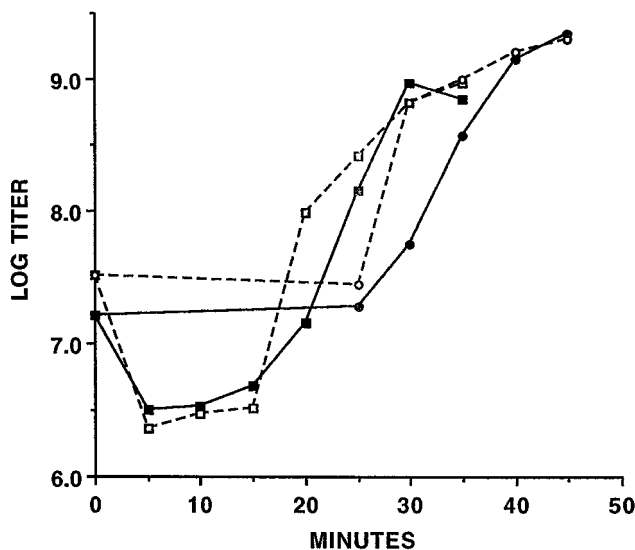


FIG. 7. One-step growth curves of T7 *I.7::λ cos Δ10-NB1*[T7] (■, ●) and T7 *I.7::λ cos Δ10-NB1*[λ] (□, ○) in IJ1133(pTP166) containing the gene 10-complementing plasmid pSW5. The multiplicity of infection was 0.1, and phage titers were determined with (■, □) or without (●, ○) artificial lysis with chloroform.

cos sequences with respect to the normal T7 genome was determined by restriction enzyme analyses, and the direction of ejection of T7 DNA from the λ virion could then be predicted from the known polarity of λ DNA ejection (9, 33, 43). The C+B *DpnI* fragment is detectable within 1 min and reaches maximum intensity within 2 min after unmethylated T7 *I.7::λ cos Δ10-NB1*[λ] infects IJ1133(pTP166). The C+B fragment corresponds to the last part of the genome to enter the cell, and thus the entire T7 DNA molecule must have been exposed to Dam within 1 min, the increase in intensity of bands during the second minute simply reflecting an asynchrony of infection. A similar result is obtained when the orientation of λ *cos* DNA in the T7 genome is reversed; in this construction, the last DNA to enter the cell following ejection from a λ particle corresponds to the *DpnI* D and E fragments, and these are detected within 1 min of infection (data not shown). It is therefore the mode of ejection of DNA from the phage T7 particle that is solely responsible for slow methylation at GATC sites by Dam.

T7 *I.7::λ cos Δ10-NB1*[λ] grows well in gene 10-containing λ^s cells, giving a normal burst with a shorter eclipse period than a normal T7 infection (Fig. 7). It seemed likely that the phage may be able to develop faster because its genome entered the cell faster, and it was therefore of interest to know whether the time course of phage protein synthesis was normal after infection by T7 *I.7::λ cos Δ10-NB1*[λ]. The proteins gp0.3, gp0.7, and gp1 appear at their normal time (Fig. 8); these class I products are synthesized from RNAs made by *E. coli* RNA polymerase, which likely transcribes from the A1, A2, and A3 promoters almost immediately after infection by either normal T7 or T7[λ] phage. However, synthesis of the class I proteins is not shut off normally and continues unabated for at least 18 min after infection by T7[λ]. One minute after addition of T7[λ] to cells, the culture was diluted 10-fold (see Materials and Methods), making it somewhat unlikely that the failure to shut off class I protein synthesis is entirely due to an asynchrony of infection. However, the basis for the continued class I protein synthesis after infection by T7[λ] is not known.

Synthesis of class II proteins begins a little earlier after

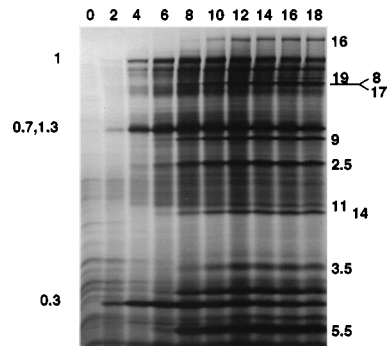


FIG. 8. Protein synthesis during T7 *I.7::λ cos Δ10-NB1*[λ] infection of UV-irradiated IJ1133(pTP166). Cells were infected at a multiplicity of 10. The time after infection that the 1-min pulse-labeling with [³⁵S]methionine was terminated is shown above each lane (in minutes). Gene numbers of several T7 proteins are indicated.

infection by T7[λ] than by normal T7; gp2.5 is made by 4 min, and gp5.5 is made by 6 min. Surprisingly, however, several class III proteins (e.g., gp8 through gp19) appear close to their normal time after infection (Fig. 8). The normal shutoff of class II protein synthesis also does not occur after infection by T7[λ]; presumably, continual synthesis of T7 RNA polymerase (a class I protein) prevents its inactivation by gp3.5, the gene product responsible for shutoff (22, 23).

Since the entire T7 genome had been ejected into the cell by T7[λ], it was expected that the stronger class III promoters would compete with class II promoters for the enzyme when T7 RNA polymerase was first made and was present in limiting amounts. Nevertheless, it is clear that some class II proteins first appear at the same time as (some even earlier than) many of the class III proteins. The idea that T7 class II promoters are weaker than class III promoters has stemmed largely from the low efficiency of transcription of the class II region of T7 DNA *in vitro*. However, the time course of protein synthesis directed by T7[λ] is more consistent with a high level of transcription over the class II region *in vivo* even when class III promoters are present, suggesting that some class II promoters are at least as strong as their class III counterparts *in vivo*. McAllister and coworkers tentatively reached a similar conclusion as they compared the relative amounts of class II and class III RNAs isolated from infected cells with those synthesized *in vitro* (19, 20, 22).

Rate of T7 DNA entry in the absence of transcription. Treating cells with rifampin prior to infection by T7 almost completely inhibits methylation of the infecting genome (Fig. 2B). However, incomplete inhibition of *E. coli* RNA polymerase by rifampin, with consequent synthesis of the rifampin-insensitive T7 enzyme, made it difficult to assess how much of the phage genome could enter the cell in the absence of transcription. In order to determine the extent of genome entry in the complete absence of transcription, methylation of the mutant 4101 was studied. The major deletion in 4101 removes all the early promoters for *E. coli* RNA polymerase and genes 0.3 through 1; unless T7 RNA polymerase (gp1) is provided by the host cell, the 4101 genome is not transcribed to any significant extent (24). However, although 4101 is deleted for all the early promoters for *E. coli* RNA polymerase, additional potential promoters in the late region of T7 have been inferred both from inspection of the nucleotide sequence of T7 DNA (13) and from the lethality of certain fragments of T7 DNA when cloned into pBR322 (42). Some of these potential promoters conceivably could participate in transcription-coupled entry of

T7 DNA. Entry of the 4101 genome was therefore monitored in both the presence and absence of rifampin.

In the presence of 500 μg of rifampin per ml, the first GATC site, at position 2794 in 4101 DNA, is methylated and is first detectable as a *DpnI* fragment (B_{4101} band) about 15 min after infection, although the amount of this fragment that is detected continues to rise for about 50 min of infection (Fig. 9A). Methylation at the second GATC site (position 8837, D-E Δ_{28} band) is detected at 30 or 40 min, reaching a maximum at 60 min, but even after 150 min of infection, the AFC band persists and only traces of the 21-kb A band can be detected. (It is shown below that treating cells with 500 μg of rifampin per ml for 150 min does not prevent T7 DNA from traversing the membrane.) These data suggest that in the absence of transcription, entry of all 4101 genomes stops after more than 9 kb but less than 30 kb of DNA has entered the cell. However, the increase in intensity of the B_{4101} band with time of infection suggests that in the absence of transcription, the entry of many infecting genomes is at least temporarily stopped by sequences within the first 2.8 kb of DNA. The average rate of entry by this transcription-independent mechanism is estimated from the times of first appearance of individual *DpnI* bands to be less than 5 bp/s, the asynchrony of the process precluding a more precise determination.

In the absence of rifampin, entry of the first 2.8 kb of the 4101 genome requires about the same amount of time as when the drug is present (Fig. 9B). However, the D-E Δ_{28} band appears earlier than in the presence of rifampin, and as judged by the appearance of bands A and C, the entire 4101 DNA molecule can enter the cell and be methylated by *Dam* after 40 min of infection. That complete entry occurs only in the absence of rifampin indicates that *E. coli* RNA polymerase does transcribe parts of the 4101 genome. The first predicted promoter to enter the cell lies 3,684 bp from the left end of the 4101 genome; the appearance of the D-E Δ_{28} band so soon after the B_{4101} band becomes visible suggests that this promoter is active in vivo and can catalyze transcription-coupled entry of the 4101 genome.

The complete entry of the genomes of 4101 and wild-type T7 is dependent on transcription. However, Moffatt and Studier described strain D502 as a T7 mutant for which genome entry and transcription were uncoupled; at least 37% of the D502 genome was shown to enter the cell in the absence of transcription (24). The D502 genome contains a small deletion of positions 343 to 808 that removes the ϕOL promoter for T7 RNA polymerase, the A1, A2, and A3 promoters for *E. coli* RNA polymerase, and sequences that normally arrest genome entry following ejection from the phage particle. As a consequence of its deletion, D502 cannot express gene $\theta.3$ but grows well in cells lacking a host type I restriction system.

In a normal infection, the methylation pattern of the D502 genome is similar to that of wild-type T7 except that the first GATC site (at position 7840 because of the deletion in D502) is recognized slightly earlier, the B_{D502} band appearing by 5 min of infection (data not shown). When transcription is inhibited by rifampin, the B_{D502} band still appears at 5 min, the D and E bands by 7 min, and the A and C bands by 12 min (Fig. 9C). Thus, the entire D502 genome can enter the cell in the absence of transcription. The overall rate of entry of the D502 genome in the absence of transcription, estimated from the time of first appearance of *DpnI* fragment bands, is about 50 bp/s. This is about 10-fold faster than the estimated rate of entry for the initial region of the 4101 genome.

In Fig. 9D, it is shown that treating cells with 500 μg of rifampin per ml for 10 min (standard conditions) or 160 min prior to infection allows complete entry of D502 DNA within

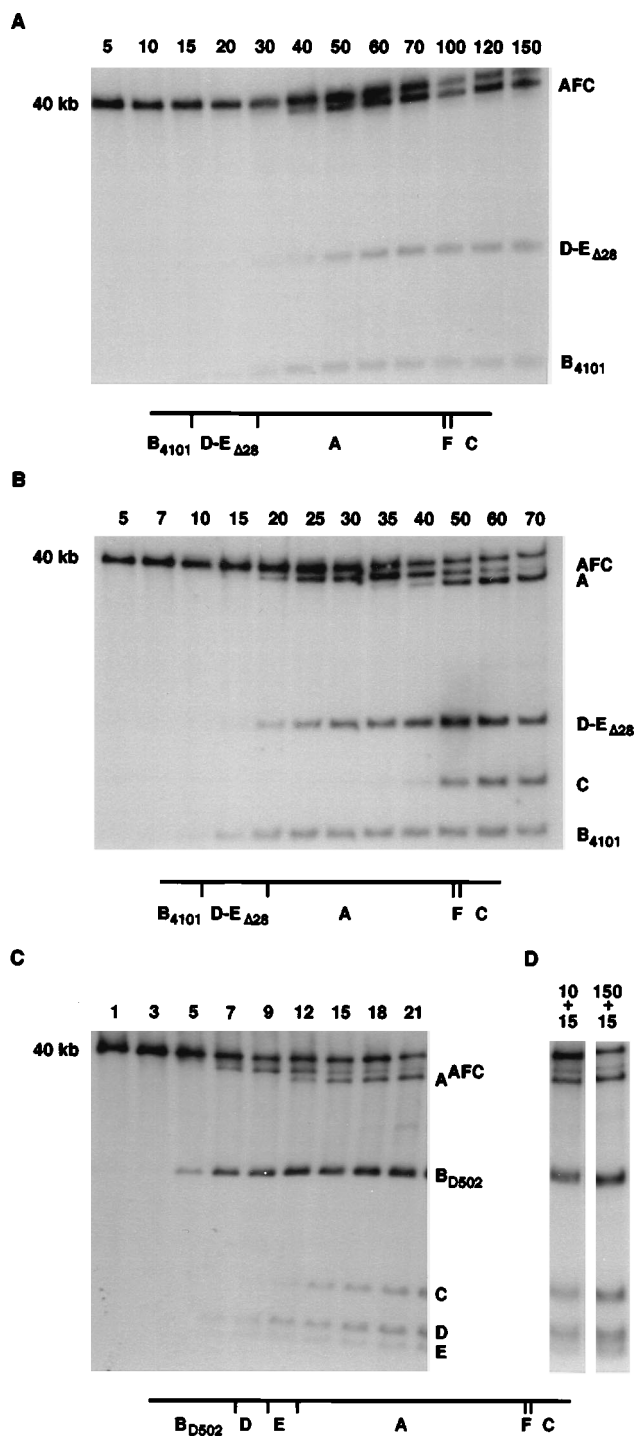


FIG. 9. Time course of methylation of 4101 (A and B) and D502 (C) DNA during infection of IJ1133(pTP166) in the absence (B) or presence (A and C) of 500 μg of rifampin per ml added 10 min prior to infection. The multiplicity of infection was 0.4 for each phage. The time after infection that each sample was analyzed is shown above each lane (in minutes). (D) IJ1133(pTP166) was treated with 500 μg of rifampin per ml for 10 or 160 min before addition of D502; samples were analyzed after 15 min of infection.

15 min. Part of the culture used in this experiment was infected with 4101 (Fig. 9A); the failure of the 4101 genome to completely enter the cell within 150 min is therefore not due to nonspecific effects of rifampin.

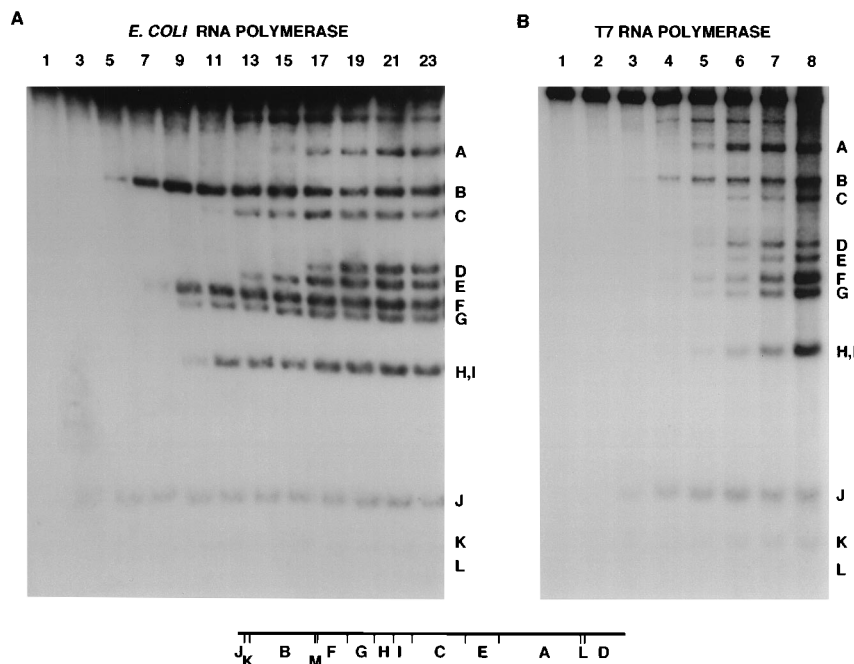


FIG. 10. Time course of methylation of Dam12 DNA during infection at a multiplicity of 5. The time after infection that each sample was analyzed is shown above each lane (in minutes). (A) IJ1133(pTP166) pretreated with 200 μ g of chloramphenicol per ml. (B) The T7 gene *I*-containing strain IJ1134(pTP166) pretreated with 200 μ g of rifampin per ml.

Rate of genome entry of a T7 mutant containing additional GATC sites. The distribution of GATC sites in wild-type T7 DNA precludes detailed measurements of the kinetics of genome entry. Additional GATC sequences were therefore introduced into T7 DNA at six different sites, resulting in phage Dam12. In addition to the extra GATC sites, T7 Dam12 contains a deletion that removes genes *0.4* through *0.7* and a small insertion within gene *10*. Dam12 grows normally, but, probably because of one of these two mutations, the first 20% of its genome enters the cell about 2 min faster than that of the wild type. Dam12 has been used to measure the kinetics of genome entry in a normal infection, in chloramphenicol-treated cells, and in cells that contain T7 RNA polymerase prior to infection. Figure 10A shows the kinetics of methylation of the Dam12 genome in chloramphenicol-treated cells, in which entry is catalyzed primarily by *E. coli* RNA polymerase. The first GATC site, at position 836, is methylated by 3 to 5 min, allowing *DpnI* to produce the J fragment. The B fragment appears by 5 min, indicating that *E. coli* RNA polymerase has read through the early transcription terminator TE. The remaining *DpnI* fragments appear sequentially, corresponding to their positions in the Dam12 genome, the rightmost fragment appearing about 17 min after infection.

The leftmost promoter ϕOL for T7 RNA polymerase is at position 405, upstream of the A1, A2, and A3 promoters for *E. coli* RNA polymerase, and can be used to catalyze genome entry when the T7 enzyme is present in the cell prior to infection. Figure 10B shows the kinetics of methylation of the Dam12 genome in rifampin-treated cells, in which entry is catalyzed primarily by T7 RNA polymerase. The J band, which appears when the GATC site at position 836 is first methylated, is first detectable 2 to 3 min after infection, and only 5 min are required to translocate the entire Dam12 genome into the cell. No difference in the rate of entry of the Dam12 genome was detected when gene *I*-containing cells were treated with both rifampin and chloramphenicol prior to infection (data not

shown), indicating that no phage protein other than RNA polymerase affects the rate of DNA translocation from the phage head into the bacterial cell.

The times of appearance of individual *DpnI* bands shown in Fig. 10, together with those of IJ1133(pTP166) infected by Dam12 in the absence of antibiotics, were used to estimate the kinetics of genome entry under different conditions. After 3 min of infection in the presence of chloramphenicol, when *E. coli* RNA polymerase is bringing the phage DNA into the cell, the rate of entry of the Dam12 genome becomes almost constant, at about 45 bp/s (Fig. 11). This rate of genome entry is in reasonable agreement with estimates of the rate of RNA chain elongation in *E. coli* (2, 5, 17, 29, 40). Similarly, the rate of genome entry catalyzed by T7 RNA polymerase can be calcu-

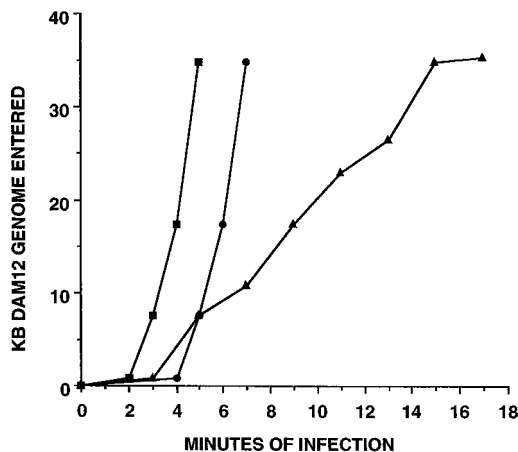


FIG. 11. Rates of entry of Dam12 DNA into cells estimated from the experiment shown in Fig. 10. ●, normal infection; ▲, *E. coli* RNA polymerase catalyzed; ■, T7 RNA polymerase catalyzed. KB, kilobases.

lated to be 200 to 300 bp/s, a value comparable to the estimated average rate of transcription in vivo and in vitro (7).

DISCUSSION

The slow entry of phage T7 DNA into the bacterial cell can be assayed by measuring the times of Dam methylation at sequential GATC sites on the infecting genome. Overexpression of *dam* from a multicopy plasmid is required to achieve complete methylation of T7 DNA, probably because the rates of phage DNA replication and packaging are too fast for the normal levels of methylase activity produced from the chromosomal copy of *dam*. The assay is insensitive to the multiplicity of infection and, unlike assays of T7 genome entry employed previously, can be used under conditions that include normal, productive infections. The various steps in the process of translocating DNA from the phage capsid across the cell membranes into the cell cytoplasm can then be studied in more detail than has previously been possible. Although only entry of T7 DNA and, to a lesser extent, λ DNA has been examined, Dam methylation of infecting phage DNA may be an appropriate assay to study genome entry for many other phages.

The entry of T7 DNA into the cell is normally coupled to transcription, first by *E. coli* RNA polymerase and later by the T7 enzyme. Blending experiments (45), measurements of degradation of labeled T7 DNA by the type I restriction enzymes *EcoB* and *EcoK* (24), and methylation assays all show that rifampin inhibits efficient internalization of an infecting wild-type T7 genome. In the presence of chloramphenicol, which blocks synthesis of T7 proteins, complete entry of the T7 genome occurs as a result of transcription by *E. coli* RNA polymerase reading through the early terminator TE and other natural terminator sequences in T7 DNA. Entry of the entire genome is delayed under these conditions by the slower rate of elongation of *E. coli* RNA polymerase than of the T7 enzyme (6, 7); conversely, when T7 RNA polymerase is present in the cell before infection, the rate of genome entry is faster than normal. Presumably, as each RNA polymerase transcribes, it pulls the phage DNA from the capsid into the cell. The estimated rates of transcription catalyzed by *E. coli* and T7 RNA polymerases, and the respective estimated rates of genome entry catalyzed by each enzyme, are comparable, indicating that translocation of T7 DNA across the cytoplasmic membrane is not a rate-determining step.

The T7 particle causes ejection of the leading end of the genome into the cell, where RNA polymerase normally recognizes at least one of the three major promoters located at positions 498, 626, and 750 on the T7 genome (13). Thus, at least 498 bp must be directly ejected from the phage head and into the cell. The pattern of Dam methylation of 4101 DNA in the absence of transcription suggests that entry of some genomes into the cell is arrested before 2.8 kb of DNA has translocated across the cell membranes. The most likely cause of arrest is a DNA sequence near the left end of the genome (see below) first identified by Moffatt and Studier (24). However, arrest of genome entry by this sequence is not totally efficient, and at least 8.8 kb, although definitely less than 30.6 kb, of some 4101 genomes enter the cell in the absence of transcription. A more precise determination of the extent of DNA penetration, and of the sequences that stop translocation of the 4101 genome after more than 8.8 kb of DNA has entered the cell in the absence of transcription, will require introducing more GATC sites into the 4101 genome. This estimate of the extent of transcription-independent entry is considerably higher than those made previously (24, 45), but the differences

are likely trivial consequences of the high sensitivity of the assay employed in this study and of the very slow rate of DNA entry in rifampin-treated cells.

In contrast to the slow entry of 4101 DNA in the presence of rifampin, that of D502 DNA is fast. D502 was first described as a T7 mutant whose DNA can enter the cell in a process not coupled to transcription, and it was suggested that the DNA sequence between positions 343 and 393 (corresponding to the left ends of the deletions in the D502 and 4101 genomes, respectively) serves to arrest the normal entry of T7 DNA (24). The results presented here are in accord with this suggestion; the rate of entry of the complete D502 genome is at least 10-fold faster than that of the part of 4101 DNA that enters in the absence of transcription. In the absence of transcription, entry of the D502 genome occurs synchronously, whereas that of the initial regions of the 4101 or sRK836 genome is not. This difference is consistent with the idea that D502 contains a deletion of the sequences that, during infection by wild-type phage, slow the rate of genome ejection from the capsid as they pass through the membrane into the cell.

Alternatively, the D502 deletion may alter the ejection machinery to give a faster initial rate of ejection from the phage particle. Thus, whereas the genomes of most T7 strains may be ejected from the phage capsid at a rate of about 5 bp/s until transcription accelerates the rate of entry to about 40 bp/s, the D502 genome is ejected at about 50 bp/s. This idea is supported by the observation that ejection of T7 DNA from a λ particle is not detectably slowed or arrested as the sequences that correspond to the D502 deletion enter the cell. Furthermore, if most T7 genomes are ejected into the cell at about 5 bp/s, about 100 s would be required for the A1 promoter for *E. coli* RNA polymerase to enter the cell. Transcription from the A1 promoter of the next 800 bp of DNA and translation of the resulting *o*.3 RNA should require only about 20 s. The estimated time required for the initial appearance of gp0.3 after T7 infection is thus about 2 min, exactly that found following infection by sRK836 (Fig. 5). However, to determine whether either idea provides an accurate description of the mechanism of entry of T7 DNA into the cell requires further study of the process.

It is interesting that the sequences that arrest translocation of T7 DNA after its ejection from a T7 capsid have no apparent effect on DNA translocation after ejection from a λ particle. The orientation of the arresting sequences with respect to the direction of genome translocation does not seem to be important (Fig. 6 and data not shown), but a number of other explanations can be imagined.

(i) The T7 DNA "arrest sequence" may only slow the rate of, rather than stop, genome translocation across the membrane. The rapid rate of λ DNA ejection (>50 kb/min) may indeed be slowed as the arrest sequence traverses the membrane, but the change in rate is not detectable by these experiments.

(ii) The DNA sequences that first enter the cell after infection by T7[λ] are those of λ *cos* and are single-stranded. The sequences at the ends of T7 DNA are different from those of λ DNA, and the ends of T7 DNA are also double-stranded. Perhaps the 160-bp direct repeats at the ends of the T7 genome are also important in the arrest of T7 DNA translocation into the cell.

(iii) A mutation in the last duplex base pair of the λ chromosome (the first base pair to enter the cell) causes a severe defect in phage growth. It is thought that a defective interaction between the tail protein gpH* and the mutant base pair prevents both proper ejection and routing of the DNA from the capsid into the cell (44). gpH* is ejected from the phage

particle and becomes resistant to proteases after "infection" of liposomes; these findings led to the suggestion that gpH* associates with the cell membrane to help form a transmembrane hole suitable for DNA entry (28). It is not known whether T7 codes for a protein with a function analogous to that of gpH*, but a membrane channel used by T7 DNA in entering the cell is likely to consist of components different from those used by λ DNA. Recognition of the specific T7 DNA arrest sequences by the transmembrane hole made by λ is therefore an improbable event.

It is not clear why Dam methylation at position 836, after infections by sRK836 or Dam12, should be delayed relative to transcription over that site when transcription does not appear to interfere with methylation. Preliminary data indicate that deletion of the A1, A2, and A3 promoters and insertion of a GATC site at position 445 do not alleviate the delay in methylation and that *lac* repressor cannot access this region of T7 DNA at the same time as RNA polymerase (14a). A similar delay before degradation by type I and type III restriction nucleases occurred was also noted previously by Moffatt and Studier (24). Of the possible mechanisms that they envisaged to explain the selective recognition of T7 DNA early after infection, the idea that the incoming T7 DNA enters a cellular compartment that is accessible to RNA polymerases but not to several other proteins is perhaps the most appealing. Such a hypothetical compartment may contain bacterial components and/or phage proteins that are ejected into the cell together with the DNA. If phage proteins are involved, it is easy to imagine that they have evolved specifically to allow selective accessibility of cellular enzymes to the phage genome.

It is interesting that 4101 DNA appears to be stable in infected cells; there is no reduction in the intensity of bands on the autoradiogram for more than 20 min after the entire genome has entered the cell and been methylated by Dam (Fig. 9B). Thus, 4101 DNA is resistant to degradation by RecBCD and other cellular nucleases. It is possible that sufficient gp5.9, the T7 protein that inhibits RecBCD (16), is made in 4101-infected cells, but a second possibility is that the 4101 genome never becomes accessible to RecBCD or other nucleases. The linear 4101 genome is also not degraded in cells containing the type I restriction enzyme *EcoB* or *EcoK* (24).

It has been suggested that the slow entry of the T7 genome is an important element in the control of gene expression (21); this is likely to be true, but perhaps not quite in the way envisaged. The most obvious effect of rapid genome entry that occurs when a λ virion is used to eject T7 DNA into the cell are that class I and class II gene expression is not shut off. Furthermore, the normal sequential expression of class II and class III genes is largely preserved even though both are in the cell and presumably equally accessible to T7 RNA polymerase after it has been made from early transcripts.

Both transcription and translation of class I genes are shut off during a normal T7 infection. Transcription catalyzed by *E. coli* RNA polymerase is inhibited by gene *0.7* (3, 4, 31). One possibility for the continued synthesis of class I proteins in the T7[λ] infection is that the normal mode of ejection of T7 DNA is important for gp0.7 action. A second possibility is that, unlike during a normal T7 infection, T7 RNA polymerase transcribes the early region of T7 DNA, producing RNAs that continue to be translated. Transcription could initiate either at ϕOL , at the beginning of the early region, or at class III promoters, since the early region of T7 DNA is fused to the right end of the normal T7 genome in T7[λ] (see Fig. 6). T7 RNAs are, however, relatively stable *in vivo*, and some form of translational regulation likely participates in the normal shut-off of class I protein synthesis. Evidence for translational dis-

crimination against *0.3* mRNA by late RNAs has been reported (37–39). The continued synthesis of class II proteins suggests that late mRNA synthesis could be aberrant in T7[λ] infections, but there is no direct evidence for or against this idea.

Only T7 RNA polymerase (gp1) is required for transcription of the class II and class III genes of T7, the two classes being distinguished by the preferential continued synthesis of class III RNAs (22) and proteins (40). T7 RNA polymerase is present in limiting amounts when it is first made after infection by T7[λ], and it was therefore anticipated that class III genes would be preferentially expressed. However, synthesis of the most abundant class II proteins occurs at the same time as, perhaps even earlier than, that of the most abundant class III proteins. Although there is some evidence that class II promoters are stronger *in vivo* than *in vitro* and may thus effectively compete with class III promoters (19, 20, 22), the intracellular structure of T7 DNA after ejection from a λ particle may also account for the early synthesis of class II proteins. T7 *1.7::\lambda cos \Delta 10-NB1* DNA has the sticky ends of λ DNA when packaged in a λ particle; thus, it should circularize and become supercoiled after infection. Circular T7 DNA has in fact been detected in cells infected by T7[λ] (14), and it has been shown that the difference in class II and class III promoter strength seen *in vitro* can disappear when supercoiled templates are employed (8, 15). Circularity of phage DNA following infection by T7[λ] would also allow transcription from class III promoters to encompass both the class I and class II regions, since there is no terminator at the end of the class III genes in T7 DNA.

ACKNOWLEDGMENTS

We thank A. Rosenberg and F. W. Studier for providing T7 strains and plasmid pAR5378, M. Marinus and M. Gold for bacterial strains and plasmids, K. Tedin for the *lac*-T7 gene 1 construct, and New England Biolabs for ER1868. We also thank three anonymous reviewers for their thoughtful comments.

This work was supported by Public Health Service grant GM32095 from the National Institutes of Health. L.R.G. was supported in part by a predoctoral fellowship from the National Science Foundation.

REFERENCES

1. Beck, P. J., and I. J. Molineux. 1991. Defective transcription of the right end of bacteriophage T7 DNA during an abortive infection of F plasmid-containing *Escherichia coli*. *J. Bacteriol.* **173**:947–954.
2. Bremer, H., and D. Yuan. 1968. RNA chain growth-rate in *Escherichia coli*. *J. Mol. Biol.* **38**:163–180.
3. Brunovskis, I., and W. C. Summers. 1971. The process of infection with coliphage T7. V. Shutoff of host RNA synthesis by an early phage function. *Virology* **45**:224–231.
4. Brunovskis, I., and W. C. Summers. 1972. The process of infection with coliphage T7. VI. A phage gene controlling shutoff of host RNA synthesis. *Virology* **50**:322–327.
5. Casjens, S., and M. B. Adams. 1985. Posttranscriptional modulation of bacteriophage P22 scaffolding protein gene expression. *J. Virol.* **53**:185–191.
6. Chamberlin, M. J. 1982. Bacterial DNA-dependent RNA polymerases, p. 61–86. *In* P. D. Boyer (ed.), *The enzymes*, vol. 15. Academic Press, New York.
7. Chamberlin, M., and T. Ryan. 1982. Bacteriophage DNA-dependent RNA polymerases, p. 87–108. *In* P. D. Boyer (ed.), *The enzymes*, vol. 15. Academic Press, New York.
8. Chapman, K. A., and R. R. Burgess. 1985. Construction of bacteriophage T7 late promoters with point mutations and characterization by *in vitro* transcription properties. *Nucleic Acids Res.* **15**:5413–5432.
9. Chattoraj, D. K., and R. B. Inman. 1974. Location of DNA ends in P2, 186, P4, and lambda bacteriophage heads. *J. Mol. Biol.* **87**:11–22.
10. Condreay, J. P., and I. J. Molineux. 1989. Synthesis of the capsid protein inhibits development of bacteriophage T3 mutants that abortively infect F plasmid-containing strains. *J. Mol. Biol.* **207**:543–554.
11. Condreay, J. P., S. E. Wright, and I. J. Molineux. 1989. Nucleotide sequence and complementation studies of the gene 10 region of bacteriophage T3. *J. Mol. Biol.* **207**:555–561.

12. **Davanloo, P., A. H. Rosenberg, J. J. Dunn, and F. W. Studier.** 1984. Cloning and expression of the gene for bacteriophage T7 RNA polymerase. *Proc. Natl. Acad. Sci. USA* **81**:2035–2039.
13. **Dunn, J. J., and F. W. Studier.** 1983. Complete nucleotide sequence of bacteriophage T7 DNA and the locations of genetic elements. *J. Mol. Biol.* **166**:477–535.
14. **García, L. R., and I. J. Molineux.** 1995. Incomplete entry of bacteriophage T7 DNA into F plasmid-containing *Escherichia coli*. *J. Bacteriol.* **177**:4077–4083.
- 14a. **García, L. R., and I. J. Molineux.** Unpublished observations.
15. **Ikeda, R. A., A. C. Lin, and J. Clarke.** 1992. Initiation of transcription by T7 RNA polymerase at its natural promoters. *J. Biol. Chem.* **267**:2640–2649.
16. **Li, L.** 1992. Ph.D. dissertation. State University of New York at Stony Brook.
17. **Manor, H., D. Goodman, and G. Stent.** 1969. RNA chain growth rates in *Escherichia coli*. *J. Mol. Biol.* **39**:1–29.
18. **Marinus, M. G., A. Poteete, and J. A. Arraj.** 1984. Correlation of DNA adenine methylase activity with spontaneous mutability in *Escherichia coli* K-12. *Gene* **28**:123–125.
19. **McAllister, W. T., and C. L. Barrett.** 1977. Hybridization mapping of restriction fragments from the early region of bacteriophage T7 DNA. *Virology* **82**:275–287.
20. **McAllister, W. T., and R. J. McCarron.** 1977. Hybridization of the in vitro products of bacteriophage T7 RNA polymerase to restriction fragments of T7 DNA. *Virology* **82**:288–298.
21. **McAllister, W. T., C. Morris, A. H. Rosenberg, and F. W. Studier.** 1981. Utilization of bacteriophage T7 late promoters in recombinant plasmids during infection. *J. Mol. Biol.* **153**:527–544.
22. **McAllister, W. T., and H.-L. Wu.** 1978. Regulation of transcription of bacteriophage T7. *Proc. Natl. Acad. Sci. USA* **75**:804–808.
23. **Moffatt, B. A., and F. W. Studier.** 1987. T7 lysozyme inhibits transcription by T7 RNA polymerase. *Cell* **49**:221–227.
24. **Moffatt, B. A., and F. W. Studier.** 1988. Entry of bacteriophage T7 DNA into the cell and escape from host restriction. *J. Bacteriol.* **170**:2095–2105.
25. **Molineux, I. J.** 1994. T7 bacteriophage, p. 1388–1396. *In* R. G. Webster and A. Granoff (ed.), *Encyclopedia of virology*. Academic Press, New York.
26. **Morrison, T. G., D. D. Blumberg, and M. H. Malamy.** 1974. T7 protein synthesis in F' episome-containing cells: assignment of specific proteins to three translational groups. *J. Virol.* **13**:386–393.
27. **Pao, C. C., and J. F. Speyer.** 1973. Order of injection of T7 bacteriophage DNA. *J. Virol.* **11**:1024–1026.
28. **Roessner, C. A., and G. M. Ihler.** 1984. Proteinase sensitivity of bacteriophage lambda tail proteins gpJ and pH* in complexes with the lambda receptor. *J. Bacteriol.* **157**:165–170.
29. **Rose, J., R. Mosteller, and C. Yanofsky.** 1970. Tryptophan messenger ribonucleic acid elongation rates and steady-state levels of tryptophan operon enzymes under various growth conditions. *J. Mol. Biol.* **51**:541–550.
30. **Rosenberg, S. M., M. M. Stahl, I. Kobayashi, and F. W. Stahl.** 1985. Improved *in vitro* packaging of coliphage lambda DNA: a one strain system free from endogenous phage. *Gene* **38**:165–175.
31. **Rothman-Denes, L. B., S. Muthukrishnan, R. Haselkorn, and F. W. Studier.** 1973. A T7 gene function required for shut-off of host and early T7 transcription, p. 227–239. *In* C. F. Fox and W. S. Robinson (ed.), *Virus research*. Academic Press, New York.
32. **Saigo, K.** 1975. Polar DNA ejection in bacteriophage T7. *Virology* **65**:120–127.
33. **Saigo, K., and H. Uchida.** 1974. Connection of the right-hand terminus of DNA to the proximal end of the tail in bacteriophage lambda. *Virology* **61**:524–536.
34. **Sambrook, J., E. F. Fritsch, and T. Maniatis.** 1989. *Molecular cloning: a laboratory manual*, 2nd ed. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y.
35. **Schmitt, C. K., P. Kemp, and I. J. Molineux.** 1991. Genes *1.2* and *10* of bacteriophages T3 and T7 determine the permeability lesions observed in infected cells of *Escherichia coli* expressing the F plasmid gene *pifA*. *J. Bacteriol.* **173**:6507–6514.
36. **Shinder, G., and M. Gold.** 1988. The Nu1 subunit of bacteriophage lambda terminase binds to specific sites in *cos* DNA. *J. Virol.* **62**:387–392.
37. **Strome, S., and E. T. Young.** 1978. Translational control of the expression of bacteriophage T7 gene *0.3*. *J. Mol. Biol.* **125**:75–93.
38. **Strome, S., and E. T. Young.** 1980. Chemical and functional quantification of gene *0.3* messenger RNA during T7 infection. *J. Mol. Biol.* **136**:417–432.
39. **Strome, S., and E. T. Young.** 1980. Translational discrimination against bacteriophage T7 gene *0.3* messenger RNA. *J. Mol. Biol.* **136**:433–450.
40. **Studier, F. W.** 1972. Bacteriophage T7. *Science* **176**:367–376.
41. **Studier, F. W.** 1975. Genetic mapping of a mutation that causes ribonuclease III deficiency in *Escherichia coli*. *J. Bacteriol.* **124**:307–316.
42. **Studier, F. W., and A. H. Rosenberg.** 1981. Genetic and physical mapping of the late region of bacteriophage T7 DNA by use of cloned fragments of T7 DNA. *J. Mol. Biol.* **153**:503–525.
43. **Thomas, J. O.** 1974. Chemical linkage of the tail to the right-hand end of bacteriophage lambda DNA. *J. Mol. Biol.* **87**:1–9.
44. **Xu, S., and M. Feiss.** 1991. The last duplex base-pair of the phage lambda chromosome. *J. Mol. Biol.* **220**:293–306.
45. **Zavriev, S. K., and M. F. Shemyakin.** 1982. RNA polymerase-dependent mechanism for the stepwise T7 phage DNA transport from the virion into *E. coli*. *Nucleic Acids Res.* **10**:1635–1652.