Incomplete Entry of Bacteriophage T7 DNA into F Plasmid-Containing *Escherichia coli*

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The penetration of bacteriophage T7 DNA into F plasmid-containing *Escherichia coli* cells was determined by measuring Dam methylation of the entering genome. T7 strains that cannot productively infect F-containing cells fail to completely translocate their DNA into the cell before the infection aborts. The entry of the first 44% of the genome occurs normally in an F-containing cell, but the entry of the remainder is aberrant. Bypassing the normal mode of entry of the T7 genome by transfecting naked DNA into competent cells fails to suppress F exclusion of phage development. However, overexpression of various nontoxic T7 *1.2* alleles from a high-copy-number plasmid or expression of T3 *1.2* from a T7 genome allows phage growth in the presence of F.

There are many known instances in which the growth of an infecting phage is prevented by the presence of another, sometimes unrelated, parasite in the infected cell. Prophages or plasmids can prevent subsequent phage infections by disparate mechanisms, including alteration of cell surface receptors (lysogenic conversion), prevention of genome penetration leading to periplasmic degradation of the superinfecting DNA (superinfection exclusion), cytoplasmic degradation of infecting DNA (restriction), and immunity. In these cases, the mechanism by which growth inhibition of the incoming parasite is achieved is at least moderately well understood. More complex situations arise when phage infections are aborted midway through their infection cycle. Chief among these are the exclusion of T4 *rII* mutants by λ *rex*⁺ lysogens; of T7 and T5 by the conjugative plasmids F and ColIb, respectively; of T4 by constitutive *lit* mutants of the cryptic element *e14*; and of other lambdoid phages by *sieB* of P22 or λ and by λ *rex* (for reviews, see references 10 and 17). All of these abortive infections are characterized by a plethora of physiological dysfunctions that accompany the inhibition of phage growth; in most cases, the primary cause of the abortive infection has not been distinguished from associated effects. A detailed model has been proposed recently to explain *rex* exclusion of T4 *rII* mutants: during T4 *rII* infections, the RexA protein activates the membrane-spanning RexB, forming an ion channel that results in loss of membrane potential and cellular energy (25). Although the model is largely consistent with extant data (33), there are no experiments yet reported that demonstrate a RexB-containing, depolarizing ion channel.

Perhaps the best known abortive infection understood at a molecular level is that in which *e14 lit* and T4 gene 23 cooperate to cleave EF-Tu, preventing translation of late T4 proteins (40). It is interesting and may also be significant that two other exclusion systems also inactivate components of the translational apparatus. *prf*-mediated exclusion of T4 *pnk* or *rli* mutants is caused by irreparable cleavage of a host tRNA^{Lys} (1), and interference with peptidyl-tRNA hydrolase activity results in exclusion of phage λ by *Escherichia coli rap* mutants (21).

Following T7 infection of F-containing (i.e., male) cells, the initial events of adsorption and early gene product synthesis

appear to be normal (10, 17). As the infection progresses, an interaction between certain gene products of T7, F, and the host occurs which causes all viral and cellular macromolecular processes to halt. More or less concurrently, the integrity of the infected cell's membrane weakens, resulting in a rapid efflux of nucleotides to the surrounding medium. However, mutants of T7 that grow normally in the presence of F still elicit nucleotide leakage from infected cells (18, 30). Thus, leakiness of membranes is merely an effect associated with T7-infected cells and does not cause the infection to abort. F-containing cells infected by one of these T7 mutants must therefore continue to synthesize sufficient ATP and other energy-rich compounds to sustain phage development. As the T7 infection aborts, infected cells die but do not lyse, and few if any phage particles are released. It has been speculated that F evolved this phage exclusion system to restrict T7-like phages from spreading through a population already colonized by the plasmid. Following an abortive infection by T7, the demise of only one cell among a population of male cells can be regarded as an altruistic death.

Three proteins are known to be directly involved in the F-mediated abortive infection: the products of T7 genes *1.2* and *10* (18) and the F plasmid protein PifA (7-9, 20, 26, 31). The combination of PifA and either of the T7 proteins is sufficient to cause cell death (30, 31), and synthesis of gp1.2 in a PifA-containing cell has been shown to cause all of the physiological defects associated with the abortive phage infection (17, 29). Null mutations in *pifA* render the F-containing cell fully permissive for a wild-type T7 infection (4, 20, 30). Likewise, T7 mutants which contain a missense or null mutation in gene *1.2* and two missense mutations in gene *10* grow efficiently in cells which harbor wild-type F (18).

PifA has no known biochemical properties, and its only recognized function is the exclusion of the majority of T7-like phages. Gene *10* encodes the major capsid protein for morphogenesis of the phage particle (34), and gene *1.2* encodes a protein which inhibits a cellular dGTP triphosphohydrolase (12, 22, 23). Gene *1.2* is essential only for phage growth in mutants (*optA1*) of *E. coli* that overexpress the dGTPase (27, 38). However, there is no evidence that these properties are important in the abortive infection. Although (in the absence of gene *1.2*) null mutations in gene *10* prevent the physiological defects associated with the abortive infection, mutations in other genes that preclude assembly of gp10 into head-like structures do not alleviate the defects (6, 18). Furthermore,

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among T7 strains that grow in the presence of F, several code for mutant gene *l.2* products that maintain anti-dGTPase activity (18).

In contrast to T7, the closely related phage T3 grows normally on male cells (6, 19, 35). The ability of T3 to productively infect F-containing *E. coli* normally depends on synthesis of gp1.2 (6, 19). Infections of male cells by T3 *l.2* null mutants display the same characteristic dysfunctions as infections by wild-type T7. For these T3 mutants, interactions between gp10 and PifA cause the infection to abort. However, after infection by wild-type T3, gp1.2 protects against the toxicity of gp10 and PifA (6). Genes *l.2* and *l.10* of T7 and T3 lie in equivalent positions in their respective phage genomes, and each gene product exhibits substantial homology to its counterpart in the other phage (2, 32); gene *l.10* of one phage complements gene *l.10* mutants of the other (7). It is likely, therefore, that the T3 proteins have similar activities to their T7 counterparts, with the exception that the interaction of T7 gp1.2 with PifA is lethal to the cell.

The initial cellular defect that ultimately leads to the death of the abortively infected cell has yet to be distinguished from subsequent events. One characteristic of the abortive infection is that the overall level of gene expression from the right end of the genome is severely depressed (3–5, 37, 39). The class III genes are situated in this area of the genome; they encode proteins that are involved in particle assembly, DNA packaging, and lysis (34). The most pronounced defect is the inability to produce full-length class III RNA transcripts from the rightmost 30% of the phage genome. The 5' ends of RNAs initiating from the ϕ 13 and ϕ 17 promoters can be visualized, but the normal 3' ends of these transcripts cannot be detected (3). Entry of the T7 genome into the cell is from left to right on the genetic map (24) and is coupled to transcription (11, 16, 41); thus, the failure to detect normal transcripts synthesized from the right end of the phage DNA during an abortive infection suggests that this region of the genome might fail to enter the cell.

In order to characterize F exclusion of T7 in more detail and to determine whether genome entry is associated with the primary dysfunction of the abortively infected cell, we have measured the extent of phage DNA entry into male cells. We also show that T3 *l.2*, when part of a T7 genome, allows phage growth in male cells and that high gene dosages of mutant T7 *l.2* alleles are required for T7 to avoid exclusion.

MATERIALS AND METHODS

Bacteria, plasmids, and phages. The *E. coli* K-12 strain IJ511 (Δ lacX74 galK2 galT22 supE44 hsdS) and the F42 derivatives F'lac (Kn) and F42-P3 (a pifA mutant of F42) have been described previously (30). The optA1 mutation present in HR44 (27) was transduced into IJ511 with P1 vir by using tonA2 as a selectable marker; the transductant is called IJ511optA1. GM2163 (dam-13::Tn9 dcm-6 hsdR2 mcrA mcrB leuB6 his-4 thi-1 ara-14 lacY1 galK2 galT22 xyl-5 mtl-1 rpsL136 tonA31 tsx-78 supE44) and the Dam methylase-overproducing plasmid pTP166 were gifts of M. G. Marinus (15). The T7 gene *l.2* plasmids pCS5 (1.2W31C), pSG1 (1.2Y28N), and pSG4 (1.2R37G) are derivatives of pUC plasmids (31) and are identical except for the point mutation; T7 gene *l.2* is expressed both from the vector lac promoter and from the phage ϕ 1.3 promoter. Plasmid pK173, which was constructed by C. K. Schmitt (29), is similar but contains T3 gene *l.2* and T3 ϕ 1.3.

T7 wild type, ST16 [Δ (1.1-1.2)], HS7 [(1.2)_{UGA}], MM10 (10-A2D 10-A228G), and R122 (ST16,MM10) and the T3 mutant Δ 2 [Δ (1.05-1.2)] are from the laboratory collection and have been described before (6, 18). The construction of Dam12 is described in the accompanying paper (11); other phages are described in the text.

Assays of phage genome entry. Genome entry was assayed by the methylation assay described in the accompanying paper (11). Briefly, cells were grown at 30°C to a density of 2×10^8 /ml and, except where stated, infected with phage at a multiplicity of 0.1. At various intervals, portions of the culture were added to an ice-cold ethanol-phenol mixture (2% [vol/vol] phenol, 75% [vol/vol] ethanol, 8 mM EDTA, 20 mM sodium acetate [pH 5.2]) to stop the infection, and DNA was

isolated and digested with *DpnI*. After electrophoresis in agarose gels, DNAs were transferred by capillary action to a nylon membrane and hybridized with T7 [³²P]DNA. DNA was labeled with [α -³²P]dCTP together with the Klenow fragment of DNA polymerase I and random-sequence oligonucleotides as primers. However, the intensities of bands in these Southern hybridizations are not proportional to the sizes of the respective DNA fragments. Under the conditions employed, which are standard and in common use, transfer of different DNA fragments from the gel to the membrane is not equally efficient, even for fragments of similar size and even after partial depurination of DNA by acid treatment prior to denaturation. In addition, labeled probes were shown not to represent all T7 DNA sequences equally. The lack of probe uniformity appears to be due to variations in hybridization and/or extension of short primers on a T7 DNA template; different batches of random hexamer and heptamer primers have been used, but all resulted in comparable deviations in band intensities from those expected theoretically.

Transfection of T7 DNA. Cells were made competent by treatment with ice-cold 50 mM CaCl₂. Approximately 2×10^8 cells in 0.1 ml of 50 mM CaCl₂ were added to 0.5 μ g of T7 DNA, and the mixture was incubated at 0°C for 30 min to allow DNA uptake. The cells were then heat shocked at 45°C for 3 min and plated with IJ511 cells as the indicator. In a control experiment, cells were treated with 500 μ g of rifampin per ml for 5 min prior to their being made competent. After incubation with T7 DNA in the presence of rifampin, cells were gently centrifuged, resuspended in buffer containing 1 μ g of DNase I per ml, and incubated at 30°C for 10 min in order to destroy extracellular DNA prior to heat shock and plating. The efficiency of infective center formation was reduced by treatment with rifampin, probably because of cell killing by the drug, but was unaffected by the presence of DNase I.

RESULTS

Entry of the T7 genome into F-containing and F⁻ cells.

Figure 1A shows the appearance of wild-type T7 *DpnI* DNA fragments during a productive infection of Dam-overproducing *E. coli*; under these conditions, the latent period is about 30 min. During the first 5 min of the infection, only a 40-kb band corresponding to the full-length genome is apparent. As the infection progresses, the intensity of this band decreases, but it does not completely disappear; at late times, this band probably represents the genomes of phages that irreversibly adsorbed but failed to eject their genome into the cell. After 7 min of infection, an 8.3-kb band (B) appears, signifying that at least 21% of the T7 genome has penetrated the cell. After 9 min, bands D and E appear, and after 12 min, the appearance of bands A and C demonstrates that at least 90% of the infecting genome has become accessible to Dam methylase.

An increase in the intensity of all *DpnI* bands and the appearance of the concatemer junction (band CB) at 15 min are indicative of T7 DNA replication, although most of the increase in DNA mass occurs between 21 and 27 min. Bands DE and CF are formed when Dam methylation is normal at other GATC sites but does not occur at positions 11516 and 36087, respectively, on the T7 genome. These two sites are thought to be less reactive to Dam in vivo (11). Cell debris becomes visible between 27 and 30 min, but because all methylation experiments performed in this study involve low-multiplicity infections, the time of cell lysis could not be determined precisely.

The overall pattern of methylation of T7 DNA during an abortive infection of an F-containing host differs substantially from that seen in a permissive infection. Bands B, D, and E appear normally during the first 9 min of infection, but a significant proportion of the infecting molecules are not methylated at the two rightmost GATC sites, resulting in the persistence of band AFC (Fig. 1B). This result suggests that a fraction of the phage genomes do not enter the cell before the infection begins to abort. However, since the A and C bands can be detected by 12 min, at least 90% of some genomes does enter the male cell. There is very little replication of wild-type T7 DNA following infection of the male cell; there is no significant increase in the intensity of individual *DpnI* bands, and the concatemer junction fragment is not detectable until about 24 min after infection, a time when permissively infected cells

FIG. 1. Autoradiogram of a Southern hybridization, depicting *DpnI*-digested DNA from (A) IJ511(pTP166) and (B) IJ511(pTP166)/F'lac(Kn) infected by wild-type T7. The time after infection that each sample was analyzed is shown above each lane (in minutes). The left-hand gel in panel A was exposed eight times longer than the right-hand gel. See text for details.

are close to lysing. Thus, even those genomes that completely enter the male cell are able to be replicated only at very low efficiency. It was conceivable that the events leading to initiation of DNA replication in an F-containing cell might be responsible for the abortive infection. However, preventing DNA replication by infecting either F⁻ or F⁺ cells with a gene 5 (DNA polymerase) deletion mutant did not change the pattern of methylation of the parental DNA from that of wild-type T7 (data not shown).

The conclusions that can be drawn from this experiment are consistent with those of earlier studies on exclusion of T7 and T7-like phages by F (4, 5, 13, 14, 18, 20, 37, 39). The early class I proteins are made normally; their genes all lie on the *DpnI* B fragment, and the GATC at the end of this fragment is methylated normally. It is generally agreed that the major defect in gene expression affects the late class III region of the phage, and methylation of the two GATC sites in this region is defective. Incorporation of thymidine into T7 DNA is greatly inhibited, although the infecting genomes are not degraded, and there is little change in the amount of methylated T7 DNA available for hybridization to a T7 DNA probe throughout 30 min of the abortive infection.

FIG. 2. Autoradiogram of a Southern hybridization, depicting *DpnI*-digested DNA from IJ511(pTP166) and its F'lac(Kn) derivative infected at a multiplicity of 0.5 by the T7 mutant Dam12. The time after infection that each sample was analyzed is shown above each lane (in minutes). The presence or absence of F'lac(Kn) is indicated by + and -, respectively. The 12-min lanes were scanned with a densitometer to provide estimates of the amounts of DNA in each *DpnI* fragment. For each band, the amount of DNA detected in the infection of the F' cell was divided by that in the F⁻ cell. The ratio obtained for the first *DpnI* fragment to enter the cell (J) was factored to unity to correct for unequal efficiencies of penetration of phage DNA into the two cell types; other ratios were then normalized with the same correction factor. Thus, 38% of the Dam12 genomes infecting F' cells were methylated by Dam to yield the rightmost *DpnI* fragment D. The approximate positions of genes 1.2, 6, and 10 on the *DpnI* map of the Dam12 genome are indicated.

In order to measure the average extent of entry of a T7 genome into F-containing cells with more precision, methylation of Dam12 DNA was measured. Dam12 is a T7 mutant that contains six additional GATC sites in its genome (11); in a permissive infection, all GATC sites on the infecting Dam12 DNA molecule are methylated between 7 and 12 min (Fig. 2), prior to the onset of DNA replication. The nonuniformity of radioactive label in the randomly labeled T7 DNA used as a probe (see Materials and Methods) precluded a simple quantitation of the amount of DNA in each *DpnI* band during infection, and the extent of genome entry into a male cell was therefore estimated relative to the extent of genome entry into an F⁻ cell.

Entry of Dam12 DNA into the F-containing cell appears to be normal for the first 44% of the genome; the DNA fragments corresponding to *DpnI* bands J through H/I appear at equimolar concentrations. Thus, DNA between the genetic left end of Dam12 and gene 6 enters the cell before the infection begins to abort. However, *DpnI* fragments that lie to the right of the I fragment appear in steadily decreasing molar amounts. At 12 min after infection of the F' cell, a time when the genome is completely internalized in a permissive infection, the rightmost D fragment is represented by only about 40% of the genomes that initiated cellular penetration. This result indicates that in 60% of the infected F-containing cells, the infection begins to abort while the phage genome is being translocated into the cell.

Both gene 1.2 and gene 10 prevent total methylation of the phage genome during infection of F-containing cells. Expression of either of gene 1.2 or 10 is sufficient to trigger F exclu-

FIG. 3. Autoradiogram of a Southern hybridization, depicting *DpnI*-digested DNA from IJ511(pTP166)/F' *lac*(Kn) infected by (A) MM10 (*10-A2D 10A228G*) and (B) ST16 [$\Delta(1.1-1.2)$]. The time after infection that each sample was analyzed is shown above each lane (in minutes).

sion of wild-type T7 (18). To determine whether only one of these genes is responsible for incomplete methylation of T7 DNA in male cells, the time course of methylation was measured for the *1.2* deletion mutant ST16 and the gene *10* mutant MM10 (the double mutant ST16,MM10 is known as R122 and grows in F-containing cells). In both permissive (not shown) and nonpermissive infections (Fig. 3), the kinetics of methylation of ST16 and MM10 DNAs are indistinguishable from those following infection by wild-type T7 DNA. Incomplete Dam methylation of the genomes of both mutants implies that expression of wild-type *1.2* or *10* initiates the abortive infection that can prevent the phage genome from completely entering the cell.

A characteristic of F-mediated abortive infections is the leakage of intracellular nucleotides into the culture medium. It is therefore possible that *S*-adenosylmethionine, the cofactor for Dam methylase, also leaks from the cell, thereby prevent-

FIG. 4. Autoradiogram of a Southern hybridization, depicting *DpnI*-digested DNA from IJ511(pTP166)/F' *lac*(Kn) infected by R122 [$\Delta(1.1-1.2)10-A2D 10-A228G$]. The time after infection that each sample was analyzed is shown above each lane (in minutes).

ing methylation of entering T7 DNA. This idea was tested by assaying methylation of a T7 mutant that grows on F⁺ cells and whose genome must therefore completely enter the cell; such mutants still allow leakage of intracellular nucleotides during infection (18). Methylation of the first GATC site of the T7 mutant R122 occurs 7 to 9 min after infection of an F-containing cell, and methylation of the remainder of the parental R122 genome is complete by 12 min (Fig. 4), essentially the same time as in a permissive infection by T7⁺. The concatemer junction (band CB) of R122 DNA and an increase in the intensity of all *DpnI* bands of the phage genome are noticeable only after 18 min, indicating that replication is delayed in the F-containing cell. However, the increase in the amounts of DNA that are susceptible to cutting by *DpnI* demonstrates that Dam can methylate (and therefore the intracellular levels of *S*-adenosylmethionine in the cell are adequate) R122 DNA at times after infection when nucleotide leakage from infected cells is readily detected (18, 30). Thus, the membrane damage that is associated with a T7 infection of an F-containing strain does not prevent Dam methylation of DNA.

Transfection of T7 DNA into F-containing cells does not bypass F exclusion. The mode of DNA uptake by *E. coli* made competent by using calcium chloride is not understood; however, it appears to be fundamentally different from T7 DNA entry into the cell during a fundamental infection. At 0°C, T7 DNA is taken up by competent cells in the presence of rifampin; cellular internalization of T7 DNA by transfection therefore appears to be independent of transcription of the phage genome.

In order to distinguish the effect that F exclusion exerts on translocation of a complete T7 genome out of the capsid into the cell from subsequent events, we transfected competent cells with purified phage DNA and measured the efficiencies of infective center formation. Relative to transfections with R122 DNA, which grows in cells containing F, transfections with DNA from wild-type T7 or the mutants ST16 and MM10 were less efficient. The relative transfection efficiencies were 1 for R122(ST16,MM10), 0.32 for T7⁺, 0.29 for ST16 [$\Delta(1.1-1.2)$], and 0.32 for MM10 (*10-A2D 10-A228G*), calculated as the ratio

TABLE 1. Complementation by plasmids expressing mutant T7 gene *1.2* or T3 gene *1.2*

Phage	Relative efficiency of plating on IJ511/F' <i>lac</i> containing T7 or T3 gene <i>1.2</i> plasmid ^a :				
	pUC18	pCS5 (T7 <i>1.2W31C</i>)	pSG1 (T7 <i>1.2Y28N</i>)	pSG4 (T7 <i>1.2R37G</i>)	pK173 (T3 <i>1.2</i> ⁺)
T7 ⁺	<10 ⁻⁷	1.1	0.54	0.87	0.26
ST16 [$\Delta(1.1-1.2)$]	<10 ⁻⁷	1.04	0.43	1.02	0.48
T3 $\Delta 2$ [$\Delta(1.05-1.2)$]	<10 ⁻⁷	0.80	0.94	1.02	0.87

^a Relative to that on the female strain IJ511(pUC18).

of infective centers on IJ511/F'*lac* to those on its *pif* derivative, normalized to the value for R122 DNA. Since transfection of T7 DNA did not alleviate F exclusion of T7 in a single cycle of phage growth, inhibition of the normal process of entry of a T7 genome into the cell is more likely a consequence of the abortive infection rather than the initial target of F exclusion.

It should be noted that F⁻ inhibition of infective center formation by T7 is far less complete than inhibition of T7 plaque formation in a standard plating assay. The threefold reduction in infective center formation in the transfection assay is comparable to that found in one-step growth experiments with the same phages and host cells (data not shown). The efficiency of T7 plaque formation on IJ511/F'*lac* relative to that on the isogenic F⁻ strain IJ511 is <10⁻⁸, but this value is the result of many rounds of phage growth on the plate.

Nontoxic mutant T7 gp1.2 can prevent F-mediated abortive infection. The gene *1.2* mutations W31C, Y28N, and R37G were found in phages that overcome F exclusion; the mutations abolish the toxicity associated with wild-type gp1.2 in cells expressing *pifA* (18, 31). Phages that carry only a *1.2* missense mutation are still fully excluded by F; however, the presence of these nontoxic *1.2* alleles on a high-copy-number plasmid permits wild-type T7 and *1.2* mutants to grow in male cells (Table 1). Thus, the mutant gp1.2 produced from these plasmids possesses an activity that prevents not only the phage-encoded wild-type gp1.2 but also gp10 from interacting with PifA. In regard to the latter, overexpression of mutant T7 gene *1.2* has the same effect on allowing T7 growth in male cells as normal levels of expression of T3 gene *1.2* have on allowing wild-type T3 to grow in the same F-containing cells.

Overexpression of mutant T7 gene *1.2* also allows complementation of a T3 *1.2* deletion mutant for growth in male cells, and overexpression of T3 gene *1.2* similarly complements T7 (Table 1). T7 gene *1.2* on the above pUC derivatives is expressed from both the *lac* promoter of the plasmid and the phage ϕ 1.3 promoter. Thus, gp1.2 should be present in the cell before phage infection and also be synthesized at high levels after T7 infection. T3 gene *1.2* is also expressed from the vector *lac* promoter in pK173 and can also be expressed from T3 ϕ 1.3. However, T3 promoters are not efficiently recognized by T7 RNA polymerase, and T3 gp1.2 is not made at comparably high levels after T7 infection as the mutant T7 gp1.2. Consequently, complementation of T7 growth by high levels of T3 gp1.2 is not as efficient as complementation by high levels of nontoxic T7 gp1.2. Nevertheless, these observations suggest that the properties of T3 gp1.2 and the mutant T7 gp1.2 proteins in preventing gp10 from triggering the abortive infection are similar, except that the T7 proteins are less efficient and are needed at higher concentrations.

Expression of T3 *1.2* from the T7 genome allows T7 to grow on F-containing cells. In order to determine whether a single copy of T3 gene *1.2* can allow T7 to grow in the presence of F, the gene was cloned into the unique *Bcl*I site (within the nonessential gene *1.7*) of wild-type and *1.2* mutant T7 DNAs

and also into the unique *Bam*HI site associated with the deletion in ST16 DNA. In phage SW2, T3 gene *1.2* can be transcribed by *E. coli* RNA polymerase as part of the polycistronic early RNA and from both T7 ϕ 1.1 promoters; in RG1, RG2, and RG3, T3 gene *1.2* expression is from the ϕ 1.1, ϕ 1.3, ϕ 1.5, and ϕ 1.6 promoters.

The plating efficiencies of RG1 and RG3 on *optA1* mutant cells are essentially the same as those of wild-type T7 and RG2 (Table 2), indicating that sufficient expression of T3 gene *1.2* occurs from its position in T7 gene *1.7* to inhibit the host dGTPase. In contrast, the lower plating efficiency of SW2 on *optA1* mutant cells suggests that expression of T3 gene *1.2* from the same promoters that normally transcribe T7 genes *1.1* and *1.2* is not adequate to overcome the effects of dGTPase overexpression. Cloning a DNA fragment containing genes *1.1* and *1.2* at the same position as the gene *1.2* insert of SW2 produces a phage that plates normally on *optA1* mutants (data not shown), suggesting either that T3 gene *1.1* affects growth on *optA1* mutant cells or that wild-type levels of T3 gene *1.2* expression require coexpression of gene *1.1*. However, although translation of T7 gene *1.2* has been shown to be coupled to translation of gene *1.1* (28), comparable regulatory elements are not obvious in the T3 nucleotide sequence (32).

All four hybrid phages plate orders of magnitude better than wild-type T7 on F-containing cells (Table 2). SW2 is least able to avoid F exclusion, a result that again reflects poor expression of T3 gene *1.2*, since addition of the *optA1* mutation almost completely prevents plaque formation. However, RG1, RG2, and RG3 all plate moderately well in the presence of F even when the dGTPase is overexpressed. On cells containing F, the plating efficiency of RG1 and RG2 is two- to threefold lower than that of RG3. The most likely reason for this difference is the lack of T7 gene *1.1* (a gene of unknown function) in RG3, suggesting that T7 gp1.1 may interfere with the activity of T3 gp1.2 in preventing F exclusion. Nevertheless, it is clear that the presence of a single copy of T3 gene *1.2* in a T7 genome is sufficient to allow the phage to escape from F exclusion.

TABLE 2. T3 *1.2* expressed from the T7 genome can protect from F exclusion

Phage	Relative plating efficiency ^a (avg burst size)		
	IJ511/F' <i>lac</i>	IJ511 <i>optA1</i>	IJ511 <i>optA1</i> /F' <i>lac</i>
T7 ⁺	<10 ⁻⁷	1.00	<10 ⁻⁷
ST16 [$\Delta(1.1-1.2)$]	<10 ⁻⁷	<10 ⁻⁵	<10 ⁻⁷
HS7 (<i>1.2</i> _{UGA})	<10 ⁻⁷	<10 ⁻⁵	<10 ⁻⁷
RG1 (HS7 <i>1.7::1.2</i> _{T3})	0.26 (9)	0.72	0.19
RG2 (T7 <i>1.7::1.2</i> _{T3})	0.37 (11)	0.88	0.20
RG3 (ST16 <i>1.7::1.2</i> _{T3})	0.65 (23)	0.72	0.42
SW2 (ST16 <i>Bam</i> HI:: <i>1.2</i> _{T3})	0.17 (10)	0.04	2.8 × 10 ⁻⁴

^a Relative to that on IJ511. Numbers in parentheses are average burst sizes (phage particles per cell) in one-step growth experiments.

DISCUSSION

T7 DNA is not fully methylated during an infection of a *dam*-overexpressing male *E. coli*, implying that the infection aborts before all infecting genomes completely penetrate the F-containing cell. Since methylation is only an indirect assay of genome entry, it is possible that Dam methylation is inhibited, and inhibition of methylation is simply one of the many physiological dysfunctions that are triggered by the abortive infection. However, entry of the T7 genome into the cell is not a passive process and is largely dependent on transcription. Since all macromolecular processes in the cell, including transcription, are inhibited during phage exclusion, it is likely that entry of the entire length of every infecting genome is also inhibited. This result is consistent with the failure to detect full-length transcripts originating from the ϕ 13 late promoter after infection of an F-containing cell, and it was also concluded that there was no specific site where the entry process was blocked (3). In this study, 60% of infected cells were shown to exhibit incomplete methylation of the infecting phage DNA, but there is no specific point on the genome beyond which methylation does not occur. As the class III, or late, region of the genome is being brought into the cell, the transcriptional capacity of all cells declines steadily, reducing the rate or efficiency of genome entry. If this decline occurs at different rates in each individual cell, the end result is complete genome entry in some cells but only partial entry in others.

It is unlikely that transcriptionally coupled entry of T7 DNA is the immediate target of the *pif* exclusion system, since some genomes do become fully methylated and transfection of T7 DNA does not prevent phage exclusion. More likely is that the overall capacity of the cell to transcribe T7 DNA is compromised by the interaction of *pifA* and gene *1.2* or gene *10* and that this can prevent complete genome entry. However, whether this interaction affects the transcription complex directly or whether inhibition of the transcription complex is a result of *pifA* and *1.2* (or *10*) interacting with another component of the cell is not known.

The genomes of not only wild-type T7 but also those of the gene *1.2* mutant ST16 and the gene *10* mutant MM10 do not become fully methylated in infected male cells. Expression of either wild-type gene *1.2* or gene *10* therefore causes *pif* exclusion to inhibit transcription before genome entry is complete. The slow polar mode of T7 DNA entry allows gp1.2 (an early, class I protein) to be synthesized before gp10 (a late, class III protein), and it might therefore be thought that an infection by a *1.2*⁺ phage should abort earlier than an infection by a *1.2* mutant phage. However, substantial inhibition of methylation of the infecting genome does not occur until the class III genes are being transcribed as they enter the cell. Previous work has indicated that in the presence of PifA, gp1.2 is less toxic to the cell than gp10 (31). The results in this report are in agreement with this observation and thus suggest that the toxicity of gp1.2 is not manifest immediately after its synthesis in an infected male cell. Conversely, the toxicity of gp10, as judged by the inhibition of infecting genome methylation, appears to coincide with its synthesis. The rapid effects of gene *10* expression may simply reflect the fact that gp10 is the most abundant phage protein found in infected cells and therefore must be synthesized at extremely high rates.

How T7 genes *1.2* and *10* interact with *pifA* is not yet known. Overexpression of mutant gene *1.2* allows T7⁺ to grow in cells containing F, suggesting that the nontoxic proteins not only compete with wild-type gene *1.2* for a target but also prevent gene *10* from interacting with the same target. In this regard, T3 gene *1.2* may simply be more efficient at preventing this

interaction, because cloning a single copy of the gene onto a T7 genome is sufficient to prevent F from inhibiting phage growth. Whether the immediate target of genes *1.2* and *10* is *pifA* and a *1.2(10)-pifA* complex interferes with the function of a cellular gene(s) or whether *1.2(10)* and *pifA* independently interact with and together inhibit this gene(s) is unclear. The recent isolation of *E. coli* mutants (36) that survive the combination of each phage gene and *pifA* should help differentiate these possibilities and should also provide new insights into the mechanism of T7 exclusion by F.

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REFERENCES

- Amitsur, M., I. Morad, D. Chapman-Shimshoni, and G. Kaufmann. 1992. HSD restriction-modification proteins partake in latent anticodon nuclease. *EMBO J.* **11**:3129-3134.
- Beck, P. J., S. Gonzalez, C. L. Ward, and I. J. Molineux. 1989. Sequence of bacteriophage T3 DNA from gene 2.5 through gene 9. *J. Mol. Biol.* **210**: 687-701.
- 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.
- Britton, J. R., and R. Haselkorn. 1975. Macromolecular synthesis in T7 infected F⁺ cells. *Virology* **67**:264-275.
- Condit, R. C., and J. A. Steitz. 1975. F factor-mediated inhibition of bacteriophage T7 growth: analysis of T7 RNA and protein synthesis *in vivo* and *in vitro* using male and female *Escherichia coli*. *J. Mol. Biol.* **98**:31-43.
- 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 cells. *J. Mol. Biol.* **207**:543-554.
- 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.
- Cram, D., A. Ray, and R. Skurray. 1984. Molecular analysis of F plasmid *pif* region specifying abortive infection of T7. *Mol. Gen. Genet.* **197**:137-142.
- Cram, H. K., D. Cram, and R. Skurray. 1984. F plasmid *pif* region: Th1725 mutagenesis and polypeptide analysis. *Gene* **32**:251-254.
- Duckworth, D. H., J. Glenn, and D. J. McCorquodale. 1981. Inhibition of bacteriophage replication by extrachromosomal genetic elements. *Microbiol. Rev.* **45**:52-71.
- García, L. R., and I. J. Molineux. 1995. Rate of translocation of bacteriophage T7 DNA across the membranes of *Escherichia coli*. *J. Bacteriol.* **177**:4066-4076.
- Huber, H. E., B. B. Beauchamp, and C. C. Richardson. 1988. *Escherichia coli* dGTP triphosphohydrolase is inhibited by gene 1.2 protein of bacteriophage T7. *J. Biol. Chem.* **263**:13549-13556.
- Linial, M., and M. H. Malamy. 1970. Studies with bacteriophage ϕ II: events following infection of male and female derivatives of *Escherichia coli* K-12. *J. Virol.* **5**:72-78.
- Linial, M., and M. H. Malamy. 1970. The effect of F factors on RNA synthesis and degradation after infection of *E. coli* with phage ϕ II. *Cold Spring Harbor Symp. Quant. Biol.* **35**:263-268.
- 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.
- 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.
- Molineux, I. J. 1991. Host-parasite interactions: recent developments in the genetics of abortive phage infections. *New Biol.* **3**:230-236.
- Molineux, I. J., C. K. Schmitt, and J. P. Condreay. 1989. Mutants of bacteriophage T7 that escape F restriction. *J. Mol. Biol.* **207**:563-574.
- Molineux, I. J., and J. L. Spence. 1984. Virus-plasmid interactions: mutants of bacteriophage T3 that abortively infect plasmid F-containing (F⁺) strains of *Escherichia coli*. *Proc. Natl. Acad. Sci. USA* **81**:1465-1469.
- Morrison, T. G., and M. H. Malamy. 1971. T7 translational control mechanisms and their inhibition by F factors. *Nature (London) New Biol.* **231**:37-41.
- Murgola, E. J., and G. Guarneros. 1991. Ribosomal RNA and peptidyl-tRNA hydrolase: a peptide chain termination model for lambda bar RNA inhibition. *Biochimie* **73**:1573-1578.
- Myers, J. A., B. B. Beauchamp, and C. C. Richardson. 1987. Gene 1.2

- protein of bacteriophage T7. *J. Biol. Chem.* **262**:5288–5292.
23. Myers, J. A., B. B. Beauchamp, J. H. White, and C. C. Richardson. 1987. Purification and characterization of the gene *1.2* protein of bacteriophage T7. *J. Biol. Chem.* **262**:5280–5287.
 24. Pao, C. C., and J. F. Speyer. 1973. Order of injection of T7 bacteriophage DNA. *J. Virol.* **11**:1024–1026.
 25. Parma, D. H., M. Snyder, S. Sobolevski, M. Nawroz, E. Brody, and L. Gold. 1992. The rex system of bacteriophage λ : tolerance and altruistic cell death. *Genes Dev.* **6**:497–510.
 26. Rotman, G. S., R. Cooney, and M. H. Malamy. 1983. Cloning of the *pif* region of the sex factor and identification of *pif* protein product. *J. Bacteriol.* **155**:254–264.
 27. Saito, H., and C. C. Richardson. 1981. Genetic analysis of gene *1.2* of bacteriophage T7: isolation of a mutant of *Escherichia coli* unable to support the growth of T7 gene *1.2* mutants. *J. Virol.* **37**:343–351.
 28. Saito, H., and C. C. Richardson. 1981. Processing of mRNA by ribonuclease III regulates expression of gene *1.2* of bacteriophage T7. *Cell* **27**:533–542.
 29. Schmitt, C. K. 1989. Ph.D. dissertation, University of Texas at Austin, Austin, Tex.
 30. 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.
 31. Schmitt, C. K., and I. J. Molineux. 1991. Expression of gene *1.2* and gene *10* of bacteriophage T7 is lethal to F plasmid-containing *Escherichia coli*. *J. Bacteriol.* **173**:1536–1543.
 32. Schmitt, M. P., P. J. Beck, C. A. Kearney, J. L. Spence, D. DiGiovanni, J. P. Condreay, and I. J. Molineux. 1987. Sequence of a conditionally essential region of bacteriophage T3, including the primary origin of DNA replication. *J. Mol. Biol.* **193**:479–495.
 33. Snyder, L., and G. Kaufmann. 1994. T4 phage exclusion mechanisms, p. 391–396. In J. D. Karam (ed.), *Molecular biology of bacteriophage T4*. American Society for Microbiology, Washington, D.C.
 34. Studier, F. W. 1972. Bacteriophage T7. *Science* **176**:367–376.
 35. Studier, F. W. 1979. Relationships among different strains of T7 and among T7 related bacteriophages. *Virology* **95**:70–84.
 36. Wang, W.-F., and I. J. Molineux. Unpublished observation.
 37. Whitaker, P. A., Y. Yamada, and D. Nakada. 1975. F-factor-mediated restriction of bacteriophage T7: synthesis of RNA and protein in T7-infected *Escherichia coli* F⁻ and F⁺ cells. *J. Virol.* **16**:1380–1390.
 38. Wurgler, S. M., and C. C. Richardson. 1990. Structure and regulation of the dGTP triphosphohydrolase from *Escherichia coli*. *Proc. Natl. Acad. Sci. USA* **87**:2740–2744.
 39. Young, E. T., and R. C. Menard. 1975. Analysis of the template activity of bacteriophage T7 messenger RNAs during infection of male and female strains of *Escherichia coli*. *J. Mol. Biol.* **99**:167–184.
 40. Yu, Y. N., and L. Snyder. 1994. Translation elongation factor Tu cleaved by a phage-exclusion system. *Proc. Natl. Acad. Sci. USA* **91**:802–806.
 41. 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.