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Genetics

Virus–plasmid interactions: Mutants of bacteriophage T3 that abortively infect plasmid F-containing (F^+) strains of *Escherichia coli*

(deletions/gene *1.1*/gene *1.2*/*optA*/bacteriophage T7)

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ABSTRACT **Bacteriophage T7 and many closely related phages abortively infect plasmid F-containing (F^+) strains of *Escherichia coli*. However phage T3, which is also closely related to T7, grows normally in F^+ hosts. Mutants of phage T3 that, like T7, are subject to F-mediated restriction have been isolated. These T3 mutants lack or are defective in one or both of two genes that are nonessential for phage growth in F^-, wild-type strains. Our results show that the products of phage T3 gene *1.1* or *1.2*, or both, are essential for growth and suggest that the comparable phage T7 genes are naturally defective in their ability to counteract the inhibitory effects of F-encoded proteins.**

Bacteriophage T7 provides a classical example of a virus–host interaction that is profoundly influenced by a plasmid resident in the host cell. There are many reports describing the inhibition of virulent phage growth in cells that harbor plasmids or prophages (for a review, see ref. 1). Some of the mechanisms underlying this inhibition of growth of the infecting phage have been well established and include resistance to adsorption, superinfection exclusion, and nucleolytic degradation by restriction enzymes. Less well-understood is the mechanism, or mechanisms, of inhibition of what has been described as an abortive infection. Abortive infections are those in which the early stages of the infectious process appear normal but subsequent events are grossly aberrant. Amongst the best known of these are the inhibition of growth of T-even, rII mutant phage in cells lysogenic for phage λ, the colicinogenic plasmid-induced inhibition of phages T5 and BF23, and the F-mediated inhibition of T7 and related phages. The last of these is the subject of this report.

Bacteriophages T3 and T7 are closely related members of the classic T-series of bacteriophages. They possess a comparable mode of gene expression and their genetic maps have been aligned (2, 3). The DNAs of the two phages extensively cross-hybridize, although there are distinct and separate regions of nonhomology (4). Complementation studies indicate that the corresponding gene products of phages T3 and T7 have the same primary biological role (5).

One of the significant differences between phages T7 and T3 is that, whereas T7 infections of a "male" strain of *Escherichia coli* (i.e., a strain that contains the plasmid F) are unproductive, T3 infections of these strains result in normal phage growth (6). When T7 infects male strains, macromolecular synthesis is prematurely terminated and no progeny phage are made, although the host bacteria are killed during the abortive infection (1). This phenomenon has been extensively studied, but no consensus explanation as to its cause has been achieved (1, 7).

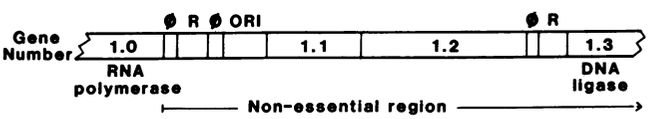

FIG. 1. Schematic diagram of part of the early region of phage T7 (adapted from refs. 15 and 16); that of phage T3 is thought to be similar (14). φ, promoter recognized by the phage RNA polymerase; R, RNase III processing site; ORI, origin of DNA replication.

At least one (8) and possibly more (9, 10) genes (*pif*) on the F plasmid are responsible for the inhibition of phage T7 growth. The *pifA* gene product has recently been identifed (10), but the biochemical properties of the protein are not yet known. Mutant F plasmids that are defective in the *pif* gene products do not inhibit T7 growth. The inhibitory effects of the wild-type F gene product(s) can be alleviated also by allele-specific *rpsL* (streptomycin-resistant) mutations (11). In this case, the inhibitory effects can be reimposed by additional *rpoB* (rifampicin-resistant) mutations (11).

In contrast to the above chromosomal or F plasmid mutations that allow phage T7 to productively infect male strains of *E. coli*, no simple mutants of T7 have been isolated that can overcome the inhibitory effects of F gene products (12). Therefore, we took a different approach to obtaining variants of T7 that could productively infect male strains and recently have reported the characterization of T7–T3 recombinants that are able to grow in F plasmid-containing (F^+) strains (12). Two distinct regions, presumably derived from T3, were essential for productive growth of the hybrid phage on male strains of *E. coli*.

One of these regions encompasses the right part of gene *1* through the left part of gene *1.3*; the second is located between genes *3* and *4* (12). The hybrid phage can rescue phage T7 from the abortive infection; thus, *trans*-acting products are important (13). The gene products in the first of these T3-derived regions of the hybrid phage are class 1, or early, proteins and, because this region of T3 has been shown to be organized in the same way as T7 (14), should include only the phage RNA polymerase, DNA ligase, and the products of genes *1.1* and *1.2* (Fig. 1). It is this region on which we focus here.

RNA polymerase (product of gene *1*; gp1) is an essential phage protein (2). It transcribes approximately 85% of the phage DNA and, thus, is necessary for all late gene expression. Genes *1.1*, *1.2*, and *1.3*, though normally nonessential for phage growth, are part of this 85% region and, thus, are expressed both early and late. DNA ligase (product of gene *1.3*; gp1.3) is only required when the host cell is also ligase-deficient (14). Phage T7 gene *1.2* is required for productive growth only on *optA* mutant hosts (17). When mutants of T7

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Abbreviations: bp, base pair; gp1, gp1.1, gp1.2, products of genes *1*, *1.1*, *1.2*.

Table 1. Bacterial strains

Designation	Genetic characteristics	Ref.
011′	Thy$^-$, Gal$^-$, Str^R, *supE44*	18
011′L1	as 011′, but *lig*$^-$	19
B	prototroph	18
BL2	as B, but *lig*$^-$	19
B837	Met$^-$, Gal$^-$, $r_B^-m_B^+$	20
B837*supE44*	as B837, but su_{II}^+	See text
HR23	*thr*-1, *leu*-6, *his*-1, *arg*H1, *lys*-25, *lac*Y1, *mal*A1, *xyl*-7, *ara*-13, *mtl*-2, *gal*-6, *pur*E43, *ton*A2, *thi*-1, *str*-9 λ^R	17
HR40	as HR23, but *optA1*	17
993F$^-$	Met$^-$, Gal$^-$, $r_K^-m_K^-$	12

F$^+$ or F42 (F′*lac*) derivatives of the strains listed are indicated in the text by showing F$^+$ or F′*lac* after the strain number.

that do not express the product of gene *1.2* (gp1.2) infect *optA* mutant hosts, phage-directed DNA and RNA synthesis cease prematurely, newly synthesized DNA is rapidly degraded, but protein synthesis appears to be unaffected (17). The properties of gene *1.1* are not known because no requirement for gp1.1 in T3 or T7 infections has yet been demonstrated.

In this report we describe the properties of some mutants of phage T3 that abortively infect male strains of *E. coli*. The properties of these mutant phage indicate that gene *1.1* or *1.2*, or both, play a crucial role in the ability of wild-type phage T3 (T3$^+$) to grow on male strains of *E. coli*. These studies further suggest that T3$^+$, together with these T3 mutants, can serve as a model system for an understanding of why phage T7 is subject to F-mediated inhibition of growth.

MATERIALS AND METHODS

***E. coli* and Phage Strains.** The bacterial strains used in these studies are listed in Table 1. The *supE44* allele was introduced into strain B837 by P1 phage transduction using *zbf-507*::Tn*10* (originally present in strain JW1071 from J. Wechsler) as a selectable marker. *E. coli* strain 993F$^+$ (12) was the original source of the F$^+$ plasmid that was introduced into several other strains. F42(F′*lac*) was from our collection. T3$^+$ and the T3 ligase-deletion mutants LG102, LG114, and R14 (14) were provided by F. W. Studier. T3 amber mutations N108 and H233 (3) were from R. Hausmann. Deletion mutants Δ1–Δ4 were isolated after enrichment by heating a lysate of T3$^+$ at 58°C for 2 hr as described (14). Surviving phage were screened for their ability to grow on 993F$^-$ and 993F$^+$. Of 600 plaques tested, 4 contained phages that failed to grow on 993F$^+$. The mutants M21a and O103 were isolated as phages that failed to grow on 993F$^+$ and HR40 (*optA1*), respectively. Both phages could be reverted to give a wild-type phage phenotype, but they failed to complement each other; nor could either of them complement the Δ2 deletion mutant for growth on male or on *optA* mutant strains. A more detailed description of these point mutants will be given elsewhere.

Phage Growth. All phage manipulations were performed at 30°C and were performed as described for phage T7 by Studier (18) with *E. coli* strain 011′ as the standard permissive host for all strains.

Restriction Endonuclease Digestions. *Hpa* I (New England BioLabs) was used as directed by the manufacturer to digest purified phage DNAs. Fragments were separated by electrophoresis on 1% agarose or 5% acrylamide gels and visualized under UV light after staining with ethidium bromide.

Kinetics of DNA, RNA, and Protein Synthesis. *E. coli* strains HR23, HR23F′*lac*, and HR40 (*optA1*) were grown at 30°C in M9 medium, supplemented with 0.4% glucose and all essential nutrients, to a cell density of 2×10^8 per ml. Cultures were then infected at a multiplicity of 10, and at various times aliquots were removed and added to tubes containing 0.25–1 μCi (1 Ci = 37 GBq) of [*methyl*-^{3}H]thymidine, [5-^{3}H]uracil, or a mixture of ^{14}C-labeled amino acids. After 20 sec at 30°C, synthesis was terminated by the addition of 0.3 M trichloroacetic acid, and acid-insoluble radioactivity was measured in a liquid scintillation counter.

RESULTS

Mutants of Phage T3 That Are Restricted by F-Containing Strains of *E. coli*. Productive growth on F-containing strains of *E. coli* of a T7–T3 recombinant phage only occurred when the region spanning the right part of gene *1*, genes *1.1* and *1.2*, and the left part of gene *1.3* was of T3 origin (12). Genes *1.1–1.3* are part of a normally nonessential region of phage T3 (14); therefore, it seemed possible that deletion mutants of T3 that lack this region would fail to grow on F-containing hosts. Mutants with this phenotype were in fact isolated and were shown to be deletion mutants by restriction enzyme analysis.

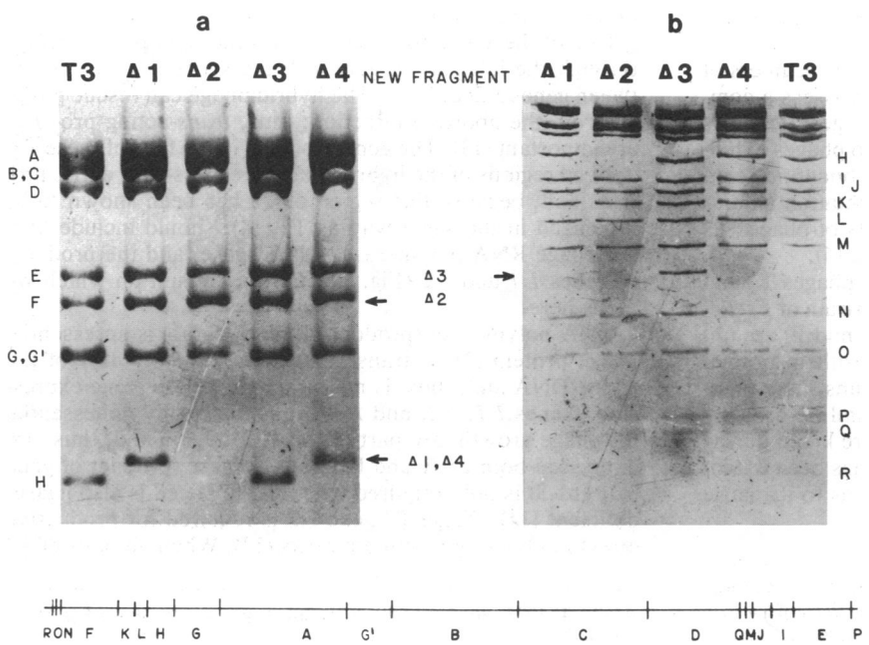

FIG. 2. *Hpa* I digestion of DNA isolated from phage T3 deletion mutants. T3 DNA was isolated from mutant phages, digested with *Hpa* I, and analyzed by electrophoresis in 1% agarose gels (*a*) or 5% acrylamide gels (*b*). The *Hpa* I map of T3 is that derived by Bailey *et al.* (21), modified to include the extra fragment seen in the Luria strain of T3 (6, 12).

Hpa I digestion and electrophoresis on agarose and acrylamide gels of DNA isolated from the four deletion mutants (Δ1–Δ4) are shown in Fig. 2. DNA from the Luria strain of $T3^+$ leads to a slightly different *Hpa* I restriction enzyme pattern than that of the Hausmann strain of $T3^+$ (6, 12). In particular, the band marked G,G′ in Fig. 2 consists of two DNA fragments whose positions in the T3 genome have been located (12, 21). All four deletions reduced the relative intensity of this doublet band and were shown to affect the G but not the G′ fragment by additional analyses (not shown). The fusion fragments are indicated in Fig. 2; those of the Δ1 or Δ4 deletions appeared to be the same, whereas that of the Δ2 deletion was detected by an increase in relative intensity of the band containing the F fragment. The deletion Δ3 only affected the mobility of fragment G; the others must extend closer to the coding sequences of gene *1* because they also affected the H fragment (21).

A summary of the extent of the four deletions isolated as part of this work and the results from *Hpa* I digestion (electrophoretic data not shown) of the ligase deletion mutant LG102 are presented in Table 2. Also included in this table are the data obtained by Bailey *et al.* (21), who studied other T3 ligase deletion mutants. It should be noted that, whereas the mutants Δ1–Δ4 were isolated from the Luria strain of T3, the other mutants were isolated from the Hausmann strain of T3, and it is quite likely that mutants Δ1 and Δ3 contain deletions that are closely equivalent, perhaps identical, to those present in mutants LG123 and R14, respectively.

Despite the fact that different phenotypes were used to isolate the two collections of deletion mutants—i.e., those isolated here and those of Studier and Movva (14)—it is clear that they are similar. The complete phenotypes of several of these mutants overlap, and both have deleted part of the *Hpa* I H or G fragments, or both, of $T3^+$. These fragments contain sequences of the right part of gene *1* and extend rightwards through the genes *1.1*, *1.2*, and *1.3* and beyond the early transcription terminator (14, 21).

However, gene *1* is essential for phage growth and cannot be severely affected by these deletions. In order to determine which phage genes are required for productive infections of male strains, the T3 deletion mutants were tested for their growth characteristics on wild-type but F-containing hosts and also on mutant F^- hosts that restrict phage defective in either gene *1.2* or *1.3*.

Table 3 shows that there are three classes of deletion mutant phage: the first is defined by mutant Δ2, which failed to plate on male strains and on the *optA* mutant but contains DNA ligase; the second (LG102 and LG114) failed to grow only on ligase-deficient hosts; and the third (Δ1, Δ3, and R14) was defective on all the tester strains. The mutant Δ2 contains DNA ligase but failed to grow on male strains of *E. coli*, whereas LG102 and LG114 are lacking DNA ligase but productively infected male strains. Because restriction enzyme analysis showed that the Δ2 deletion lies to the left of the ligase deletions (Table 2 and Fig. 2), a factor that is essential for growth of T3 on males must be either gene *1.1* or *1.2*, or both. All of those deletion mutants that failed to grow on F plasmid-containing hosts also failed to grow on *optA* mutants; therefore, it is possible that gp1.2 is necessary for phage growth on both strains.

We recently have isolated a number of point mutants of T3 that are unable to grow on male strains, or independently, on *optA* mutants, and data on one typical mutant of each type are given in Table 3. M21a was originally isolated as a phage that could not productively infect male strains, but it is clear that it also fails to grow on the *optA1* mutant. Conversely, O103 was isolated as a phage that failed to grow on *optA* mutant hosts, and it fails to grow on male strains. This is only a preliminary characterization of these point mutants, but it suggests that T3 gp1.2 is, in fact, required for growth on male strains of *E. coli*. However, we cannot yet exclude the possibility that the mutations in M21a and O103 are both in gene *1.1* but are also polar on gene *1.2* expression. A T7 gene *1.1* mutation was in fact shown to be polar on gp1.2 expression (22), and further analysis of M21a and O103 (and similar phages) is necessary to positively identify the altered gene.

Table 4 shows that the deletion mutant Δ2 could complement, and also be complemented by, both the gene *1* amber mutant H233 and by LG102, a ligase deletion mutant. Thus, the abortive infection of male strains of *E. coli* by these

Table 2. Analysis of DNA from deletion mutants of bacteriophage T3

Mutant	Phenotype*	*Hpa* I fragment missing	Size of new fragment, bp	Estimated size of deletion, bp
Δ1,Δ4	$M^-O^-L^-$	H,G	1490	2000
Δ2	$M^-O^-L^+$	H,G	2645	845
Δ3	$M^-O^-L^-$	G	470	1690
LG102	$M^+O^+L^-$	G	950	1150
LG114†	$M^+O^+L^-$	G		
R14†	$M^-O^-L^-$	G	400	1700
LG123†		H,G	1400	1900

*See Table 3. M^+ refers to growth on males; an M^- phage is restricted. Similarly, O^+ and L^+ refer to growth on *optA* mutants and *lig*$^-$ mutants, respectively.
†See Bailey *et al.* (21).

Table 3. Plating efficiencies of phage T3 mutants on various host strains

Phage strain	Relative* plating efficiency on *E. coli* strains				
	B	BL2(*lig*$^-$)	HR23	HR23F′*lac*	HR40(*optA1*)
$T3^+$	1.0	1.0	0.9	0.9	0.9
Δ1	0.7	$<10^{-7}$	0.8	$<10^{-7}$	1×10^{-4}
Δ2	0.8	0.7	0.9	$<10^{-7}$	3.2×10^{-4}
Δ3	0.9	$<10^{-7}$	0.8	$<10^{-7}$	2.6×10^{-4}
LG102	0.9	$<10^{-7}$	0.8	0.7	0.8
LG114	1.0	$<10^{-7}$	0.9	0.8	0.8
R14	0.7	$<10^{-7}$	0.7	$<10^{-7}$	2.1×10^{-4}
M21a	0.8	0.7	0.8	1.5×10^{-6}	7×10^{-4}
O103	0.9	0.9	1.0	2.0×10^{-6}	4×10^{-3}

*Relative to *E. coli* strain 011′.

Table 4. Complementation of mutant Δ2 by a gene *1* amber mutant and a ligase deletion

Tester strain	Relative number of phage		
	H233 (amber) with LG102(M^+L^-)*	H233 (amber) with Δ2 (M^-L^+)	Δ2 (M^-L^+) with LG102(M^+L^-)
B837*supE44*	1 (44.8)	1 (73.2)	1 (62.4)
B837*supE44* F^+	1.02	0.41	0.54
B837 (*sup*$^+$)	0.47	0.55	1.04
B837 F^+	0.45	0.008	0.54
011′L1 (*supE44*,*lig*$^-$)	0.56	1.00	0.43
BL2 (*sup*$^+$,*lig*$^-$)	0.06	0.40	0.42
BL2 F^+	0.06	0.010	$<1 \times 10^{-5}$

*M^+ and L^+ refer to the phenotype of productive growth on male strains and ligase-deficient hosts, respectively. M^- and L^- phages fail to productively infect these strains. Each phage was added to BL2 (F^+) (a nonpermissive host for all three phage strains) at a multiplicity of 10; 10 min later the infected cells were diluted 10^4-fold and incubated at 30°C for 60 min before plating on the various strains. The numbers in parenthesis represent burst sizes (progeny phage per infective center) after correcting for unadsorbed phage.

phage T3 mutants is *trans*-recessive, as is the case with phage T7 (13).

It has been proposed (23) that it is the right end of phage T3 gene *1* (RNA polymerase) that is required for T7–T3 hybrid phages to grow on male strains of *E. coli*. However, mutant Δ2 complements a gene *1* amber mutant in a mixed infection of an F-containing host (Table 4); therefore, the deletion mutant must encode an RNA polymerase that retains activity in the presence of F-encoded proteins. If a difference between phages T3 and T7, as regards their ability to grow on male strains of *E. coli*, does in fact reside in the RNA polymerase, it cannot by itself be sufficient.

Inhibition of Macromolecular Synthesis During the Abortive Infection. In order for phage T3 mutants to serve as a model system for the abortive infection of phage T7, it is necessary to show that these mutants exhibit similar physiological defects. As T3 gene *1.2* may be required for growth not only on male strains of *E. coli* but also on *optA* mutants, it was originally thought that infections of these strains by gene *1.2*$^-$ mutants may show comparable defects.

Fig. 3 shows that all macromolecular synthesis was prematurely terminated after a male cell was infected by phage T3 mutant Δ2. These kinetics resemble those of T7$^+$ infections of F-containing strains (13) and indicate that the T3 deletion mutant is phenotypically equivalent to wild-type T7 in this abortive infection. Furthermore T3 Δ2-infected *optA* mutants exhibit the same defects in nucleic acid synthesis as do T7 *1.2*$^-$-infected *optA* mutants (17). Thus phages T3 and T7 gene *1.2* appear to provide the same activity that is necessary for productive phage infections of *optA* mutants.

It is clear, however, that biochemical differences exist between mutant phage T3 infections of *optA* and male hosts. F-encoded proteins effectively terminate all macromolecular synthesis directed by T3 Δ2, whereas the *optA* mutant protein affects nucleic acid synthesis but not protein synthesis. This difference may simply be due to independent developmental blocks being imposed by the *optA1* mutation or by F-encoded proteins. However, it is probable that T3 gene *1.2* is required for productive infections of both *optA* mutants and male strains (Table 3). The observation that F-encoded proteins cause a more severe inhibition of T3 Δ2-directed macromolecular synthesis than does the *optA1* mutant protein (Fig. 3) may be a consequence, therefore, of both gene *1.1* and gene *1.2* being essential for productive phage infection of male strains of *E. coli*.

DISCUSSION

We have described the isolation and characterization of some mutants of bacteriophage T3 that fail to grow on *E. coli* strains that harbor an F plasmid. Infection of male strains by these mutant phage leads to the same biochemical defects as are seen after infection of the same strains by wild-type phage T7; thus, these data support the conclusion that F-mediated restriction is exerted through the same mechanism(s) in both phage systems. No simple mutants of T7 that productively infect male strains of *E. coli* have yet been isolated; in fact, this type of T7 mutant may not exist (12). In contrast, wild-type T3 grows normally on F plasmid-containing hosts, whereas the mutant T3 strains described here do not. These mutants are amenable to genetic studies; thus, it should prove possible to define all of the T3 genes necessary to overcome the potentially deleterious effects of F-encoded proteins. Thus, the T3 system should prove to be a valuable model for exploring the question of why T7 is a female-specific phage.

Either or both of genes *1.1* and *1.2* are necessary for phage T3 to grow on male strains of *E. coli*. Because the homologous region of T7 encodes similar proteins but this phage is restricted in cells that contain the F plasmid, we propose that

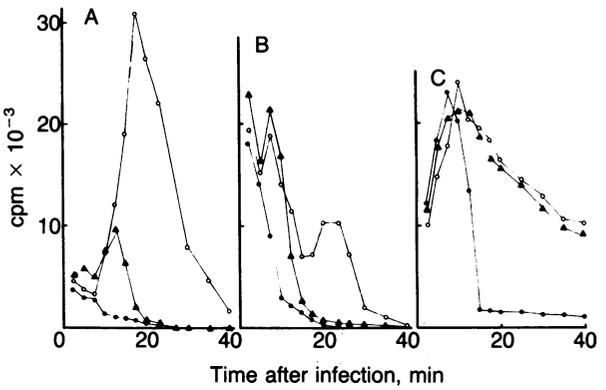

FIG. 3. Time course of the rates of DNA (*A*), RNA (*B*), and protein (*C*) synthesis in mutant Δ2-infected cells. The rates of synthesis were measured by pulse-labeling cells at various times after infection. ○, HR23; ●, HR23F′*lac*; ▲, HR40(*optA1*).

the products of T7 genes *1.1* and/or *1.2* are defective in the presence of F-encoded proteins and, in addition, that the enzymatic activities of these normally nonessential proteins are required for productive phage growth in normal male strains. The enzymatic properties of gp1.1 or gp1.2 of either phage T3 or T7 are still unknown. Gene *1.2* of T7 has been shown to be involved in DNA replication (17), and, by analogy with some mutants of phage T4, it has been suggested that gp1.2 may possess helicase activity (24), but this has not yet been confirmed. It is clear, however, that at least one of T3 gp1.1 and gp1.2 must either simply be resistant to the effects of F-encoded proteins or have additional properties to those of their T7 counterparts. The latter possibility is not without precedent in comparisons of T3 and T7 proteins in that only the 0.3 gene product of T3 possesses *S*-adenosylmethionine hydrolase activity, whereas the primary function of both T7 and T3 gp0.3 is to inactivate the type 1 restriction-modification enzyme of the host (14, 25).

It has recently been proposed that the difference between phages T3 and T7 as regards their growth on male strains is due to a difference in the right end of gene *1*, the phage RNA polymerase (23). This is not necessarily in conflict with the data presented here because it is possible that both essential (gene *1*) and normally nonessential (genes *1.1* and/or *1.2*) are required to overcome F-mediated restriction. However, the parental origin of gene 1.1 and gene 1.2 was not examined in the recombinants used by Kruger *et al.* (23). It is possible that all the T7–T3 phages that overcome F-mediated restriction contain T3-derived sequences not only for the right end of gene *1* but also for the adjacent genes *1.1* and *1.2*. Thus, the conclusion (23) that it is the phage RNA polymerase that determines the susceptibility or resistance to F-restriction should be considered speculative until more direct evidence is obtained.

The mechanism or mechanisms by which the F plasmid exerts its inhibitory effects on phage T7 or on the phage T3 mutants described here is obscure, at least in part because these abortive infections have such pleiotropic, sometimes apparently conflicting, phenotypes. Malamy and co-workers (9, 26) have maintained that a combination of T7 infection and an F-encoded (*pifA*$^+$) protein results in a defect in translational capacity of the infected cell due to the inactivation of a ribosomal component. In this context, one function of T3 gp1.1 and/or gp1.2 could be to prevent this inactivation from occurring, perhaps by displacing the *pifA*$^+$ protein from the ribosome. The equivalent phage T7 protein presumably would not have this capacity. In contrast, Chakrabarti and Gorini proposed that the block of T7 growth in a male strain is exerted somewhere at the level of early, host-catalyzed transcription (11). The experiments that led to this conclu-

sion did not depend on the expression of any phage gene. Therefore, we suggest that suppression of the inhibitory potential of the *pifA*$^+$ protein by an *rpsL* mutation should render gene *1.1* and gene *1.2* of T3 or T7 nonessential for productive phage growth in these male strains. The suppression of F-mediated restriction by a streptomycin-resistance mutation is presumably exerted by an alteration in the host cell, perhaps due to a change in the rate of translation of *pif* or other cellular mRNAs by the altered ribosome. Alternatively the *pif* gene product(s) may not interact with a streptomycin-resistant ribosome and, thus, not be able to interfere with phage T7 gene expression.

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