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Abortive Infection of F-Plasmid-Containing *Escherichia coli* Cells by Bacterial Virus T7 Is Determined by the Right End of T7 Gene 1

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Phage T7 infects male (F-plasmid-carrying) *Escherichia coli* cells abortively, whereas the closely related phage T3 grows normally. The inability or ability of phage to replicate in male host cells depends on whether the right end of gene 1 (coding for the phage-specific RNA polymerase) consists of T7 or T3 DNA base sequences.

It has been known for a long time that bacteriophage T7 does not replicate in *Escherichia coli* cells which carry a fertility (F) plasmid (14). That is why T7 is termed a female-specific phage. The molecular mechanism of the incompatibility between the F plasmid and T7 phage development has not been elucidated and remains controversial despite numerous papers on the subject (see the reviews of Condit [4], Duckworth et al. [5], and Krüger and Schroeder [12]). Originally it was assumed that T3, a close relative of T7, was also female specific (19, 22), but it then turned out that the supposed T3 phage under study (19, 22) was in fact T7 (20), and neither Hausmann (8) nor Studier (20) observed an inhibition of T3 growth in male *E. coli* cells.

We have looked for the reason why the two phages differ so remarkably in their ability to replicate in F^+ cells. It was decided to compare the properties of T3 and T7 to identify the gene(s) responsible for the abortive outcome of T7 infection.

Into the DNA restriction- and modification-negative strain *E. coli* WA921, designated *E. coli* 0 by us (13), we introduced the F^+ or the F′ *lac* plasmid. Table 1 shows that the efficiency of plating (EOP) of T7 in these male cells was depressed by two orders of magnitude compared with the F^- strain. Furthermore, the normal plaque morphology seen on *E. coli* 0 was disturbed on F-plasmid-carrying cells, where miniplaques appeared. Replating of T7 plaques isolated from F^+ cell lawns did not improve on the growth of T7 in F^+ cells; i.e., an F^+-specific phage modification did not take place. In contrast to T7, T3 grew equally well on all the tested host strains, and there was no F-plasmid-mediated exclusion of T3 (Table 1).

Lysogenic conversion of bacteria is known to cause resistance (inability to adsorb) towards T3 and T7 (18). However, the inability of T7 to grow in F-carrying cells is not related to adsorption. T7 adsorbed efficiently to male and female *E. coli* 0 cells (data not shown). Also, there was no intracellular degradation of T7 DNA in F-carrying cells, i.e., no DNA restriction (Fig. 1), but rather an abortive infection. These data agree with previously published observations (5, 8).

To find out which of the T7 genes known to differ greatly from its T3 counterpart could be responsible for the exclusion of T7, we tested two T7-like mutants of T3 for their ability to grow in male cells: (i) T3*sam*$^-$, a gene 0.3 mutant which, like T7, does not induce *S*-adenosylmethionine hydrolase (7, 10, 13); and (ii) $T3h_W$, a mutant (probably gene 17) with T7-like host range and immunogenicity (11). These T3 mutants, however, replicated normally in F-carrying cells (Table 1), so other genes must be responsible for the different behavior of T3 and T7 in male hosts. A third difference between T3 and T7 is gene 1. The DNAs of T3 and T7 hybridize poorly in the gene 1 area, and the gene 1-coded RNA polymerases exhibit distinct template specificities (12).

We then checked the ability of T3 × T7 recombinants to replicate in male host cells. These recombinants were isolated and characterized by Beier et al. (1). Fig. 2a shows the results. The property shared by all recombinants capable of growing in male cells is the T3 origin of their gene 1. The complete gene 1 and neighboring genes on both sides may be T3 specific (R8, R60, R71), or the T3 portion of the recombinant DNA may extend into gene 1 from either the left (R12, R30) or the right (R55). However, the recombinant R39, in which T3 sequences

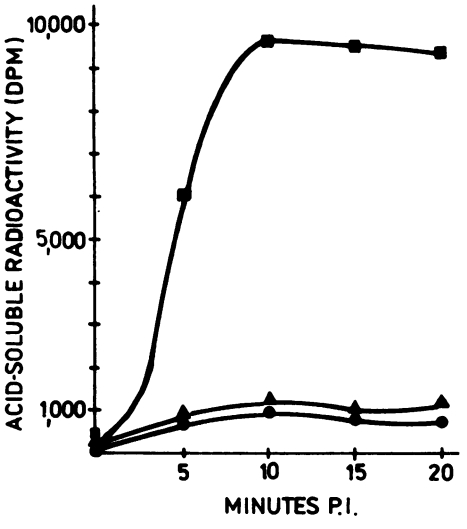

FIG. 1. Fate of [^{3}H]DNA-labeled T7/D111 in male cells. [^{3}H]thymidine-labeled phages were grown and purified as described (13). To demonstrate DNA degradation in restricting cells, log-phase cells were concentrated to 10^9 cells per ml in nutrient broth, infected with a multiplicity of infection of 0.1 phage per cell, and incubated at 37°C. At 0, 5, 10, 15, and 20 min postinfection, 0.9 ml of the infected mixture was added to 0.1 ml of ice-cold 50% trichloroacetic acid and kept cold for 30 min. The acid-precipitable fraction of the samples was then removed by centrifugation, and the radioactivity of the supernatant was counted. The total radioactivity of phage DNA in a 0.9-ml sample was 18,000 dpm; nearly 75% of phage (13,500 dpm) adsorbed to the cells after 5 min. The specific radioactivity of phage was 2×10^{-4} dpm/PFU. The *ocr*$^-$ mutant T7/D111 was used instead of wild-type T7 because the DNA of *ocr*$^-$ mutants is restricted in *E. coli* B (13), serving as a positive control in this experiment. Results are shown for infection of *E. coli* strains 0 (●), 0(F′ *lac*) (▲), and B (■).

extend into gene 1 from the left, is excluded in F^+ cells (Fig. 2a).

To identify which part of gene 1 must originate from T3 if the recombinants should replicate normally in F^+ cells and which part of T7 gene 1 leads to exclusion, we examined the growth of T3 × T7 recombinants in gene 1 (isolated and mapped by Beier and Hausmann [2]) in *E. coli* 0, 0(F^+), and 0(F′ *lac*). Figure 2b shows that all recombinants that have the left region and up to 90% (R33 and R39) of gene 1 from the T3 genome and the whole right part from T7 are unable to reproduce in male cells.

Thus, the critical DNA sequence which is decisive for the ability of the phage to grow in male cells must be localized at the right end of gene 1. This is the sequence determining the template specificity of RNA polymerases (2). Nevertheless, template specificity and F^+ exclusion seem not to be necessarily correlated: in contrast to R33 and R39, which possess T7 template specificity (2) and which (like T7) are excluded (Fig. 2b), the recombinant R12 (also exhibiting T7 template specificity [1]) is capable of reproducing in F^+ cells (Fig. 2a).

Recently, Gómez and Galicia (6) described a T7 mutant able to replicate in male *E. coli* cells which is altered in seven genes. The authors did not identify which of these mutations is essential for growth in F^+ cells and which are just the result of the severe procedure of mutagenesis. Our results show that an alteration of the right side of gene 1 is sufficient to enable T7 to replicate in F-plasmid-carrying cells.

Through what mechanism does the phage RNA polymerase decide on the outcome of infection in male cells? During T7 infection of F^+ cells, phage-specific RNA polymerase is synthesized and late mRNAs appear, suggesting that T7 RNA polymerase does not lose its catalytic power; T7 DNA synthesis, however, is completely blocked (5, 8). The primary initiation of T7 DNA replication depends on a functional T7 RNA polymerase (9, 17, 21). One could hypothesize that in the presence of the F plasmid, T7 RNA polymerase (unlike T3 RNA polymerase) is unable to fulfill its role in primary initiation. The absence of DNA synthesis explains why the infection is abortive. Various authors have described abnormalities in late T7 transcription or translation (5) which may be directly or indirectly caused by the dysfunction of the T7 RNA polymerase in F^+ cells. A deficiency of late T7 proteins, which are necessary for T7 DNA replication, could also contribute to the lack of T7 DNA synthesis in F^+ cells.

It remains to be discovered by what mechanism the F plasmid gene(s) (localized at the 38.3 F position [15]) influence the function of T7 RNA polymerase and how the inhibiting mechanism can be neutralized (3, 16) by certain chromosomal mutations of *E. coli*.

TABLE 1. Replication of T7, T3, and mutants in male and female *E. coli* 0 cells

Phage · grown on *E. coli* strain	Relative EOP on *E. coli* strain[a]:		
	0	0(F^+)	0(F′*lac*)
T7 · 0	1	0.01[b]	0.01[b]
T7 · 0(F^+)	1	0.01[b]	0.01[b]
T3 · 0	1	1	1
T3*sam* · 0	1	1	1
T3h_W · 0	1	1	1

[a] EOPs determined in parallel on the host strains indicated are expressed as fractions of the EOP on the permissive strain *E. coli* 0.

[b] Miniplaques (approximately 0.3 mm in diameter).

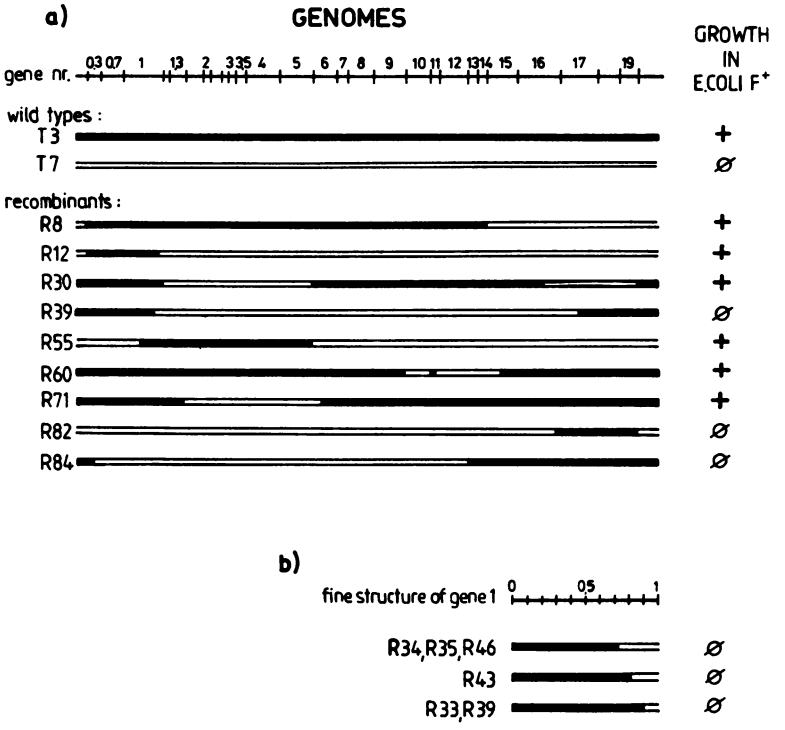

FIG. 2. Growth of T3 × T7 recombinants in male *E. coli* cells. (a) Recombinants isolated by Beier et al. (1); (b) gene 1 recombinants isolated by Beier and Hausmann (2). Symbols: ——, T3 genome segments; ═══, T7 genome segments; +, relative EOP on *E. coli* 0(F^+) and 0(F′ *lac*) cells of 0.8 to 1.0 (where EOP on *E. coli* 0 = 1), the same plaque size as on *E. coli* 0; ∅, relative EOP of 0.01, obligate miniplaques on *E. coli* 0(F^+) and 0(F′ *lac*).

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