Adenosine 5'-Triphosphate Leakage Does Not Cause Abortive Infection of Bacteriophage T7 in Male Escherichia coli

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galU and rpsL mutations restore plating efficiency of bacteriophage T7 in male Escherichia coli without suppressing leakage of adenosine 5'-triphosphate pools.

Bacteriophage T7 grows normally on F- strains of Escherichia coli, but infection is abortive in male strains (F+, F', Hfr). At 8 to 10 min after infection of male cells a rapid decrease in the rate of phage protein and RNA syntheses occurs. At the same time ATP starts leaking out of the cell as a result of permeability lesions in the host membrane. It has been suggested that infection is abortive for lack of ATP and other triphosphates during late protein and RNA syntheses (2, 4).

This model is, however, contradicted by the observation that ATP leakage continues in a strain containing pifA, an F-factor mutation, whereas plating efficiency of T7 is substantially restored (1). According to Blumberg et al. (1), the presence of the pifA+ gene product in a male host specifically affects translation of T7 mRNA. In this study we measured ATP leakage in two other mutants in which the F-mediated inhibition of T7 is suppressed. One strain, DEV2, is a streptomycin-resistant mutant which contains an rpsL mutation; the other strain, DEV6, contains a galU mutation (see Table 1).

Chakrabarti and Gorini (3) first reported that the F-mediated inhibition of T7 is suppressed in certain streptomycin-resistant mutants of E. coli. We have independently selected streptomycin-resistant mutants from DEV1 [a lacZ(Am) derivative of E. coli K-12 Hfr KL16]. Readthrough at the lacZ(AM) codon results in 0.4% residual β-galactosidase activity in DEV1. Different rpsL alleles restrict, or lower, readthrough at a terminator codon to various extents, as previously shown by Gorini (5). Our results indicate that plating efficiency of T7 is restored only in strain DEV2, which contains the most restrictive rpsL allele. This allele, named rpsL221, resembles the strA1 allele of E. coli B: readthrough is severely restricted and cannot be restored by the addition of streptomycin (5).

Strain DEV6, a derivative of DEV1 containing a galU mutation, was isolated in a bizarre way. We noted that DEV1 was killed efficiently by seeding plates simultaneously with T7 and Qβ, a male-specific RNA phage. T7 alone is not effective because of the F-mediated inhibition; Qβ alone is not effective because it lacks a strong lysis function. The mechanism of this synergistic effect is not yet understood. When 107 cells of DEV1 were plated on fresh tryptone plates (10 g of tryptone, 5 g of NaCl, and 10 g of agar per liter) seeded with 107 T7 and 3 × 106 Qβ, about 300 surviving clones appeared after 1 to 2 days of incubation at 37°C. Approximately 3% of these had acquired a Gal- Gal+ phenotype, being unable to grow on galactose or on glycerol and galactose as sole carbon sources. We concluded that this phentoype is caused by a galU mutation on the basis of the following evidence. (i) Conjugation between DEV6 and AB3282 selecting Trp+ yielded over 90% Gal- Gal+ recombinants, as expected for galU but not for other gal loci (9). One of these was named DEV8. (ii) DEV6 could be transduced at low frequency to Gal+, using bacteriophage φ80. Such low-frequency transduction of galU by φ80 has been previously demonstrated (9). (iii) The receptor site for T7 attachment involves the lipopolysaccharide on the outer membrane of E. coli (7). In a strain containing galU the lipopolysaccharide is deficient in two glucose residues, as no UDP glucose is made. This deficiency is expected to interfere with T7 adsorption. We found that T7 adsorption is reduced by 90 to 95% in both DEV6 and DEV8.

When T7 was plated on DEV6, small T4-size plaques were obtained with a plating efficiency of 5 to 10%. This suggested that T7 plating efficiency was at least partially restored in spite of the adsorption deficiency. To separate these effects, a T7h (host range) mutant was selected on DEV9, a T7-resistant derivative of W3110. T7h appeared at a frequency of 10-8. One of these was purified and used for further studies. We were unable to obtain high-titer lysates of T7h with Studier's procedure (10). This difficulty was overcome by concentrating 1-liter lysates 500-fold by polyethylene glycol precipita-
The resulting T7h plated with normal efficiency on DEV6 and DEV8. In addition, plaque sizes were comparable. We concluded that galU suppresses the F-mediated inhibition of T7. This conclusion is supported by the observation that the parental phenotype is fully restored in Gal+ revertants obtained from DEV6. Mutants containing galU deletions were also obtained in this study, suggesting that the observed effect is not limited to a special subclass of galU alleles.

Leakage of ATP pools after T7 or T7h infection was assayed with firefly lantern extracts, essentially as described by Condit (4), except that samples were immediately filtered through a 0.45-nm Millex filter, so that only ATP released in the medium was measured. Results are shown in Fig. 1. It is clear that ATP leakage is not suppressed in either DEV2 or DEV6. A slight delay in the onset of ATP leakage was consistently observed with DEV2. Differences in ATP levels observed among the Hfr strains at later times fell within experimental error.

The mechanisms by which rpsL221 and galU suppress the F-mediated inhibition of T7 remain unclear. Specific activity of UTP-α-1-phosphate uridylyltransferase (EC 2.7.7.9), the gene product of galU, was determined by method I of Hansen et al. (6). It is normal in DEV2 and less than 10% in DEV6. It has been suggested that various pleiotropic effects of the rpsL221 type of allele might be the result of a slower elongation rate during translation of mRNA (12). We have found that the elongation rate in DEV2 is indeed slower than in DEV1, as determined by the kinetics of β-galactosidase induction (8) (data not shown). Regarding the effect of galU, one possibility is that the F-mediated inhibition of T7 requires UDP glucose, which is absent in DEV6. Alternatively, the lipopolysaccharide deficiency in DEV6 probably forces T7 and T7h to enter the host through other receptor sites. The F-mediated inhibition is perhaps dependent on the specific attachment and entry via the lipopolysaccharide site.

More importantly, among four independent mutations which suppress F-mediated inhibition of T7 (pifA, pifAB, rpsL221, and galU) only one (pifAB) also suppresses leakage of ATP pools (1). This suggests that abortive infection of T7 in male cells and leakage of ATP pools may not be causally related after all.

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LITERATURE CITED


