Plasmid pR386 Renders *Escherichia coli* Cells Restrictive to the Growth of Bacteriophage T4 *unf* Mutants

RICHARD E. HERMAN AND D. PETER SNUSTAD*

Department of Genetics and Cell Biology, College of Biological Sciences, University of Minnesota, St. Paul, Minnesota 55108

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The introduction of the F1 incompatibility group plasmid pR386 Tc into several common laboratory strains of *Escherichia coli* rendered them restrictive to the growth of bacteriophage T4 *unf* mutants, which are defective in unfolding the host genome. The growth inhibition was temperature dependent. The single mutant *unf39* $\times$ 5 exhibited an efficiency of plating of $<10^{-8}$ at 27°C. However, at 37°C, complete growth inhibition occurred only when host DNA degradation was also absent.

The nucleoids of *Escherichia coli* cells are rapidly unfolded beginning at 3 min after infection with bacteriophage T4 (13, 16). The results of experiments using chloramphenicol to inhibit protein synthesis indicate that the T4-induced unfolding of the *E. coli* chromosomes is dependent on the presence of one or more proteins synthesized between 2 and 3 min after infection (13). The simplest explanation of these results is that the protein involved is the product of the *unf* gene, since mutations in this gene have been identified and shown to result in a deficiency of the T4-induced unfolding of the host nucleoid (13). However, there is no direct proof that the *unf* gene product is a protein. Subsequently, *unf* mutants were isolated on the basis of their ability to permit survival of T4 multiple mutants which synthesize cytosine-containing phage DNA (14). These mutants were called *alc* mutants (allows late transcription on cytosine-containing DNA). Many *alc* mutants are also *Unf*⁻ (11), and the 19 *unf* mutants that we have isolated are all *Alc*⁻ (15). Moreover, we have never observed complementation between *unf* and *alc* mutations (15, unpublished data). Thus, the *unf* and *alc* mutations appear to identify the same gene.

The *unf**alc* gene product is known to be involved in the shutoff of late RNA synthesis from cytosine-containing T4 DNA (14) and from phage λ DNA (7). It also may be involved in the shutoff of host RNA synthesis (11, 15). Pettijohn and Hecht (8) demonstrated that treatment of *E. coli* cells with rifampin results in unfolding of the nucleoids, suggesting that ongoing transcription is necessary to maintain the structure of the folded genomes. It thus seemed likely that T4 mutants that failed to induce the unfolding of the host nucleoids (13) might also fail to shut off host transcription. This was reported to be the case

by Sirotkin et al. (11). However, in subsequent studies, the shutoff of host transcription was found to be complete by 9 min after infection with *unf* or *alc* mutants (15, P. Dennis, personal communication). In these studies, the only detectable effect of the *unf* mutations on RNA synthesis was a slightly slower switchover from host RNA synthesis to phage RNA synthesis. Thus, the role of the *unf**alc* gene product in the shutoff of host RNA synthesis remains unclear.

The *unf**alc* gene product is apparently unessential, since many *unf**alc* mutants are viable under a wide variety of conditions. However, the growth of certain *unf**alc* mutants is temperature sensitive (11). It is possible, therefore, that the *unf**alc* gene product is essential and that the nontemperature-sensitive *unf**alc* mutations are leaky. A confounding factor in interpreting the phenotypes of *alc* mutants is that T4 phage forced to grow with cytosine-containing DNA accumulate a high frequency of secondary mutations (E. Kutter, personal communication). Since *alc* mutants can only be selected under conditions in which phage T4 DNA contains cytosine, their phenotypes may be due in part to secondary mutations. Even though our original *unf* mutations were identified on the basis of their inability to unfold the host nucleoid, the procedures involved are quite laborious. Clearly, it would be desirable to have a simple selection procedure for the identification of *unf**alc* mutations and for monitoring their presence in phage strains which does not require the growth of T4 phage under conditions where its DNA contains cytosine rather than 5-hydroxymethylcytosine.

In this paper, we report the results of experiments which show that the introduction of plasmid pR386 into several common laboratory strains of *E. coli* renders them restrictive to the

TABLE 1. Efficiencies of plating of T4 phage mutants with deficiencies in host DNA degradation, nuclear disruption, and unfolding of the host nucleoid on *E. coli* S/6/5(pR386)

Genotype ^a	Efficiency of plating ^b	
	27°C	37°C
T4D ⁺	0.07	0.47
<i>nd28</i> × 6	0.08	0.58
<i>rPT8</i> × 2	0.02	0.16
<i>unf39</i> × 5	<10 ⁻⁸	0.02
<i>rPT8</i> × 2 <i>nd28</i> × 6	0.04	0.18
<i>nd28</i> × 6 <i>unf39</i> × 5	<10 ⁻⁸	1 × 10 ⁻⁷
<i>rPT8 unf39</i>	<10 ⁻⁸	2 × 10 ⁻⁸
<i>rH23 unf39</i> × 5	<10 ⁻⁸	1 × 10 ⁻⁷
<i>rA105 unf39</i> × 5	<10 ⁻⁸	0.02
<i>rPT8</i> × 2 <i>nd28</i> × 6 <i>unf39</i> × 5	<10 ⁻⁸	<10 ⁻⁸
<i>rH23 nd28</i> × 6 <i>unf39</i> × 5	<10 ⁻⁸	<10 ⁻⁸
<i>rH23 ndd98 nd28</i> × 6 <i>unf39</i> × 5	<10 ⁻⁸	<10 ⁻⁸
<i>rA105 nd28</i> × 6 <i>unf39</i> × 5	<10 ⁻⁸	1 × 10 ⁻⁶
<i>rA105 ndd98 nd28</i> × 6 <i>unf39</i> × 5	<10 ⁻⁸	<10 ⁻⁸

^a *nd28* × 6 is in gene *denA*; it is deficient in T4 endonuclease II and, thus, in host DNA degradation (10, 17). *rPT8*, *rH23*, and *rA105* are T4B mutants which carry deletions extending from within the *rIIA* gene into the unessential "D" region adjacent to the *rIIB* gene. *rPT8* × 2 is a T4D derivative of *rPT8* (12). The *rPT8* deletion extends through genes D1 (function unknown), *denB* (T4 endonuclease IV), *pla*⁻262 (function unknown), and into *ndd* (nuclear disruption) (2, 4, 9, 12). Deletion *rH23* extends through gene D1 into gene *denB*; deletion *rA105* extends only into gene D1 (2, 4, 9, 12). *ndd98* is an hydroxylamine-induced mutation deficient in the ability to induce nuclear disruption (12). *unf39* × 5 is defective in the T4-induced unfolding of the host nucleoid (13); it has also been shown to be an *alc* mutation (15).

^b In all cases, the efficiency of plating of the same strain on *E. coli* S/6/5 was set at 1.0. All values are the averages of four plates each on S/6/5 and S/6/5(pR386). All platings were done with EHA top layer agar and EHA bottom layer agar as previously described (13).

growth of *unfalc* mutants at 27°C. In addition, the pR386-containing *E. coli* strains are restrictive to the growth of multiple mutants deficient in both host DNA degradation and unfolding of the host nucleoid at all temperatures tested.

Plasmid pR386 was obtained from the Plasmid Reference Center, Stanford University, in *E. coli* strain J53F⁻(λ) *pro-22 met-63* (1). It is an F-like, conjugative R plasmid in the F1 incompatibility group and confers tetracycline resistance to host cells (3, 5). Plasmid pR386 has been transferred by the procedure described by Miller (6) into *E. coli* strains C600, B/5, B834 *r⁻ m⁻*, S/6/5, K-12 Yale, and CR63. In all of these strains, plasmid pR386 renders the host cells restrictive to the growth of the host DNA degradation-deficient, unfoldase-deficient multiple mutants at all temperatures. The complete restriction of this host nucleoid modification-deficient multiple mutant in *E. coli* strain S/6/5(pR386) is documented in Table 1 and Fig. 1. The *unf39* × 5 single mutant plates with an efficiency of about 10⁻² on S/6/5(pR386) at 37°C. The loss of *rIIA*, *rIIB*, and D1 functions does not lower the plating efficiency (*rA105 unf39* × 5). When either endonuclease II (*nd28* × 6 *unf39* × 5 and *rA105 nd28* × 6 *unf39* × 5) or endonuclease IV (*rH23 unf39* × 5) is also absent, the plating efficiency decreases by at least four orders of magnitude to 10⁻⁶ to 10⁻⁷. When both endonuclease II and endonuclease IV are absent, the plating efficiency is below the detectable level (<10⁻⁸). In the presence of endonuclease IV, the absence of nuclear disruption will also cause a plating efficiency of <10⁻⁸ in an endonuclease II-deficient strain (*rA105 ndd98 nd28* × 6 *unf39* × 5). We do not presently understand the reasons for these interactions.

Our most important observation is that plas-

FIG. 1. Spot test illustrating the restriction of growth of *unf39* × 5 by pR386. The phage stocks were all adjusted to 10⁶ phage per ml and small drops placed on each indicator strain in quadruplicate. The indicator strains were grown in H broth, and platings were done on EHA agar as previously described (12). Plasmid pR386 is very stable in *E. coli* strain S/6/5. We have grown overnight cultures and indicator cultures for several months in the absence of tetracycline without loss of the plasmid. The various phage genotypes are described in footnote a of Table 1.

mid pR386 causes host cells to be restrictive to the growth of *unf* single mutants at 27°C (Fig. 1, Table 1). Whereas the exact efficiency of plating varies slightly from strain to strain (for example, the efficiency of plating of T4D⁺ ranges from 0.4 to 0.6 at 37°C on the various plasmid-containing strains), the same pattern of restriction of *unf* mutants has been observed in all six *E. coli* strains examined to date.

We have used two means to demonstrate that the restriction of the *Unf*⁻ strains is due to the presence of the *unf* mutation and not some other uncharacterized mutation present in these

FIG. 2. Sucrose gradient analysis of the nucleoids isolated by folded genome conditions from (A) uninfected *E. coli* S/6/5 cells and from *E. coli* S/6/5 cells at 5 min after infection with (B) the host DNA degradation-deficient, nuclear disruption-deficient, unfoldase-deficient multiple mutant *rPT8* × 2 *nd28* × 6 *unf39* × 5, (C) the host DNA degradation-deficient, nuclear disruption-deficient, unfoldase-proficient strain *rPT8* × 2 *nd28* × 6, and (D) an unfoldase-proficient revertant of *rPT8* × 2 *nd28* × 6 *unf39* isolated on the basis of its ability to form plaques on *E. coli* strain S/6/5(pR386). Detailed descriptions of these phase strains are given in footnote a of Table 1. [³H]thymidine-labeled cells were grown and infected in Fraser glycerol medium as previously described (13). [¹⁴C]thymidine-labeled T4 phage were prepared as previously described (13) and added to one tube in each centrifugation as a reference marker (arrows). Sucrose gradients of 10 to 40% were prepared and analyzed by the procedure described earlier (13). The gradients were collected from the bottom; thus, fraction 1 is the bottom of the gradient and fraction 19 is the top of the gradient. ○, ³H-labeled host DNA; ●, ¹⁴C-labeled intact T4 phage marker.

FIG. 3. Rates of adsorption of *Unf*⁺ and *Unf*⁻ T4 phage to *E. coli* strain S/6/5(pR386) at 30°C. Details of the protocol used have been described earlier (13). ○, T4D⁺; ▲, *unf39* × 5; and □, *rPT8* × 2 *nd28* × 6 *unf39* × 5.

strains. (i) When *unf* mutants are plated on plasmid pR386-containing *E. coli* strains, plaques form with a frequency of about 10⁻⁹. Ten independent plaques which formed when *rPT8* × 2 *nd28* × 6 *unf39* was plated on *E. coli* S/6/5(pR386) at 37°C were punched out, diluted, and replated on S/6/5(pR386), and one high titer stock was grown from each independent isolate. All 10 were assayed for their ability to unfold the host nucleoid by our pour test assay (13) and found to be *Unf*⁺ revertants. Figure 2 shows the results of a sucrose gradient folded genome analysis of one of these revertants. (ii) Five independently isolated *unfalc* mutants (*unf39* × 5, isolated by our pour test procedure (13); *alc1*, isolated by Snyder et al. with the *alc* selection procedure (14); and three independent *unfalc* mutants isolated in our lab by the *alc* selection procedure [15]) have all been shown to be unable to grow in host strains containing plasmid pR386 (data not shown).

The *unfalc* mutants adsorb to plasmid pR386-containing host cells with normal kinetics (Fig. 3). Thus, defective adsorption is not responsible for the restricted growth of *unfalc* mutants in the presence of pR386. Experiments on the mechanism of inhibition of growth of *unfalc* mutants are in progress. Clearly, the ability to study the *unfalc* single mutants under conditions where they exhibit conditionally lethal phenotypes will greatly facilitate future studies on the function of the *unfalc* gene product.

The plasmid pR386-conferred growth inhibition of host nucleoid modification-deficient T4 mutants, but not of wild-type T4, suggests that postinfection plasmid gene expression may be responsible for the restricted growth of these T4 mutants. Perhaps the rapid shutoff of pR386 gene expression induced by wild-type T4 is essential for its growth in the presence of the plasmid. If so, this would illustrate an interesting competition between plasmids and viruses for control of intracellular niches.

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