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Genetics

Mutants of T_7 bacteriophage inhibited by lambda prophage

(phage T_7 and lambda interaction/r_{II} function/ligase suppression/*rex* gene/DNA packaging)

CHIA C. PAO AND J. F. SPEYER

Genetics and Cell Biology, U-131, The University of Connecticut, Storrs, Conn. 06268

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ABSTRACT **Mutants in gene 20, a new T_7 gene, cannot grow on *rex*$^+$ λ lysogens. Gene 20$^-$ mutants suppress in double mutants the phenotype of T_7 ligase negative mutations, but not vice versa. Amber 20$^-$ mutants have been obtained. There are differences between these T_7 mutations and the similar T_4 r_{II} mutations. There are host mutations which permit T_7 20$^-$ mutants to grow on λ$^+$ lysogens. T_7 DNA synthesis on normal λ$^+$ lysogens infected with 20$^-$ mutants is essentially normal, but the DNA is not packaged. The gene 20 protein is active in *in vitro* complementation and probably used late in infection for DNA packaging into phage heads.**

A wide range of phenotypic effects are produced by the presence of plasmids or lysogenic phage in cells. Among these are many examples where the resident episome or prophage prevents the growth of infecting phages or episomes. Well understood is the repression of infecting λ DNA in λ lysogens (1) or the cleavage of such λ DNA in P_1 lysogens by P_1 restriction enzymes (2). In different cases, a limited number of different mechanisms are likely to apply. Other, less understood examples of interference with superinfecting phage by plasmids include the inability of phage T_5 and BF23 to replicate in *Escherichia coli* harboring Col Ib factor (3, 4) and the effect of the F factor of male cells on the growth of female specific phage such as T_7 (5). The best known example due to prophage is the growth restriction of r_{II} mutants of phage T_4 or T_2 when they infect λ lysogenic cells (6). The mechanism of this interference is obscure. It is known that the *rex* gene of λ is required, and this is the only known role of that gene (7, 8). The role of the T_4 r_{II} proteins is unknown. They are required early in infection, and their absence is characterized by these phenotypes. In *E. coli* B strains, r_I or r_{II} or r_{III} mutants do not exhibit lysis inhibition; this is responsible for their characteristic plaque morphology (6). In K_{12} cells only r_I mutants lack lysis inhibition. In K_{12} (λ) lysogens there is an absolute requirement for r_{II} function. T_4 r_{II} mutants phenotypically suppress gene 30$^-$ T_4 mutants (ligase$^-$). Double mutants (r_{II}^-/30$^-$) of T_4, unlike 30$^-$ mutants, can grow on nonlysogenic *E. coli* provided that these hosts are not ligase-defective (9–11). Evidently, the host's ligase suffices in the infections with the double mutant phage.

Recently, mutants in T_5 have been found that resemble the r_{II} mutants of T_4 in that they cannot grow on *rex*$^+$ λ lysogens (12). These T_5 mutants (*lr*, lambda-repressed) are located in gene I, and are a special class of gene I mutants, required only for growth on λ lysogens. Amber mutants of gene I are unable to grow on either lysogenic or nonlysogenic cells and show that the gene I product is needed for the injection of the whole T_5 DNA strand and for the breakdown of host DNA. It is not known where the *lr* mutants of gene I are blocked in their infection of λ lysogens, or if there is a relationship with ligase. In T_5 phage no ligase-less mutants are known.

We report here mutants in gene 20, a new T_7 gene. Although T_7-infected cells do not exhibit lysis inhibition, in other ways the phenotype of gene 20$^-$ mutants resembles that of the T_4 r_{II} mutants: T_7 20$^-$ mutants do not grow on λ *rex*$^+$ lysogens and also suppress the phenotype of phage ligase mutations. The map position and other properties suggest that gene 20 acts late in infection, unlike gene I and r_{II} of T_5 and T_4, respectively. A reasonable hypothesis is that all these genes affect a DNase.

MATERIALS AND METHODS

Bacterial and Phage Stocks. *E. coli* W3110, Su$^-$, CR 34 Su$^+_{II}$ were gifts of Dr. B. Bachmann and used in most experiments as nonpermissive and permissive hosts. Various lysogens were constructed with λ mutants in these hosts; where we indicated λ resistant, λ^R, derivatives were used. Other amber suppressor strains used were N720, Su$^+_{III}$ from Dr. M. Gottesman; X7026N, Δ(*lac, pro*) SupE (Su$^+_{II}$), NalA, *thi*$^-$ and ECO Su$^+_{II}$ from Dr. J. Miller; and unmapped amber suppressors obtained by us from W3110 *trp*$^-_{am}$, *his*$^-_{am}$ as prototrophic revertants or as φ80p Su$^+_{III}$ lysogens. Ligase-deficient *E. coli* and their parent were a gift from Dr. J. Karam and were all derivatives of FF7059; N1624 *lig*$^+$, N1626 *lig*$_4$ and N2668 *lig*$_{ts7}$ (13). The λ strains used were either gifts some time ago from Dr. A. D. Hershey or recent gifts from the following scientists: Dr. N. Franklin: W1485 (λ N$^-_{96A}$, cI^{857} *ind*$^-$, *rex*$^+$) and W1485 (λ N$^-_{96A}$, cI^{857} *ind*$^-$, *rex*$^-$); Dr. G. Gussin: λ *rex*$^-_{5a}$, λ *rex*$^-_{3a}$, λ *rex*^-_Q (8); Dr. W. Dove: 594 (λ cI^{857}) (14). T_7 phage and the various amber mutants used were a gift of Dr. W. Studier. The gene 20$^-$ (*−rbl*) mutants and double mutants were constructed in this laboratory.

Media have been described: TBMM (15), M9S and L broth (16). Diluent for T_7 phage is 50 mM Tris·HCl pH 8.0, 1 M NaCl, 10 mM $MgCl_2$.

Chemicals. 2-Aminopurine and spermidine were obtained from Calbiochem Co. [^{3}H]Thymine was purchased from New England Nuclear Co.

Isolation of the T_7 *rbl* (Repressed by Lambda) Mutants. T_7 phage were mutagenized by a single cycle of growth in CR34 in M9S containing 1 mg/ml of 2-aminopurine. The mutagen was added to the growing cells 2–3 hr before the addition of the phage. Suitable dilutions of the mutagenized T_7 lysate were plated on a layer of soft agar with W3110, λ^R on top of a layer of soft agar containing W3110 (λ$^+$), λ^R on L agar plates. Use of λ^R derivatives prevents infection of the nonlysogen with λ from the lysogenic cells. Two independent T_7 *rbl* mutants were isolated from turbid plaques from these double layer plates, *rbl-1* and *rbl-2*. Both turned out to be amber mutants suppressible by Su$_{II}$, but not Su$_{III}$ cells. The two *rbl* mutants were shown to be at different sites in

Table 1. Host range of T_7*rbl* mutant

Host strains	T_7*rbl*	T_7^+	T_7*am9*	T_4r_{II}- *A105*	T_4- *amB272*	λc	λv	434	φ80	21
W3110	+	+	—	+	—	+	+	+		
W3110(λ+)	—	+	—	—	—	—	+	+		
W1485	+	+	—	+	—	+	+	+		
W1485(λ+)	—	+	—	—	—	—	+	+		
CR34,Su_{II}^+	+	+	+	+	+	+	+	+		
CR34(λ+),Su_{II}^+	+	+	+	—	+	—	+	+		
X7026N,Su_{II}^+	+	+	+	+	+	+				
X7026N(λ+),Su_{II}^+	+	+	+	—	+	—				
ECO,Su_{II}^+	+	+	+	+	+	+				
ECO(λ+),Su_{II}^+	+	+	+	—	+	—				
N720,Su_{III}^+	+	+	—	+	+	+	+			
N720(λ+),Su_{III}^+	—	+	—	—	+	—	+			
W3110(λcI^{857}*ind*-*rex*$_1$)	+	+		+		—				
W3110(λcI^{857}*ind*-*rex*+)	—	+		—		—				
CR34(λcI^{857}*ind*-*rex*$_1$),Su_{II}^+	+	+		+		—				
CR34(λcI^{857}*ind*-*rex*+),Su_{II}^+	+	+		—		—				
W3110(λ*rex*$_{5a}$)	+	+		+		—	+			
CR34(λ*rex*$_{5a}$),Su_{II}^+	+	+		+		—	+			
W3110(λ*rex*$_{30a}$)	+	+		+		—	+			
CR34(λ*rex*$_{30a}$), Su_{II}^+	+	+		+		—	+			
W3110(λ*rex*$_Q$)	+	+		+		—	+			
CR34(λ*rex*$_Q$),Su_{II}^+	+	+		+		—	+			
W3110(434)	+	+		+		+	+	—		
W3110(λi^{434})	+	+		+		+	+	—		
W3110(21)	+	+		+		+			+	—
W3110(λi^{21})	+	+		+		+			+	—
W3110(φ80)	+	+		+		+			—	
W3110(φ80iλcI^{857})	—	+		—		—			+	

Bacterial cells were grown to about 2.0 × 10^8/ml in nutrient broth, and 0.2 ml of cells was poured onto agar plates to make a lawn. Phage was diluted to about 10^6/ml, and drops of about 0.03 ml were spotted on the lawn. Results were tabulated after a 6-hr incubation at 37° except for temperature-sensitive lysogens, which were incubated at 30° and tabulated after overnight incubation. T_7*am9* is an amber mutant in gene 16 (coded for a tail protein) and T_4*amB272* is an amber mutant in gene 23 (coded for major head protein). T_4r_{II}*A105* is a deletion mutant of T_4 in cistron *A* of r_{II} gene.

the same gene by their ability to recombine and their failure to complement with each other in crosses on W3110, λ^R and W3110 (λ^+), λ^R, respectively. One mutant, *rbl-1*, was used for mapping.

Mapping and Crosses. The crosses between T_7 *rbl* and various T_7 amber mutants were done by the procedures of Studier (17). *E. coli* CR34 was used for crosses and to measure total progeny; wild-type recombinants were measured with W3110 (λ^+), λ^R as plating bacteria.

Measurement of Burst Size. Exponentially growing cultures of W3110, λ^R or W3110 (λ^+) λ^R at titers of about 2 × 10^8/ml in M9S were infected at a multiplicity of infection of 10. Three minutes later, cells and unabsorbed phage were separated by centrifugation (7000 × *g* for 3 min). The cells were resuspended and infectious centers were plated immediately on W3110, λ^R. For phage growth, the infected cells were diluted in M9S 10,000-fold; after 60 min at 37°, they were treated with chloroform and phage were assayed by plating on W3110, λ^R. For measurements of burst size in the presence of spermidine, this chemical was added to 10 mM just prior to infection and also to the dilution medium.

RESULTS

Characterization

Four T_7 *rbl* mutants were isolated from two independently mutagenized lysates. One mutant was leaky and two were identical; thus, only two *rbl* mutants were obtained. They were at different sites in the same gene because they recombine but do not complement on W3110, λ^R and W3110 (λ^+), λ^R, respectively. Table 1 shows the plaque-forming ability of T_7 *rbl-1* on various hosts. In this test the ability to form single plaques on a lawn, or the clearing by a drop containing 10^4–10^5 phage is observed; both methods gave the same positive or negative results. These results show clearly that it is the *rex*$^+$ gene that is responsible for the inability of the *rbl* mutants to grow in λ lysogens. Poor adsorption by λ^+ lysogens of T_7 *rbl* is not the reason for this lack of growth. Table 2 shows absorption is normal; λ^+ lysogens are killed by *rbl*

Table 2. Percent survival of host cells after infection with T_7^+ or T_7*rbl*

Min after infection \ Host cells	W3110, λ^R		W3110(λ^+), λ^R	
	T_7^+	T_7*rbl*	T_7^+	T_7*rbl*
5	0.52	0.29	1.31	1.79
10	0.31	0.32	0.86	1.66
Actual multiplicity of infection	6.0	7.0	4.3	5.5

Bacterial cells were grown to 2.0 × 10^8/ml in M9S. Appropriate amount of phage was added to prewarmed bacterial cells. Aliquots were drawn at times indicated and plated for survival cells.

Table 3. Effect of spermidine on the growth of T_7 *rbl* mutant

Phage	Spermidine (10 mM)	Host cells (burst size, phage per infected center) W3110, λ^R	W3110(λ^+), λ^R
T_7^+	—	143	90
	+	117	78.7
T_7 *rbl*	—	126	1.0
	+	107	0.9
T_4^+	—	181	200
	+	90.2	85.7
$T_4 r_{II}A105$	—	210	<1.0
	+	141	68.5

mutants as easily as by T_7^+ phage. Table 3 compares the burst size of T_7^+ and T_7 *rbl* on λ^+ lysogens and nonlysogens. In λ^+ lysogens, the burst size of T_7 *rbl* is less than 1% of normal and cells do not lyse at the usual time. It is normal on nonlysogens, and thus *rbl* is not essential on these hosts. In its inability to grow on λ^+ lysogens, the *rbl* mutants resemble the T_4 r_{II}^- mutants. The r_{II} mutants can grow on λ^+ lysogens if Mg^{++} or spermidine is added (18, 19). Therefore, this was tried with the T_7 *rbl* mutants, but as shown in Table 3 that does not work for the *rbl* mutants.

The restriction of growth of T_7 *rbl* mutants by W3110 (λ^+) was seen on a wide variety of λ^+ lysogens. Apparently, a host factor is involved because a mutant of W3110 was obtained which, as a λ^+ lysogen, will grow T_7 *rbl* mutants. This mutant of W3110 is not an amber suppressing *E. coli* strain. Both *rbl* mutants obtained here were judged to be amber mutants suppressible by several Su_{II}^+ (glutamine) but not by Su^+_{III} (tyrosine) strains lysogenic for λ^+ prophage. In spite of this similarity, *rbl-1* and *rbl-2* are located on different sites but in the same gene.

Mapping of the *rbl* mutation

The genes of T_7 can be grouped into three classes. Each is characterized by time of expression and relatedness of function (20). Class I and II genes seem to be mainly concerned with the control of gene expression and with DNA metabolism, respectively. Class III genes are expressed late and specify phage particle proteins and proteins involved in phage assembly. Genes in each class are clustered on the T_7 genetic map and the colinear DNA. The genes are numbered in linear consecutive sequence from left to right, early to late (20), and enter the cell in infection in that order (21).

It is quite probable that some T_7 genes have not yet been characterized by mutants; in T_7-infected cells some 30 new proteins are made (22) although only about 25 genes are known. Since the *rbl* mutation phenotype is seen only on lysogens, it was missed in previous searches for T_7 mutants and may be in a new gene. The *rbl* mutants complemented amber mutants in all T_7 genes tested. Crosses with T_7 amber mutants put it near gene 19, the right-most gene on the map. Two-factor crosses with genes in the right end of the map produced these recombination frequencies, which represent twice the percent of wild-type recombinants among total progeny: 16^- to 19^- = 10%, 17^- to 19^- = 10%, 12^- to *rbl* = 31%, 16^- to *rbl* = 18%, 17^- to *rbl* = 17%, and 19^- to *rbl* = 5%. The T_7 amber mutants 9, 290, 182, and 10 were used in genes 16, 17, 18, and 19, respectively (17).

In a three-factor cross, T_7 *rbl* was crossed with the double mutant $17^-/19^-$, and concurrent two-factor crosses were also performed. The recombination frequencies were: $17^-/19^-$ to 18^- = 1%, $17^-/19^-$ to *rbl* = 10%, 17^- to *rbl* = 20%, and 19^- to *rbl* = 8%. In another cross 18^- was crossed with the double mutant 19^-/*rbl*; the recombination frequency was 7%. Fifty recombinants able to grow on W3110 (λ^-), Su^- were analyzed: only one was T_7 *rbl*, the rest were T_7^+. These results prove that *rbl* is to the right of the mutants in the DNA processing genes, 18 and 19. The *rbl* mutation is in gene 20, a new gene, and not in gene 19 for the following reasons. The amber *rbl* mutation has a unique phenotype, and it complements mutants in twenty other genes tested. With gene 19, complementation was done not only by plate spot tests, but also in liquid culture: W3110 (λ)Su^- co-infected by both 19^- and *rbl* phages produce a normal burst size.

Suppression of ligase-less mutants by the *rbl* mutation

In T_4 phage, ligase-less mutants (gene 30^-) are phenotypically suppressed by r_{II}^- in double mutants. Since the T_7 *rbl* mutation (20^-) resembles the T_4 r_{II}^- mutation, it was reasonable to look for a similar suppression in T_7 phage. This expectation was fulfilled. Unlike the 30^- mutants of T_4, the ligase-less T_7 mutants (1.3^-) can grow on normal *E. coli* cells; the host ligase can suffice for T_7 replication. Only on ligase-defective *E. coli* are ligase-negative T_7 mutants unable to grow (23). The amber mutant HA13, gene 1.3^-, was used in the subsequent experiment. A direct test of suppression of HA13 by a 20^- mutation would be to construct the double mutant by a cross. However, the expected phenotype of the double mutant is like that of the 20^- parent. Therefore, such double mutants were sought among the spontaneous revertants of HA13 on lawns of ligase-deficient host cells. Among 3–5% of these revertants are of this type, and unlike HA13, can no longer grow on λ^+ lysogens; they have the *rbl* phenotype. One such presumed 1.3^-/*rbl* double mutant was studied further. From it 30 revertants able to grow on W3110 (λ^+) were isolated. They occurred with a frequency of about 10^{-4}, and all behaved like HA13. They were able to grow on the temperature-sensitive, ligase-defective strain N2668 at 30° but not at 37°. To confirm the 1.3^-/*rbl* nature of the double mutant it was crossed with T_7^+ phage, and HA13 was found with a 20% frequency in the progeny. Thus, the *rbl* mutation suppresses the phenotype of T_7 ligase-less mutants. The particular *rbl* mutation in the double mutant was not isolated because its phenotype resembles the double mutant. It, too, appeared to be an amber because the 1.3^-/*rbl* mutant grows on CR34 (λ^+) Su^+_{II}, and was judged to be in gene 20 by complementation tests with amber mutants in genes 17, 18, 19, and 20, on lawns of W3110 (λ) Su^- cells.

DNA synthesis and other properties

Fig. 1 shows [^{3}H]thymine incorporation by lysogens and nonlysogens infected with T_7^+ and T_7 20^- mutants. As can be seen, the accumulation of thymine into acid-insoluble material is almost normal in lysogens and normal in nonlysogens infected with T_7 20^- phage.

This DNA formed in T_7 20^- infected λ^+ lysogens remains completely DNase-sensitive when the cells are lysed with chloroform. Cells infected with T_7^+ and lysed the same way have at least half the thymine label in DNA that is resistant to DNase. Presumably, this label is in DNA inside phage heads; it is DNase-sensitive if it is first heated to 100°.

These results suggest that gene 20 is involved in DNA packaging like the adjacent genes 18 and 19. Further work may reveal how this is done. It is known that T_7 DNA is rep-

licated to a concatemer of mature DNA and then cut to size as it is packaged into phage heads (25, 26).

Preliminary experiments with crude extracts indicate that the gene 20 product is required in the conversion of noninfectious precursors into T_7 phage. These experiments were modeled after those by Sadowski on gene 19 (27). Lysed W3110 (λ^+), λ^R previously infected with T_7 20^- were supplemented with extracts from cells infected with T_7 $5^-/19^-$ and incubated. Extracts from uninfected cells, or cells infected with T_7 20^- or T_7 $1^-/5^-/19^-$, served as controls. A 300- to 1000-fold increase in viable phage, due to the gene 20 protein, was observed above the controls (unpublished results). Phage progeny were found to be all T_7 20^-. The triple amber mutant T_7 $1^-/5^-/19^-$ is a control because late gene transcription depends on T_7 RNA polymerase, gene I. This result supports the assignment of 20 as a late gene and may be an assay for the purification of the gene 20 protein.

DISCUSSION

The results show a new gene in T_7 phage, gene 20. This is a late gene, as judged from its map position, its apparent role in DNA processing, and from the fact that its gene product can be used to make viable T_7 by cell-free complementation. The product of gene 20 is a protein since amber mutants were found. The gene 20 protein is essential only in infections of λ *rex*$^+$ lysogens. Several different λ *rex*$^-$ lysogens, four in all, were tested and all permit growth of T_7 20^- mutants. In this lack of growth on λ *rex*$^+$ lysogens, the T_7 20^- mutants are like the T_4 r_{II}^- mutants and the *lr* mutants in gene I of T_5 phage. The T_7 20^- mutants further resemble the T_4 r_{II}^- mutants in that they can suppress phage ligase mutations. However, there are these differences between T_7 20^- and T_4 r_{II}^- mutants: unlike the r_{II} mutants, gene 20^- mutants are not suppressed by Mg^{++} ion or spermidine. Gene 20 is a late gene, r_{II} is early. DNA synthesis in λ^+ lysogens infected by T_7 20^- is normal except for a defect in packaging, whereas in T_4 r_{II}^- infection phage DNA synthesis begins and soon stops. Last, there are host mutations that as λ^+ lysogens allow the growth of T_7 20^- but not of T_4 r_{II}^- phage. We do not know whether these differences signify a completely different mode of action. Unfortunately, nothing is known about the role played by the r_{II} gene. It seems almost certain that gene 20 has a role in DNA packaging. Since gene 20 is unessential in nonlysogens and λ *rex*$^-$ lysogens, that role likely can be filled by a host enzyme. If so, perhaps nonlysogenic *E. coli* mutants can be found in which T_7 growth requires gene 20.

The map position of gene 20 lengthens the right end of the T_7 map. We got no deletions of gene 20 by methods (heat resistance) that select deletions in the left end of the T_7 map (28). Possibly, this negative result might indicate essential parts in the T_7 genome beyond gene 20.

Since ligase mutations are suppressed by r_{II} mutations, and as ligase and nuclease perform opposite roles, it has been proposed that the r_{II} function is a nuclease (9, 10). This is not unreasonable because in T_4 phage the ligase mutation can also be suppressed by a mutation in a gene for T_4 endonuclease (29). In T_7 phage ligase mutations suppress T_7 endonuclease I mutations and vice versa (30). A nuclease function for the gene 20 protein, for example, the T_7 endonuclease II (31, 32), would fit the DNA maturation role but there is no evidence for this. If this is so, then the λ *rex* protein might be an antagonist of nuclease action. One can find support for that in the observation that λ *rex*$^+$ lysogens are partially protected from DNA breakdown induced by colicin action (33)

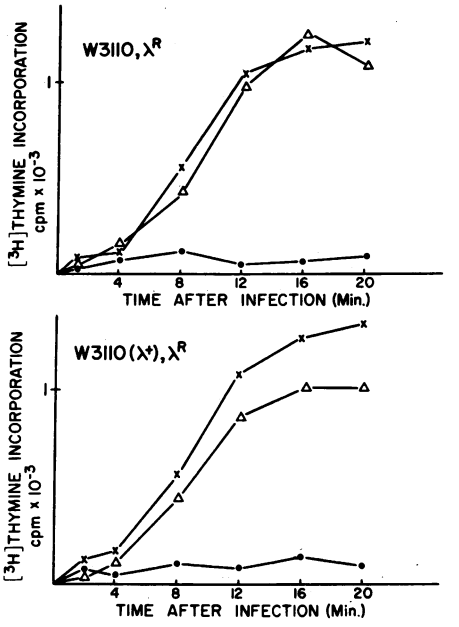

FIG. 1. Phage DNA synthesis after infection of W3110, λ^R and W3110(λ^+), λ^R by T_7^+ and T_7*rbl*. Exponentially growing cultures (2.0×10^8 cells per ml) were UV-irradiated to inhibit the synthesis of bacterial DNA. [^{3}H]Thymine (New England Nuclear, Boston, Mass.) was added to a final specific activity of 40 μCi/ml. Phage was then added to a multiplicity of 10. After incubation for various times at 37°, samples were taken and assayed for acid-precipitable radioactivity. Experimental details are in ref. 24. ×, T_7^+; △, T_7*rbl*; and ●, uninfected cell.

or T_5 infection (12). These speculations may be erroneous, although they suggest experimental tests.

It should be mentioned that the *rex*$^+$ phenotype has been observed in lysogens other than λ (34). Thus, the r_{II}-like genes, found in T_2, T_4, T_5, and now in T_7 phage, are probably widespread because they confer a broader host range to the lytic phage.

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