Regulation of λ rex Expression After Infection of *Escherichia coli* K by Lambda Bacteriophage

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The ability of *Escherichia coli* K to support bacteriophage T4rII replication starts to decline at 3 to 6 min after infection by λ . This inhibition appears to depend on expression of the λ rex⁺ gene, since little inhibition was observed following infection by a λ rex mutant or by a hybrid bacteriophage λ i⁴³⁴ which lacks a functional rex gene. For promotion of the synthesis of rex product, cII⁺ and N⁺ genes are required and can act *trans*, whereas cY⁺, also required, must be *cis* to a rex⁺ gene. These genes presumably play a role in the transcription of the cI-rex operon because they are also known to be required for repressor (cI product) synthesis. Functional cIII, O, P genes are not necessary for ample rex product synthesis. We also observed full rex expression after infection by λ susC mutants, suggesting that rex and repressor are separate gene products and that repressor is not required for inhibition of T4rII replication. We also report experiments with a rex mutant that is not leaky when in a lysogen but is sufficiently leaky shortly after infection to cause inhibition of T4rII replication.

Wild-type T4 bacteriophage can propagate in lambda lysogens of *Escherichia coli* K cells [*E. coli* K(λ)] but T4rII mutants cannot (4). The rII mutants can, however, grow in lysogens of the hybrid phage λ i⁴³⁴, a phage constructed to contain the immunity region of phage 434 and all other genes from λ (22). This suggested that the inhibition of rII is caused by the gene that codes for the lambda repressor, the product responsible for lambda immunity. Damage to the host genome at the specific insertion site of λ was ruled out as a possible factor in rII inhibition by experiments with λ hybrids. Regardless of insertion site, ability to inhibit rII was a property linked tightly to the λ repressor gene (27). More recent evidence has shown that the repressor gene itself is not involved. Inhibition of rII growth is caused by a product of the lambda rex gene, a gene which functions independently of the repressor gene (19), but is part of the same operon (15, 39).

This paper is concerned with "early" rex expression as measured by the inhibition of T4rII replication that occurs shortly after cells of *E. coli* K are infected with lambda. Inhibition occurs after infection by λ (41) but not after infection by λ i⁴³⁴ (33). If various λ mutants are used instead of λ wild type, their effect or lack of effect on T4rII inhibition should reveal which lambda genes must act prior to the expression of rex. For example, it has been reported that infection with λ cII mutants does not cause early T4rII inhibition

(33). Since it was known that cII gene action precedes the expression of the cI repressor gene (21), the results indicated that the cII gene product is required for expression of the operon that encompasses the cI and rex genes. In our experiments, we infected cells with lambda and at various times thereafter superinfected with T4 phage. Since superinfection with T2 (35) or T4 (37) turns off further lambda-directed syntheses, we should be able to measure the timing of rex product biosynthesis by observing whether or not inhibitory material is present at the time of superinfection with T4rII. This measure assumes that the observed rII inhibition is an uncomplicated function of rex expression. It has been reported, however, that some cI mutants—in contradistinction to most cI mutants—do not inhibit rII replication. The interpretation was that the combined action of rex and repressor is required for rII inhibition (33). An alternate interpretation is that the aberrant mutants were polar and turned off translation of rex. We tested the requirement for cI product by use of λ sus mutants and found that repressor is not required for rII inhibition. Therefore, our procedure should be specific for detection of the rex gene product. We have utilized the procedure to determine which λ genes must act for efficient expression of the rex gene and to measure the time after infection when rex product can be first detected.

MATERIALS AND METHODS

Bacteriophage. Table 1 and Fig. 1 present the λ phage strains that were used. Wild-type T4B (r^+) and T4Br1993 (rII) were sent by S. Benzer. The rII mutant is a long deletion in the A gene. Plkc was obtained from I. P. Crawford.

Bacteria. The source and derivation of our nonlysogenic *E. coli* K-12 strain has been described (7). It is su^+ for all the *sus* mutants used in this study. *E. coli* B came originally from R. Herriott, and the *E. coli* strains C600 (su^+) and W3350 (su^-) came from A. Campbell and carried his stock numbers 691 and 173, respectively. *E. coli* 594 (su^-) was received from E. Signer and recommended by him as very restrictive for λ *sus* mutants. *E. coli* W1485 came from I. P. Crawford.

Media. Tryptone broth contained 1% tryptone and 0.1 M NaCl; 2X broth and 4X broth had double and quadruple the concentrations of tryptone broth. Phage dilution fluid (PDF) contained 0.1 M NaCl, 1 mM $MgCl_2$, and 0.1 mM $CaCl_2$. Irradiation buffer contained 10 mM potassium phosphate buffer, pH 7.0, 1 mM $MgSO_4$, and 0.1 mM $CaCl_2$. Soft agar for bacteriophage titration (1) and plating agar contained, respectively, 0.7% and 1.5% agar in tryptone broth.

Preparation of phage-clear mutants of λ . Plate lysates from single-picked plaques were used to infect cultures of C600 in tryptone broth enriched with 1 μ g of thiamine per ml and 10 mM $MgSO_4$. The phage was purified by differential centrifugation, treated with deoxyribonuclease, and then subjected again to differential centrifugation. After banding the phage in $CsCl$ ($\rho = 1.52$, 18 hr 30,000 rev/min) and dialysis against buffer containing 10 mM tris (hydroxymethyl) aminomethane (Tris) buffer, pH 7.5, in PDF, one-tenth volume of 1% peptone was added, and the phage was stored over 2 to 3 drops of chloroform. In our studies of rII inhibition, we did not use λ preparations over 6 months old. Occasionally, older preparations of λ^+ caused slower and lesser inhibition of rII replication.

Temperate λ . New stocks were plated on *E. coli* C600 or K-12, and the central turbid growth of a single plaque was streaked on tryptone-agar. Phage was obtained from the cloned lysogens by ultraviolet (UV) irradiation. Further purification of the lysate was as described above for clear lambda mutants.

Our preparation of T4 phage by differential centrifugation (17) has been described (7). P1 phage was prepared by plate lysates on *E. coli* W1485, with all media containing 2.5 mM $CaCl_2$. *E. coli* K (P1) lysogens were freshly isolated before use (3).

Standard experimental conditions: adsorption of λ . An overnight culture of cells was diluted 10-fold in fresh broth and aerated at 37 C for 2 to 2.5 hr. The cells in 3 ml of broth at 10^9 to 1.5×10^9 cells/ml were washed twice in 3 ml of water by centrifugation and then suspended in 10 ml of 20 mM $MgSO_4$ (16). After aeration at 37 C for 30 min, the suspension was sampled for viable cells, and the bacteria were infected at a multiplicity of infection (MOI) of 6 to 10 with lambda freshly diluted in 20 mM $MgSO_4$. Aeration at 37 C was continued for 20 min while adsorption occurred. Samples for survivors were taken 15 min after

TABLE 1. Source of lambda bacteriophages

Type	Phage	Source	Reference
λ Defectives	λ N53, λ O8, λ P3	A. Campbell	8
λ cII	λ co ₁ , λ c68	A. D. Kaiser	21
	λ c2002	R. Thomas	6
λ Y	λ c2001	R. Thomas	6
	λ c42	R. Thomas	21
	λ c17 ^a	R. Thomas	31
λ x (Non defective)	λ c50	R. Thomas	21
λ cI	λ c60	A. D. Kaiser	21
	λ sus6, λ sus9	M. Lieb	
λ rex	λ rex293	G. Gussin	24 ^b
	λ rex30	This lab	
λ cIII	λ c67	A. D. Kaiser	21
	λ am611	E. Signer	
λ b2		N. C. Franklin	26
λ vir		A. Campbell	20
λ^+	λ PAPA	D. A. Goldthwait	10
λ i ⁴³⁴		Dorothy Frazer	22
λ i ⁴³⁴ c17 ^{+c}		This lab	

^a Type λ c17h was sent. The h marker was crossed out with λ^+ . The c17 was verified by backcross to λ c17h.

^b R. Friedman isolated *rex*293. We received a preparation carrying his original designation, λ go. A. D. Kaiser (*personal communication*) verified that the λ go phage, sent from his laboratory to G. Gussin and thence to us, is indeed *λrex*293.

^c A hybrid reconstructed to contain λ^+ genome from the c17⁺ marker rightward.

addition of λ , and colonies of surviving bacteria were replicated on plates seeded with C600 to test whether the survivors had been infected with λ . In experiments with *λsus* mutants, those surviving bacteria that had been infected with λ could be detected by replication on plates seeded with C600 (λ i⁴³⁴). In complementation experiments with heteroimmune phages, the survivors were tested with appropriate lysogens. The figure and legends present the per cent of input cells that were not infected by λ .

Superinfection with T4. Zero time for λ infection is defined as the time when one-third volume of 4X broth is added to the adsorption mixture. No λ DNA is injected prior to the addition of broth (25, 35). Immediately after addition of broth, 1 ml of mixture was withdrawn, 0.5 ml to be infected with 10^7 T4r⁺ phage and the other 0.5 ml with 10^7 T4rII phage. Seven minutes after addition of T4 phage, the cells were diluted 10^5 -fold, and the final dilution was shaken a further 53 min at 37 C. Progeny T4 phage was plated on *E. coli* B and again plated after treating the lysate with chloroform. Samples of λ -infected cells, taken 3, 6, 9, 15, and 30 min after the addition of 4X broth, were superinfected with T4 in similar fashion.

FIG. 1. Map location of λ mutants. The gene order in a segment of the lambda genome is taken from Eisen *et al.* (13) but is not drawn proportional to map distances. The allele order in Y and the relative positions of the cII mutants c2002 and c68 are from Brachet and Thomas (6). A reversed order of c17 and c42 (24) has been retracted. We do not know the position of co_1 other than its location in cII (21). Mutant c50 is placed in the 434 nonhomology region (14, 22). According to a personal communication from M. Lieb, *sus9* is in the left half of cI and *sus6* is to the right of c71 (not used here), a mutant tightly linked to c60 in the right half of cI (31). *Rex* mutant 293 maps between v2 and the cI gene (24). Our *rex* mutant 30 is located between N53 and c60 (this paper) and is provisionally placed in *rex* because of its phenotype in lysogens. Our λ virulent is of uncertain origin but is probably at least a triple mutant at the v1, v3, and v2 sites (32).

For purposes of burst-size calculation, the number of T4-infected cells in the one-step growth cycle was estimated from the input phage titer and a Poisson correction for cells infected with more than one phage particle. The calculations were verified by control experiments with anti-T4 serum and direct measurements of infected cells. We also observed that the yield of progeny phage was not significantly altered by treating with chloroform at the end of the growth cycle. Except for Fig. 2 where actual burst sizes of T4r⁺ and T4rII are shown, the data are presented as ratios of rII-r⁺ burst sizes. Furthermore, the data were normalized by arbitrarily setting the rII-r⁺ ratio to 1.0 at zero time. This was done to minimize systematic errors that might arise from imprecise titrations or consistently different adsorption of the original r⁺ and rII phages.

Recombination. Parental lambda phages at a MOI of 3 to 6 each were adsorbed to washed, starved cells in 20 mM MgSO₄ as described above. After the 20-min adsorption period, the cells were irradiated with 300 ergs of UV per mm² to stimulate recombination and to reduce lysogeny. Then the cells were diluted 10⁴-fold in tryptone broth and aerated for 2 hr, and the progeny were diluted and plated on C600.

λ rex30. This mutation was obtained fortuitously during a search for cII mutants. *E. coli* K (λ^+) cells were induced with 600 ergs of UV per mm², aerated in broth at 37 C for 35 min, mutagenized for 20 min at 37 C with 100 μ g of nitrosoguanidine per ml in Tris-maleate buffer, pH 6.0 (buffer composition in reference 2), and again aerated in broth till lysis. Several cII mutants, characterized by complementation assay (21), were collected, and lysogens were made from them. One of the lysogens had the *rex* phenotype. In a cross of the mutant phage with λ c160, 9/9 c⁺ recombinants had the *rex* phenotype, indicating that the *rex* mutation is to the left of c60 or closely linked to it. One of the c⁺ recombinants was saved as λ rex30. In a cross of λ rex30 with λ i⁴³⁴N53, 9/9 λ i⁴³⁴N53 recombinants had the *rex* phenotype. The recombinants were obtained from different recombination mixtures to collect independent isolates. The two crosses locate λ rex30 between N53 and c160. A complementation test indicated that the *rex*30 and *rex*293 mutations

are in the same gene. The double lysogen K-12 su⁺ (λ N53*rex*30, λ rex293) supported T4rII growth as well as single lysogens of either λ rex mutant.

λ i⁴³⁴c17⁺. λ i⁴³⁴O8 was crossed with λ c17 to yield λ i⁴³⁴c170⁺ and the latter was crossed with λ^+ to yield λ i⁴³⁴c17⁺. In these crosses, which have low recombination frequencies, we were concerned about the authenticity of the λ i⁴³⁴c17 recombinants because approximately 0.1% c mutants appear when λ i⁴³⁴ is "selfed" by our usual recombination procedure. We therefore collected and tested several independent recombinants. No c⁺ recombinants were observed in matings of the independent isolates with each other or with λ c17. Failure to recombine with λ c17 could have occurred if the clear mutation were in the heterologous immunity region of λ i⁴³⁴, but crosses of λ i⁴³⁴c17 with λ^+ yielded nearly equal numbers of λ c and λ i⁴³⁴c⁺ recombinants, proving that the c mutation was outside the region of nonhomology. In a three-point cross of λ i⁴³⁴c17 by λ c42, 14/16 selected c⁺ recombinants carried λ immunity, data which confirm the mapping of c17 to the left of c42 (6). A final verification of out λ i⁴³⁴c17 isolates was their lack of replication inhibition (30); that is, they could replicate in a C600 (λ i⁴³⁴) lysogen with the aid of λ i^h helper phage.

RESULTS

The kinetics of early T4rII inhibition are shown in Fig. 2. At zero time for λ infection, the burst sizes of r⁺ and rII were usually equal or nearly so. Three to 6 min after λ infection, the rII burst size fell and continued to fall thereafter. Inhibition appears to depend on the expression of λ genes in that infection of K-12 (P1) does not cause rII inhibition. This corresponds to the fact that infecting λ deoxyribonucleic acid (DNA) breaks down rapidly in the restrictive lysogen K-12 (P1) (11). Although our experiments with K-12 (P1) are flawed by the lack of data to prove that the cells were infected with λ , the experiments with λ i⁴³⁴ also demonstrate that rII inhibition depends

FIG. 2. Changes in burst size of T4 after infection of host with λ^+ or λi^{434} . Left, T4rII; right, T4r $^+$. Symbols: (X) *E. coli* K infected with λ^+ , (●) K(PI) infected with λ^+ , (○) K infected with λi^{434} . Uninfected by λ : K-12 plus λ^+ , 5%; K-12 (PI) plus λ^+ , not measured; K-12 plus λi^{434} , <1%.

on lambda gene expression. Furthermore, the observation that λi^{434} does not cause rII inhibition indicates which lambda region is involved. Since the hybrid phage λi^{434} contains all lambda genes except for the heterologous immunity region from phage 434 (22, see Fig. 1), the difference between the effects of λ and λi^{434} can be ascribed to the immunity region. An easily formed hypothesis is that the *lex* gene, located in the immunity region, is responsible for early rII inhibition.

The rII burst size after λ^+ infection did not fall to zero. We asked whether our pretreatment of host cells might affect the *rex* $^+$ or *rex* $^-$ phenotype. In tests of lysogens, it did not. Twice-washed lysogens starved for 50 min in MgSO_4 gave the expected phenotypes when they were superinfected with T4 at various times after addition of broth. At 37°C, the burst sizes of T4r $^+$ in K-12 (λi^{434}), K-12(λ^+), and K-12 (*lex*30) ranged between 140 and 160, whereas the burst sizes of T4rII were, respectively, 120, < 0.05, and 110. Because our conditions did not alter the inability of T4rII to grow in K-12 (λ^+), we next asked whether the T4rII was growing only in cells uninfected by λ . We did a single-burst experiment with λ^+ -infected K-12 cells superinfected with T4rII at 30 min. The results indicated that the progeny T4rII had come exclusively from a small population of cells, most

probably those uninfected by λ . An average of 2.22 T4rII-infected cells was distributed to each of 102 tubes. At the end of the latent period, the entire contents of each tube were plated. If all the cells could support T4rII replication, only 11% of the plates would be expected to have no plaques. On the other hand, because 7.6% of the cells had escaped infection by λ , 84.5% of the plates would have no plaques if the rII progeny came exclusively from those cells. We found 83/102 or 82.5% of the plates to have zero plaques, and the remainder to have sufficient plaques to account for the average T4rII yield.

cI. The repressor gene, cI, is also located in the immunity region, and it has been suggested that repressor participates in early rII inhibition (33). The data of Fig. 3 indicate that repressor is not required for early rII inhibition. It is especially noted that rII inhibition occurs after infection of *E. coli* 594 with the nonsense mutant λclsus6 . Since transcription and translation of the cI-*rex* operon proceeds from right to left (39), the location of the *sus6* mutation should allow synthesis of only a very short segment of the repressor molecule. Still, just as with the other cI mutants, rII inhibition occurred.

cII, cIII. The cII $^+$ and cIII $^+$ genes act early after infection to promote lysogeny (21) but are not

FIG. 3. Effect of *cI* mutation on *rII* inhibition. Changes in *rII*-*r*⁺ burst-size ratios after (●) λ cI60 infection of *E. coli* K, (■) λ cI*sus*9 infection of *E. coli* 594, (▲) λ cI*sus*6 infection of 594. Uninfected by λ : experiment with λ *sus*6, 2.5%; *sus*9, 1.2%; c60, not measured.

needed nor expressed in an established lysogen (5). Since *cI* and *rex* are in the same operon, the stimulation of early repressor synthesis by *cII*⁺ and *cIII*⁺ (12, 34) leads to the expectation that early *rex* function would also require prior expression of *cII*⁺ and *cIII*⁺ genes. The data in Fig. 4 indicate that the λ *cII*⁺ gene is required for *rII* inhibition; prior infection with λ *cII* mutants does not inhibit T4*rII* replication. The difference between λ ⁺ and λ *cII* was observed also in *E. coli* C600 and W3350 and with two other T4*rII* mutants (r638, a deletion of the *rIIB* gene, and r1272, a complete deletion of the *rIIB* genes A and B). In contrast to the effect of *cII*⁺, the λ *cIII*⁺ gene apparently is not required for early *rII* inhibition. After infection with either of two λ cIII mutants, c67 or am611, the extent of *rII* inhibition (Fig. 4) was similar to that with wild-type lambda. The validity of this surprising result should be tested further with *cIII* deletions.

N, O, and P genes. Because *rII* inhibition is observed so soon after infection, only early lambda genes can be involved. Three essential genes, N, O, and P, are expressed immediately after induction or infection (see reference 9 for summary). Among these genes, only gene N has

been implicated in the induction of *cI*-*rex* (36). This agrees with the data in Fig. 5. Inhibition of *rII* was observed with O and P mutants but much less inhibition was found after infection with a λ N mutant. Thus, the O⁺ and P⁺ genes are not required for *rII* inhibition, but N⁺ must play some role.

Y mutants. Clear mutants in this region can complement for lysogeny with homoimmune *cII* and *cIII* mutants but do not complement with most *cI* mutants (6), with heteroimmune *cII* (6), or *cIII* mutants (15). Consequently, it has been postulated that Y⁺ is a promoter site for expression of *cI*-*rex* (15). We should expect that Y mutants would not inhibit *rII* replication, and, furthermore, because Y⁺ turns on the *cis* *cI*-*rex* operon only, no complementation should be observed between λ Y mutants and λ i⁴³⁴, whose Y⁺ region is not connected in *cis* to a functional *rex* gene. These expectations are realized by the data of Fig. 6. Prior infection with λ cY2001 causes no *rII* inhibition, and λ cY42 elicits significantly less inhibition than λ wild type. Double infection with a λ cY mutant plus λ i⁴³⁴ does not result in the complementary production

FIG. 4. Effect of λ *cII* and *cIII* mutations on *rII* inhibition. (■) Average (AVE) of changes in *rII*-*r*⁺ burst-size ratios after infection of *E. coli* K with *cII* mutants λ c68, λ c67, or λ c2002. Crosses (×) represent values obtained with each mutant at 30 min. (●) Average (AVE) of changes in *rII*-*r*⁺ burst-size ratios after infection of *E. coli* K with λ cIII67, and *E. coli* 594 with λ cIIIam611. Crosses represent individual values at 30 min. Uninfected by λ : experiment with λ 2002, 2.5%; c68, 5%; c67, 5%; am611, 2.7%.

FIG. 5. Effect of λ N, O, and P mutations on rII inhibition. Changes in rII- r^+ burst-size ratios after infection of W3350 by (■) λ N53, (●) λ O8, (▲) λ P3. Uninfected by λ : λ N, 14%; λ O, 20%; λ P, 13%.

of material that can inhibit T4rII replication. This is seen clearly with λ cY2001 plus λ i⁴³⁴. In the experiments with λ cY42 plus λ i⁴³⁴, only the 30-min samples offer any possible indication of complementation in that slightly more rII inhibition was caused than by λ cY42 alone. The λ cY mutants do however complement with homoimmune cII and N mutants. Table 2 shows that λ cY2001 and λ cII2002, neither one of which by itself causes significant inhibition, can together cause rII inhibition. Similar complementation data were found with λ cY2001 and λ N53. Furthermore, not only do λ cII and λ N mutants complement with λ Y mutants, but they also complement with λ i⁴³⁴ and with each other. This agrees with experiments which have shown that cII⁺ (5) and N⁺ (40) are not immunity specific and can act *trans*, whereas Y⁺ is immunity specific and acts *cis* (6, 15). Thus, the development of rII inhibition parallels the requirements for repressor production. Since repressor is not involved in rII inhibition (Fig. 3), genes cII⁺, N⁺ and Y⁺ must be required for expression of the cI-*rex* operon.

Other λ mutants. The cI7 mutation is also located in the Y region but differs from other Y mutants in its failure to complement with any other clear plaque mutants. Its inability to ly-

sogenize is caused by constitutive production of O⁺ and P⁺ gene products (30) and not by inability to produce cI product. As expected with a phage that can express the cI-*rex* operon, rII inhibition occurs (Table 3). The mutant λ c50, located in the immunity-specific X region, complements with Y and cII mutants but not with most cI mutants (R. Thomas, *personal communication*). It was of interest to determine whether the c50 mutation prevents early expression of the cI-*rex* operon. From its location in the X region, it was conceivable that c50 might result in a particularly potent *cro* product (13, 29) or a poor promoter for cI-*rex* expression. The data in Table 3, however, show that the clear plaque phenotype of c50 is not caused by a failure to express the cI-*rex* operon. Table 3 also shows that rII inhibition occurs after

FIG. 6. Effect of cY mutants on rII inhibition and failure of λ cY to complement with λ i⁴³⁴. Changes in rII- r^+ burst-size ratios after infection of *E. coli* K with (○) λ cY2001 or (□) λ cY42, and after infection of W3350 with (●) λ cY2001 plus λ i⁴³⁴ or (■) λ cY42 plus λ i⁴³⁴. Uninfected by λ : λ cY2001, 3.4%; λ cY42, 5.3%; λ cY2001 plus λ i⁴³⁴, 0.5%; λ cY42 plus λ i⁴³⁴, 0.4%.

TABLE 2. Complementation between λ mutants to inhibit T4rII replication^a

Phage	rII-r ⁺ burst size at times after λ infection	
	15 Min	30 Min
λ N53 + λ cY2001	0.42	0.28
λ N53 + λ cII2002	0.18	0.09
λ N53 + λ i ⁴³⁴	0.21	0.12
λ cII2002 + λ cY2001	0.27	0.28
λ cII2002 + λ i ⁴³⁴	0.14	0.10

^a W3350 was the host for all experiments except that *E. coli* K was used for cII plus Y complementation. Cells were infected with both λ phages at a multiplicity of infection (MOI) of 5 to 7 each and superinfected 15 or 30 min later with T4 at a MOI of 0.1. Uninfected survivors of λ infection: cII plus Y, 3%. Uninfected or singly infected survivors: N plus Y, 15%; N plus cII, 12%; N plus i⁴³⁴, 14%; cII plus i⁴³⁴, 5%.

TABLE 3. Lack of effect of some λ mutations on rII inhibition^a

Phage	rII-r ⁺ burst size at times after λ infection	
	15 Min	30 Min
λ c17	0.24	0.05
λ c50	0.37	0.14
λ b2	0.17	0.08
λ vir	0.21	0.04

^a Host was *Escherichia coli* K. Uninfected by λ : c17, 5%; c50, 2.5%; b2 and vir, not measured.

infection with λ b2 and λ vir. λ b2 makes repressor but cannot integrate into the host chromosome. λ vir is mutant at operator sites so that cI repressor cannot turn off early operons that participate in vegetative growth. It is clear that the constant element for rII inhibition is ability to express the cI-*rex* operon.

λ rex. All the data thus far indicate that *rex*⁺ is responsible for early rII inhibition. It remains to be shown that λ rex mutants do not cause inhibition. Figure 7 shows that λ rex293, as expected, is not inhibitory, but it is also seen that λ rex30 is! Which mutant presents the correct picture of *rex* action? λ rex30 might be leaky, or, on the other hand, λ rex293 might behave like a reduced immunity (*rim*) mutant and have lowered expression of the cI-*rex* operon (38). Neither possibility was confirmed in tests that unfortunately could be carried out only in lysogens of the two mutants rather than directly after infection. Since the T4rII plating efficiency was unimpaired by lysogeniza-

tion with either *rex* mutant, we could detect no leakiness of either *rex* mutant. The test for reduced immunity is to observe whether N⁺ or O⁺ function of a lysogenized phage is not completely repressed and can therefore provide sufficient complementing activity to support replication of a heteroimmune λ i⁴³⁴N or O mutant. The plating efficiency of λ i⁴³⁴N or λ i⁴³⁴O was the same on lysogens of λ rex30, λ rex293, and λ ⁺ in *E. coli* 594 (ca. 0.1%), indicating that reduced expression of the cI-*rex* operon underlay neither *rex* phenotype of our mutants.

Most of our data implicate *rex* in early rII inhibition. However, we did want to eliminate the possibility that the different effects of λ ⁺ and λ i⁴³⁴ were caused by genetic differences that lay outside the immunity region. The original λ i⁴³⁴ was made by crossing phage 434 with λ cY42 (22). Perhaps, since λ c17 inhibits T4rII replication and λ c42 does not, a segment of the Y gene located between c17 and c42 is required for rII inhibition. Accordingly, λ i⁴³⁴c17⁺ was made, but it also failed to inhibit rII replication, and, in experiments similar to those of Fig. 6, it doesn't complement with λ cY42 any better than does λ i⁴³⁴. We are left with the original interpretation that *rex*⁺ causes early rII inhibition.

FIG. 7. Effect of *rex* mutants on rII inhibition. Changes in rII-r⁺ burst-size ratios after infection of *E. coli* K at 37 or 39°C with (■) λ rex293 or (●) λ rex30. Uninfected by λ : λ rex293, 4%; λ rex30, 6%.

DISCUSSION

It would appear from the data in Fig. 2 that the burst size of T4rII at zero time after λ i⁴³⁴ infection is twice that after λ infection. If this were true, it would suggest that T4 does not immediately turn off lambda-directed synthesis. However, in repeated experiments with λ , λ i⁴³⁴, and the various λ mutants, we found that the zero-time burst sizes were independent of the infecting lambda phage and ranged between 50 and 150. Presumably, the physiological state of the host cells could be altered significantly by small changes in the pretreatment of the cells. Presentation of the data as ratios of rII-r⁺ has the effect of using the r⁺ burst size to control for changes in the physiological state of the host. Whereas the actual burst sizes varied considerably, the rII-r⁺ ratios were quite constant from one repeat experiment to another. Because the data are reduced to ratios, it should be pointed out that the λ -infected cells supported T4r⁺ replication over the entire experimental period. We think 30 min is close to the permissible time limit because in approximately one-fourth of the experiments with λ c mutants we observed a 25 to 50% drop in T4r⁺ burst size between 15 and 30 min.

If we defer consideration of experiments with λ rex30, the data indicate that the *rex* gene product is responsible for early rII inhibition. Direct and quantitative measurements of repressor synthesis have shown that the cI part of the cI-*rex* operon is turned on at 3 min after infection (12, 34), approximately the same time that we can detect rII inhibition. Factors necessary for the expression of repressor are also required for the appearance of rII inhibition. For promotion of the synthesis of both repressor and *rex* product, cII⁺ (12, 34) and N⁺ (36) are required and can act *trans*, whereas cY⁺ must be *cis* (12, 34). A further correlation with repressor synthesis is that there is no role for cI product in the promotion of early expression of the cI-*rex* operon (34). Furthermore, neither λ i⁴³⁴ nor λ rex293, both of which lack *rex*, can cause early rII inhibition. In sum, when *rex* can be made, early rII inhibition occurs.

Our data indicate that cIII⁺ is not necessary for rII inhibition even though it is known to stimulate repressor synthesis (12, 34). However, 15 to 20% of the normal amount of repressor is synthesized in the absence of cIII⁺ (12, 34). Insofar as we only measure whether there is enough *rex* product to inhibit T4rII, the amount made without cIII⁺ may be sufficient. Another possibility is that our infection conditions obviate the need for cIII⁺. Not only do we observe full rII inhibition with cIII mutants, but the lysogenization frequency—a crude measure of repressor synthesis—

is near that of wild-type λ (*data not presented*). This could be related to the physiological state of the host cell, a factor known to participate in the decision between lysogeny and lysis (18, 28). In our procedure, the host cells are washed twice with water (an excellent way to remove acid-soluble material) and then starved by aeration at 37°C in 20 mM MgSO₄ for a total period of 50 min. Thereby, the energy-depleted cells should have high levels of cyclic adenosine monophosphate (AMP) which, in turn, would promote efficient lysogenization (18). Since starvation eliminates or reduces the need for cIII⁺ in expression of the cI-*rex* operon, it should be interesting to examine whether the cIII⁺ product is concerned with cyclic AMP function.

Our data with *susC* mutants indicate that repressor is not involved in early rII inhibition. We may also infer that cI and *rex* are independent genes. Both conclusions depend, of course, on the assumption that the *sus* mutants are not leaky in *E. coli* 594. This point is not certain. In Fig. 3, we noted a delayed appearance of rII inhibition after infection with λ susC mutants. If it is assumed that the mutants are slightly leaky, the delay could be a reflection of the additional time required for accumulation of an amount of cI product adequate for rII inhibition. On the other hand, our interpretation is that the *susC* mutants would be expected to be somewhat polar and thereby delay the accumulation of *rex* gene product. If we are correct, some data in the literature need to be reinterpreted. The observation that some cI mutants do not cause early rII inhibition was interpreted to mean that repressor participates in rII inhibition (33). If, instead, the explanation is that this class of cI mutants is polar and turns off *rex* expression, the existence of these mutants provides further proof that cI and *rex* are in the same operon. Another indication that repressor participates in rII inhibition came from work with temperature-sensitive cI mutants (41). When cells carrying class I temperature-sensitive mutants (cI-tsI) were heated, they immediately became capable of supporting rII replication, but cells carrying cI-tsII mutants did not. Perhaps, the results indicate a variable effect of heat on the *rex* product, variations dependent on host strain, medium, energy supply, age of cells, or infection conditions. Similar experiments in another laboratory showed much less inactivation of inhibitory material, and the correlation of inactivation with the class of cI mutants apparently was the reverse of that cited above (23). Furthermore, heating caused a significant increase in the transmission coefficient for T4rII even when the host cells harbored a temperature-resistant prophage repressor (23).

If all our data indicate that *rex* is responsible for early rII inhibition, how can we account for the inhibition observed with λ rex30? Perhaps the simplest explanation is that there is far more expression of the cI-*rex* operon after infection than in a lysogen. It has been shown that repressor synthesis can proceed in an infected cell at 85 times the rate in a lysogen (34). The defect in *rex*30 may be detectable only in a lysogen where little *rex* material is produced.

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