JOURNAL OF VIROLOGY, Oct. 1972, p. 760-765
Vol. 10, No. 4

Isolation and Properties of *rex*⁻ Mutants of Bacteriophage Lambda

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Received for publication 26 May 1972

Twenty-five *rex*⁻ mutants of phage lambda have been isolated. Three of the mutants, including one amber mutant, map at three distinct sites within the *rex* region of the lambda genetic map. The existence of the amber mutant provides further evidence that *rex* and *c*I are distinct genes, since it seems to be identical to wild-type lambda in its ability to establish or maintain lysogeny.

Lysogenization of *Escherichia coli* by the temperate bacteriophage lambda requires the presence of a repressor, the product of the phage *c*I gene, which directly (18) or indirectly (8) prevents expression of almost all lambda genes. Two genes that are expressed are the *c*I gene itself, since the repressor must be present continuously to maintain the lysogenic state (8, 20), and the *rex* gene, which is defined by its effect on the multiplication of *r*II mutants of phage T4. T4*r*II mutants cannot multiply in a lysogen for a λ*rex*⁺ prophage (3), but *rex*⁻ mutants can be selected on the basis of their inability to prevent multiplication of T4*r*II phage (14; and R. Friedman, Ph.D. thesis, Cambridge Univ., 1967). Since mutants selected in this way do not seem to be defective in any λ function, the role of *rex* in the life cycle of the phage may be incidental. On the other hand, *rex* and *c*I are adjacent in the λ immunity region (*see* Fig. 1) and are coordinately expressed during infection (2) and in the lysogenic state (9, 10). These facts suggest that *rex* may play an important, if not always essential, role in lysogeny.

In this paper, we describe the isolation of 25 new *rex*⁻ mutants. Mapping data show that at least three of the mutants are located at distinct sites within the *rex* gene. One of the mutants is a nonsense mutant, defined by sensitivity of its Rex⁻ phenotype to the action of the *Su*II⁺ amber suppressor (13, 19). The existence of this mutant provides further evidence that the *rex* and *c*I genes are in fact distinct and confirms the presumption that the *rex* gene specifies a protein.

In the suppressed bacterial host, the nonsense mutation is temperature-sensitive. It is phenotypically *rex*⁺ at temperatures between 25 and 30 C, but *rex*⁻ at temperatures above 35 C.

MATERIALS AND METHODS

Bacteria and bacteriophage. Table 1 lists the bacteriophage and bacterial strains used in these studies. The derivation of additional strains is described where necessary.

Media. T broth contains per liter of distilled water: tryptone (Difco), 10 g.; NaCl, 2.5 g.; KCl, 2.5 g. For phage assays, bottom agar contains 10 g of B agar (Difco) and top agar contains 6 g of agar per liter of T broth.

Assays. Phage and infectious centers were assayed by plaque formation using the agar layer method (1).

Mutagenesis and isolation of mutants. Cultures of WNQ8 (*see* Table 1) were grown at 32 C to a density of about 10^8 cells/ml in T broth. Nitrosoguanidine (*N*-methyl-*N'*-nitro-*N*-nitrosoguanidine) was added to a concentration of 10 μg/ml with continued aeration for 7 min. The cells were then diluted 1:1,000 in fresh T broth, and incubation was continued for 3 hr at 32 C.

Mutagenized cultures were spread on agar plates together with 10^5 T4*r*II1589 phage and incubated overnight at 32 C. Nibbled colonies (initiated by cells sensitive to T4*r*II infection) were screened by spot-testing for the ability to support T4*r*II multiplication and for immunity to superinfection by λ*c*I60*b*2. All mutants reported in this paper are phenotypically *c*I⁺ *rex*⁻ on the basis of these tests.

Recovery of rex⁻ mutations from WNQ8. The *rex*⁻ lysogens, WNQ8 (λ*Nsus*7 *Nsus*53 *c*I857 *Od*58 *rex*⁻), were superinfected with λ*imm*434 at a cell density of 2×10^8 cells/ml and a multiplicity of five phage per cell. Phage were allowed to adsorb in 0.01 M $MgSO_4$ for 20 min at room temperature, after which the cells were diluted 1:10 in T broth and incubated for 60 min at 37 C. *N*⁺*O*⁺ recombinants having lambda immunity (able to plate on *imm*434 lysogens) were then screened for their *rex*⁻ and *c*I857 phenotypes. Phage with the genotype *c*I⁺*rex*⁻ were obtained by crossing *cIsus*14 and *rex*⁻*c*I857 phage.

Infection of lysogens with T4rII 1589. Strain 594 and its lysogens of *rex*⁻ mutants were grown to a density of 2×10^8 cells/ml in T broth. To one ml of culture were added: NaN_3, 0.002 M; tryptophan, 40 μg/ml; and T4*r*II1589 or wild-type T4 to a multiplicity of two to five phage per cell. After 10 min at 32 C for phage adsorption, T4 antiserum was added to a final *K* value of 0.5/min (1); the cultures were incubated

TABLE 1. *Bacteria and phage strains*

Strain	Pertinent properties	Source	Reference
Bacterial			
W3102	*Su*⁻	H. Eisen	
594	*Su*⁻ = W3350 *Sm*ʳ	A. Kaiser	
WNQ8	*Su*⁻ = W3350 (λ*cI857Nsus7Nsus53Od58*)[a]	H. Eisen	9, 10
CA-150	*Su*⁻	J. Beckwith	5
CA-161	*Su*II⁺	J. Beckwith	5
C600	*Su*II⁺	M. Meselson	6, 19
CR63	*Su*I⁺, T4 indicator strain	R. Epstein	11, 19
Phage			
λ *bio*24-5*nin*5	Deletion of part of *rex*; (*see* Fig. 1)[b]	F. Blattner	17
λ *bio*16-3*nin*5	Same as above[b]	F. Blattner	17
λ *bio*t-75*nin*5	Same as above[b]	W. Szybalski	12
λ wild-type		A. Kaiser	
λ *imm*434	λ-434 Hybrid (*see* Fig. 1)[c]	H. Eisen	16
λ *vir*	Able to infect a lysogen[d]	A. Kaiser	15
λ *cI60b2*	Subject to λ immunity[d]	D. Dussoix	*See* 8
T4 wild-type		R. Epstein	
T4*r*II1589	Deletion joining *r*II A and *r*II B cistrons	R. Epstein	4

[a] *cI857* is a temperature-sensitive mutation in *cI* (20); the term *sus* is used to indicate that a mutation is suppressor-sensitive (6).
[b] *nin*5 is a mutation that enables *bio* transducing phage to form plaques (7).
[c] The hybrid was isolated by Tomizawa.
[d] Used to test presumptive lysogens.

for an additional 10 min, diluted into T broth by a factor of 10^5, and assayed immediately for infectious centers and surviving bacteria. After 90 min of incubation with aeration at 32 C, the diluted cultures were assayed for progeny phage on CR63.

Phage adsorption under these conditions was very poor. On the average, only about 75% of the cells were actually infected, and in most cases less than half the infecting phage were adsorbed. We could not enhance these percentages by concentrating the cells before adsorption, allowing greater time for adsorption, changing the growth medium, or by omitting NaN_3. With bacterial strains other than 594, there was even less adsorption. We were unable to select phage strains that adsorbed more efficiently.

Mapping rex⁻ mutations. Recombinants were selected in crosses between λ*rex*⁻*cI857* and λ*bio nin*5 deletion mutants. Phage were added to W3102 in 0.01 M $MgSO_4$ at a cell density of about 2×10^8/ml and a phage multiplicity of five of each parent. After 20 min at room temperature for adsorption, infected cells were diluted 1:10 into T broth at 37 C. After 60 min, progeny phage were plated on W3102 at 42 C. Turbid plaques, formed by recombinants arising from an exchange between *cI857* and the end of the *bio* deletion, were identified in the background of clear plaques. (The *bio nin*5 parents form clear plaques because of the deletion of the *cIII* gene.) Lysogens formed by each recombinant were isolated and spot-tested for their *rex* genotype to determine the frequency of *rex*⁺ phage among the turbid recombinants.

Heat inducibility of rex⁻ cI857 lysogens. The effect of *rex*⁻ mutations on the heat inducibility of *cI857* lysogens was tested by incubating W3102 lysogens for 15 min at the indicated temperatures. After incubation, a 0.1-ml portion was added directly to W3102 plating bacteria and plated without preadsorption. Plates were incubated at 32 C, and the number of plaques was taken to be the number of cells induced during the 15-min incubation period at the indicated temperature.

Materials. T4 antiserum having a *K* value of 600/min was purchased from Gateway Immunosera Co., Cahokia, Ill. (In some experiments, antiserum provided by F. Eiserling was used.) Nitrosoguanidine was purchased from Aldrich Chemical Co., Milwaukee, Wis.

RESULTS

Properties of mutants. Table 2 lists the burst sizes obtained in one-step growth of T4*r*II1589 and wild-type phage after infection of lysogens of three of the *rex*⁻ mutants isolated according to the procedure described above. In each case, the T4*r*II phage yield on infection of a *rex*⁻ lysogen is comparable to the yield on the corresponding nonlysogenic strain and is in marked contrast to the T4*r*II phage yield on infection of the wild-type (*rex*⁺) lysogen. The percentage of cells that scored as infectious centers, indicating the pro-

TABLE 2. *Infection of rex⁻ lysogens by T4rIII589*

Infected strain	Percent infectious centers after infection by		Phage yield/cell[a] after infection by	
	T4	T4rII 1589	T4	T4rII1589
594 Nonlysogenic	87	62	134	122
594 (λ *rexQ*)	92	59	109	134
594 (λ *rex5a*)	95	75	110	173
594 (λ *rex30a*)	79	50[b]	100	107
594 (λ *rex⁺*)	75	0.5	104	2.6×10^{-3}

[a] Phage yield per cell to which phage was added. The phage yield per infectious center can be obtained by dividing by the corresponding value for the percentage of infectious centers given in the first two columns.

[b] In other experiments with 594 (λ *rex30a*), this percentage was as high as 94; the low value observed in this experiment is therefore a reflection of poor phage adsorption rather than of restriction of T4rII1589 multiplication. Multiplicities of infection: T4, 4.0; T4rII1589, 2.3.

duction of at least one progeny phage particle in the initially infected cell, was in each case comparable to the percentage obtained on infection of the corresponding nonlysogen. Thus, by these two criteria, there is no residual *rex* function expressed in lysogens of the three mutants tested.

Spot tests of T4rII1589 on C600 lysogens of the 25 *rex⁻* mutants isolated in these studies indicated that only one of them, *rex*Q, was suppressible by the *Su*II amber suppressor contained in C600. Quantitative estimation of T4 burst sizes in *Su*II⁺ lysogens of *rex*Q prophage is difficult because none of the *Su*II⁺ bacterial strains we have tested adsorbs T4 efficiently. Nevertheless, the results of single-burst experiments in the *Su*II⁺ strain CA-161 and in the closely related (but not precisely isogenic) *Su⁻* strain CA-150 clearly demonstrate that *rex*Q is an amber mutation (Table 3). At 26 C in strain CA-161, it is phenotypically *rex⁺*, effectively preventing T4rII multiplication; but at the same temperature, it is *rex⁻* in the unsuppressed strain CA-150, in which T4rII multiplication is normal.

In addition to its suppressor-sensitivity, the *rex*Q mutation is temperature sensitive when suppressed (Table 3). At 41 C, it is *rex⁻* even in CA-161. These results are confirmed by assays of relative efficiencies of T4rII plaque formation at 30, 35, and 40 C in *rex* lysogens (Table 4). Note that *rex*Q is phenotypically *rex⁺* only at 30 C in the *Su*II⁺ strain CA-161. Since temperature sensitivity is observed in both C600 and CA-161 lysogens, it is most likely due to some property of the amber suppressor *Su*II and not to physiological properties of the strains used. One possibility is that the suppressed *rex*Q protein, containing glutamine at the site of the amber mutation (19), is itself temperature sensitive. Alternatively, T4rII multiplication may be differentially affected at high and low temperatures by the reduced levels of *rex* protein synthesized in suppressed strains, since the frequency of suppression by *Su*II is less than 30% (13, 19).

TABLE 3. *Phage yields of T4rII1589 on infection of SuII⁺ and Su⁻ lysogens of λ rexQ at 26 and 41 C*

Bacterial S strain	*Su*II⁺ allele	Phage yield/cell[a] after infection at	
		26 C	41 C
CA-161	*Su*II⁺	24	17
CA-150	*Su⁻*	23	20
CA-161 (λ)	*Su*II⁺	0.02	0.1
CA-150 (λ)	*Su⁻*	0.004	0.01
CA-161 (λ *rex*Q)	*Su*II⁺	0.1	12
CA-150 (λ *rex*Q)	*Su⁻*	12	21

[a] Phage yield per cell to which phage were added. Experiments were performed as described, except that cells were grown and infected at the indicated temperatures and that cells infected at 26 C were incubated for 135 min after infection before assaying. Phage yields are low because of poor phage adsorption and a consequent low percentage of infectious centers. For CA-161, the frequencies of infectious centers were 31% (26 C) and 39% (41 C); for CA-150, the frequencies were 26% (26 C) and 29% (41 C).

TABLE 4. *Relative efficiencies of plating of T4rII589 on SuII⁺ and Su⁻ lysogens of λ rexQ at various temperatures*

Bacterial strain	*Su*II⁺ allele	Temp[b] (C)		
		30	35	40
CA-161	*Su*II⁺	1.0[a]	1.2	1.2
CA-150	*Su⁻*	0.7	1.1	1.1
CA-161 (λ *rex*Q)	*Su*II⁺	0.003	1.0	1.2
CA-150 (λ *rex*Q)	*Su⁻*	0.95	1.2	1.2

[a] Relative efficiencies of plating are normalized to a value of 1.0 for the number of plaques obtained on plating T4rII1589 on CA-161 at 30 C. Efficiencies of plating of wild-type phage, normalized in the same way, ranged from 0.95 to 1.2.

[b] Plating bacteria were grown at the indicated temperature.

Mapping of rex⁻ mutations. The three *rex*⁻ mutants used in these experiments were mapped with λ*bio* transducing phage in which the *bio* "deletions" had been shown to extend into the *rex* region, but not beyond it into *c*I (17; W. Szybalski, *personal communication*). Turbid recombinants from crosses of the type λ*bio nin*5 × λ*rex*⁻*c*I857 (*see* Table 1 and Fig. 1) were screened for their *rex* genotype. If no *rex*⁺ turbid recombinants could be recovered from a cross, it was concluded that the *bio* deletion included the particular *rex*⁻ site. The three deletion mutants used define four regions of the *rex* gene (Fig. 1) and the results of nine crosses (Table 5) define the following map order: *bio*24-5 end point, *rex*5a, *bio*16-3 end point, *rex*Q, *biot*-75 end point, *rex*30a.

In a similar cross, the *rex*⁻ mutant (*go*293) of Friedman was concluded to be in the same region (region II, Fig. 1) as *rex*5a (17).

We have placed *rex*30a to the left of *cIsus*14 because the evidence is convincing that *c*I and *rex* are distinct genes. In a three-factor cross, λ*cIsus*14 × λ*rex*30a*c*I857, 13 of 14 *c*I⁺ recombinants were *rex*⁻. These data are consistent with, but do not prove, the assumption that *rex*30a is to the left of *cIsus*14.

Effects of rex⁻ mutations on lysogeny. The possibility that *rex*⁻ mutants are affected in their ability to maintain lysogeny was investigated by examining the efficiency of heat induction of *rex*⁻*c*I857 lysogens during a 15-min incubation at various temperatures. The results of these experiments (Fig. 2) demonstrate that the frequencies of heat induction are similar, if not identical, in lysogens of *rex*⁺*c*I857 and *rex*⁻*c*I857 phage. The small differences observed are no greater than those observed with separate isolates of the same *rex*⁺*c*I857 lysogen.

Thus, within the limits of sensitivity of this kind of assay, the *rex* locus does not affect the thermal stability of lysogens of a temperature-sensitive prophage. Studies of frequencies of lysogenization yield similar results; that is, no difference between

TABLE 5. *Fraction of* λ *rex*⁺ *phage among turbid recombinants in crosses of the type* λ *bio nin*5 × λ *rex*⁻ *cI857*

bio Parent	*rex*⁻ Parent		
	λ *rex*5a*c*I857	λ *rex*Q*c*I857	λ *rex*30a*c*I857
λ *bio*24-5*nin*5	3/19[a]	8/38	19/26
λ *bio*16-3*nin*5	0/30	6/25	14/32
λ *biot*-75*nin*5	0/25	0/27	12/26

[a] For each entry, the denominator indicates the number of turbid recombinants examined (see Materials and Methods) and the numerator indicates the number of recombinants that were *rex*⁺.

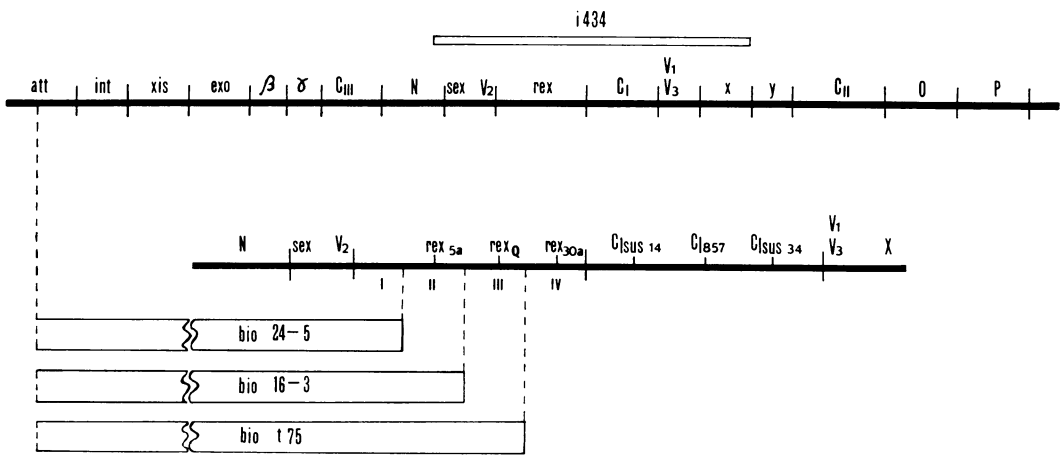

FIG. 1. *Map location of rex region of lambda. The rex gene can be subdivided into four regions (I–IV) based on the positions of the right-hand end points of the bio deletions, all of which have as their left end point the center of att. The boundaries between v2 and rex, between rex and cI, and between v1v3 and cI are not precisely defined. The v2 mutation is in the operator to which repressor binds to block transcription leftward from sex; v1v3 are mutations in the operator to which repressor binds to block transcription rightward from x. The immunity region is defined by the region of nonhomology between λ and 434 (indicated by* i^{434}*). The cIsus mutants were obtained from M. Lieb. Her unpublished data and ours indicate that cIsus14 is farthest to the left in cI, and that cIsus34 is farthest to the right. Att is the region in which recombination between the bacterial chromosome and the lambda deoxyribonucleic acid molecule occurs during integration of the prophage. cII and cIII are required for the establishment of lysogeny, but not for its maintenance. (See reference 8.)*

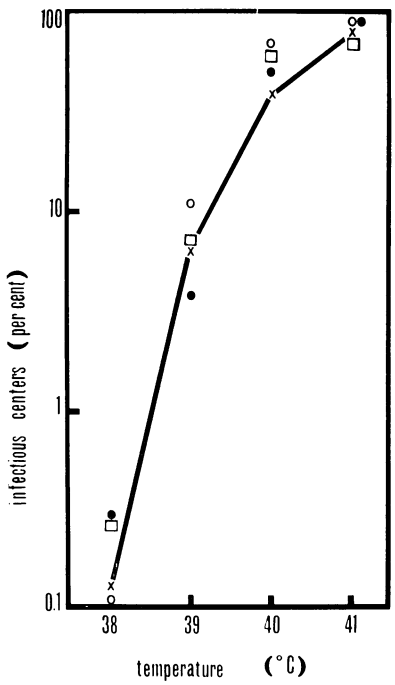

FIG. 2. *Heat-inducibility of rex⁻cI857 lysogens at various temperatures. Cells heat-induced during a 15-min incubation period at the indicated temperatures were assayed as infectious centers by plaque formation at 32 C on C600. Symbols: ×, induction of W3102 (λcI857); ●, induction of W3102 (λrexQcI857); ○, induction of W3102 (λrex5acI857); □, induction of W3102 (λrex30acI857). The line connects the points for the induction of the λcI857 lysogen. Sampling error at 39, 40, and 41 C is approximately indicated by the size of the symbols used; at 38 C, error is greater because the number of plaques was small.*

efficiency of lysogenization by rex^+ and rex^- phage is observed.

DISCUSSION

The fact that the amber mutant *rex*Q is phenotypically cI^+ clarifies several points about the relation between *c*I and *rex*. First, together with the finding that at least one *c*I amber mutant retains normal *rex* function (2), the existence of *rex*Q demonstrates unambiguously that *rex* and *c*I are distinct genes. These findings support Kayajanian's similar conclusion based on the existence of *bio* deletion mutants that are rex^-cI^+.

Second, *rex* cannot be essential to lysogenization under conditions normally used in studies of lambda. Because rex^- mutants selected for their inability to block T4*r*II multiplication need not be defective in the lambda-specific function of *rex*, only a nonsense mutant can be presumed to be truly rex^- in terms of the role of *rex* in the lambda life cycle.

Finally, if an interaction between *rex* protein and the repressor occurs, it cannot be obligatory either for repression or for the prevention of T4*r*II multiplication.

The possibility that the *rex* and *c*I genes were not distinct, or that they specified two polypeptides that interacted in some way, was first inferred from experiments of Tomizawa and Ogawa (21). In these experiments, incubation of cultures at 47.5 C simultaneously inactivated a temperature-sensitive (*cItsI-1*) repressor and abolished normal *rex* function. Recently, however, K-K. Mark (Szybalski, *personal communication*; Mark and Szybalski, *manuscript in preparation*) has found that the effect on *rex* function depends on the abnormally high temperature used and not on the inactivation of the repressor. According to Mark's results, inactivation of the *cItsI-1* repressor at 42 C does not affect *rex* function.

None of these results provides a clue to the way in which *rex* protein blocks the multiplication of T4*r*II phage. The fact that *rex*Q is temperature-sensitive in the permissive host CA-161 offers a way of investigating this question in more detail.

ACKNOWLEDGMENTS

This investigation was supported by Public Health Service research grant 1-R01-AI-10019 from the National Institute of Allergy and Infectious Diseases and by Public Health Service Biomedical Sciences support grant FR-07035 from the Division of Research Facilities and Resources.

We are grateful to H. Eisen, A. Kaiser, F. Blattner, and W. Szybalski for providing some of the phage and bacterial strains used in these studies, and to L. Astrachan and J. F. Miller for communication of some of their results prior to publication.

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