Phage Lambda Mutants Deficient in r_{II} Exclusion

Abstract. *The lambda gene responsible for* r_{II} *exclusion is distinct from other lambda genes and lies between the* N *and* C_I *genes on the genetic map.*

Phage T4 r_{II} mutants do not propagate on *Escherichia coli* lysogenic for phage λ (*1*). Exclusion of r_{II} mutants does not occur in cells lysogenic for the hybrid phage λ_i^{434} that contains almost all of the λ genome except for a small region including the C_I gene which comes from the related phage 434 (*2*). This result suggests that the r_{II} exclusion property of λ is governed by a region in or near the λ C_I gene. However, it has not yet been possible to determine whether the r_{II} exclusion factor is actually distinct from the λ repressor, the product of the C_I gene. Recent studies suggest there may be such a distinction. Cells lysogenic for some λ mutants that produce a temperature-sensitive repressor can be placed at a temperature at which the repressor becomes inactivated while r_{II} exclusion remains unaffected (*3*). This report describes λ mutants deficient in r_{II} exclusion (*rex*$^-$). The properties of the λ *rex*$^-$ mutants further indicate that the *rex* locus is distinct from the C_I gene and lies between the *N* and C_I genes.

The λ *rex*$^-$ mutants were selected by a slight modification of the method used by Benzer and Champe (*4*) to obtain bacterial suppressors of r_{II} point mutants. A mutagenized culture of 200 to 300 cells and approximately 10^5 r_{II} phage particles were spread with a glass rod on a tryptone agar plate (*5*). Cells have the opportunity to multiply for several generations before becoming infected with the phage. The rare cells that do not exclude r_{II} phage form nibbled colonies, while the remaining cells form normal round colonies. To assure that the *rex*$^-$ mutants would not be specific for only certain alleles of r_{II} mutants, the selection procedure employed an r_{II} mutant, r_{II}*Ed41*, deleted for the entire r_{II} region. Preliminary studies demonstrated that nibbled colonies of *E. coli* B (λ) cells are more distinctive than those of K12 (λ) cells when spread with T4 r_{II}^+ phage. Therefore, a strain of B, Bc834 (λ), which is a mutant unable to restrict λ phage (*6*), was used. To facilitate mapping of the *rex* locus the λ prophage contained the following three markers: *susN*$_{96A}$, *ind*$^-$, and the temperature-sensitive C_I mutation, *ts857*. Mutagenesis of the Bc834 (λ *susN*$_{96A}$ *ind*$^-$ *ts857*) cells was accomplished by treatment with nitrosoguanidine as described by Adelberg *et al.* (*7*). Most nibbled colonies proved to be cured of λ. The prophage present in nibbled lysogenic colonies was transferred to the K12 strain C600 for study.

Of the four λ *rex*$^-$ mutants independently isolated in this way, one, *rex*$_1$, was further characterized. The efficiency of plating of r_{II}*Ed41* and r_{II}*N21*, a point mutant, on a lysogen of this mutant was the same as that on nonlysogenic C600 cells. The following parameters were essentially the same for the *rex*$_1$ mutant and the corresponding *rex*$^+$ phage: (i) plaque morphology (including the degree of turbidity of plaques), (ii) percentage of cells (picked from the center of a fully developed plaque) that were lysogenic (> 90 percent), (iii) rate of spontaneous curing ($< 10^{-4}$ per cell division) and induction ($< 10^{-8}$ per cell division) of lysogens, and (iv) burst size (about 50). The three other λ *rex*$^-$ mutants were similar to λ *rex*$_1$ with respect to at least the first two of these parameters. Apparently, a mutation in the *rex* locus can drastically alter the r_{II} exclusion properties of λ, yet have no effect on phage multiplication or on the function of the C_I gene. Therefore, the *rex* locus seems to involve a gene separate from the C_I gene and all other genes vital for phage multiplication.

The *rex*$_1$ locus was mapped by a series of multiple factor crosses involving markers in the *rex*, *N*, and C_I genes. The relative positions of the markers used are described in the segment of the λ genetic map of Fig. 1.

The crosses were performed as follows. Exponentially growing cultures of cells lysogenic for one of the parental phages were centifuged, and the cells were resuspended in a buffer of 0.01*M* $MgSO_4$ and 0.001*M* tris (*p*H 7.2). The second parental phage was added at an input multiplicity of 0.2 to 0.5. After incubation of the cultures at 37°C for 15 minutes, antiserum was added for a 10-minute treatment to inactivate the unadsorbed phage. Cells were then diluted 100-fold into tryptone broth at 42°C. After 20 minutes, samples were taken and incubated, with aeration, at 37°C until the cells had time to burst. For the cross involving the *t27* marker, the cells were induced by ultraviolet light before superinfection. The lysates were chloroformed and plated on the nonpermissive strain W3350 at 42°C. The desired $N^+C_Its^+$ or N^+C_Its recombinants were selected as turbid or clear plaques, respectively. Lysogens of such recombinants were tested for their *ind* phenotype by inducibility after ultraviolet irradiation, and tested for their *rex* phenotype by the ability of a streak of the cells to grow across r_{II} phage spread along a line on a tryptone plate. Only one recombinant was studied per sample of superinfected cells to assure that each recombinant tested was the result of an independent recombination event. Control studies indicated that the frequency of recombination that gave the relevant genotypes was at least 50 times greater than the rate of spontaneous mutation to the same geno-

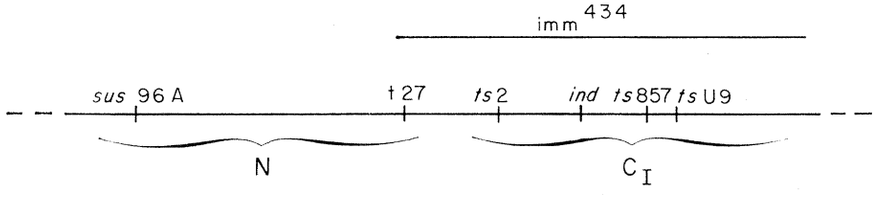

Fig. 1. Genetic map of a segment of the λ genome. The map distances are only approximate. Phage carrying the *N* gene mutations are unable to multiply. The *sus*$_{96A}$ mutation is suppressed by C600. The C_I *ts* mutants produce a temperature-sensitive repressor. The *ind*$^-$ mutant is not inducible by ultraviolet light. The *imm*^{434} segment denotes the region of nonhomology between λ and i^{434}.

Table 1. Relative number of *rex*$^-$ and *rex*$^+$ genotypes among recombinants. The parental phage in parentheses was used as a prophage.

Cross	Recombinant	Number of recombinants
(*rex*$^-_1$ *sus*$_{96A}$ *ind*$^-$ *ts857*) × *tsU9*	N^+ *ts*$^+$ *ind*$^+$ *rex*$^+$	7
	N^+ *ts*$^+$ *ind*$^+$ *rex*$^-$	1
(*sus*$_{96A}$ *ts2*) × *rex*$^-_1$ *ind*$^-$ *ts857*	N^+ *ts*$^+$ *ind*$^+$ *rex*$^+$	1
	N^+ *ts*$^+$ *ind*$^+$ *rex*$^-$	13
(*t27*) × *rex*$^-_1$ *sus*$_{96A}$ *ind*$^-$ *ts857*	N^+ *ts857 ind*$^-$ *rex*$^+$	0
	N^+ *ts857 ind*$^-$ *rex*$^-$	10

type. The results of the crosses given in Table 1 strongly indicate that the *rex* gene lies between the *N* and C_I genes.

It should be noted that the identification of the *rex* locus as a separate gene demonstrates that the C_I gene is not the only λ gene to be expressed in the lysogenic state.

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References and Notes

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2. A. D. Kaiser and F. Jacob, *Virology* **4**, 509 (1957).
3. S. Kasatiya and M. Lieb, *J. Bacteriol.* **88**, 1585 (1964); J.-I. Tomizawa and T. Ogawa, *J. Mol. Biol.* **23**, 277 (1967).
4. S. Benzer and S. Champe, *Proc. Nat. Acad. Sci. U.S.* **47**, 1025 (1961).
5. Media: Tryptone broth contains 0.5 percent NaCl and 1 percent Bacto-tryptone; for plates, 1.2 percent agar was used for the bottom layer and 0.75 percent for the top layer.
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8. Supported by NIH grant No. AI 7956-01. I thank Drs. Margaret Lieb, Ron Sederoff, and William Wood for donations of strains, and Mrs. Mary Vickers for help in characterizing recombinants.

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Renin-Like Enzyme in the Adrenal Gland

Abstract. *The rabbit adrenal gland contains an enzyme which reacts with renin substrate to form a vasopressor polypeptide, probably angiotensin I. In view of the strong effects of angiotensin on secretion of aldosterone and catecholamine, this finding suggests that there may be an intra-adrenal mechanism for the control of adrenal secretions.*

The finding (*1, 2*) that tissues other than the kidney contain renin or a renin-like enzyme suggests that the renin-angiotensin system is possibly a local hormone system (*2, 3*). Although the adrenal gland has not been surveyed in this regard, there is evidence that it may contain renin. Goormaghtigh and Handovsky (*4*) found modified smooth muscle cells, similar to the juxtaglomerular cells of the renal afferent arterioles, in the capsule of the adrenal gland. Granzer (*5*) found an enzyme in adrenal homogenates which degraded renin substrate. However, evidence that angiotensin was one of the degradation products was not presented. These observations, along with the demonstrations that angiotensin causes the release of medullary catecholamines (*6*) and aldosterone (*7*), led to a study of adrenal tissue for an enzyme capable of releasing angiotensin.

Four experiments, each requiring five normal adult rabbits, were performed as follows. Immediately after killing an animal with an intravenous overdose of pentobarbital (60 mg/kg), the adrenal glands were removed aseptically, weighed, and then homogenized with mortar and pestle. Distilled water was added (10 ml per gram of wet tissue); this suspension was frozen and thawed twice in a slurry of dry ice and acetone, and then tissue debris was removed by centrifugation at 2000 rev/min at 4°C for 30 minutes. The supernatant was dialyzed at 4°C against 5 liters of Na_2HPO_4-citric acid buffer, *p*H 2.6 (*8*) for 24 hours, 5 liters of distilled water for 6 hours, and then 5 liters of 3 m*M* disodium ethylenediaminetetraacetate (EDTA) for 24 hours. At the end of this time, if the contents of the dialysis sac were not free of catecholamines, dialysis was continued against 5 liters of 1 m*M* sodium phosphate buffer, *p*H 7, for 24 hours. The precipitate that formed during dialysis was removed by centrifugation at 2000 rev/min at 4°C for 30 minutes.

The supernatant (5 ml) was mixed with 5 ml of soybean trypsin inhibitor (*9*) (1 mg/ml), 5 ml of 40 m*M* EDTA, 0.5 ml of 0.4*M* dimercaprol (BAL) (*10*), and 10 ml of renin substrate that was prepared as described (*11*). Soybean trypsin inhibitor and EDTA were in 0.1*M* sodium phosphate buffer, *p*H 6.0, with chlorhexidine gluconate (*12*) (0.01 percent weight to volume). The final *p*H of the reaction mixture was 6.0. When prepared in this manner, the reaction mixture was free of kallikrein, angiotensinase, and converting-enzyme activities (*13*).

The mixture was incubated at 37°C for 20 hours. The reaction was stopped by the addition of 5 ml of trichloroacetic acid (20 percent weight to volume). After 60 minutes at 4°C, precipitated protein was removed by centrifugation. The clear, colorless supernatant was saved, and the precipitate was washed with 5 ml of 3-percent trichloroacetic acid and then again centrifuged. The combined supernatants were extracted three times with two volumes of diethyl ether. The aqueous layer was neutralized to *p*H 7.0 with 2*N* NaOH and then dried in a rotary evaporator to about 4 ml. This solution was extracted twice with 4 ml of *n*-butanol, previously equilibrated with 0.05*M* sodium phosphate buffer, *p*H 7.0. The butanol extracts were combined, and a portion (6 ml) was applied as the upper phase in the first tube of a ten-transfer countercurrent train. The countercurrent system developed was *n*-butanol for the upper phase and 0.05*M* sodium phosphate buffer (*p*H 7.0) for the lower phase (*14*). The upper phase was two volumes (6 ml per tube) in respect to the lower phase.

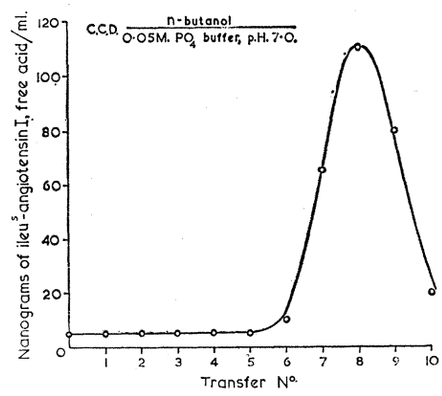

Fig. 1. Distribution of the vasopressor product of the reaction of adrenal enzyme with renin substrate.

At each step, angiotensin was assayed; the criterion was the change in the mean arterial blood pressure of the anesthetized rat treated with pentolinium (*15*). 5-Isoleucine-angiotensin I (200 ng/ml) or 5-valine-angiotensin II-β-amide (*16*) (100 ng/ml) was used as the assay standard.

Results of a countercurrent distribution are shown in Fig. 1. The apparent distribution coefficient of the activity peak was 2, and it corresponded to that of 5-isoleucine-angiotensin I. In this system, 5-isoleucine-angiotensin II, 5-valine-angiotensin II, epinephrine, norepinephrine, and vasopressin have distribution coefficients of 0.2 or less. Recovery of angiotensin in transfer tubes 7, 8, and 9 was 53 percent of that applied to the countercurrent-distribution train and 38 percent of that in the original incubation mixture.

The vasopressor material in the aqueous phase of transfer tubes 7, 8, and 9 was incubated at 37°C, *p*H 7.8 (0.01*M* tris HCl buffer) with trypsin or chymotrypsin (*17*). The ratio of enzyme to substrate was 1 : 1 by weight. Reactions were stopped by heating samples in a boiling water bath