*A DELETION ANALYSIS OF PROPHAGE LAMBDA AND ADJACENT GENETIC REGIONS**

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Communicated by Charles Yanofsky, August 12, 1968

The attachment site of the λ prophage on the chromosome of *Escherichia coli* K-12 is between the *gal* operon and the *bio* region.[1] (Symbols used for genes of *E. coli* are given in Taylor and Trotter.[6]) The order of prophage markers is a cyclic permutation of that in the vegetative phage.[2–5] Recently, linkage to the *gal-bio* region has been shown for several other markers: *aroG*, *aroA*, *chlA*, and *uvrB*.

Chlorate is toxic to *E. coli* K-12 under anaerobiosis. The toxic compound is probably not chlorate *per se* but rather chlorite or some other reduction product of chlorate that arises through the agency of an enzymatic system capable of reducing nitrate as well as chlorate. Mutants that are resistant to chlorate and are also defective in nitrate reductase activity can be isolated.[7] The mutants were classified in three groups—*chlA*, *chlB*, and *chlC* which are linked to *gal*, *mtl* and *try*, respectively.[8] The *chlA* locus was provisionally mapped between *gal* and *bio* by crossing *chl-s* and *chl-r* strains marked in the *gal* and *bio* regions.[7]

The present report describes more definitive mapping of the *chlA* locus, as well as other markers in this region, including a new locus for chlorate resistance (*chlD*). Mapping of these loci included the use of bacterial deletion mutants, some of which penetrate the λ prophage. Some properties of the prophage deletions that are relevant to the general biology of phage λ are described.

Materials and Methods.—The strains of *E. coli* K-12 used in these experiments are listed in Table 1.

Suppressor-sensitive (*sus*) mutants of phage λ were previously described.[17] Some new *sus* mutants (of cistrons N, O, R, E, G, H, and J) isolated from a virulent stock of λ (by D. Court, G. Lorick, and S. Adhya, unpublished results) were also used when possible because they showed more efficient marker rescue. The λ*dg* transducing phages were isolates that will be described in a later communication.[4] The λ*db* transducing phages were isolated by G. Kayajanian.[9] Phages λ*b2 c* (used in the test for prophage integration) and wild-type λ were from the stock collections of A. Campbell.

Isolation of chl-r mutants: Single colony isolates of strain CA2441 were grown in tryptone broth to about 10^8 cells/ml, and aliquots were plated on chlorate agar plates (8 gm of nutrient broth, 15 gm of agar, 2 gm of glucose, and 2 gm of $KClO_3$ per liter; glucose and $KClO_3$ were autoclaved separately). The plates were incubated for 24 hr at 37°C inside a desiccator under an atmosphere of 90% N_2 and 10% CO_2. The colonies were then enlarged by incubation overnight in the presence of air. Colonies were then purified by two restreakings on chlorate agar. Prophage deletions were isolated in the same manner by picking *chl-r* mutants from a lysogenic strain (R894) and printing them onto a sensitive indicator (R594). Colonies that gave no visible halo of lysis after exposure to ultra-violet light (240 ergs/mm²) and overnight incubation were purified and tested for prophage markers.

Because the strain R894 used to isolate prophage deletions already carried two point mutations in the *gal* operon, mutants were tested for deletions in the *gal* operon by crossing the *F⁻str-r* deletion mutants with Hfr *str-s* strains that carry known *gal⁻* point mutations (H1052, H1061, and H1060) and by looking for F⁻ *gal⁺ str-r* recombinants.

TABLE 1. *Bacterial strains.*

Strain	Properties	Origin or reference
CA2441	HfrH *lac⁻try⁻thi⁻str-s*	R. Thomas
R594	F⁻ *galT-1 galK-2 str-r*	Reference 13
W602	F⁻ *gal⁻bio⁻leu⁻thi⁻str-r*	Reference 1
R894	F⁻ *galT-1 galK-2 str-r* (λ)	Lysogenization of R594
R902	F⁻ *gal⁻bio⁻leu⁻thi⁻str-r chlA-r*	Chlorate-resistant mutant of W602
R823	F⁺ *gal⁺/galT-1 galK-2 str-s*	Strain 15-68 in reference 13
R891	F⁺ *gal⁺bio⁺chlA⁺/gal⁻bio⁻chlA-4 leu⁻ thi⁻str-r*	Cross R902 × R823
MS5061	F⁻ (*gal-*λ)*Δ5061 his str-r*	Reference 4
H1052	HfrH *galE-95 str-s his⁻thi⁻*	Hfr derivative of SA95 of reference 4
H1061	HfrH *galE-1061 str-s thi⁻*	Strain PL2 of Buttin (Ref. 20)
H1060	HfrH *galK-138 str-s thi⁻*	Strain H138-1 of Buttin (Ref. 20)
C600	F⁻*thr⁻leu⁻thi⁻lac⁻tonA-r*	Reference 18
R174	F⁻*thr⁻leu⁻thi⁻lac⁻tonA-r* (λ*imm 434*)	Lysogenization of C600
R903	F⁻*galT-1 galK-2 str-r* (λ*cI-t1 susA-11*)	D. Court

Nitrate reductase activity[19] was tested by stabbing the culture into tubes of nitrate agar and incubating overnight. (Nitrate agar was the same as chlorate agar except that $KClO_3$ was replaced with KNO_3.) Then 0.5 ml of 0.8% sulfanilic acid in 5 *N* acetic acid was added, followed by 0.1 ml of 0.5% α-naphthylamine in 5 *N* acetic acid. Appearance of an intense red color indicates the production of nitrite by nitrate reductase. Nutritional markers were scored by replication on appropriately supplemented minimal media. Sensitivity to ultraviolet light (UV) was tested by printing streaks of bacterial strains on tryptone agar and exposing them to 400 ergs/mm² of UV. The amount of growth after overnight incubation was much less for UV-sensitive than for UV-resistant strains.

Transductions were performed as described by Kayajanian.[9] Prophage integration in hosts with or without a normal *att*λ region was tested by the method of Gottesman and Yarmolinsky.[11] Deletion of the *att*λ region should prevent stable lysogenization by wild-type λ, so that wild-type λ in a deletion host behaves in this assay system like a λ*int* mutant infecting a normal host.

Results.—Chlorate-resistant mutants were isolated from strain W602 (*gal⁻-bio⁻*). The frequency of such mutants in a log phase culture was about 10^{-5}. Among 50 mutants tested, 48 showed no detectable nitrate reductase activity. The other two exhibited some activity, but less than the wild-type strain.

Several methods were used to map these mutants.

(a) *Specialized transduction:* Each transducing variant of λ phage is thought to contain a connected block of bacterial genes.[2] An unknown gene can be precisely located from a study of specialized transduction. If the gene is between *gal* and λ, for example, it should be present in every *dg* stock. In fact, none of the 48 completely defective mutants acquired nitrate reductase activity when transduced for *gal*. Three λ*dg* phages (λdg⁺, λdgA, and λdgD) independently isolated from a wild-type lysogen[4] were used. Transduction for *bio* was performed on two of these mutants by using three λ*db* stocks. Two of these (M3-29 and M36-3) carried all known *bio* markers, whereas the third (M58-2) was broken within the *bio* gene cluster.[12] Of these three *db*'s only M3-29 contained the nitrate-reductase (chlorate-resistance) gene: some of the *bio⁺* transductants ob-

tained with this λ*db* gave a positive nitrate-reductase test, were chlorate-sensitive, and yielded on induction HFT-transducing lysates. Two *bio*$^-$ segregants from one transductant tested were also chlorate-resistant and nitrate reductase-defective. Since Puig *et al.*[7] found that their *chlA* mutants were not transducible by λ*dg* phage but were closely linked to the *gal-bio* region, we classify the above two *chl-r* mutations of our collection as *chlA*. The transduction studies indicate the gene order *gal-att*λ*-bio-chlA*.

The two *chl-r* mutants of strain W602 that were partially defective in nitrate reductase activity behaved differently. When these strains were transduced for *gal* by λ*dg*, they became chlorate-sensitive and nitrate reductase-positive. This result was obtained with two λ*dg* phages. One (λ*dg*$^+$) carried the complete *gal* operon. The other (λdgA) was broken within it. Transduction by λ*db* did not affect the chlorate resistance or nitrate reductase activity of these two mutants. We conclude that these mutants belong to a genetic locus between *gal* and *att*λ. We call this locus *chlD*. The partial block in nitrate reductase activity is a property of the locus rather than of the two mutants tested: a previously described mutant of *E. coli* (MS5061) that carries a deletion which extends from within the *gal* operon to within the λ prophage (and therefore is missing the entire *chlD* locus) also proved to be chlorate-resistant and partially defective for nitrate reductase. Transduction of this strain for λ*dg* restored both characters to their wild-type state.

(b) *Segregation from partial diploids:* The partial diploid strain R891 was derived from one of the two mutants assigned above to the *chlA* locus. It is heterozygous for the genes *gal*$^+$*bio*$^+$*chlA*$^+$/*gal*$^-$*bio*$^-$*chlA-r*. The frequency of unselected markers among stable *gal*$^+$ and *gal*$^-$ haploid segregants was scored. The results are shown in Table 2. As observed previously in similar two-factor

TABLE 2. *Segregation pattern from diploid strain R891 (gal*$^+$*bio*$^+$*chlA*$^+$/*gal*$^-$*bio*$^-$*chlA-r).*

Segregants selected as	Unselected Markers *bio*	*chl**	Number	Per cent
gal$^+$	+	+	18	30
	+	−	18	30
	−	+	0	0
	−	−	24	40
gal$^-$	+	+	0	0
	+	−	0	0
	−	+	0	0
	−	−	55	100

* Scored by the nitrate reductase character.

experiments with *gal* and λ as markers,[13] the segregation is polarized. All *gal*$^-$ segregants are also *bio*$^-$ and *chl*$^-$, whereas all *chl*$^+$ segregants are also *gal*$^+$ and *bio*$^+$. The *bio*$^+$ segregants are all *gal*$^+$ but can be either *chl*$^+$ or *chl*$^-$, whereas *bio*$^-$ segregants are all *chl*$^-$ but can be either *gal*$^+$ or *gal*$^-$. The *gal* and *chl* markers thus behave symmetrically. The pattern is compatible only with the gene order *gal-bio-chl*. It is understandable if the segregants arise by internal recombination from a diploid that bears an internal tandem duplication,[13] although there are other possible explanations for polarized recombination.

TABLE 3. *Characterization of multiple-defective chl-r mutants from strain CA2441.*

No. of mutants	Gal utilization	λ Phage integration	Ability of desthiobiotin to replace biotin	Ability to grow without added biotin	UV resistance	Ability to grow without added phenylalanine or tyrosine
2	−	−	−	−	−	+
17	+	−	−	−	−	+
3	+	+	+	−	−	+
3	+	0	0	−	−	−
9	+	+	0	+	+	−

Among one group of *bio*⁻ mutants, desthiobiotin can satisfy the biotin requirement. Growth on desthiobiotin requires a functional *bioB* gene.[12]

As indicated in the last column, some of the mutants require phenylalanine (which can be replaced less efficiently by tyrosine). The *aroA* gene described by Pittard and Wallace[16] maps to the right of *bio*. Mutational loss of *aroA* function causes a multiple requirement for phenylalanine, tyrosine, and tryptophan. As our parent strain CA2441 already requires tryptophan, we can only test the phenylalanine and tyrosine requirements. We tentatively assume that the amino acid requirement of the last two mutant types shown is due to deletion of all or part of the *aroA* gene.

Symbols: (−) absence of property; (+) presence of property; (0) not tested.

The parent strain is (+) for all properties scored here.

(c) *Deletion mapping:* Since the frequency of spontaneous *chl-r* mutants was high and the isolation technique provided positive selection for the mutant phenotype, it was feasible to isolate large numbers of spontaneous *chl-r* mutants to see whether they might include some deletion mutants that extend into neighboring genes. Among 400 mutants isolated from strain CA2441, 49 failed to grow in minimal medium with glucose as carbon source. Thirty-four of these are further characterized in Table 3. Because of the multiple nature of their defects, we conclude that they are caused by deletion mutations of the *chlA* and/or *chlD* loci that penetrate neighboring genes to various extents. All the deletions obtained fit a single linear map, as shown in Figure 1.

The gene order shown in Figure 1 is the only one compatible with the deletion types found. Positioning of *uvrB* between *bio* and *chlA* is based on the last three deletion types shown. We have not positively established that the last type (chlorate-resistant and aromatic amino acid-requiring) really involves the *chlA* and *aroA* loci rather than other genes somewhere else on the *E. coli* chromosome. The placing of *uvrB* to the left of *chlA* is therefore still tentative.

The existence of deletions among *chl-r* mutants and the location of one locus on each side of *att*λ make feasible the isolation of prophages deleted from either terminus. The *chl-r* mutants of strain R894 (which is lysogenic for λ) were screened for inability to produce phage and then characterized for prophage marker rescue, integration of superinfecting λ, and presence of neighboring bacterial genes. Thirty-two *chl-r* defective lysogens that turned out to be deletions were studied in detail. The different types of deletions obtained are shown in Figure 2. It is clear that prophage deletions can be isolated both as *chlA* and *chlD* mutants entering the prophage from the expected terminus. All the *chlA* deletions are also UV-sensitive and *bio*⁻. The three *chlD* deletions were UV-resistant and *bio*⁺, partially defective for nitrate reductase, and transducible by λ*dg*. All three deletions penetrate the *gal* operon, and two of them have the phenotype of *aroG* mutants as well. This observation indicates that the *aroG* locus is to the left of *gal*, which is in agreement with previous mapping.[10]

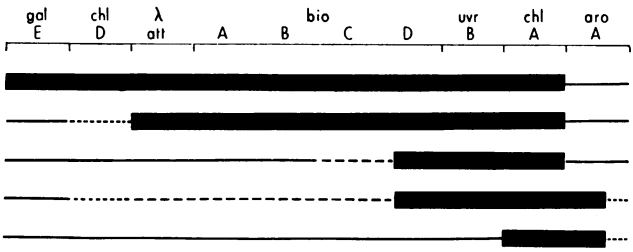

FIG. 1.—Deletion map of strain CA2441 based on the data of Table 3. The dark solid line represents the deleted segment. Dotted lines indicate regions, the presence or absence of which cannot be determined from Table 3. The location of the *chlD* locus between *galE* and *attλ* is taken from transduction studies and from an analysis of the deletions shown in Fig. 2.

Properties of prophage deletions: Bacterial deletions including all or part of a prophage are of interest for two reasons. (1) They provide a topological mapping of the prophage and adjacent bacterial regions. In this regard, the present work merely confirms what was already fairly well established about the mode of prophage attachment. All possible variations of the topological mapping technique have now been applied to the λ prophage. Both transducing phages and bacterial deletions that involve either end of the prophage and its contiguous bacterial genes can be obtained. (2) The properties of particular deletions may give some insight into the determinants of normal phage growth and lysogeny. A few facts of interest have emerged from the present studies.

(*a*) Deletions have been isolated both from the nonlysogen CA2441 and the lysogen R894, which are lacking the entire region from *gal* through *chlA* (Figs. 1, 2). Infection of these strains by phage λ gave a burst size of about 90 phage per cell. The yield from the parental strains in parallel experiments conducted concurrently was about 30 phage per cell. The significance of this quantitative difference awaits further investigation. Our only conclusion is that the *attλ* locus and neighboring genes do not play any essential role in the lytic cycle.

(*b*) The shortest prophage deletions of both *chlD* and *chlA* types produce, on induction with mitomycin C (2 μg/ml), a low yield of phage (about 10^{-6} to 10^{-5} of that produced by the wild-type lysogen, depending on the strain). These groups can be compared with the terminal deletion mutants of i^{80} h^{λ} prophage.[14] The most likely explanation is that part of the prophage terminus that plays a structural role in excision has been deleted.

(*c*) None of the prophage deletions, except those mentioned in the previous paragraph, produces any plaque-forming units on induction. However, those *chlA* deletions that carry some or all of the known J cistron markers (Fig. 2) give, on mitomycin induction, lysates that contain defective particles. When these lysates are mixed with phage tails (prepared by heat induction of strain R903), particles are made that can form plaques on a heteroimmune lysogen (R174), but not on a nonlysogen (R594). The number of plaque-forming units in the lysates after reconstitution is 10^{-5} to 10^{-3} per induced cell. Weigle[15] has demonstrated *in vitro* reconstitution of λ when phage heads are mixed with phage

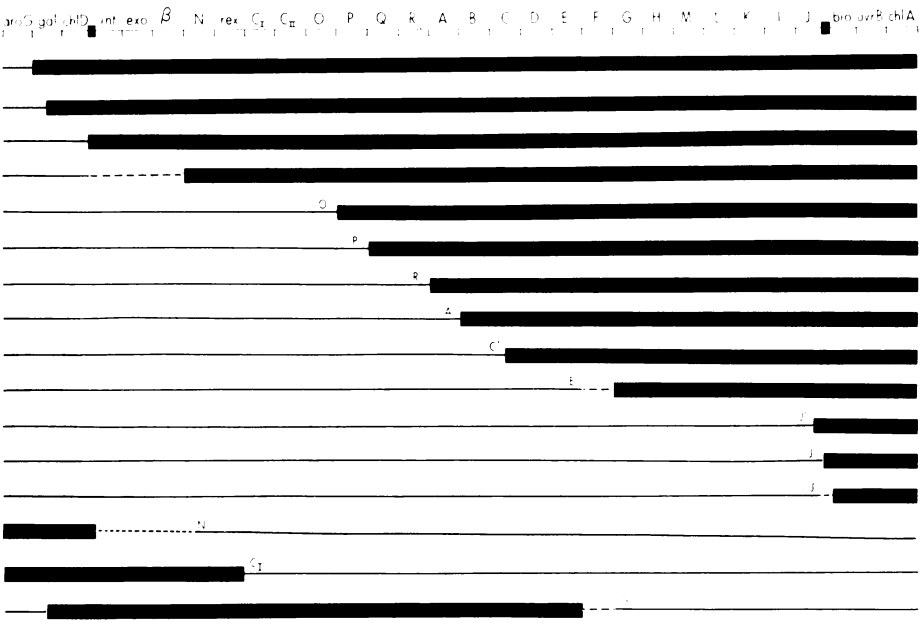

FIG. 2.—Terminal deletion mutations of prophage λ. The dark solid line represents the deleted segment, and the dotted lines indicate regions, the presence or absence of which cannot be determined from the genetic analyses that have been done. *C'* and *J'* indicate that the end point of the deletions is between known *sus* markers of the respective cistrons.

tails. Our results indicate that excision sometimes occurs even in those deletions where tail genes are missing, and that heads containing the DNA of these defective genomes are produced. The resulting particles will not form plaques on a nonlysogenic indicator because they lack a full complement of the normal tail genes. Tail function is provided by the prophage genes of the heteroimmune lysogen.

Summary.—Genetic mapping by three methods—specialized transduction, segregation from partial diploids, and isolation of deletion mutants—establishes the order shown in Figures 1 and 2 for λ prophage and neighboring genes. Of the two loci that determine chlorate resistance, the *chlA* locus is the one previously described by Puig *et al.*[7, 8] Absence of the *chlA* locus abolishes nitrate reductase activity. The *chlD* locus has not been previously reported. Some nitrate reductase activity is seen even when this locus is entirely deleted. Mutants of both loci are recessive to their wild-type alleles.

Chlorate-resistant mutants include deletions of the *chlA* or *chlD* genes. Deletions that penetrate λ prophage from either side or lack the entire *att*λ locus have been isolated.

We appreciate the help and suggestions of Don Court and Karen Killen.

* This work was supported by USPHS grant E-2862 from the Division of Allergy and Infectious Diseases. One of us (A. C.) was the recipient of a USPHS Research Career Award. Another of us (P. C.) was a USPHS predoctoral trainee.

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