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Excision of Bacteriophage Lambda from a Site in the Arabinose *B* Gene[1]

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A lambda lysogen with the prophage inserted into the arabinose *B* gene of *Escherichia coli* strain K-12 has been prepared. Induction of the phage from this lysogen yields viable phage at a frequency 4×10^{-6} that found for induction of lysogens with phage inserted at the normal attachment site. Over 30% of the phage particles induced from the insertion in *ara* are arabinose-transducing phage. The excision end points of 62 independently isolated, nondefective *araC*-transducing phage containing less than the entire *araC* gene were genetically determined and were found to be randomly distributed through the *araC* gene. The amount of arabinose deoxyribonucleic acid contained on four selected transducing phage was determined by electron microscopy of deoxyribonucleic acid heteroduplexes, providing a physical map of the *araC* gene. The efficiency with which these phage transduce *araC* and *araB* point mutations was found to be approximately proportional to the homology length available for recombination.

In *Escherichia coli* strains deleted of the normal lambda phage attachment site, the phage integrates at a reduced frequency into certain other preferred locations in the host chromosome (10). By highly selective genetic methods, very rare lysogens have been found in which lambda has integrated into the genes coding for enzymes used in arabinose utilization (4). All eight independently isolated lysogens containing lambda in the arabinose *B* gene contained the phage inserted at the same site and in the same orientation, suggesting that the sequence into which the phage is inserted weakly resembles the authentic phage attachment site. We reasoned that the site in the arabinose operon into which lambda had reluctantly integrated might be a site from which lambda also reluctantly excises. Indeed this proved to be so, and lambda was found to excise from this site at about 10^{-6} the rate it excises from the normal attachment site. Similar tendencies have been found by Shimada et al. (9-11) for lambda inserted in other secondary insertion sites.

Ordinarily lambda excises correctly, removing just itself from the chromosome. However, at a frequency of about 10^{-5} the prophage excises incorrectly from the normal attachment site, picking up bacterial deoxyribonucleic acid (DNA) from either or both sides of the attachment site (1). Improper excision products are viable unless either too much phage DNA or essential genes have been lost. Since the incorrect excision event that produces these transducing phage does not involve the phage or bacterial attachment site, it seemed likely that nondefective transducing phage would be produced at the same absolute rate from the *ara* insertion strain as they are produced from strains with phage inserted in the normal insertion site, (9-11). However, since the number of phage produced by the *ara* insertions is drastically reduced, the relative frequency of transducing phage in lysates prepared from these lysogens should be dramatically increased. Indeed, approximately 30% of these phage were able to transduce markers from the arabinose operon.

We sought to determine whether the end points of the excision are randomly located or whether they are, as in the case of the integration reactions, at certain preferred locations. A simple plate test was devised for the identification of phage that had excised from the site in the *araB* gene and carried some but not all of the *araC* gene. Sixty-two of these phage were isolated. The locations of their excision end points were mapped genetically to detect any "excision hot spots" within the *araC* gene. None were found in nondefective transducing phage.

The amount of arabinose DNA on four of the *araC* phage was determined by electron microscopy of DNA heteroduplexes. This method provided several physically located points on the genetic map of the *araC* gene. The four phage

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used in the physical mapping, as well as several used in the previous study (7), provided a set of phage with various amounts of *E. coli* DNA surrounded by lambda DNA. A measurement of the ability of these phage to transduce point mutations, as a function of the maximum amount of DNA homology existing between the phage and the cell DNA, was thus possible. The transduction frequency was found to increase with homology length up to at least several thousand base pairs.

MATERIALS AND METHODS

Media, chemicals, bacterial strains, and phage. Media and lamba suspension buffer were as described previously (3, 5). Nalidixic acid (Sigma Chemical Co.) and rifamycin (Calbiochem) plates contained 20 and 100 μg per ml, respectively, of the drugs in both the bottom and the top agar layers. Crude pancreatic deoxyribonuclease (DN-25) was from Sigma Chemical Co. Bacterial strains and phage are described in Tables 1 and 2. Bacterial strains were derived from *E. coli* K-12 and all phage contained the cI_{857} and S_7 mutations.

Characterization of phage derived from a lysogen with lambda integrated in araB. (i) Preparation of lysate. A series of independent lysates were prepared by addition of 0.05 ml of strain RFS657 or RFS1373 grown overnight in YT medium to 1 ml of fresh YT medium. Cells were grown at 32 C to a density of 6×10^8 cells per ml, induced 15 min at 42 C with vigorous shaking, and grown for 75 min at 32 C. Three drops of chloroform were added, the culture was allowed to stand 20 min at room temperature, and debris was removed by centrifugation.

(ii) Titration and plaque identification of transducing phage. The indicating strains were grown from single colonies to 10^9 cells per ml in YT medium, centrifuged, and suspended at 2×10^9 cells per ml in 0.01 M magnesium sulfate. Cells could be stored at least 4 months at 4 C. The numbers of the various transducing phage types produced by induction of RFS1373 were quantitated by diluting the lysate 100-fold and plating on strains JTL161, JTL128, or JTL157. After a 20-min absorption at room temperature, 2.5 ml of top agar was added and the mixture was poured on arabinose tetrazolium plates. After 36 h, plaques produced by normal phage were clear. Those phage carrying bacterial genes that recombined with the point mutation in the plating strain contained a few dark colonies in a clear background, whereas those that complemented the Ara⁻ cells had halos and raised red centers (4).

TABLE 1. *Phage used and generated in this work*

Phage no.	Comments/reference
λ*paraB107*	Contains *araB*, *araO*, *araI*, and part of *araC* genes, 4
λ*paraB114*	Like λ*paraB107*, 4
λ*paraC116*	Contains *araC*, *araO*, *araI*, and part of *araB* genes, 4
λ*paraBIOC159*	From cross between λ*paraB107* and λ*paraC116* Contains *araC*, *araB*, *araO*, and *araI* genes; made by John T. Lis
λ*para121* λ*para123* λ*para133* λ*para147*	Derivatives of λ*paraB114* containing deletions of some arabinose DNA, 7
λ*paraC156* λ*paraC157* λ*paraC158* λ*paraC160*	Partial *araC*-transducing phage prepared by heat induction of lambda from RFS1373 and mapped in this work

Isolation of partial araC-transducing phage. An RFS1373 lysate was diluted 100-fold with YT medium and 0.1 ml was mixed with 0.1 ml of JTL161, incubated, and poured with top agar on arabinose tetrazolium plates as above. A mixture of 0.1 ml of JTL157 and 2.5 ml of lambda top agar was then added and incubation was carried out at 35 C for 36 h. The desired plaques had a few colonies in the JTL161 layer and were either clear or showed recombination in the layer containing JTL157. These plaques were retested by picking them with a sterile toothpick and inoculating small areas on an arabinose tetrazolium plate containing rifamycin and a rifamycin-resistant derivative of strain JTL161 in the top agar, and on an arabinose tetrazolium plate containing nalidixic acid and a nalidixic acid-resistant derivative of strain JTL157 in the top agar. The antibiotics and the resistant strains prevented interference in the plaque assay by cells carried along with the phage being inoculated into the different plates. After incubation at 35 C for 36 h, transducing phage containing less than the entire *araC* gene (partial *araC*-transducing phage) showed recombinant plaques on strain JTL161 and showed clear or recombinant, but not complementing plaques on JTL157. Candidate plaques were picked with a sterile Pasteur pipette, suspended in 1 ml of lambda suspension buffer, treated with chloroform, and allowed to diffuse from the agar at room temperature for 1 h. These candidates were then retested by lightly streaking the solution containing the eluted phage over the surface of arabinose tetrazolium plates containing the indicating strains poured in the top agar. In 36 h the resultant pure plaques could be scored, picked, and eluted for the further growth of the phage.

Mapping of excision end points by ability to transduce araC point mutations. (i) Preparation of plate stocks. The candidates purified as described above were grown as plate stocks by adding 0.15 ml of the solution containing the phage to 0.1 ml of RFS1485, and pouring in soft agar on YT plates. After incubation at 37 C for 5 h, the phage were harvested by adding 2.5 ml of lambda suspension medium, scraping the top agar into a centrifuge tube, adding chloroform, and removing the debris by centrifugation. Titers were about 10^{10} phage/ml.

(ii) Transduction. Marker strains, containing *ara*-point mutations and grown to 10^9 cells per ml in YT medium containing 0.4% maltose, were centrifuged and suspended in 0.01 M magnesium sulfate. Phage, added at a multiplicity of 10, were incu-

TABLE 2. *Bacterial strains used and generated in this work*

Strain no.	Genotype	Comments/reference
JTL128	F⁻: *araB15 trp lacZ str supF*	6
JTL157	F⁻: *araC76 trp lacZ str supF ton 157*	4
JTL157 (nal^r)	F⁻: *araC76 trp lacZ str supF ton 157 nal*	Nalidixic acid-resistant derivative of JTL157; JTL24 (*str nal recA*) was mated with JTL157
JTL161	F⁻: *araB118 trp lacZ str supF ton 161*	4
JTL161 (rif^r)	F⁻: *araB118 trp lacZ str supF ton-161 rif*	Strain JTL161 P1 transduced to rifamycin resistance
RFSF′ strains	F′: *thr⁺ leu⁺ ara/ara-498 thi lac-74*	Deletion of arabinose DNA on chromosome; point mutation in *ara* DNA on episome, 6
RFS657	HFR: *ara-498* ($\lambda cI_{857}S_7$)	Lambda integrated at normal *att*λ site, 6
RFS1373	Hfr: *araD54* Δ(*gal attλ bio uvrB*) ($\lambda cI_{857}S_7$)	Lambda in *araB* gene, 4
RFS1465	F′: *thi str lig8 ton*	From D. Botstein D5404, ligase over producer, streptomycin, resistant, thiamine requiring, φ80 resistant
RFS1485	F⁻: *ara-498 supF*	Deletion of arabinose DNA

bated with 0.1 ml of cells. Transductions were done in polypropylene blocks and inoculated in assays on trays containing solidified minimal arabinose medium as described previously (6). Trays were incubated at 35 C and scored for colony growth after 40 h.

Purification of phage for electron microscopy. Four phage having excision end points occurring at widely spaced locations in the *araC* gene were prepared for electron microscopy. The ligase over-producing strain RFS1465 was grown in 500 ml of YT medium at 34 C to 2 × 10^8 cells/ml. Magnesium sulfate concentration was brought to 0.01 M by adding 5 ml of 1 M magnesium sulfate solution; phage were added at a multiplicity of infection of 3. After 30 min of growth, 5 ml of a 20% glucose solution was added and growth continued with vigorous aeration for 3 h at 34 C. The cells were centrifuged 15 min at 4,000 × *g*, suspended in 20 ml of lambda suspension buffer, and 0.2 ml of chloroform was added. Crude pancreatic deoxyribonuclease (0.1 ml of a 1-mg solution per ml in 10^{-3} N HCl) was added, the culture was incubated at 37 C for 15 min, and debris was removed by centrifugation. Usual yields were 10^{12} to 10^{13} phage per ml.

Phage were purified by consecutive descending and ascending cesium chloride block gradient centrifugations as described previously (3). DNA concentrations in the resulting phage stocks were approximately 10 mg/ml.

Heteroduplex formation and electron microscopy. Heteroduplex formation and electron microscopy were performed by the formamide technique of Davis (2) as modified by Lis and Schleif (4). Glass-distilled water was used in all solutions and all glassware was acid washed. Stock solutions were filtered through 0.45-μm membrane filters (Millipore Corp.). Samples were observed with a Phillips EM 300 electron microscope at a film (35 mm) magnification of ×3,000. Photographs were traced on a Kodak Recordax model C film reader and tracings were measured with a Kueffel and Esser 620315 map measurer. The magnification standard used in the heteroduplex measurements was the length of the shorter arm in the "double-bubble" heteroduplexes (Fig. 1 and 2).

Phage λparaBIOC159. Phage λ*paraBIOC159* was isolated as a particle carrying the entire *araC* and *araB* genes after a cross between λ*paraB107* and λ*paraC116*. There are two ways in which this cross could occur: (i) a recombinational crossover event between regions of homologous sequence would produce the normal *ara* region on the resultant phage, as shown in Fig. 1a; or a site-specific event promoted by the *int* and *xis* products occurring between the two *att* regions of the phage would produce the structure shown in Fig. 1b. Curiously, it is not necessary to know which of these two actually produced λ*paraBIOC159*, for in either case, the DNA heteroduplex between this phage and candidates of the form λ*paraB′IOC′* will yield the *ara* DNA of the candidate as duplex DNA bounded by single-stranded bubbles as shown in Fig. 2a and b. It may be noted that in the second case, a loop will occur within the central region. As we never saw such a loop in the many heteroduplex structures examined, we believe that the event producing the phage 159 was a recombinational crossover in a region of *ara* DNA homology.

Measurement of transducing frequency. Ten cultures of RFSF′17 (AraB⁻) and RFSF′75 (AraC⁻) were grown from single colonies to 5 × 10^8 cells/ml in 5 ml of YT medium containing 0.4% maltose, and suspended in 2.5 ml of 0.01 M magnesium sulfate. A 0.1-ml sample of each tube was tested on minimal arabinose plates for reversion. The culture containing the fewest Ara⁺ revertants was used in the transduction experiments.

Phage were added to 0.1 ml of cells at a multiplicity of infection of 10. The volume of the solution was adjusted to 0.3 ml with lambda suspension buffer, and the mixture was incubated at room temperature (22 C) for 15 min. Appropriate dilutions of the original mixture through lambda suspension

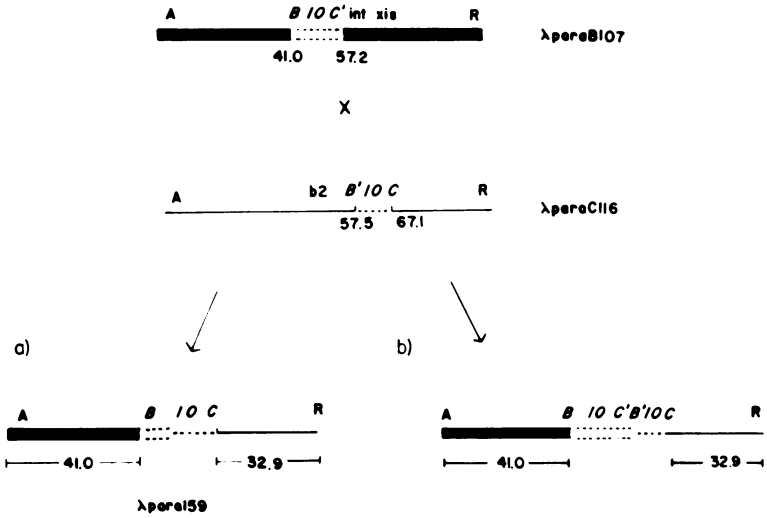

FIG. 1. *Predicted structure of λparaBIOC159, not drawn to scale, arising from parental phage λparaB107 and λparaC116 by recombination in a region of ara DNA homology (a) or, at the attλ site (b). Dotted lines indicate ara DNA. Numbers indicate the position of the ara substitution in lambda units. The B' and C' notations indicate that only a portion of the araB or araC gene is present, while the symbol OI represents the arabinose regulatory region.*

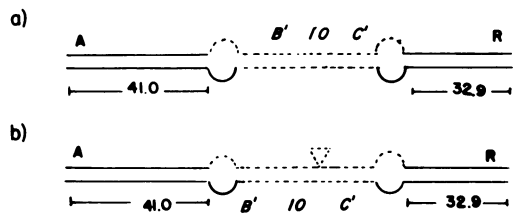

FIG. 2. *Structure of DNA heteroduplexes between λparaBIOC159 and araC-transducing phage containing a portion of the araC gene. (a) Heteroduplex between structure of Fig. 1a and partial araC-transducing phage DNA. (b) Heteroduplex between structure of Fig. 1b and partial araC-transducing phage DNA. The upper DNA strand comes from λparaBIOC159 while the lower one comes from the araC phage. Bacterial DNA is indicated with dotted lines.*

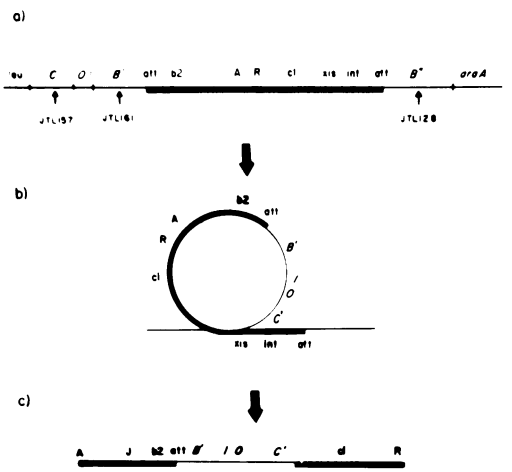

FIG. 3. *Orientation of lambda in strain RFS1373 and formation of araC-transducing phage. (a) Phage is inserted into the arabinose B gene. The thick line represents phage DNA and the thin lines represent bacterial DNA. The bacterial DNA is stretched relative to the phage DNA. Approximate positions of point mutations in strains used to select transducing phage are shown. (b) Circularization of phage DNA before excision leading to (c) formation of araC-transducing phage by improper excision of lambda (redrawn from reference 4).*

buffer were spread on minimal arabinose-B1 plates and yielded 50 to 400 colonies after 36 h at 35 C. Control plates containing phage or cells alone produced negligible background. The transduction frequency is computed as number of Ara^+ colonies/number of cells plated.

RESULTS

Transducing phage isolation. Lambda prophage has been found to excise at a reduced efficiency from abnormal chromosomal integration sites (4, 9–11). Using a strain with lambda inserted in the arabinose genes we sought to quantitate this effect and to look for possible preferred excision products. Figure 3 shows part of the arabinose operon, the orientation of lambda inserted in the *araB* gene in the strain RFS1373, and the locations of several mutations used in this work.

Lysogens containing lambda with a tem-

perature-sensitive repressor were heat induced at 42 C for 15 min, incubated at 35 C for 75 min, lysed, and titered. Phage yields per cell in lysates, obtained by induction of a lysogen containing lambda integrated at the normal *attλ* site and at a site in the arabinose *B* gene, were 7.7×10^{1} and 3×10^{-4}, respectively. Data averaged from five experiments reveals that the phage yield per cell from the strain with lambda in *araB* was 4×10^{-6} the yield from a strain with lambda inserted at the normal att^{λ} site. The frequency of transducing phage in a lysate was determined by means of the distinctive morphology on arabinose tetrazolium plates of plaques from phage that recombine with or complement *araB* or *araC* point mutations (4). Table 3 shows that 14% of the phage in a collection of nine lysates carry *ara* DNA on the *araC* gene side of the insertion, i.e., transduce JTL157 or JTL161, while 17% carry DNA from the *araA* side and transduce JTL128. Ninety per cent of the *araC* containing phage complement the *araC* point mutation in JTL157, and therefore contain more than 2,000 base pairs of bacterial DNA. The remaining 10% of *araC*-transducing phage contain only part of the *araC* gene.

Phage containing only part of the *araC* gene were sought for the remainder of these studies. Such transducing phage yield Ara$^+$ recombinant colonies when plated on the *araB* strain JTL161, but are unable to complement *araC* mutant strains to Ara$^+$. These phage were identified on a single tetrazolium indicating plate by use of a double layer of cells. AraB$^-$ cells were infected and poured in soft agar as usual. After the first agar layer had hardened, AraC$^-$ cells were poured on top in a second layer of soft agar. After the 36 h of incubation, the plaques showing Ara$^+$ recombinants in the lower layer and lacking cells complemented to Ara$^+$ in the upper layer were purified and retested separately on AraB$^-$ and AraC$^-$ strains. In all, 62 phage recombining with the *araB* point mutation of JTL161 and not complementing the *araC* mutation of JTL157 were isolated by this method.

TABLE 3. *Transducing phage in lysate prepared by induction of lambda from strain RFS1373 (lambda in araB insertion)*

Type of Plaque	No. of plaques		
	JTL157[a] *araC76*	JTL161 *araB118*	JTL128 *araB115*
Clear	1387 (85%)	1411 (86%)	1554 (83%)
Complement	215 (13%)	0	0
Recombinant	23 (1.4%)	223 (14%)	326 (17%)
Total	1625	1624	1880

[a] Titering strain.

To detect sites in the *araC* gene preferred in the excision process that produces the nondefective transducing phage, the excision end points of 62 partial *araC* phage were mapped by their ability to transduce *araC* point mutations. The mapping showed that the end points of the arabinose DNA acquired by the phage are distributed throughout the *araC* gene (Fig. 4). There is no evidence for an "excision hot spot" anywhere in the region. It should be noted, however, that the genetic map could contain distortions. The separation of genetic markers used in constructing the genetic map was based on the number of independent deletions ending between point mutations, rather than on a physical basis (6, 7). To measure the absolute amount of arabinose DNA on selected transducing phage having excision end points at various locations in the *araC* gene, we employed physical measurements of DNA heteroduplexes, as described below.

Physical mapping of the amount of arabinose DNA on transducing phage. The most convenient way to measure the amount of the *ara* region on the transducing phage was to form heteroduplexes in which the *ara* DNA was present as double-stranded DNA between two single-stranded bubbles. Examination of the structures of the phage available for forming these heteroduplexes showed that all were

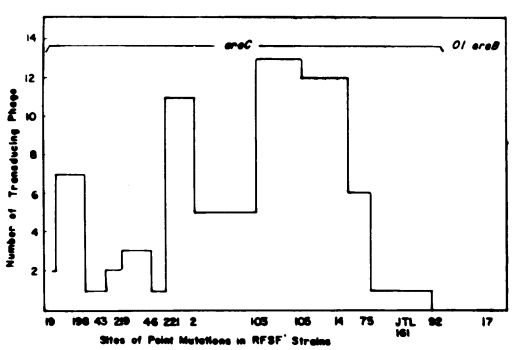

FIG. 4. *End points in the arabinose C gene of 62 lambda-transducing phage carrying a portion of the C gene. The relative positions of the araC point mutations in the RFS F' strains are shown, and the number of phage having ara DNA ending between the markers is shown. The spacing of markers is proportional to the number of discrete RFS deletions terminating between them (6).*

unsuitable, being similar to λ*paraB107* or λ*paraC116* (Fig. 1). However, a phage carrying the *araBIOC* region formed by recombination between these two phage would be satisfactory. Such a phage, λ*paraBIOC159*, was isolated and used in the measurements. Figure 2a shows the structure expected of the DNA heteroduplex between λ*paraBIOC159* and the *araC*-transducing phage to be measured. Figure 5 shows the physical map thus derived for the *araC* gene.

Transducing frequency and homology length. We used the *araC*-transducing phage described above and *araB* phage described previously (7) (Fig. 5) to determine how the maximum amount of homology between recombining molecules affects the recombination frequency. Both an *araB* and an *araC* point mutation were tested, and the results are shown in Fig. 6. The frequency with which either marker was transduced increased roughly proportionally to homology length up to about 2,000 base pairs. Phage λ*para133* containing 475 base pairs of arabinose DNA transduces the RFSF'17 marker (Fig. 6) at a lower frequency than might be expected by comparison to the rest of the data. This may result from the location of the RFSF'17 marker which is allelic to a site almost at the end of the arabinose DNA on the phage.

DISCUSSION

When a strain containing lambda inserted in the *araB* gene is induced, it yields nondefective phage at an efficiency of 4×10^{-6} compared to a strain containing lambda inserted in its normal attachment site. The reduced phage yield is consistent with a low excision efficiency resulting from a slight resemblance between the normal *att* site and the site of integration in the arabinose *B* gene. This slight resemblance is further suggested by the very low frequency of 10^{-11} with which lambda inserts into the *araB* site compared

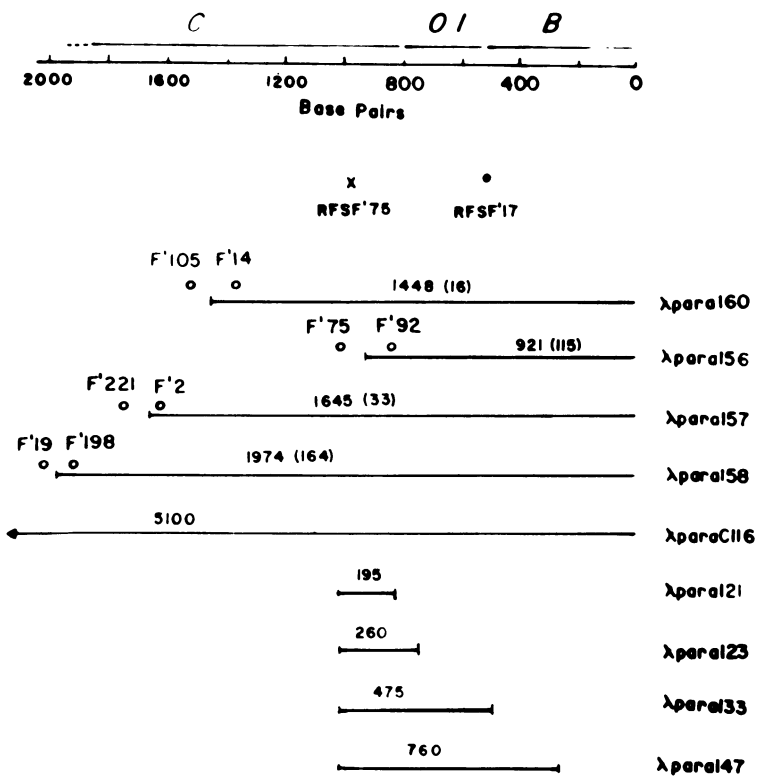

FIG. 5. *Arabinose DNA contained on various transducing phage. Phage λpara156, λpara157, λpara158, and λpara160 were isolated and arabinose DNA was measured by methods described in the text. Numbers indicate base pairs of arabinose DNA on phage. Numbers in parentheses are twice the standard error of the measurements. Other phage isolated are from Schleif and Lis (7). Position of the point mutation in the araB⁻ strain, RFSF'17 (●), and the araC⁻ strain, RFSF'75 (×), used in transducing studies are shown. The approximate locations of araC point mutations in RFSF' strains are indicated (○).*

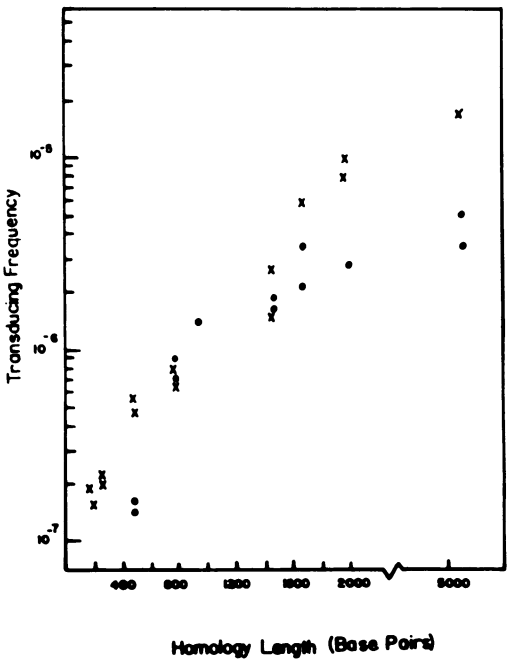

FIG. 6. *Effect of the amount of homologous DNA on the ability of arabinose-transducing phage to transduce an araB and an araC point mutation. Phage used are those indicated in Fig. 5. Strains transduced: RFSF'17 (araB⁻) (●); RFSF'75 (araC⁻) (×).*

to the normal attachment site (4).

As many as 30% of the nondefective phage in a lysate prepared by induction of lambda from the arabinose *B* gene are *ara*-transducing phage. The fraction of phage in these lysates which are transducing phage is about 10^5 times greater than the fraction of *gal*- or *bio*-transducing phage in lysates prepared by induction of lambda inserted at the normal attachment site. The high fraction of transducing phage in lysates prepared from lambda insertions in *araB* apparently reflects the severely reduced yield of wild-type phage and not an increased rate of transducing phage production.

The relationship between the frequency of insertion and the yield of wild-type phage excised from an abnormal integration site is consistent with data previously reported for lambda inserted into the *trpC* gene (8–11). Integration into the *trpC* gene occurred at a frequency of 10^{-6}. Induction of this strain yielded a burst size of viable phage of less than 1% of normal and 0.03% of the phage carried *trp* DNA to the right of the insertion. The higher fraction of normal phage in these lysates compared to that from the *araB* insertion parallels the higher frequency of insertion into the *trpC* gene. Induction of lambda from sites in *araB*, *trpC*, or att^{λ}, all yield transducing phage at roughly the same frequency per cell, 10^{-4}.

By means of a plate assay we were visually able to detect transducing phage in lysates prepared by excision of lambda from the arabinose *B* gene. Normal phage, phage containing *ara* DNA which complements a point mutation, and phage containing *ara* DNA which recombines with a point mutation in the bacterial lawn produce plaques of distinctive morphology on arabinose tetrazolium plates. About 10% of the *araC*-transducing phage in a lysate produced by induction of lambda from the arabinose *B* gene were found to contain less than the entire *araC* gene (partial *araC*-transducing phage).

Having developed a convenient method for detecting partial *araC*-transducing phage, we sought to detect excision hot spots in the approximately 1,000 base-pair region spanned by the *C* gene. The excision end points of 62 phage carrying a portion of the *araC* gene were mapped genetically and the end points in the *araC* gene were found to be randomly distributed.

The 10% of all nondefective *araC*-transducing phage that carry only part of the *araC* gene appear to arise by a random sequence-independent excision. The remaining 90%, which contain the entire *C* gene as well as DNA from beyond *araC*, could have arisen by a sequence-specific event. We therefore cannot conclude that all transducing phage in lysates prepared from the lambda in *araB* insertion are derived from random excisions.

The partial *araC*-transducing phage contain different amounts of bacterial DNA. Four were accurately characterized by electron microscopy to determine the exact amount of arabinose operon DNA carried on each. The physical map thereby determined closely agrees with the map previously constructed (Fig. 4 and 5). Thus, the genetic map contains no serious size distortions in the *araC* gene. The phage used in the mapping, plus additional phage characterized previously (7), provided a unique opportunity to correlate transduction frequency with homology length. Two point mutations were used in this study, an *araB* and an *araC* mutation. These behaved similarly, although transduction efficiency of the *araC* mutant tended to be higher.

The frequency at which either marker was transduced to Ara^+ by the different phage increased with the amount of arabinose DNA present on the phage up to 2,000 to 5,000 base pairs. An apparently low transduction frequency

of the *araB* marker by a phage containing 475 base pairs of arabinose DNA may be explained by the fact that transduction in this case would require a crossover that occurs infrequently very near the end of the bacterial DNA on the phage.

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