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The Isolation and Characterization of Plaque-forming Arabinose Transducing Bacteriophage λ

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Non-defective arabinose transducing phage, λp*ara*, were isolated in two steps: first, *Escherichia coli* strains containing rare insertions of λ DNA into the arabinose *C* or *B* genes were selected; and second, these lysogens were induced and transducing phage were selected from the resulting lysates. The approximate location of the bacterial substitution on the phage and the *ara* gene content of the substitution were determined genetically. The precise location of the substitution was determined by electron microscopy of DNA heteroduplexes.

Transducing phage, derived from the strain possessing λ inserted into the *araC* gene, carried part of the *araC* gene, the *ara* regulatory region, and all of the *araB* gene. Transducing phage, derived from eight independent strains possessing λ inserted in the same orientation and in the same position in the *araB* gene, carried a portion of the *araB* gene, the *ara* regulatory region and all of the *araC* gene. In these nine cases the *ara* DNA on the phage was immediately adjacent to the normal phage attachment site, indicating that the transducing phage were formed by the same type of abnormal excision which produces *gal* or *bio* transducing λ phage. The relative orientations of *ara* and phage genes were deduced from the topology of such excisions. One anomalous transducing phage was also characterized.

1. Introduction

The *araC* protein exerts a positive control over the expression of the *ara* operon in *Escherichia coli*. The presence of arabinose and *araC* protein results in transcription of the *ara* operon by RNA polymerase initiating in the region between the *araC* and *araB* genes and proceeding rightward across the *araB*, *araA*, and *araD* genes (Fig. 1). The positive activity of the *araC* protein has been demonstrated *in vivo* (Sheppard & Englesberg, 1967), *in vitro* in DNA-directed protein synthesizing systems (Greenblatt & Schleif, 1971; Wilcox *et al.*, 1974*a*), and in a purified transcription system in which *ara* mRNA synthesis was dependent on the addition of partially purified *araC* protein (Lee *et al.*, 1974). A repressor activity for *araC* protein has also been detected *in vivo* (Englesberg *et al.*, 1969), and *in vitro* (Greenblatt & Schleif, 1971; Wilcox *et al.*, 1974*a*).

Specialized arabinose-transducing phage have played an important role in developing our present understanding of the *ara* operon. The DNA extracted from λhy80d*ara* phage has been used in RNA–DNA hybridization assays of *ara*-specific messenger (Schleif, 1971; Wilcox *et al.*, 1971; Cleary & Englesberg, 1974; Lee *et al.*, 1974), as a DNA template in transcription or coupled transcription–translation systems, and in a study of DNA binding properties by *araC* protein (Wilcox *et al.*, 1974*b*). The

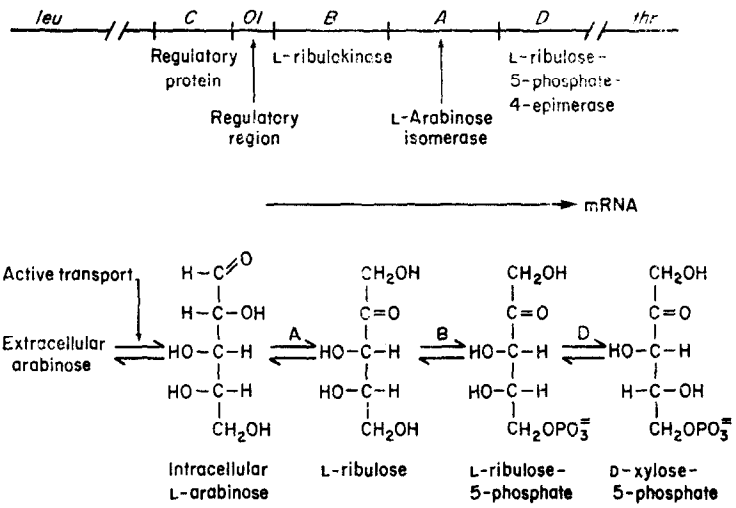

FIG. 1. The arabinose gene cluster and the enzymatic reactions performed by its structural enzymes (Englesberg, 1971).

growth of these phage has also provided a means of enriching bacterial extracts in *araC* protein. The increase in the synthesis of *araC* protein can be brought about by a gene dosage effect of the multiple DNA copies (Müller-Hill *et al.*, 1968; Abelson *et al.*, 1970), and by fusion of the gene to a strong promoter (Schleif *et al.*, 1971). We sought non-defective *ara* transducing phage suitable for more detailed protein binding studies, sequence determination, and transcription studies. In addition, these phage were used in the work described in the accompanying papers: purification of a DNA fragment containing the *ara* control region and physical and genetic studies of this region.

A major difficulty in isolating specific specialized transducing phage has been the requirement for proximity of the prophage and the desired bacterial sequence. Methods have been developed for selecting strains in which distant bacterial sequences had been moved adjacent to a phage integration site (Gottesman & Beckwith, 1969), or in which a phage integration site had been moved adjacent to a normally distant bacterial sequence (Press *et al.*, 1971). Shimada *et al.* (1972) described a useful variation of the former methods utilizing a strain deleted of the normal λ integration site. λ lysogens are found in such strains, but at about 0·5% of the usual frequency. Interestingly, the phage in these strains are found to have inserted into a variety of chromosomal locations; however, the insertion is non-random and a few secondary sites are preferred (Shimada *et al.*, 1973). Using an *attB.B'* deletion strain and coupling the selection for lysogens with a powerful selection for mutations in the arabinose genes, we have isolated insertions of λ into the *araC* and *araB* genes. This paper describes these selection methods as well as a genetic and physical characterization of plaque-forming transducing phage isolated from these insertion strains.

2. Materials and Methods

(a) *Media, bacterial strains and phage*

Media are as described previously (Schleif, 1969). Tryptone broth medium (Gottesman & Yarmolinsky, 1968) contains 0·4% maltose. Bacterial strains are described in Table 1

TABLE 1

Bacterial strains used and generated in this work

Strain no.	Genotype	Comments
JTL64	F⁻: *trp lacZ str suIII*	Strain KR3a, from M. Howe, P1 transduced to Rec⁺ by selection for methyl methane sulfonate resistance. *trp* amber suppressed to Trp⁺ by *suIII*
JTL103	F⁻: *araC1022 leu1022 lac74 tsx28*	A deletion of *araC* and *leu*, Lis & Schleif (1973)
JTL133	F⁻: *trp lacZ str suIII* (λc1857S7)	A λc1857S7 lysogen of JTL64
JTL134	F⁻: *leu trp lacZ str suIII*	Derived from JTL64, by transduction to *ara*⁺*leu*⁻ of an *ara*⁻ derivative of JTL64 using P1 grown on C600.
JTL156	F⁻· *rec48 suII* (λhy*imm*^{434} *int6 red3*)	From D. Freifelder, Lysogen of QR48, Signer & Weil (1968). Recombination-deficient
JTL157	F⁻: *araC76 trp lacZ str suIII ton157*	From JTL134 by P1 transduction to *leu*⁺ *araC*⁻ of RFSF′C76; Schleif (1972) Resistant to phage T1.
JTL161	F⁻: *araB118 trp lacZ str suIII ton161*	From JTL134 by P1 transduction to *leu*⁺ *araB*⁻ of RFSF′118, Schleif (1972)
RFSF′15	F′: *thr*⁺ *araB15 leu*⁺/*ara498 leu498 thr thi lac74*	R. Schleif (1972)
RFS1366	Hfr:*ara* D54 *Δ(gal attλ bic uvrβ)*	Derived from KS 73, Shimada *et al.* (1972) by P1 transduction to *leu*⁺ *araD* 54 (Schleif 1969)

and are derived from *E. coli* K12. Unless otherwise indicated in the text, all phage mentioned possess the cI857 and S7 mutations. Phage λhy*imm*^{434} (Schleif *et al.*, 1971) and other phage not described in the text are as previously described (Lis & Schleif, 1974).

(b) *Isolating insertions of λ into* araC *and* araB

An arabinose-sensitive strain, RFS1366 (*Δattλ araD54*) (Shimada *et al.*, 1972; Schleif, 1969) was grown at 35°C in 100 ml Tryptone broth medium to 4×10^8/ml at which time phage was added at a multiplicity of 10. After 45 min the infected cells were added to 600 ml yeast/Tryptone medium (Schleif, 1969), and 60 min later L-arabinose was added to 0·4%. Cells were grown until they reached 2×10^9/ml, about 20 h, and 0·1 ml was spread on an arabinose–tetrazolium plate previously spread with 10^9 λcI*b*2. After 36 h, λ immune, arabinose-resistant, arabinose negative colonies (0 to 100/plate, average 10/plate) were purified on yeast/Tryptone plates. Each of these was tested for its ability to produce *ara* transducing phage. Purified cells were grown in yeast/Tryptone medium to 2 to 5×10^8/ml, heat induced, grown for 3 h, lysed with $CHCl_3$, and debris removed by centrifugation. The lysates, 0·1 ml of each, were added to 10^9 cells from each of 2 testing strains, JTL103 (*araC1022 leu1022*) and RFSF′15 (*araB15*), and spread on minimal arabinose plates. Cells to be transduced were prepared as described by Lis & Schleif (1973). Candidates producing Ara⁺ transductants on either or both testing strains were used for isolation of non-defective transducing phage. Approximately 100 separate applications of this entire procedure yielded the nine independent strains listed in Table 1 containing λ inserted into the arabinose operon.

(c) *Isolation of plaque-forming transducing phage*

Transduction was scaled up a factor of 10 from that described in the previous section for each strain shown capable of producing *ara* transducing phage. The resulting 2000 transductants were resuspended together in yeast/Tryptone medium, diluted, and grown to 3×10^8/ml. The prophage in these cells were heat-induced and new lysates made. When plated at 35°C on a lawn of JTL157 (*araC76 su111*) or JTL161 (*araB118 su111*) on arabinose–tetrazolium plates, plaques formed by non-defective transducing phage whose *ara* genes complement the Ara$^-$ cells of the lawn are easily identified as they have halos and colored centers whereas non-transducers produce much clearer plaques (Plate I).

(d) *Genetic complementation test for* int red *substitutions*

A *recA*$^-$ strain, JTL156 (QR48) lysogenic for λhy*imm*^{434} *int6 red3* (from D. Freifelder, Brandeis) was grown in Tryptone broth plus 0·4% maltose at 32°C to 2×10^8/ml and infected at a multiplicity of 2·5 with phage to be tested. The culture was induced at 42°C for 10 min, grown at 32°C for 1·5 h, lysed with $CHCl_3$, and titered on a λ lysogen, JTL133. Cells infected with phage lacking the wild type *int red* region produce about 10^5-fold fewer hy*imm*^{434} phage than do those infected with either wild type or *b*2-substituted phage.

(e) *Genetic determination of arabinose markers carried on the phage*

A set of F′ *ara*$^-$ strains previously mapped against deletions (Schleif, 1972) were grown in Tryptone broth containing 0·4% maltose to 10^9/ml and infected at a multiplicity of 10. After 20 min the cells were transferred to trays or plates containing minimal arabinose medium. Cell growth was scored after 36 h.

(f) *Electron microscopy*

Heteroduplexes were made, mounted, and analyzed essentially as described by Davis *et al.* (1971). Our hyperphase contained 47% and the hypophase 17% formamide (Fisher reagent grade, pH 8·0 if diluted 1/10 with water). In place of a Petri dish of hypophase, we used a 0·8 ml drop. The top 4/5 of the drop surface is self-cleaning, obviating wiping with a Teflon bar. The 10 μl of hyperphase containing 0·005 μg of DNA sample was layered onto the surface by allowing it to flow down a glass slide (1·5 mm wide) inserted at about 30° from horizontal. Bright lighting from a 100 W bulb with reflector 2 feet above the drop greatly aids observations of hyperphase as it is added. Only spreadings in which hyperphase clearly flows over at least one-half of the surface of the drop are satisfactory. Vigorous surface swirling often accompanies these successful spreadings. Recently the glass slide has been eliminated by the use of an inverted plastic Petri plate lid (Optilux no. 1001). 5 or 6 1-cm wide scallops, evenly spaced around the perimeter, have been bent outward at 90° halfway up the side. A 0·3 ml drop of hypophase just reaches the scallop. An Eppendorf pipette containing the hyperphase is used to tease a portion of the hypophase onto the scallop and hyperphase is slowly squirted into this. If the hyperphase sinks, a new portion of hypophase is pulled onto the scallop and layering is continued with the remaining hyperphase. Usually only about 2·5 μl of hyperphase spreads onto the surface. If spreading is not initially successful, the 4 or 5 squirts possible with the 10 μl of hyperphase usually give a successful spreading. All surfaces coming into contact with the hypo- and hyperphase solutions must be scrupulously clean and dust-free. We routinely clean the Petri plate with Ivory detergent and pipettes with fresh chromic/sulfuric acid and rinse with filtered and then deionized water. Also, we avoid spreading samples in the vicinity of a running mechanical vacuum pump.

1 min after spreading with either technique the protein–DNA film is picked up with parlodion coated grids from a sector over which hyperphase was seen to spread. Adequately contrasted DNA suitably free of debris was picked at the point of greatest curvature, about one-third of the way from the drop edge to the scallop. Grids were stained with uranyl acetate and coated with a thin layer of carbon by evaporation or stained with uranyl acetate and shadowed with Pt–Pd. The Pt–Pd shadowing eliminates the need for reinforcing the parlodion by carbon coating and increases contrast especially if the uranyl acetate staining is less than optimum.

Microscopy and length measurements are described previously (Davis *et al.*, 1971); however, we find the Keuffel and Esser 620315 map measurer offers greater accuracy and a more uniform scale than model 620300.

For measurements on double-stranded DNA between 200 and 27,000 base-pairs long, in general we have found the same relationship between the length of the DNA (L) and the standard deviation (σ_D) in a set of data, i.e. $\sigma_D = 2{\cdot}6\ (L)^{\frac{1}{2}}$ (units of L in base-pairs), as was found by Davis *et al.* (1971). Thus the means of samples containing n molecules drawn from the population are normally distributed with standard deviation $\sigma_D/(n)^{\frac{1}{2}}$ and hence there is a 0·95 probability that the interval $4\sigma_D/(n)^{\frac{1}{2}}$ centered about the mean actually includes the mean of the population from which the sample was drawn. Our measurements are presented as mean $\pm 2\sigma_D/(n)^{\frac{1}{2}}$. We have not included in the error analysis the relatively small uncertainties in the length of the standards used to calibrate the magnification of each molecule measured.

Standard deviations of segments containing the left end of λ*c*I857S7 often were about a factor of 2 larger than predicted from the expression $\sigma_D = 2{\cdot}6\ (L)^{\frac{1}{2}}$. This is consistent with the observations of Thomas & Davis (1975) that a fraction of λ*c*I857S7 DNA molecules are deleted of about 3% of their length at the left end.

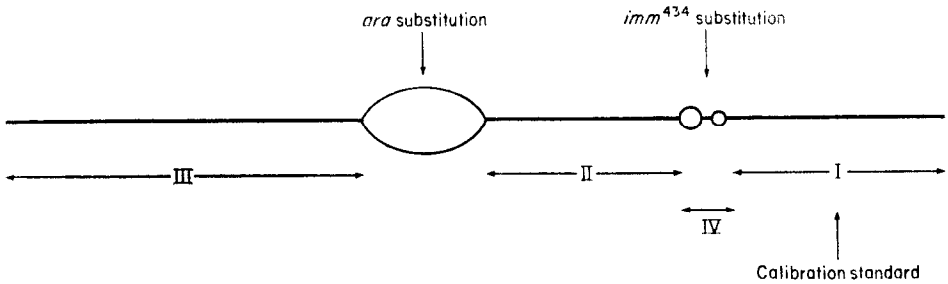

FIG. 2. A diagrammatic representation of a heteroduplex of λ*para*/λhy*imm*^{434}. Segments I, II, and III represent double-stranded DNA whose contour lengths are measured for each heteroduplex. Segment I begins at the right end of λ and ends at the beginning of the *imm*^{434} single-stranded bubble. This known length served as a calibration standard by which magnification was determined, allowing conversion of the measured length of II and III to physical lengths in λ units or base-pairs. Segment IV is the *imm*^{434} "double-bubble" substitution which serves both to define one end of the calibration standard and to identify the right end of the DNA molecule.

The internal standard used for calibration in our measurements was the length from the right end of the λhy*imm*^{434}/λ*para* heteroduplex to the beginning of the *imm*^{434} substitution (Fig. 2, segment I), and was previously determined by Westmoreland *et al.* (1969) to be 20·9 λ units in length (100 λ units = λ = 46,500 base-pairs (Davidson & Szybalski, 1971)). The left endpoint of the *ara* substitution was physically mapped by measuring the length of segment III. The right endpoint was mapped by first measuring segment II and adding to it the previously determined lengths of segments I and IV (Westmoreland *et al.*, 1969). The error reported with each endpoint location is calculated from our measurements of segment II or segment III and does not include deviations associated with the previously determined lengths of segments I and IV.

3. Results

(a) *Isolation of strains containing λ insertions in the* araC *or* araB *genes*

Phage λ lysogenizes cells deleted of the normal phage attachment site at about 0·5% the normal efficiency. The alternate, less preferred integration sites in this situation are scattered about the chromosome, although a few sites account for the great majority of insertions (Shimada *et al.*, 1973). Our preliminary studies indicated that insertions of λ into the arabinose operon were infrequent. We therefore devised a strong genetic selection for isolating such insertion strains. This was a double

selection, first, for cells lysogenic, and second, for cells whose arabinose operon was inactivated, due to the insertion of λ into it. The selection utilized an *araD* mutant. A strain containing the *araD* mutation cannot grow in the presence of arabinose due to the accumulation of the bacteriostatic substrate of the *araD* enzyme, ribulose-5-phosphate-4-epimerase (Boyer *et al.*, 1962). However, a strain with a secondary defect in the operon, for example, due to the insertion of λ into another of the *ara* genes can grow since the synthesis of the substrate is blocked. Cells of strain RFS1366 (*araD ΔattλλλΔattλ*) were infected with λ and grown in the presence of arabinose. Survivors were spread on nutrient broth plates which had been covered with λc*I*b2 and which contained arabinose and dye to indicate utilization of arabinose. Each of the small number of Ara$^-$ colonies growing after 30 hours was then tested individually for λ inserted in the *ara* genes.

A λ prophage inserted into the *ara* genes could excise incorrectly to produce phage carrying portions of the adjacent *ara* genes. Such an effect is seen when λ is inserted into its normal attachment site where incorrect excision produces phage particles carrying portions of either the *gal* or *bio* genes at a frequency of 10^{-6}/phage (Morse *et al.*, 1956; Wollman, 1963). We tested lysates made from each of our insertion candidates for their ability to transduce arabinose markers. Nine were found. Since none of the phage particles resulting from a strain with λ inserted into the *araB* gene could carry an intact *araB* gene, they would transduce *araB* mutants only by recombination. However, some phage could contain the entire *araC* gene and transduce *araC* mutants at a very much greater frequency by complementation (Fig. 3). Eight candidates produced lysates that gave 1000-fold more Ara$^+$ transductants with the *araC* than the *araB* mutant and were classified as insertions in *araB*. A single candidate gave the opposite response and was classified as an insertion in *araC*. The

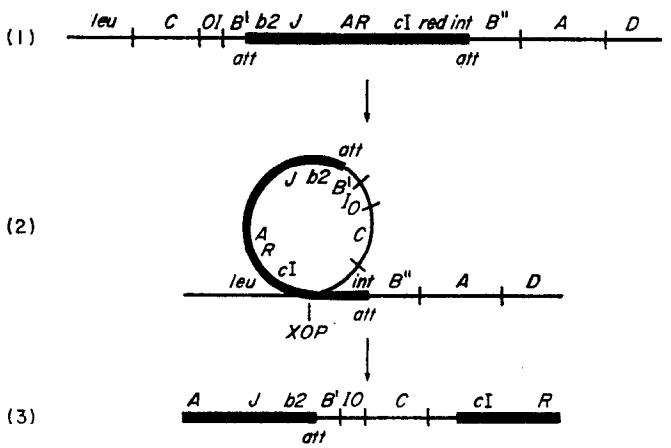

FIG. 3. Generation of a λ*araC* transducing phage from a bacterial strain containing λ inserted into the *araB* gene. Stage (1) illustrates a λ DNA inserted into the *E. coli araB* gene dividing the gene into 2 sections, *araB'* and *araB''*. The thick line represents phage DNA and the thin lines represent bacterial DNA; the bacterial DNA is enlarged in scale relative to phage DNA. Stage (2), excision shows the generation of a circle containing the essential phage genes and *araC*, *araOI*, and *araB'* bacterial DNA. A reciprocal recombination at the locus where bacterial and phage DNA cross (*XOP*) liberates the transducing phage circle from the *E. coli* chromosome and from the λ *int red* DNA. Stage (3) illustrates the vegetative map of λ*araC* phage that results from opening the circular phage at the λ *AR* sticky ends.

genetic mapping of purified transducing phage from the strains containing insertions of λ in *ara* later confirmed this preliminary determination of insertion location.

(b) *Isolation of plaque-forming transducing phage from* λ *in* ara *insertion strains*

Each of the nine strains found by the screening described above was used to isolate plaque-forming arabinose-transducing phage. Lysates made by heat induction of λ in *araB* insertion strains were used to transduce cells of JTL103 (*araC*) to Ara$^+$. Several thousand of the resulting transduced colonies were pooled and a mass lysate from them was plated on JTL157 (*araC suIII*) on complete medium containing the dye tetrazolium to indicate arabinose utilization. Ten to ninety per cent of the plaques contained arabinose-metabolizing transduced cells resulting from complementation by nondefective *ara* transducing phage (Plate I). Lysates from the strain with λ inserted in *araC* were treated identically except that RFSF'15 (*araB*) was transduced to Ara$^+$, and mass lysates were titered on JTL161 (*araB suIII*).

(c) ara *gene content of the transducing phage*

The *ara* DNA contained on the transducing phage was accurately determined by mapping with many F'*ara*$^-$ strains and the results are summarized in Figure 4. The λ*paraC* transducing phage from the eight independent insertion strains contain the entire *araC* gene, the regulatory region, and about one-third of the *araB* gene as estimated from the fine structure map of the arabinose operon (Schleif, 1972). All but one of the 11 λ*paraC* transducing phage were found to recombine with the same *araB* mutants. The anomalous phage λ*paraC110*, possessed additional DNA from the *araB* gene (Fig. 4.)

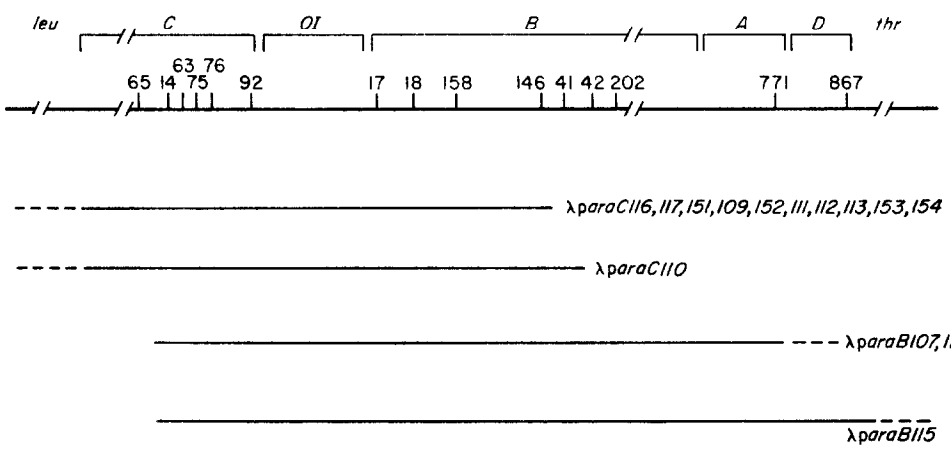

FIG. 4. Fine structure mapping of the *ara* gene content of the transducing phage. The drawing represents the *ara* gene cluster in which the regulatory region, *OI*, and portions of the adjacent *araB* and *araC* genes have been expanded in scale. The hashmarks represent a single or a cluster of point mutations previously mapped against deletions, and the spacing of point mutations was done by allotting each of the independently isolated deletions a fixed length of map (Schleif, 1972). Mutations contained in the original map which fail to revert or which contain an *araD* mutation as well as an *araC* or *araB* mutation have not been included in this map. The numbers above the hashmarks represent point mutations of the series RFSF'X, where X is the mutation number. Hashmarks and numbers in the unexpanded regions (except 771 and 867) have been omitted due to problems of overcrowding. The solid lines below the map represent *ara* DNA carried and the dashed lines represent DNA that may be carried on the indicated λ*para*-transducing phage.

The three λ*paraB* transducing phage isolated from the strain with λ inserted in *araC* carry all the *araB* gene, the control region and about one-quarter of the *araC* gene. All three possessed genetically identical amounts of the *araC* gene.

(d) *Localization of the* ara *substitution on* λpara *phage by a genetic test*

The excision of λ DNA from a lysogen normally occurs by a site-specific recombination between the left and right *att* loci producing an intact λ DNA molecule. Occasionally a λ prophage is excised incorrectly by recombination between phage and bacterial genomes producing a transducing phage (Campbell, 1962). Figure 3 illustrates an incorrect excision. In this example a λ*paraC* phage is produced from a strain which had λ inserted in *araB*. It should be noted that the process will usually delete some phage genes from the resulting transducing particle. If upon formation of the original λ in *araB* lysogen, the insertion of phage DNA was by a recombination at the normal phage *attP.P'* site, then the non-essential phage genes that could be left off the transducing phage would be adjacent to the phage *attP.P'* site, either the *b2* or the *int-red* genes. The genes deleted depend upon the orientation of λ in the original insertion. Assay for the *int* and *red* functions in the *ara* transducing phage indicates which phage genes have been replaced with the bacterial genes. This determines the orientation of the phage in the insertion as well as the relative orientations of the phage and *ara* genes on the transducing phage, if integration and excision have followed the model of Campbell (1962).

The activities of the *int* and *red* genes of each transducing phage were measured by release of imm^{434} phage following superinfection of the lysogen JTL156 (*rec48*

TABLE 2

Int-red *activities of* λpara *transducing phage isolated*

Bacteriophage	λhyimm^{434} phage rescued/0·1 ml of lysate	Comment or conclusion
λ*pbio*II	1	Control, substitution through *int-red* (Manly *et al.*, 1969)
λ*int6*	1000	Control, nonsense mutation in *int*, partial rescue activity due to *red* gene and/or partially active *int* product
λ$^+$	400,000	Control, Int$^+$ Red$^+$
λ*paraC109*	1	Behaves like λ*pbio*II, Int$^-$ Red$^-$
λ*paraC111*	1	Behaves like λ*pbio*II, Int$^-$ Red$^-$
λ*paraC116*	1	Behaves like λ*pbio*II, Int$^-$ Red$^-$
λ*paraC152*	1	Behaves like λ*pbio*II, Int$^-$ Red$^-$
λ*paraC151*	2	Behaves like λ*pbio*II, Int$^-$ Red$^-$
λ*paraC153*	2	Behaves like λ*pbio*II, Int$^-$ Red$^-$
λ*paraC154*	2	Behaves like λ*pbio*II, Int$^-$ Red$^-$
λ*paraC113*	300	Partially defective *int-red*
λ*paraC112*	2000	Partially defective *int-red*
λ*paraC117*	7000	Partially defective *int-red*
λ*paraB107*	70,000	Int$^+$ Red$^+$
λ*paraB115*	100,000	Int$^+$ Red$^+$
λ*paraB114*	200,000	Int$^+$ Red$^+$
λ*paraC110*	500,000	Int$^+$ Red$^+$

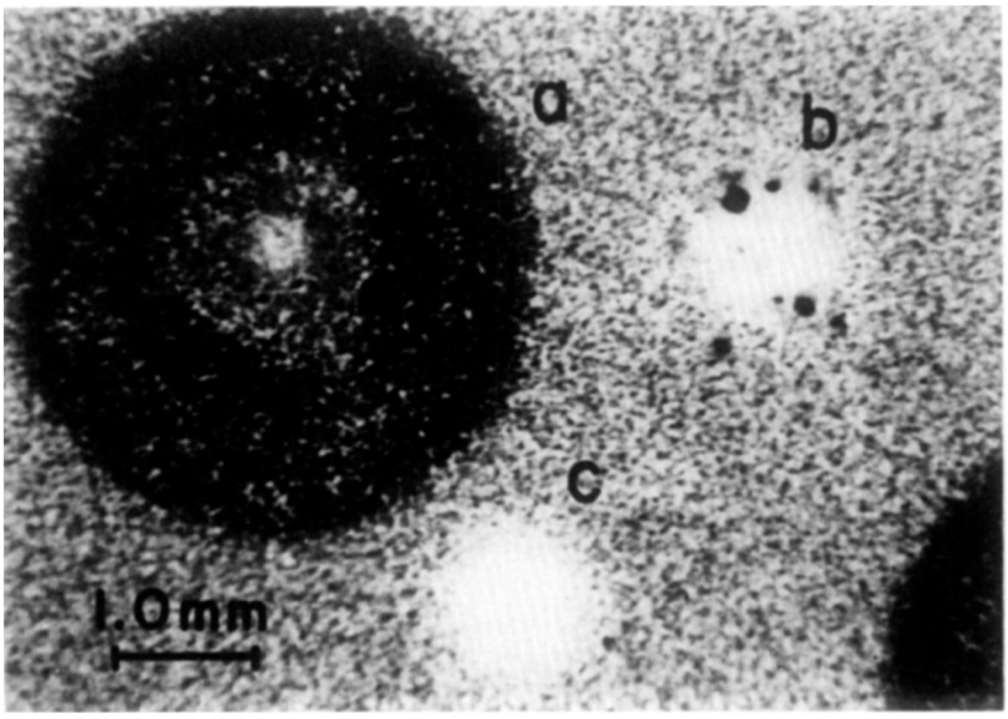

PLATE I. Transducing phage titered on JTL157 (*araC76 suI11*) arabinose tetrazolium plates. The plaques indicate complementation of the *araC* marker by λ*paraC116* (a); recombination by λ*paraB114* (b); no recombination by λ*c*1857S7 (c). The photograph was taken after incubating the plate for 40 h at 35°C.

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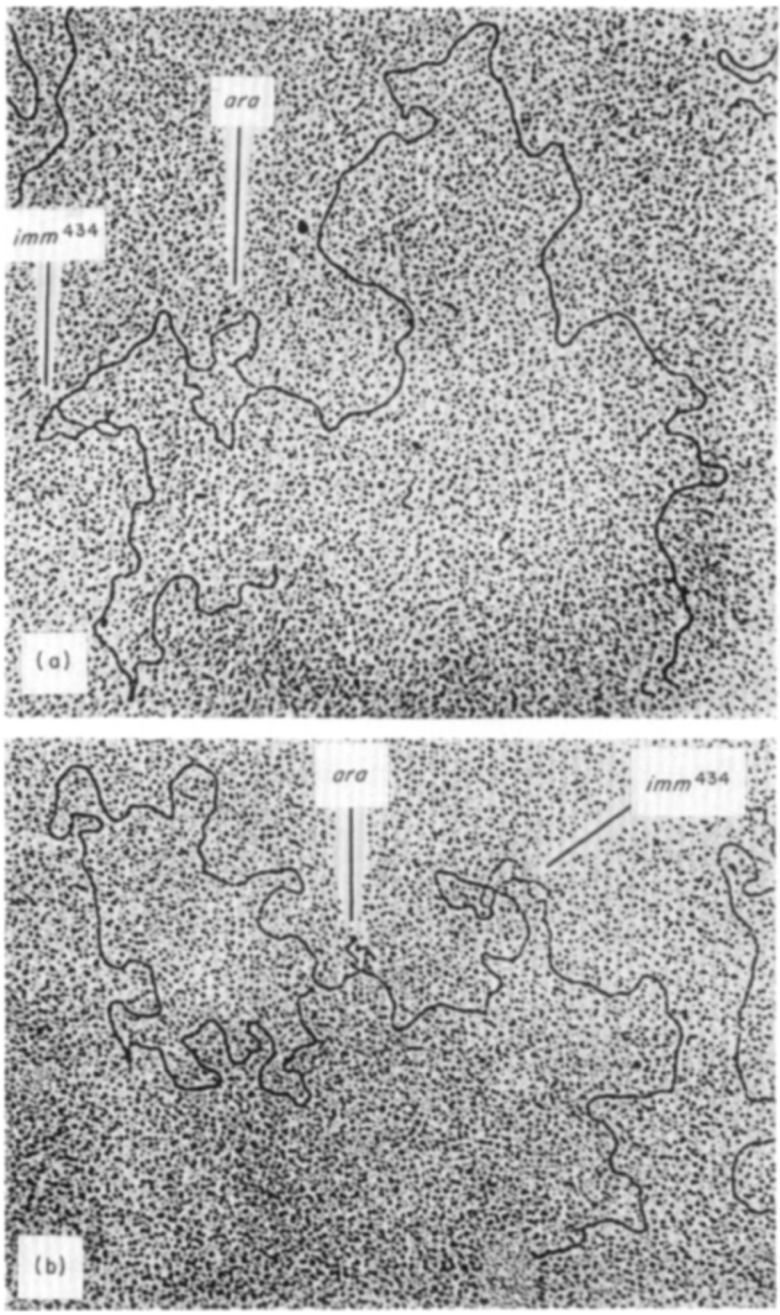

PLATE II. (a) λp*araC116*/λhy*imm*^{434} heteroduplex. The grid was prepared as described in Materials and Methods, stained with uranyl acetate, but not shadowed with platinum–palladium. Contrast of the final print was enhanced by rephotographing the original print onto high contrast copy film (Kodak film 5069) and reprinting. (b) λp*araC110*/λhy*imm*^{434} heteroduplex.

TABLE 3

Strains derived from RFS1366 by λ insertion into ara

Strain no.	Location of λ	λ*para* phage derived from each strain
RFS1368	*araC*	*B107, B114, B115*
RFS1373	*araB*	*C116, C117, C151*
RFS1414	*araB*	*C109*
RFS1415	*araB*	*C152*
RFS1416	*araB*	*C110, C153*
RFS1417	*araB*	*C111*
RFS1418	*araB*	*C112*
RFS1419	*araB*	*C113*
RFS1420	*araB*	*C154*

(λ*imm*^{434} *int red*). This prophage is defective in its ability to excise from the *E. coli* chromosome, but it can be helped to excise by a superinfecting phage that synthesizes this *int* gene product (Echols, 1970). The prophage and host are also defective in recombination systems, *red* and *rec*. However, a superinfecting phage that synthesizes the *red* gene product could recombine with the prophage moving the *imm*^{434} marker onto the superinfecting phage (Signer & Weil, 1968; Echols & Gingery, 1968). Either of these mechanisms can generate free λ*imm*^{434} phage and will be called *imm*^{434} rescue in this paper.

The production of λhy*imm*^{434} was assayed by titering the lysate following superinfection on a λ immune lysogen, so that the λ*imm* superinfecting phage could not form plaques. A superinfecting phage possessing a functional *int-red* region rescues about 10^5-fold more λhy*imm*^{434} phage than does a transducing phage deleted of this region. This test was performed with all the transducing phage and the results are summarized in Table 2. Some of the transducing phage produced an intermediate response, approximately that of λ*int6*, and have been labeled as partially defective in *int-red* function. One of these phage was physically characterized as described in the next section. Table 3 shows the phage and the strain from which each was derived.

The strain with λ inserted in *araC* produced λ*paraB* transducing phage that display an Int$^+$ Red$^+$ phenotype. All strains with λ inserted in *araB* produced at least one λ*paraC* phage that had a defective or partially defective *int-red* function. We conclude that the λ*paraC* transducing phage possess substitutions in the *int-red* region; the λ*paraB* phage do not.

(e) *Localization of the* ara *substitution on* λpara *phage by electron microscopy*

The precise location of the substitutions in the λ*para* transducing phage were obtained by electron microscope analysis of heteroduplexes of the DNA from each phage with λhy*imm*^{434}. Analysis of heteroduplexes formed between the DNA from phage λhy*imm*^{434} and λ*paraC116* showed the *ara* substitution on this phage to extend from 57·5±1·2 (n = 15) to 67·1±0·1 (n = 19) units on the λ map (Fig. 5(a), Plate II(a)). Thus the substitution begins very near or at the *attP.P′* locus at 57·4 (Davis & Parkinson, 1971) and extends through the *int* and *red* genes ending near the *gam*

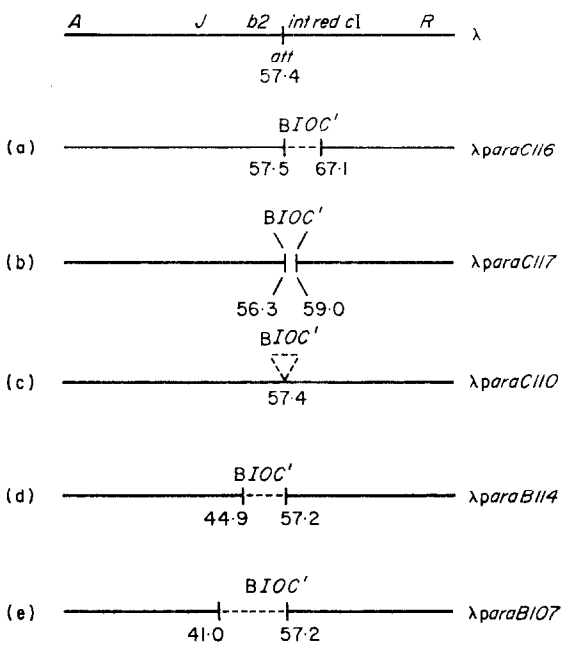

FIG. 5. Location of the bacterial substitution on the λ*para* phage. The solid lines represent the λ DNA and the dashed lines the bacterial substitution DNA for each of the 5 transducing phage that have been characterized by both electron microscopy and genetic techniques. The length of the λ DNA segments were determined by electron microscopy as described in Materials and Methods. Although the bacterial DNA in the substitution is positioned accurately with respect to the physical map of λ, its length is not drawn necessarily to scale.

gene. The *gam* gene is intact however, since λ*paraC116* grows on *recA*⁻ cells. It would not if the substitution extended into *gam* (Zissler *et al.*, 1971).

Analysis of heteroduplexes formed between λhy*imm*^{434} and λ*paraB114* shows the *ara* DNA in the transducing phage to substitute in the λ*b*2 region and to extend from 44·9±1·0 ($n = 10$) to 57·2±0·3 ($n = 10$) (Fig. 5(d)). A second λ*araB* transducing phage, λ*paraB107*, isolated from the same strain with λ inserted in *araC* contained *ara* DNA in the phage *b*2 region. The substitution extended from near the *J* gene 41·0±1·1 ($n = 5$) to 57·2±0·4 ($n = 8$) (Fig. 5(e)).

From one of the strains with λ inserted in *araB* two types of transducing phage were isolated: λ*paraC116* lacking *int* and *red* functions and λ*paraC117* which produced partially active *int-red* function, Table 2. Heteroduplex analysis demonstrated that both possessed substitutions of *ara* DNA to the right of the λ*attP.P'* locus indicating both were generated by the same type of excision event. However, less phage DNA was deleted from the phage producing partial *int-red* activity, λ*paraC117* (Fig. 5(b)). The *ara* substitution on λ*paraC117* begins near or at λ*attP.P'* and displaces only 744±107 base-pairs of λ genome to the right, from 56·3±0·9 ($n = 27$) to 59·0±0·2 ($n = 32$). This phage has 7×10^3 times more *int-red* activity than a phage like λ*pbio*II which totally lacks the *int-red* region. However, it produces at least tenfold less activity than that produced by a normal Int⁺-Red⁺ phage. Thus λ*paraC117* may lack the *int* gene or possess an *int* gene with reduced activity.

(f) *An anomalous transducing phage*

An *araC* transducing phage, λp*araC110*, possessed the peculiar property of losing the arabinose genes at a high frequency. Many of the phage in a plate stock made from a single plaque have lost the arabinose genes. This is demonstrated by the failure of 2 to 5% of the plaques to complement the *araC* bacterial lawn of JTL157 when titered on indicating plates. This phage produced wild type levels of *int-red* activities. Another transducing phage, λp*araC153*, isolated from the same insertion strain was stable, and like all the other λp*araC* phage, lacked *int-red* activity. Heteroduplex analysis of DNA from λp*araC110* and λhy*imm*^{434} (Fig. 5(c), Plate II(b)) shows that the bacterial genes do not substitute for the phage genes, but rather were added onto the phage DNA at the *attP.P′* locus. The single-stranded addition loop seen on these heteroduplexes is located at the phage *attP.P′* locus at 57·4±0·16 ($n = 14$). This phage also contains more of the *araB* gene than any of the mapped λp*araC* phage (Fig. 4). We therefore believe the phage was excised by recombination of bacterial DNA beyond the ends of the left and right *att* loci. A transducing phage so generated would possess both the *att* left and *att* right sites bordering the bacterial sequence containing the *ara* genes. Such a phage would have functional *int* and *xis* genes and their expression could catalyze specific recombination, analogous to prophage excision, between *attB.P′* and *attP.B′* sites. This would remove bacterial DNA from the phage. A phage with similar properties, λ*att*^{2}, has been previously isolated and characterized (Shulman & Gottesman, 1971).

4. Discussion

The object of the work described here was to isolate and characterize λp*ara* transducing phage containing the *ara* regulatory region. The first application of these phage, the isolation of sizeable quantities of a 1000 base-pair fragment containing the *ara* regulatory region, is described in the following paper (Lis & Schleif, 1975).

Arabinose-transducing phage have been isolated previously (Gottesman & Beckwith, 1969; Lis & Schleif, 1973; Lee *et al.*, 1974); however, many of these phage are defective and require the presence of a helper phage for propagation. The defective phage have the obvious disadvantage in that applications requiring pure transducing phage necessitate an isopycnic centrifugation purification step. Another disadvantage is that selection of deletions entering the bacterial DNA on the phage is not convenient using defective phage (Schleif & Lis, 1975).

The facility with which plaque-forming arabinose-transducing phage were isolated from the strains with λ inserted in *ara* appears to be a consequence of two factors: first, the proximity of the inserted λ phage to the genes picked up upon incorrect excision, and second, the low numbers of wild type phage produced upon induction of these lysogens. As a result, plaque-forming arabinose-transducing phage should be relatively abundant in lysates from these strains. Possibly, the reduced production of normal λ results from the difficulties the prophage genome may experience in excising from the *ara* operon. Transducing phage production from these insertion strains is about the same as it is from strains with λ inserted into its normal attachment site. This is not surprising as both events involve incorrect excisions. Consequently the effort required to isolate the rare λ in *ara* insertions was compensated

by the ease of isolating plaque-forming *ara* transducing phage from these strains. The frequency of detectable insertions of λ into the *ara* genes was estimated to be about 10^{-11} that of insertion of λ into its normal λ attachment site.

The insertion of λ into sites other than the λ*attB.B'* on the *E. coli* chromosome has been shown to be an *int*-promoted recombination event between bacteria DNA and the normal λ*attP.P'* (Shimada *et al.*, 1972,1973). In our study, five λ*para* phage, produced from heat induction of three independent insertion strains, were physically mapped by electron microscope analysis of heteroduplexes of the λ*para* phage with λhy*imm*^{434}. In all five transducing phage one end of the *ara* substitution was at, or very near the λ*attP.P'* site. This physical study indicates that λ inserted *via* the normal integration pathway into pseudo-attachment sites in the *ara* operon.

Shimada *et al.* (1972,1973) found the insertion of λ into alternative sites was non-random. The fact that eight independent strains possess λ inserted in *araB* between the closely spaced markers in strains RFSF'146 and RFSF'41 lends strong support to their conclusion. Not only did insertion occur at a specific site, or within a small region in the *araB* gene, but the orientation of the prophage was the same in all eight insertion strains as evidenced by the fact that the stable λ*para* phage, produced from all the insertions in *araB*, had bacterial DNA substituted in the *int-red* region.

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