

## Isolation and Characterization of Lambda Transducing Bacteriophages for *argF*, *argI* and Adjacent Genes

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Two genes for ornithinetranscarbamylase exist in strain *Escherichia coli* K-12, *argI*, at 85 min, and *argF*, at 7 min. In an attempt to compare the deoxyribonucleic acid material of these two genes, the lambda transducing phages carrying a portion of the *argI* region,  $\lambda$ *valS argI*,  $\lambda$ *valS*, and  $\lambda$ *valS pyrB*, and of the *argF* region,  $\lambda$ *dargF*, have been isolated. Their structure, including that of  $\phi$ 80*dargF* previously isolated, was studied by the method of heteroduplex mapping. In this paper, the results of this mapping are reported.

*Escherichia coli* strain K-12 has two genes for ornithinetranscarbamylase (OTC), *argF* at 7 min and *argI* at 85 min on the current linkage map (24). The parallelism of the regulatory expression of the two OTC genes, as well as the physicochemical resemblance of each gene product, were shown by the fact that the enzyme molecule is a trimer composed of a random assortment of the product of the two genes (11, 17). To answer the question whether this similarity of the two OTC genes might be extended to the homology of the deoxyribonucleic acid (DNA) sequence of the two operons, including the promoter-operator region, we decided to study the DNA itself.

The first step was the isolation of a specialized transducing phage for each gene. We have attempted the isolation of lambda-defective *argF* and *argI* transducing phages by the method based on the finding by Shimada et al. (20) that lambda phage tends to integrate into several new loci in the *E. coli* chromosome if the normal lambda attachment site is deleted. The subsequent induction of that lysogen may give new specialized transducing phage for the genes near to where the lambda prophage has been integrated.

This first paper will describe the isolation and physicochemical properties of some of these lambda transducing phages. In the *argI* region we have isolated *valS*, *valS argI*, and *valS pyrB* transducing phages, the latter by imposing an *argI* deletion. None of the phages carries the entire *valS argI pyrB* region although these three genes are not more than 0.2 min apart based on P1 transduction data (15). We have isolated a  $\lambda$ *dargF*, whereas in a previous work E. James and L. Gorini (unpublished data) have isolated a  $\phi$ 80*dargF*, according to the method described by Gottesman and Beckwith (13). All of these phages are characterized by

the heteroduplex mapping technique. Attempts to anneal the *argI* and *argF* genes are in progress.

### MATERIALS AND METHODS

Bacterial strains and bacteriophages are listed in Table 1. Methods for basic bacterial and bacteriophage manipulations were performed according to Miller (19) and specifically for lambda according to Gottesman and Yarmolinsky (12). Composition of media used are taken from Gottesman and Yarmolinsky. General methods for the isolation of lambda transducing phage were carried out according to Shimada et al. (20). Any departure from these methods is described in the paper. The related genetic markers are summarized in Fig. 1. Bacterial strains and bacteriophages constructed in this work are listed in Table 2.

**Purification of phage particles.** Phages were grown by inducing the appropriate lysogens. When the prophage carried the mutations *cl 857 S7* (temperature-sensitive immunity and lysis defective), heat-induced cells were centrifuged and concentrated about 50 to 100 times in 0.01 M MgSO<sub>4</sub> and then were lysed by chloroform at 18 C, followed by the addition of 5  $\mu$ g of pancreatic deoxyribonuclease per ml. The lysate, after removing cell debris, usually gave 10<sup>11</sup> to 10<sup>12</sup> plaque-forming units (PFU)/ml, which is the required concentration for the next step. If the prophage was not a lysis-defective mutant, and therefore the culture could not be concentrated before lysis, the phages could be precipitated, by adding polyethylene-glycol 6000 according to Yamamoto et al. (26), and resuspended in a small volume of 0.01 M MgSO<sub>4</sub> to get a titer of 10<sup>11</sup> PFU/ml.

About 4.0 g of solid CsCl was added to each 5.2 ml of concentrated phage suspension to adjust the initial density to  $\rho = 1.500$  g/cm<sup>3</sup>, and density gradient centrifugation was performed in a Spinco 50 Ti rotor at 25,000 rpm for at least 20 h at 8 C. The visible bands of helper and defective phage were extracted from the nitrocellulose centrifuge tube by a lateral puncture with a hypodermic needle. Each pooled fraction was subjected to another centrifugation in CsCl at 35,000 rpm for 24 h at 8 C.

TABLE 1. List of bacterial strains and bacteriophages

Strain	Genotype	Source
<b>Bacterial strain</b>		
CA8000	Hfr H B <sub>1</sub>	J. Beckwith
CA7033	Hfr H B <sub>1</sub> ( <i>proA/B argF lac</i> ) <sup>Δ</sup>	J. Beckwith
RW420	Hfr H B <sub>1</sub> ( <i>proA/B argF lac</i> ) <sup>Δ</sup> ( <i>gal attλ wrB</i> ) <sup>Δ</sup>	M. Gottesman
KS302	Hfr H B <sub>1</sub> ( <i>gal attλ bio</i> ) <sup>Δ</sup>	M. Gottesman
RW262	<i>tonA suIII<sup>-</sup> mel</i>	N. Kelker
NP29	F <sup>-</sup> <i>valS</i> (ts) (λ)	F. Neidhardt
UL101	<i>valS</i> (ts)( <i>proA/B argF lac</i> ) <sup>Δ</sup> <i>argA strA rel</i> (λ <sup>-</sup> )	P. Primakoff
BC22	Hfr H B <sub>1</sub> ( <i>proA/B argF lac</i> ) <sup>Δ</sup> <i>pyrB</i> ( <i>λCl 857 S7 b515 b519 xis</i> )	Our collection
PM8	F <sup>-</sup> B <sub>1</sub> ( <i>proA/B argF lac</i> ) <sup>Δ</sup> <i>argI his met aro argR11 Sm<sup>R</sup> λ<sup>R</sup> malA</i> (φ80i <sup>λ</sup> <i>cl 857</i> ) (φ80i <sup>λ</sup> <i>cl 857 dargF</i> )	Our collection
<b>Bacteriophages</b>		
λy199	<i>λCl 857 S7 xisam6 b515 b519</i>	M. Gottesman
λb2c		M. Gottesman
λh80 del9c		M. Gottesman
λy271	<i>λp proA/B cl 857 int</i>	R. Weisberg
λvir		R. Wolf
λCl 857 S7		J. Beckwith
φ80i <sup>λ</sup> <i>cl 857</i> (hybrid 5)		Our collection
φ80 vir		Our collection
φ80c		Our collection
λO am 29 <i>cl 60</i>		A. Torriani
λP am 3 <i>cl 60</i>		A. Torriani
λP am 80 <i>cl 60</i>		A. Torriani
λR am 216 <i>cl 60</i>		A. Torriani

The particle number was estimated by determining the transducing activity or the PFU and by absorbancy at 260 nm. One unit of absorbancy at 260 nm was equal to  $4 \times 10^{11}$  particles/ml.

The approximate density of the transducing phages was measured on the above-mentioned CsCl equilibrium density gradient using λb2c ( $\rho = 1.491$  g/cm<sup>3</sup>) and λCl 857 S7 ( $\rho = 1.508$  g/cm<sup>3</sup>) as internal markers (4).

**DNA preparation.** The phage suspension was dialyzed against 0.01 M tris(hydroxymethyl)-aminomethane (Tris)-hydrochloride and 0.01 M ethylenediaminetetraacetic acid/trisodium (pH 8.0) for 30 min through a freshly made collodion bag (3). The DNA was deproteinized at room temperature by adding an equal volume of buffer-saturated phenol and by mixing with gentle rotatory motion for 30 min and for another 10 min with one more volume of chloroform-isoamylalcohol (24:1 mixture). After short centrifugation, the phenol chloroform phase was pipetted off and the aqueous phase was treated twice more in the same fashion. The final aqueous phase was extensively dialyzed against 1,000 volumes of 0.01 M Tris-hydrochloride + 0.01 M ethylenediaminetetraacetic acid/trisodium (pH 8.0) for 24 h (three changes, at least) at 4 C. The concentration of DNA was determined by absorbancy at 260 nm, 1 U being equal to 50 μg/ml. The contamination of protein or phenol was carefully checked by scanning the ultraviolet absorption profile from 230 to 300 nm.

**Heteroduplex mapping.** Heteroduplex formation and visualization of DNA under the electron microscope was performed according to Davis et al. (7) with the modification of Tye et al. (25).

The two DNA samples (5 μg/ml of each in 0.01 M

Tris-hydrochloride, pH 8.6) were mixed in equal amounts and denatured by adding 1/50 volume of 5 N NaOH (> pH 11). After 30 min at 18 C, the solution was neutralized by 6/51 volume of a solution containing 1 M Tris-hydrochloride (pH 7.4) and 0.9 M HCl. Then the DNA was renatured slowly in the presence of the same volume of formamide (50% final concentration) at 18 C for 18 h, followed by an addition of 6/114 volume of 1 M Tris-hydrochloride (pH 8.6) and incubation for another 22 h at 18 C. A 120-μl volume of DNA solution was added to 2 μl of a cytochrome c solution (2 mg/ml of water) and spread by the formamide technique using 20% formamide in 0.01 M Tris-hydrochloride (pH 8.6) as a hypophase. DNA on the parlodion membrane grid was stained by 0.025 mM uranylacetate in 95% ethanol, shadowed by platinum palladium, and examined in a JELCO 100B electron microscope (25).

In the later study, the phage particle was used as DNA source instead of the extracted DNA and the results were quite satisfactory, as reported previously by Davis et al. (7).

## RESULTS

**Preparation of the strains.** *E. coli* K-12 contains two genes, *argF* and *argI*, coding for the same enzyme OTC. This peculiar situation requires some special care in preparing the necessary strains. The strain donating the transducing phage should not only have a deletion in *attλ* but also be deleted for that one of the two genes *argI* or *argF*, which we are not interested in. The recipient strain for the lysate

should be deleted in both *argF* and *argI* to avoid Arg<sup>+</sup> revertants.

(i) **Preparation of the *argI*<sup>Δ</sup> *argF*<sup>Δ</sup> strain (AD1).** Since the *argF* gene is located between *proA/B* and *lac*, any strain carrying a *proA/B*

*lac* deletion also provides the deletion of the *argF* gene. Therefore, the Hfr H strain CA7033 (*proA/B lac*)<sup>Δ</sup>, from the J. Beckwith collection, carries an *argF* deletion. Using ampicillin enrichment (19) without prior mutagenesis, we

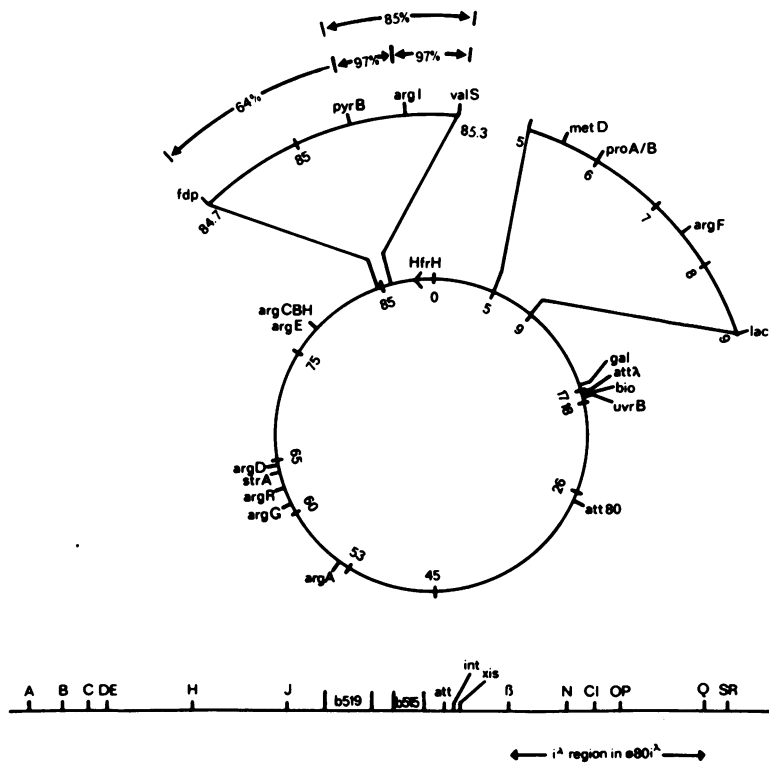


FIG. 1. Abridged genetic map of *E. coli* K-12 and bacteriophage lambda. The *E. coli* genetic map is taken from Taylor and Trotter (24) with the modification that the *proB* locus is moved to a place close to the *proA* locus, according to the recombination data by Broda (1), and according to the experiments with phage P22 by Hoppe and Roth (14). The P1 co-transduction frequency for *pyrB argI valS* loci is taken from the data by Jacoby (15). The lambda phage map is taken from the heteroduplex mapping data by Davidson and Szybalski (4).

TABLE 2. List of bacterial strains and lambda transducing phages constructed in this study

Determinants	Genotype	Parent	Parent of transducing marker	Parental phage
Bacterial strain				
AD1	Hfr H ( <i>proA/B argF lac</i> ) <sup>Δ</sup> <i>argI</i> <sup>Δ</sup>	CA7033		
AD9	<i>argI</i> <sup>Δ</sup> ( <i>gal attλ bio</i> ) <sup>Δ</sup> Sm <sup>R</sup>	Cross between KS302 and AD1 Sm <sup>R</sup>		
AD92-55	<i>argI</i> <sup>Δ</sup> ( <i>gal attλ bio</i> ) <sup>Δ</sup> ( <i>proA/B</i> ) Sm <sup>R</sup>	AD9 <i>proA/B</i> <sup>-</sup> (λy199)		
AD92-61	<i>argI</i> <sup>Δ</sup> ( <i>gal attλ bio</i> ) <sup>Δ</sup> ( <i>proA/B</i> ) <sup>Δ</sup> Sm <sup>R</sup>	AD9 <i>proA/B</i> <sup>-</sup> (λy199)		
Bacteriophage				
λ <i>dargI valS</i>	<i>cI</i> 857 <i>S7 xis</i> (A... <i>att</i> ) <sup>Δ</sup>		RW420	λy199
λ <i>pvalS2</i>	<i>cI</i> 857 <i>S7 xis b515 b519</i>		RW420	λy199
λ <i>dvalS1</i>	Not tested		RW420	λy199
λyk11 (λ <i>dpyrB valS</i> )	<i>b515 b519 (att ... R)</i> <sup>Δ</sup>		AD9	λ <i>pvalS2</i>
λyk14 (λ <i>dpyrB valS</i> )	<i>b515 b519 (att ... cI)</i> <sup>Δ</sup> <i>S7</i>		AD9	λ <i>pvalS2</i>
λyk611 (λ <i>dargF</i> )	( <i>att ... P</i> ) <sup>Δ</sup> <i>S7</i>		AD92-61	λy271
λyk5512 (λ <i>dargF</i> )	( <i>att ... R</i> ) <sup>Δ</sup>		AD92-55	λy271

selected some Arg<sup>-</sup> derivatives of strain CA7033, which were able to grow in citrulline but not on ornithine. They did not contain any OTC activity and the lesion was more than 90% co-transducible by P1 with *valS* or with *pyrB*. They were therefore *argI*<sup>-</sup>, and one of these strains, AD1, did not revert and was taken as an *argI*<sup>Δ</sup> (*proA/B argF lac*)<sup>Δ</sup>.

(ii) **Preparation of the strain *argI*<sup>Δ</sup> *attλ*<sup>Δ</sup> (AD9).** Strain AD1, (*proA/B argF lac*)<sup>Δ</sup> *argI*<sup>Δ</sup>, was made Sm<sup>R</sup> by spontaneous mutation. Since it is an Hfr, it was grown for 48 h in rich medium to produce an F<sup>-</sup> phenocopy and used as a recipient in a cross with strain KS302 [Hfr H (*gal attλ bio*)<sup>Δ</sup>] as donor. Among 80 Pro<sup>+</sup> Sm<sup>R</sup> recombinants, which were also Arg<sup>+</sup>, one was Gal<sup>-</sup> Bio<sup>-</sup>, presumably having incorporated the (*gal attλ bio*) deletion. The mating was interrupted after 30 min; therefore there was no chance that Arg<sup>+</sup> could derive from the *argI*<sup>+</sup> of the donor, since this gene would only be transferred after 85 min with Hfr H donor. Therefore, the strain AD9 is *argI*<sup>Δ</sup> (*gal attλ bio*)<sup>Δ</sup> Sm<sup>R</sup>.

(iii) **Preparation of strains *argI*<sup>Δ</sup> *pro*<sup>Δ</sup> *attλ*<sup>Δ</sup> (AD92-55 and AD92-61).** The preparation of this strain is based on the fact that, in an *attλ*<sup>Δ</sup> strain, 2 to 3% of the lambda lysogens are at the *pro* site (22). The strain AD9 [*argI*<sup>Δ</sup> (*gal attλ bio*)<sup>Δ</sup> Sm<sup>R</sup>] was infected by a lambda phage with defective excision (we used λy199: *cl 857 S7 b515 b519 xis6*) at a multiplicity of infection of 10. The lysogens were isolated by plating on EMB-O plates, seeded with λh80 *del9c*, and incubating at 30 C. These plates were directly replicated on plates containing: minimal medium plus B1, biotin, and glucose; tryptone and B1; and tryptone and B1 seeded with λ*vir*. They were incubated at 30, 42, and 30 C, respectively. The colonies which failed to grow on all three plates were those which had the λy199 phage inserted in some structural gene, were killed by the λy199 induction at 42 C, and were not simply lambda resistant (were killed by λ*vir* at 30 C). They were purified from the EMB-O plate and 10 colonies out of about 500 were identified as Pro auxotrophs caused by the insertion of λy199 phage in the *pro* gene. Each auxotroph was grown overnight to 10<sup>9</sup> cells/ml, spread on preheated (42 C) tryptone plates, and incubated for 2 days at 42 C. The survivors should be either Pro<sup>+</sup> because they lost the prophage by spontaneous curing, or Pro<sup>-</sup> because by defective excision they lost at least part of the *pro* gene and either the whole phage genome or at least that part of it which is responsible for the temperature sensitivity (*cl 857*). Actually, we found very few Pro<sup>+</sup> colonies, probably because the *xis*<sup>-</sup> (amber) was strongly restricted by the Sm<sup>R</sup> muta-

tion in the host. Two of the Pro<sup>-</sup> were used as a *pro*<sup>Δ</sup> and called AD92-55 and AD92-61.

(iv) **The strain carrying *argF*<sup>Δ</sup> *attλ*<sup>Δ</sup>.** This was kindly provided by M. Gottesman. It is the strain RW420 Hfr H (*proA/B argF lac*)<sup>Δ</sup> (*gal attλ uvrB*)<sup>Δ</sup>.

**Preparation of the mixed lysate.** Strain RW420 [Hfr H (*proA/B argF lac*)<sup>Δ</sup> (*gal attλ uvrB*)<sup>Δ</sup>] was grown overnight and resuspended into 0.01 M MgSO<sub>4</sub> at 5 × 10<sup>8</sup> cells/ml, and λy199 (*cl 857 S7 b515 b519 xis6*) was allowed to adsorb for 30 min at 18 C at a multiplicity of infection of 10. Because the natural *attλ* site was deleted, the phage was forced to integrate at places elsewhere in the chromosome. A 0.05-ml amount of a 10<sup>-2</sup> dilution of this mixture was spread on EMB-O plates seeded with 10<sup>9</sup> λh80 *del9c* to kill the non-lysogenized cells. On this plate the lysogenized colonies appear pink (12). After 2 days of incubation at 30 C, 200 to 300 pink colonies per plate appeared. Six thousand colonies were picked up carefully to avoid excessive recovery of λh80 *del9c* phage from the plate and were resuspended together into broth. More than 80% of this cell mixture was temperature sensitive at 42 C, indicating the existence of λy199 phage integrated somewhere. One liter of the culture grown up to 2 × 10<sup>8</sup> cells/ml at 30 C was heat induced at 42 C for 45 min. After an additional 2 h of vigorous aeration at 33 C, the cells were centrifuged, resuspended into 5 to 10 ml of 0.01 M MgSO<sub>4</sub>, and lysed by chloroform in addition to deoxyribonuclease (5 μg/ml). After low-speed centrifugation to eliminate cell debris and chloroform, a mixed lysate was obtained containing different specialized transducing lambda phages. The PFU of this mixed lysate was determined using strain RW262 *suIII*<sup>+</sup> *tonA*<sup>-</sup> as an indicator. The titer found was of the order of 10<sup>12</sup> PFU/ml.

**Isolation of *argI* transducing phage.** A culture of AD1 [*argI*<sup>Δ</sup> (*proA/B argF lac*)<sup>Δ</sup>], lysogenized by λ*cl 857 S7*, was resuspended at 5 × 10<sup>9</sup> cells/ml in 0.01 M MgSO<sub>4</sub>, and the mixed lysate was adsorbed at a multiplicity of infection of 20. About 10<sup>9</sup> cells were spread on minimal plates (no arginine) and incubated at 30 C for 2 or 3 days. The 32 Arg<sup>+</sup> colonies obtained from 5 × 10<sup>9</sup> cells plated were grown in broth and induced at 42 C, and the lysates were tested using the same AD1 lysogenic for λ*cl 857 S7* as recipient. One lysate gave rise to an HFT for *argI* (*argF* would not have been carried by the lysate because that gene was already deleted in strain RW420, originating the mixed lysate). As the *argI* gene is known to be co-transduced by P1 at 97% with *valS* and *pyrB* (15), we next examined *valS* and *pyrB* transducing ac-

tivity of this *argI* transducing phage by testing the lysate on either strain NP29 [*valS*(ts),  $\lambda^+$ ] or strain BC22 (*pyrB*<sup>-</sup>), and by looking for survivors at 42 C in the case of strain NP29 and for uracil prototrophs at 30 C in the case of strain BC22. Actually this *argI* transducing phage was found to suppress the temperature-sensitive *valS* activity at 42 C. Although we did not confirm this by any biochemical test of either *argI* or *valS* gene activity, the transduction data strongly suggested that this phage in fact carries both *argI* and *valS* genes. On the other hand *pyrB* transducing activity was not detected in this *argI valS* phage. Therefore the region of *E. coli* chromosome picked up by this  $\lambda$ *dargI valS* specialized transducing phage is different from that of P22 specialized transducing phage for *argI* and *pyrB* (16).

**Isolation of *valS* transducing phage.** We could isolate this phage directly in the following way. Strain NP29 [*valS*(ts),  $\lambda^+$ ] was used as recipient, adsorbed with the mixed lysate, plated on nutrient agar, and incubated at 42 C overnight. The cells which grew were those which received *valS*<sup>+</sup>. After ultraviolet induction they were tested for HFT ability for *valS*<sup>+</sup> using the same strain NP29 as recipient. In this way 11 out of 21 colonies obtained from  $3 \times 10^9$  cells were proven to carry a *valS* transducing phage. Moreover, seven out of those 11 transducing phage lines were characterized as a plaque-forming type. None of these *valS* transducing phages found in this way show *argI* transducing activity. One of these,  $\lambda$ *pvalS2*, was used in this work.

**Isolation of *pyrB* transducing phage.** When a culture of Hfr H strain CA8000 *att* $\lambda^+$  strain lysogenized by  $\lambda$ *pvalS2* phage was heat induced and the lysate was tested on strain BC22 (*pyrB*<sup>-</sup>), it was found that the lysate often contained *pyrB* transducing activity at very low frequency, suggesting that some integration occurred at the *valS* site. When these *Pyr*<sup>+</sup> transductants were heat induced again, they produced an HFT lysate for *pyrB*, *argI*, and *valS*. But this HFT activity was so unstable (when the lysate was kept overnight at 4 C it did not show any more HFT activity the next day) that the transducing phage line could not be cloned. The instability of those phage particles may be attributed to the size of lambda DNA carrying *pyrB argI valS* which could be too large to be packaged in the lambda head. Therefore, it might be possible to obtain a stable *pyrB* transducing phage, if the size of DNA is shortened by deleting *argI*. The protocol for the isolation of *pyrB* transducing phages was the following.

Strain AD9 [*argI*<sup>Δ</sup> (*gal att* $\lambda$  *bio*)<sup>Δ</sup> *argF*<sup>-</sup>] was lysogenized by  $\lambda$ *pvalS2* phage so that the integration occurred into the *valS* site by recombination. A culture of this lysogen was heat induced and the lysate was used to transduce strain BC22 selecting on minimal plates (no uracil). All transductants were checked for their ability to generate HFT lysates. Two out of 17 colonies obtained from  $10^8$  cells gave HFT lysates ( $\lambda$ yk11 and  $\lambda$ yk14) for *pyrB* and *valS*. As expected, *argI* transducing activity was completely missing.

**Isolation of *argF* transducing phage.** The lambda transducing phage for *argF* was isolated in a way similar to that used for the  $\lambda$ *dpyrB* phage. The procedure was as follows. Strains AD92-55 and AD92-61 (*argI*<sup>Δ</sup> *pro*<sup>Δ</sup> *att* $\lambda$ <sup>Δ</sup>) were lysogenized by the  $\lambda$ y271 phage [ $\lambda$ *proA/B*, which is known to complement any (*proA/B*)<sup>Δ</sup> strain]. These lysogens, which became *Pro*<sup>+</sup>, were heat induced and the lysates were tested as to whether they contained *argF* transducing phage using strain AD1 (lysogenized by  $\lambda$ *cI 857 S7*) as a recipient. Three out of 12 colonies appearing on the selective plates (no arginine), obtained from  $2 \times 10^9$  phages from the AD92-55 lysate, and one out of three colonies obtained from  $2 \times 10^9$  phages of the AD92-61 lysate gave HFT lysates for *argF*. Lysate  $\lambda$ yk611 derived from strain AD92-61 and  $\lambda$ yk5512 derived from strain AD92-55 were saved for further study.

**The determination of the transducing phage density.** The density of each transducing phage was measured by a CsCl equilibrium density gradient, using  $\lambda$ *cI 857 S7* ( $\rho = 1.508$  g/cm<sup>3</sup>) and  $\lambda$ b2c ( $\rho = 1.491$  g/cm<sup>3</sup>) as internal markers. The results are summarized in Fig. 2.

The density of the  $\lambda$ *dargIvalS* and the  $\lambda$ *dvalS1* is not very different from that of the parental strain  $\lambda$ y199, whereas that of  $\lambda$ *pvalS2* is heavier, suggesting that the quantity of DNA increased about 10%. Since  $\lambda$ y199 phage has about 9.7% less DNA than the wild-type lambda DNA because of the two deletions of non-essential genes, *b515* and *b519* (6), the  $\lambda$ *pvalS2* phage has a density similar to that of the wild type ( $\lambda$ *cI 857 S7*) as shown in Fig. 2.

The density of the two  $\lambda$ *dvalS pyrB* obtained independently ( $\lambda$ yk11 and  $\lambda$ yk14) was found to be the same and similar to that of their parental phage  $\lambda$ *pvalS2*. By contrast, the density of  $\lambda$ yk611 was greater than that of the parental phage  $\lambda$ y271, whereas the other,  $\lambda$ yk5512, was less dense. Since these two  $\lambda$ *dargF* transducing phages were derived from  $\lambda$ y271 lysogens of two independently isolated *proA/B*<sup>Δ</sup> strains, AD92-55 and AD92-61, they might have originated in different ways.

**Heteroduplex mapping.** (i)  $\lambda$ *dargI valS*.

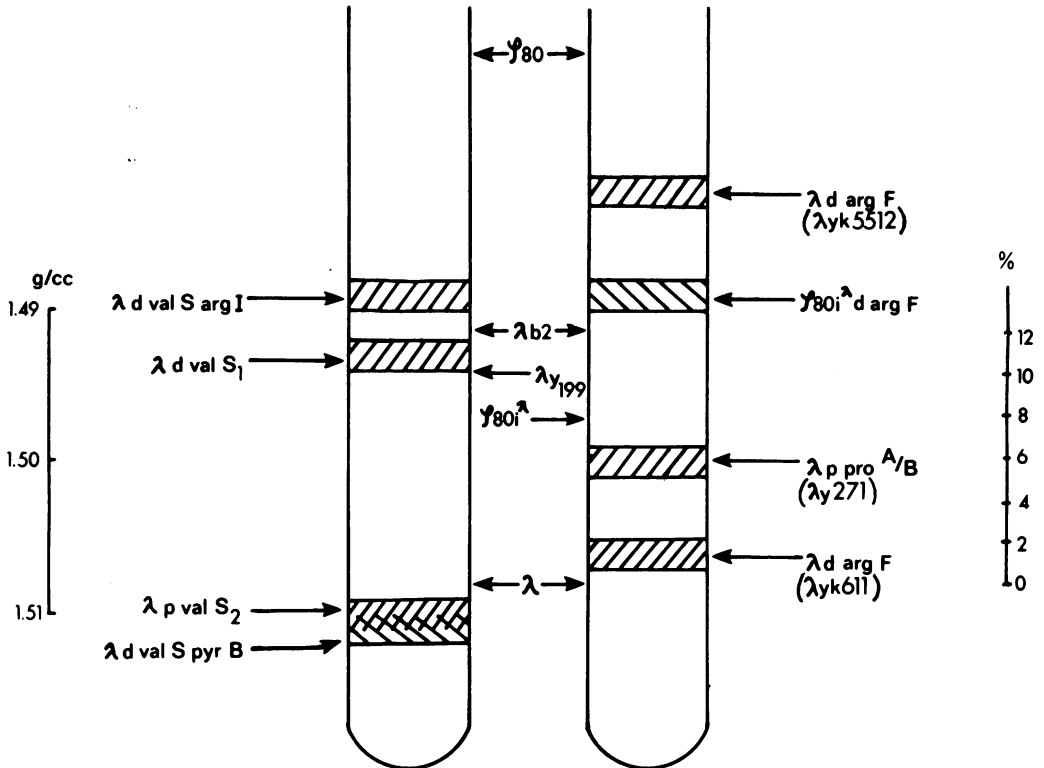


FIG. 2. The density of transducing phages. The density of each transducing phage was measured by CsCl density gradient equilibrium centrifugation as described. The results were superimposed into two sets for visualizing the density of transducing phage particles relative to each parental phage. The scale shown at the left side of the figure expresses the density of CsCl, deduced from the position of the two markers,  $\lambda b2c$  ( $\rho = 1.493 \text{ g/cm}^3$ ) and  $\lambda cI 857 S7$  ( $\rho = 1.508 \text{ g/cm}^3$ ). Since the CsCl density gradient was performed in the fixed angle rotor, the density in the upper and lower part of the tube (outside the scale) was no longer linear. The other scale at the right indicates the percentage loss of DNA length from the wild-type lambda phage ( $\lambda cI 857 S7$ ), according to the calculation given by Davis and Davidson (5).

Figure 3 shows the electron micrograph of heteroduplex DNA molecules formed between  $\lambda dargI valS$  phage and wild-type lambda phage. The conclusion that the foreign bacterial DNA has replaced the lambda phage genes for late function from A through *att*, at the left side of *att*, is based on the following two considerations. (i) The parent of  $\lambda dargI valS$  is the phage  $\lambda y199$  which carries two deletions, *b515* and *b519*, at the left side of *att*. If this side were present in the  $\lambda dargI valS$ , then the heteroduplex with lambda wild type should show two loops of insertion-deletion. But it does not. (ii) The phage  $\lambda dargI valS$  still contains the heat-labile immunity of the parent  $\lambda y199$  (*cI 857*) which is known to be located at the right side of *att*, between the *N* and *O* gene. The location of *att* itself is assigned by measuring the double strand length from one end. The foreign DNA corresponds to about 0.4 min equivalent of the *E. coli* genome and provides enough space to accommodate the *valS* and *argI* genes without

altering the total length of DNA of the transducing phage, which should be about the same as that of  $\lambda y199$ , as suggested by the density measurement.

(ii)  $\phi 80i^{\Delta dargF}$ .  $\phi 80i^{\Delta dargF}$  phage prepared from the strain PM8 was also examined by the heteroduplex method with  $\phi 80vir$  DNA. Figure 4 shows that the structure of  $\phi 80i^{\Delta dargF}$  is not different from that of  $\lambda dargI valS$ . The replacement was also found in the late genes: from gene 4, corresponding to gene D of lambda phage (8), through *att* of  $\phi 80$  phage. The right part of the figure shows the expected non-homology between  $\phi 80 vir$  and  $\phi 80i^{\Delta}$  (8). These heteroduplex mapping data are consistent with the results of the marker rescue experiments (E. James and P. Lombroso, personal communication).

(iii)  $\lambda pvalS2$ . The DNA of one of the lambda plaque-forming *valS* phages was examined by heteroduplex mapping with lambda wild-type DNA. Surprisingly, we found three bubbles of insertion-deletion, as shown in Fig. 5. Undoubt-

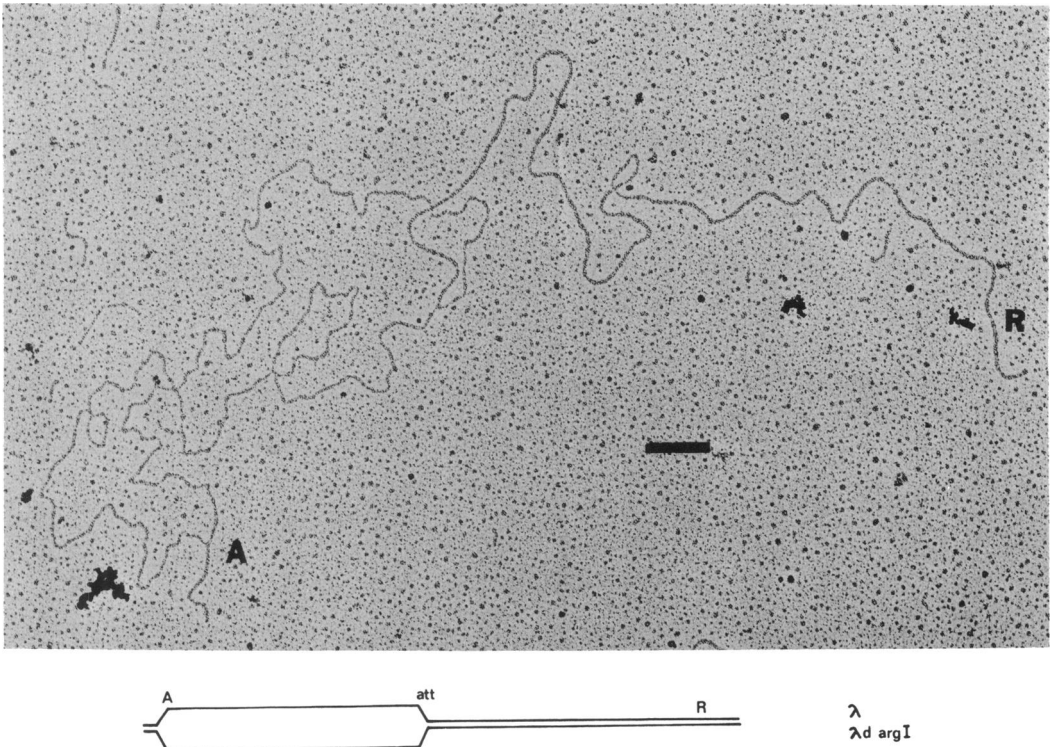


FIG. 3. Electron micrograph of heteroduplex DNA between  $\lambda$ dargI valS and wild-type lambda phage. Bar represents 0.3  $\mu$ m. The linear drawing at the bottom of the heteroduplex picture was composed by measuring several heteroduplex molecules. The exact length of double strand is shown in each case but the single-strand region is not proportional to actual length.

edly, because of their size and position, the two bubbles from the left end correspond to the deletion of *b519* and *b515*, respectively, which originally existed in  $\lambda$ y199 parental phage.

The third big bubble located around the *att* site seems to be the *valS* gene. Therefore the *valS* gene was not incorporated as a result of a substitution of phage DNA by host DNA, but rather host DNA is an addition inserted at the *att* site. The size of the insertion is about 5,000 base pairs which corresponds to a little in excess of the *valS* gene (3,000 base pairs at least since the molecular weight of valyl-transfer RNA synthetase is 100,000). Furthermore, this *valS* insertion is matched well with the size of the DNA lost by *b519* and *b515* deletions (4,500 base pairs), so that the  $\lambda$ pvalS2 phage should have regained a quantity of DNA similar to wild-type lambda phage, as suggested already by the density of this phage. The complementation test for *int* and *xis* function (kindly carried out by R. Weisberg) showed that all the seven  $\lambda$ pvalS phages isolated here had intact *int* and *xis* genes; that is, the *valS* insertion is located outside of the *int* gene.

(iv)  $\lambda$ dvalS pyrB. The two independently isolated  $\lambda$ dvalS pyrB phages,  $\lambda$ yk11 and  $\lambda$ yk14, were found to carry different sizes of *E. coli* chromosomal substitution, although they could not be distinguished by their density as shown above. The substitution in  $\lambda$ yk11 (Fig. 6a) is from *att* to the *R* gene (only the cohesive end remains at the righthand extremity) and in  $\lambda$ yk14 (Fig. 6b) from *att* to the *ci* gene. In each case the substitution is in the right arm, because the *b519* and *b515* deletions can be observed in the heteroduplex with lambda wild-type DNA, and because no heat-inducible immunity (*ci* 857) is detected. The heteroduplex between  $\lambda$ yk11 and  $\lambda$ yk14 (Fig. 6c) indicates that the difference between the two DNA is observed only at the right terminus of the substitution part of the above-shown heteroduplex. This means that the excision of prophage in each case occurred at different points.

(v)  $\lambda$ dargF. The two  $\lambda$ dargF phages,  $\lambda$ yk611 and  $\lambda$ yk5512, which showed a large difference in density, were examined by the heteroduplex mapping technique. Figure 7a shows the DNA heteroduplex between  $\lambda$ yk611 and wild-type lambda. The location of substitution was found

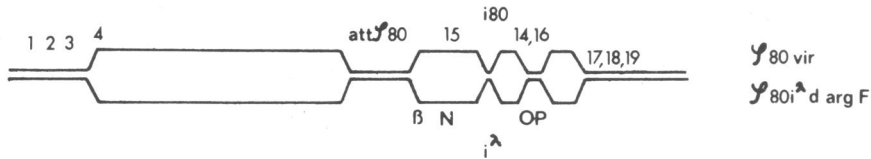
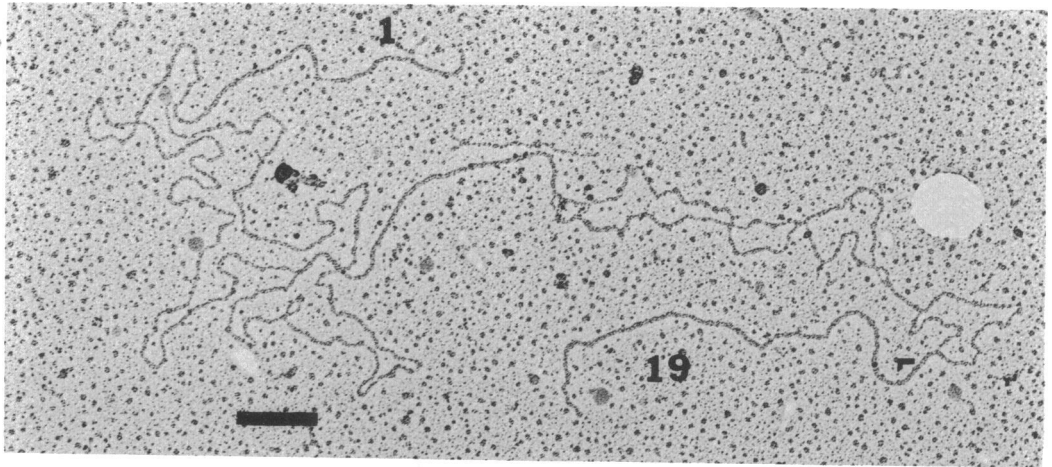


FIG. 4. Electron micrograph of heteroduplex DNA between  $\phi 80i^+dargF$  and  $\phi 80$  vir phage. Bar represents 0.3  $\mu m$ .

to be from *att* to the *P* gene of the right arm of the lambda phage genome, so that the phage carries the structural genes, *A-J* and *Q,S,R*. (See the marker rescue experiments.)

On the other hand, Fig. 7b shows the heteroduplex between  $\lambda k5512$  and lambda wild-type DNA. The substitution here was found to be up to the *R* gene so that only the cohesive end remains at the right extremity.

Figure 7c shows the heteroduplex between  $\lambda k611$  and  $\lambda k5512$  DNA; two regions of non-homology were observed: one, of the substitution type, with unequal amounts of DNA in each strand, and the other, of the insertion deletion type, at the right extremity. The unmatched regions indicate that the extent of the deletion between *proA/B* and *argF* of strain AD92-55 and of strain AD92-61 was in fact different, and also that the excision point of prophage was not the same in each case. During the preparation of large phage lysates it was noticed that both phages have lysis-defective phenotypes. This is reasonable in  $\lambda k5512$  since the *S* and *R* genes are not present in the transducing phage, whereas some additional explanation is needed for  $\lambda k611$  phage which apparently carries these genes.

**Marker rescue experiments.** Marker rescue was attempted in phages  $\lambda dpyrB$  *valS* ( $\lambda k11$  and  $\lambda k14$ ) and  $\lambda dargF$  ( $\lambda k611$  and  $\lambda k5512$ ) using as hosts strain UL101 [*valS(ts)*] and strain AD1 (*argF<sup>+</sup> argI<sup>+</sup>*), respectively. The

results are presented in Table 3. It is seen that (i) all four lysogens are sensitive to  $\lambda h80$  *del9c* indicating that none of the four phages carries the lambda gene *cI*; (ii)  $\lambda k11$  does not complement with amber derivatives of genes *O,P,S,R* indicating that these genes are missing; (iii)  $\lambda k14$  complements with *O,P,R* showing that these genes are present. Concerning the *S* gene, which is located between *P* and *R* and therefore should also be present, the negative result can be explained by the fact that the *S* gene is present but as a negative allele (*S7*), because the parent phage  $\lambda y199$  carries *S7*. (iv)  $\lambda k611$  does not complement *O,P,S* but complements *R*. According to the heteroduplex mapping it should also carry *S*. This implies that *S* is also present as a negative allele (*S7*), although the parent phage  $\lambda y271$  is *S<sup>+</sup>* (see Discussion). (v)  $\lambda k5512$  does not carry any of these genes.

These results are consistent with the heteroduplex mapping.

## DISCUSSION

In principle it should always be possible to isolate a lambda transducing phage for any given gene by simply selecting for lambda phages integrated within 0.5 min from the gene in question on the *E. coli* genetic map. In the case of the *argI* gene, there are two genes located on either side that are 97% co-transducible by P1: *valS* and *pyrB*. However, a *valS<sup>-</sup>* ( $\lambda^+$ ) lysogen, i.e., with a *valS* gene split by lambda



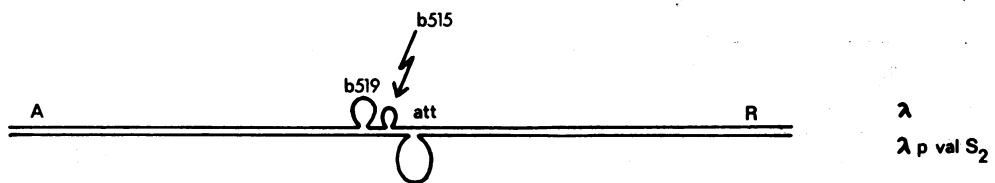
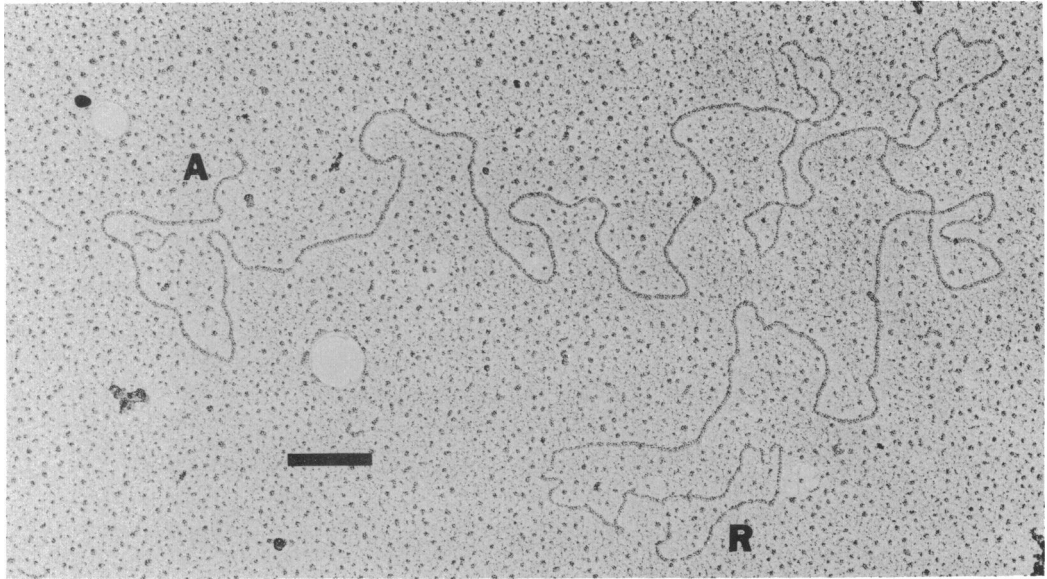


FIG. 5. Electron micrograph of heteroduplex DNA between  $\lambda pvalS_2$  and wild-type lambda phage. Bar represents  $0.3 \mu m$ . The single-strand loop of heteroduplex DNA in the drawing shown at the bottom does not represent actual length but relative size.

and therefore inactivated, is not viable since the *valS* final product, i.e., valyl-transfer ribonucleic acid, cannot be provided from outside. A *pyrB*<sup>-</sup> ( $\lambda^+$ ) lysogen, on the other hand, is difficult to select because of heavy cross feeding of the *pyrB*<sup>-</sup> cells in the presence of *pyrB*<sup>+</sup>. Therefore, neither of the two markers on either side of *argI* could be used for selecting an *argI* transducing lambda phage.

Another way to isolate *argI* transducing phages is in two steps as follows. First, by lambda integration into *argI*, an *argI*<sup>-</sup> ( $\lambda^+$ ) lysogen is isolated, which by induction should give either *valS* or *pyrB* transducing phage in the usual way. Next, this phage is forced to lysogenize a cell which is *att* $\lambda^{\Delta}$ . In this way, by recombination at the *valS* or *pyrB* locus, the phage will be integrated close to *argI*. Finally, these lysogens can be induced to obtain new lines of transducing phages carrying nearby markers, in our case *valS argI* or *pyrB argI*  $\lambda$  transducing phages. However, in the particular case of *argI* we did not succeed in performing the first step. By using RW420 *att* $\lambda^{\Delta}$  (*proA/B argF lac*)<sup>Δ</sup> as recipient of  $\lambda y199$ , we were unable

to isolate any *argI*<sup>-</sup> ( $\lambda^+$ ) auxotrophs among 8,000 independent lysogens, even after the enrichment for arginine auxotrophs by the ampicillin method. So the construction of *argI*  $\lambda$  transducing phage from a defined lysogen was abandoned.

A third possibility remained based on the fact that, according to Shimada et al. (21), about 97% of lambda secondary site lysogens showed no detectable change for the host cell such as nutritional requirement or phage resistance. Therefore many integration sites should exist in between genes so that *valS argI pyrB* transducing phages can be isolated if the lambda integration occurred just nearby. Therefore we decided to collect about 6,000 independent lysogens and to induce them in a lysogen mixture. The forthcoming mixed lysate should contain a variety of lambda-specialized transducing phages for every *E. coli* genetic marker, and one has only to select *valS*<sup>+</sup>, *argI*<sup>+</sup>, and *pyrB*<sup>+</sup> transductants. In this way, *valS* and *valS argI* transducing phages were successfully isolated.

The last two phages,  $\lambda dpyrB valS$  and  $\lambda dargF$ , were obtained by the recombination

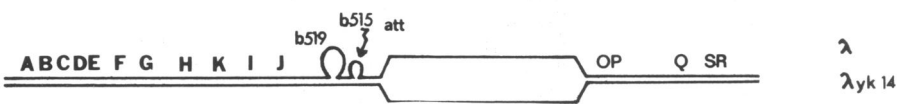
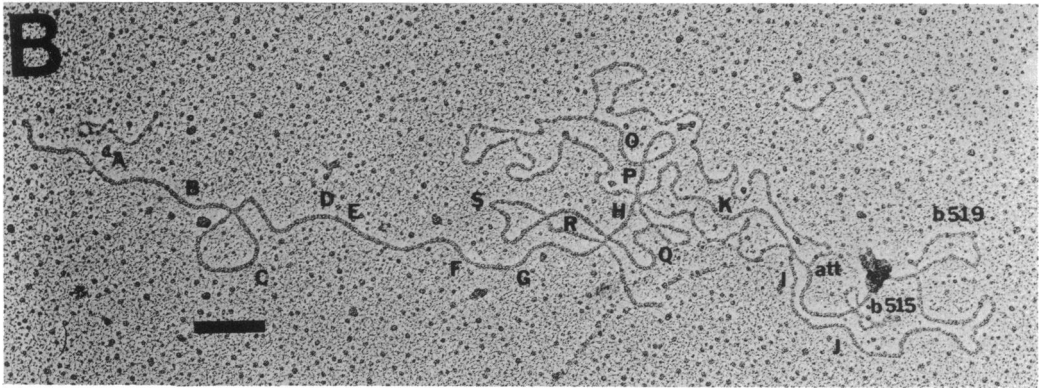
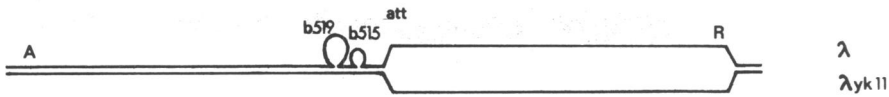
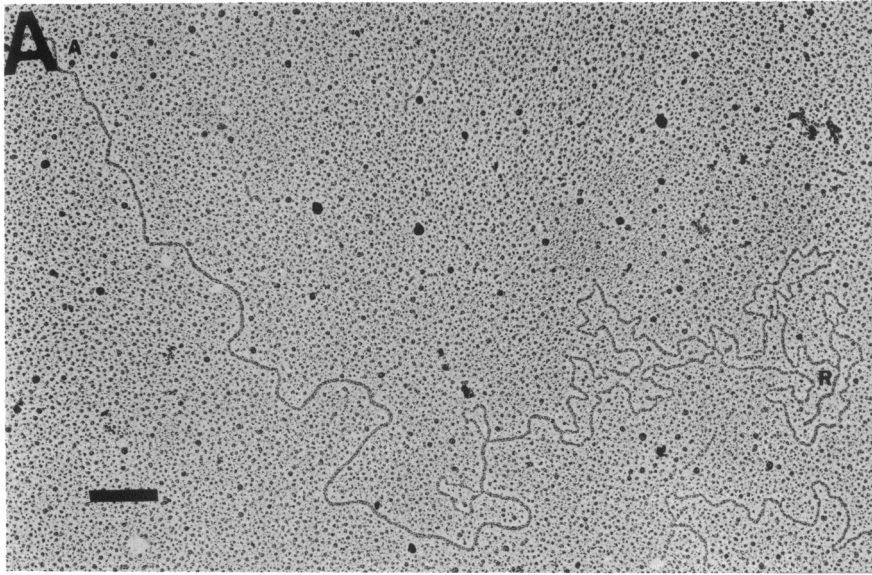


FIG. 6. (A) Electron micrograph of heteroduplex DNA between  $\lambda$ dpyrB valS ( $\lambda$ yk11) and  $\lambda$ cI 857 S7 phage. Bar represents 0.3  $\mu$ m. (B) Electron micrograph of heteroduplex DNA between  $\lambda$ dpyrB valS ( $\lambda$ yk14) and  $\lambda$ cI 857 S7 phage. Bar represents 0.3  $\mu$ m. (C) Electron micrograph of heteroduplex DNA between  $\lambda$ yk11 and  $\lambda$ yk14 phages. Bar represents 0.3  $\mu$ m.

method. The  $\lambda$ prvaIS and  $\lambda$ proA/B phage were forced to lysogenize an att $\lambda$  recipient in which the distance between valS and pyrB or proA/B and argF was shortened by inserting a deletion between the two markers: argI $\Delta$  for valS pyrB and a small proA/B $\Delta$  for proA/B argF. Espe-

cially in the latter case, where the distance of proA/B to argF as given in the literature is about 1.5 min, it is quite natural that no argF transducing phage was found in the lysate of the proA/B $^-$  ( $\lambda^+$ ) lysogen. The proA/B $\Delta$  strain, selected from proA/B $^-$  ( $\lambda^+$ ) lysogen, still con-

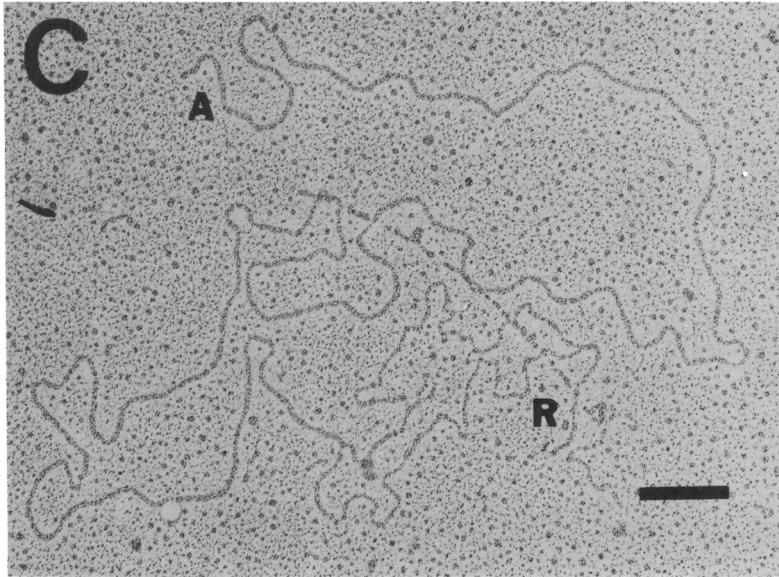


FIG. 6C

TABLE 3. Marker rescue test

Lysogen				Complementation <sup>a</sup>				
Phage	Host	Selective marker	Sensitivity to $\lambda h80 del9c$	$\lambda O am 29$	$\lambda P am 3$	$\lambda P am 80$	$\lambda S am 7$	$\lambda R am 216$
$\lambda dpyrB valS$	UL101	$valS^+$	Sensitive	-	-	-	-	-
$\lambda yk11$	UL101	$valS^+$	Sensitive	+	+	+	-	+
$\lambda dargF$	AD1	$argF^+$	Sensitive	-	-	-	-	+
$\lambda yk611$	AD1	$argF^+$	Sensitive	-	-	-	-	-

<sup>a</sup> Complementations were carried out by spot tests. The solution of lambda phage ( $10^7$  particles/ml) carrying an amber mutation in the gene in question was applied to a lawn of each lysogen at 42 C. Clear lysis indicates positive complementation (+).

tained part of the *proA/B* region and/or part of the lambda prophage genes. In this way the phage  $\lambda proA/B$  used for lysogenizing such a *proA/B*<sup>3</sup> strain was able to find the homology necessary for recombination and integration at the *proA/B* site. The method was successful in both cases for obtaining  $\lambda dpyrB valS$  and  $\lambda dargF$  phages.

Provided that no complicated events occurred during the isolation of these transducing phage lines, such as an inversion of genes or multiple deletions in one step, a heteroduplex mapping study will offer a plausible model for how each one of them arose.

First of all, in the case of  $\lambda pvalS2$  phage, the parental phage  $\lambda y199$  should have been integrated to the left or to the right of the *valS* gene in strain RW420 at a site located within 2,000 base pairs from the *valS* gene, because the *valS* insertion in  $\lambda pvalS2$  phage consists of, at most, 5,000 base pairs of *E. coli* genome in total and, of these, 3,000 base pairs correspond to the *valS* structural gene itself.

The possible orientation of the *valS* gene in the  $\lambda pvalS2$  phage can be deduced from the heteroduplex mapping of the  $\lambda dpyrB valS$  phages which have been derived from  $\lambda pvalS2$ . Since both of the  $\lambda dpyrB valS$  phages,  $\lambda yk11$

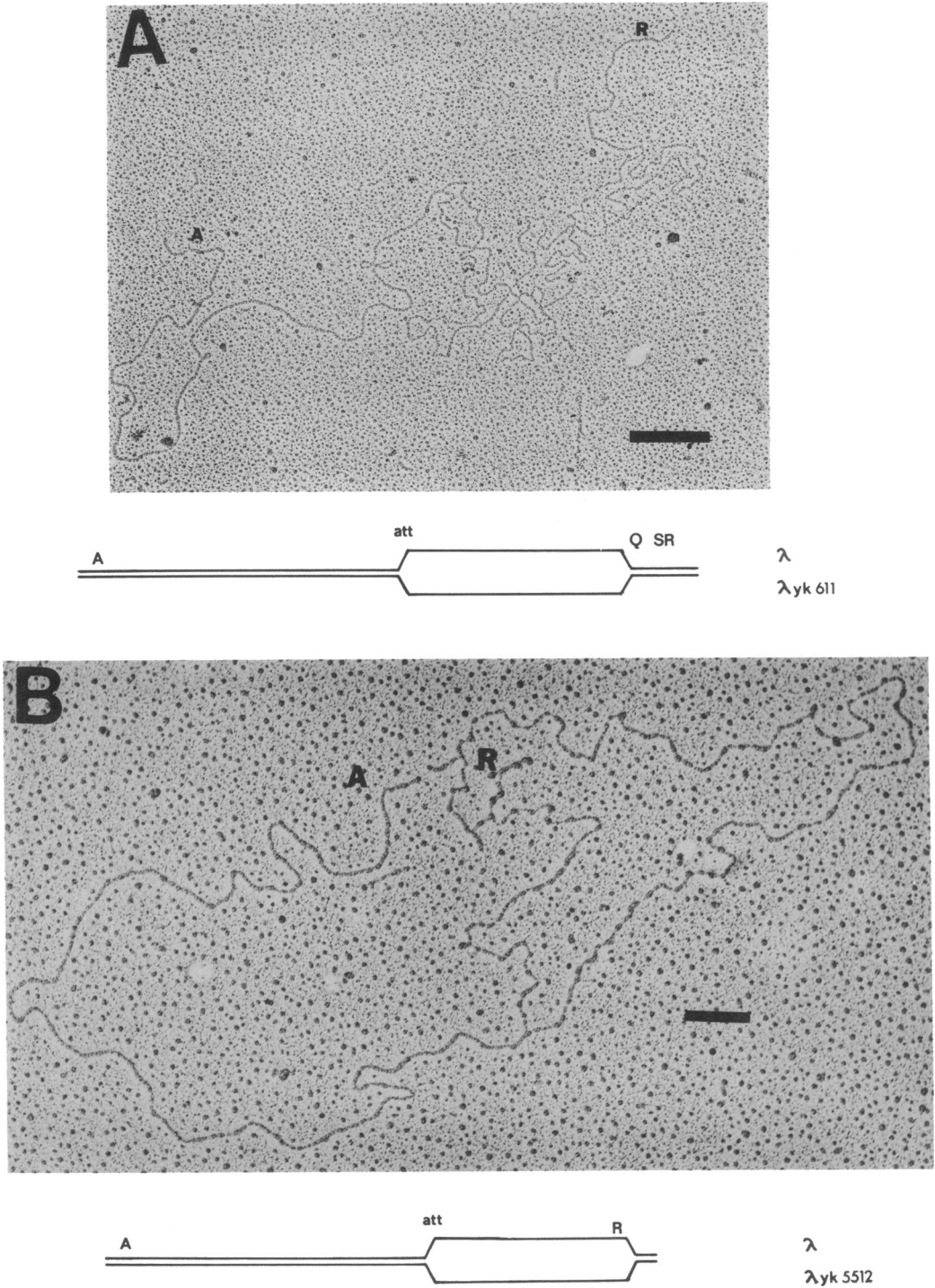
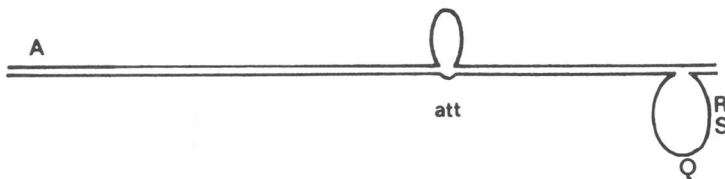
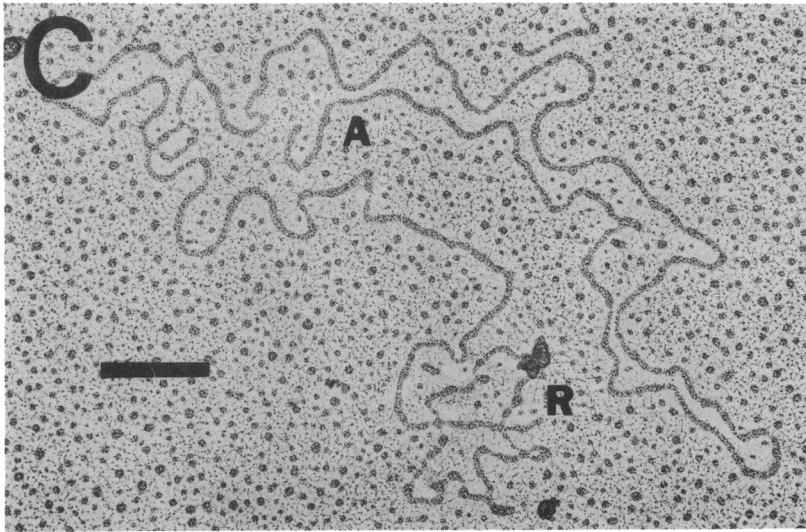


FIG. 7. (A) Electron micrograph of heteroduplex DNA between  $\lambda$ dargF ( $\lambda$ yk611) and  $\lambda$ cI 857 S7 phage. Bar represents 0.3  $\mu$ m. (B) Electron micrograph of heteroduplex DNA between  $\lambda$ dargF ( $\lambda$ yk5512) and  $\lambda$ cI 857 S7 phage. Bar represents 0.3  $\mu$ m. (C) Electron micrograph of heteroduplex DNA between  $\lambda$ yk611 and  $\lambda$ yk5512 phages. Bar represents 0.3  $\mu$ m.



$\lambda$ yk 5512

$\lambda$ yk 611

FIG. 7C

and  $\lambda$ yk14, have a substitution in the right arm (from *att* through *ci*), the  $\lambda$ *pv*als2 phage must have been integrated into the AD9 strain by recombination at the *valS* gene with the resulting prophage orientation given in Fig. 8. Therefore, the original  $\lambda$ *pv*als2 phage should also have the structure shown in Fig. 8. If the  $\lambda$ *pv*als2 configuration would be the opposite, another step of prophage deletion or rearrangement would be required to obtain the  $\lambda$ yk11 and  $\lambda$ yk14 structure. The structural difference between  $\lambda$ yk11 and  $\lambda$ yk14 can be accounted for by supposing that the prophage excision occurred at two different places, so that the unmatched loop in the heteroduplex DNA between  $\lambda$ yk11 and  $\lambda$ yk14 (Fig. 6C) must contain the *OPQSR* genes of the lambda phage in one strand and the *E. coli* chromosome beyond the *pyrB* gene in the other strand.

In the case of the  $\lambda$ *dargI valS* phage, heteroduplex mapping data show that replacement of phage genes occurred in the left arm. Therefore, the integration site of the  $\lambda$ y199 parental phage into RW420 should have occurred in one of two possible ways: *-pyrB-J . . . A R S Q ci N int-argI valS-*; or *-pyrB argI valS-int N ci Q S R A . . . J-*.

However, preliminary heteroduplex mapping between  $\lambda$ *pv*als2 and  $\lambda$ *dargI valS* did not show

any *valS* DNA duplex, so that the orientation of the *valS* genes in the two transducing phages must be opposite to each other. This observation excludes the first structure because in this case the *valS* gene in the  $\lambda$ *dargI valS* transducing phage should have the same orientation as the *valS* gene in  $\lambda$ *pv*als2 phage.

The distance between the *valS* gene and the integration site of the prophage in the second structure should be about 0.3 min, calculated as follows. The total substitution of *E. coli* genes in the  $\lambda$ *dargI valS* transducing phage corresponds to about 0.4 min; the distance between *argI* and *valS* is at maximum 0.1 min, which corresponds to about 5,000 base pairs which accommodates both *argI* (1,000 base pairs) and *valS* (3,000 base pairs); the distance from *argI* to *pyrB* is also less than 0.1 min so that, if the above-mentioned distance between the *valS* gene and the integration site would be less than 0.3 min, the transducing phage should also carry the *pyrB* gene and we know that this is not the case.

It should be noted that the appearance of the  $\lambda$ *dargI valS* transducing phage was very low ( $10^{-11}$ ) so that alternative explanations cannot be ruled out easily, including the possibilities of gene inversion or two simultaneous deletions during prophage excision.

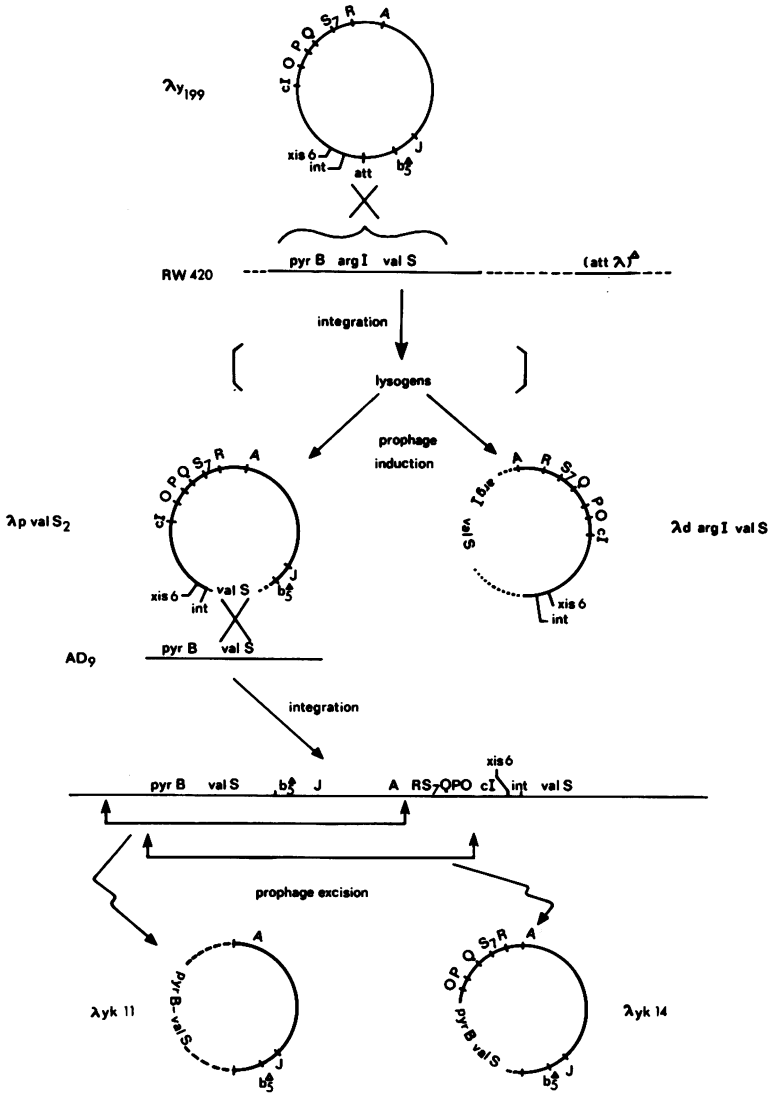


FIG. 8. Sequences of events involved in construction of transducing phages for *argI valS*, *valS*, and *pyrB valS*. Details of each step are described in the text. The order of letters of genetic markers, conserved in lambda phage as it is in the *E. coli* chromosome, indicates the orientation of the genes so that *pyrB* is transcribed from right to left (23), the same as *argI* (15). The orientation of the *valS* and *argF* genes is not known but this rule is kept. *b5*<sup>+</sup> indicates the wild-type structure of the *b515*, *b519* region, whereas *b5*<sup>A</sup> indicates the two deletions of *b515* and *b519*.

Any model proposed for the formation of the  $\lambda_{dargF}$  phages should take into account that the two phages we have studied,  $\lambda_{k611}$  and  $\lambda_{k5512}$ , are lysis defective. This is particularly difficult to explain for  $\lambda_{k611}$  because from the heteroduplex data this phage should carry the *S* and *R* genes. The simplest model is the following. Only two types of phages were employed for the construction of  $\lambda_{k611}$  phage. One,  $\lambda_{proA/B}$  ( $\lambda_{y271}$ ) from which  $\lambda_{k611}$  was directly obtained, is *S*<sup>+</sup>. The other,  $\lambda_{y199}$  phage

used for isolating the *proA/B* deletion in the AD9 strain, is in fact *S*<sup>-</sup>. Therefore, the *S*<sup>-</sup> phenotype must be derived from the  $\lambda_{y199}$  phage genome. We propose that the *proA/B* deletion strain, AD92-61, may have still kept as cryptic prophage a part of the  $\lambda_{y199}$  phage genome, including the *S7* gene but not the *ci* 857 gene. This is not an uncommon situation (2, 9, 10, 18). This model is shown in Fig. 9. The opposite orientation of  $\lambda_{y199}$  prophage into the *proA/B* gene is unlikely because, in one step,

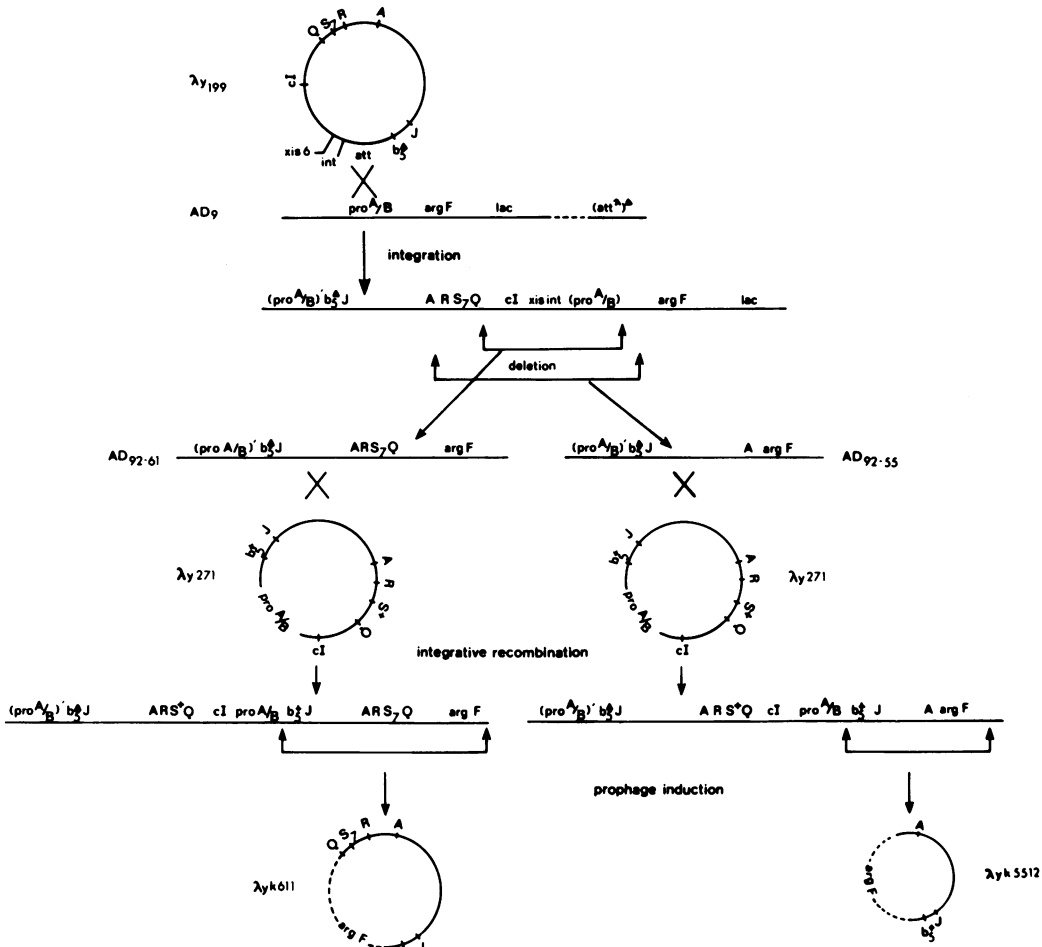


FIG. 9. Sequence of events involved in construction of  $\lambda$  *dargF* transducing phage. Details are explained in the text and markers are expressed in the same manner as in Fig. 8.

not only should *cI* 857 be deleted, but also part of the *proA/B* gene to shorten the distance between the cryptic phage and the *argF* gene without losing the *S7* marker. Subsequently, the  $\lambda$  *proA/B* ( $\lambda$ 271) phage was integrated into the AD92-61 strain. From the fact that  $\lambda$  *yk611* phage carries *S7*, one may deduce that  $\lambda$ 271 *proA/B b515+ b519+ S+* phage was integrated into the cryptic prophage *b515- b519- S7* by an integrative recombination at a marker between *b515 b519* and *S* to obtain *S7* progeny ( $\lambda$  *yk611*). The heat induction of the AD92-61 lysogen provides the *argF* transducing phage ( $\lambda$  *yk611*) by cutting at the arrowed points in such a way that the DNA structure of the phage includes the *S7* gene.

Since the orientation of the *argF* gene in the  $\lambda$  *yk5512* phage is the same as in  $\lambda$  *yk611* (see the heteroduplex between the two  $\lambda$  *dargF* phages in

Fig. 7C), the above-mentioned scheme is also applicable to  $\lambda$  *yk5512* with the assumption that the AD92-55 strain carries as cryptic prophage a more deleted phage genome which excludes *S7*. The process is shown on the right side of Fig. 9.

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