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Isolation and Characterization of the Specialized Transducing Bacteriophages *ϕ80dargF* and *λh80cI857dargF*: Specific Cleavage of Arginine Transducing Deoxyribonucleic Acid by the Endonucleases *Eco*RI and *Sma*R

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The directed transposition of *argF* to the *tonB* locus of the *Escherichia coli* chromosome and the subsequent isolation of the specialized transducing phage *ϕ80dargF* is described. The structure of this phage has been determined. A hybrid *λh80cI857dargF* phage has been constructed. Deoxyribonucleic acid isolated from these and their parent bacteriophages has been specifically cleaved by the endonucleases *Eco*RI and *Sma*R; the unique deoxyribonucleic acid fragments thus obtained have been resolved and analyzed by electrophoresis in agarose gel.

An important genetic tool for the study in vitro of the regulation of gene expression is the availability of a specialized transducing phage carrying and replicating the gene under study in large amounts. This tool has been of great value in studies concerning the arabinose (11), galactose (25), lactose (2), and tryptophan (39) operons. Gottesman and Beckwith (10) described a general procedure for directed gene transposition in order to isolate specialized transducing bacteriophages for any *Escherichia coli* gene. In particular, they described the isolation of the specialized bacteriophage *ϕ80dara* from a strain in which the arabinose operon had been transposed to the *att80* region.

To examine the regulation of the arginine biosynthetic pathway, we have transposed *argF* to the *att80* region of the *E. coli* chromosome and have isolated the specialized transducing bacteriophage *ϕ80dargF* from this directed transposition strain. The specialized transducing bacteriophage *λh80cI857dargF* has been constructed. We show in this work that phage genes in the left arm have been replaced by deoxyribonucleic acid (DNA) of bacterial origin. In consequence, a new and unique procedure for the resolution of the DNA strands of this bacteriophage has been developed (29). Sens and James (30) have shown that DNA isolated from *λh80cI857dargF* is a good template for in vitro transcription of *argF* messenger ribonucleic acid (mRNA). The rate of mRNA synthesis is biphasic at 7 min, presumably due to read-through from a downstream phage promoter. We now describe the specific cutting of DNA isolated from these arginine-transducing phages by the endonucleases *Eco*RI and *Sma*R and propose the use of such specific DNA fragments for in vitro transcription of *argF* without read-through from downstream phage promoters.

MATERIALS AND METHODS

Bacteria and phage strains. The genotype and origin of the *E. coli* strains used in this work are listed in Table 1, and the locations of the relevant markers on the genetic map are shown in Fig. 1. The derivation of the strains constructed is outlined in Table 2.

Media and buffer solutions. L(A-N) liquid medium and agar (2%) plates (33) were used for general growth of bacteria; bacterial strains used for preparing phage stocks were grown in L liquid medium (20).

Selection plates contained minimal medium A (5), supplemental growth factors as required, 2% agar, and 0.5% glucose or lactose as carbon source. Supplements were used at the following concentrations (μg/ml): adenine, 20; L-arginine, 100; L-citrulline, 100; L-histidine, 30; L-leucine, 80; L-methionine, 30; L-ornithine, 100; L-proline, 50; thiamine, 10; L-threonine, 50; thymidine, 10; L-tryptophan, 25; uracil, 20.

Lactose fermentation was scored on tetrazolium (TZ) plates (24). Plates containing 5-bromo-4-chloro-3-indoyl-β-D-galactoside were used to score *lac* character on minimal medium (4). For selecting against streptomycin-sensitive strains, streptomycin sulfate (500 μg/ml) was added to minimal or rich medium. *ϕ80* was titrated on H plates in H top agar (0.65%) (10). F top agar (0.65%) was used for plating cells on minimal medium (22).

A-N buffer contained the following (g/100 ml): K_2HPO_4, 0.7; KH_2PO_4, 0.3; Na_3 citrate·$2H_2O$, 0.05; $MgSO_4 \cdot 7H_2O$, 0.01.

T_1 buffer was 6×10^{-4} M $MgSO_4$, 5×10^{-4} M

TABLE 1. *Bacterial strains and bacteriophages used*

Strains or bacteriophages	Genotype	Origin
Hfr strains		
CA8000	*thi*	J. Beckwith
KL1699	*thi recA1*	B. Low
CA7033	*thi (lac proB proA argF)*^{r}	J. Beckwith
F^{-} strains		
GL1	*thi thr leu argF argI gal lac* λ^{-} λ^{r} *strA*	N. Glansdorff
GL2	*thi leu argF proA strA argI adeK lac mal*	N. Glansdorff
X181a	*pyrC trp* λ^{r} *strA*	J. Beckwith
LS539	*thi (lac pro argF)*^{r} *trp*^{r} *att80*^{r} *tonB his mal*	L. Soll
F′ strains		
X178	Col V/B^{+} *pyrC his*	J. Beckwith
E7129	F′$_{TS114}$ *lac*$^{+}$ *proB*$^{+}$ *proA*$^{+}$ *argF*$^{+}$*/(lac proB proA argF)*$^{\nabla}$ *strA*	J. Miller and J. Beckwith
Bacteriophages		
ϕ80		Our collection
ϕ80v		Our collection
ϕ80h		J. Beckwith
λh80cI$_1$857 (ϕ80 att80iλ)		M. Z. Fiandt
ϕ80 sus mutants		L. Soll

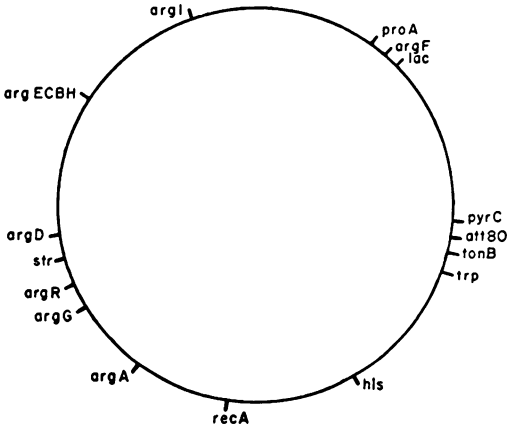

FIG. 1. *E. coli linkage map according to Taylor and Trotter (35).*

$CaCl_2$, 6 × 10^{-3} M tris(hydroxymethyl)aminomethane (Tris)-hydrochloride (pH 7.3), and 0.1% (wt/vol) gelatin.

Mating procedure: flask technique. Donors were grown with aeration in L(A-N) medium with glucose at 37 C to a density of about 10^{8} cells/ml. Strains carrying a temperature-sensitive episome were grown at 30 C. Growth was then continued without aeration to a density of about 2 × 10^{8} cells/ml. All matings were performed at 37 C without agitation in a 125-ml Erlenmeyer flask containing a total volume of 1 ml. Recipient bacteria in early stationary phase were in 10-fold excess, and approximately 4 × 10^{7} donor cells were used in each conjugation experiment.

Mating was interrupted by diluting 10-fold into A-N buffer followed by agitation using a Vortex mixer for 30 s. Further dilutions were made in A-N buffer enriched with 10 ml of L medium per 100 ml and with 0.2% glucose; appropriate dilutions were spread on selective minimal plates.

Mating procedure: multiple mating technique. Donors were grown to early log phase as described for the flask technique. Matings were performed at 37 C in a well device containing 20 individual sample positions using a total volume of about 100 μl. After a period of between 1 and 1.5 h, all 20 samples were transferred simultaneously to minimal plates with a steel disk containing 20 stainless steel screws matching the 20 holes in the well device. Males were counterselected by streptomycin. Male and female controls gave no grown or incipient hazy growth after 20 h, with no further growth evident after 48 h. Recombinants from Hfr/F^{-} matings gave confluent growth in 20 to 40 h.

Preparation of phage stocks. Bacteriophage *ϕ80v* and *ϕ80h* were propagated by the plate procedure. Titers of between 2 × 10^{11} and 4 × 10^{11} phage/ml were obtained. Transducing phage were prepared in one of the following ways. A double lysogen for *ϕ80dargF* and *ϕ80h* was grown in L medium to a density of 3 × 10^{8} bacteria/ml; the culture was centrifuged and the pellet was resuspended in half the original volume of 0.1 M $MgSO_4$. The suspension was then irradiated in 5-ml portions in an open petri dish for 50 s with a 15-W germicidal lamp (General Electric G15 T8) at a distance of 69 cm. When large volumes were induced, 125-ml portions were irradiated in a Pyrex dish (12 by 18 inches [about 30.5 by 45.8 cm]) for 60 s. The irradiated culture was added to an equal volume of double concentration of L medium containing 0.5% glucose, and the cells were incubated at 37 C in the dark with vigorous agitation for 3 h. A double lysogen for *λh80cI857dargF* and *λh80cI857* was grown in L medium at 30 C to a density of 3 × 10^{8} bacteria/ml; the culture was transferred to a temperature of 42 C for 15 min after which time the temperature was reduced to 37 C and the culture was incubated with vigorous aeration for 2 to 3 h until extensive lysis was evident. Chloroform was then added to the culture (0.5 ml/100 ml) and agitation was continued for 5 min. The culture

TABLE 2. *Bacterial strains constructed*[a]

Strains	Genotype	Origin
EJ4	*thi leu argF proA strA argI lac his*	GL2, nitrosoguanidine mutagenesis for *his, ade*$^+$
EJ10	*thi leu argF proA strA argI lac recA1*	KL1699 × EJ14 selected for *his*$^+$ recombinant, scored for *recA*
EJ11	*thi (lac proA proB argF)*^{r} *his strA argI*	CA7033 × EJ4 selected for *leu*$^+$ recombinants, scored for *proA*
EJ48	*thi (lac proA proB argF)*^{r} *his strA argI (ϕ80h)*	EJ11 lysogenized with *ϕ80h*
EJ29	*thi (lac proA proB argF)*^{r} *(F'lac*$^+$ *proA*$^+$ *proB*$^+$ *argF*$^+$*) tonB*	Directed transposition from E7129
EJ32	*thi (lac proA proB argF)*^{r} *(F'lac*$^+$ *proA*$^+$ *proB*$^+$ *argF*$^+$*) tonB (ϕ80h)*	EJ 29 lysogenized with *ϕ80h*
EJ38	*F' thi argI (lac pro argF)*^{r} *trp*^{r} *att80*^{r} *tonB his mal*	LS539, nitrosoguanidine mutagenesis for OTC$^-$
EJ39	*F'thi argI (lac pro argF)*^{r} *trp*^{r} *att80*^{r} *tonB his mal str*^{r} *(ϕ80h)*	EJ38 lysogenized for *ϕ80h* and streptomycin-resistant spontaneous mutation
EJ55	F'_{TS114} *trp*$^+$ *att80*$^+$*/thi arg (lac pro argF) att80 trp tonB his mal*	RK 505 × EJ38 selected *trp*$^+$ recombinant
EJ81	*thi thr leu argF lac strA argI (λh80C*$_1$*857)*	GL1 lysogenized with *λh80cI857*
EJ82	*thi thr leu argF lac strA argI (ϕ80dargF)*	Single defective lysogen from GL1
EJ83.1	*thi thr leu argF lac strA argI (λh80C*$_1$*857) (λh80C*$_1$*857dargF)*	EJ81 transduced with *λh80cI857dargF*

[a] Only the pertinent phenotype is reported.

was centrifuged at 16,000 × *g* for 10 min and the supernatant fluid was stored over chloroform.

Preparation of colicin. Colicin lysates were prepared from strain X178 as described for the preparation of *ϕ80* transducing bacteriophage except that growth was allowed to proceed for 4 to 4.5 h after induction by irradiation with ultraviolet (UV) light. Chloroform was removed from colicin lysates by gentle aeration prior to storage.

Construction of strains lysogenic for temperate phages. The recipient strain was grown with aeration in L(A-N) with glucose to a density of 5×10^8 cells/ml, and a volume of 0.1 ml was spread on an L(A-N) plate. Drops of lysate containing between 10^7 and 5×10^8 phage were spotted on the bacterial lawn and the plate was incubated overnight at 30 C. Colonies growing in the clean areas of lysis were picked, purified by streaking to single colonies, and tested for lysogeny.

Selection of *tonB* mutants. Overnight cultures (2.5 ml) were centrifuged to concentrate the cells. The supernatant was removed and discarded. The pellet was resuspended in the remaining medium (about 200 μl), 300 μl of *ϕ80v* (minimum titer 2×10^{11} phage/ml) was added, and the mixture was blended in a Vortex mixer gently for about 5 s. The phage was allowed to adsorb at 37 C for 15 min, and the cells were again concentrated by centrifugation; the supernatant was discarded. A 300-μl of volume of colicin V/B was then added, and the mixture was incubated further for 15 min. A 200-μl volume was spread on TZ plates and incubated at 42 C for 36 h.

Concentration of phage and cesium chloride centrifugation. Phage preparations were concentrated by adding 10% polyethylene glycol and 2.43% sodium chloride with gentle agitation (38); the further purification of phage has been described (29).

Transduction with *ϕ80h* transducing phage. A stationary-phase culture of the strain which was lysogenic for the helper phage *ϕ80h* was concentrated by centrifugation and resuspended in the original volume of 0.1 M $MgSO_4$ as described by Signer (32) and aerated for 1 h. Approximately 2×10^9 cells were used and infected with putative *ϕ80dargF* at a multiplicity of 20; phage were allowed to adsorb for 20 min at 37 C and the mixture was plated in 2.5 ml of F top agar on selective minimal plates.

Screening of strains for HFT lysates. Strains to be tested were patched onto L plates and incubated at 37 C for 4 h, when perceptible growth had occurred. Plates were then exposed to UV radiation for 50 s as described in "Preparation of phage stocks" and incubated for an additional 2 h at 37 C after each dish had been wrapped in aluminum foil to exclude light. Plates were then replica plated (18) onto a lawn of Sm^r recipient bacteria spread on a selective minimal plate. HFT liquid lysates were subsequently obtained by returning to the original induced patch on the L plate and growing a 5-ml culture in L broth followed by UV induction as described in "Preparation of phage stocks."

Curing of episomes. The F' was removed from diploid strains, when necessary, by treatment with acridine orange by the method of Hirota (14).

Genetic analysis of the transducing phage (marker rescue). Strain EJ82, a single defective lysogen of *ϕ80dargF*, and strain CA8000 were grown overnight in L medium, diluted 100-fold into fresh media, and permitted to grow at 37 C until a cell density of 2×10^8/ml was reached. A 1-ml volume of strain CA8000 was added to 2.5 ml of H top agar and thoroughly mixed, and drops were distributed in a regular geometric pattern over the surface of a prewarmed H plate. After the drops had solidified, a loop of strain EJ82 was spotted onto each mound

followed by a loop of various *ϕ80* amber mutants (27). After the spots had dried, the plate was given a 20-s exposure to UV irradiation as described. The plates were incubated overnight at 34 C; mutants which gave a clear spot indicated complementation with strain EJ82 and, therefore, the presence in the *ϕ80dargF* prophage of the gene for which the *ϕ80sus* mutant is defective.

Construction of strain EJ10 carrying *recAl*⁻. The *recAl* allele was crossed into the ornithine transcarbamylase (OTC)-negative (OTC⁻) strain PA260-R9 by crossing with strain KL1699 using the standard mating procedure as described in "Mating procedure" selecting for His⁺ recombinants. Approximately 100 recombinants were purified by streaking to single colonies. Each recombinant was tested for UV sensitivity by spreading approximately 5×10^3 cells on each of two L(A-N) plates; one was exposed to UV radiation for 15 s and both plates were then incubated at 37 C overnight in the dark. Strains carrying the *recAl*⁻ allele gave 0 to 20 colonies on the plate which had been exposed to UV compared with about 5×10^3 on the control plate; *recAl*⁺ strains gave about 5×10^3 colonies on both plates.

Preparation of endonucleases *Eco*RI and *Sma*R. *Eco*RI was purified by the procedure of B. K. Allet (personal communication) and was demonstrated to be a homogeneous protein preparation as judged by electrophoresis in a sodium dodecyl sulfate acrylamide slab gel by the procedure of Laemmli (17). *Eco*RI activity was determined by detecting the extent of specific cutting of λ DNA (using electrophoresis in agarose gel, as described below) after incubation with various quantities of the enzyme. The endonuclease *Sma*R was prepared by the procedure of Mulder (personal communication) and enzyme activity was determined by the same general protocol as that utilized in the case of *Eco*RI.

Digestion of DNA with *Eco*RI and *Sma*R. DNA was extracted from bacteriophage, freshly banded in a cesium chloride gradient, by the method of Miller et al. (23). For digestion with *Eco*RI the incubation mixture contained 50 μg of DNA, 90 mM Tris-hydrochloride (pH 7.5), 10 mM $MgCl_2$, and 50 units of *Eco*RI in a total volume of 250 μl. (One unit of *Eco*RI or *Sma*R is defined as that amount of enzyme which will specifically digest 1 μg of λ DNA in 30 min.) Incubation was permitted to proceed for 60 min at 37 C. The mixture was cooled to 4 C, extracted once with phenol saturated with 10 mM Na_2 ethylenediaminetetraacetic acid (EDTA) (pH 8.0), and dialyzed exhaustively against 10 mM Tris-acetate (pH 8.0).

For digestion with *Sma*R, 50 μg of DNA was incubated in a mixture containing 15 mM Tris-hydrochloride (pH 9.0), 15 mM KCl, 6 mM $MgCl_2$ and 50 units of *Sma*R enzyme in a total volume of 250 μl. Conditions of digestion, phenol extraction, and dialysis of DNA were as described for digestion with *Eco*RI.

Electrophoresis in agarose gel. A modification of the procedure of Sharp et al. (31) was used for electrophoretic resolution of digested DNA samples. Electrophoresis buffer was 40 mM Tris, 20 mM sodium acetate, and 1 mM Na_2 EDTA adjusted with glacial acetic acid to pH 8.0.

DNA samples with the cohesive ends of the phage DNA together were prepared by annealing for 3 h at 45 C in dialysis buffer (10 mM Tris-acetate, pH 8.0) containing 0.1 M NaCl. To prepare DNA samples with cohesive ends separated, samples were heated at 65 C for 10 min in dialysis buffer and rapidly cooled to 0 C.

DNA samples (0.3 μg) were adjusted to 20 μl with electrophoresis buffer containing 10% sucrose and 0.02% bromophenol blue and layered into preformed slots (0.4 by 0.5 cm) in an 0.8% agarose gel slab (0.5 by 13 by 20 cm). Electrophoresis was carried out at a potential difference of 2.4 V/cm for 10 h at approximately 20 C.

To visualize the DNA, gels were extruded into electrophoresis buffer containing 0.5 μg of ethidium bromide per ml and irradiated with short wavelength UV light. Gels were photographed using a red filter (Tiffen 25A) on Tri-X Pan Kodak film with a 60-s exposure.

Determination of the molecular weight of DNA fragments. The endonuclease *Eco*RI produces six specific fragments of λ DNA. These fragments were utilized as standards for the estimation of the molecular weight of other DNA species. The molecular weights of various λ fragments reported by Thomas and Davis (36) were utilized in this work (13.7×10^6, 4.74×10^6, 3.73×10^6, 3.48×10^6, 3.02×10^6, and 2.13×10^6). The values reported for the molecular weights of fragments smaller than 5×10^5 may be subject to a relatively large error as their size was determined by extrapolation from larger standards.

RESULTS

These experiments involve the manipulation and study of one of the two genes, each coding individually for the enzyme OTC in *E. coli* K-12. One of these genes, *argF*, maps near *proA* (8) at about 7 min on the chromosome map; the other, *argI* is located at about 84 min between *pyrB* and *valS* and is 98% co-transducible with both of these markers (15).

Test for the presence of gene *argF*⁺ in the F'_{TS114} *lac*⁺ *proB*⁺ *proA*⁺ episome. Since the map location of *argF* is between *proA/B* and *lac*, it was expected that the F'_{TS114} episome carrying *pro*⁺ and *lac*⁺ should also carry *argF*⁺. We have shown that this is the case by crossing it (using strain E7129) into strain EJ10 which is *proA argF lac argI recAl* and separately selecting either for Arg⁺ or Pro⁺ sexductants. When scored for Pro⁺ and Arg⁺, respectively, they were all found to be Arg⁺ Pro⁺.

Integration of F'_{TS114} *lac*⁺ *proB*⁺ *argF*⁺ *proA*⁺ into the *tonB* locus. The directed transposition of F'_{TS114} *lac*⁺ *proB*⁺ *argF*⁺ *proA*⁺ was performed as described by Gottesman and Beckwith (10). The diploid strain E7129 carries a *lac argF pro* deletion in the endogenote but an intact *argI* gene. It was, therefore, impossible to follow the Arg phenotype during these experiments; however, it was assumed that

Lac$^+$ strains at 42 C which were also Pro$^+$ would also be *argF*$^+$.

One hundred ten broth cultures from independent colonies of this strain were treated with *φ80v* and colicin V/B and spread on Lac TZ plates at 42 C. Approximately 2 × 10^9 cells were spread on each selection plate and incubated for 36 h. Every culture gave rise to between 20 and 500 *tonB* mutants and, of these, zero to about 50% were Lac$^+$ at 42 C. Colonies in which the episome was stably integrated were white to pale yellow, whereas colonies in which the episome was not stably integrated were red or sectored red.

Temperature-resistant white Lac$^+$ colonies were picked from each selection plate (up to 10 per plate) and streaked to single colonies on Lac TZ plates at 42 C; those which remained temperature stable were tested for the site of integration of the episome.

Testing for the integration site of F'_{TS114} *lac*$^+$ *proB*$^+$ *argF*$^+$ *proA*$^+$. Strains with stably inserted episomes should be Hfr strains except in those cases in which an essential sex factor is inactivated by the crossover event leading to insertion; such defective Hfr strains are discarded by the test procedure used in these experiments.

Strains to be tested were mated, using the multiple mating technique, with strain X181A *(pyrC trp strA)* at 37 C for 1.5 h. Recombinants *trp*$^+$ or *pyrC*$^+$ were detected by replica plating the mating mixture onto glucose-minimal agar containing streptomycin, thiamine, and either uracil or tryptophan. Confluent growth in one selection with zero growth or a few individual colonies on the corresponding spot of the second selection was the criterion for reexamination of the corresponding strain using conventional mating procedures. Three directed transposition strains were found donating *pyrC* early and *trp* late. In subsequent crosses, it was also found that all of them donated *argF*$^+$ and *proA*$^+$ early and *lac*$^+$ late. We believe the structure of these strains (EJ29) to be *pyrC-att80-tonB'-(proA,argF)*-F-*lac-tonB-trp*.

Isolation of *φ80dargF* transducing bacteriophage. A low-frequency transducing lysate was prepared from strain EJ32 (strain EJ29 lysogenized with *φ80h*) as described in Materials and Methods, and 10^{12} phages were tested for transduction of an OTC$^-$ strain. We used as a recipient strain EJ48 which carries an *argF*^{v} and an *argI* point mutation and which was made *φ80h* lysogenic so that a multiplicity of infection of 20 could be used. Arginine-independent colonies which grew on the selective plate were purified by streaking to single colonies and then tested for Hft character using the plate technique (see Materials and Methods). Lysates prepared from two of these colonies were found to transduce *argF*$^+$ at high frequency.

The transducing titer obtained from different lysates was usually between 2 × 10^{-2} and 10^{-3} of the phage titer as described by Gottesman and Beckwith (10) and Press et al. (26). Equilibrium density gradient centrifugation in cesium chloride indicated that transducing phage was present in lysates at concentrations of about 20 to 30% of the total phage titer and its density was approximately 8 mg/ml lighter than that of *φ80h*.

The transducing particles were shown to be *φ80dargF* by the following criteria. (i) Strain EJ48 or a *tonB* mutant of that strain was transduced to Arg$^+$ by the lysates, whereas a strain resistant to *φ80h (tonA)* was not. (ii) Extracts from Arg$^+$ transductants possess OTC activity. Furthermore, the *argF* gene carried by *φ80dargF* is intact because the arginine gene carried on the *φ80dargF* prophage is under the control of the *argR* gene product to approximately the same extent as the normal chromosomal *argF* gene (Fig. 2). Escape synthesis of OTC occurs approximately 15 min after heat induction. This indicates that there is insufficient holo-repressor present in the cells to maintain repression during phage multiplica-

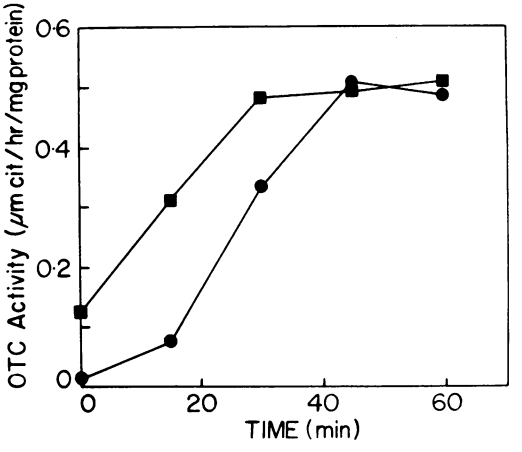

FIG. 2. *Time course of OTC synthesis under conditions of repression and derepression subsequent to inactivation of the cI gene product in a lysogen carrying λh80cI857 and λh80cI857dargF (EJ83.1). Induction of the prophages was initiated by shifting the growth temperature from 30 to 42 C for 10 min when the cultures had reached a density of approximately 2 × 10^8 cells/ml. After this period the temperature was reduced to 30 C, samples of 1.0 ml were removed from each culture at appropriate times, and the concentration of OTC was determined as described by Gorini (9).*

tion or, possibly, that some expression of *argF* may result from read-through from a downstream phage promotor during lytic development. (iii) A strain, EJ55 (derived from RK505), carrying F'_{TS114} *trp*$^+$ *att80*$^+$ in an OTC$^-$ *(trp att80)*$^\nabla$ background was transduced to OTC$^+$ by the phage. The resulting OTC$^+$ character was temperature sensitive and *argF*$^+$ segregated with *trp*$^+$ showing that *ϕ80dargF* was integrated in the episome. Both *argF*$^+$ and *trp*$^+$ were sexduced with high frequency to a strain (EJ39) carrying *argI, argF, trp,* and lysogenic for *ϕ80h*.

Structure of the *ϕ80dargF* phage. The structure of the *ϕ80dargF* genome was determined genetically by marker rescue tests utilizing a set of *ϕ80* mutants (27) and a strain carrying *ϕ80dargF* as a single defective prophage. These experiments indicated that the event which led to the production of this specialized transducing phage resulted in the replacement by bacterial genes of the late phage genes 4 through 11, at least, of *ϕ80* as follows: -1-2-3-*argF*-*att80*-15-*imm*$_{\phi 80}$-14-16-17-18-19-. The structure of *ϕ80dargF* is similar to *ϕ80dara* (28) and to *ϕ80dgnd his* (R. E. Wolf, Jr., personal communication), suggesting that a common specific event (or events) may have occurred in the isolation of each of these phages. The events leading up to the formation of these genomes were similarly rare; the frequency was approximately 5×10^{-12} in each case.

Construction of *λh80cI857dargF*. The strain EJ82 which contains the *ϕ80dargF* prophage was grown in L medium to about 2×10^8 cells/ml. A 1-ml volume of the culture was concentrated by centrifugation, resuspended in 1 ml of 0.1 M $MgSO_4$, and incubated at 37 C for 30 min. The cells were again concentrated by centrifugation, resuspended in the remaining liquid, infected with an equal number of *λh80cI857* phage in 0.2 ml of 0.1 M $MgSO_4$, and incubated at 37 C for 15 min. The mixture was then irradiated with UV light as described, 1 ml of L medium was added, and the culture was shaken vigorously at 34 C for 3 h. After lysis by chloroform, the bacterial debris was removed and the lysate containing *λh80cI857dargF* recombinants used to transduce the strain GL1 lysogenic for the helper phage *λh80cI857* (strain EJ81) to Arg$^+$. Transductants were purified and tested for release of active phage after thermal induction using the plate method described in "Concentration of phage and cesium chloride centrifugation." The strain EJ83-1 was thus obtained as a stable, temperature-sensitive double lysogen.

Specific digestion of λ, *ϕ80*, *λh80cI857*, *ϕ80dargF*, and *λh80cI857dargF* with *Eco*RI and *Sma*R. The specific cutting patterns obtained by the action of *Eco*RI are shown in Fig. 3 and those obtained with the *Serratia marscesens* endonuclease (*Sma*R) are presented in Fig. 4. The data obtained from Fig. 3 are presented in diagrammatic form in Fig. 5 to facilitate their interpretation. The fragments carrying λ or *ϕ80* cohesive ends are readily identified by comparison of the fragment patterns determined in low and high salt. Molecular weights of the various *Eco*RI fragments and identification of fragments originating from homologous regions in λ, *ϕ80*, and *λh80cI857* are summarized in Table 3. The map location of *Eco*RI cutting sites in λ and *ϕ80* (Fig. 6) correspond to those previously described by Helling et al. (13). The position assigned to λ fragment 2 in the map of *λh80cI857* (*ϕ80att80i*$^\lambda$) is in agreement with the electron micrograph mapping of DNA heteroduplexes by Fiandt et al. (7). Using these data, we are therefore able to deduce *Eco*RI fragment maps of the arginine transducing phages.

Fragment patterns produced after digestion of DNA isolated from these phages by *Sma*R are shown diagrammatically in Fig. 7. The molecular weight of *Sma*R-generated fragments are summarized in Table 4. The location of the specific cutting sites recognized by *Sma*R, for these phages, is shown in Fig. 8. These were deduced in the following manner. The presence of *ϕ80* fragment 1 in the digest of *ϕ80dargF* serves to locate this fragment, which has a *ϕ80* cohesive end, as the right terminal one, whereas the difference in fragments *ϕ80*-4 and *ϕ80dargF*-3, which also have a cohesive end, locate these as the left terminal fragments. A similar situation occurs between *λh80cI857* and *λh80cI857dargF*. The difference in left terminal fragments is explained by the substitution of bacterial information in the left arm of these transducing phages, a conclusion previously reached from analysis of *Eco*RI cleavage patterns and the marker rescue data presented herein. Since this difference in fragments is relatively small (see Table 4) and an error in molecular weight determination could possibly lead to a different interpretation of the data, co-electrophoresis was used to verify the inequality between fragments *ϕ80*-4 and *ϕ80dargF*-3 and between *λh80cI857*-4 (*ϕ80*-4) and *λh80cI857dargF*-3 (data not shown). The technique of co-electrophoresis was also useful in verifying equality of fragments originating from different bacteriophage DNAs and was used when deemed necessary in conjunction

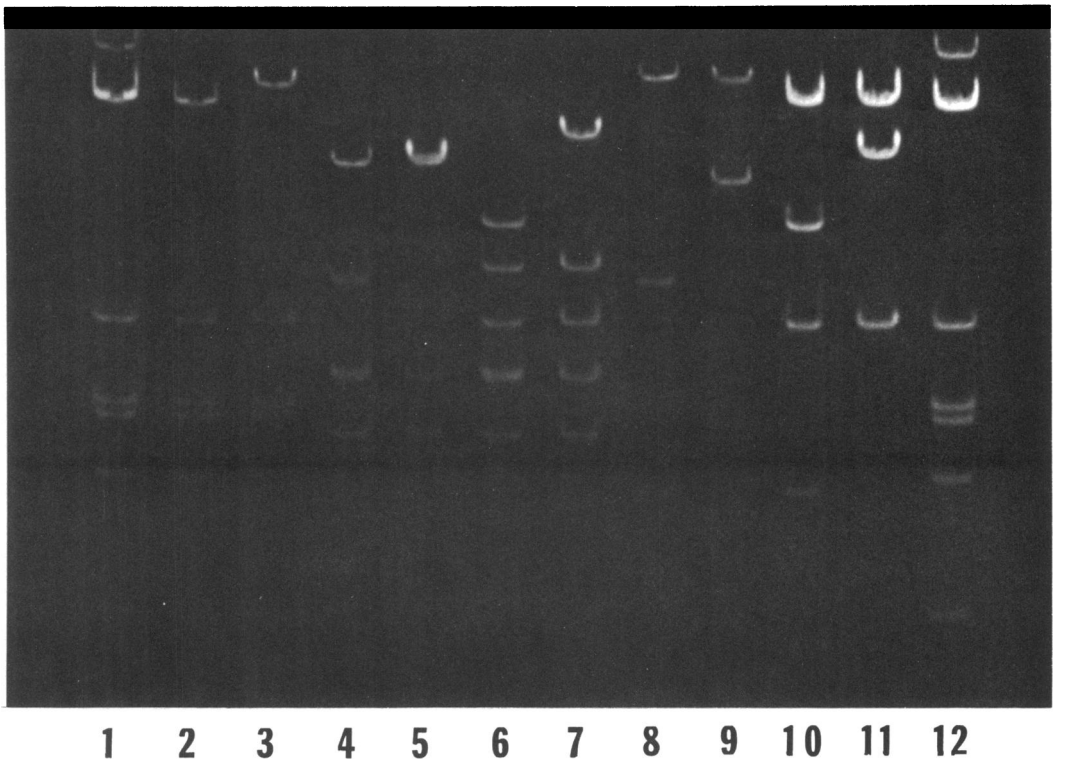

FIG. 3. *Agarose gel electrophoresis of EcoR1 digested parental and arginine-transducing phage DNAs. From left to right: 1, Molecular weight standards (native λ plus EcoR1 λ fragments); 2 and 3, λ (h,s); 4 and 5, φ80 (h,s); 6 and 7, λh80cI857 (h,s); 8 and 9, φ80dargF (h,s); 10 and 11, λh80cI857dargF (h,s); 12, same as 1. Abbreviations: h, sample was heated to 65 C prior to electrophoresis; s, sample was annealed in 0.1 M NaCl as described in text. In all gels electrophoresis was from top (cathode) to bottom (anode).*

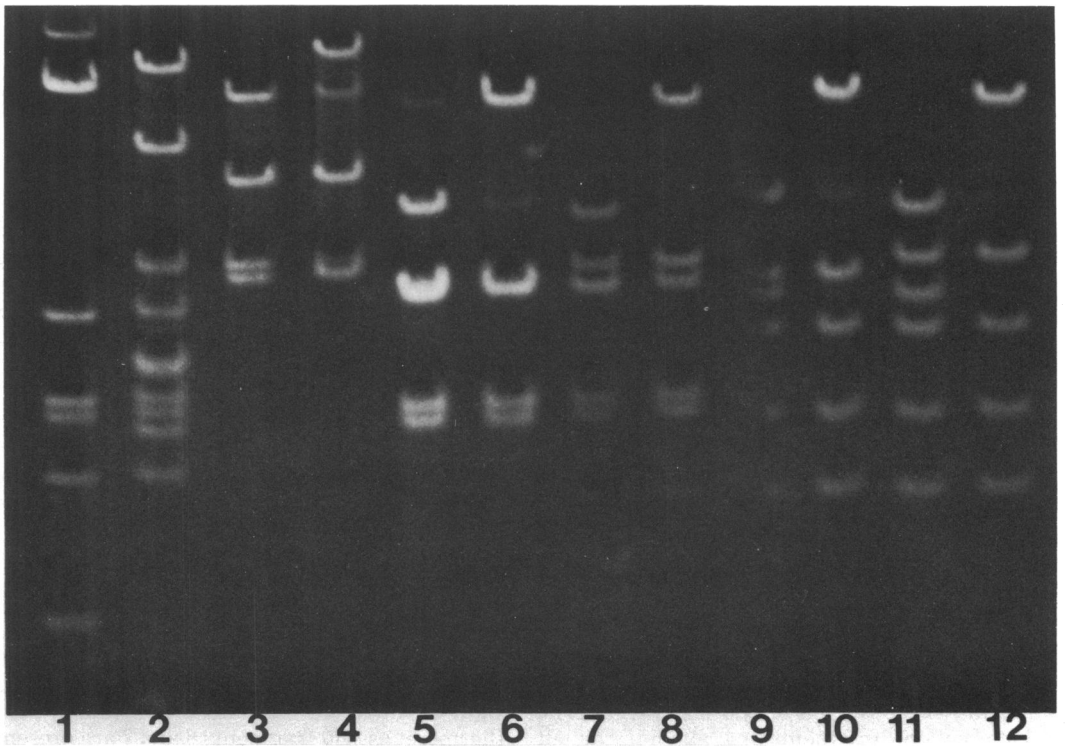

FIG. 4. *Agarose gel electrophoresis of SmaR digested parental and arginine-transducing phage DNAs. From left to right: 1, same as 1 in Fig. 3; 2, λ digested with EcoR1 (s) plus φ80 digested with EcoR1 (h); 3 and 4, λ (h,s); 5 and 6 φ80 (h,s); 7 and 8, λh80cI857 (h,s); 9 and 10, φ80dargF (h,s); 11 and 12, λh80cI857dargF (h,s). Abbreviations are as defined in legend to Fig. 3.*

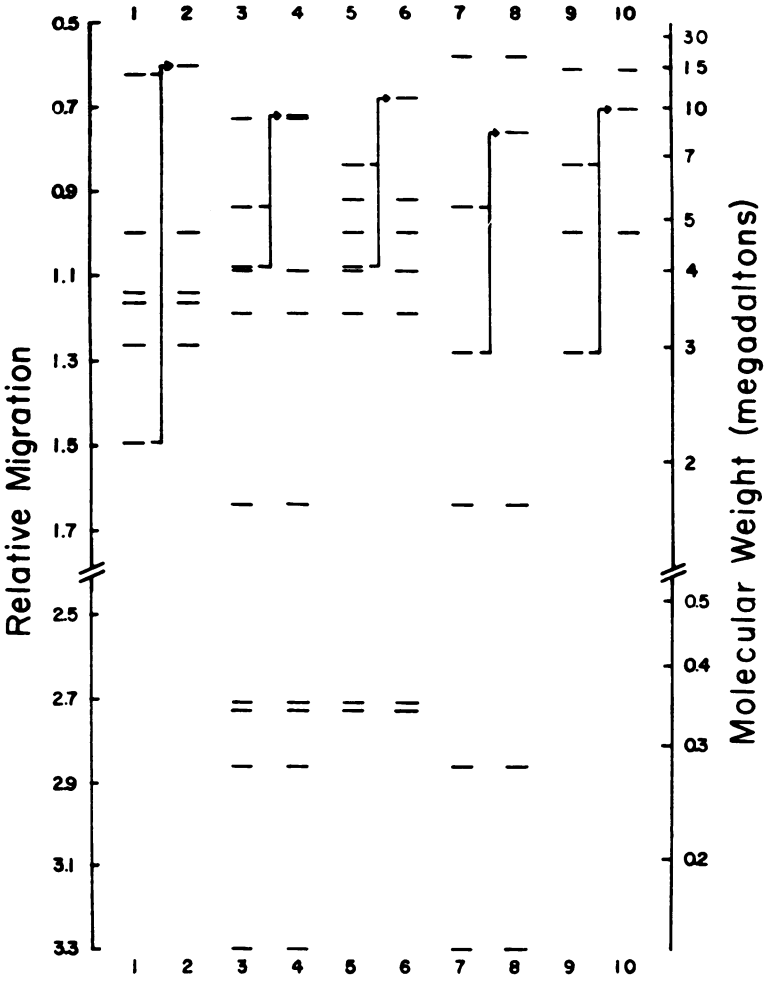

FIG. 5. *Diagrammatic representation of the resolution of EcoRI digests shown in Fig. 3. From left to right: 1 and 2, λ (h,s); 3 and 4, φ80 (h,s); 5 and 6, λh80cI857 (h,s); 7 and 8, φ80dargF (h,s); 9 and 10, λh80cI857dargF (h,s). Abbreviations are as defined in legend to Fig. 3. The location of the various fragments is a direct function of their migration relative to λ EcoRI fragment 2. Arrows indicate which fragments are joined together by the phage DNA cohesive ends subsequent to annealing as described in text. The molecular weight and relative migration of DNA fragments smaller than 0.4 × 10⁶ was determined by electrophoresis of 1-μg samples of digested DNA for 5 h.*

with the data in Tables 3 and 4 to insure the correct interpretation of fragment patterns.

The presence of *φ80* fragments 2, 6, and 7 in digests of DNA isolated from *φ80dargF* indicates that these fragments must originate from the right arm and to the left of *φ80*-1. The replacement of *φ80*-2 by λ-4 in *λh80cI857* and the change in the right terminal fragment between *φ80* and *λh80cI857* due to the insertion of λ genetic information in the right arm of the hybrid phage serve to locate *φ80* fragment 2 immediately to the left of *φ80* fragment 1. The location of *φ80* fragments 3, 4, and 5 in the left arm is indicated by the absence of these fragments in *φ80dargF* in which bacterial DNA has replaced phage information in the left arm; consequently, *φ80* fragments 6 and 7 must lie immediately to the left of fragment 2.

The assignment of fragment 8 in the map of *λh80cI857* has been determined by the following reasoning. This fragment is also present in digests of DNA isolated from the specialized transducing phage *λh80cI857dargF*, which clearly indicates that fragment 8 must lie to the right of *λh80cI857* fragments 3, 4, and 5. Furthermore, as *φ80* fragments 6 and 7 are present

TABLE 3. *Molecular weights of EcoR1 fragments*

Source of DNA	Mol wt of *Eco*R1 fragments ($\times 10^{-6}$)[a]										Summation of mol wt	Reported mol wt of DNA
	1	2	3	4	5	6	7	8	9	10		
λ[b]	13.7[c]	4.74	3.73	3.48	3.02	2.13[d]					30.8	30.8 (3)
φ80	9.2	5.3[d]	4.05[c]	3.95	3.35	1.7	0.35	0.34	0.28	0.14	28.7	28.3 (7)
λh80cI857	6.8[d]	5.7	4.75 (λ-2)	4.05[c] (*φ80*-3)	3.95 (*φ80*-4)	3.35 (*φ80*-5)	0.35 (φ80-7)	0.34 (*φ80*-8)			29.3	27.7 (37)
φ80dargF	18	5.3[d] (*φ80*-21)	2.95[c]	1.7 (*φ80*-6)	0.28 (*φ80*-9)	0.14 (*φ80*-10)					28.4	
λh80cI857dargF	14	6.8[d] (*λh80cI857*-1)	4.75 (λ-2)	2.95[c]							28.5	

[a] Average standard deviation = 0.05 × 10^6 molecular weight as determined by measurement of migration of each fragment, relative to λ standards, in three electrophoretic experiments.
[b] Molecular weights were calculated by electron microscopy as reported by Thomas and Davis (36).
[c] Left end.
[d] Right end.

TABLE 4. *Molecular weights of SmaR fragments*

Source of DNA	Mol wt of *Sma*R fragments ($\times 10^{-6}$)[a]									Summation of mol wt	Reported mol wt of DNA
	1	2	3	4	5	6	7	8	9		
λ	12.4[b]	7.6	5.4[c]	5.2						30.6	30.8 (3)
φ80	6.6[c]	4.9	4.9	4.9[c]	3.6	3.45	0.80			29.1	28.3 (7)
λh80cI857	6.3[c]	5.2 (λ-4)	4.9 (*φ80*-2/3)	4.9[b] (*φ80*-4)	3.6 (*φ80*-5)	3.45 (*φ80*-6)	0.80 (*φ80*-7)	0.17		29.3	27.7 (37)
φ80dargF	6.6[c] (*φ80*-1)	4.9 (*φ80*-2/3)	4.75[b]	4.3	3.45 (*φ80*-6)	2.85	0.80 (*φ80*-7)	0.42		28.1	
λh80cI857dargF	6.3[c]	5.2 (λ-4)	4.75[b]	4.3	3.45 (*φ80*-6)	2.85	0.80 (*φ80*-7)	0.42	0.17 (*λh80cI857*-8)	28.2	

[a] Average standard deviation = 0.05 × 10^6 molecular weight, as determined by measurement of migration of each fragment, relative to λ standards, in three electrophoretic experiments.
[b] Left end.
[c] Right end.

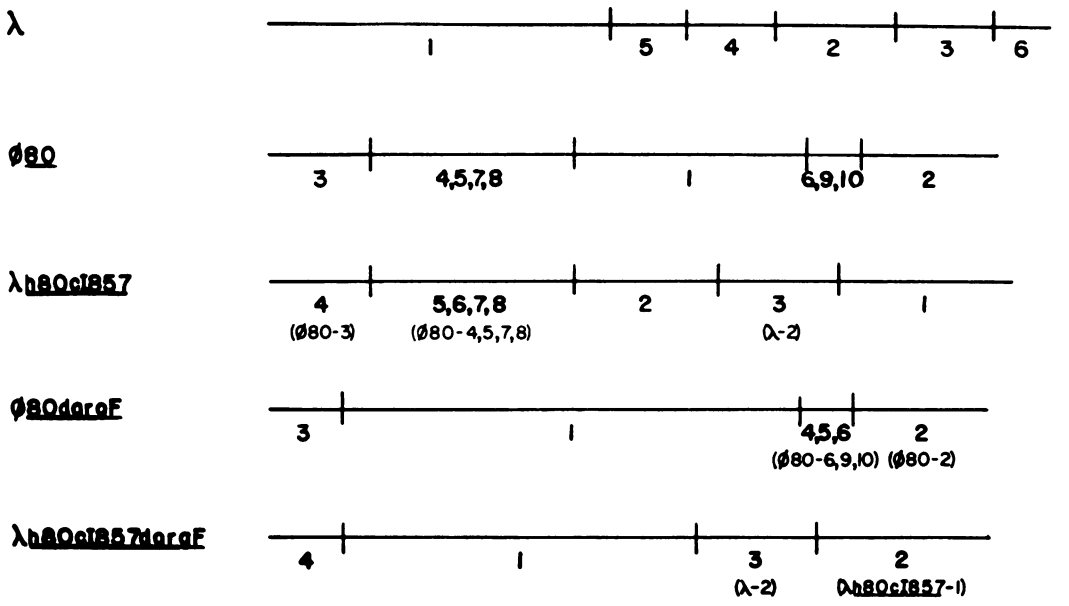

FIG. 6. *EcoRI cleavage maps for parental and arginine-transducing phages. The position of EcoRI-generated λ fragments and identification of ϕ80 terminal fragments have been previously reported (13).*

in *ϕ80*, *λh80cI857*, *ϕ80dargF*, and *λh80cI857dargF*, fragment 8 must result from the addition of a λ cutting site to the right of *ϕ80* fragments 6 and 7. The observation that *λh80cI857* fragment 2 is, in fact, λ-4 indicates that it has a λ cutting site at each end. The right-hand λ cutting site on *λh80cI857* fragment 2 is accounted for by the change in fragment 1 between *ϕ80* and *λh80cI857*. We may, therefore, deduce that fragment 8 is adjacent to the right end of fragments 6 and 7 and contains the leftward junction of *ϕ80* and λ genetic information.

Deduction of the *Sma*R fragment map for λ was aided by digestion of λDNA with both restriction enzymes: a portion containing 10 μg of *Sma*R-digested λDNA (50 μl) was adjusted to pH 7.5 by the addition of 5 μl of 1 M Tris-hydrochloride (pH 7.5), and 2 μl of 0.1 M $MgCl_2$ was added. Ten units of *Eco*RI was added and the λDNA was digested as described. This resulted in the production of a large fragment having a molecular weight of 12.4×10^6, identical to that obtained by digestion with *Sma*R alone, together with additional smaller fragments (data not shown). This fragment was shown to have a λ cohesive end and is approximately 1.3×10^6 molecular weight smaller than *Eco*RI λ fragment 1 which has previously been identified as the left terminal fragment (13). The right terminal fragment is therefore deduced to be fragment 3. The presence of λ fragment 4, a fragment originating from the immunity region of λ (7), in digests of *λh80cI857* and *λh80cI857dargF*, serves to locate λ fragment 4 adjacent to λ fragment 3. These data lead to the *Sma*R fragment map for λ. The data shown in Fig. 8 are consistent with the conclusions previously reached from analysis of *Eco*RI cleavage patterns and the marker rescue data presented herein.

Samples of λDNA specifically cleaved by *Eco*RI were annealed at 0 C as described by Tanaka and Weisblum (34) and then incubated with polynucleotide ligase for 18 h at 0 C. This treatment resulted in the formation of concatemers of high molecular weight and also in the formation of covalently closed circles (data not presented). Similar treatment of λDNA with *Sma*R annealing and incubation with polynucleotide ligase at 0 C did not result in the formation of oligomeric DNA structures or closed covalently linked circles.

DISCUSSION

The specialized transducing bacteriophages *ϕ80dargF* and *λh80cI857dargF* have been shown to be good templates for the in vitro synthesis of *argF*-specific mRNA and for the regulation of such synthesis by the interation of the specific arginine holo-repressor (30). The synthesis of *argF* mRNA by DNA isolated from these phages is under the control of the *argF* promoter-operator-start region for the first 7

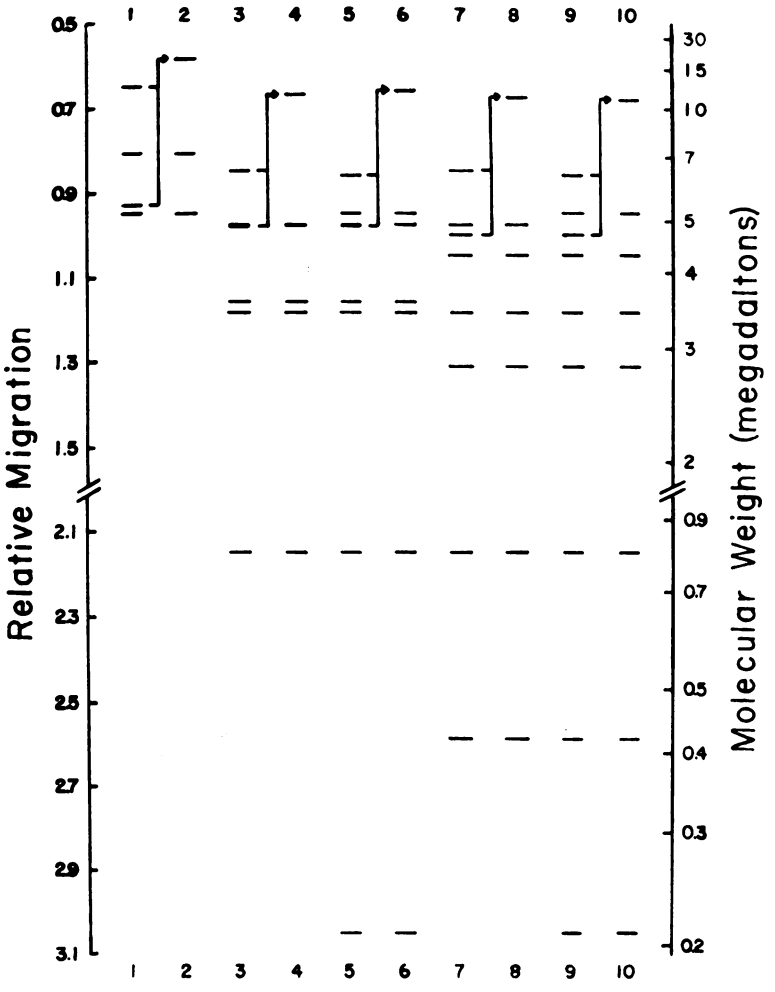

FIG. 7. *Diagrammatic representation of the resolution of SmaR digested parental and arginine-transducing phage DNAs. From left to right: 1 and 2, λ (h,s); 3 and 4 φ80 (h,s); 5 and 6, λh80cI857 (h,s); 7 and 8, φ80dargF (h,s); 9 and 10, λh80cI857dargF (h,s).*

min of synthesis; however, at that time there is a significant increase in the rate of synthesis of *argF* mRNA, presumably due to read-through from a powerful downstream phage promoter (30). It was reasoned, therefore, that studies of in vitro regulation of both *argF* mRNA synthesis and cell-free translation of *argF* would be facilitated if DNA carrying *argF* were specifically separated from DNA carrying major phage promoters. A study was consequently performed concerning the specific cleavage of DNA isolated from *φ80dargF* and *λh80cI857dargF* with the restriction endonucleases *Eco*RI and *Sma*R. The cutting patterns shown in Fig. 6 are consistent with the electron microscope mapping data of Kikuchi et al. (16) and the marker rescue data presented herein. The hybrid arginine-transducing phage has λ genetic information from β to beyond P as the λ-2 fragment is present in *Eco*RI digests of *λh80cI857dargF*. The major left and right phage promoters are carried on this fragment (1). The *Eco*RI fragment maps deduced from this work for *φ80* and *λh80cI857* are essentially in agreement with those proposed by Helling et al. (13) except that our data place the small *φ80* fragments 9 and 10 in the right arm of the phage between fragments 1 and 2. These small fragments cannot lie in the region between φ80 fragments 3 and 1 because they are present in *Eco*RI digests of the specialized transducing phage *φ80dargF* in which phage information has been replaced by bacterial genes extending from beyond the left of the

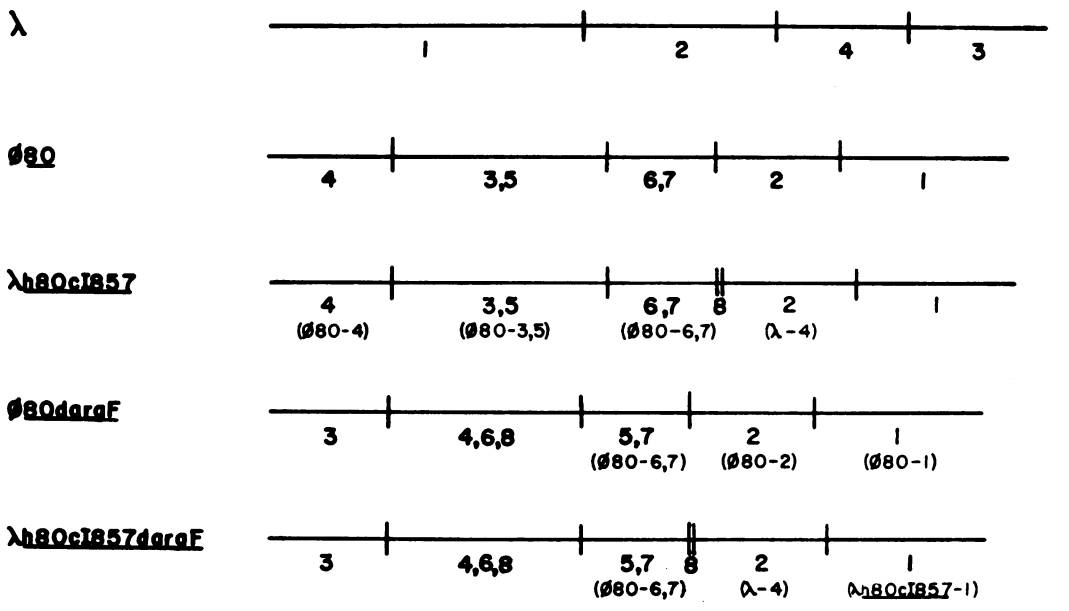

FIG. 8. *SmaR cleavage maps for parental and arginine-transducing phage DNAs.*

cutting site of *ϕ80* fragment 3 to the right of the cutting site between *ϕ80* fragment 1 and *ϕ80* fragments 4, 5, 7, and 8. Fragments 9 and 10 are absent in the specialized transducing phage *λh80cI857dargF* as the *ϕ80* cutting sites between *ϕ80dargF* fragments 1 and 2 have been removed and replaced by λ genetic information. Our data confirm that *ϕ80* fragments 7 and 8 lie in the region mapped by Helling et al. (13). Our data cannot rule out the possibility that *ϕ80* fragments 9 and 10 are different from *ϕ80dargF* fragments 9 and 10 and that the latter are coincidentally the same size as those obtained from *ϕ80* but are derived from bacterial information. This will be determined by asking if the same subset of fragments are obtained by digesting fragments 9 and 10 from *ϕ80* and *ϕ80dargF* with another restriction enzyme such as *Hind*II.

The precise structure of the cutting site for the endonuclease *Sma*R has not been determined (R. Roberts, personal communication); the data presented in this work suggest that the degree of specificity of *Sma*R is similar to that of *Eco*RI as the frequency of cutting for λ or *ϕ80* DNA is approximately the same. The DNA sequence recognized by *Sma*R is different from that recognized by *Eco*RI. Whereas *Eco*RI introduces staggered nicks four bases apart (12) which may be covalently joined by polynucleotide ligase after annealing at 0 C (21), such treatment of λDNA with *Sma*R followed by annealing at 0 C and incubation with polynucleotide ligase did not result in the covalent joining of the DNA fragments. The possibility that this observation is due to contamination of the *Sma*R preparation with a 5′ single-strand-specific exonuclease, phosphatase, or other inhibitors of ligation was excluded since covalent joining of fragments bearing λ cohesive termini (λ*Sma*R fragments 1 and 3) was observed under these conditions. These data suggest that *Sma*R cuts both DNA strands at the same site or with staggered nicks no further apart than 3 bases.

The data presented in this work concerning the action of the endonucleases *Eco*RI and *Sma*R on DNA isolated from these arginine-transducing phages clearly indicate that it is possible to isolate the *argF* gene from the major phage promoters p_R and p_L. In the case of the hybrid phage, *λh80cI857dargF*, these major phage promoters are on *Eco*RI fragment λ-2 whereas *argF* lies on either fragment 1 or 4 or it may be cut at the cutting site between these fragments (the precise location of *argF* has not yet been determined).

In the case of *ϕ80dargF*, the major phage promoters are presumed to lie in *ϕ80* fragment 6 and again will be separated from the *argF* gene carried on fragment 1 or 3 (or both).

The site in the left arm of the phage where the inserted bacterial DNA is cut by *Sma*R is approximately 2,800 base pairs to the right of the corresponding site for *Eco*RI. This clearly indicates that, should the *argF* gene be cut by

one of these restriction enzymes, it is not cut by the other because the subunit molecular weight of ornithine transcarbamylase is approximately 35,000 (19), which requires less than 1,000 base pairs of DNA.

It is, therefore, clear that the specific cleavage of either of these transducing phages with one or other of the endonucleases *Eco*RI or *Sma*R will provide the opportunity of isolating a DNA molecule substantially enriched for the *argF* gene and with the major phage promoters removed. We are now isolating such fragments by preparative electrophoresis in agarose gel for use in performing in vitro experiments concerning the regulation of gene expression in the arginine biosynthetic regulon.

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