

## Isolation and Characterization of a Plaque-Forming Lambda Bacteriophage Carrying a ColE1 Plasmid

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A plaque-forming  $\lambda imm^{434}$  bacteriophage carrying the entire genome of colicinogenic factor E1 has been isolated and characterized. This phage,  $\lambda imm^{434}ColE1$ , can lysogenize as a stable plasmid within a recombination-deficient *Escherichia coli* cell that lacks the normal attachment site for  $\lambda$  phage. Furthermore, it has been found that  $\lambda imm^{434}ColE1$  phage carrying amber mutations in the *O* and *P* genes of the  $\lambda$  genome, i.e.,  $\lambda imm^{434}OamPamColE1$ , behaves as a plaque-forming phage, and this finding suggests that the ColE1 factor DNA permits replication of the DNA of the plaque-forming phage.

Colicinogenic factor E1 (ColE1) DNA replication is sensitive to rifampin (5) and is dependent on *polA* (12) and some *dna* gene functions (6). Furthermore, ColE1 replicates extensively in the absence of net protein synthesis (4). Very little, however, is known about the role of ColE1 genes in the replication of its own DNA. The existence of a large number of ColE1 DNA copies in a cell makes its genetic analysis extremely difficult.

Recently a hybrid plasmid was constructed by in vitro recombination between ColE1 DNA and a DNA fragment of *Escherichia coli* with genes governing guanine synthesis derived from a transducing phage (18, 23). The hybrid, named ColE1-*cos* $\lambda$ -*guaA*, replicates as a stable plasmid. Moreover, the hybrid plasmid can be packaged within a  $\lambda$  phage coat when *E. coli* cells carrying the plasmid are infected by  $\lambda$  (7). However, the ColE1-*cos* $\lambda$ -*guaA* transducing phage cannot form plaques on *E. coli*. This communication describes the isolation and characterization of a plaque-forming phage which carries the entire genome of ColE1. Using this phage, we have obtained results suggesting that the association of the ColE1 replicon with  $\lambda$  can compensate for a lack of *O* and *P* gene function in  $\lambda$ .

### MATERIALS AND METHODS

**Bacterial and phage strains.** Bacterial strains are all derivatives of *E. coli* K-12 and are listed in Table 1. Bacteriophage  $\lambda$ ,  $\lambda imm^{434}$ , and  $\lambda Pam3$  are from our stock collection.  $\lambda imm^{434}c$  and  $\lambda imm^{434}Oam29Pam3$  were obtained from K. Matsubara.

**Media and enzymes.** PBB medium was used for bacterial growth (17). Colony counts and plaque assays were performed on PBB agar plates. Casamino agar

supplemented with xanthine (20  $\mu$ g/ml) and biotin (0.04  $\mu$ g/ml) were used for the *guaA* gene transduction assay (18). EMBO agar was described by Matsubara (15). [<sup>3</sup>H]thymidine was obtained from The Radiochemical Centre, Amersham, England (5 Ci/mmol) and was used for labeling DNAs (17). *EcoRI* restriction endonuclease was prepared by the method of Yoshimori (Ph.D. Thesis, University of California at San Francisco, San Francisco, 1971). *HindIII* restriction enzyme was a generous gift from M. Takanami of Kyoto University.

**Transduction procedure.** The method for transduction has been described previously (7).

**Density-gradient centrifugation.** Solid CsCl was added to phage lysates to give a refractive index of 1.380.  $\lambda para$  was added as a density reference. Centrifugation and collection of samples were carried out as described in the previous paper (7).

**Detection of plasmid DNA by cesium chloride-ethidium bromide density-gradient centrifugation.** The method used here was described before (17).

**Preparation of plasmid DNA and phage DNA.** Bacteria containing ColE1 recombinant plasmids were grown in 10 liters of PBB medium to a density of 180 Klett units, then chloramphenicol was added (100  $\mu$ g/ml) and incubated for another 15 h at 37°C. The cells were harvested and washed with 20 mM tris(hydroxymethyl)aminomethane-hydrochloride and 1 mM ethylenediaminetetraacetic acid (pH 7.0). The plasmid DNA was extracted and purified according to the methods reported previously (17).

The phage particles were purified by banding in a CsCl density gradient, and their DNAs were extracted with phenol and then dialyzed against 10 mM tris(hydroxymethyl)aminomethane-hydrochloride and 1 mM ethylenediaminetetraacetic acid (pH 7.0).

**Agarose gel electrophoresis.** Agarose gel solutions were prepared by dissolving agarose in a tris(hydroxymethyl)aminomethane-acetate buffer containing ethidium bromide (22). A 150-ml portion of the solution was autoclaved and poured into the horizontal slab gel apparatus (14 by 21 cm). About 1 to 0.5  $\mu$ g of DNA from an *EcoRI* or *HindIII* enzyme

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TABLE 1. *Bacterial strains*

Strain	Relevant genotype <sup>a</sup>	Source or reference
C600S	<i>thi lac su</i> <sup>+</sup>	Sato et al. (21)
KS1616	HfrH $\Delta$ (gal-att $\lambda$ -bio) $\Delta$ ( <i>guaA-B</i> )	Shimada et al. (23)
TM96	KS1616 (ColE1- <i>cos</i> $\lambda$ - <i>guaA</i> )	Mukai et al. (18)
KS1963	HfrH att $\lambda$ <sup>+</sup> $\Delta$ ( <i>guaA-B</i> )	Fukumaki et al. (7)
KS1944	KS1963 <i>recA</i>	Fukumaki et al. (7)
KS1915	KS1616 <i>rpsL</i>	This work
KS2008	KS1915 <i>recA</i>	This work
JG138	<i>polA1 rha thy lacZ</i> (Am)	Campbell et al. (3)
CGSC785	<i>groN</i>	Georgopoulos (8)

<sup>a</sup> Abbreviations used: *su*<sup>+</sup>, carrying nonsense suppressor; att $\lambda$ , attachment site for  $\lambda$ ;  $\Delta$ , deletion; *cos* $\lambda$ , cohesive ends of  $\lambda$  phage DNA. The other genetic symbols are those used by Bachmann et al. (1).

digest was adjusted to 8% sucrose in 50 to 100  $\mu$ l and layered in a gel slot. Electrophoresis of the 0.8% (wt/vol) agarose gels was carried out at 20 mA for 15 h at room temperature. Photographs were taken as reported previously (19).

## RESULTS

**Isolation of  $\lambda$  phage carrying a ColE1 plasmid.** The general principles of the isolation of  $\lambda$  phage carrying the ColE1 DNA have been described elsewhere (24). The ColE1-*cos* $\lambda$ -*guaA* plasmid was constructed by ligation of *Eco*RI-digested DNA of ColE1 and  $\lambda$ *pgua* DNA (18, 23). Strain TM96, which carries the ColE1-*cos* $\lambda$ -*guaA* plasmid, was lysogenized by  $\lambda$ *imm*<sup>434</sup> phage. Most of the lysogens carried the  $\lambda$ *imm*<sup>434</sup> DNA within ColE1-*cos* $\lambda$ -*guaA* plasmids, because this strain lacks the normal  $\lambda$  attachment site, and because there is extensive homology between  $\lambda$  DNA and the ColE1-*cos* $\lambda$ -*guaA* plasmid. When these lysogens were induced with mitomycin C, the lysates contained the  $\lambda$ *imm*<sup>434</sup> phage and ColE1-*cos* $\lambda$ -*guaA* transducing phage particles as previously reported (see Fig. 1) (7). Using these lysates, we looked for new transducing phages carrying the  $\lambda$ *imm*<sup>434</sup> *guaA* gene and the ColE1 DNA. These phages could be expected to be produced by illegitimate recombination as shown in Fig. 1 (24). The selection for the phage was done as follows: KS1915 cells were mixed with mitomycin C-induced lysates of TM96( $\lambda$ *imm*<sup>434</sup>) at a phage multiplicity of less than 10<sup>-2</sup> per cell, incubated at 30°C for 30 min, and plated on Casamino agar plates. GuaA<sup>+</sup> colonies grown on these plates were replica plated onto EMBO agar seeded with 10<sup>7</sup> particles of  $\lambda$ *imm*<sup>434</sup>c phage. The transductants, which were phenotypically both *imm*<sup>434+</sup> and *guaA*<sup>+</sup>, were 1,000 times less numerous than GuaA<sup>+</sup> colonies. They were divided into two phenotypic groups. Among three independent GuaA<sup>+</sup> and  $\lambda$ *imm*<sup>434+</sup> transductants, one was ColE1-*imm*<sup>+</sup> and the other two were ColE1-*imm*<sup>-</sup> transductants. When the ColE1-*imm*<sup>-</sup> transductant was induced by mitomycin C treatment, lysates contained the plaque-forming  $\lambda$

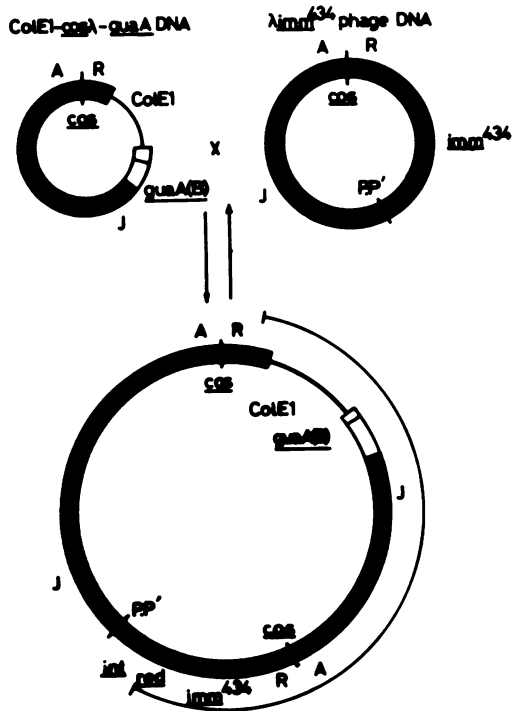


FIG. 1. Integration of  $\lambda$ *imm*<sup>434</sup> DNA into ColE1-*cos* $\lambda$ -*guaA* DNA and Ter-dependent packaging of  $\lambda$ *imm*<sup>434</sup> and ColE1-*cos* $\lambda$ -*guaA* transducing particles. The mechanism of formation of these transducing phages is shown by Fukumaki et al. (7) and Shimada et al. (24). The portion of the largest circular DNA diagrammed that is presumed to give rise to  $\lambda$ *imm*<sup>434</sup> ColE1 is indicated by the line outside the circle (—). Both ends of this molecule represent approximate sites of an abnormal excision. The thick lines represent the  $\lambda$  genome; thin lines, ColE1 DNA; and double thin lines, a fragment of *guaA*(B) genes in the bacterial chromosome.

phage which can transduce *guaA*<sup>+</sup>, ColE1-*imm*<sup>+</sup>, and *imm*<sup>434+</sup> genes to KS1915 cells. This phage was designated as  $\lambda$ *imm*<sup>434</sup>ColE1.

**General properties of  $\lambda$ *imm*<sup>434</sup>ColE1.** We have examined the plating efficiency of

$\lambda imm^{434}$ ColE1 phage on various indicators (Table 2). It could plate efficiently on KS1963, KS1915, KS1944, and KS2008 as well as on C600S. The plaque-forming titer was approximately the same as that of the *guaA* transducing unit. These results indicate that a *recA* function and an *att $\lambda$*  site do not affect plating efficiency and *guaA* transduction. On the other hand,  $\lambda imm^{434}$ ColE1 could plate neither on a  $\lambda imm^{434}$  lysogen nor on *groN* bacteria, suggesting the dependency of  $\lambda imm^{434}$ ColE1 phage plaque formation on the intact phage N gene functions.

To determine the density of this phage, CsCl density gradient centrifugation was carried out by using  $\lambda rapa$  as a reference.  $\lambda imm^{434}$ ColE1 banded at a density of 1.499, indicating net loss of approximately 6.8% of the length of  $\lambda$  phage DNA (2) (data not shown).

$\lambda imm^{434}$ ColE1 lysogenized very efficiently within an *att $\lambda$* -deleted *recA* cell (Table 2), and from this fact we expected that the phage genome was replicating as a plasmid. To test this point, KS2008 cells lysogenic for  $\lambda imm^{434}$ ColE1 were labeled with [ $^3$ H]thymidine, and cells were lysed and centrifuged in a cesium chloride-ethidium bromide solution (Fig. 2). The denser and minor peak showed the presence of covalently closed circular DNA molecules. This fraction was able to transform  $Ca^{2+}$ -treated KS1915 to  $\lambda imm^{434+}$ , *guaA* $^+$ , and ColE1-*imm* $^+$  cells. In addition, the closed circular DNAs were accumulated after adding chloramphenicol as shown in Fig. 2b, while nonlysogenic KS2008 contained no chloramphenicol-amplifiable plasmids (data not shown). Since  $\lambda dv$  plasmid DNA is not accumulated in the presence of chloramphenicol (16), this indicates that the plasmid DNA can replicate utilizing the ColE1 replication functions.

Genetic markers of  $\lambda imm^{434}$ ColE1 were examined by marker rescue tests. It was found that

$\lambda imm^{434}$ ColE1 carried all the late genes of  $\lambda$  phage and a region responsible for  $\lambda$  DNA replication and regulation. It also carried *red* and *gam* genes, but did not exhibit *int* and *xis* gene functions (data not shown). These results suggested that the  $\lambda imm^{434}$ ColE1 DNA contains at least the *red* genes through the ColE1-*imm* region, as shown in Fig. 1.

An analysis of these hybrid DNAs by restriction enzyme cleavage supported the presence of a whole ColE1 DNA in  $\lambda imm^{434}$ ColE1. Figure 3 shows the restriction patterns of  $\lambda imm^{434}$ ColE1 DNA digested with *EcoRI* or *HindIII* endonuclease.  $\lambda imm^{434}$ ColE1 DNAs which were prepared from the mature phage or from plasmid were used in this experiment. Each molecule (lanes d and e, Fig. 3) generated two additional DNA fragments in comparison with those produced from  $\lambda imm^{434}$ DNA (lane b, Fig. 3) by *EcoRI* digestion. The size of "B" fragment (Fig. 3) was in good agreement with that of ColE1 DNA derived from ColE1-*cos $\lambda$* -*guaA* plasmid DNA. This was expected since ColE1 DNA is bounded by *EcoRI* sites on each side, because it was ligated to ColE1 and  $\lambda$  *guaA* DNA through *EcoRI* sites when the ColE1-*cos $\lambda$* -*guaA* plasmid was constructed (7, 18). The "C" fragment contains a DNA sequence generated in the process of an illegitimate recombination (see Fig. 1). The cleavage maps of *EcoRI* fragments are shown in Fig. 4. Restriction analyses made with *HindIII* showed that  $\lambda imm^{434}$ ColE1 had only two sites susceptible to *HindIII* (Fig. 3), as expected from previous results in which the ColE1-*cos $\lambda$* -*guaA* DNA gave no *HindIII* site (14). These results are consistent with those obtained after digestion with *EcoRI*. Thus, it is strongly suggested that  $\lambda imm^{434}$ ColE1 DNA carries a whole ColE1 DNA, as shown in Fig. 1.

**A plaque-forming ability of  $\lambda imm^{434}$ -ColE1 defective in O and P genes.** All results

TABLE 2. Efficiencies of plating of  $\lambda imm^{434}$ ColE1 and  $\lambda imm^{434}$ OamPamColE1 on various indicator strains

Indicator strain	Relevant properties	$\lambda imm^{434}$ ColE1		$\lambda imm^{434}$ OamPamColE1	
		Plaque-forming ability <sup>a</sup>	<i>guaA</i> -transducing ability/ plaque-forming ability <sup>b</sup>	Plaque-forming ability <sup>a</sup>	<i>guaA</i> -transducing ability/ plaque-forming ability <sup>b</sup>
KS1963		$4.0 \times 10^{-1}$	$3.5 \times 10^{-2}$	$3.7 \times 10^{-1}$	$1.1 \times 10^{-1}$
KS1944	<i>recA</i>	$7.2 \times 10^{-1}$	$1.2 \times 10^{-2}$	$1.7 \times 10^{-1}$	$1.3 \times 10^{-1}$
KS1915	$\Delta att\lambda$	$8.4 \times 10^{-1}$	$8.6 \times 10^{-2}$	$4.7 \times 10^{-1}$	$8.0 \times 10^{-2}$
KS2008	<i>recA</i> $\Delta att\lambda$	$6.1 \times 10^{-1}$	$5.5 \times 10^{-2}$	$4.7 \times 10^{-1}$	$7.2 \times 10^{-2}$
JG138	<i>polA</i>	$3.1 \times 10^{-1}$	NT	$<1.9 \times 10^{-8}$	NT
TM96	ColE1 <i>cos<math>\lambda</math></i> - <i>guaA</i>	$1.8 \times 10^{-4}$	NT	$<6.6 \times 10^{-8}$	NT
TM174	Lysogenic for $\lambda imm^{434}$	$<5.0 \times 10^{-8}$	$6.6 \times 10^{-1}$	$<6.6 \times 10^{-8}$	$2.7 \times 10^{-1}$
CGSC785	<i>groN</i>	$<2.0 \times 10^{-8}$	NT	$<1.9 \times 10^{-8}$	NT

<sup>a</sup> Efficiency of plating on C600S is taken as 1.

<sup>b</sup> Plaque-forming ability was measured on C600S. The transducing procedures are described in the text. Starved cultures of KS1915 were used as recipient cells. NT, Not tested.

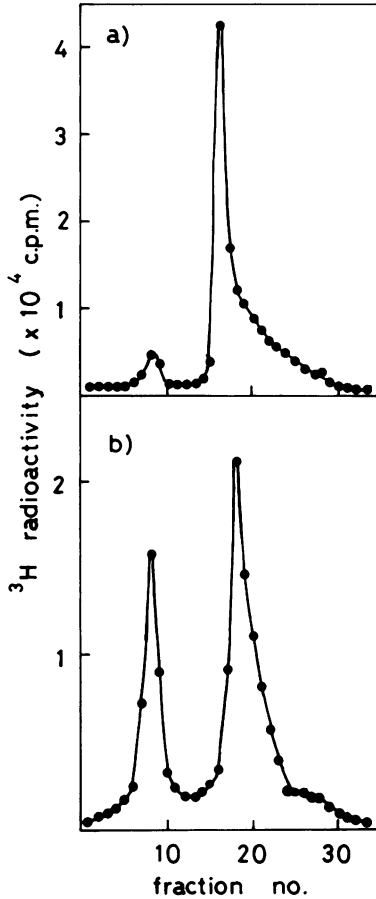


FIG. 2. Cesium chloride-ethidium bromide density gradient centrifugation of whole DNA extracted from  $\lambda imm^{434}ColE1$  lysogenic cells. KS2008( $\lambda imm^{434}ColE1$ ) was grown to  $2 \times 10^8$  cells/ml in 5 ml of Casamino medium. It was divided into two equal portions. Each sample was labeled with [ $^3H$ ]thymidine (6  $\mu Ci/ml$ ) for 7 h (a) in the absence of and (b) in the presence of chloramphenicol (100  $\mu g/ml$ ). Subsequent experimental procedures were as described by Matsubara et al. (17).

presented above indicated that  $\lambda imm^{434}ColE1$  phage is carrying a whole ColE1 DNA and an autonomously replicating region of the  $\lambda$  genome. It was interesting to examine whether the ColE1 factor could mediate replication of  $\lambda$  phage when the *O* and *P* gene functions of the  $\lambda$  genome were eliminated. To answer this question, the following experiments were performed.

$\lambda imm^{434}Oam29Pam3$  was lysogenized into the *att $\lambda$* -deleted C600S carrying the ColE1-*cos $\lambda$* -*guaA* plasmid, and the lysogens were induced with mitomycin C as described above. These lysates were plated onto *Su*<sup>-</sup> bacteria (KS1616),

and plaque-forming phages were selected, on the assumption that the ColE1 replicon can substitute for *O* and *P* gene functions of  $\lambda$  phage genome. The turbid plaques were obtained at frequencies less than  $10^{-6}$  per plaque-forming phage measured on *Su*<sup>+</sup> bacteria. These phages formed small plaques on the *Su*<sup>-</sup> bacteria and were able to transduce ColE1-*imm*<sup>+</sup>, *guaA*<sup>+</sup>, and *imm*<sup>434</sup> genes to KS1915 cells. One of the transductants was purified, and the plating efficiency of  $\lambda cI857Pam3$  on this lysogen was measured by using C600S as a control. It was less than  $2.1 \times 10^{-6}$  on the lysogen and was  $1.0 \times 10^{-1}$  on KS1915( $\lambda imm^{434}$ ) cells. The results indicate that this lysogen still retains at least an amber mutation in the *P* gene.

This phage was named  $\lambda imm^{434}OamPamColE1$ . Table 2 also summarizes the plating efficiency of  $\lambda imm^{434}OamPamColE1$  on various indicators. Unlike the  $\lambda imm^{434}ColE1$ ,  $\lambda imm^{434}OamPamColE1$  could not plate on *polA* strain (=JG138) or on TM96 strain, which carries ColE1-*cos $\lambda$* -*guaA* plasmid. Failure in plating on the *polA* mutant suggests the participation of the ColE1 factor as a mediator of replication for plaque formation, and the failure in plating on bacteria carrying ColE1-*cos $\lambda$* -*guaA* plasmid may result from incompatibility between the two different kinds of hybrid ColE1 DNAs. These results indicate that ColE1 provides replication functions for the  $\lambda imm^{434}OamPamColE1$  phage.

$\lambda imm^{434}OamPamColE1$  phage lysogenized the *att $\lambda$* -deleted *recA* cell efficiently and stably, suggesting that  $\lambda imm^{434}OamPamColE1$  exists as an extrachromosomal element in the cytoplasm. To examine this point, KS2008( $\lambda imm^{434}OamPamColE1$ ) lysogens were labeled with [ $^3H$ ]thymidine, and whole DNAs were extracted and analyzed by cesium chloride-ethidium bromide isopycnic centrifugation. Covalently closed circular DNAs were detected and accumulated in the presence of chloramphenicol, as observed with  $\lambda imm^{434}ColE1$  (data not shown). The presence of a ColE1 DNA was confirmed by digesting these plasmid DNAs with *EcoRI* and by subsequent electrophoretic analysis of the DNA fragments in agarose gel (Fig. 5). Cleavage patterns were similar to those of  $\lambda imm^{434}ColE1$  as shown in Fig. 3, and we concluded that the  $\lambda imm^{434}OamPamColE1$  DNA consists of ColE1 DNA, a part of  $\lambda imm^{434}$  DNA, and a DNA fragment of *E. coli* for guanine synthesis.

## DISCUSSION

A plaque-forming  $\lambda$  phage carrying a whole ColE1 plasmid has been isolated by applying in vivo genetic techniques. This phage could lysogenize within *att $\lambda$* -deleted *E. coli* hosts as a

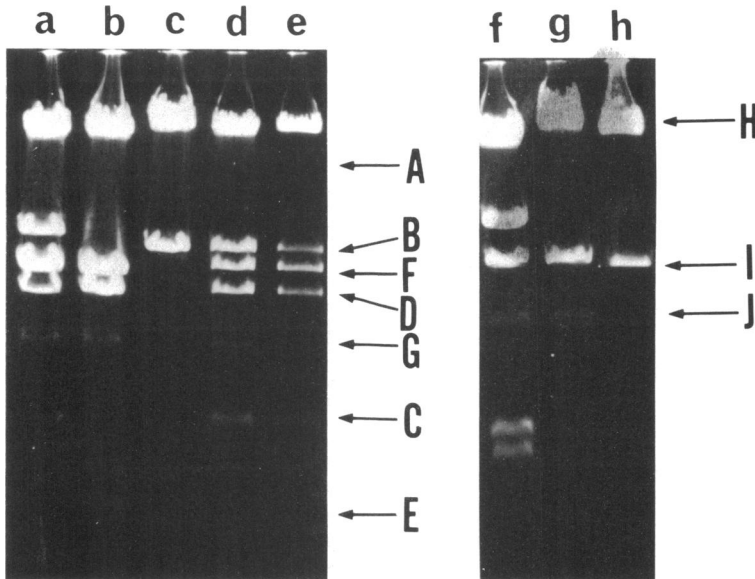


FIG. 3. Agarose slab gel electrophoresis of the *EcoRI* and the *HindIII* digests of  $\lambda imm^{434}ColE1$  DNA. Approximately 1  $\mu g$  of the DNA digested with each enzyme was loaded in each gel slot. The gels were run and illuminated with short-wave-length UV light as described in the text. *EcoRI* digestion: (a)  $\lambda cI857$  DNA, (b)  $\lambda imm^{434}$  DNA, (c) *ColE1-cos $\lambda$ -guaA* DNA, (d)  $\lambda imm^{434}ColE1$  phage DNA, (e)  $\lambda imm^{434}ColE1$  plasmid DNA. *HindIII* digestion: (f)  $\lambda imm^{434}$  DNA, (g)  $\lambda imm^{434}ColE1$  phage DNA, (h)  $\lambda imm^{434}ColE1$  plasmid DNA. Fragments cleaved by *EcoRI* are lettered from A to G. *HindIII* fragments are lettered from H to J. Note that the (d) and (g) profiles, which show fragments of DNA from phage particles, contain DNA fragments G and J from the right end of the phage genome, respectively. The (e) and (h) profiles of fragments of plasmid DNA do not contain the terminal fragments G and J.

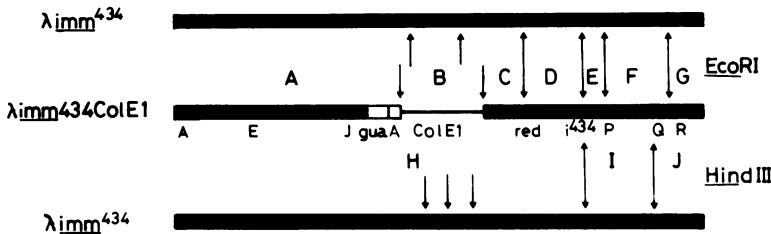


FIG. 4. Physical map of *EcoRI* and *HindIII* cleavage sites on  $\lambda imm^{434}ColE1$  phage DNA. Fragments cleaved by *EcoRI* or *HindIII* are lettered from left to right.

stable plasmid. Furthermore, we found that  $\lambda imm^{434}ColE1$  phage carrying amber mutations in the *O* and *P* genes of the  $\lambda$  genome still behaves as a plaque-forming phage and replicates by means of the *ColE1* replicon. The isolation of this type of phage indicates that replication of  $\lambda$  can occur by utilizing the replicatability of *ColE1* plasmid covalently connected with the phage genome.

Most  $\lambda$  lysogens examined so far have the  $\lambda$  phage genome within the host chromosome. However, the  $\lambda N^-$  mutant phage can lysogenize as a plasmid (13, 25). We have demonstrated

that the  $\lambda imm^{434}ColE1$  phage is maintained as a plasmid by the functions of the *ColE1* plasmid, because this phage DNA is accumulated as covalently closed circular DNA after the addition of chloramphenicol, as reported for *ColE1* plasmid (4). The major difference between the two is that  $\lambda imm^{434}ColE1$  lysogens were killed by induction with mitomycin C and plaque-forming phages were induced by this treatment, whereas  $\lambda N^-$  phage lysogens did not produce any plaque-forming phages after mitomycin C treatment.  $\lambda imm^{434}ColE1$  has characteristics similar to P1 phage, which can exist as a plasmid in the cy-

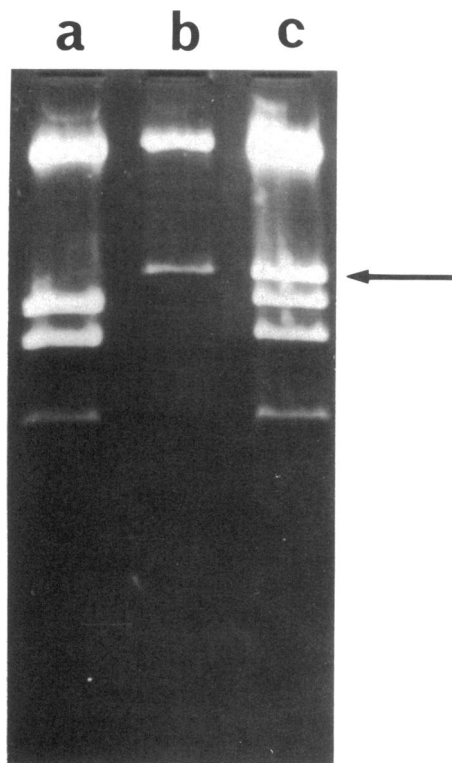


FIG. 5. Agarose gel electrophoretic pattern after digestion of  $\lambda imm^{434} OamPamColE1$  plasmid DNA with *EcoRI* endonuclease. DNAs used are: (a)  $\lambda imm^{434} c$ , (b) *ColE1-cos $\lambda$ -guaA*, (c)  $\lambda imm^{434} OamPamColE1$ . Procedures are described in the text and Fig. 3. Arrow indicates the fragment with the same mobility as *ColE1* DNA.

toplasm and as a plaque-forming phage (11).

Our  $\lambda imm^{434} OamPamColE1$  phage plated on *polA*<sup>+</sup> host, but it could not form a plaque on hosts carrying a *polA* mutation.  $\lambda imm^{434} ColE1O^+P^+$  recombinants, which were isolated by infecting  $\lambda bio11$  onto KS2008( $\lambda imm^{434} OamPamColE1$ ) bacteria, plated on *polA* hosts as efficiently as on *polA*<sup>+</sup> hosts (unpublished data). This result suggests that the functions of *ColE1* DNA can be used to replicate this phage when the phage has defects in the *O* and *P* cistrons (20).  $\lambda imm^{434} Oam29Pam3$  phage could not make plaques on bacteria carrying *ColE1*, indicating that the products of the *ColE1* plasmid cannot complement the functions of the *O* and *P* genes in *trans* (unpublished observation).

$\lambda imm^{434} OamPamColE1$  could not plate on a  $\lambda imm^{434}$  lysogen or a *groN* host, and this indicates that the plaque formation depends on the intact *N* gene function. On the other hand,  $\lambda imm^{434} OamPamColE1$  efficiently transduced

the *guaA*<sup>+</sup> gene to a  $\lambda imm^{434}$  lysogen, suggesting that the maintenance of the  $\lambda imm^{434} OamPamColE1$  genome as a plasmid is not dependent upon the presence of an intact *N* gene function.

$\lambda imm^{434} ColE1$  phage could not plate on *groP* bacteria (unpublished data), which are unable to support growth of all lambdoid phages (9). If the replication depended on *ColE1* but not on  $\lambda$ , it might plate on a *groP* mutant. However, failure in plating on *groP* does not contradict the *ColE1*-dependent replication, because *ColE1* DNA replication depends to some extent on *dnaB* function, which is identical to the product of the *groP* gene (10).

We have to mention here one possibility that we cannot eliminate by our present data. As we selected for plaque formation by  $\lambda imm^{434} OamPamColE1$  using a *su*<sup>-</sup> host, we cannot be sure that *ColE1* can substitute for the *O* and *P* gene functions without further mutations in the  $\lambda$  genome or in *ColE1* DNA. However, the hybrid phage still carries a mutation in *P* gene at least, and the hybrid phage DNA molecules accumulate within lysogenic cells in the presence of chloramphenicol as covalently closed circular DNAs.

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