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Lambda Bacteriophage-Mediated Transduction of ColE1 Deoxyribonucleic Acid Having a Lambda Bacteriophage-Cohesive End Site: Selection of Packageable-Length Deoxyribonucleic Acid

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An in vitro recombinant ColE1-*cos*λ deoxyribonucleic acid (DNA) molecule, pKY96, has 70% of the length of λ phage DNA. The process of λ phage-mediated transduction of pKY96 generated a small amount of transducing phage particles containing ColE1-*cos*λ DNA molecules of 80 or 101% of the length of λ phage DNA, in addition to those containing original pKY96 DNA molecules. The newly isolated larger plasmid DNAs were transduced 100 times more efficiently than pKY96 DNA. Their structures were compared with that of a prototype pKY96 DNA, and the mechanism of the formation of these molecules is discussed.

An in vitro recombinant DNA molecule, pKY96 (former name, ColE1-*cos*λ-*guaA*), exists as a stable monomer plasmid within *Escherichia coli* K-12. It contains a whole ColE1 DNA and a bacterial gene for the *guaA* enzyme (=XMP aminase) together with a part of the λ genome, R through J:(R-*cos*λ-A-F-J)$^+$ (10, 18). Its molecular weight is estimated to be about 21.6 ± 0.2 × 10^6 (15), corresponding to 70% of that of λ phage DNA. The λ phage-mediated specialized transduction of pKY96 DNA is reported by Fukumaki et al. (10).

Previous papers on λ phage morphogenesis report very inefficient packaging of the monomer circular λ DNA within λ phage particles (6, 11, 23) and note that 75% of the length of λ phage DNA is the shortest molecule that produces plaques of nearly normal size (J. S. Parkinson, cited in reference 26). We, therefore, estimated the molecular sizes of pKY96 DNAs packaged within λ phage particles by analyzing the density of transducing particles. We found that the majority of the transducing particles contained whole pKY96 DNA molecules (28), whereas a minority of *guaA*-transducing particles contained DNA molecules having 80 or 101% of the length of λ phage DNA.

What is the significance of the generation of the *guaA*-transducing particles containing DNA molecules of that length? To elucidate this, we isolated ColE1-*cos*λ plasmid DNAs of 80 or 101% of the length of λ phage DNA by infecting these *guaA*-transducing particles onto *E. coli* K-12 *recA* cells. We compared their structures with that of a pKY96 plasmid DNA and discuss the mechanism of their formation. We also examined the effect of DNA size on the efficiency of transduction of ColE1-*cos*λ plasmid DNAs.

MATERIALS AND METHODS

Bacterial and bacteriophage strains and plasmids. The bacterial and bacteriophage strains used are listed in Table 1. *E. coli* K-12 KS1616 was deleted of *gal-att*λ-*bio* and *guaA-guaB* regions of an *E. coli* chromosome (19). KS2127, which has λBAM within the normal λ attachment site, was isolated as described previously (28).

All the plasmids used in this work are derivatives of ColE1 and carry cohesive end sites of the λ phage genome (*cos*λ).

Media. Polypeptone bonito extract broth medium and agar were used to grow and titrate bacteria and phage (10). PB medium was same as the polypeptone bonito extract broth medium, except that it contained 1 g instead of 10 g of bonito extract per liter (17). Minimal agar and minimal medium were supplemented with 20 μg of xanthine per ml, when necessary (10).

Phage stocks. Phage lysates containing ColE1-*cos*λ DNA-transducing particles were prepared by the plate method or by heat induction of λ*c*I857 lysogens (20, 28).

***GuaA* gene transduction.** A 0.1-ml amount of KS1616 cell suspension in 0.01 M $MgSO_4$ and 0.1 ml of diluted phage lysates were mixed and incubated for 30 min at 30°C. The mixtures were plated on minimal agar supplemented with 20 μg of xanthine per ml, and the plates were examined after incubation for 2 days at 30°C (28).

CsCl density gradient analysis. Phage lysates

TABLE 1. *Bacterial and bacteriophage strains*[a]

Strain	Description	Source or reference
Bacteria		
HfrH	*sup*$^+$	20
KL16-99	HfrKL16 *recA1*	20
KS1616	HfrH Δ(*gal-bio*) Δ(*guaB-A*)	19
KS1963	KS1616 (*gal-bio*)$^+$	This work
KS2008	KS1616 *recA*	28
KS1944	KS1963 *recA*	10
KS2127	KS1944 (λBAM)	This work
TM96	KS1616(pKY96)	18
KS1941	KS1944(pKY96)	28
KS2128	KS1941 (λBAM)	28
Bacteriophage		
λcI857		22
λ*bio*69	Δ(*int-red*) (ρ = 1.511 g/cm^3)	12
λBAM	Δ(*int-c*III)	7
λ$i^{21}b2bio11$	(ρ = 1.481 g/cm^3)	28

[a] del, Deletion. Other genetic symbols are those used by Bachmann et al. (1) for *E. coli* and by Szybalski and Herskowitz (24) for λ. All λ phages whose immunity is not given carry the *c*I857 temperature-sensitive *c*I mutation.

were mixed with a CsCl solution and centrifuged for 15 h at 23,000 rpm at 10°C in a Spinco model L ultracentrifuge in a type 39 swinging rotor (28).

Preparation of plasmid DNAs. ColE1-*cos*λ DNAs were accumulated by incubating cells in a minimal medium supplemented with 20 μg of xanthine per ml in the presence of 100 μg of chloramphenicol per ml (15). Extrachromosomal DNA was extracted and purified as described previously (17).

Analysis of DNA molecules by electron microscopy. Heteroduplexes were prepared by the procedure of Yamagishi et al. (29), and the length of DNA was measured as described previously (15).

Enzymes and electrophoresis in agarose gel. Restriction enzymes *Eco*RI and *Sma*R were prepared as described elsewhere (15, 25). Purified plasmid DNAs were digested with restriction enzymes, and the DNA fragments were subjected to agarose gel (0.8%) electrophoresis as described elsewhere (15).

Transformation and selection of transformants. Bacteria were made competent for transformation by the procedures described by Lederberg and Cohen (14). For transformation, 0.01-ml volume of chilled DNA samples in 0.001 M EDTA and 0.01 M Tris, pH 7.0, was added to 0.1 ml of the competent cells in cold 0.1 M $CaCl_2$, and the mixture was incubated for 60 min at 0°C. This reaction mixture was diluted 10-fold with a minimal medium, and a sample (0.5 ml) was spread over the minimal agar supplemented with 20 μg of xanthine per ml to isolate the GuaA$^+$ transformants. The plates were examined after incubation for 2 days at 30°C.

Labeling of DNAs packaged within phage particles. Cells carrying λcI857 prophage were grown to about 2 × 10^8 cells per ml in 10 ml of PB medium containing 1% Casamino Acids and 2% glucose at 30°C. The cells were then induced by shifting the temperature to 42°C for 10 min, followed by shaking for 5 min at 37°C before labeling (9, 13). A 10-ml portion of the heat-pulsed cells was supplemented with 2 mg of uridine and 10 μg of thymidine and was labeled by adding 0.05 mCi of [^{3}H]thymidine, followed by shaking for 120 min at 37°C. Lysis was completed with a few drops of $CHCl_3$, and free DNAs were digested by adding 5 μg of pancreatic DNase per ml and incubating at 37°C for 30 min. After the phages were precipitated, the pellets were resuspended in 3 ml of 0.01 M $MgSO_4$ and 0.01 M Tris, pH 7.8, to be used for the CsCl density gradient analysis. After centrifugation, radioactivity was measured in a liquid scintillation counter (2).

The specific infectivity of particles was expressed as the ratio of plaque-forming or *guaA*-transducing activity to the number of physical chromosomes present. The number of chromosomes present was expressed as the amount of ^{3}H label in the peak corrected for DNA content by dividing by the chromosome length (9).

RESULTS

Analysis of *guaA*-transducing particle density. The density of *guaA*-transducing particles found in the heat-induced lysates of KS2128 was analyzed by CsCl density gradient centrifugation (Fig. 1a; 28). About 90% of the total *guaA*-transducing particles showed a broad peak near a density of 1.467 g/cm^3 (Fig. 1a), which corresponded to λ phage particles containing 68% of the length of λ phage DNA. This may be taken as proof that most of the pKY96 DNAs were packaged into λ phage particles as a whole (28). In addition, we noted the presence of at least two minor peaks at densities of 1.485 and 1.510 g/cm^3 (Fig. 1a).

We infected the *guaA*-transducing particles with densities of 1.485 or 1.510 g/cm^3 onto KS1616 *recA*(=KS2008) cells and purified several independently isolated ColE1-*imm*$^+$ GuaA$^+$ transductants to investigate the generation mechanism of these particles. Each representative transductant was named as described in Table 2 and carried R through J of the λ phage genome, similar to that of pKY96.

We prepared phage lysates after infecting λ*bio*69 onto UK23, UK551, UK552, and UK546 cells and determined the density of *guaA*-transducing particles contained in them (Fig. 1b, c, and d). Density distribution patterns of *guaA*-transducing particles obtained from UK23 and of those found in the heat-induced lysate of KS2128 were similar (cf. Fig. 1a and b). The *guaA*-transducing particles obtained from UK551 formed one major peak at a density of 1.485 g/cm^3 (Fig. 1c). The value 1.485 is identical to that of fraction B (Table 2). We gained similar results with those obtained from UK552. The *guaA*-transducing particles obtained from

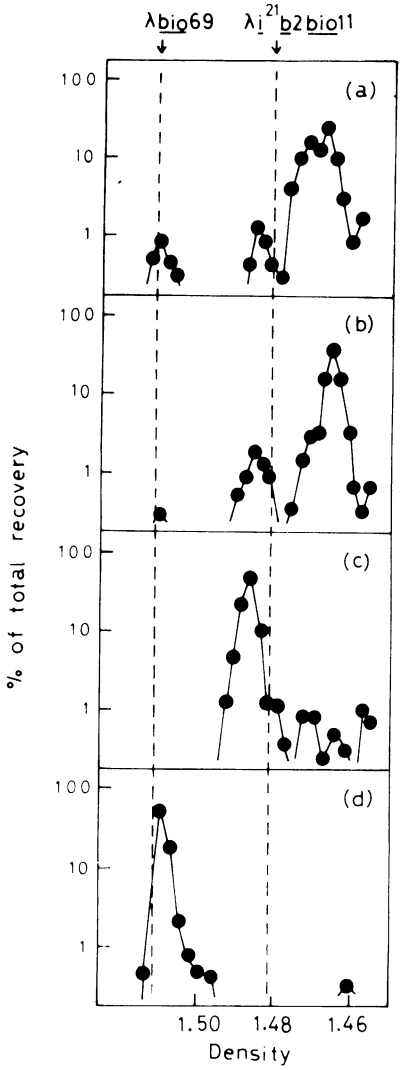

FIG. 1. *CsCl density gradient analysis of guaA-transducing particles. Lysates were obtained by heat induction of (a) KS2128 (28) or by infecting λcI857bio69 onto (b) UK23, (c) UK551, or (d) UK546. (●) Percentage of total transducing activity; 100% corresponds to: (a) 6.9 × 10⁴, (b) 1.6 × 10⁴, (c) 1.8 × 10⁵, and (d) 9.5 × 10⁴. The densities of λcI857bio69 (ρ = 1.511 g/cm³) and λi²¹b2bio11 (ρ = 1.481 g/cm³), added as density references, are indicated by the dotted lines.*

UK546 presented a major peak at a density of 1.510 g/cm³ (Fig. 1d), identical to that of fraction C (Table 2).

Molecular nature of plasmid DNAs present within transductants. Newly isolated plasmid DNAs were accumulated to examine their properties by incubating the above-described transductants (Table 2) in the presence of 100 μg of chloramphenicol per ml. Their molecular weights were estimated by measuring the contour lengths of the DNA molecules in electron micrographs, relative to that of ColE1 DNA (4.2 × 10⁶ daltons; 3) (Table 3). No apparent polymers were found among them. The two differently determined molecular weights of ColE1-*cos*λ DNAs agreed: one by electron micrographs and one by the density estimation of the *guaA*-transducing particles (Tables 2 and 3). It is, therefore, fairly certain that ColE1-*cos*λ DNAs present within transducing phage particles are injected into recipient cells and exist as stable plasmids without changing their molecular size.

Structures of ColE1-*cos*λ plasmid DNAs were studied further by digestion with restriction enzymes and analysis of the resulting fragments by

TABLE 2. *Fractions of guaA-transducing particles prepared from heat-induced lysates of KS2128*

Fractions	Density (g/cm³)[a]	Mol wt		GuaA⁺ transductant[d]	Plasmid
		× 10⁶[b]	%[c]		
A	1.467	20.9	68	UK23	pKY23
B	1.485	24.6	80	UK551	pKY551
				UK552	pKY552
C	1.510	31.1	101	UK546	pKY546

[a] The density of each fraction was calculated from Fig. 1a by refractometer readings of drops collected from the gradient and the position of the peak of marker λi²¹b2bio11 phage (ρ = 1.481 g/cm³).

[b] Molecular weight of DNAs packaged within *guaA*-transducing particles was estimated by the density of the particles.

[c] The molecular size of λ⁺ phage DNA (i.e., 3.08 × 10⁷ daltons; 4) was set as 100%.

[d] ColE1-*imm*⁺ GuaA⁺ transductants were isolated after infecting fractions A, B, and C onto KS1616 *recA* (=KS2008) cells.

TABLE 3. *Molecular weights of newly isolated ColE1 plasmid DNAs determined by electron microscopy*

Plasmid	Mol wt (× 10⁶)[a]	No. of open circular DNA molecules measured
pKY96	21.6 ± 0.2[b]	52
pKY23	21.0 ± 0.6[c]	20
pKY551	24.7 ± 0.6	20
pKY552	25.0 ± 0.8	20
pKY546	31.0 ± 1.1	20

[a] Molecular weights of plasmid DNAs were estimated by measuring the contour lengths of the molecules in electron micrographs relative to that of ColE1 DNA (4.2 × 10⁶; 3).

[b] From Maeda et al. (15).

[c] From Umene et al. (28).

agarose gel electrophoresis (Fig. 2). The electrophoretic patterns showed no differences between DNA fragments of pKY23 and parental pKY96 created by *Eco*RI or *Sma*R digestion. (cf. Fig. 2bI and cI and Fig. 2bII and cII). Thus, we concluded that pKY23 and pKY96 DNAs were identical (28).

Agarose gel electrophoresis patterns of DNA fragments produced by treating pKY551 DNAs with *Eco*RI, *Sma*R, or *Eco*RI and *Sma*R are shown in Fig. 2 (dI to dIII). The *Eco*RI digests yielded three new fragments, eX1, eX2, and eX3, other than those found in the *Eco*RI digests of pKY96 DNA, but they did not produce a fragment e2 (cf. Fig. 2dI and bI); the *Sma*R digests yielded a new fragment, sX2 (cf. Fig. 2dII and bII), and the *Eco*RI and *Sma*R digests yielded four new fragments, esX1, esX2, esX3, and esX4 (cf. Fig. 2dIII and bIII). Fragment eX3 was identical to fragment esX4. On the basis of these results, the structure of the pKY551 DNA molecule is proposed as shown in Fig. 3b: it contains a whole pKY96 DNA and a DNA fragment whose origin is not known. The DNA fragment of unknown origin has a molecular weight of about 3.5×10^6 and it has one *Sm*R and two *Eco*RI restriction sites.

Figure 2e shows agarose gel electrophoresis patterns of DNA fragments produced by treating pKY552 DNAs with *Eco*RI, *Sma*R, or *Eco*RI and *Sma*R. The *Eco*RI digests yielded a new fragment, eY2 (cf. Fig. 2eI and bI); the *Sma*R digests yielded a new fragment, sY2 (cf. Fig. 2eII and bII); and the *Eco*RI and *Sma*R digests yielded two new fragments, esY2 and esY3 (cf. Fig. 2eIII and bIII). Thus, the structure of pKY552 DNA, which consists of a pKY96 DNA and a DNA fragment of unknown origin, should be as illustrated in Fig. 3c. The DNA fragment of unknown origin has a molecular weight of about 3.5×10^6, and it has one *Sma*R and one *Eco*RI restriction site.

Figure 2f shows the agarose gel electrophoresis patterns of DNA fragments produced by treating pKY546 DNAs with *Eco*RI, *Sma*R, or *Eco*RI and *Sma*R. The *Eco*RI digests yielded one new fragment, eZ1, besides those found in the *Eco*RI digests of pKY96 DNA (cf. Fig. 2fI and bI), and the *Sma*R digests yielded one new fragment, sZ1 (cf. Fig. 2fII and bII). The sum of molecular weights of all the *Sma*R fragments was about 3.0×10^6 less than that estimated by electron micrographs. The *Eco*RI and *Sma*R digests yielded one new fragment, esZ1; this fragment was identical to sZ1. The sum of molecular weights of all the *Eco*RI and *Sma*R digests was also about 3.0×10^6 less than that estimated by electron micrographs. This discrepancy can be explained by assuming that the plasmid DNA molecule contains two copies of *Sma*R fragments, s2 and s3. Accordingly, we propose the molecular structure of pKY546 DNA as shown in Fig. 3d, and it explains that the molecule is generated by headful packaging of dimerized pKY96 DNA. An electron microscopic examination of a heteroduplex molecule of *Bam*I-cleaved pKY96 and pKY546 DNAs revealed a single-stranded insertion loop bounded on each side by duplex DNA (Fig. 4). The total length of the duplex DNA was almost identical to that of pKY96 DNA. The result of the electron microscopic analysis is consistent with the proposed structure of the pKY546 DNA molecule.

Effect of DNA length on the efficiency of transduction. The transduction of pKY96 DNAs having 70% of the length of λ phage DNA is a strange phenomenon, because λ phage DNA less than 75% of the length of a wild-type λ phage DNA is not packaged efficiently (9, 26). We examined the relation between the length of ColE1-*cos*λ plasmid DNAs and the efficiency of transduction to clarify the effect of DNA length on the process of transduction. pKY23, pKY551, and pKY546 DNAs were introduced into Ca^{2+}-treated KS2127 cells, and ColE1-*imm*$^+$ GuaA$^+$ transformants were isolated. One transformant from each DNA was heat induced, and the *guaA*-transducing activity of phage lysates was measured (Table 4). ColE1-*cos*λ DNAs of 80 or 101% of the length of λ phage DNA were transduced about 100 times more efficiently than those of 70%.

Infectivity of λ phage particles carrying ColE1-*cos*λ DNAs. We introduced pKY551 or pKY96 DNA into Ca^{2+}-treated KS1616 *rec*$^+$ (=KS1963) cells by isolating ColE1-*imm*$^+$ GuaA$^+$ transformants and lysogenized these transformants with λ*c*I857. The yield of *guaA*-transducing particles was high in the presence of *rec, red,* and *gam* functions (Table 5; 28). Using these strains, we compared the physical number of the particles carrying ColE1-*cos*λ DNAs with the number determined by a biological assay. Phage lysates labeled with [^{3}H]thymidine were prepared from UK90, and the phages were fractionated by CsCl density gradient centrifugation (Fig. 5a). Two peaks appeared in radioactivity detection as had been expected; one corresponded to the plaque-forming activity and the other corresponded to the *guaA*-transducing activity. The specific infectivity of the λ*c*I857 particles was 1.49×10^6 plaque-forming activity·length/cpm (see Materials and Methods). The same calculation for the *guaA*-transducing particles gave a value of 3.27×10^5

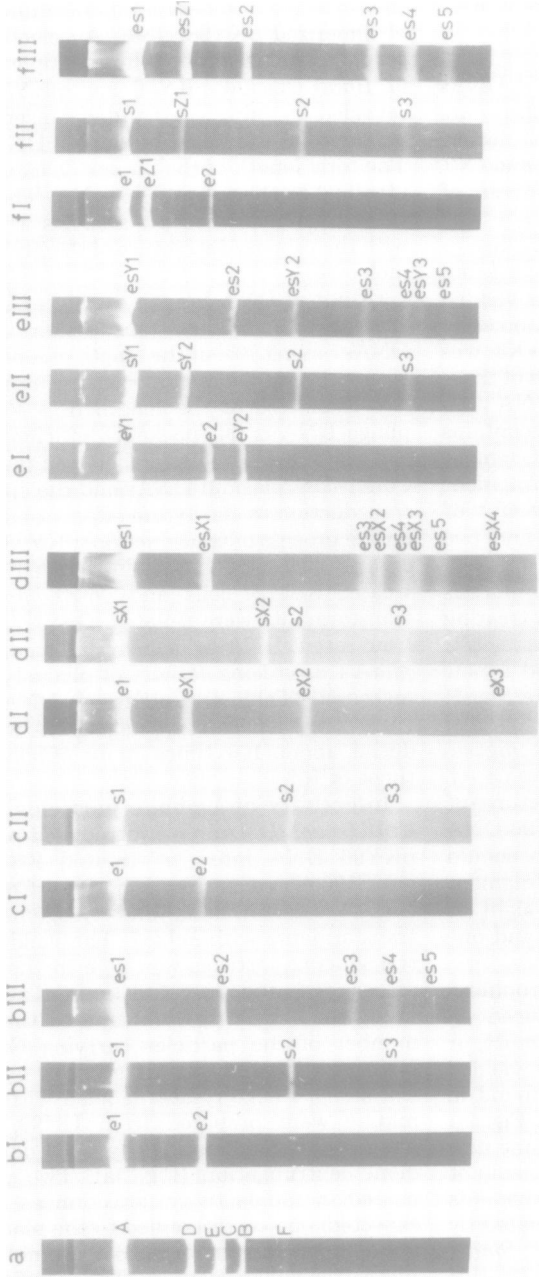

FIG. 2. *Agarose gel electrophoresis of DNA fragments produced by treating ColE1-cosλ plasmid DNAs with restriction endonucleases. Samples containing digested DNA were applied to a 0.8% agarose slab gel and were subjected to electrophoresis at 20 mA/gel for 20 h at room temperature. DNA fragments obtained after digestion with EcoRI, SmaR, or EcoRI and SmaR simultaneously are numbered with the prefixes e, s, and es, respectively. The molecular weight (×10⁶) of each DNA fragment was estimated by using a reference curve constructed with EcoRI hydrolysates of λ phage DNA (27). (a) λ phage DNA digested with EcoRI. Molecular weights of the fragments were: A, 13.7; D, 4.74; E, 3.73; C, 3.48; B, 3.02; and F, 2.18. (b) pKY96 DNA digested with EcoRI (bI), SmaR (bII), or EcoRI and SmaR (bIII). Their molecular weights were: e1, ca. 17.5; e2, 4.2; s1, ca. 18.5; s2, 2.0; s3, 1.0; es1, ca. 15.0; es2, 3.5; es3, 1.3, es4, 1.0, and es5, 0.7 (15). (c) pKY23 DNA digested with EcoRI (cI) or SmaR (cII). No difference was found between pKY96 and pKY23 (28). (d) pKY551 DNA digested with EcoRI (dI), SmaR (dII), or EcoRI and SmaR (dIII). Fragments which were not present in digests of pKY96 DNA are numbered with the prefix X. Their molecular weights were: eX1, 5.4; eX2, 1.9; eX3, 0.4; sX1, 19.6; sX2, 2.5; esX1, 4.5; esX2, 1.2; esX3, 0.9; and esX4, 0.4. (e) pKY552 DNA digested with EcoRI (eI), SmaR (eII), or EcoRI and SmaR (eIII). Fragments which are not present in digests of pKY96 DNA are numbered with the prefix Y. Their molecular weights were: eY1, 17.9; eY2, 3.0; sY1, 16.5; sY2, 5.6; esY1, 15.6; esY2, 2.1; and esY3, 0.9. (f) pKY546 DNA digested with EcoRI (fI), SmaR (fII), or EcoRI and SmaR (fIII). Fragments which are not present in digests of pKY96 DNA are numbered with the prefix Z. Their molecular weights were: eZ1, 9.5; sZ1, 6.5; and esZ1, 6.5.*

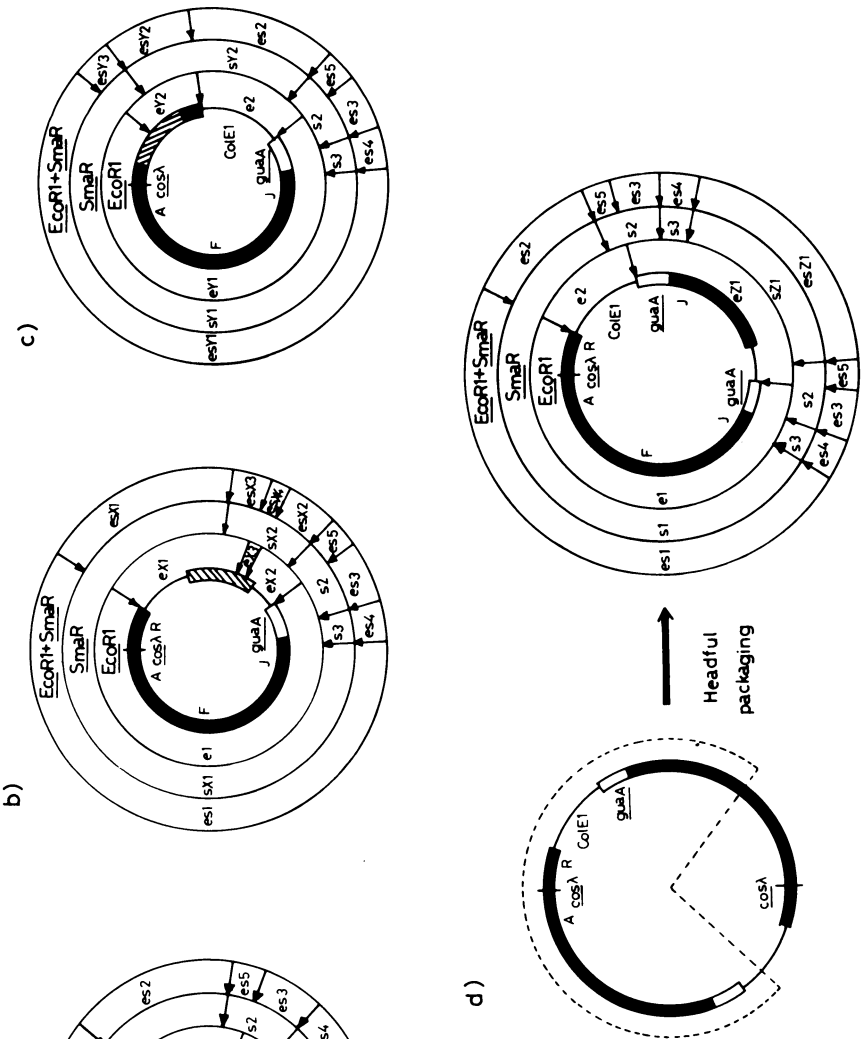

FIG. 3. *Structures and a model of the generation of ColE1-cosλ derivatives. Arrows indicate restriction endonuclease EcoRI or SmaR cleavage sites. The thin, single line represents a ColE1 DNA; the thick line, a part of the λ genome; and the double line, a fragment of a bacterial chromosome. R, A, F, J, and cosλ are λ phage gene markers (24) and guaA is a bacterial gene marker (1). Other symbols are same as described in the legend to Fig. 2. (a) Structure of pKY96 DNA (10, 15). (b) Structure of pKY551 DNA. The hatched double line represents an inserted DNA Fragment of unknown origin. (c) Structure of pKY552 DNA. The hatched double line represents an inserted DNA fragment of unknown origin. (d) Structure and a model of the generation of pKY546 DNA. This molecule is proposed to be generated by the headful packaging of dimerized pKY96 DNA as indicated by dotted line.*

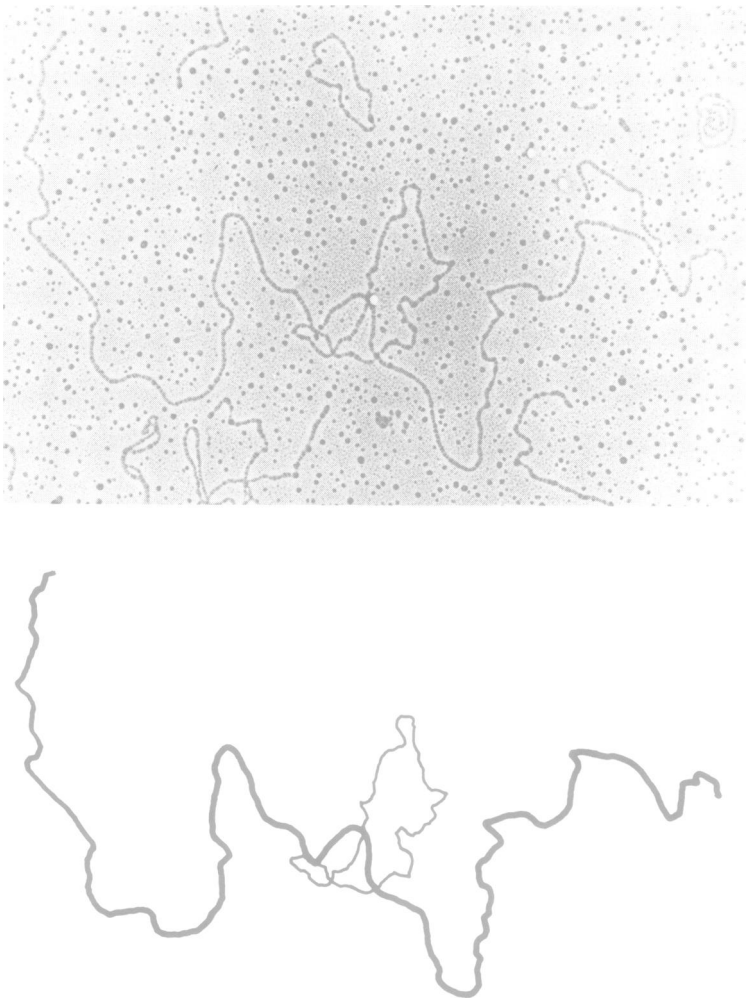

FIG. 4. *An electron micrograph of a heteroduplex molecule between pKY96 and pKY546 DNA. pKY96 DNA had only one BamI-sensitive site (15), and pKY546 DNA was expected to have only one BamI-sensitive site (Fig. 3d). BamI-cleaved pKY96 and pKY546 DNAs were heteroduplexed and spread for electron microscopy by the formamide technique (29). The region of nonhomology is seen as a loop of single-stranded DNA. The small circular molecule is an fdRFII DNA molecule which was included as an internal marker (4.09×10^6 daltons; 29).*

guaA-transducing activity·length/cpm. Accordingly, the specific infectivity of the *guaA*-transducing particles carrying DNAs of 70% of the length of λ phage DNA was about 0.2 of the reference λ phage. This result shows that a considerable fraction of the *guaA*-transducing particles containing DNAs of 70% of the length of λ phage DNA is not infectious (9).

A ^{3}H-labeled lysate of UK108 was also prepared, and the phage was fractionated by banding in CsCl (Fig. 5b). Radioactivity detection showed two peaks, one corresponding to the plaque-forming activity and the other to the *guaA*-transducing activity. The specific infectivity of the λ*c*I857 particles was 1.21×10^6 plaque-forming activity·length/cpm and that of the *guaA*-transducing particles was 1.29×10^6 *guaA*-transducing activity·length/cpm. These two close values indicate that the *guaA*-transducing particles carrying DNAs of 80% of the length of λ phage DNA are fully infectious.

DISCUSSION

We studied the process of λ phage-mediated transduction of ColE1-*cos*λ DNA molecules having 70% of the length of λ phage DNA; the results are as follows.

(i) ColE1-*cos*λ DNAs of 70% of the length of

TABLE 4. *Efficiency of transduction of ColE1-cosλ plasmid DNAs of various lengths*

Strain[a]	Plasmid	Mol wt of plasmids ($\times 10^6$)[b]	PFU/ml[c]	*guaA*-transducing activity/ml
UK66	pKY23	21.0	8.3×10^2	4.4×10^5
UK65	pKY551	24.7	6.2×10^2	7.2×10^7
UK64	pKY546	31.0	8.0×10^2	7.2×10^7

[a] These strains were constructed by isolating ColE1-*imm*$^+$ GuaA$^+$ transformants after introducing each ColE1-*cos*λ DNA into Ca^{2+}-treated KS2127 cells. *E. coli* K-12 KS2127, which is lysogenized with λBAM, is a *recA* derivative of KS1616.

[b] Molecular weights of ColE1-*cos*λ plasmid DNAs were estimated by electron microscopy (Table 3).

[c] Phage lysates were prepared by heat induction of λBAM lysogens (28).

λ phage DNA were transduced with about 1% of the efficiency of those of 80 and 101% of the length of λ phage DNA (Tables 4 and 5).

(ii) A considerable fraction of phage particles carrying DNAs of 70% of the length of λ phage DNA were not infectious (Fig. 5), whereas those carrying DNAs of 80% were fully infectious.

(iii) ColE1-*cos*λ DNA molecules of 80 or 101% of the length of λ phage DNA, in addition to those of the original 70%, were packaged within λ phage particles during the process of packaging those of 70% (Fig. 1a).

These results all indicate that ColE1-*cos*λ DNA molecules of 70% of the length of λ phage DNA are too short to be transduced efficiently. Again, the results shown in Fig. 5 reveal that a considerable fraction of phage particles carrying DNAs of 70% of the length of λ phage DNA were not infectious. These pKY96 DNAs could have been somehow damaged during their packaging process so that they either could not be injected efficiently or could not form circular plasmid DNAs readily after injection.

On the other hand, DNAs ranging from 105 to 80% of the length of λ phage DNA were packaged with constant efficiency (9). We found that when ColE1-*cos*λ DNAs are less than 50% of the length of λ phage DNA, oligomers are packaged within λ phage particles. It was reported that ColE1-*cos*λ DNAs of 29% were packaged within λ phage particles as trimers, and those of 41 or 49% were packaged as dimers (28). These results led us to the conclusion that pKY96 DNAs, which have 70% of the length of λ phage DNA,

TABLE 5. *Yield of guaA-transducing activity in the presence of all recombination functions*

Strain[a]	Plasmid	PFU/ml[b]	*guaA*-transducing activity/ml
UK90	pKY96	3.2×10^9	7.2×10^7
UK108	pKY551	9.4×10^9	3.3×10^9

[a] These strains are *rec*$^+$ derivatives of KS1616, which are deleted of a part of the guanine operon. They are lysogenized with λ*c*I857 and carry ColE1-*cos*λ plasmids.

[b] Phage lysates were prepared by heat induction of λ*c*I857 lysogens (28).

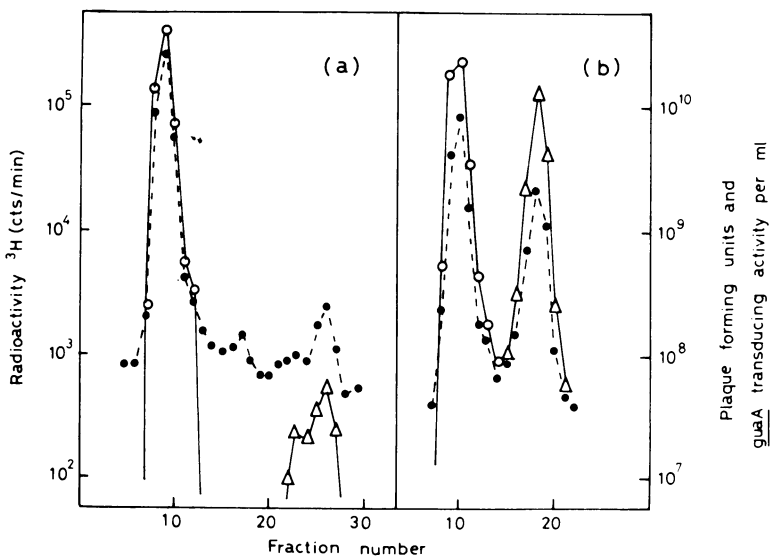

FIG. 5. *CsCl density gradient profiles of ^{3}H-labeled phage lysates obtained after heat induction of (a) UK90 = rec$^+$ (λcI857) (pKY96) and (b) UK108 = rec$^+$ (λcI857) (pKY551) (see Table 5). Symbols: (●) ^{3}H radioactivity; (○) PFU; (△) guaA-transducing activity.*

are too long to be packaged within λ phage particles as dimers and are also too short to be packaged efficiently as monomers.

It was also observed that *guaA*-transducing phage particles containing DNA molecules of 80 or 101% of the length of λ phage DNA were produced during the process of packaging of DNA molecules having 70% (Fig. 1a). We interpret this as follows: ColE1-*cos*λ DNA molecules carrying an additional DNA segment were selected preferentially during the process of packaging, because the addition augments the efficiency of packaging of ColE1-*cos*λ DNA molecules (Tables 4 and 5, Fig. 5).

We isolated transductants carrying ColE1-*cos*λ plasmid DNA molecules larger than the parental pKY96 DNA after infecting denser *guaA*-transducing particles onto *E. coli recA* cells (Table 2). A series of duplication derivatives of the λ phage were isolated on the basis of the increased buoyant density (5, 8). Therefore, an analysis of the buoyant density of phage particles gives us a chance to isolate new phages or new plasmids. By using the transduction procedures, Maeda et al. (16) observed that an ampicillin resistance transposon (Tn*3*) is translocated from R1*drd* onto the pKY96 to form a pKY96::Tn*3*. Thus, λ phage-mediated transduction of ColE1-*cos*λ derivatives is a useful method for isolating new plasmid DNAs.

The length of a mature λ phage DNA is determined not only by the capacity of a phage head, but also by the distance of the two specific cutting sites (cohesive end sites; 5, 6, 11, 21, 23). Sternberg and Weisberg (21) reported that the λ phage particle could package a headful length of prophage and host DNA and suggested that a sequence-specific nuclease (Ter) cut one end (cohesive end) of prophage DNA and the other end of DNA was cut non-specifically. The pKY546 DNA is probably a product of the packaging of dimer pKY96 DNA by the headful mechanism (Fig. 3d). It naturally follows that the length of pKY546 DNA was determined not by the distance between two specific cutting sites but by the capacity of the phage head. If this is the case, generation of pKY546 DNA molecules suggests the presence of a novel packaging mechanism in which λ capsids measure the length of packageable DNA with a *cos*λ in the middle of the molecule.

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