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Genetics

Specialized transduction of colicin E1 DNA in *Escherichia coli* K-12 by phage lambda

(XMP aminase/packaging/cohesive end)

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ABSTRACT **Genetic studies were made on *E. coli* K-12 TM96, which carries recombinant molecules constructed by *in vitro* combination of colicin E1 DNA and a DNA fragment of *E. coli* for guanine synthesis derived from transducing phage. The recombinant molecules existed as stable plasmids within the cell and contained genes for colicin E1 immunity and the *guaA* enzyme (xanthosine 5′-monophosphate aminase) together with a part of the λ genome, R through J: (R-A-F-J)$^+$. A block of the λ genome, *int* through Q, was not detected in the recombinant molecule. Thus, this recombinant molecule was named ColE1-*cos*λ-*guaA*, and the specialized transduction of the ColE1-*cos*λ-*guaA* DNA into various *E. coli* K-12 cells by λ phage was described. Lysates prepared by lytic infection of λ phage onto TM96 or by induction of TM96(λ) lysogens contained transducing particles which could transduce *gua*-deleted *E. coli* to stable *guaA*$^+$ cells. These transductants were proved to have similar genetic properties as those of TM96. The frequency of transduction was not affected by the presence of an attachment site for λ, prophage λ, colicin E1 plasmids, or the *recA* property within *gua*-deleted recipient cells. Transducing particles were resistant to EDTA treatment and most of them had an average density of about 1.472. This value corresponds to that of λ phage particles, which contain about 72% of the length of λ DNA.**

Colicinogenic factor E1 (ColE1) is an *Escherichia coli* plasmid that directs the production of a specific antibiotic protein. The plasmid DNA has a molecular weight of 4.2×10^6 (1), corresponding to about 14% of that of the well-studied bacteriophage λDNA. The ColE1 plasmid exists as multiple copies, in contrast to the F factor or R factor. The number of plasmid DNAs in a cell is carefully controlled, probably, by the function(s) of the host cell together with that of plasmid DNA itself. The mechanism of maintenance of a stable level of plasmid DNA is an interesting subject. It is reported that the replication of ColE1 DNA depends on host *dnaA* (2) and *polA* (3) functions but not on the *dnaE* function (4), and that ColE1 DNA initiates and completes a round of semiconservative replication in a crude *in vitro* system (5). However, the role of ColE1 genes in controlling the replication of its DNA is poorly understood. The large number of ColE1 DNA copies within a cell makes genetic analysis very difficult.

This paper describes the specialized transduction of a recombinant molecule, which carries a complete ColE1 genome joined *in vitro* with a part of the λ phage genome and a fragment of an *E. coli* chromosome for guanine synthesis, in *E. coli* K12 by λ phage. This system makes it possible to introduce the established phage genetics into genetic study of ColE1 DNA replication.

MATERIALS AND METHODS

Strains. The bacterial strains used are listed in Table 1. *E. coli* K-12 TM96, which carries the *in vitro* ColE1-*guaA* recombinant, was obtained from Dr. T. Mukai and is described in ref. 6. Most of the phage strains used are listed in (7). λp*guaBA*504(λp*gal* type transducing phage) was isolated from heat-induced lysates of KS504 (λ*c*I857 within *guaB* lysogens) (10). BF23 phage cannot kill ColE1 resistant cells, but its growth is not affected by the presence of ColE1 immunity (14).

Media. Polypeptone bonito extract broth (PBB) medium (see ref. 15) was used for bacterial growth. PBB agar was used for phage and bacterial assays. Minimal agar was supplemented with 0.1 μg/ml of biotin and thiamine and, where required, 20 μg/ml of xanthine. These media have been described previously (7, 15). Suspensions of bacteria and phages were diluted with 0.01 M $MgSO_4$. Lysogens were isolated on EMB-O agar (7).

Phage Assays and Phage Stocks. Free phage was assayed as described previously (7). Phage stocks were obtained by the plating method or by induction of λ lysogens (see legend to Table 3).

Measurement of Transducing Ability. Overnight cultures of various recipients in PBB supplemented with 0.1% maltose, were centrifuged and the cells were resuspended in 0.4 volume of 0.01 M $MgSO_4$ and aerated for 1 hr at 37°. The final transduction mixture in a volume of 0.2 ml had a phage multiplicity of less than 10^{-2} per cell. After incubation for 30 min at 30°, to allow adsorption of phage, the mixture was plated on minimal agar supplemented with 20 μg/ml of xanthine. Plates were examined after incubation for 2 days at 30° or at 37°.

ColE1 Immunity Test. Crude ColE1 protein was prepared from Kp482 as described in ref. 16. This preparation contained about 1600 colicin units (17) per ml. The presence of ColE1 immunity was determined by spotting full grown cultures onto plates of PBB agar overlayered with 0.2–0.5 ml of crude ColE1 preparation. Growth at this spot after 12 hr of incubation indicated that the cell was either immune or resistant to ColE1 protein. BF23 phage was used to distinguish ColE1 resistant cells from ColE1 immune cells. Cultures of Kp482 which contains ColE1 plasmid, and of KS1616 were always used as controls.

Detection of ColE1 Protein Production. Colicin production by single colonies was assayed by stabbing samples of each colony into a fresh plate and incubating the plate at 37° for 12 hr. The surface of this plate was then exposed to chloroform vapor for 10 min to kill the bacteria. The plate was then overlayered with 2.5 ml of soft agar containing 5×10^7 indicator bacteria, either HfrH or HfrH(λ$^+$), and incubated overnight at 37°. The position of colicinogenic colonies was seen as a clear zone in the turbid lawn of indicator bacteria.

Estimation of *Int* Function and Measurement of EDTA-Sensitivity of Phage λ. The methods used were as described in previous papers (18, 19).

Enzyme Assays. Crude enzyme extracts were prepared (20)

Abbreviations: ColE1, colicinogenic factor E1; PBB medium, polypeptone bonito extract broth.

Table 1. Bacterial strains

Strain	Relevant genotype*	Source or reference
C600	λ^s *supII*$^+$	R. Appleyard (see ref. 7, 8)
KL16-99	Hfr KL16 *rec A* str^s	B. Low (see ref. 7, 8)
Kp482	F$^+$, ColEl, str^s	T. Miki
HfrH	λ^s *sup*$^-$ str^s	W. Hayes (see ref. 7, 8)
KS302	HfrH (*gal-att*λ-*bio*)^{del}	K. Shimada *et al.* (9)
KS504	KS302 (λcI857 within *guaB*)	K. Shimada *et al.* (10)
KS1616	KS302 (*guaA-guaB*)^{del}	From KS504, K. Shimada *et al.* (10)
TM96	KS1616 (ColEl-*cos*λ-*guaA*$^+$)	T. Mukai *et al.* (10)
PL1072	W3110 *guaB*52 *guaA*$^+$	P. Lambden and W. Drabble (11)

* Genotypes include: *am* = sensitive to amber suppressors; *sup* = inability to support growth of λ*am* mutants; str^S, str^R = sensitivity or resistance to streptomycin; *del* = deletion; *att*λ = attachment site for λ; *cos*λ = cohesive ends of λ phage DNA; ColEl = colicinogenic factor El; PFU = plaque-forming unit; TDA = *guaA* transducing ability. The other genetic symbols are those used by Taylor and Trotter (12) for *E. coli* and by Szybalski and Herskowitz (13) for λ.

and the *guaA* (xanthosine 5′-monophosphate aminase) and *guaB* (inosine 5′-monophosphate dehydrogenase) enzymes were assayed as described elsewhere (11).

CsCl Density Gradient Centrifugation of Lysates. Volumes of 2.66 ml of phage lysates were mixed with 2.00 g of CsCl in a centrifuge tube. After adjusting the ρ of the mixture to 1.495 at 25°, the tube was filled up with paraffin. Centrifugation was performed in a Hitachi model no. 55P-2 centrifuge in a type no. 39 swinging bucket rotor at 23,000 rpm for 20 hr at 5°. After centrifugation, the bottom of the tubes was punctured and fractions of four drops were collected.

Centrifugation with Ethidium Bromide-Buoyant CsCl Density Gradients of Sarkosyl Lysates from Cells Treated with Chloramphenicol. Chloramphenicol was added at a final concentration of 30 μg/ml to exponentially growing bacteria in 5 ml of PBB containing 1% casamino acid at 37°, and incubation was continued for 150 min. Molecules of ColE1 DNA replicate extensively in the absence of net protein synthesis (21). Then, [^{3}H]thymidine, deoxyadenosine, and thymine were added at final concentrations of 7 μCi/ml, 200 μg/ml and 1 μg/ml, respectively. After 2 hr of incubation, the cells were collected by centrifugation, washed twice, and resuspended in 0.5 ml of 0.025 M EDTA–0.04 M Tris·HCl buffer at pH 8.5. Additional experimental procedures were performed as described in ref. 15. After centrifugation (37,000 rpm for 36 hr) in a Beckman model L centrifuge with a number 50 rotor, the bottom of the tubes was punctured and fractions of 15 drops were collected. Radioactivity was assayed in a liquid scintillation counter.

RESULTS

General properties of *E. coli* carrying *in vitro* ColE1-*guaA* recombinant molecules

Mukai *et al.* (6) reported construction of recombinant molecules by *in vitro* combination of ColE1 DNA and a gene fragment of *E. coli* for guanine synthesis derived from λp*guaBA*504 transducing phage (10). This hybrid molecule can replicate autonomously in *E. coli*, carries the fragments of both parental species, and expresses ColE1 immunity and *guaA* gene function (6).

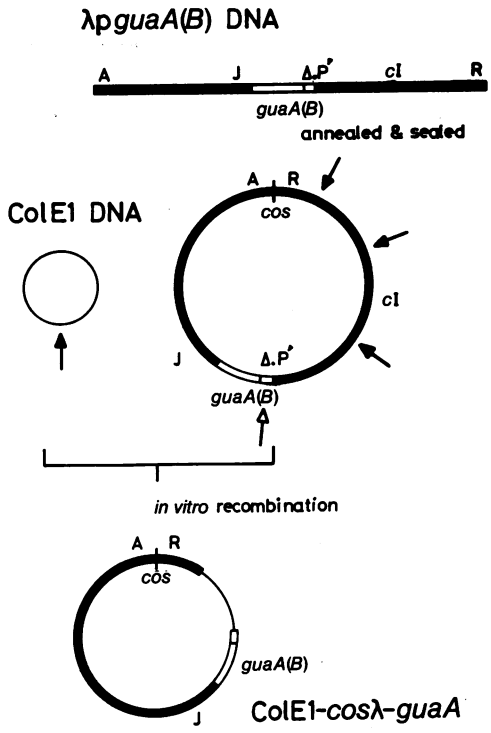

FIG. 1. Structure of the *in vitro* ecombinant ColE1-*cos*λ-*guaA* molecule. DNA extracted from λp*gua*BA504 transducing phage was fused by the cohesive ends, sealed by DNA ligase, and digested with *Eco*RI restriction enzyme. ColE1 DNA was also treated with *Eco*RI. A mixture of the fragments of phage DNA and ColE1 DNA, formed by treatment with *Eco*RI, was incubated with *E. coli* DNA ligase. Aliquots of the ligated mixtures were incubated with Ca^{++}-treated *E. coli* KS1616 cells and *guaA*$^+$ transformants were picked up. One of the transformants (TM96) was studied. Detailed information on construction is given in ref. 6. The approximate *Eco*RI cleavage sites are indicated by arrows (17). The open arrow indicates a putative *Eco*RI site within the *guaB* region. The light lines represent ColE1 DNA, the heavy lines λ genome, and the double lines a fragment of bacterial chromosome including *guaA* and a part of the *guaB* genes. A, R, J, cI, Δ.P′, and *cos* are phage markers and *guaA* and *guaB* are bacterial markers.

The properties of TM96 which carries the recombinant molecule were further characterized and are summarized as follows (see Table 2 and Fig. 1): (*a*) analysis of residual prophage markers showed the presence of prophage genes R-A-F-J and the absence of the N-cI-P-Q region. (*b*) No significant *int* expression was detected. *Int* activity was measured using λ*att*^{2} phage (18). After lytic infection of EDTA-sensitive λ*att*^{2}*int am*29 phage onto C600 (known *sup*$^+$), KS1616 (known *sup*$^-$), and TM96 (*sup*$^-$, ColE1-*guaA*$^+$) the ratios of EDTA-resistant phages were found to be 0.89, 0.031, and 0.029, respectively. These results indicate the absence of *int* protein within TM96. (*c*) The integration frequencies of λcI857 on TM96*recA* and on KS1616*recA* were measured and found to be similar. This result suggests the absence of a Δ.P′ structure on the recombinant molecule. (*d*) Under *gua* repression, the specific activity of the *guaA* enzyme was about 30-fold higher in this strain than in a *guaB*$^-$*guaA*$^+$ point mutant strain (PL1072).

The λp*gua*BA504 DNA molecule was degraded into four fragments by digestion with the restriction enzyme *Eco*RI (6). We judge from the report of Thomas and Davis (22) that λp*guaBA*504 DNA should have at least three restriction sites (see Fig. 1). The other two restriction sites of λDNA are sub-

Table 2. General properties of TM96 carrying *in vitro* ColEl-*cos*λ-*gua A* recombinant molecules

Condition*	Cell response KS1616	TM96
(a) Growth on minimal agar	—	—
+ xanthine	—	+
+ guanine	+	+
(b) Sensitivity to ColEl protein	Sensitive	Resistant
Sensitivity to BF23 phage	Sensitive	Sensitive
Production of ColEl	—	—
(c) λ phage gene: J	—	+
E	—	+
A	—	+
R	—	+
Q	—	—
P	—	—
cI	—	—
N	—	—
int	—	—
Δ.P′	—	—

* Growth on minimal agar was tested by streaking starved cultures of each strain on appropriate plates. Xanthine and guanine were each added at concentrations of 20 μg/ml. Sensitivity to ColEl protein and production of ColEl protein were tested as described in the *Materials and Methods*. The sensitivity of *E. coli* strains to BF23 phage was tested by cross-streaking overnight cultures on PBB agar against BF23 at a phage density of 10^9/ml. Complementation of λ*am*s was performed as described previously (7).

stituted by *gua* genes. Therefore, the formation of four fragments on *Eco*RI treatment suggests the presence of one new restriction site within the *guaB* region (indicated by a open arrow in Fig. 1). All these results enabled us to conclude that this hybrid molecule carries a part of the λ genome, R through J: (R-A-F-J)$^+$ and lacks the region, *att*P′ through Q: (*att*P′-*int*-N-*c*I-P-Q)^{del}. Henceforth, this plasmid molecule is named ColE1-*cos*λ-*guaA*.

Transduction of ColE1-*cos*λ-*guaA* plasmid DNA by phage λ

Lysates of TM96 obtained after lytic infection with λ*c*I857 were found to contain activity to transduce KS1616 to *guaA*$^+$ (Table 3). When λ lysogenic derivatives of TM96 were induced by a temperature shift or mitomycin-treatment, the lysates contained similar activity at higher level (Table 3). λp*guaBA*504 phage itself, cannot transduce KS1616 to *guaA*$^+$, because this strain is deleted of an *att*λ and *guaA-guaB* region (10). Thus, the *guaA*$^+$ transduction of KS1616 by phage lysates prepared on TM96 seems to be the result of plasmid formation by the function of ColE1 DNA which has been connected *in vitro* with the *guaA*$^+$ gene. The frequency of the *guaA* transduction was not affected by the presence of an *att*λ, prophage λ, *recA*, or ColE1 plasmids in *gua*-deleted recipient cells (Table 4). We could not detect any transductants when we used λ resistant *gua*-deleted strains as recipients (data not shown).

Table 4. Effect of recipient cells on *guaA* transduction

Recipient strain no.	Relevant properties *att*λ	λ*papa*	ColEl	*recA*	Relative efficiency of *guaA* transduction
KS1616	—	—	—	+	1.0
KS1963	+	—	—	+	0.57
KS1944	+	—	—	—	0.55
KS1966	—	—	+	+	0.94
KS1968	+	—	+	+	0.42
KS1970	+	+	—	+	0.58
KS1971	+	+	—	—	0.32

A mitomycin C-induced lysate of TM96 (λ*papa*) was used as the phage source. All the recipient cells are derivatives of KS1616 (see Table 1) and were constructed by mating KS1616 with HfrH, KL16-99, or Kp482 (see Table 1) as described in (7). The procedures used of transduction were described in the *Materials and Methods*. A relative frequency of 1.0 corresponds to 6.0×10^{-2} transductants per plaque-forming unit. There was no significant difference in the plating efficiencies of λ*vir* on these strains.

The presence of ColE1 plasmids in recipient cells did not affect the frequency of ColE1-*cos*λ-*guaA* transduction (Table 4, compare KS1966 and KS1968 with KS1616), suggesting that two kinds of ColE1 DNAs, i.e., ColE1 DNA and ColE1-*cos*λ-*guaA* DNA, may exist compatibly within a cell. To clarify this point, we tested the ability of *guaA*$^+$ transductants isolated from two different *E. coli* strains, which carry ColE1 plasmids, to produce ColE1 protein. Twelve independent *guaA*$^+$ transductants isolated from KS1968 were purified. Six of them retained the ability to produce ColE1 protein. Similarly, 12 *guaA*$^+$ transductants were picked up from KS1966, and 10 of these were found to be able to produce ColE1 protein. These

Table 3. *guaA*-transducing ability found in the phage lysates prepared on TM96

Source of phage lysate	Plaque-forming ability/ml	*guaA*-transducing ability/ml	Transducing ability/ plaque-forming ability
Chloroform treated TM96*	$<10^0$	$<10^0$	—
Lytic infection of TM96 with λ*c*I857†	3.1×10^8	1.7×10^6	5.5×10^{-3}
Heat-induction of TM96 (λ*c*I875)‡	1.9×10^9	2.7×10^7	1.4×10^{-2}
Heat-induction of TM96 (λ*c*I857p*gal*8)‡	1.3×10^8	4.2×10^7	3.3×10^{-1}
Mitomycin C-induction of TM96 (λ*papa*)§	1.2×10^9	7.2×10^7	6.0×10^{-2}

Phage lysates were prepared as follows:

* Five milliliters of overnight culture of TM96 in PBB were treated with 0.1 ml of chloroform.

† Starved TM96 culture (0.2 ml) was infected with 0.1 ml of λ*c*I857 (10^7/ml). After adsorption at 30° for 20 min, it was plated on PBB in soft agar and incubated at 40°. After incubation for 5 hr, 5 ml of 0.01 M $MgSO_4$ and 0.5 ml of chloroform were added and the plates stood for 10 min. The lysates were collected with soft agar.

‡ TM96 was lysogenized with λ*c*I857 or λ*c*I857p*gal*8 (23). These lysogens were purified and grown in 5 ml of PBB at 30°. Cultures in the exponential phase of growth were supplemented with $MgSO_4$ (final concentration = 0.01 M), shifted to 40° for 20 min, incubated at 37° for 2 hr with shaking, and then treated with chloroform.

§ TM96 was lysogenized with λ*papa*. One of the lysogens was purified and inoculated into 5 ml of PBB at 37°. Cultures in the exponential phase were mixed with mitomycin C (final concentration = 2 μg/ml) and incubated further for 3 hr. Then 0.1 ml of chloroform was added. All these lysates were centrifuged to remove bacteria, agar, and other debris. Plaque-forming ability was measured on C600. The transduction procedures were as described in the *Materials and Methods*. Starved cultures of KS1616 were used as recipient cells.

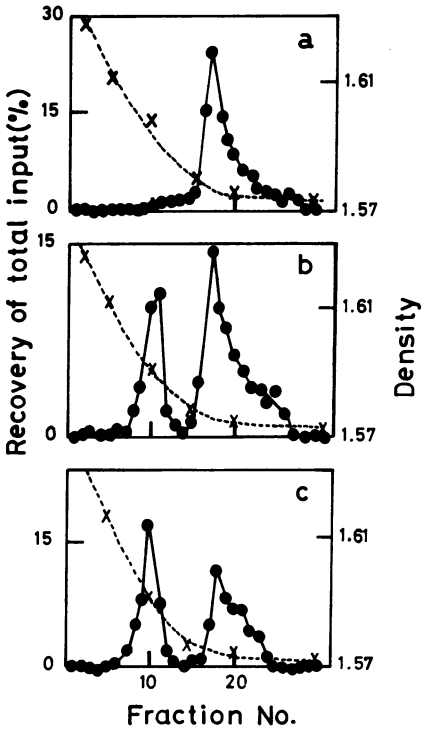

FIG. 2. Centrifugation profiles with ethidium bromide-CsCl density gradients of [^{3}H]thymidine labeled DNAs extracted from (a) KS1616, (b) TM96, and (c) one of the ColE1-*cos*λ-*guaA*$^+$ transductants. For experimental procedures, see *Materials and Methods*. One hundred percent corresponds to (a) 770 cpm, (b) 1800 cpm, and (c) 4200 cpm, respectively. —●—, recovery of total input (%); --×--, gradient density at 25°.

results suggest that there is weak incompatibility between ColE1 and ColE1-*cos*λ-*guaA* plasmid DNAs.

Properties of the *guaA*$^+$ transductants of KS1616

Twelve independent *guaA*$^+$ transductants of KS1616 were picked up, purified, and inoculated into PBB. These purified transductants were used for further analysis, and their properties were studied and can be summarized as follows: (*a*) all these transductants were sensitive to λ phage. (*b*) They could complement λ*am* A, F, J, and R. However, they could not complement λ*am* N, P, or Q. (*c*) They were resistant to ColE1 protein and were sensitive to BF23 phage. Accordingly, all these transductants have ColE1 immunity. (*d*) None of them could produce ColE1 protein. (*e*) Four of the transductants together with the parent strains were labeled with [^{3}H]thymidine in the presence of deoxyadenosine and 30 μg/ml of chloramphenicol. After 3 hr of incubation, their DNAs were extracted and analyzed by centrifugation with ethidium bromide-buoyant CsCl gradients (see *Materials and Methods*). The results, shown in Fig. 2, indicate the presence of two peaks of radioactivity in the extract of one of the transductants (Fig. 2c). The peak of denser material represents covalently closed circular DNA and the peak of lighter material represents chromosomal DNA. Two similar peaks are observed in an extract of the original TM96 (Fig. 2b), whereas an extract of KS1616 showed one peak corresponding in position to chromosomal DNA (Fig. 2a). The extract of the other three transductants also gave two bands of DNA on centrifugation: one at the position of closed circular DNA and the other at that of chromosomal DNA (data not shown). These results demonstrate the presence of plasmid DNAs in these four independently isolated *guaA*$^+$ transductants.

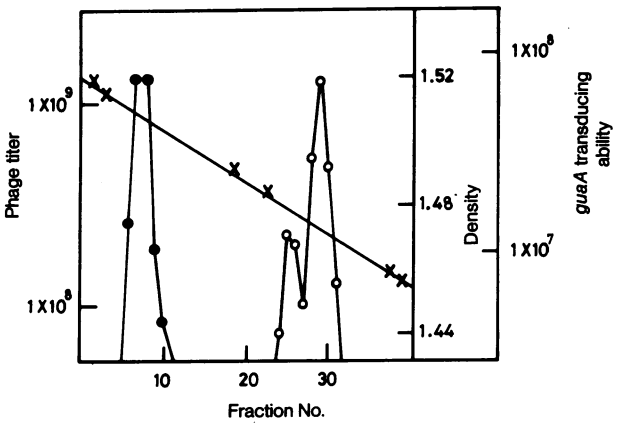

FIG. 3. Profile of a lysate obtained after mitomycin C-induction of TM96 (λ*papa*) on centrifugation with CsCl density gradients. The lysate contained 1.2 × 10^9/ml of plaque-forming units and 7.2 × 10^7/ml of transducing ability. For experimental procedures, see *Materials and Methods*. —●—, plaque-forming ability per ml; —○—, transducing ability per ml; —×—, gradient density at 25°.

Properties of the transducing ability

The molecular weight of ColE1-*cos*λ-*guaA* DNA was estimated to be about 2.1 × 10^7 by agarose gel electrophoresis (6). This value indicates that ColE1-*cos*λ-*guaA* plasmid DNAs exist as monomers. If this plasmid DNA is actually transduced by λ phage particles, then the *guaA* transducing ability can be expected to be EDTA-resistant. Inactivation of phage λ in the presence of 0.01 M EDTA is largely dependent on the DNA content of the λ head (24). Accordingly, we tested the EDTA sensitivity of the transducing particles found in mitomycin C-induced lysates of TM96 (λ*papa*). After treatment with EDTA for 30 min at 37°, 6.1 × 10^{-1} of transducing ability was recovered, whereas only 1.3 × 10^{-3} of plaque-forming unit remained. This indicates that the DNA in transducing particles is much smaller than that in plaque formers. Analysis of the transducing ability after CsCl density gradient centrifugation of the above lysates confirmed this point (see *Materials and Methods*). As shown in Fig. 3, plaque forming ability was distinctly separated from transducing ability. Material with plaque-forming ability exhibited a density of about 1.508, which corresponds to that of the wild type, whereas the peak of material with transducing ability was at a density of about 1.472. This material represents λ phage particles which contain approximately 72% of the length of λ DNA.

DISCUSSION

Lysates of λ phage made on TM96, which carries ColE1-*cos*λ-*guaA* plasmids, were found to contain ColE1-*cos*λ-*guaA* transducing phage particles. *GuaA*$^+$ transductants of KS1616, isolated after infecting with these particles, showed similar genetic properties as those of TM96, so far as examined.

The mechanism of formation of these transducing particles is an interesting problem. Our present working model is shown schematically in Fig. 4. After lytic infection of λ phage onto TM96, λDNA recombines with the ColE1-*cos*λ-*guaA* plasmid. The recombinant molecule carries two λ*cos*es, as shown in Fig. 4 (25, 26, 28) and is expected to be a good substrate for terminase-promoted packaging (27). This scheme explains the relatively high level of transducing ability of lytic lysates. However, the lysates prepared by lytic infection of λ*c*I857 *int*$^-$*red*$^-$ onto TM96 *recA* contained similar transducing ability (data not

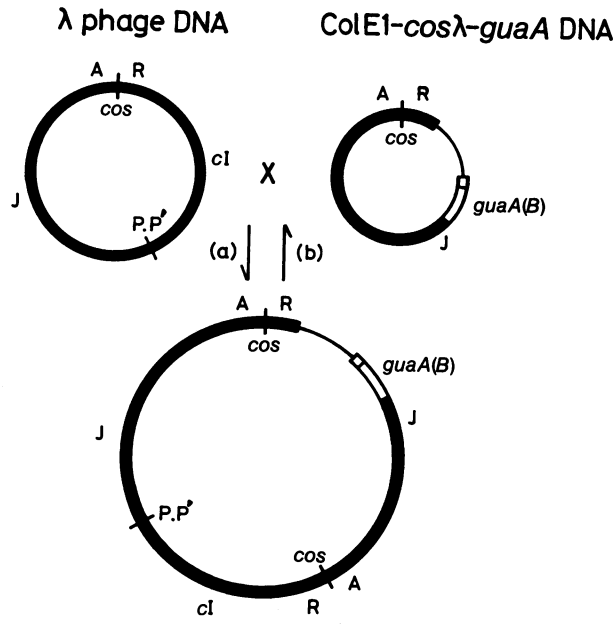

FIG. 4. Ter dependent formation of ColE1-*cos*λ-*guaA* transducing particles. Reaction (a) is a general recombination between two homologous DNAs and is promoted by host Rec function (possibly Red or Ter of phage λ). The recombinant molecule carries two λ*cos*es. Reaction (b) is promoted by Ter and is coupled with packaging (27). The heavy lines represent λ genome, the light lines ColE1 DNA and the double lines a fragment of bacterial chromosome including the *guaA* and a part of the *guaB* gene. A, R, J, cI, P.P', and *cos* are phage markers and *gua* is a bacterial marker.

shown) suggesting that this recombination is not dependent upon the host *rec*$^+$ function, phage *red*, or *int* functions.

When λ phage is lysogenized onto TM96, the lysogens most probably carry λDNA within ColE1-*cos*λ-*guaA* plasmids, because this strain is deleted of the normal λ attachment site (7), and because there is much homology between λDNA and ColE1-*cos*λ-*guaA* plasmid DNA. After induction of TM96(λ) lysogens, efficient Ter cutting is expected (25, 26, 28). As a result of Ter cutting, one complete λ genome and one ColE1-*cos*λ-*guaA* plasmid DNA are packaged into separate phage heads. The former is an EDTA-sensitive plaque-former and the latter is an EDTA-resistant *guaA* transducing particle.

The molecular weight of ColE1-*cos*λ-*guaA* plasmid DNA was estimated by agarose gel electrophoresis to be about 2.1×10^7 (6), corresponding to 68% of that of normal phage DNA. The density of transducing particles (Fig. 3) suggests that these particles contain about 72% of the length of λDNA. The λ phage head can package 109% of the λDNA length into its head without losing any plaque-forming ability (29). This means that the transducing particles studied in this work have enough room to pack about 40% more λDNA (i.e., approximately 1.3×10^7 daltons). Accordingly, the ColE1-*cos*λ-*guaA* recombinant molecule seems to be a good vehicle for gene engineering. When a genetically uncharacterized DNA fragment is connected with ColE1-*cos*λ-*guaA* DNA, it can exist as a part of stable plasmid DNA and can be packaged within a λ phage particle, if it is less than 1.3×10^7 in size.

On the other hand, the *cos*λ-*guaA* fragment of the recombinant molecule seems to be a convenient tool for genetic studies on various replicons, such as the F factor and R factor. When an essential part of these replicons is joined to *cos*λ-*guaA* DNA, the recombinant replicon can be studied by the methods of phage genetics.

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