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Formation of Oligomeric Structures from Plasmid DNA Carrying *cos*λ That Is Packaged into Bacteriophage Lambda Heads

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Plasmids that carry *cos*λ, the region necessary for λ phage packaging and that are as small as four kilobases in size can be packaged into λ phage heads in head-to-tail tandem oligomeric structures. Multimeric oligomers as large as undecamers have been detected. Oligomer formation depends upon the products of *red* and *gam* of λ, and the general recombination occurs between different plasmids that share homologous DNA regions. The packaging efficiency of plasmids depends on its copy number in cells and its genome size. Upon injection into a cell, the DNA establishes itself as a plasmid in a tandem structure. When such a plasmid in a high oligomeric structure is used as the source of packaging DNA, the packaging efficiency of the plasmids is elevated. The oligomers are stable in *recA* cells, whereas they drift toward lower oligomers in *recA*$^+$ cells.

Plasmids having a λ cohesive end site (*cos*λ) can be packaged into λ phage heads. Upon injection and penetration, the DNA becomes a plasmid. Such plasmids are called "cosmids" (4), "phasmids" (12), and "packageable plasmids" (10).

We have determined the minimal DNA region around *cos*λ necessary for packaging, and we observed that an 85-base-pair (bp) DNA region containing *cos*λ is the structure indispensable for λ packaging. An additional 75-bp region located next to it stimulates the packaging efficiency (12a).

In addition to the requirement for *cos*λ, DNA to be packaged in λ phage heads should have a size in the range of 78% (38 kilobases [kb]) to 105% (51 kb) of wild λ DNA (49 kb) (7, 8). Thus, Umene et al. (18) studied plasmids whose sizes were 40 to 47% of the length of λ DNA and have shown that they are packaged as dimers; a plasmid 28% of the length of λ DNA was packaged as a trimer. Some of the packageable plasmids that we used for determination of the active *cos*λ region were much smaller than these. For instance, pCOS-9 (12a) was only 4.0 kb, or 8.2% of the size of λ DNA. Packaging of these small plasmids is most likely to be associated with high oligomer formation. These considerations prompted us to study the mode of oligomer formation.

In this paper, we report that formation of such high oligomers occurs at high efficiency, dependent upon the λ-promoted recombination system and rolling circle replication, and that the packaged DNA is in a head-to-tail oligomeric structure.

MATERIALS AND METHODS

Bacterial and bacteriophage strains. All of the bacterial strains used in this paper are derivatives of *Escherichia coli* K-12, C600 *recA41* (*su2*$^+$ *recA41 thr leu thi*) and LE392 (*sul*$^+$ *su2*$^+$ *metB gal hsdR*) were obtained from H. Ogawa and A. Shimizu, respectively. Of the bacteriophage strains, λ *c*I857 β113 γam210 *S*am7 was a gift from H. Ogawa; λ γam210 and λ α314 were gifts from K. Shimada (18). Other amber mutant phage derivatives were gifts from H. Yamagishi (14). All other strains have been described elsewhere (12a).

Plasmids. The packageable plasmids used in this paper are listed in Table 1.

Electron microscopy. DNA samples were prepared for electron microscopy as described by Yamagishi et al. (20). The mean contour lengths of DNA in this paper were obtained by measuring more than 50 molecules.

CsCl density gradient centrifugation assay of λ phage lysates. Lysates (10 ml) containing Ap^r transducing particles were made by thermoinduction of λ *c*I875 lysogen (12a) and treated with DNase and chloroform. After the particles were sedimented by centrifugation with a Beckman no. 30 rotor for 90 min at 27 krpm, the pellets were suspended in 1.4 ml of PB medium (12a), and 1.65 ml of CsCl solution saturated at 4°C was added. The samples were centrifuged in a Hitachi RPS40-T-2 swing rotor at 23,000 rpm for 15 h at 4°C. After the run, 25 fractions were collected from the bottom of the tubes, and plaque-forming particles and Ap^r transducing particles in each fraction were assayed.

Extraction of bulk cellular DNA. Km17 cells carrying plasmid pCOS-1 were lysogenized with λ *c*I857*S*am7,

TABLE 1. Packageable plasmids

Plasmid	Properties and references	Molecular size (kb)
pCOS-1	pBR322(11)-based recombinant plasmid (12a); the *Eco*RI-*Bam*HI region of pBR322 is replaced by a 9.0-kb fragment (93.1 to 11.4% of λ) of λ phage DNA (16); Ap^r	12.8
pCOS-19	pBR322-based recombinant plasmid (12a); has a 223-bp *Hae*III fragment containing *cos*λ inserted in the *Bam*HI site; Ap^r	4.6
ppBest322	pBR322-based recombinant plasmid (12a); has the same fragment as pCOS-19 inserted in the *Bal*I site; Ap^r and Tet^r	4.6
ppACYC184 (*cos*)	pACYC184(3)-based recombiant plasmid (12a); has the same fragment as pCOS-19 inserted in the *Bam*HI site; Cm^r	4.2
pTM1	ppBest322-based recombinant plasmid; has a 3.2-kb *Sau*3A fragment from pSC138 (17) inserted in the *Bam*HI site; Ap^r	7.8
pTM2	Similar to pTM1, except that it has a 10-kb fragment from pSC138; Ap^r	14
pTM3	Similar to pTM1, except that it has a 0.3-kb fragment from pSC138; Ap^r	4.9
ppλdv	λdv1-based recombiant plasmid carrying *cos*λ and λ arms; packaged as a monomer (1, 13); Gua^+	38.5
ppF	pSC138 (miniF)-based recombinant plasmid carrying *cos*λ and λ arms; packaged as a monomer (10); Ap^r	40.3
pp1	λdvHBAp-based recombinant plasmid (Miwa, in preparation); packaged as a monomer; Ap^r and Km^r	39.2
pp1*Pam80*	Similar to pp1, except that it carries a *P* amber80 mutation in λ phage region (Miwa, in preparation); Ap^r and Km^r	39.2

which carries an additional mutation either in one of the late genes or in the general recombination genes. The cells were grown to 2×10^8 cells/ml at 30°C, heated at 43°C for 10 min, and then incubated at 37°C. At various times, samples (2 ml) of the culture were withdrawn. To each was added 0.1 ml of 1 M Tris-hydrochloride (pH 8.0), 0.2 ml of 0.2 M EDTA (pH 8.0), and 0.1 ml of 1% lysozyme, and then they were cooled on ice for 20 min. Next, 2 ml of 0.75% sodium Sarkosynate in 40 mM Tris-hydrochloride (pH 8.0) and 25 mM EDTA (pH 8.0) (TE buffer) was added, and the samples were held on ice for 10 min, followed by incubation at 65°C for 8 min. Then they were incubated with 0.4 g of predigested pronase E at 37°C for 2 h. The lysates were mixed twice with phenol (2 ml) saturated with TE buffer. The aqueous solutions were precipitated twice by adding 2 volumes of ethanol. The DNAs were suspended in 20 μl of 10 mM Tris-hydrochloride (pH 8.0) and 1 mM EDTA (pH 8.0); 5 μl of each DNA sample was subjected to in vitro λ packaging experiments (2).

RESULTS

Plasmid DNA in oligomeric form is packaged in λ particles. Two plasmids, pCOS-1 and pCOS-19, were used for packaging studies as representatives of large and small genomes, respectively.

Cells carrying the plasmids were lysed by inducing prophage, and the lysates were subjected to a CsCl density gradient centrifugation. As these plasmids carry an *amp* (ampicillin resistance) gene, Ap^r transducing particles in the lysates were taken as representing packaged plasmids.

A lysate of cells carrying pCOS-1, whose size is 12.8 kb, yielded two bands of Ap^r transducing particles along with an infectious λ phage band (Fig. 1a). The size of DNA was deduced from the density of the particles (6) as well as by examination of the length of phenol-extracted DNA by electron microscopy. In the heavier peak (fractions 5 to 7) the size was 50.0 kb, which corresponds to 102% of the length of wild λ phage DNA. In the lighter peak (fractions 15 and 16), it was 39.2 kb, or 80% of λ DNA. These figures correspond to four and three times the length of pCOS-1 DNA. Circular DNA molecules were not detected in the DNA extracted from particles. They must be covalently linked linear duplexes having no structure depending on hydrogen bonding for coherence, as heating at 65°C for 10 min did not change the DNA size. The DNA ends of the transducing particles in fractions 15 and 16 could be annealed by keeping overnight at 45°C. λ phage DNA has three *Sma*I cutting sites (16), whereas pCOS-1 plasmid DNA has none (12a). DNA obtained from the transducing particles was treated with *Sma*I, but there were no cleavage products, as examined under an electron microscope. Thus, Ap^r transducing particles carry only the plasmid DNA.

A lysate of cells carrying pCOS-19, whose size is 4.6 kb, yielded three broad peaks of Ap^r transducing particles (Fig. 1b). Their DNA lengths as judged from buoyant density were 50, 46, and 40 kb, respectively, which correspond to 11, 10, and 9 times the length of pCOS-19 DNA. Unfortunately, the number of Ap^r transducing

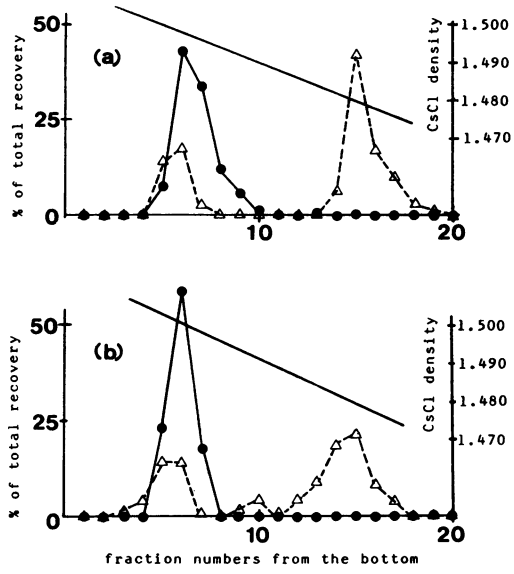

FIG. 1. CsCl density gradient profiles of lysates obtained by thermoinduction of Km17 (λ *c*I857) cells carrying (a) pCOS-1 and (b) pCOS-19 plasmids. Symbols: △, percentage of total Ap^r transducing particles, 100% corresponds to (a) 2.3×10^{10} and (b) 1.5×10^8; ●, percentage of total plaque-forming particles, 100% corresponds to (a) 1.5×10^{10} and (b) 1.3×10^{10}. Lines represent CsCl density. From buoyant density of the particles, the size of the DNA relative to that of wild-type λ phage DNA in fractions 5 and 15 were 105% and 80%, respectively.

particles was so small that DNA could not be obtained for direct measurement by electron microscopy. However, they must be oligomeric DNA as shown by the following experiments.

Tandem plasmid oligomers detected in *recA* transductants. From studies with buoyant densities, the transducing particles in the pCOS-1 or pCOS-19 lysate were expected to contain tetrameric or undecameric plasmids, respectively. We made transductants using these plasmids, and prepared plasmid DNA from transductants for further studies.

recA cells, in which oligomeric plasmids were stable (11), were infected with packaged pCOS-1 (fraction 6 in Fig. 1a) or pCOS-19 (fraction 5 in Fig. 1b). Both of the resultant Ap^r colonies were found to carry a band of large plasmid DNA over 40 kb in size as measured by electrophoresis (Fig. 2a). We named them pCOS-2 and pCOS-20, respectively. Upon *Eco*RI cleavage of these DNAs, each plasmid produced only one DNA fragment, indistinguishable from that derived from the respective parental plasmids (Fig. 2b). Digestion with *Hpa*II, which cleaves these plasmid DNAs at several sites, produced the same fragment pattern as that from the parental plasmids (data not shown). These results indicate that the DNA molecules in packaged particles are in a covalently bound, head-to-tail oligomeric structure and suggest that the size of DNA is determined by packaging capacity. Both ends of this DNA molecule may be in λ cohesive termini (10, 19).

Genes needed for formation of packageable oligomeric plasmid DNA. Small plasmids must oligomerize before or during the process of λ packaging. We examined the genes needed for oligomer formation as well as packaging. We thought that the oligomers would be formed, at least in part, by a recombination process, because two different plasmids sharing homologous regions recombine to form an oligomeric molecule (see below).

We first tested the *recA* gene, which is the major recombination gene of the host cell. In *recA*$^+$ cells, up to 20% of our plasmid DNAs were in dimeric form, whereas in *recA* cells, all of the plasmids were in monomeric form (data not shown). On the other hand, the packaging efficiencies of pCOS-1 and pCOS-19 were unchanged in both *recA* and *recA*$^+$ cells. B12 and Km17 cells were used as *recA*$^+$ and *recA* cells, respectively. Cells lysogenic for λ *c*I857, each carrying one of the two plasmids, were thermoinduced and Ap^r transducers and plaque formers in the lysates were measured. Packaging efficiencies, obtained by dividing the number of Ap^r transducers by the number of plaque formers, were as follows: for pCOS-1, 5×10^{-1} and 9×10^{-2} for *recA*$^+$ and *recA* cells, respectively; for pCOS-19, 2×10^{-3} and 3×10^{-3} for

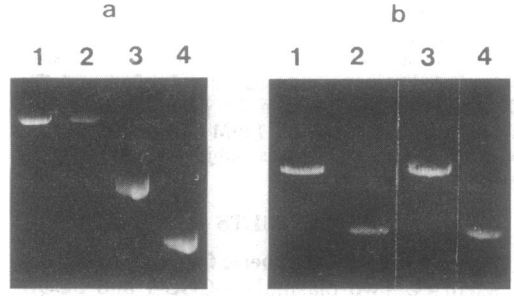

FIG. 2. Agarose gel electrophoresis of (a) closed circular DNA and (b) DNA fragments cleaved by *Eco*RI. Lanes: 1, pCOS-2; 2, pCOS-20; 3, pCOS-1; and 4, pCOS-19. Without cleavage by *Eco*RI, pCOS-2, prepared from the *recA* cells by infection with pCOS-1 (fraction 6 of Fig. 1a), is larger than the parental plasmid. Similarly, pCOS-20, prepared from pCOS-19 (fraction 5 of Fig. 1b), is larger than pCOS-19. Panel b shows that restriction fragment patterns are the same before and after the transduction, independent of plasmid size.

recA$^+$ and *recA* cells, respectively. This result confirms that of Umene et al. (18), who showed that formation of transducing particles that carry oligomeric DNA is not related to the *recA* gene function.

To see whether λ phage gene function is needed to form oligomers, we extracted DNA from λ-infected cells carrying pCOS-1 and subjected it to in vitro packaging. Figure 3 shows that the time course of formation of Ap^r transducing particles parallels that of plaque-forming particles. The latter process is also known to depend on concatemer (oligomer) λ DNA formation (5). It may be that phage DNA and plasmid DNA are oligomerized by the same mechanism. DNA extracted from noninfected cells or from cells at the time of λ infection did not give rise to packageable plasmids according to in vitro assays.

Results with λ phages having amber mutations are shown in Table 2. Phage late genes (16), such as *A* (a gene responsible for formation of the λ cohesive end), *B* (a gene for head-tail connector), *D* (a gene for major decorated head protein), and *E* (a gene for major head capsid protein subunit) did not affect formation of packageable DNA molecules. On the other hand, mutations in *redα*, *redβ*, or γ reduced formation of packageable plasmid DNA molecules. A double mutant in *redβ* and γ could not form any measureable transductants. The *redα* and *redβ* gene products stimulate general recombination of λ phage (16). The γ gene product inhibits *recBC* nuclease of host cells and promotes the rolling circle mode of λ DNA replication in the late stage (16). Some of these genes might also be involved in other functions as well, although we know very little about them. Recently, Feiss et al. (9) showed that oligomer formation depends on the γ gene function, but not on the *redβ* gene function. The difference between their and our observations is discussed below.

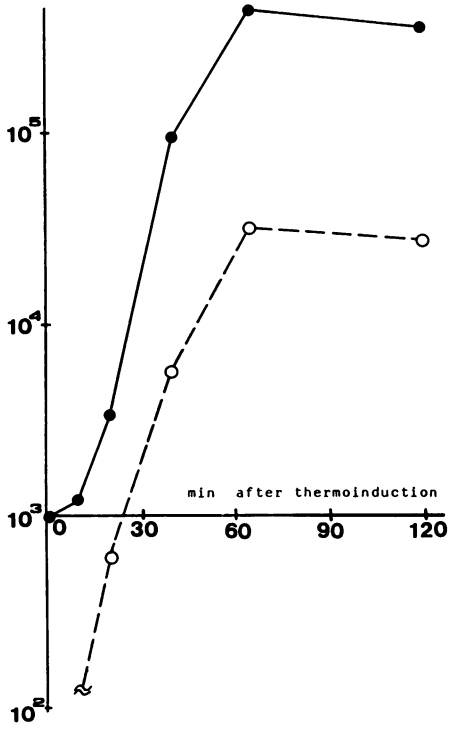

FIG. 3. Formation of Ap^r transducers as a function of time. Symbols: ○ and ●, Ap^r transducers and plaque formers, respectively. Km17 (λ *c*I857) cells carrying pCOS-1 were withdrawn at the indicated times after thermoinduction. Bulk cellular DNA was extracted and subjected to in vitro λ phage packaging. Ap^r transducers and plaque formers were assayed by using B12 (λ) and B12, respectively, as indicators. Ordinate: The number of packaged particles originating from DNA in 1 ml of culture.

TABLE 2. Phage genes needed for converting pCOS-1 into packageable form[a]

Amber mutation	Packaged particles[b]		Packaging efficiency[c]
	Ap^r transducers	Plaque formers	
Aam32	1.7×10^3	1.2×10^5	1.4×10^{-2}
Bam10	1.2×10^3	7.6×10^4	1.6×10^{-2}
Dam15	4.8×10^3	2.4×10^5	2.0×10^{-2}
Eam4	2.4×10^3	1.6×10^5	1.5×10^{-2}
β113γam210	<10	6.5×10^4	$<1.5 \times 10^{-4}$
α314	1×10^2	2.3×10^5	4.3×10^{-4}
β113	30	1.9×10^5	1.6×10^{-4}
γam210	70	2.0×10^5	3.5×10^{-4}
Wild	3.4×10^3	2.6×10^5	1.3×10^{-2}

[a] Km17 cells carrying pCOS-1 and lysogenic for λ *c*I857 with an indicated amber mutation were thermoinduced. After incubation for 2 h, bulk cellular DNA was extracted and used in in vitro packaging assays.

[b] The number of packaged particles originating from DNA in one ml of culture. The number of Ap^r transducers and plaque formers were assayed by using B12(λ) and LE392 as indicators, respectively.

[c] Number of Ap^r transducers divided by the number of plaque formers.

Sites of recombination. If the general recombination system of λ indeed takes part in plasmid oligomer formation, it should occur within homologous DNA regions. To test this, we analyzed transductants consisting of two different plasmids, packaged in a single phage particle.

ppBest322 has *amp* and *tet* (tetracycline resistance) genes, and ppACYC184(*cos*) has a *cam* (chloramphenicol resistance) gene. They carry *cos*λ and are compatible in host cells. ppBest322 and ppACYC184(*cos*) share three homologous regions: a cloned 223-bp λ fragment containing *cos*λ, a left portion of the *tet* gene

(the R region) (3, 15; 12a). The latter two portions are in an integral form in ppBest322, but are separated in ppACYC184(*cos*) by the insertion of the 223-bp λ fragment (Fig. 4a). It is expected that crossover events within the three homologous regions give rise to six DNA products as represented in Fig. 4b. The products resulting from a cross in the L region or the R region are the recombinants of interest. However, they are not easily discriminated from each other. The resultants of a cross in the *cos*λ region include both recombinants and products of cohesion by some mechanism using λ cohesive ends. Therefore, this group of "recombinants" does not necessarily reflect the real recombination event. Altogether, these "recombinants" can be detected by analyzing restriction fragments, as they exclusively yield characteristic 1.4 or 3.1-kb fragments upon digestion with *Eco*RI and *Bam*HI. Recombinants crossed in the L region or the R region do not give rise to these fragments (Fig. 4b).

Km17(λ *c*I857) cells carrying ppBest322 and ppACYC184(*cos*) plasmids were thermoinduced and lysed, and drug-resistant transducers made in vivo were measured by using B12(λ) as an indicator. The following numbers of transducing particles (per milliliter of culture) were obtained: Ap^r transducers, 3.0×10^6; Cm^r transducers, 6.9×10^6; Ap^r Cm^r transducers, 1.3×10^6; Plaque formers, 7.0×10^9. As the multiplicity of infection with transducers was too low to obtain double-infected cells, Ap^r Cm^r transductants are the results of one particle. Patterns of cleavage by *Eco*RI and *Bam*HI of plasmid DNAs in the resulting Ap^r Cm^r transductants are shown in

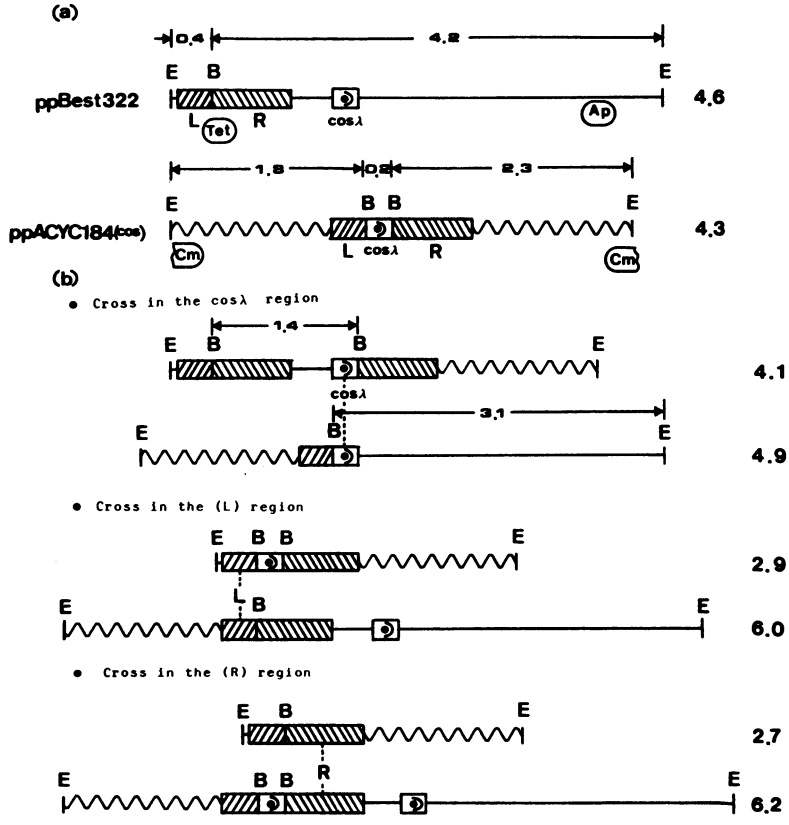

FIG. 4. Crossing sites between ppBest322 and ppACYC184(*cos*). (a) Three homologous regions between the two plasmids, along with restriction sites of *Eco*RI (E) and *Bam*HI (B) are shown. One homologous region is the cloned *cos*λ fragment. Another homologous region is the *tet* gene (hatched areas, R and L). In ppACYC184(*cos*) this region is separated into two by insertion of the *cos*λ fragment. pBR322 DNA region in ppBest322 and pACYC184 DNA region in ppACYC184(*cos*) are represented by thin and wavy lines, respectively. The sizes of DNA fragments are shown in kilobases. Tet, Ap, and Cm are shown as locations of genes which determine resistance to these drugs. (b) Schematic representation of *Eco*RI-digesting DNA fragments of crossing sites in the *cos*λ region and the R and L regions of the *tet* gene. The sizes of these fragments are shown at the right portion. Only the recombination at the *cos*λ fragment can create different-sized fragments (1.4 and 3.1 kb) upon digestion with *Eco*RI and *Bam*HI.

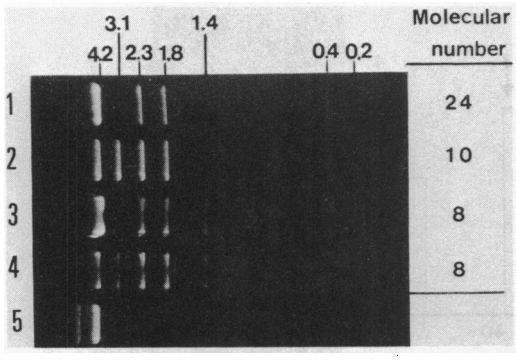

FIG. 5. Restriction fragment patterns of Ap^r Cm^r plasmids obtained by recombination of ppBest322 and ppACYC184(*cos*). The plasmid DNAs in transductants were digested with *Eco*RI and *Bam*HI (lanes 1 through 4) or with *Eco*RI (lane 5). The sizes of DNA fragments are shown above in kb; 50 such plasmids were examined, and 4 representative types are demonstrated (lane 1 to 4). The number of recombinant plasmids classified in each type is shown in the right portion of the figure. The same plasmid DNA as used in lane 1 was digested with *Eco*RI (lane 5). A 0.2-kb band was visible in samples 1 through 4 in gels, but was not reproduced in photocopies.

Fig. 5. All of them have the 0.2-, 0.4-, 1.8-, 2.3-, and 4.2-kb fragments expected to arise from ppBest322 and ppACYC184(*cos*). The cleavage patterns are classified into four types: lane 1 has no fragments other than those mentioned above, lane 2 has an additional 3.1-kb fragment, lane 3 has an additional 1.4-kb fragment, and lane 4 has both 3.1- and 1.4-kb fragments (Fig. 5). The type in lane 1 represents a recombinant DNA resulting from recombination in either the L region or the R region. This is clearly demonstrated by the *Eco*RI cleavage pattern (Fig. 5, lane 5), because it has about 4.5-, 6-, and 2.7-kb fragments, the latter two of which are expected from the crossover sites in the L region or the R region. The types in lane 2 and 3 each have at least one crossover site in the *cos*λ region. Because recombinant plasmids in the recipient cell are in circular forms, at least two crossover sites between the two parental plasmids are expected in the molecule. The cleavage patterns demonstrate that each of those types have another crossover site that can not be detected in the present assay. It is likely, however, that the second crossover site is located within the R or L region. The type in lane 4 results from reciprocal crossing over in the *cos*λ region.

A total of 50 plasmids were examined, and 24 of them were of the type in lane 1. In other words, at least half of the crossover events between ppBest322 and ppACYC184(*cos*) occurred within the R and L homologous regions, not in the *cos*λ region. There were 10 and 8 recombinants of the types in lane 2 and 3, respectively, and there were 8 that had undergone reciprocal crossing over in the *cos*λ region. Therefore, we conclude that recombination events occur in all homologous DNA regions of the plasmids and play important roles in oligomer formation in cells infected with λ phage. This recombination must be catalyzed by the general recombination of λ.

Oligomeric states change from high to low oligomers in *recA*$^+$ cells. To examine the stability of the oligomeric plasmids in *recA*$^+$ cells, the same experiments to obtain pCOS-2 and pCOS-20 plasmids in *recA* cells were repeated with *recA*$^+$ cells as the recipient. The plasmids in the resulting transductants were named pCOS-2-A and pCOS-20-A, respectively. In contrast to the results obtained with *recA* transductants, these DNAs were heterogeneous in size as measured by electrophoresis (data not shown). Length measurements by electron microscopy revealed that pCOS-2-A and pCOS-20-A DNA preparations consisted of heterogeneously sized plasmid molecules ranging in size from 1 to 4 units of pCOS-1 and 1 to 8 units of pCOS-19, respectively (Fig. 6). Restriction fragment patterns of these plasmid DNAs were indistinguishable from those of the respective parental plasmids, pCOS-1 and pCOS-19 (data not shown). Thus, in *recA*$^+$ cells the plasmids are also in head-to-tail oligomeric form, though the extent of oligomerization drifts toward lower oligomeric states. Because this drift occurs only in *recA*$^+$ cells, it seems to be catalyzed by the cellular *recA* recombination system (11).

Change in packaging efficiency of once-packaged plasmids. We measured the in vivo packaging efficiency of pCOS-20 plasmids which had been once packaged, injected into *recA* cells, and kept as oligomerized plasmid. Table 3 shows

TABLE 3. In vivo packaging efficiencies of plasmids in primary and secondary transductants[a]

Plasmid[b]	Ap^r transducers	Plaque formers	Packaging efficiency
pCOS-19	2.2×10^7	6.7×10^9	3.7×10^{-3}
pCOS-20	4.2×10^8	5.0×10^9	8.4×10^{-2}
pCOS-20-A	2.1×10^8	3.0×10^9	7.0×10^{-2}
pCOS-20-20	3.9×10^8	8.0×10^9	4.8×10^{-2}

[a] Phage lysates were prepared from Km17 or B12 cells carrying the indicated plasmids and lysogenic for λ *c*I857. The numbers (per milliliter of lysate) of Ap^r transducers and plaque formers were assayed by using B12(λ) and B12, respectively, as indicators.

[b] pCOS-19 is the parental plasmid. pCOS-20 was obtained from an Ap^r transductant of Km17 (*recA*) cells after infection with packaged pCOS-19. pCOS-20-A was obtained similarly, except that B12 (*recA*$^+$) cells was used. pCOS-20-20 was obtained in Km17 cells upon infection of packaged pCOS-20.

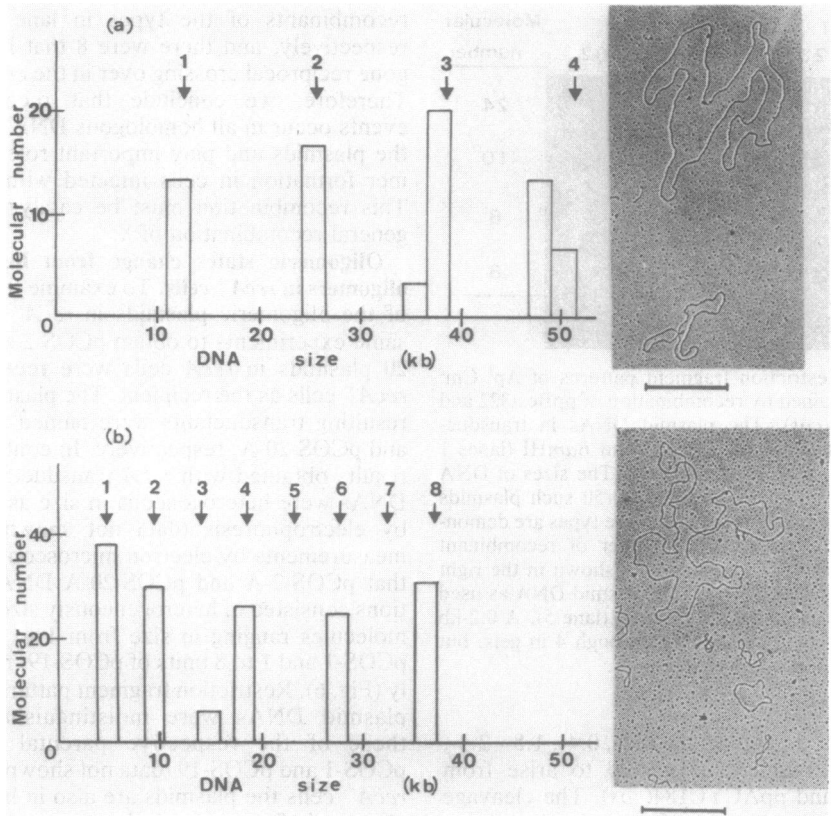

FIG. 6. Histograms showing the size distribution of plasmid DNA molecules in *recA*$^+$ transductants and electron micrographs. *recA*$^+$ cells (B12) were infected with packaged pCOS-1 in fraction 6 of Fig. 1a (tetrameric form) (a) or pCOS-19 in fraction 5 of Fig. 1b (undecameric form) (b), and Ap^r transductants were selected. One each of the transductant clones was chosen to prepare plasmid DNA (pCOS-2-A, pCOS-20-A), and the sizes of DNAs were measured by electron microscopy. Arrows with numbers respresent the lengths of integral units of pCOS-1 and pCOS-19. Electron micrographs show representative molecules in each preparation. Bars, 1 μm.

that the packaging efficiency was 1 order of magnitude higher than that of the parental pCOS-19. The same figure was obtained with pCOS-20-A prepared similarly in *recA*$^+$ cells. From cells carrying pCOS-20, a second-cycle lysate was made. One of the transductants in *recA* cells, pCOS-20-20, showed unchanged packaging efficiency (Table 3). Copy numbers of plasmids pBR322, pCOS-19, pCOS-20, pCOS-20-A, and pCOS-20-20 were all the same—about 30 copies per chromosome in monomer units (data not shown). We infer that the 10-fold elevation of packaging efficiency observed with once- and twice-packaged plasmids reflects the higher oligomeric structure of DNA. Recently, a similar observation was reported by Feiss et al. (9).

Plasmid DNA content in a cell and packaging efficiency. While working with low-copy plasmids, we observed that a derivative of miniF, 13.7 kb in size and with a copy number of about two, could not be packaged even though it carried a 223-bp λ fragment with the *cos*λ sequence (12a). On the other hand, ppF, whose origin of replicon and copy number are the same as that of miniF, but whose size is larger, can be packaged (10). Thus, for packaging, plasmids carrying *cos*λ must form oligomers of appropriate size. This process depends upon genome size as well as copy number.

A series of packageable plasmids different in size was constructed, and their packaging efficiencies were measured. pTM1, pTM2, and pTM3 are ppBest322-based plasmids. They have the same copy number as pBR322, but differ in genome size (Table 1). The relationship between the packaging efficiencies and genome size of these plasmids is shown in Table 4. The packaging efficiency increased as the genome size of the plasmids increased. Feiss et al. (9) independently have made a similar observation.

ppλdv, pp1, and pp1*P*am80 are derivatives of

TABLE 4. Relationship between plasmid genome size and packaging efficiency[a]

Plasmid	Genome size (kb)	Packaging efficiency
pTM3	4.9	7×10^{-4}
pTM1	7.8	6×10^{-3}
pTM2	14	4×10^{-2}

[a] See footnotes to Table 3.

λdv, and ppF is a derivative of miniF. These plasmids all carry *cos*λ and have nearly the same genome size (about 40 kb; Table 1). They were all packaged in phage heads as monomers. However, the copy numbers of these plasmids differed. Table 5 shows that the packaging efficiency was elevated as copy number increased.

From these results, we conclude that the packaging efficiency of plasmids carrying *cos*λ depends on the cellular content of plasmid DNA, which is related to plasmid genome size multiplied by copy number.

DISCUSSION

In this paper we demonstrated that plasmids carrying *cos*λ, which is only 4.0 kb in size, can be packaged in λ phage head in oligomeric form. These plasmids must be oligomerized before packaging. Any plasmids carrying *cos*λ can be packaged when the plasmid DNA content in a cell, expressed as the copy number multiplied by the genome size, is appropriate size, probably larger than about 40 kb.

Packaging efficiency of plasmids is high when the copy number is high (Table 5). Furthermore, the packaging efficiency increases almost exponentially as the genome size increases (Table 4). Comparing a monomeric, high-copy-number plasmid carrying *cos*λ and the same plasmid in oligomeric, low-copy-number state, as seen with once-packaged plasmid, shows that the latter has higher packaging efficiency, even though the DNA content is about the same in the two cases (Table 3). Thus, the genome size has more effect on packaging efficiency than the copy number.

TABLE 5. Relationship between plasmid copy number and packaging efficiency[a]

Plasmid	Copy number[b]	Packaging efficiency[c]
ppλdv	39	0.8
pp1	14	0.3
pp1*Pam80*	5	0.05
ppF	1	0.01

[a] The lysates of C600 *recA41* cells carrying the indicated plasmids were prepared by infecting with λ*imm*21*c*I4 phage at a multiplicity of 10 and incubating until lysis.

[b] The copy numbers of these plasmids were measured by centrifugation in CsCl-ethidium bromide and are represented in monomer units of the plasmids per chromosome.

[c] The number of *Gua* transductants (1) when ppλd was used, and the number of Ap^r transductants whe other plasmids were used.

Upon injection into cells, the packaged DNA becomes a plasmid in oligomeric form. Its structure is stable in *recA* cells, but the oligomers are converted to low multimers in *recA*$^+$ cells. So far, head-to-tail structures are the only forms detected in oligomeric plasmids. We forced head-to-head oligomers to form from a pair of compatible plasmids, pCOS-9 and ppACYC-184(*cos*), which both carry the *cos*λ sequence (12a). The pCOS-9 carries an *amp* gene, and ppACYC184(*cos*) carries a *cam* gene. In this pair, the cloned regions around *cos*λ are oriented oppositely, in conjunction with a *tet* gene located nearby. If recombination occurs, an Ap^r Cm^r-transducing particle would be expected to appear. However, this combination did not give rise to an Ap^r Cm^r transductant, whereas the other combination of two plasmids carrying *cos*λ in the same direction formed recombinants (see above). The head-to-head recombinant plasmids might not have been made, or might have been made but were not stable enough to survive in the recipient cells.

Plasmid oligomer formation needed the products of *red*α, *red*β, and γ genes of λ phage. Umene et al. (18) have reported that packaged plasmids appear in the absence of any known recombination functions. However, since they used a large plasmid that can be packaged in a monomeric form, in contrast to our system where the plasmids are small and require formation of oligomeric molecules before packaging, their result must be taken to mean that the packaging process itself is free from recombination.

Recently Feiss et al. (9) independently demonstrated that plasmid packaging depends on only the γ gene product. The γ gene product is believed to play a role in production of rolling circles (5). If the rolling circle replication occurs in plasmids, the products will be high-oligomeric DNA. Proof of production of this structure has not been available, as plasmid DNA replication cannot be blocked without impairing phage DNA replication and recombination activity. We have shown that such plasmids as ColE1, λdv, and P15A having the *cos*λ sequence can be packaged in oligomeric forms (12a). If the rolling circle replication is the primary cause of oligomer formation, we must assume that λ phage has the ability to divert replication of the plasmids in the same cell into the rolling circle mode. This is theoretically possible, but remains unestablished.

Feiss et al. (9) also suggested that *red*β function may be irrelevant, an observation which is contradictory to ours. However, it remains to be made clear whether the general recombination function of λ was totally destroyed in their system, where only one *red*β mutant phage was employed in supressor-plus cells or prophage-lysogenic cells.

If the general recombination system of λ in fact acts in plasmid oligomer formation as expected, we can predict that a plasmid that does not carry *cos*λ will also be able to form oligomers. This was tested by growing λ phage in cells carrying two compatible plasmids, ppBest322 and pACYC184, which share the *tet* gene in common, but the former is a Cm^s plasmid carrying the *cos*λ sequence, whereas the latter is a Cm^r plasmid and does not carry the *cos*λ sequence. The recombination was expected to give rise to Cm^r packageable plasmids. Such recombinants in fact appeared at high frequency, approaching one-third of the packaging efficiency of ppACYC184(*cos*) and ppBest322. Thus, we conclude that the general recombination promoted by *red*α and *red*β of λ plays a role, at least in part, in plasmid oligomer formation, regardless of whether or not the plasmids have the *cos*λ sequence.

Although occurrence or nonoccurrence of rolling circle replication of plasmids in λ-growing cells is far from clear, it would take part in oligomer formation as described by Feiss et al. (9). Therefore, it is fair to say that plasmid oligomer formation in λ phage growth occurs by both rolling circle replication and general recombination of λ.

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