JOURNAL OF VIROLOGY, Sept. 1975, p. 479-485
Vol. 16, No. 3

Printed in U.S.A.

In Vitro Construction of Different Oligomeric Forms of λdv DNA and Studies on Their Transforming Activities

KENICHI MATSUBARA, YASUYUKI TAKAGI,* AND TSUNEHIRO MUKAI

Department of Biochemistry, Kyushu University School of Medicine, Fukuoka 812, Japan

Received for publication 25 March 1975

Plasmid λdv1, which is in a dimeric form, was converted to a linear monomer duplex by the action of EcoRI restriction endonuclease that incises at a unique site in this plasmid genome. The resulting products were then joined by *Escherichia coli* DNA ligase to produce molecules with various oligomeric forms, and from these monomeric, dimeric, or trimeric circular molecules were purified. By transformation of cells with these DNAs, clones were obtained that carried λdv1 in a monomeric or dimeric form. The former type of clones have not been generated in vivo, except for one in a different host strain, and carriers of trimeric or tetrameric λdv1's have not been obtained so far. It was observed that a considerable fraction of these oligomeric circular DNAs were converted to lower oligomers (e.g., from trimer to dimer) during transformation. The characteristics of the monomeric λdv1 carriers obtained were compared with those of dimeric λdv1 carriers. The stabilities of the plasmids of the two forms were the same. However, the monomeric plasmid carriers were less tolerant to λvir phage infection and perpetuated about 30% less plasmid genomes in monomer units. Furthermore, dimeric plasmid carriers appeared spontaneously and accumulated in cultures of the monomeric λdv1 carriers.

Plasmid λdv (17) is an autonomously replicating fragment of the bacteriophage λ genome, consisting of only a few genes and genetic structures that act in the process of replication and transcription (2; K. Matsubara, manuscript in preparation) (Fig. 1). Recent studies have shown that this plasmid DNA molecule consists of two physically indistinguishable units (3, 5, 14), and that these dimer molecules undergo cycles of replication (K. Matsubara and T. Mukai, J. Biochem., in press). Many other similar plasmids, prepared from λ or related phages, including λimm^{434}, λimm^{21}, and φ80, were also found to have a dimeric structure (K. Matsubara and Y. Otsuji, submitted for publication).

Several cases are known of autonomously replicating units which are found in monomeric or oligomeric states; in polyoma *tsa-a* transformants, a series of oligomers appear upon lowering the temperature (9). The oligomeric state of mitochondrial DNA of leukocytes has been discussed in relation to leukemia (6) and to physiological conditions of the cells (22). Bacterial plasmid Col E1 is in a monomeric state in normal *Escherichia coli* cells, but many oligomeric forms are found when the cells are incubated in the absence of protein synthesis or when the plasmid is transferred to *Proteus mirabilis* cells (11). A segment of an R factor is believed to amplify under certain conditions, e.g., when cultured in the presence of an antibiotic (for a review see reference 7). Nothing is known, however, about the mechanism of interconversion of different molecular forms of a genome, or how a certain oligomeric form is chosen as the predominant species in a given cell.

Recently, Hobom and Hogness (14) discovered strains in which the plasmid λdv1 is perpetuated either in a monomeric or a higher oligomeric form, and suggested that the interconversion of the forms takes place as a result of recombination. However, these plasmids occurred in RecA$^+$ cells, and how they had been obtained was obscure. Therefore, it is possible that they were mutant plasmids.

We constructed λdv1 molecules in different oligomeric forms in an in vitro system, by incising this plasmid DNA at a single site per monomer unit using EcoRI endonuclease (13) and joining the "cohesive ends" with DNA ligase (20). The resulting molecules were then used to transform cells (12). In this way, strains that perpetuate monomeric λdv1 were obtained and their properties were examined.

MATERIALS AND METHODS

Bacteria and phage strains. The bacterial strains used were derivatives of *E. coli* K-12. Strain km605,

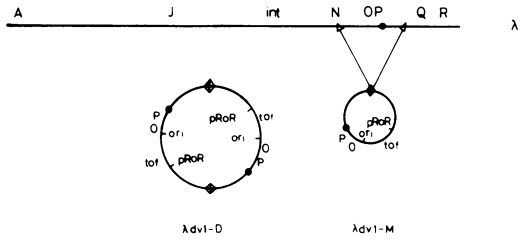

FIG. 1. *Genetic map of λ and λdv1. The upper line represents the λ genome (10), and the lower two circles represent, respectively, λdv1 in the dimeric and monomeric forms. The λdv1 genomes are drawn at twice their physical size relative to that of the λ genome. Triangles show the sites of illegitimate recombination, and small circles show the sites of action of EcoRI. For definition of symbols, see Campbell (4).*

which carries λdv1 in the dimeric form, and km723, which does not carry plasmids, have been described previously (17). km960 (*str his recA1 su⁻ gal*^{del} *thy*) is a thymine-requiring derivative of km723.

$\lambda cI_{90}nin_7$, constructed by a cross, is a clear plaque-forming mutant of λ (16) that carries a deletion mutation, nin_7 (8). This phage does not give rise to λN⁻ type plasmids (23) because the nin_7 mutation suppresses the N⁻ phenotype (8). $\lambda h^{80}cI_{90}nin_7$ is a hybrid of $\lambda cI_{90}nin_7$ and φ80. Use of a mixture of these two phages excludes the possibility of the appearance of bacterial receptor mutants among survivors of infected cells (18). λv2v1v3, referred to as λ*vir* in this paper, has been described previously (21).

Media. PB medium contained (per liter): 10 g of polypeptone (Daigo Eiyo Co. Ltd.), 1 g of bonito extract (Katayama Chemicals), and 2.5 g of NaCl, adjusted to pH 7.2 with NaOH. PBB medium was the same as PB, except that it contained 10 g of bonito extract. PBB agar was PBB with 1.5% agar. TS, TE, and TD media, respectively, represent 0.02 M Tris-hydrochloride (pH 8.0) containing 0.8% NaCl, 0.01 M Tris-hydrochloride (pH 7.0) containing 1 mM EDTA. and 0.04 M Tris-hydrochloride, pH 8.5, containing 0.02 M EDTA. SSC contained 0.15 M NaCl and 0.015 M sodium citrate.

Enzymes. EcoRI and ATP-dependent DNase from *Micrococcus luteus* were prepared by the methods of Yoshimori (Ph.D. thesis, University of California at San Francisco, San Francisco, Calif., 1971) and Anai et al. (1), respectively. DNA ligase from *E. coli* was a generous gift from Yasuhiro Anraku of Tokyo University.

Preparation of λdv1 DNA. km605 bacteria were grown in 10 liters of PBB to a density of 2×10^9 cells/ml and then harvested and washed with TS. The wet pellet (20 g) was suspended in 120 ml of TD, incubated with 20 mg of lysozyme at 0 C for 20 min, and then mixed with 100 ml of water at 0 C. Then 45 ml of 5% sodium lauryl sulfate was added with gentle mixing, and the viscous lysate was centrifuged at 30,000 rpm for 30 min at 2 C to obtain a clear, nonviscous supernatant. This was treated with one-half volume of phenol, and nucleic acids in the buffer layer were precipitated by adding 2 volumes of isopropanol. The precipitate was dissolved in 40 ml of 1× SSC containing 0.35% sodium sarkosynate and then mixed with 40 ml of 5 M NaCl. After standing at 0 C for 2 h rRNA was precipitated by centrifugation at 10,000 rpm for 10 min at 5 C. The remaining nucleic acids were precipitated with 2 volumes of ethanol, dissolved in 25 ml of 1× SSC containing 0.35% sodium sarkosynate, and incubated with 10 mg of pancreatic RNase at 37 C for 60 min. The mixture was again treated with an equal volume of phenol saturated with 0.05 M Tris-hydrochloride (pH 8.0) and the nucleic acids in the buffer layer were precipitated by addition of 2 volumes of isopropanol. The precipitate was dissolved in 20 ml of TE containing 0.35% sodium sarkosynate and subjected to CsCl-ethidium bromide isopycnic centrifugation by the method of Radloff et al. (22). The lower band was recentrifuged and then freed from dye by repeated shaking with isopropanol saturated with CsCl-water. Finally, the preparation was dialyzed against TE.

Sedimentation analyses of ^{3}H-labeled plasmid DNA. Bacteria were grown in 7 ml of PB to a density of 5×10^8 cells/ml and then labeled with [^{3}H]thymidine (4 μCi/ml) for 90 min in the presence of 200 μg of uridine per ml. The labeled cells were collected, washed twice with TS, and suspended in 1 ml of TD. The mixture was kept at 0 C for 30 min with 100 μg of lysozyme and then mixed with 0.3 ml of 5% sodium lauryl sulfate and incubated at 37 C for 15 min. The lysate was centrifuged at 35,000 rpm for 10 min at 20 C, and the resulting clear supernatant was mixed with 60 C phenol (1 ml). The nucleic acids in the buffer layer were precipitated by adding 2 volumes of ethanol, dissolved in 0.6 ml of 0.2× SSC, and kept at 90 C for 6 min. After heating, the mixture was quickly cooled by shaking in an ice bath, mixed with 0.25 ml of 20× SSC, and filtered through a Sartorius MF30 nitrocellulose membrane filter. Aliquots (100 μl) of this DNA were immediately applied to a 4.6-ml sucrose density gradient in 0.02 M Tris-hydrochloride (pH 7.5)-2 mM EDTA-1 M NaCl and centrifuged in a Hitachi ultracentrifuge in an RPS40T rotor at 37,000 rpm for 3.5 h. Then drops were collected from the bottom of the tubes directly onto Toyo no. 23 filter papers (3 by 3 cm), dried, and counted.

Isopycnic centrifugation analysis of the content of plasmid DNA by CsCl-ethidium bromide density gradient centrifugation. Cells were labeled with [^{3}H]thymidine and lysed as described in the previous section, except that 2 ml of culture was used. The washed cells were then suspended in 1.5 ml of TD and incubated with 400 μg of lysozyme at 0 C for 20 min. An equal volume (1.5 ml) of 0.75% sodium sarkosynate in TE was then added, and the lysate was incubated at 65 C for 8 min. Then it was incubated with 300 μg of predigested Pronase at 37 C for 1 h. The lysate was adjusted to 4.76 ml, mixed with 5 g of CsCl and 0.5 ml of an aqueous solution of 4.76 mg of ethidium bromide per ml, and subjected to isopycnic centrifugation (22) at 36,000 rpm for 36 h at 20 C using a Spinco no. 50 preparative rotor. The contents of each tube were fractionated into about 35 fractions,

and the trichloroacetic acid-insoluble radioactivity was counted using a toluene-2,5-diphenyloxazole liquid scintillation system.

RESULTS

Action of EcoRI on λdv1 DNA molecules. Jackson et al. (15) and Berg et al. (3) showed that λdv120 DNA molecules are split into two identical fragments upon incubation with EcoRI. This was confirmed with λdv1 DNA by sedimentation analysis. The components CC and OC in Fig. 2A represent, respectively, circular DNA in the covalently closed twisted form and the open circular form. When the DNA was incubated with a limited amount of EcoRI, an intermediate product appeared together with some smaller component (Fig. 2B). The former component is the linear dimer, and the latter the linear monomer (LM), judging from their sedimentation velocities. Upon extensive digestion all the DNA was converted to the LM (Fig. 2C).

Joining the ends of molecules with DNA ligase. The ends of EcoRI-treated DNA molecules can cohere (13, 20), and the coherent structures become joined covalently upon treatment with DNA ligase (20). Treatment of LM DNA with *E. coli* DNA ligase yielded a heterogeneous population of linear and circular molecules of various oligomeric forms (Fig. 3).

An ATP-dependent DNase from *M. luteus* (1) degrades linear, but not circular, DNA irrespective of whether or not the circular molecules have nicks (24). Therefore, to remove linear DNA, the ligated DNA preparation was treated with this enzyme and then sedimented through a sucrose density gradient. Circular molecules in monomeric, dimeric, and trimeric forms are seen in Fig. 3. These components will be referred to as cM1, cM2, and cM3, respectively.

Transformation. λdv DNA in the circular form can transform Ca^{2+}-treated $RecA^-$ cells to new carriers of λdv (12). The efficiency of transformation is about 10^{-6}/molecule. No linear molecules are able to transform under the conditions used with $RecA^-$ cells as recipients.

The sucrose fractions were assayed for transforming activities. Peaks of transforming activity corresponding to cM1, cM2, and cM3 molecules were separated (Fig. 4A). Each of these components was purified by recentrifugation (Fig. 4B-D) and added to Ca^{2+}-treated cells. It was observed that the efficiency of transformation was the same with all the oligomeric forms (Table 1). One simple outcome of this experiment might have been that the different oligomers transformed with equal molar efficiency. Instead, the result was that they are equally efficient per unit weight. A simple explanation for the facts is not available at present.

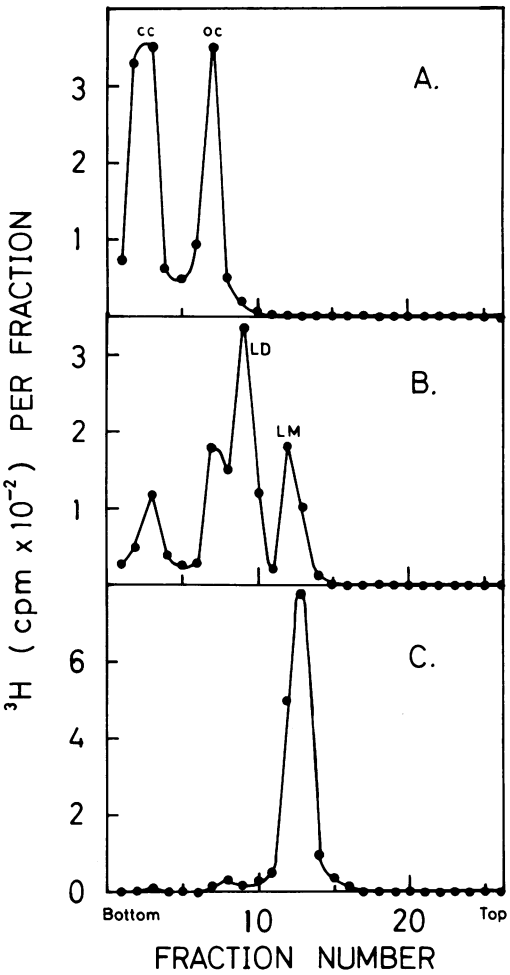

FIG. 2. *Sucrose gradient profiles of λdv1 DNA before and after treatment with EcoRI. ^3H-labeled λdv1 DNA in 0.1 M Tris-hydrochloride (pH 7.3)-10 mM $MgSO_4$ was incubated at 37 C for 30 min without enzyme (A) or with an amount of EcoRI sufficient to hydrolyze two-thirds of the restriction sites (B). (C) is the same with six times more enzyme. The reaction was terminated by adding EDTA (final concentration, 20 mM), and 0.1-ml aliquots were sedimented through 4.6 ml of a 5 to 20% sucrose gradient in 20 mM Tris-hydrochloride (pH 7.5)-2 mM EDTA, and 1 M NaCl using a Hitachi swinging type rotor RPS4OT at 37,000 rpm for 4 h at 20 C. Then fractions were collected from the bottom of the tubes onto Toyo no. 50 filter papers (3 by 3 cm), dried, and counted in a liquid scintillation counter using the toluene-2,5-diphenyloxazole system. CC, OC, LD, and LM represent, respectively, covalently closed twisted circles, nicked open circles, linear dimers, and linear monomers.*

Plasmid DNA in the transformants. The size of plasmid DNA in the transformants was

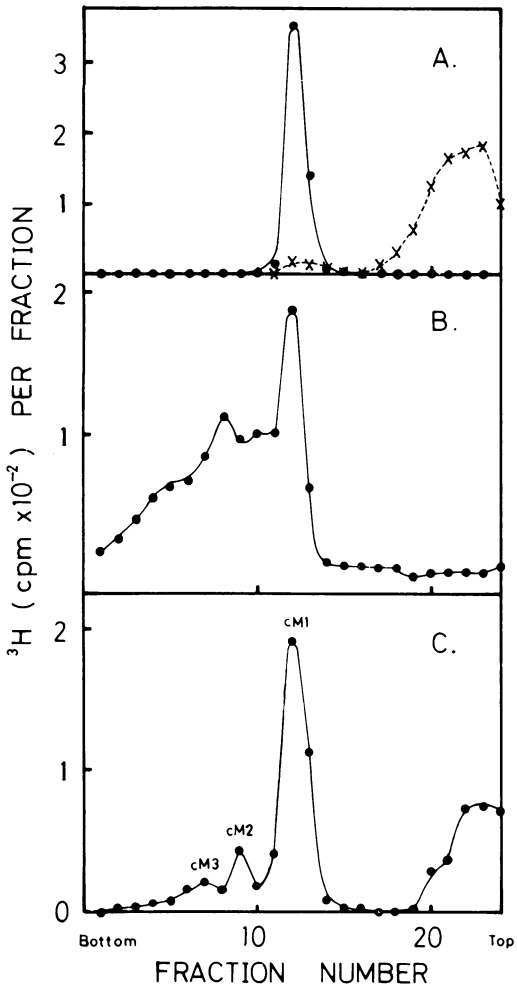

FIG. 3. *Sucrose gradient sedimentation profiles of ligated DNA. λdv1 DNA was converted to the linear monomeric form by extensive treatment with EcoRI and then treated with phenol and dialyzed against TE. Aliquots of this DNA (0.35 E_{260} units) were incubated with E. coli ligase in 30 mM Tris-hydrochloride (pH 8.0) 4 mM $MgCl_2$, 1 mM EDTA, 10 mM $(NH_4)_2SO_4$, and 0.025 mM nicotinamide adenine dinucleotide at 5 C for 10 h. An equal amount of ligase was added again, and the incubation was continued for an additional 12 h. The total volume of the reaction was 100 μl. Half of this sample was saved (B), and the other half was treated with M. luteus ATP-dependent DNase (1) (C) and sedimented through sucrose gradients as described in the legend to Fig. 2. (A) Control samples (LM DNA) not treated with ligase, with (×) and without (O) ATP-dependent DNase treatment. cM1, cM2, and cM3 indicate circular molecules in the monomeric, dimeric, and trimeric form, respectively.*

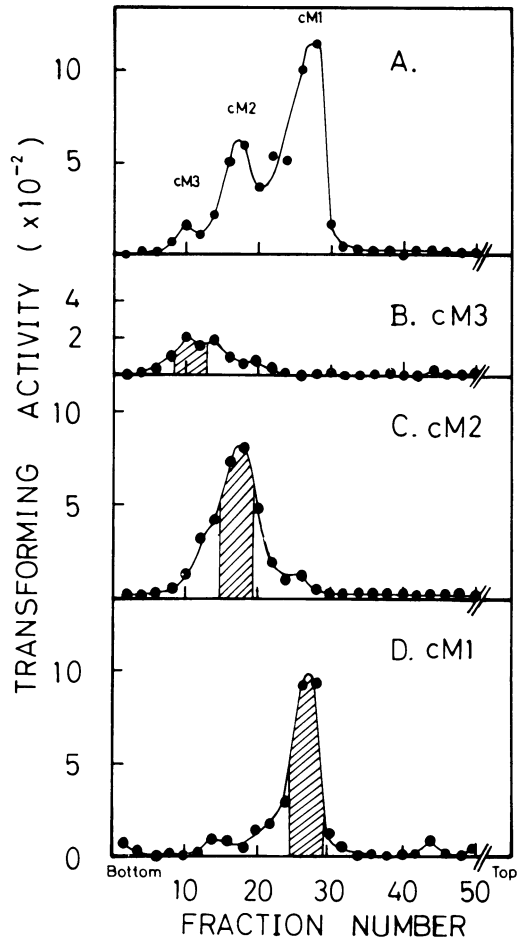

FIG. 4. *Sucrose gradient centrifugation profiles of transforming λdv1 DNA circularized in vitro. (A) LM DNA was prepared from ^{3}H-labeled λdv1 by extensive treatment with EcoRI, and 0.15 E_{260} units of this DNA was ligated in 100 μl of reaction mixture, as described in the legend to Fig. 3. It was then treated by M. luteus ATP-dependent DNase in 230 μl of reaction mixture and sedimented through 16.6 ml of a 5 to 20% sucrose gradient in 20 mM Tris-hydrochloride (pH 7.5)-2 mM EDTA using a Hitachi ultracentrifuge swinging rotor RPS25 at 25,000 rpm for 22.5 h at 20 C. Then 90 fractions were collected from the bottom of the tubes, and aliquots (50 μl) of relevant fractions were assayed for transforming activity. (B, C, D) Fractions 9–11, 16–18, and 26–28, respectively, in the top figure were separately pooled and concentrated by dialysis versus Carbowax and then versus 10 mM Tris-hydrochloride (pH 7.0)-1 mM EDTA. Each of the pooled samples was then resedimented, and its transforming activity was assayed as described above. Sedimentation was from right to left. The fractions shown in the hatched areas in the second centrifugation profiles were pooled and used as purified cM3, cM2, and cM1 in subsequent studies.*

examined by sucrose gradient centrifugation. The transformants by cM1 (monomeric circular λdv1) were found to carry monomer circles (Fig. 5B), which have only been generated once in vivo in λdv1 (14). The six independent transformants tested were all carriers of monomeric

TABLE 1. *Efficiency of transformation by purified circular oligomers*[a]

Donor DNA	Transforming activity/ml ($\times 10^{-3}$)	E_{260} units/ml ($\times 10^{-3}$)	Transforming activity/ E_{260} units ($\times 10^{5}$)
cM3	8.0	9.4	8.5
cM2	31	43.3	9.2
cM1	50	52.8	9.5

[a] Purified cM1, cM2 and cM3 preparations were prepared in the experiments shown in Fig. 4. Aliquots of these preparations were taken for transforming activity assays, and the remainder were taken for measurement of trichloroacetic acid-insoluble radioactivity. From the latter the E260 units were calculated using the known specific radioactivity. Numbers are averages of values in three independent assays.

λdv1. The monomeric plasmids are referred to as λdv1M. A small amount of dimeric DNA was present in cultures of λdv1M carriers. This problem will be discussed later.

Dimer-sized DNA was found in three clones of transformants by cM2 DNA (Fig. 5A). These plasmids are referred to as λdv1D. They were indistinguishable from in vivo generated λdv1. However, seven other transformants by the same DNA preparation were found to be carriers of monomeric plasmids. Purified cM2 DNA was used for transformation, so the monomeric plasmids must have been derived from the dimeric DNA taken up into the recipient cell, presumably at an early stage. In another experiment, about 80% (17/20) of the transformants by purified cM2 were found to be carriers of monomeric plasmids.

Almost all (66/68) the transformants by purified cM3 were found to carry dimeric plasmids, not trimeric plasmids. (The two exceptions carried monomeric plasmids.) The experiment on purification and transformation was repeated two more times. The results were similar; namely, among 23 transformants examined, 22 and 1 were found to carry dimeric and monomeric plasmids, respectively. In another experiment, linear dimer DNA was used as starting material, in place of LM DNA, and cM4 molecules were purified. Forty transformants with this DNA were examined, and all were found to carry dimeric plasmids. Thus, attempts to prepare strains carrying λdv1 in higher oligomeric forms were unsuccessful.

The DNA of monomeric plasmid λdv1M obtained above was converted to LM DNA by EcoRI. Upon ligation followed by sucrose gradient sedimentation analyses, this DNA preparation behaved indistinguishably from the LM DNA prepared from the dimeric λdv1D DNA.

Properties of the transformants. The characteristics of transformants carrying λdv1M and λdv1D were compared. The stabilities of the carrier states were similar, and in marker rescue tests (19) both carriers donated the same set of genes as those of λdv1, indicating that their genetic constitutions were identical (Table 2). Upon extensive treatment by EcoRI, both DNAs yielded the same product, namely, LM DNA.

A difference was noted in susceptibilities to infection by bacteriophage λ*vir*; λdv1M carriers were more susceptible to infection than λdv1D carriers (Table 2).

The amounts of plasmid DNA relative to chromosomal DNA in carriers of λdv1M and λdv1D were 12.4 and 17.2%, respectively. This indicates that the former cells perpetuate about 79 independently replicating molecules per chromosome, whereas the latter perpetuate about 55 molecules. The total number of λdv genomes in dimer carriers is 109 in monomer units, which is 38% more than that in monomer carriers.

Conversion of monomer carriers to dimer

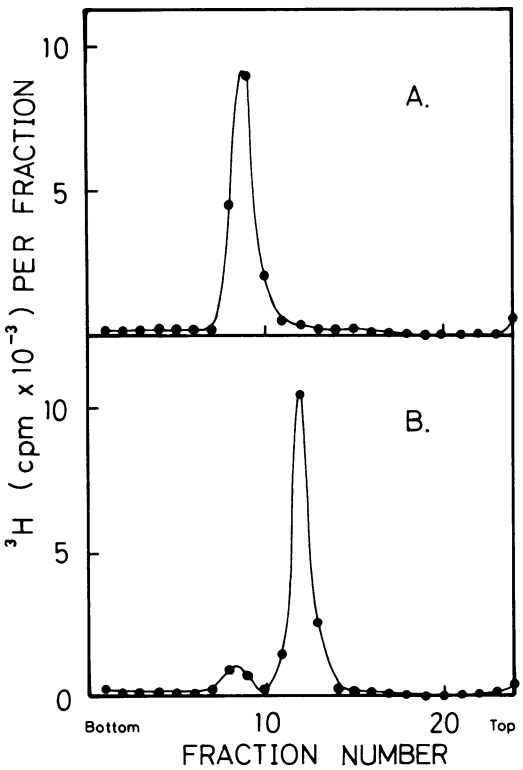

FIG. 5. *Sedimentation profiles of plasmid DNA in the covalently closed circular twisted form prepared from a transformant clone by cM2 DNA (A) and a transformant clone by cM1 DNA (B). For experimental procedures, see Materials and Methods.*

TABLE 2. *Characteristics of λdv1M and λdv1D carriers*

Characteristic	λdv1M carriers	λdv1D carriers
Stability of plasmid (%)[a]	<0.5, <0.5, <0.5, <0.5, 0.7	<0.5, <0.5, <0.5, 1.0, <0.5
Marker rescue[b]	$A^-J^-N^-(oLcIoR)^+O^+P^+Q^-R^-$	
Tolerance to λ*vir* infection (%)[c]	24, 31, 15, 17	94, 100, 79, 95
Content of plasmid DNA (%)[d]	10.5, 14.0, 12.5, 13.4, 11.6 (avg 12.4)	15.3, 20.4, 15.4, 17.9 (avg 17.2)

[a] The frequencies of appearance of λdv$^-$ segregants, determined as the fractions of cells that had lost plasmids after overnight growth in PBB, were assayed by scoring the number of λ-sensitive colonies (18).

[b] Marker rescue experiments were done as described previously (19). For location of the genes on the λ genome, see Fig. 1. + and − denote the presence and absence, respectively, of the marker on the plasmid genome.

[c] Exponentially growing plasmid carrier cells (2×10^8/ml) in PB medium containing maltose (0.2%) and $MgCl_2$ (1 mM) were infected with λ*vir* at a multiplicity of infection of 10, kept at 0 C for 30 min, and then incubated at 37 C for 25 min and assayed for colony formers. Numbers are ratios of the numbers of colony formers before and after infection.

[d] The ratios of plasmid DNA in the covalently closed circular twisted form to the total bacterial DNA as determined by CsCl-ethidium bromide isopycnic centrifugation (see Materials and Methods). The number of λdv1 genomes in λdv1M carriers is 79 in monomer units and in λdv1D carriers it is 109 in monomer units, assuming that the molecular weights of the λdv1 monomer and the *E. coli* chromosome are 4.75×10^6 (5) and 3×10^9, respectively.

carriers. DNA prepared from λdv1M carrier cells always contained some dimers (Fig. 5B). A λdv1M carrier clone was subjected to successive dilution and cultivation, and the DNAs prepared from the cultures were examined. After 32 generations of growth from a single cell, 5.4% of the total plasmid DNA was found in the dimeric form. Upon subsequent cycles of dilution and growth, the percentage increased to 12.0, 22.3, and 31.6% after 68, 107, and 147 generations, respectively. The final culture was composed of monomeric λdv carrier clones or dimeric λdv carrier clones. No cells were found carrying both forms of plasmids simultaneously. These findings may indicate that dimers which appear spontaneously in cultures have a selective advantage over monomers within the intracellular pool. The dimer clones presumably outgrow the monomer carrier clones during cycles of dilution and cultivation.

DISCUSSION

In this work dimeric plasmid λdv1 generated in vivo was converted to monomeric linear molecules (LM DNA) by the action of EcoRI, and the products were joined to produce molecules in various oligomeric forms.

Circular monomeric DNA made in vitro (cM1 molecules) transformed cells and yielded clones which carried monomeric plasmids. This finding clearly demonstrates that at least one-half the in vivo generated dimeric λdv1 DNA molecules has a "complete" set of genes and genetic structures.

The circular dimer DNA (cM2 molecules) made in vitro transformed cells and yielded dimeric plasmid carriers. These plasmids were indistinguishable from λdv1 generated in vivo. No transformants carrying trimeric or tetrameric plasmid DNA were obtained on transformation by purified cM3 or cM4 molecules. It is not clear at present whether clones carrying such plasmids are unstable or whether the frequency of their appearance is too low for their detection. The failure to obtain oligomeric λdv does not necessarily contradict reports on the finding of oligomers (14, 19), as they appeared in RecA$^+$ cells and as the possibility has not been ruled out that these natural oligomers were mutants.

In many transformants, the oligomeric forms of the plasmid after stabilization were lower than those used for transformation. For example, 80% of the transformants by cM2 molecules carried monomeric λdv, and 97% (128/131) of the transformants by cM3 or cM4 were carriers of dimeric plasmids. Therefore, RecA$^-$ cells must have some mechanism(s) to reduce the oligomeric form of transforming plasmid DNA. Assuming that the genes are arranged as *tof*-O-P (represented as TOP) and TOPTOP on LM DNA and dimeric λdv1, respectively (5), the frequency with which the "correct" sequences TOPTOP and TOPTOPTOP appear in cM2 and cM3 preparations will be 50 and 25%, respectively. It may be that molecules of the "wrong" sequence, e.g., TOPPOT, give rise to molecules of a lower oligomeric form. (This does not exclude the possibility of reduction in size of some correctly arranged dimers or trimers.)

Several results were obtained by comparing the characteristics of strains that carry monomeric or dimeric plasmids. The average number of independently replicating plasmid copies per chromosome was 79 for monomeric λdv1 car-

riers and 55 for dimeric λdv1 carriers. Therefore, the number of these plasmid copies in a cell is not determined by limitation or saturation of the number of available "sites" for replication. Rather, this and other works (14; K. Matsubara and Y. Otsuji, submitted for publication) suggest that the number of λdv genomes in a monomer unit tends to be constant. However, the monomer genome content of dimeric λdv1 is 110, which is 37% higher than the value for monomeric λdv. This difference might be the basis for the difference in tolerancy to infection by phage λ*vir*, as the level of autorepressor (*tof* gene product) which may be proportional to the gene dosage is assumed to control tolerance (18).

Small amounts of dimers were always detected in the λdv1M DNA preparation. Their origin in the RecA$^-$ cell is not yet clear, but the present results strongly suggest that the plasmids in the dimeric form, once produced, outgrow those in the monomeric form. The original dimeric λdv1 is likely to have been selected similarly. The product of the reverse process, namely, the monomeric λdv carriers, would not have been detected, even if they were produced spontaneously, because such carriers have a growth disadvantage.

These observations seem to indicate that the most probable oligomeric form in the steady state is determined by a balance between at least two factors: the need to maintain a certain level of genomes for production of the appropriate amounts of gene product for controlled replication, and the effect of the accumulation of independently replicating particles on the metabolism of the carrier cell.

ACKNOWLEDGMENTS

This work was supported in part by a grant from the Ministry of Education of Japan and by Public Health Service grant GM 12052 from the National Institute of General Medical Sciences.

Grateful acknowledgment is due to T. Nakamura for his technical assistance.

LITERATURE CITED

1. Anai, M., T. Hirahashi, and Y. Takagi. 1970. A deoxyribonuclease which requires nucleoside triphosphate from *Micrococcus lysodeikticus*. J. Biol. Chem. **245**:767-774.
2. Berg, D. E. 1974. Genes of phage λ essential for λ*dv* plasmids. Virology **62**:224-233.
3. Berg, D. E., D. A. Jackson, and J. E. Mertz. 1974. Isolation of a λ*dv* plasmid carrying the bacterial *gal* operon. J. Virol. **14**:1063-1069.
4. Campbell, A. 1971. Genetic structure, p. 13-44. *In* A. D. Hershey (ed.), The bacteriophage lambda. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
5. Chow, L. T., N. Davidson, and D. E. Berg. 1974. Electron microscope study of the structures of λ*dv* DNAs. J. Mol. Biol. **86**:69-89.
6. Clayton, D. A., and J. Vinograd. 1969. Complex mitochondrial DNA in leukemic and normal human myeloid cells. Proc. Natl. Acad. Sci. U.S.A. **62**:1077-1084.
7. Clowes, R. 1971. Molecular structure of bacterial plasmids. Bacteriol. Rev. **36**:361-405.
8. Court, D., and K. Sato. 1969. Studies on novel transducing variants of λ: dispensability of genes *N* and *Q*. Virology **39**:348-352.
9. Cuzin, F., M. Vogt, M. Dieckmann, and P. Berg. 1970. Induction of virus multiplication in 3T3 cells transformed by a thermosensitive mutant of polyoma virus. J. Mol. Biol. **47**:317-333.
10. Davidson, N., and W. Szybalski. 1971. Physical and chemical characteristics of lambda DNA, p. 45-82. *In* A. D. Hershey (ed.), The bacteriophage lambda. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
11. Goebel, W., and D. R. Helinski. 1968. Generation of higher multiple circular DNA forms in bacteria. Proc. Natl. Acad. Sci. U.S.A. **61**:1406-1413.
12. Hashimoto, T., and K. Matsubara. 1974. Transformation of Ca^{++}-treated recA derivative of *Escherichia coli* K12 by λ*dv* DNA. Jpn. J. Genet. **49**:97-101.
13. Hedgpeth, J., H. M. Goodman, and H. W. Boyer. 1972. DNA nucleotide sequence restricted by the RI endonuclease. Proc. Natl. Acad. Sci. U.S.A. **69**:3448-3452.
14. Hobom, G., and D. S. Hogness. 1974. The role of recombination in the formation of circular oligomers of the λ*dv*1 DNA. J. Mol. Biol. **88**:65-87.
15. Jackson, D. A., R. H. Symons, and P. Berg. 1972. Biochemical method for inserting new genetic information into DNA of simian virus 40: circular SV40 DNA molecules containing lambda phage genes and the galactose operon of *Escherichia coli*. Proc. Natl. Acad. Sci. U.S.A. **69**:2904-2909.
16. Kaiser, A. D. 1957. Mutations in a temperate bacteriophage affecting its ability to lysogenize *Escherichia coli*. Virology **3**:42-61.
17. Matsubara, K. 1972. Plasmid formation from bacteriophage λ as a result of interference by resident plasmid λ*dv*. Virology **47**:618-627.
18. Matsubara, K. 1974. Preparation of plasmid λdv from bacteriophage λ: role of promoter-operator in the plasmid replicon. J. Virol. **13**:596-602.
19. Matsubara, K., and A. D. Kaiser. 1968. λ*dv*: an autonomously replicating DNA fragment. Cold Spring Harbor Symp. Quant. Biol. **33**:769-775.
20. Mertz, J. E., and R. W. Davis. 1972. Cleavage of DNA by RI restriction endonuclease generates cohesive ends. Proc. Natl. Acad. Sci. U.S.A. **69**:3370-3374.
21. Ptashne, M., and N. Hopkins. 1968. The operators controlled by the lambda phage repressor. Proc. Natl. Acad. Sci. U.S.A. **60**:1282-1287.
22. Radloff, R., W. Bauer, and J. Vinograd. 1967. A dye-buoyant-density method for the detection and isolation of closed circular duplex DNA: the closed circular DNA in HeLa cells. Proc. Natl. Acad. Sci. U.S.A. **57**:1514-1521.
23. Signer, E. R. 1969. Plasmid formation: a new mode of lysogeny by phage λ. Nature (London) **223**:158-160.
24. Takagi, Y., K. Matsubara, and M. Anai. 1972. A deoxyribonuclease which requires nucleoside triphosphate from *Micrococcus lysodeikticus*. IV. The mode of DNA hydrolysis. Biochim. Biophys. Acta **269**:347-353.