In vitro Packaging of Foreign DNA into Heads of Bacteriophage T1

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SUMMARY

The isolation of a collection of 44 morphologically T1-like phages is described. It is shown that these phages share some similarity with T1 in terms of cross-inactivation with anti-T1 serum, particle proteins and DNA packaging in vitro by the headful process. Virion DNA extracted from these phages was treated with T1 in vitro packaging extracts and the reaction mixtures were tested for the formation of infectious phage particles. The packaging efficiencies observed varied from about 1 to 100% of that of virion T1 DNA. Phage \( \lambda \) virion DNA was packaged with an efficiency of between 0.01 and 2% (5 \( \times 10^3 \) to 3 \( \times 10^3 \) p.f.u./\( \mu \)g DNA), the shorter deleted derivative \( \lambda \) L47 being packaged more efficiently than normal length \( \lambda \) CI857 DNA. Virion DNA from phages T3 and T7 was also packaged at an efficiency similar to that for \( \lambda \). The in vitro packaging of T1 DNA requires the presence of the pac sequence which initiates headful packaging from a concatemeric precursor. The high efficiency of packaging DNA from some of the T1-like phages may indicate the presence of similar packaging sequences. However, in the case of \( \lambda \) L47, which is known not to contain such a sequence, the in vitro DNA packaging reaction must occur by a secondary pathway unrelated to the headful mechanism.

INTRODUCTION

The double-stranded linear DNA molecules extracted from T1 phage particles have a molecular weight of 31 \( \times 10^6 \) and consist of three to four circular permutations of the nucleotide sequence (Gill & MacHattie, 1976). Each molecule is terminally repetitious for about 6% of the genome sequence and there is a slight heterogeneity of molecular weight. This form of DNA structure is generally considered to reflect a mechanism of DNA packaging which excises headfuls of DNA from a concatemeric substrate (Streisinger et al., 1967). Headful packaging is initiated at a specific sequence, the pac site, and proceeds along the concatemer packaging headfuls of DNA until the substrate is exhausted (Tye et al., 1974). While the cleavage which initiates packaging (pac cleavage) is specifically associated with the pac site, all subsequent cleavages (headful cleavages) depend only on head-filling and are not defined in terms of nucleotide sequence. T1 concatemers are about three to four genome equivalents in length and this produces the limited number of permutations observed for T1 virion DNA (MacHattie & Gill, 1977; Ritchie & Joicey, 1978; Ramsay & Ritchie, 1980, 1983).

The series of events occurring in the course of an infection in vivo are closely interrelated and therefore not easily discerned in isolation of each other. Thus, in order to study individual features of the packaging process at a molecular level, we have developed an in vitro packaging system. This is based on extracts of non-permissive (sup°) Escherichia coli B cells infected with an amber mutant defective in phage DNA synthesis but proficient in the synthesis of T1 structural proteins and other morphogenic functions. This system packages exogenously supplied T1 virion DNA to form infectious particles by a mechanism which we believe involves pac cleavage of a concatemeric intermediate, as occurs in vivo. This belief is based on observations that T1 virion DNA is packaged into infectious particles much more efficiently by extracts containing
active products of T1 genes 3-5 and 4 than by extracts defective in either gene product and that in the presence, but not the absence, of active gene 3-5 and 4 products the virion DNA molecules are converted to concatemers (Ritchie et al., 1984; Liebeschuetz et al., 1985). The use of an in vitro system permits direct comparison of the packaging efficiency of T1 virion DNA with that of other forms of DNA substrate. By attempting to package non-T1 DNA substrates, it is envisaged that comparison of relative packaging efficiencies with known properties of the DNA molecules will provide further information about the DNA substrate requirements of the T1 packaging mechanism.

We report here the results of packaging experiments using the T1 in vitro packaging system and three kinds of heterologous DNA substrates: (i) the DNAs of several virulent phages whose capsid morphology is indistinguishable from T1 but whose genomes differ from T1 to various degrees, (ii) DNA from the temperate phage λ, whose particle morphology and genome length are similar to T1, but whose genome is not circularly permuted, and (iii) the DNAs of coliphages T3 and T7 whose capsids are morphologically quite different from that of T1 [they are smaller and have a short tail (for review, see Hausmann, 1976)] and whose genomes are only 80% of the length of T1 genomes.

The results show that all the substrates were packaged into phage heads to form infectious phase particles. However, the packaging efficiencies varied considerably and were generally lower than for T1 DNA. These results, taken with those of J. Liebeschuetz & D. A. Ritchie which demonstrate the in vitro packaging of plasmid DNA (unpublished data), suggest that two different T1 packaging mechanisms exist, a high efficiency mechanism requiring the T1-specific pac sequence and a lower efficiency mechanism which packages any type of DNA.

**METHODS**

**Phages.** T1 type 3 (Ramsay & Ritchie, 1982) was used as the wild-type strain. For preparing packaging extracts T1 am5am20, which carries mutations in genes 1 and 2 essential for T1 DNA synthesis, was used. The 44 morphologically T1-like phages, all of which grow on E. coli B, were newly isolated from sewage and hitherto undescribed.

Phage λ CI857 is wild-type in all genes except that it codes for a temperature-sensitive lambda repressor. λ L47 is a standard cloning vector which carries the nin5 deletion (Loenen & Brammar, 1980), and therefore lacks the esp site necessary for efficient in vivo packaging by T1 coats (Drexler, 1984). Phages D20, T7 and T3 were from our collection.

**Bacteria.** E. coli B was the non-suppressor (sup°) host for T1 and E. coli KB-3 (supE) was the suppressor host for T1 am mutants. E. coli K12 W3110 was the standard host for λ. λ CI857 was isolated from the lysogenic E. coli strain G11000. E. coli KB-3/1 was used as the T1-resistant host.

**Anti-T1 serum.** Rabbit antiserum against T1 was obtained by weekly subcutaneous injections of 1010 purified phage particles over a 4 week period.

**Preparation of DNA.** Phage λ CI857 was propagated by heat induction of an exponential culture of the E. coli lysogen G11000. Other phage stocks were made by infection of exponential cultures of E. coli B. All phages were purified from lysates using the method given by Maniatis et al. (1982) for phage λ. DNA was extracted from purified phage stocks with phenol, dialysed extensively in TE buffer (0.01 M-Tris, 0.001 M-EDTA, pH 7.4), precipitated with ethanol and resuspended in TE to a final concentration of about 100 µg/ml.

**Preparation of T1 extracts for in vitro packaging.** Packaging extracts were prepared with the T1 am5am20 mutant and E. coli B as described by Davison et al. (1984), except that the incubation period in prewarmed H broth was 12 to 15 min.

For packaging reactions 0.5 µg DNA in TE buffer, 50 µg lysozyme, 2.5 µl 20 x incubation mixture (0.08 M-spermidine, 0.06 M-2-mercaptoethanol, 0.1 M-MgCl2, 0.12 M-Tris–HCl pH 7.4) and 5 µl ATP (10 mM, pH 7.5) were added to 30 µl samples of the frozen packaging extracts. The final volume was 50 µl. Reaction mixtures were incubated for 1 h at 37°C, then diluted to 100 µl T2 buffer (Hershey & Chase, 1952) and titrated for infectious phage.

**Protein analysis.** Characterization of phage-coded proteins was by SDS–polyacrylamide slab gel electrophoresis and autoradiography; this was carried out as described previously (Korsten et al., 1979; Mertens & Hausmann, 1982).

**Restriction endonuclease digestion.** Phage DNA (0.5 µg) was digested in a reaction volume of 25 µl for 2 to 4 h at 37°C. The following endonucleases were used as recommended by the supplier: BglI and BglII (P-L Biochemicals) and EcoRI (Boehringer-Mannheim).
DNA ligation. Two μg λ DNA was precipitated with ethanol, resuspended in 20 μl ligase buffer and incubated overnight with 0·5 units T4 DNA ligase (as recommended by the supplier, Boehringer-Mannheim).

Agarose gel electrophoresis. Samples of 30 μl containing 0·5 μg DNA were analysed by electrophoresis in horizontal agarose (Sigma, Type 1) slab gels of dimensions 265 × 165 × 5 mm. Electrophoresis buffer contained 89 mM-Tris base, 89 mM-boric acid, 2·5 mM-EDTA with a final pH of 8·3. Gels were run overnight at 60 V. They were stained and photographed as described by Ramsay & Ritchie (1980). The negatives of gels with DNA fragments of T1-like phages were scanned using a Joyce-Loebl double beam scanning microdensitometer.

Molecular weights of T1-like phage DNA fragments were determined using BglI and BglII fragments of T1 type 3 DNA and EcoRI fragments of λ DNA as standards using a plot of distance migrated against the reciprocal of the molecular weight. T1-like DNA restriction fragments are referred to alphabetically in decreasing order of molecular weight.

Electron microscopy. A drop of a phage suspension in T2 buffer was placed on a copper grid. The sample was negatively stained with 2% uranyl acetate, pH 4·6.

RESULTS

Characterization of virulent phages with T1-like capsids

As described in Methods, we have isolated a series of 44 virulent phages which grow on E. coli B and whose capsid morphology is indistinguishable by electron microscopy from that of phage T1 (Fig. 1). Some of these phages were further characterized by the following four additional criteria: (i) staining pattern of virion polypeptides separated by SDS-PAGE, (ii) autoradiographic pattern of intracellular phage-coded polypeptides separated by SDS-PAGE, (iii) restriction endonuclease cleavage pattern of virion DNA and (iv) patterns of cross-reaction with anti-T1 serum.

Fig. 2 shows the profiles of virion polypeptides for a series of these phages and it is apparent that many similarities exist in terms of the total polypeptide complement, their relative abundances and molecular weights. This result adds credibility to our belief that these phages share common genetic properties. The patterns of intracellular phage-coded polypeptides showed a much greater degree of variation (Fig. 3).

The serological findings indicated only a limited degree of antigenic similarity. Phages 103 and D20 cross-reacted with anti-T1 serum with heterologous K values of 30% and 10% respectively, when compared with the homologous K value of 25 (Adams, 1959). This result suggests a relatively close genetic relationship of these two phages to T1, at least in terms of coat protein structure, whereas the other phages are not closely related by this criterion. In the case of D20 this would have been predicted in view of its capacity to form genetic recombinants with T1 (Trautner, 1960). The remaining phages showed no serological cross-inactivation.

A representative group of these phages was selected for restriction endonuclease analysis using the enzymes EcoRI, BglI and BglII. Examples of the restriction fragment patterns are shown by microdensitometer tracings in Fig. 4. Little, if any, similarity in band profile could be found among the members of this group either with each other or with T1. However, for phages Hi, 103, 150, 168, 171, 172, 174 and KD9 we observed submolar bands in the restriction patterns, as is the case for T1. An earlier study by Ramsay & Ritchie (1982) has also shown that phage D20 shares this property. These submolar bands did not represent partial digestion reactions since they were reproducible at different restriction enzyme concentrations. It is very likely, therefore, that they reflect, as in the case of T1 itself, the cleavage patterns of virion DNA populations containing limited circular permutations of DNA sequences. This would imply a headful mode of DNA packaging for these phages.

In vitro packaging of DNA from coliphages with T1-like capsids

In the course of these assays several different batches of extract were used which showed some variation in the efficiency of packaging of T1 DNA. The packaging efficiencies of heterologous DNAs were therefore determined in conjunction with a homologous control for the same batch of extract and are expressed as percentages of that control. The batch-to-batch variation in packaging efficiency for a given heterologous substrate was comparable to that for the homologous substrate, T1. The results showed clearly that all the heterologous DNAs were
Fig. 1. Electron micrographs of phages (a) T1, (b) 102, (c) 174, (d) 150, (e) 168 and (f) Hi (negatively stained with 2% uranyl acetate; bar marker represents 50 nm). All the T1-like phages investigated were indistinguishable from T1 by electron microscopy. Note the presence of four tail fibres, a feature not previously described for T1.

packaged by the T1 extracts although the efficiency of packaging varied considerably from phage to phage (Table 1). DNA from phages Hi, 172 and 150 was packaged with an efficiency very close to that of the homologous T1 system. At the other end of the range, phages 102 and D20 showed efficiencies of 1% or less while the remainder were in the 10 to 30% range.

In vitro packaging of λ DNA

The genome of phage λ can be packaged in vivo by T1 particles to form transducing phages able to transmit infectious λ to recipient cells (Drexler, 1970; Drexler & Christensen, 1979). According to Drexler (1984) this is possible because of the existence on the λ chromosome of a base sequence, called espλ, which is similar to the T1 pac site and which therefore can initiate
Fig. 2. Electrophoretic banding patterns of the coat proteins of phage T1 and some T1-like phages. Phages marked with an asterisk were used for the in vitro packaging studies. For comparison, the capsomere pattern of T7, the T7 relative 126, and several additional T1-like phages not referred to, are displayed on the same slab gels. Electrophoresis (150 V, 3 h) was in an SDS–polyacrylamide gradient (10 to 18%) and the protein bands were stained with Coomassie Brilliant Blue. Some of the major T7 capsomere proteins (left) are identified according to their genes (see Dunn & Studier, 1983); the terminology for the T1 particle proteins (right) is that of Martin et al. (1976) with the corresponding molecular weights ($\times 10^{-3}$) given in parentheses.
Fig. 3. Patterns of intracellular proteins coded for by T1 and the T1-like phages whose DNAs were packaged into T1 heads. *E. coli* B cells were u.v.-irradiated and infected with 5 to 10 phage particles/cell. [35S]Methionine was added after 1 min and incubation was continued at 37 °C for 15 min. The cells were disrupted and proteins subjected to SDS-PAGE, on a 10 to 18% gradient slab gel. Dried gels were autoradiographed to visualize the phage-coded proteins synthesized throughout the latent period. The pattern of T7-coded proteins is displayed for comparison with some selected T7-coded gene products indicated (left) as molecular weight references (see Dunn & Studier, 1983).
In vitro packaging of foreign DNA by phage T1

(a) Hi DNA cut with BglI; (b) 172 DNA cut with BglII.

Fig. 4. Restriction patterns of two of the T1-like phages used for in vitro DNA packaging. Data are presented as microdensitometer tracings of negatives of photographs of DNA digests electrophoresed on agarose gels. Peaks are lettered in order of decreasing molecular weight from left to right with the submolar bands marked with the letter s. (a) Hi DNA cut with BglI; (b) 172 DNA cut with BglII.

packaging by the T1 headful mechanism. Drexler & Christensen (1979) also proposed an esp site on the E. coli chromosome, situated between the gal operon and the att2 site which would be responsible for the enhanced efficiency of T1 transduction of bacterial markers, including the λ prophage, in the neighbourhood of this site.

On the basis of this information we reasoned that the T1 packaging system might package λ DNA and that the ability to package could depend on the presence of the λ esp site. This was tested by comparing the packaging of two λ DNA substrates, λ CI857 which carries the λ esp site and λ L47 which is deleted for the nin5 region known to include the esp2 site. Since initiation of packaging at the esp2 site could inactivate a monomer molecule we included as substrates (i) a standard preparation of λ DNA which will contain a proportion of annealed cos sites and therefore comprises a mixture of linear monomers, circular monomers and concatemers, (ii) ligated λ DNA which contains a high proportion of concatemers and (iii) heat-treated λ DNA (5 min at 70 °C) which comprises exclusively linear monomers.

The results (Table 2) show that both λ CI857 and λ L47 substrates are packaged to form infectious phages, albeit at low frequency and that, contrary to expectation, λ L47 is packaged up to 100-fold more efficiently than λ CI857. Neither ligation nor heat treatment of the substrate had any apparent effect. These results would appear to exclude a role for the λ esp site in the in vitro packaging reaction and a more important factor may be the length of the genome in view of the 20% size reduction of λ L47 DNA resulting from its development as a DNA cloning vector.

In vitro packaging of T3 and T7 DNA

The DNA of phages T3 and T7 have not been tested for their ability to be transduced by T1 and there is no information available for the presence of sites comparable to esp2.
Table 1. In vitro packaging efficiencies of DNA of T1-like phages

<table>
<thead>
<tr>
<th>Heterologous phage DNA</th>
<th>Packaging extract batch no.</th>
<th>P.f.u./μg DNA</th>
<th>Relative to T1 DNA (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hi</td>
<td>1</td>
<td>3.3 × 10⁴</td>
<td>5</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>3.1 × 10⁴</td>
<td>52</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>2.8 × 10⁴</td>
<td>34</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>3.2 × 10⁴</td>
<td>39</td>
</tr>
<tr>
<td>171</td>
<td>1</td>
<td>1.4 × 10⁴</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>9.2 × 10³</td>
<td>15</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>7.3 × 10³</td>
<td>9</td>
</tr>
<tr>
<td>172</td>
<td>2</td>
<td>3.1 × 10⁴</td>
<td>52</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>4.1 × 10⁴</td>
<td>50</td>
</tr>
<tr>
<td></td>
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<td>6.3 × 10⁴</td>
<td>115</td>
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<td>10</td>
</tr>
<tr>
<td></td>
<td>1</td>
<td>6.5 × 10⁴</td>
<td>9</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>2.4 × 10⁴</td>
<td>29</td>
</tr>
<tr>
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<td>1.0 × 10²</td>
<td>0.2</td>
</tr>
<tr>
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<td>5</td>
<td>4.8 × 10²</td>
<td>0.9</td>
</tr>
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<td>6</td>
<td>7.7 × 10⁴</td>
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<td>6.0 × 10⁴</td>
<td>109</td>
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<td>58</td>
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<td>7</td>
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<td>9.4 × 10³</td>
<td>17</td>
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</tr>
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</tr>
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<td>0.3</td>
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* The unit is p.f.u./μg DNA in a 50 μl reaction volume. All values are converted to the percentage of the T1 DNA packaging efficiency. Control platings of undiluted reaction mixtures from which either DNA or extract was omitted routinely gave zero plaques. This is equivalent to <1 p.f.u./μg DNA.

With a molecular weight of 25 × 10⁶ (40 kb) the virion DNA molecules are considerably shorter than for λ CI857 (50 kb) but of equivalent size to λ L47 (40-6 kb). Table 2 shows that T3 and T7 virion DNA molecules can also be packed by T1-infected cell extracts and at an efficiency intermediate between those of λ CI857 and λ L47. In order to ascertain that these phages constructed in vitro consisted of T3 or T7 genomes within T1 heads, a sample of the reaction mixture was treated with anti-T1 serum. Anti-T1 serum treatments which reduced the infectivity of T1 phage particles to about 1% of the initial titre reduced the titres of the T3 and T7 phages constructed in vitro to 30% (phage 168 particles constructed in vitro showed an intermediate sensitivity with 7% surviving phage). This observation that the constructed T3 and T7 phages, unlike normal T3 and T7 particles, were inactivated by anti-T1 serum clearly demonstrates that the capsids were T1-derived. The phages isolated from plaques produced by the packaging reaction were shown by electron microscopy to have the characteristic T3/T7 particle morphology and the DNA isolated from these phages had the BglII restriction patterns of standard T3 and T7 virion DNA molecules. Both these observations confirm that the T1 packaging system will package these heterologous DNA substrates.
In vitro packaging of foreign DNA by phage T1

Table 2. In vitro packaging efficiencies of lambda, T3 and T7 DNA*

<table>
<thead>
<tr>
<th>Heterologous phage DNA</th>
<th>Packaging extract batch no.</th>
<th>Packaging efficiency† P.f.u./μg DNA Relative to T1 DNA (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>λ CI857</td>
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<td></td>
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<tr>
<td></td>
<td>7</td>
<td>4.2 x 10^4</td>
</tr>
<tr>
<td>Heated</td>
<td>1</td>
<td>7.6 x 10^4</td>
</tr>
<tr>
<td>Ligated</td>
<td>6</td>
<td>7.4 x 10^4</td>
</tr>
<tr>
<td>λ L47</td>
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<td></td>
</tr>
<tr>
<td>Untreated</td>
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* For details, see Table 1.
† The unit is p.f.u./μg DNA in a 50 μl reaction volume. All values are converted to the percentage of the T1 packaging efficiency.

DISCUSSION

In vivo, it has been shown that T1 can transduce certain bacterial genes (Drexler, 1970) and entire genomes of phage λ (Drexler, 1984). In this paper, we have used a T1 in vitro packaging system to compare quantitatively the packaging efficiency of T1 DNA with that of heterologous phage DNA substrates.

One category of heterologous substrates comprised a collection of virulent phages having capsid structures indistinguishable from T1 as judged by electron microscopic analysis. DNA substrates extracted from these phages were packaged at efficiencies of about 1 to 100% that of a homologous T1 DNA substrate. No correlation was observed between the degree of apparent phylogenetic relatedness and packaging efficiency for different genomes. For example, phage D20 showed a rather strong similarity of intracellular protein patterns with T1, but its DNA was packaged very poorly into T1 heads, whereas DNA from phages Hi, 150, 168, 172, 174 and KD9 was packaged nearly as efficiently as T1 DNA, although the corresponding patterns of intracellular proteins revealed a more distant relationship to T1. It is likely that in vitro packaging of DNA from T1 relatives at comparable efficiencies to T1 is achieved by the same mechanism as for T1, that is by pac cleavage of a concatemeric intermediate (Ritchie et al., 1984; Liebeschuetz et al., 1985). Consistent with this view, all of the T1 relatives tested gave rise to submolar species of restriction fragments implying that they are packaged by such a mechanism in vivo. Variations in the packaging efficiencies of T1 relatives in vitro may reflect differences in the presumptive pac sites of these phages and the degree to which they are recognized by the T1 pac cleavage function.

Phage λ is less closely related to T1 than the first category of heterologous substrate but is better characterized. The finding that λ L47 DNA, which lacks the λ pac-like site esp, was packaged about 100 times more efficiently than λ CI857 DNA which contains this site, seems to exclude a role for esp in packaging of λ DNA into T1 heads in vitro. Instead, we propose that a secondary packaging mechanism exists in vitro, whereby genome-length DNA can be
encapsidated directly and not via a concatemeric intermediate. Consistent with this proposal, it was found that ligation of λ DNA prior to treatment with extracts did not increase packaging efficiency either for λ L47 or λ CI857. It may be that the optimum length of DNA molecule for encapsidation by this secondary mechanism is less than that for the headful mechanism which would explain why λ L47 (80% of T1 genome length) is packaged more efficiently than λ CI857 (100% of T1 genome length). As far as we are aware, this is the first suggestion that a phage which normally utilizes the headful packaging mechanism can in some circumstances encapsidate less than one headful of DNA.

The third category of heterologous substrates comprised DNA from phages T3 and T7 which have genome lengths of 80% that of T1. DNA substrates extracted from these phage were packaged at efficiencies of 0-03 to 1-5% that of T1, intermediate between that of λ CI857 and λ L47. It is possible that the packaging extracts convert virion DNA from T3 and T7 to concatemers which are subsequently cleaved at pac-like sites as yet uncharacterized. However, the finding that λ L47 DNA, a molecule of similar size to T3 and T7 DNA, can apparently be encapsidated directly in a pac-independent process, makes this rationalization seem relatively implausible, and we think it more likely that T3 and T7 DNAs are also packaged by the secondary mechanism mentioned above. The observation that the particles formed by packaging T3 and T7 (also 168) DNA molecules into T1 heads were less sensitive than T1 phages to inactivation by anti-T1 serum may reflect differences in particle structure imposed by the different DNA substrates.

Our results can be compared with those from similar experiments on two other phage systems. It has been found that in a P22 in vitro packaging system both homologous and heterologous DNA substrates are encapsidated directly, without concatemer formation, in a pac-independent process (Strobel et al., 1984; W. Behnisch & H. Schmieger, personal communication). A study of heterologous DNA packaging in a phage λ in vitro packaging system (Hohn, 1975) showed that DNA substrates from λ-related φ80 and φ21 were packaged at about 100% and 1% respectively of the efficiency of the homologous substrate, whereas phage P2 DNA was not packaged at detectable efficiency. Additionally, we have found that T1 virion DNA cannot be packaged into λ heads in vitro (J. Liebeschuetz & D. A. Ritchie, unpublished). The variation in packaging efficiency between φ21 and λ DNA substrates has been related to λ terminase recognition of a DNA sequence, close to but not within cos, containing two base pairs at which φ21 and λ have diverged (Feiss et al., 1981). The available evidence therefore suggests that the λ in vitro packaging mechanism has very specific DNA substrate requirements, and that sequence-independent packaging of heterologous DNA is not possible.

The picture emerging from the T1 in vitro packaging system shows elements in common with both the P22 and λ systems, but does not closely resemble either. We think that the homologous substrate and close relatives are packaged by sequence-specific pac cleavage of a concatemeric precursor. We also propose that there exists a secondary mechanism whereby DNA is encapsidated directly. It is probable that this mechanism does not require specific DNA substrate sequences and tolerates variations in substrate length.

The differences observed between the T1 and P22 in vitro DNA packaging systems, namely the ability of the former to package some substrates via a concatemeric intermediate, may reflect the relative abilities of the two systems to form concatemers rather than a fundamental difference in packaging specificity. For T1 we believe that concatemers are formed by a recombinational mechanism both in vivo and in vitro, whereas for P22 it is probable that concatemers are formed in vivo by rolling circle replication which may not be reproducible in vitro.

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