In Vitro Assembly of Bacteriophage Lambda Heads
(morphogenesis/DNA condensation/in vitro complementation)

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ABSTRACT The assembly of plaque-forming particles in cell-free extracts of induced lambda lysogens was observed two ways. (i) DNA isolated from a λ-related phage, 434 for example, is added to an extract of an induced λ lysogen, and plaque-formers with the genotype of the added DNA are detected. (ii) One extract from an induced λ lysogen that carries an amber mutation in one of the head genes (A, B, C, D, or E) is mixed with one carrying an amber mutation in a different head gene; an increase in the number of λ plaque-formers is found over that in either extract alone. These plaque-forming particles have the properties of normal plaque particles. They are resistant to DNase, although DNase added to an extract before addition of DNA prevents their appearance; they have a sensitivity to neutralizing antibody and a specificity of adsorption to bacteria characteristic of the source of the extract, but they have the genotype of the added DNA; and they have about the same buoyant density as phage particles.

Mutants in genes B, C, or D can donate DNA to the phage formed by complementation between extracts of different mutants, but mutants in genes A or E cannot. Complementation occurs between a pair of extracts only if one (or both) is a DNA donor. This observation suggests a tentative pathway for head assembly: that the products of genes A and E act before those of B, C, and D.

After the germinal discovery of Edgar and Wood (1) that bacteriophage T4 heads, tails, and tail fibers can join together in vitro, many steps in bacterial virus assembly were worked out. But the problem of how DNA-containing phage heads are assembled has remained unsolved. How is DNA cut, condensed, and packaged inside a protein coat? Phage λ may be a good subject for experimental exploration of this problem because its isometric head contains relatively few proteins, six perhaps (2), its head genes cluster and saturate the left end of the phage chromosome (3, 4), and several assembly reactions in vitro have already been demonstrated (5-7).

Eight proteins are required for λ head assembly: the protein products of seven phage genes, A, W, B, C, D, E, and F, (8) and the product of the bacterial gro E gene (9, 10). DNA molecules isolated from phage particles have two short, single-stranded ends of unique sequence (11). The precursor to this DNA is a polymer of more than two phage-sized units that lacks cohesive ends (12, 13). Two of the eight required proteins, the F and W proteins, act after DNA is cut and packaged, because W- and F- mutants accumulate morphologically normal heads that contain cohesive-ended monomers (7). These heads, however, cannot join to tails. Mutants in any of the other six genes, A, B, C, D, E, or gro E, accumulate precursor or precursor-like DNA (14-16, 9), as if DNA cutting depended on prior functioning of the products of all these genes. A challenging problem in head assembly is to explain how cutting and packing of DNA is coupled to aggregation of head protein subunits. As an initial experimental step toward the solution of this problem, we wish to report evidence for the in vitro assembly of λ heads.

MATERIALS AND METHODS

Bacteria, Phage, and DNA. All bacterial strains used for preparation of extracts were derived from the Su- prototrophic Escherichia coli K12 strain W3101 (17). A series of heat-inducible lysogens were prepared by infection of W3101 with each of the following strains of phage: λ cts577 Sam7, λ Aam1 cts857 Sam7, λ Aam32 cts857 Sam7, λ Bam10 cts857 Sam7, λ Bam427 cts857 Sam7, λ Cam42 cts857 Sam7, λ Dam15 cts857 Sam7, and λ Eam4 cts857 Sam7. DNA was extracted with water-saturated phenol from λimm 434 cts56 Sam7, φ80(19), and λimm21 (21 by 1 of ref. 20).

Preparation of Extracts. The procedure is an adaptation of that used by Wickner et al. (18) to prepare extracts active in DNA synthesis. Lysogenic bacteria were grown in H broth (containing per liter: 8 g of nutrient broth, 5 g of Bactopeptone, 1 g of glucose, 5 g of NaCl) to a density of 8 × 106 bacteria per ml. Then they were heat-induced by warming to 46°C for 15 min, with aeration. Bacteriophage were allowed to develop for 40 additional minutes at 37°C, by which time, determined by experiment to be the period of most rapid completion of phage particles, the bacterial mass had tripled. The culture was chilled on ice, and the bacteria were collected by centrifugation at 10,000 × g for 15 min at 5°C. The bacterial pellet was immediately suspended in 10% sucrose-50 mM Tris-HCl (pH 7.4) (200 ml of culture in 0.4 ml of sucrose-Tris). Aliquots of 0.1-0.6 ml were then quick-frozen and stored in liquid nitrogen. These frozen bacteria were stable for at least 1 month.

Frozen bacteria were thawed rapidly at 35°C, cooled to 0°C, and, for each 0.1 ml of bacteria, 5 μl of egg-white lysozyme (1 mg/ml in 0.25 M Tris-HCl (pH 7.4)) were added. The thawed bacteria were lysed by exposure to lysozyme for 30 min at 0°C and addition of 10 μl of the following mixture: 6 mM Tris-HCl (pH 7.4), 15 mM ATP, 18 mM MgCl2, 60 mM spermidine-HCl, 30 mM 2-mercaptoethanol. The lysed suspension was sedimented at 40,000 × g for 20 min at 5°C, and a resulting amber-colored, slightly opalescent supernatant was removed with a micropipet. Because of its instability, the supernatant, called "the extract," was kept at 0°C and was used within 30 min.

Abbreviation: PFU, plaque-forming units.
**Results**

**Head Assembly with Exogenous DNA.** If DNA isolated from λ imm 434 phage particles is added to a concentrated cell-free extract of an induced λ lysogen, then plaque-forming particles carrying 434 DNA are produced (Table 1). The extracts are prepared from a λ lysogen 40 min after thermal induction, at which time the bacteria are producing complete phage particles at maximum rate. The extracts contain about 10⁹ complete λ per ml, but 434 DNA-containing particles are specifically assayed by plating the incubation mixture for plaques on a λ lysogen. Because of the specificity of the λ repressor present in these bacteria, this assay has the capacity to detect one 434 particle against a background of more than 10⁹ λ particles.

The data presented in Table 1 also show that the number of 434 plaque-forming units (PFU) increases with amount of 434 DNA added, at a rate of about one plaque per 10⁶ DNA molecules. Pancreatic DNase added before phage DNA destroys the plaque-forming activity. Six times the amount of DNase used in the experiment reported in Table 1 is routinely added at the end of the incubation with DNA. Therefore, the extract converts the plaque-forming capacity of 434 DNA from a DNase-sensitive to a DNase-resistant state. The reaction is stimulated by ATP and is dependent upon addition of spermidine. Spermidine may help DNA condense (23), and it is required for λX174 DNA synthesis in similar extracts (24). In another experiment, 434 DNA was added to an extract of (mock-) induced nonlysogenic bacteria, but no 434 PFU were observed. Thus, some phage-specific product(s) must be present in the extract, a property that distinguishes these experiments from reports of λ multiplication in vitro (25, 26).

The buoyant density of the 434 PFU was measured in an equilibrium density gradient (upper panel, Fig. 1). Complete λ phage particles present in the extract before addition of 434 DNA serve as a density reference, but as confirmation that these λ had not been altered in some way as a result of the extraction procedure, λ ctλ+S phage was added to the assembly mixture in CsCl just before centrifugation. These phage form turbid plaques at 40° on Su− bacteria and can, therefore, be distinguished from endogenous λ ctλs857 Sam 7, which cannot plate on Su− bacteria and which form clear plaques on Su+ bacteria at 40°. The ratio of turbid to clear plaque-formers was about constant across the λ peak, showing that endogenous λ had normal buoyant density.

λ imm 434 phage form a band with its mode four fractions up-gradient from that of λ phage (bottom panel, Fig. 1). This occurs because imm434 DNA is 2% shorter than λ DNA but is enclosed in the same amount of protein as λ (27). The 434 PFU from an assembly mixture (Fig. 1, top panel) band more broadly than imm434 phage and have a mode 11 fractions up-gradient from λ, indicating an average 1% lower density than imm434 phage particles. Because, as will be shown below, the PFU contain an entire molecule of added DNA, their lowered density can be explained by the presence of 4% more than the normal phage content of protein. Perhaps a small but variable amount of protein is trapped inside a head as it forms in an extract.

Additional evidence that exogenous DNA can be encapsulated by extracts is presented in Table 2. Phage ϕ80, though related to λ, differs from it antigenically and in host range (28). Antibodies, raised in rabbits by inoculating them with purified λ, neutralize λ but not ϕ80. Lambda adsorbs well to phage T1-resistant strains of K12, symbolized K12/T1, but ϕ80 adsorbs poorly. DNA extracted from purified particles of phage ϕ80 was mixed with an extract of induced λ Su− (λ ctλs857 Sam7) bacteria, and ϕ80 plaque-formers were generated. As shown in Table 2, PFU from the assembly reaction were inactivated by antisera against λ at about the same rate as the endogenous λ present in the same mixture and many times faster than ϕ80 phage. The ϕ80 PFU proved to adsorb faster to K12/T1 than to K12, as did λ, whereas ϕ80 phage adsorbed more slowly to K12/T1 than to K12. Subsequently, the ϕ80 PFU were propagated on K12 Su3+ bacteria and, as shown by the last line of Table 2, as a result they regained the antisera resistance and adsorption properties of ϕ80 phage. Therefore, the ϕ80 PFU generated in an assembly mixture behave like ϕ80 DNA encapsulated in λ head and tail proteins provided by the extract. After propagation these phage regained ϕ80 adsorption and antibody specificity because they possessed ϕ80 genes.

**Head Assembly with Endogenous DNA.** If extracts of induced lysogens for amber mutants in head genes A, B, C, D, or E are mixed by pairs, then complementation between mutants in different genes can be observed. As shown in Table 3, the mixtures yield more plaque-forming particles than either of the component extracts alone. Only the A− plus E− mixture does not exceed its background, while the most efficient mixtures rise 80-times higher than their background. The ability to complement does not seem to depend on the particular amber mutants chosen: Δamber11 and Δamber19 have the same complementation pattern. So do Δamber10 and Δamber427 (data not shown).

As expected, these plaque-forming particles have the immunity specificity of λ: they form plaques on nonlysogenic
Su3+ bacteria, on Su3+(434), but not on Su3+(λ). No exogenous DNA has been added; as shown by their immunity specificity, the plaque-formers arise from DNA endogenous to the extracts. To determine the buoyant density of the plaque-formers, they were sedimented to equilibrium in CsCl solution. Comparison of the middle and bottom panels of Fig. 1 shows that the λ plaque-formers generated by the mixed extracts have the same average buoyant density, measured relative to added imm434 phage particles, as complete λ phage particles. Though the A− and C− extracts contain some λ plaque-formers before mixing, these are less than 10% of the total. In the experiment shown in the middle panel of Fig. 1, more than 50% of the λ plaque-formers added to the CsCl solution were recovered, and all of them were in the single λ peak shown. Therefore, at least 50% of the plaque-formers in that peak were generated by complementation.

Although both members of each pair of complementing extracts contain phage DNA, usually only one of them donates DNA to the phage particles formed by complementation. In the A− plus B− extract complementation, for example, all 75 of the progeny phage tested had a B− genotype, indicating that DNA was contributed by the B− extract to the phage formed by complementation. In general, extracts of B−, C−, and D− serve as DNA donors; extracts of A− and E− do not. A− or E− particles found in the complementation mixtures can be accounted for by the background plaque-forming activity of unmixed extracts.

Exogenous DNA can also be encapsulated by mixed extracts. As shown in Table 3, plaque-forming particles with 434 immunity specificity are formed from added imm434 DNA. The encapsulation of exogenous DNA is strongly correlated with the in vitro encapsulation of endogenous DNA: those pairs of extracts that complement for endogenous DNA encapsulate added imm434, with the possible exception of B− plus E−.

To test whether genetic recombination occurs between exogenous and endogenous DNA, we added λimm21 DNA to a mixture of an extract of a λ Aam11 clts857 Sam7 lysogen with an extract of a λ Cam42 clts857 Sam7 lysogen. The assembly mixture was plated on Su3+ bacteria at 42°, and the turbid plaque-formers were picked and purified on Su3+ bacteria. Finally, they were tested for immunity specificity and for their ability to form plaques on Su− bacteria. All 100 independent plaques tested proved to be λimm21 Aam+ Cam− Sam+. These genes span 95% of the λ genome, suggesting that the added imm21 DNA molecules were encapsulated intact.

**DISCUSSION**

The experiments reported here can be explained by the in vitro assembly of phage heads. To what extent can they be explained in other ways? For example, might the plaque-forming particles be DNA molecules adventitiously coated with protein from the concentrated extract? Probably not. The properties of these particles—their DNase resistance, buoyant density, sensitivity to neutralizing antibodies, and capacity to adsorb to certain bacteria—are those of phage particles. Assuming, then, that the reaction products are phage particles, might they be arising in the extract from surviving bacteria or spheroplasts infected with the added DNA or with DNA from a complementing extract? Probably not, for two reasons. First, bacteria and spheroplasts should have been removed by the centrifugation at 40,000 × g that precedes the use of the extracts. Second, the pattern of complementation differs from that observed in cells. Whereas in cells any pair of mutants in different genes complement each other (3), in extracts A− fails to complement E−.

Whereas mixedly infected cells yield a mixture of both in-
fecting genotypes, in extracts complementation of \( A^- \) or \( E^- \) with \( B^- \), \( C^- \), or \( D^- \) yields only \( B^- \), \( C^- \), or \( D^- \) offspring. (A few \( A^- \) or \( E^- \) particles are formed, but these are accounted for by the background activity of the \( A^- \) or \( E^- \) extracts.)

If it be granted that phage heads are being assembled in vitro, then the pattern of complementation suggests a pathway for head assembly. As a rule, the more steps required to complete an assembly process in vitro, the less efficient is that process. For example, gene \( F \) controls the last step in \( \lambda \) head assembly and gene \( W \) controls the next to last (7).

Under the same in vitro conditions, the conversion of \( W^- \) heads to complete phage particles is two to three orders of magnitude less efficient than conversion of \( F^- \) heads to complete phage (7). For this reason the failure of \( A^- \) or \( E^- \) extracts to donate DNA to the phage particles formed by complementation suggests that the reactions governed by the products of \( A \) and \( E \) occur before those governed by \( B, C, \) and \( D \).

There is evidence that the \( A \) and \( E \) proteins interact directly with \( \lambda \) DNA. Brody (submitted to Virology) has recently described a soluble form of \( E \) protein that binds DNA. \( E \) protein eventually makes up 75% of the mass of protein in the complete head (29, 30). Gene \( A \) appears to specify an enzyme that cleaves polymeric head precursor DNA into cohesive-ended monomers. Extracts of induced lysogens for mutants in genes \( B, C, D, \) or \( E \) contain, but mutants in gene \( A \) lack, an activity that, in crude extracts, produces molecules with cohesive ends from a mixture of dimeric and monomeric, covalently-closed \( \lambda \) DNA circles (31). The cohesive ends have a unique specific sequence (11), and the \( A \) protein must recognize that sequence, presumably by binding to it. However, if \( A \) protein cuts the cohesive ends, then

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<th>Table 2. Antigenic and adsorption specificity of plaque-formers assembled in vitro</th>
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<td>Fraction not neutralized by antiserum against ( \lambda )</td>
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<tr>
<td>( \phi )80 PFU</td>
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<td>( \lambda ) phage</td>
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<td>( \phi )80 phage</td>
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<td>( \phi )80 PFU after propagation</td>
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30 \( \mu \)l of \( \phi \)80 DNA, \( A_{365} = 5.2 \), was mixed with 100 \( \mu \)l of extract of \( Su^- \) (\( \lambda \) c7ts57 Sam7) and processed as described in Methods. After treatment with DNase, the mixture contained 1.6 \( \times 10^2 \) \( \phi \)80 PFU assayed on \( Su^+ \) \( \lambda \) and 3.7 \( \times 10^2 \) \( \lambda \) assayed on \( Su^+ \) bacteria. To one aliquot of this mixture, antiserum from rabbit against \( \lambda \) was added at a final dilution of 1/200. After 20 min at 25\(^\circ\), the mixture was assayed for the number of surviving \( \lambda \) and \( \phi \)80. Control \( \phi \)80 phage was treated separately. \( \phi \)80 PFU were purified and propagated through single plaque growth on \( Su^+ \) bacteria. To a second aliquot of the mixture, \( K12Su^+ \) bacteria were added, and to a third, \( K12Su^+ / T1 \) bacteria. After these mixtures were incubated for 15 min at 35\(^\circ\), the bacteria were sedimented to a pellet (at 5\(^\circ\)), and the unadsorbed phage remaining in the supernatant fluid were assayed. From the fraction of unadsorbed phage, the (pseudo-) first order rate of adsorption was calculated.

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<th>Table 3. Complementation between extracts of induced lysogens for ( \lambda ) head mutants</th>
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<td>Extracts mixed</td>
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<td>( A^- + B^- )</td>
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<td>( A^- )</td>
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<td>( E^- )</td>
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Lysogens carrying mutations in different head genes were grown, induced, and extracted. 50 \( \mu \)l of the extracts were mixed and then incubated for 80 min at 35\(^\circ\). DNAse, then 100 \( \mu \)l T broth were added. Finally 100 \( \mu \)l of each mixture (about half of the total volume) was plated on the \( Su^+ \) bacterial strain, Ymel. The number of plaques observed is given in the second column. The particular mutant alleles used were Asm11, Bam427, Cam42, Dam15, and Eam4. Plaques were picked; the phage were purified on \( Su^+ \) bacteria and tested for genotype by plating on a series of \( Su^- \) (odg \( imm434 \)) strains (22). \( S^- \) phage are able to form plaques on the \( dg \) lysogen that lacks the entire head region. To each of a similar set of mixtures of extracts, 0.11 \( \mu \)g of \( imm434 \) DNA was added. After incubation and DNase treatment, the entire volume of each complementation mixture was plated on \( Su^+ \) \( \lambda \). The number of plaques observed are recorded in the last column. The mutant alleles used were Asm32, Bam10, Cam42, Dam15, and Eam4. For purposes of comparison, the same amount of \( imm434 \) DNA added to an extract of a lysogen for \( \lambda \) c7ts57 Sam7 would have given 10\(^4\) plaques.

There is a paradox because, as indicated in Table 3, A gene function is required to assemble phage particles from exogenous DNA that already has cohesive ends. To resolve this paradox, we would like to suggest that A protein binds DNA at the cohesive end sequence as one of the initial steps in head assembly, but that it does not cleave the DNA until the entire head is formed. This notion might also help to explain why cohesive-ended monomers do not accumulate in bacteria infected with the head mutants \( A^- \), \( B^- \), \( C^- \), \( D^- \), or \( E^- \) (12, 14–16), how a DNA end is correctly positioned in the head to pass through the tail, and how the sequence of the cohesive end can influence phage particle assembly (32).

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