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PROCESSING AND ASSEMBLY OF THE HEAD OF BACTERIOPHAGE LAMBDA

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When phage DNA is added to an extract of an induced lambda lysogen, complete phage particles are made that contain the added DNA. The DNA substrate for packaging is a covalently joined polymer of several phage units. Unjoined units must first be joined by DNA ligase in the extract. Therefore DNA cutting is a necessary part of the DNA packaging reaction. The protein product of gene A, called A protein, behaves like the enzyme that cuts DNA and is a necessary component of the extract.

Three of the head proteins preassemble into a spherical shell that subsequently combines with DNA. These shells are made of E protein, the major protein of a finished head, and they can be the sole source of that protein. They also contain a few molecules of two processed proteins, fused C–E and cleaved B. The processing may be essential for assembly because other shells that contain C protein not fused and B protein uncleaved are less than 1% as active.

Protein A and DNA first react with the protein shells, then D protein, the second most abundant head protein, is added. These new observations are combined with published data to develop a comprehensive view of λ head assembly.

A macromolecular assembly process becomes irreversible if the covalent structure of the constituent subunits is altered after assembly has taken place. The head of bacteriophage lambda contains DNA and several proteins. During head assembly, the primary structures of three of the proteins and the DNA are altered by specific processing reactions. The purpose of this article is to show how processing is a necessary part of assembly.

Let us first consider the final state of the structure we wish to build. The λ head is a shell of protein covering a single molecule of DNA. Figure 1 shows these structures after negative staining. Some particles have lost their DNA as evidenced by entrance of stain into the head. The protein shell is 55 nm in diameter and since it encloses a DNA molecule 15,000 nm long, the DNA must be folded back and forth some 250 times. The surface of the head is covered by a regular array of bumps and the pattern of "fingers" described by Williams and Richards (1) is evident on some of the particles.

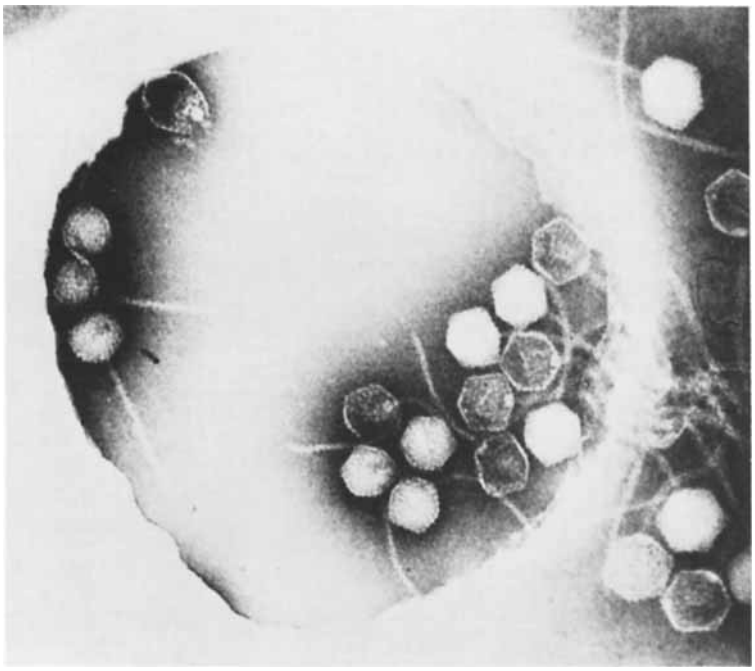

Fig. 1. Bacteriophage λ, negatively stained with phosphotungstic acid (× 130,000).

HEAD ASSEMBLY IN CELL-FREE EXTRACTS

Phage heads can be formed and added phage DNA can be packaged in a cell-free extract of an induced λ lysogen (2). Because the experiments described below utilize this in vitro packaging reaction it is necessary to describe the principle of its operation. An extract is prepared from an induced λ lysogen at the time phage are being formed at maximum rate. DNA is added to the extract and, after an incubation for assembly, newly formed infectious particles are counted by plaque assay.

To distinguish phage particles formed in the extract from those present in the cells at the time of extraction, one can add DNA from phage 434 hy to the extract of an induced λ lysogen and look for phage particles that contain 434 hy DNA. Because 434 hy resembles λ in every way except for the specificity of its repressor and operators (3), properties that are irrelevant to assembly, the 434 hy DNA competes with the λ DNA in the extract for head proteins. By virtue of the difference in repressor specificity, 434 hy phage particles can be detected in the presence of an excess of λ particles. Phage particles that contain 434 hy DNA form plaques on indicator bacteria that are lysogenic for λ and contain λ repressor; those particles that contain λ DNA do not form plaques.

PROCESSING DNA

The DNA molecule isolated from a complete head is a duplex with a unique base sequence. As depicted in Fig. 2, the ends of the molecule project out as two

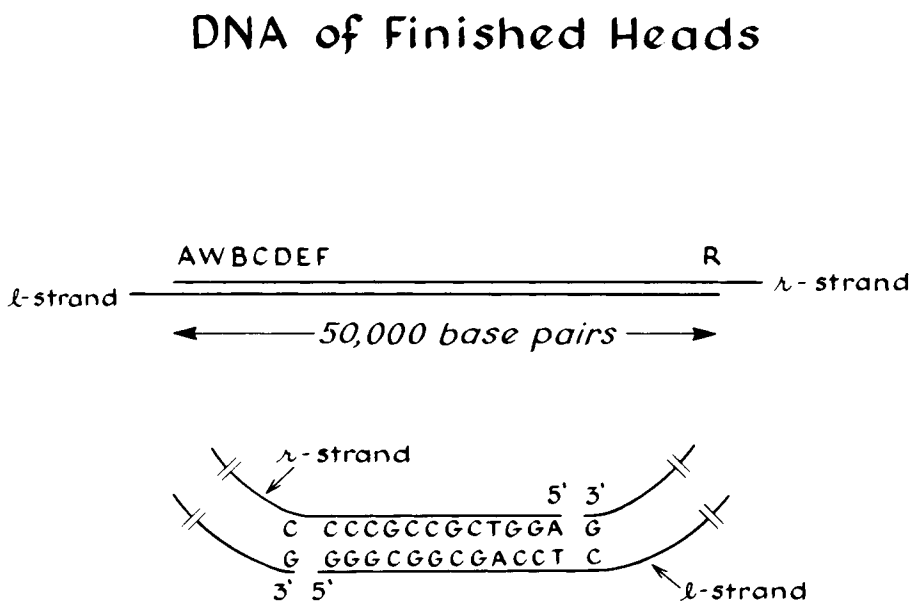

Fig. 2. Structure of λ DNA as isolated from bacteriophage particles. Head genes A through F are located at the left end. R at the right end is the gene for the phage endopeptidase (14). Below: the cohesive ends are shown enlarged and base paired. The sequence is based on that of Wu and Taylor (4).

5′ terminated single strands, 12 bases long, and having complementary sequences that allow the ends to cohere to each other (4).

As shown in Table I, molecules of the type found in phage particles, i.e., linear monomers with free cohesive ends, are not efficient precursors for heads assembled in extracts. Shortly after λ DNA injects into a bacterium, its ends cohere and are covalently closed (5, 6). Molecules of that sort, covalently closed circular monomers, are also poor precursors. The best substrate tested in Table I is a polymer formed by the joining of several molecules through their cohesive ends. Syvanen (7) has shown that DNA ligase in the extracts closes the missing phosphodiester bonds in these polymers and that this closure is a prerequisite for the high activity of the polymers. Though possibly surprising at first view, these observations are in accord with in vivo experiments showing that polymers, not monomers, are produced by λ DNA replication and are precursors to the DNA in complete heads. For example, when head protein assembly is blocked, λ DNA accumulates in the cell in polymeric form (8–11). Moreover, phage particles are not formed if phage DNA replication is altered in such a way as to permit formation of monomer but not polymer λ DNA inside cells (12, 13).

TABLE I. DNA Substrate for Packaging*

DNA	Plaques per 100 ng DNA
Linear monomers	50
Covalently closed circular monomers	3
Circular and linear polymers	14,000

*DNA was extracted with phenol from phage 434 hy that had been purified by equilibrium density gradient sedimentation. To prepare circular and linear polymers, DNA at a concentration of 40 μg/ml in 0.13 M KCl, 1 mM EDTA, 10 mM Tris, pH 8, was annealed by heating at 47° for 2 hr, then at 42° for 2 hr. To prepare linear monomers, the annealed DNA was heated to 70° for 10 min, cooled quickly to 0°, then assayed. Covalently closed circular monomers were prepared according to Wang (26).

The cell-free extract was prepared from induced W3101 su^- (λ cIts857 Sam 7). Extraction and incubation were as described by Kaiser and Masuda (2).

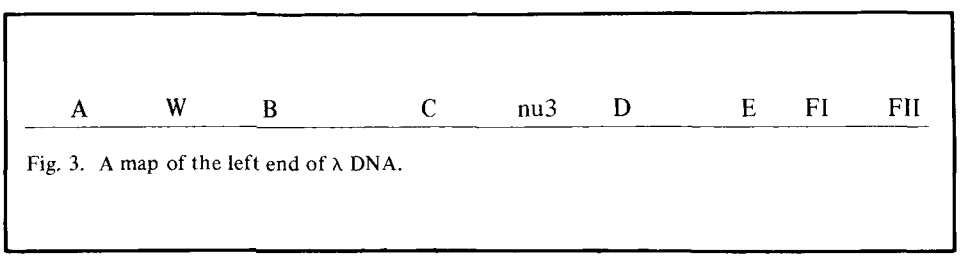

Fig. 3. A map of the left end of λ DNA.

GENES AND PROTEINS OF THE λ HEAD

Figure 3 is a genetic map for the left 8,000 base pairs of the mature λ DNA molecule (14). Nine genes whose products are directly required for head assembly are located there. The protein products of seven of these genes have been identified either in phage particles or in extracts of infected cells, as indicated in Table II. The aggregate molecular weight of the seven proteins is 295,000, corresponding to about

TABLE II. Protein Composition of Heads and Petit Lambda*

Protein	Mol. Wt.	Complete Heads	A⁻ Petit	groE Petit
E	38,000	+	+	+
Cleaved B	56,000	+	+	–
Fused C–E	31,000;	+	+	–
	29,000	+	+	–
D	11,000	+	–	–
FII	11,000	+	–	–
B	62,000	trace	–	+
C	64,000	–	–	+
nu3	19,000	–	–	+
A	90,000	–	–	–

*Data summarized from (17, 18). The protein products of gene W and of the newly recognized gene FI (27) have not yet been identified. The composition and properties of A⁻ petit λ are the same as wild-type petit λ (18).

8,000 base pairs of coding DNA. In view of the need to accommodate the protein products of the two remaining genes, it seems likely that almost all of the head genes in this region have been identified. In λ, genes with related functions are clustered (15), and no head genes have been found outside the "head region". Thus the λ head is a protein–nucleic acid assembly for which all or almost all of the necessary components have been recognized.

Two sorts of processed proteins are found in the completed head (Table II). One is a cleavage product of the protein specified by gene B, from which about 6,000 daltons have been removed (16). There are about 12 molecules of cleaved B per head. The second is a pair of proteins formed by covalent fusion of the proteins specified by gene C and that specified by gene E followed by cleavage of both the C and E parts (17). There are also about 12 molecules of fused C–E per head. Two phage proteins needed for head assembly are not incorporated into the completed head: the products of the A and nu3 genes (Table II). The bulk of the protein mass of the head (95%) is made up of the products of genes D and E. There are 420 molecules of each of these proteins interspersed in a mosaic pattern. Antibodies to E protein and antibodies to D protein can be seen attached over the entire head surface (18). Williams and Richards (1) have proposed an arrangement of 140 trimers of E protein, plus 60 hexamers and 12 pentamers of D protein on an icosahedral T=7 lattice that accounts for both the chemical data and the electron microscope images of λ heads.

HEAD ASSEMBLY FROM PETIT LAMBDA

Whenever λ grows, in addition to phage particles there appear small particles, devoid of DNA and tail, called petit lambda (19). Figure 4 is an electron micrograph of a mixture of λ and petit λ. Petit λ is smaller, less angular than the finished head, and contains E but no D protein (16, 20). The first idea was that the petit λ was a by-product of head assembly (19), but Hendrix's finding (summarized in Table II) that it contained the processed proteins, cleaved B and fused C–E, suggested instead that petit λ might be a precursor. A second type of petit λ was found after λ infection of a bacterial mutant called groE that specifically blocks head assembly (21). GroE petit λ contains E protein, the unprocessed forms of B and C proteins, and an additional pro-

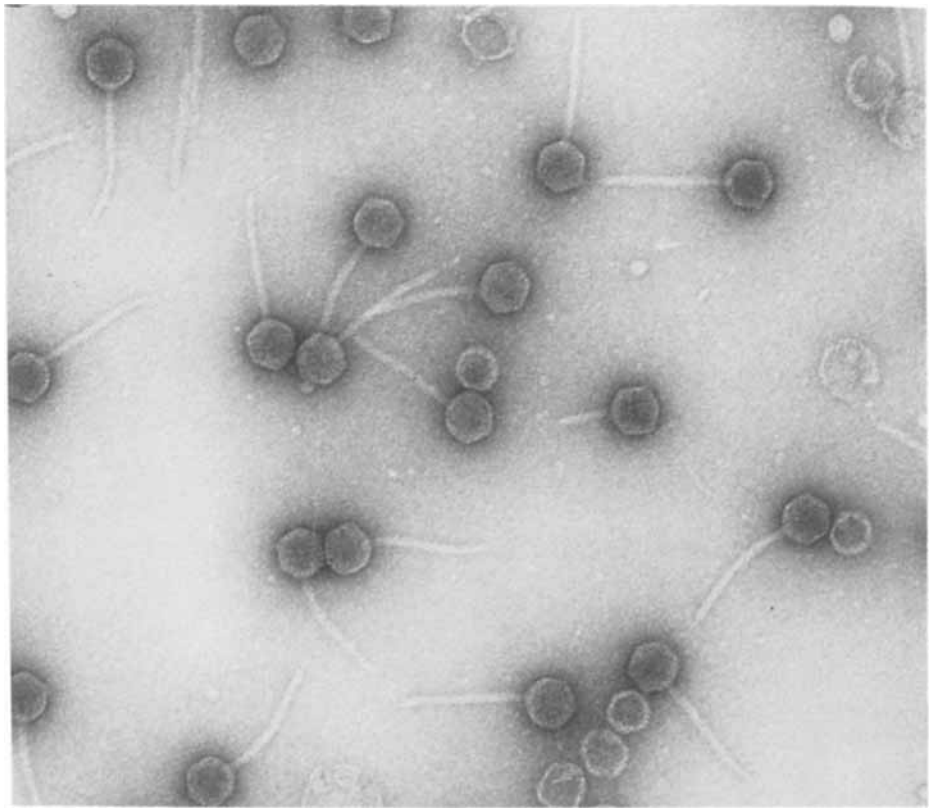

Fig. 4. Petit λ and bacteriophage λ, negatively stained with uranyl acetate (X 130,000).

tein likely to be the product of the gene nu3 (Table II).

The cell-free extracts described above were used to test whether petit λ is a precursor to the head. If purified petit λ is added to an extract prepared from a lysogen for λ Eam$^-$, then the only source of the major head protein, E protein, is petit λ. As shown in Table III, phage particles are indeed formed from such a mixture. All three components, petit λ, DNA, and extract are required. The reaction also requires ATP and spermidine (2). During the course of the reaction, DNA is converted from a DNase sensitive to a DNase resistant state, indicating its enclosure in a protein coat. The reaction product has the buoyant density of a phage particle showing that it has the proper DNA to protein ratio. These results imply that petit λ can be an efficient precursor of normal phage heads.

If, instead of A$^-$ or wild-type petit λ (which have the same composition) one uses petit λ prepared from infected groE bacteria, ten thousand times fewer phage are formed (Table III). Since complete heads contain the processed proteins and since A$^-$ petit λ has the processed proteins while groE petit λ has unprocessed proteins plus nu3 protein, it appears that head proteins must be processed before petit λ reacts with DNA. The low but significant level of activity observed with groE petit λ (Table III) suggests that unprocessed petit λ may be the precursor to the processed form.

When the processed form of petit λ gives rise to phage particles, some of the head gene products no longer need be added to the assembly mixture. As shown in Table IV, phage particles are formed from extracts that lack B, C, E, or nu3 proteins,

TABLE III. Head Assembly from Petit Lambda*

Components	Plaques
Complete	1.8×10^6
Minus petit λ	<10
Minus DNA	<10
Minus extract	<10
groE petit λ instead of A^- petit λ	1.2×10^2
Plus DNase at t=60 min	8×10^5
Plus DNase at t=0 min	<10

*Complete system contains 10^9 purified A^- petit λ, 10^9 molecules 434 hy DNA, and extract of 5×10^7 cells in a total volume of 5 μl. The extract was prepared from induced W3101 su^- (λ Eam4 cIts857 Sam7) and was incubated as described by Kaiser and Masuda (2).

or that are defective for groE. These proteins must therefore be involved only in the assembly of the active form of petit λ. The same conclusion is also suggested by the chemical analyses of λ and petit λ (16). The gene products which must be supplied by the extract to convert petit λ into a head, namely A, D, W, and F proteins and tails, must therefore be required later in the assembly pathway.

This is already known to be true for W and F proteins and tails. Working backward through the assembly pathway, tails are added to finished heads to form infective phage particles (22). F protein is the connector between head and tail; it gives the head its specificity for tail joining (23). W protein must react with the head before it will accept F protein (24). Consequently the reactions with W protein, F protein, and tails are obligatorily ordered. The head precursor for these three terminal reactions contains λ DNA monomers with cohesive ends and has the same morphology as a finished head (24). Thus we are left to explain how A protein, D protein, and DNA combine with petit λ to cut DNA and to yield this precursor particle.

TABLE IV. Protein Requirements for Assembly from Petit Lambda*

Extract Lacking	Present in Petit	Assembly Titer
B	+	5.2×10^6
C	+	9.2×10^6
E	+	1.2×10^6
nu3		7.5×10^5
groE		3.4×10^5
A	–	<10
D	–	<10
W	–	<10
F	–	60
tails	–	<10

*Assembly conditions are described in footnote to Table III. Extracts lacking a particular protein were prepared from an induced su^- lysogen for an amber mutant in the appropriate phage gene. An extract lacking an active bacterial groE protein was prepared from groE 140 (λ cIts857 Sam7).

THE ROLES OF A AND D PROTEINS

Which combines first with DNA and petit λ, A or D protein? Assuming that penetration of DNA into petit λ would protect DNA from hydrolysis by DNase, petit λ was added to DNA and an extract containing either both A and D proteins, or only A protein, or only D protein. Following a short incubation, pancreatic DNase was added, then the missing component (D or A protein) with W, F, and tails. The results shown in Fig. 5, are that A and D together convert added DNA to a highly resistant form; D protein alone gives very little resistance; whereas A protein alone gives an intermediate degree of DNase resistance. This result is most easily explained if, as suggested in Fig. 6, A protein reacts first with DNA and petit λ, allowing DNA to enter petit λ and become sequestered from external DNase. DNA is not cut in this initial reaction with A protein and petit λ because polymeric DNA accumulates in bacteria infected with λ D^- mutants (11).

Electron micrographs of negatively stained particles show petit λ to be smaller than a finished head (19; cf Fig. 4). This suggests that petit λ must expand to accept DNA and the role of D protein could be to fill holes opened by expansion. The Williams-Richards arrangement of D and E protein subunits in a complete head fits the idea that D protein enters into a preexisting matrix of E protein. In the final structure each hexamer or pentamer of D protein is completely surrounded by trimers of E protein, whereas the trimers of E protein form a self-connected surface (1). The idea that D protein is added after DNA has entered petit λ also explains why full-sized heads are not formed in thymine-starved λ infected bacteria (19), even though DNA is not required for the continued stability of the head (Fig. 1).

Since polymeric DNA is the precursor to monomeric DNA in the finished head, DNA must be cut in staggered fashion to give the unique 12 base long, single stranded ends (Fig. 2). Cutting occurs after D protein has been added because the next step requires W protein and the heads that form in λ W^- infected bacteria contain cut DNA (24). A protein is believed to be the enzyme or part of the enzyme that cuts DNA because crude extracts of induced lysogenic bacteria that contain A protein but lack C, D, E, FI, and FII proteins cut covalently closed circular λ DNA to produce cohesive ended molecules (25). That reaction is stimulated by ATP. Thus it seems likely that A protein binds its specific sequence to initiate the reaction between DNA and petit λ but does not cut the DNA until D protein has been incorporated into the nascent head. A protein must be released at this point because it is not included in the finished head (23).

In conclusion, Fig. 6 summarizes the role we believe each of the proteins plays in head assembly. First E protein assembles into a spherical shell with smaller amounts of B, C, and nu3 proteins. Then under the influence of the bacterial groE protein, B protein is cleaved, C is fused to E protein, the fused molecules are cleaved, and nu3 protein is expelled. The resulting protein shell then reacts with polymeric DNA and A protein, DNA enters, the shell expands, and D proteins fills the holes thus created. This triggers A protein to cut the DNA. Finally W protein can react, F protein adds and a tail joins to complete the structure.

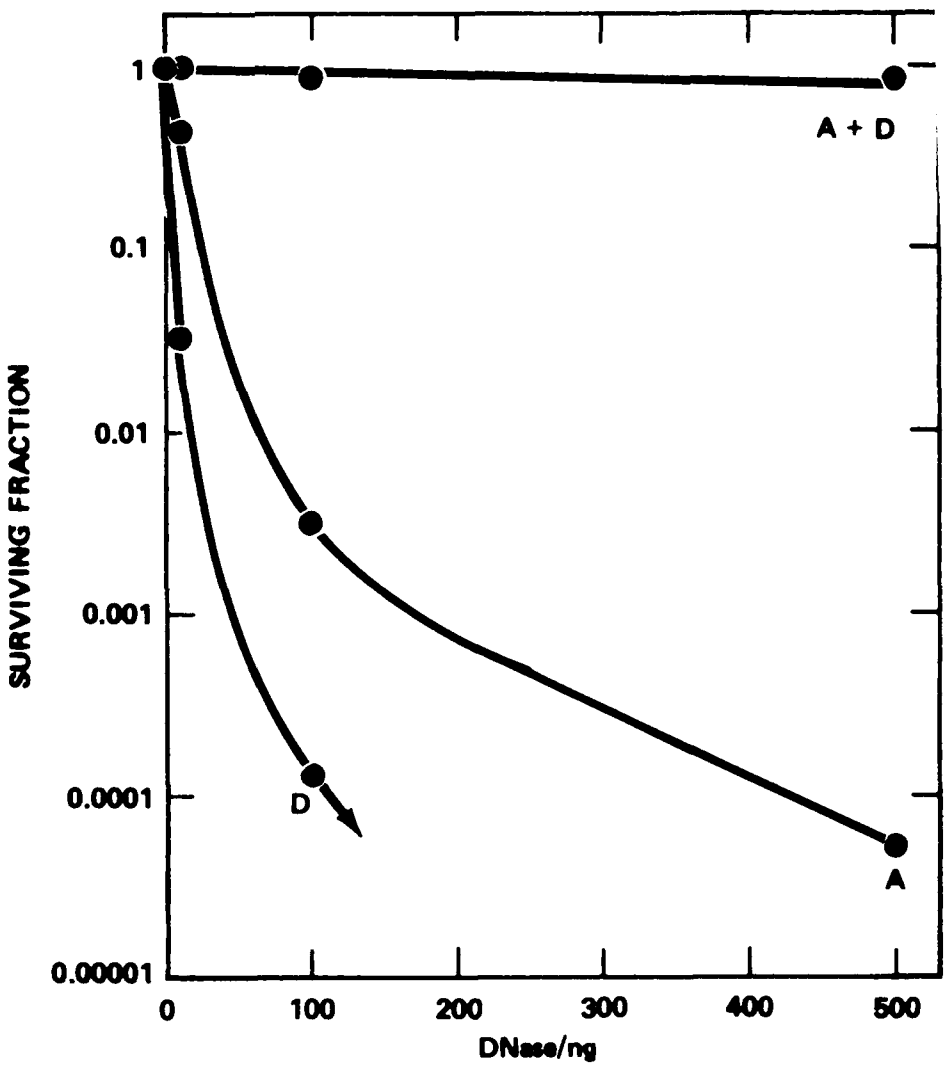

Fig. 5. Sequence of reactions with A and D proteins. To a mixture of petit λ and 434 hy DNA was added *either* an extract of an induced lysogen for λ Dam⁻ cIts857 Sam7 as a source of A protein (middle curve), *or* an extract of an induced lysogen for λ Aam⁻ cIts857 Sam 7 as a source of D protein (lower curve), *or* both extracts (upper curve). After 10 min at 37° Pancreatic DNase I in the concentration given on the abscissa was added. Immediately, the complementary extract was added as a source of D or A protein, and of W protein, F protein and tails; the incubation was continued for 60 min and the mixture assayed for number of 434 hy phage formed. The ordinate gives the ratio of the number of plaque-forming phage produced in the presence of a given concentration of DNase to the number produced when only DNase diluent was added.

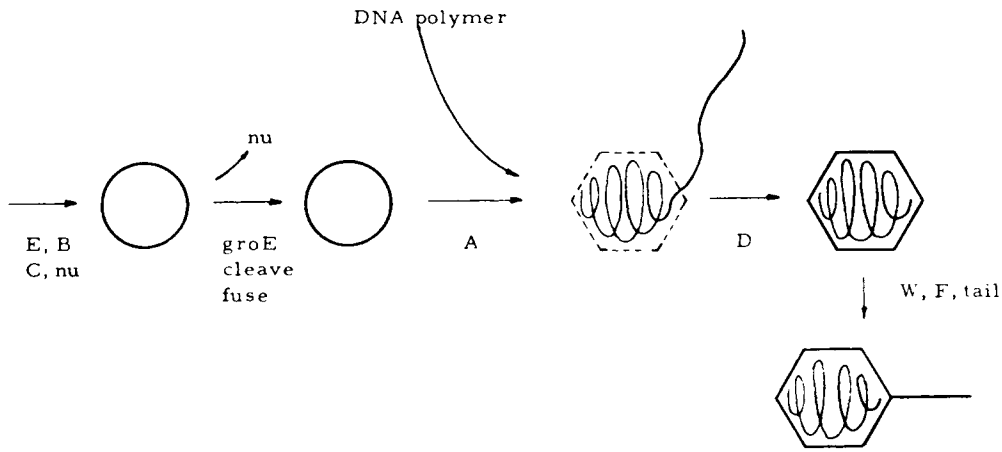

Fig. 6. Sequence of head assembly reactions. Circles represent petit λ. Other components are defined in the text.

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REFERENCES

1. Williams, R. C., and Richards, K. E., J. Mol. Biol. (in press).
2. Kaiser, D., and Masuda, T., Proc. Nat. Acad. Sci. U.S. 70:260 (1973).
3. Ptashne, M., Nature 214:232 (1967).
4. Wu, R., and Taylor, E., J. Mol. Biol. 57:491 (1971).
5. Young, E. T., and Sinsheimer, R. L., J. Mol. Biol. 10:562 (1964).
6. Bode, V. C., and Kaiser, A. D., J. Mol. Biol. 14:399 (1965).
7. Syvanen, M., J. Mol. Biol. (in press).
8. Weissbach, A., Bartl, P., and Salzman, L. A., Cold Spring Harbor Symp. Quant. Biol. 33:525 (1968).
9. Kiger, J. A., and Sinsheimer, R. L., J. Mol. Biol. 40:467 (1969).
10. Skalka, A., "The Bacteriophage Lambda," p. 535. Cold Spring Harbor Laboratory (1971).
11. Wake, R. G., Kaiser, A. D., and Inman, R. B., J. Mol. Biol. 64:519 (1972).
12. Stahl, F. W., McMilin, K. D., Stahl, M., Malone, R. E., and Nozu, Y., J. Mol. Biol. 68:57 (1972).
13. Enquist, L. W., and Skalka, A., J. Mol. Biol. 75:185 (1973).
14. Davidson, N., and Szybalski, W., "The Bacteriophage Lambda," p. 45. Cold Spring Harbor Laboratory (1971).
15. Dover, W. F., "The Bacteriophage Lambda," p. 297. Cold Spring Harbor Laboratory (1971).
16. Hendrix, R. W., and Casjens, S. R., J. Supramol. Struct. 2:329 (1974).
17. Hendrix, R. W., and Casjens, S. R., Proc. Nat. Acad. Sci. U.S. 71:1451 (1974).
18. Hendrix, R. W., and Casjens, S. R., J. Mol. Biol. (in press).

19. Karamata, D., Kellenberger, E., Kellenberger, G., Terzi, M., Path. Microbiol. 25:575 (1962).
20. Casjens, S., Hohn, T., and Kaiser, A. D., Virology 42:496 (1970).
21. Georgopoulos, C. P., Hendrix, R. W., Casjens, S. R., and Kaiser, A. D., J. Mol. Biol. 76:45 (1973).
22. Weigle, J., Proc. Nat. Acad. Sci. U.S. 55:1462 (1966).
23. Casjens, S. R., (in preparation).
24. Casjens, S., Hohn, T., and Kaiser, A. D., J. Mol. Biol., 64:551 (1972).
25. Wang, J. C., and Kaiser, A. D., Nature New Biol., 241:16 (1973).
26. Wang, J. C., J. Mol. BIol. 43:263 (1969).
27. Boklage, C. E., Wong, E. C., and Bode, V. C., Genetics 75:221 (1974).
28. Parkinson, J. S., and Davis, R. W., J. Mol. Biol. 56:425 (1971).