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Packaging of Genomes in Bacteriophages: A Comparison of ssRNA Bacteriophages and dsDNA Bactgeriophages

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Packaging of genomes in bacteriophages: a comparison of ssRNA bacteriophages and dsDNA bacteriophages

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In complex DNA bacteriophages like λ, T4, T7, P22, P2, the DNA is packaged into a preformed precursor particle which sometimes has a smaller size and often a shape different from that of the phage head. This packaging mechanism is different from the one suggested for the RNA phages, according to which RNA nucleates the shell formation.

The different mechanisms could be understood by comparing the genomes to be packaged: single stranded fII RNA has a very compact structure with high helix content. It might easily form quasispherical structures in solution (as seen in the electron microscope by Thach & Thach (1973)) around which the capsid could assemble. Double stranded phage DNA, on the other hand, is a rigid molecule which occupies a large volume in solution and has to be concentrated 15-fold during packaging into the preformed capsid, and the change in the capsid structure observed hereby might provide the necessary DNA condensation energy.

Bacteriophages containing single stranded (ss) RNA and those containing linear double stranded (ds) DNA are assembled according to different mechanisms (figure 1). The ssRNA of bacteriophages fII (fII is used for the group of strongly related bacteriophages f2, fr, M12, MS2, R17 (Hohn & Hohn 1970)) and Qβ coassembles with the capsid subunits, the dsDNA of bacteriophages such as P22, λ or T4 is filled into a preformed capsid (prehead, prohead) (articles in this book by King *et al.* 1976; Hohn, Wurtz & Hohn 1976; Kellenberger 1976; Laemmli 1976). The different packaging mechanisms can be understood by considering the physical properties of the genomes to be packaged. ssRNA can exist in solution as a compact quasi-spherical molecule, dsDNA is a rigid and extended molecule which occupies a large volume in solution and has to be condensed many-fold for packaging.

Reviews have been published concerning some aspects of the subject of this contribution. These should be consulted for further details and references (Hohn & Hohn 1970; Kozak & Nathans 1972; Hindley 1973; Weissmann *et al.* 1973; Zinder 1975).

The evidence for the packaging mechanisms

Most of the evidence for dsDNA packaging into a protein prehead (figure 1*b*) is presented elsewhere in this book (King *et al.* 1976; Hohn *et al.* 1976; Kellenberger 1976). For additional illustration, table 1 shows that the formation of DNA-free bacteriophage λ preheads, as assayed by their ability to package added DNA, is independent of DNA synthesis.

In contrast, all attempts to package ssRNA into preformed RNA bacteriophage capsids have failed so far (Stavis & August 1970). This could mean that the appropriate precursor capsid has not yet been found or that RNA forms a core around which the capsid assembles, whereby the capsid protein might codetermine the final shape of the core. Evidence for this

co-assembly of capsid protein and RNA stems from *in vitro* self-assembly experiments: The RNA bacteriophage capsid will form *in vitro* in the absence or in the presence of RNA. The RNA-free assembly, however, seems not to be the physiological mechanism, since (1) the speed of particle formation is highly increased in presence of RNA (figure 2), (2) particles can be formed from solutions of much lower protein concentration in the presence of RNA

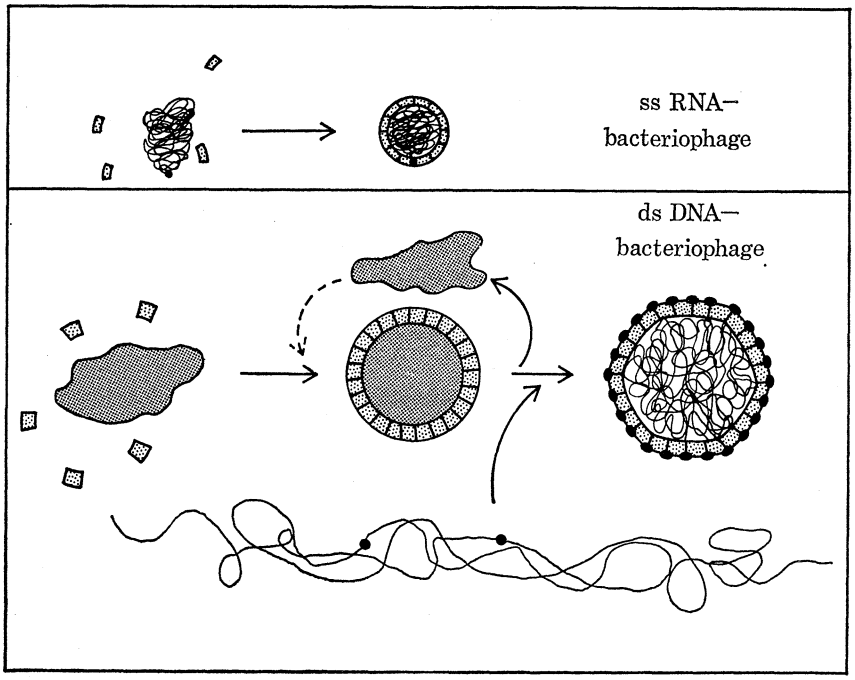

FIGURE 1. Genome packaging in bacteriophages. (*a*) Capsid formation and genome encapsidation is coupled. The single stranded RNA in bacteriophages fII and Qβ acts as a core and initiator of capsid formation. (*b*) Capsid formation and genome encapsidation is uncoupled. A number of bacteriophages build preheads with the aid of core protein. During maturation the core protein is released and double stranded DNA is packed into the capsid.

TABLE 1. BACTERIOPHAGE LAMBDA PREHEADS IN LYSATES DEFECTIVE IN DNA SYNTHESIS

BHB 2385 thy⁻ is a thymidine auxotrophe carrying a λ prophage. It was induced and grown for 60 min in media supplemented with different amounts of thymidine. After harvesting mature bacteriophages were plated on a suitable indicator. Preheads were tested after *in vitro* complementation with heteroimmune DNA by selective plating. (Details: Hohn *et al.* 1975.)

strain	condition	phage produced % p.f.u.	prehead produced % complementation
BHB 2385 thy⁻	no thymidine	3	76
	0.2 μg/ml Thy	10	100
	0.5 μg/ml Thy	30	89
	40 μg/ml Thy	100	52

than in its absence (figure 3), (3) particles formed in the presence of RNA are more uniform than those formed in absence of RNA: In the absence of RNA, several types of abnormal particles are assembled such as double shells (Hohn 1969) and multishells (Schubert & Franck 1971; Oriel & Cleveland 1970). In the presence of RNA mainly normal sized capsids containing RNA are formed. Thus RNA acts as nucleating agent for capsid formation and becomes packaged.

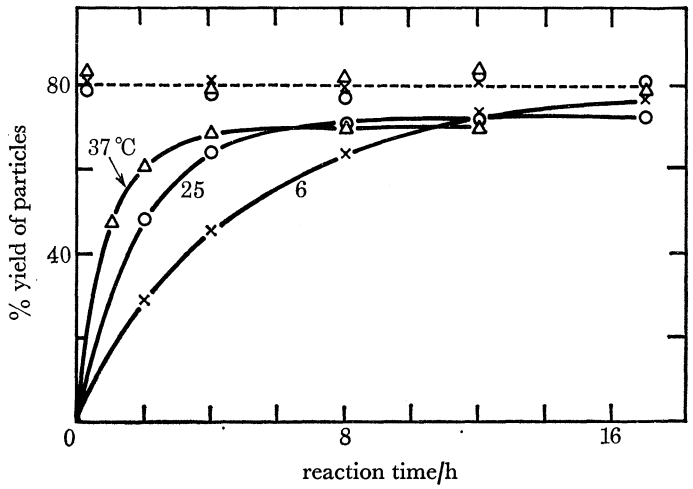

FIGURE 2. Kinetics of the self-assembly of fII defective particles and fII protein particles. – – – –, defective particles in the presence of RNA; ——, protein particles in the absence of RNA: (×) at 6 °C, (○) at 25 °C, (△) at 37 °C. Yield is defined as the percentage of protein in sucrose gradients sedimenting between 50 and 80S (Hohn 1969*b*).

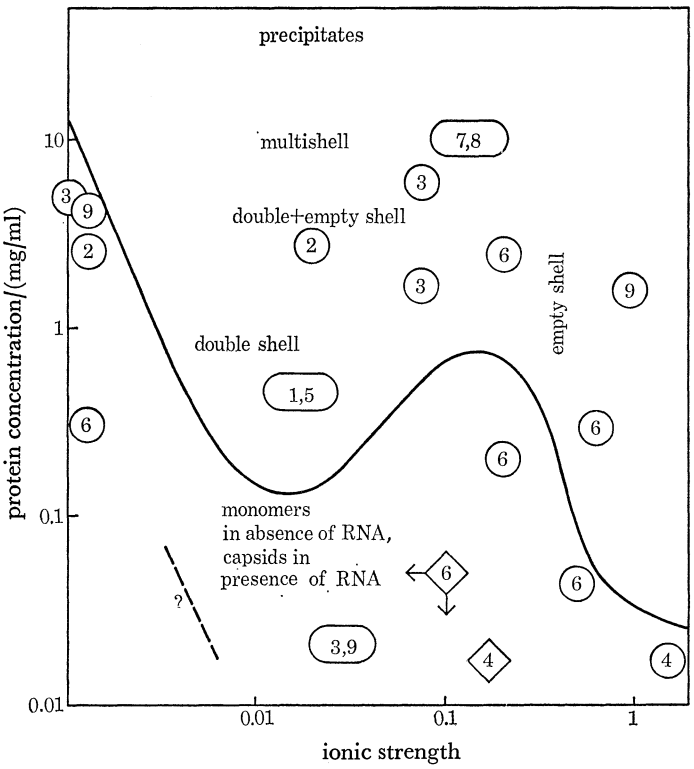

FIGURE 3. Diagram summarizing the conditions at which various assemblies of fII coat protein forms. Numbers refer to experiments reported by Hohn 1969*a* (1); Rohrmann & Krüger 1970 (2); Schubert & Franck 1971 (3); Hohn 1967 (4); Hermann *et al.* 1968 (5); Matthews & Cole 1972 (6); Oriel & Cleveland 1970 (7); Oriel, Lindsey & Schueneman 1971 (8); Zipper, Kratky & Schubert 1971 (9). ○, experiments in absence of RNA, ◇, experiments in presence of RNA.

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

Genome condensation

Genomes packaged in bacteriophage capsids, either ssRNA or dsDNA, occupy a volume which is of the same order of magnitude as that of biomolecules in crystals, i.e. about 3×10^3 nm^3 for each 10^6 molecular mass (table 2). As free molecules in solution and probably in bacteria, they occupy larger volumes (table 2). In the following some properties of genomes in solution are described in relation to their ability to condense to the volume occupied in phage.

TABLE 2. VOLUME REQUIRED BY BIOMOLECULES OF MOLECULAR MASS 10^6

	$\frac{V}{\text{nm}^3}$	references
in crystal		
40 molecules chymotrypsinogen	2.8×10^3	Matthews 1968
40 molecules tRNA	2×10^3; 5×10^3	Young *et al.* 1969, Johnson *et al.* 1970
inside bacteriophage capsids		
fII RNA	4×10^3	Zipper *et al.* 1971
1/30 of λ DNA	3.2×10^3	Kistler *et al.*, in prep.
1/27 of P22 DNA	3×10^3	Earnshaw *et al.*, 1976
in buffer		
fII RNA at pH = 7 I = 0.1	12×10^3	Siegers *et al.* 1973; Zipper *et al.* 1975
fII RNA at pH = 3.8 I = 0.1	1.7×10^3	Siegers *et al.* 1973
1/30 of λ DNA at pH = 8 I = 0.1	8×10^6	†
on electron microscopical grid		
fII RNA stained with uranyl acetate	$\sim 10^3$	Thach & Thach 1973
inside bacteria		
1/2.5 × 10^3 of *E. coli* chromosome	2×10^5	Ryter 1968, Worcel & Burgi 1972

† Calculated via the Stokes radius (r_s): $\frac{V = 4r_s^3\pi}{3}$; $r_s = \frac{M\,(1-\bar{\nu}\rho)}{6\pi\,\eta SN}$ ($M = 3 \times 10^7$) (Davidson & Szybalsky 1971), $\bar{\nu} = 0.53$ cm^3/g (value for *E. coli* DNA; Worcel & Burgi 1972), $\eta = 10.08 \times 10^{-4}$ Pa s, $S = 32 \times 10^{-13}$ cm/s (Davidson & Szybalsky 1971), $\rho = 1$ g/cm^3.

Bacteriophage fII ssRNA, a molecule of 1.15×10^6 (Boedtker & Gesteland 1975) probably has a specific secondary and tertiary structure. Hypochromicity data allow the estimate that 70% of the bases are involved in base pairing (Boedtker 1968); the RNA sequence, which is now totally known, can be arranged into plausible secondary structures as was shown for the coat protein cistron (figure 4) (Min You, Haegeman, Ysebaert & Fiers 1972; Fiers *et al.* 1975). That these hypothetical RNA hairpins accord with reality is indicated by the yield of very specific RNA fragments after digestion with limiting amounts of RNase (discussed in Boedtker & Gesteland 1975). Compared with protein molecules, however the tertiary structure of RNA might be more flexible and capable of being modified in its structure by certain regulatory proteins. Thus, binding of coat protein, replicase or A protein (figure 5; references in the legend) might shift the RNA tertiary structure to that suitable for one or the other of its working modes: the mode for replication, various modes for regulated translation and the mode for nucleating capsid formation. The best studied example is the binding of the coat protein to the β initiation hairpin (marked in figure 4). In this case the binding of the protein slows down the melting of the hairpin 100-fold (Gralla, Steitz & Crothers 1974). Phage RNA can thus be seen as a dynamic molecule restricted in its movements by its selfmade environment of proteins.

The secondary and tertiary structure make the fII RNA a fairly compact molecule. Thus at neutral pH and physiological ionic strength, the volume occupied is only three times larger

than the space available inside the phage capsid. In acidic solution fII RNA exists in an even more condensed state because the repulsive negative charges are neutralized. This compact state is probably the one observed by Thach & Thach (1973) in the electron microscope after staining with (acidic) uranyl acetate. fII capsids are negatively charged outside, hydrophobic at the subunit–subunit interaction sites and positively charged on the inner side (Matthews & Cole 1972). It is conceivable that these positive charges on the capsid subunits will neutralize some of the negative charges of the RNA during assembly and thus bring it to the most compact form. On the other hand the RNA causes a high local concentration of capsid protein and thus 'catalyses' the capsid assembly. Thus final RNA condensation and capsid formation are mutually dependent processes.

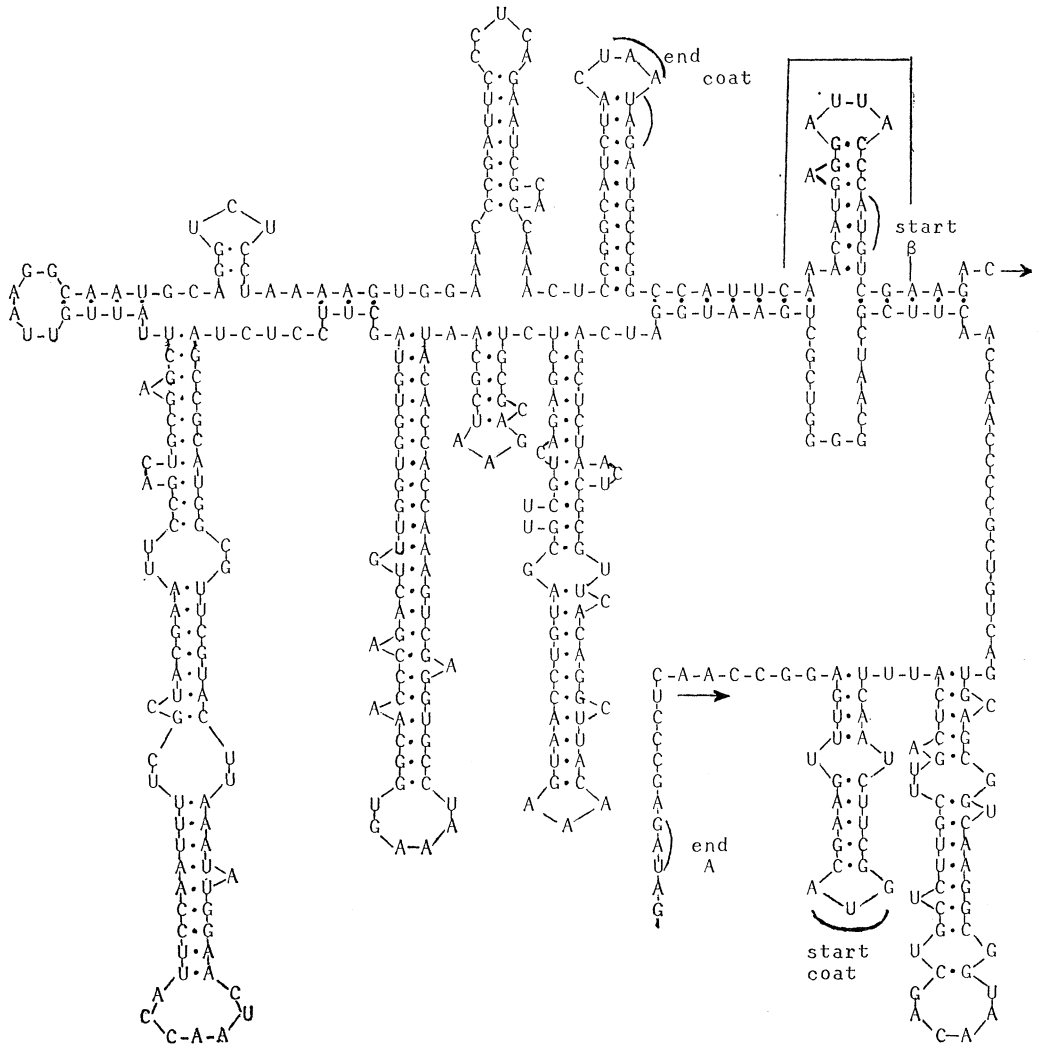

FIGURE 4. Plausible secondary structure of the coat protein cistron of fII-RNA. Start- and end-codons are marked. The initiation hairpin has been framed (after Min Jou *et al.* 1972).

In contrast, dissolved bacteriophage DNA, for example bacteriophage λ DNA (table 2), has a volume 4000 times higher than the space available in the capsid, if calculated from its hydrodynamic properties. This figure, however, might not apply to the conditions in the host cell since only 5 DNA molecules (volume 2.4×10^8 nm^3 each) could then fit into one bacterium

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

(volume about 1.2×10^9 nm^3; Séchaud, Ryter & Kellenberger 1959). Ryter (1968) found the diameter of the *E. coli* chromosome in this sectioned bacteria to be about 1000 nm. If assumed to be spherical, a volume of 2×10^5 nm^3 per 10^6 molecular mass could be calculated, this approximates the volume derived from the hydrodynamic properties of the 'condensed *E. coli* chromosome' in solution (Worcel & Burgi 1972). If λ DNA exists in a similar semicondensed state, 200 molecules could fit into the bacteria, close to the number actually observed. Its volume however would still be 100 times larger than the space available in the λ capsid. Consequently, final DNA condensation will require a much higher energy to compensate for the entropy decrease (volume decrease) than RNA condensation. In addition energy requirements for the kinks in the helix (Crick & Klug 1975) have to be taken into account. This energy might become available only if a stable container (the prehead) was already provided and if packaging were coupled to energy releasing reactions as discussed elsewhere in this book (Laemmli 1976; Hohn *et al.* 1976).

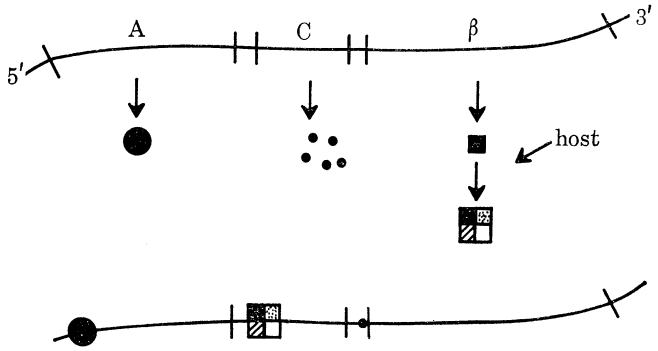

FIGURE 5. Binding of RNA phage coded proteins to RNA. Bacteriophage RNA showing the A-protein cistron (A) coat protein cistron (C) and cistron for the β subunit of the RNA-replicase (β). The β subunit forms the replicase together with three host proteins. Two of these are normally involved in tRNA binding to the ribosome (Blumenthal, Landers & Weber 1972), the other is normally the S1 protein of the ribosome (Wahba, Miller, Niveleau & Landers 1974; Inouye, Pollak & Petre 1974). All three gene products can bind to fII RNA exhibiting some form of control. The RNA polymerase can bind strongly to a site near the coat protein initiation codon. Thus it effectively blocks the entry of further ribosomse while those already bound to the RNA will finally fall off. This clears the RNA for replication which starts at another weak polymerase binding site, the 3′ end (Hindley 1973). The coat protein binds to the initiator hairpin for the β subunit (Gralla, Steitz & Crothers 1974) and thereby represses the production of replicase. The binding site for the A-protein has been chosen arbitrarily. One molecule of it finally becomes part of the phage. A-protein initiates the assembly of normal viable phage (Kaerner 1970), is needed for adsorption to the sex pili of the host, and enters the host with the RNA (Paranchych 1975). The RNA-A-protein complex alone shows some infectivity (Shiba & Miyake 1975).

As the DNA in such a mechanism does not function as a core in capsid nucleation, as does the RNA in RNA bacteriophages, this nucleating activity has been taken over by certain core proteins (see King *et al.* 1976; Hohn *et al.* 1976). These core proteins have to be removed from the prehead before the genome can be packaged.

Other viruses

Formation of functional capsids of many icosahedral RNA plant viruses, *in vitro*, depends on the presence of RNA. However, as in bacteriophage fII, the aggregation of capsid protein *per se* is not stringently dependent on it and various assemblies form in its absence. Cowpea chlorotic mottle virus (CCMV) and alfalfa mosaic virus (AMV) represent viruses with different degrees of dependence of capsid assembly on the presence of RNA as seen in *in vitro*

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

experiments. CCMV protein assembles *in vitro* to form a variety of structures such as spherical particles of different size, spiral forms, 'plates', laminar forms and polyheads. If RNA is present, mainly normal size capsids containing RNA are formed, if the RNA is fragmented, normal sized and some small particles appear (Bancroft, Wagner & Bracker 1969; Bancroft 1970). The shape and size of *in vitro* assemblies of AMV protein is highly dependent on the RNA size. Short RNA produces spherical particles or short tubes, long RNA produces elongated tubes (Lebeurier, Fraenkel-Conrat, Wurtz & Hirth 1971). Thus in these cases the coassembly mechanism (figure 1*a*) seems likely (also discussed in Hohn & Kellenberger 1976).

The situation is less clear for animal picorna viruses. Jacobson & Baltimore (1968) suggested that empty polio virus capsids are precursors to virions (prehead pathway). Ghendan, Yakobson & Mikhejera (1972) on the other hand reported some evidence that empty precursor capsids do not accumulate but that capsid protein subassemblies coassemble with RNA.

For viruses with supertwisted dsDNA such as bacteriophage PM2 (Franklin, Hinnen, Schäfer & Tsukagoshi 1976) or SV40, no predictions can be made since supertwisted DNA exists as a much more compact molecule than linear DNA.

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