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Structure and Assembly of Phage <latex>ϕ</latex>29

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Structure and assembly of phage ϕ29

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[Plate 5]

Bacteriophage ϕ29 is a small, morphologically complex, virus with a DNA of molecular mass 12×10^6.

The most likely structure of the head of ϕ29 consists of two fivefold symmetric end-caps based on $T = 1$ icosahedral symmetry, separated by an equatorial row of 5 hexamers.

The eighteen genes identified in ϕ29 genome have been mapped and, in some cases, the gene products have been identified. Five linked genes, four coding for structural proteins (G, A, E, H) and one coding for a non-structural protein (J), are essential to determine the normal shape of the capsid. Protein pJ may be a scaffolding protein.

An account of the effects of mutations in ϕ29 genes is given.

Bacteriophage ϕ29 is a small, morphologically complex *Bacillus subtilis* virus with a double-stranded DNA of only 12×10^6 (Anderson, Hickman & Reilly 1966; Talavera, personal communication). These features make attractive the study of its structure and feasible the identification of the viral genes and their role in morphogenesis. An understanding of ϕ29 assembly should help to elucidate how other viruses and complex biological structures are put together.

1. Structure of ϕ29

(*a*) *Structural components*

Bacteriophage ϕ29 consists of a prolate head with fibres, a neck and a short tail. The head has a flattened base and is about 30 nm wide and 40 nm long. The fibres radiating from the head have a length of about 14 nm and a width of 2 nm. The neck consists of two collars. The upper collar is joined to the base of the head, and the lower collar is located just below and contains twelve symmetrically attached appendages. The distance from the head base to the tail end is about 30 nm. The distal part of the tail is wider than the upper portion surrounded by the collars (Anderson *et al.* 1966).

The protein moiety of ϕ29 consists of seven polypeptides, most of which have been assigned to specific morphological components, as outlined on figure 1 (Méndez, Ramírez, Salas & Viñuela 1971; Ramírez, Méndez, Salas & Viñuela 1972; Salas, Vásquez, Méndez & Viñuela 1972). Often, protein HP2 is not well resolved from protein HP1.

The DNA of ϕ29 is interesting because a protein is involved in its circularization. A circular complex of one DNA molecule and two molecules of a DNA-associated protein (DAP) has been isolated from phage particles (Ortin, Viñuela, Salas & Vásquez 1971). DAP is closely related but not identical to the major capsid protein HP1, as shown by a comparison of the

tryptic peptides of both proteins. The DNA-protein complex is highly infectious whereas the DNA alone is virtually inactive (Hirokawa 1972), although it is capable of rescuing mutants in most of the phage cistrons (unpublished). A similar DNA–protein complex has been also found in adenovirus (Robinson, Younghusband & Bellett 1973).

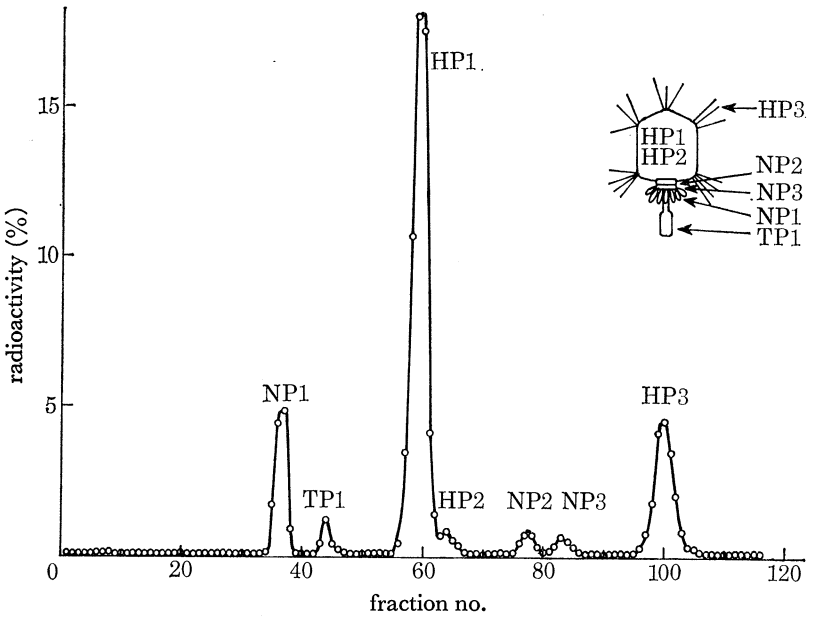

FIGURE 1. Polyacrylamide gel electrophoresis of ϕ29 proteins labelled with a mixture of [^{14}C]amino acids. Phage ϕ29 was labelled with a mixture of fourteen [^{14}C]amino acids, purified and subjected to polyacrylamide gel electrophoresis as described by Méndez *et al.* (1971). The electrophoresis was run for 24 h at 4.5 V/cm. The total count per minute in the sample was 42720. In the graph, the front (anode) is to the right.

(*b*) *Capsid geometry*

The capsid of phage ϕ29, although non-isometric, may be related to the icosahedral symmetry by elongation of a regular icosahedron, as seems to occur with the capsid of T4 (Moody 1965; Boy de la Tour & Kellenberger 1965; Branton & Klug 1975). That both phages may have a capsid geometry based on similar principles is supported by the finding of similar morphological variants.

Direct observation of surface detail on the negatively stained capsids of ϕ29 has not so far provided information on capsid geometry. However, knowledge of the number of copies of each structural protein per phage particle may provide some information upon which to propose reasonable models for ϕ29 phage structure (table 1).

DESCRIPTION OF PLATE 5

FIGURE 2. A photograph of a model showing the ϕ29 head geometry.

FIGURE 4. Electrophoresis of proteins in purified mutant particles produced by susD241 (*a*), susI44 (*b*), wild-type phage (*c*) and in lysates of u.v.-irradiated cells infected with wild-type phage (*d*). Wild-type phage and phage-infected bacteria were labelled with a mixture of [^{14}C]amino acids (Carrascosa *et al.* 1973). Mutant particles were purified in a 15–30% sucrose gradient and subjected to slab gel polyacrylamide electrophoresis in the presence of sodium dodecyl sulphate (Studier 1972; Maizel 1972). At the right of the plate are the structural proteins, labelled according with the convention indicated on figure 1, and protein pJ.

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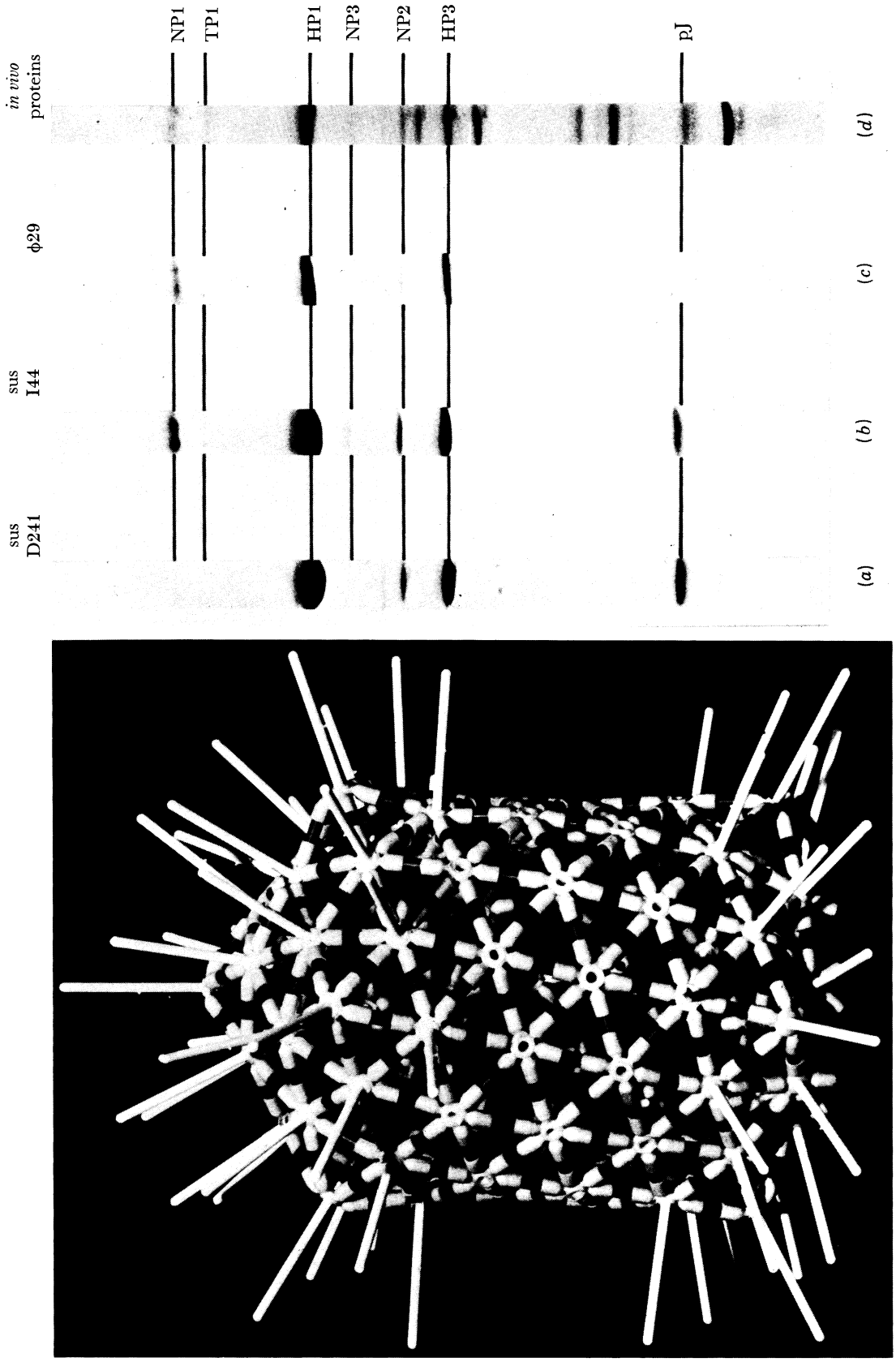

FIGURES 2 AND 4. For description see opposite.

(*Facing p.* 30)

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By assuming a basic plane hexagonal lattice p6 (Caspar & Klug 1962), the subunits of the major capsid protein HP1 can be arranged in 11 pentamers, formed by 5 subunits, and 5 equatorial hexamers, formed by 6 subunits, making a total of 85 subunits. A reasonable location for protein HP2 could be the centre of the hexamers.

Electron micrographs of ϕ29 show that the head fibres are absent on the equatorial part of the head and gathered around the upper and lower parts of the capsid (Anderson *et al.* 1966; Salas *et al.* 1972; Tosi & Anderson 1973). Since there are eleven fivefold symmetry vertices and there is more than one fibre per vertex, the minimum number of fibres would be 55 if each pentamer, formed by (5) subunits of protein HP1, contains (5) copies of protein HP3. The closeness of this value with the determined number of molecules of protein HP3 per capsid (table 1) strongly suggests that each fibre is a single molecule of protein HP3.

According to the principle of quasi-equivalence (Caspar & Klug 1962), the capsid of ϕ29 would be formed by two fivefold lattices within each end-cap, corresponding to an icosahedral $T = 1$ symmetry, separated by a band of 5 hexamers (figure 2, plate 5).

TABLE 1. NUMBER OF COPIES OF THE STRUCTURAL PROTEINS ON PHAGE ϕ29

	structural component						
	capsid			neck			tail
protein	HP1	HP2	HP3	NP1	NP2	NP3	TP1
10^{-3} × molecular mass†	54	48	28	80	40	36	71
protein mass, %‡							
(*a*) 14[^{14}C]amino acids	56.3	3.0	19.0	12.6	2.6	2.4	2.4
(*b*) 15[^{3}H]amino acids	58.2	2.8	21.1	11.1	2.4	1.8	2.0
(*c*) mean	57.2	2.9	20.0	11.8	2.5	2.1	2.2
number of subunits per phage							
(*a*) method 1§	95.3	5.4	64.3	13.3	5.6	5.2	3.4
(*b*) method 2‖	86.2	4.9	58.1	*12*	5.1	4.8	2.5
subunit composition	85	5	55	12	5(6)	6(5)	3

† From Méndez *et al.* (1971).

‡ Determined after gel electrophoresis of proteins from phage labelled with mixtures of either [^{14}C]- or [^{3}H]-amino acids as indicated in the legend to figure 1. The radioactive counts under each protein peak was assumed to be proportional to protein mass.

§ From the mass contribution of each structural protein and their molecular masses taking for the molecular mass of the protein moeity of ϕ29 a value of 9×10^6 (Rubio *et al.* 1974; Corral, Salas & Viñuela, unpublished).

‖ From the mass contribution of each structural protein and their molecular masses using as an 'internal standard' a number of 12 copies of protein NP1 per phage.

2. GENETIC CONTROL OF ϕ29 ASSEMBLY

Several collections of temperature-sensitive (ts) and nonsense suppressor-sensitive (sus) mutants have been isolated (Talavera, Jiménez, Salas & Viñuela 1971; Hagen, Zeece & Anderson 1971; Talavera, Jiménez, Salas & Viñuela 1972; Reilly, Zeece & Anderson 1973; McGuire, Pène & Barrow-Carraway 1974; Moreno, Camacho, Viñuela & Salas 1974; Mellado, Viñuela & Salas, unpublished) and many of the gene products identified (Anderson & Reilly 1974; McGuire *et al.* 1974; Camacho *et al.* 1974).

The mutants isolated in Madrid and Minneapolis have been interchanged, analysed by *cis-trans* tests and mapped by two- and three-factor crosses, resulting in a total of 18 genes

arranged in a linear genetic map (figure 3). Analysis of gene functions (Schachtele, Oman & Anderson 1970; Talavera, Salas & Viñuela 1972; Schachtele, Reilly, de Sain, Whittington & Anderson 1973; Anderson & Reilly 1974; Camacho *et al.* 1974; Carrascosa, Camacho, Viñuela & Salas 1974; McGuire *et al.* 1974) has indicated that ϕ29 genes with related functions are clustered in the viral genome.

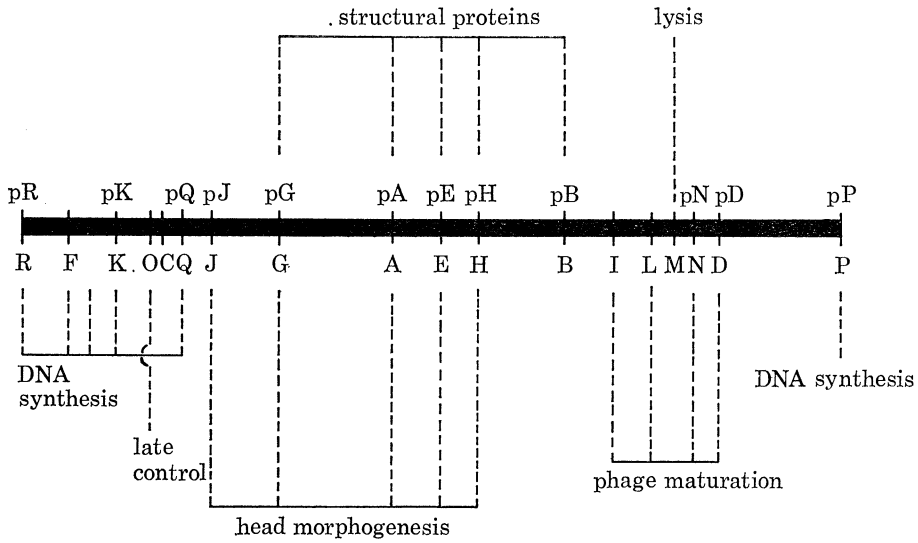

FIGURE 3. Genetic map of phage ϕ29. The capital letters indicate genes. Genetic distances have been drawn proportional to recombination frequencies. A p before a gene number refers to the identified polypeptide chain of that gene.

Cistrons R, F, C, K, Q and P. Genes R, F, C, K, Q and P control the synthesis of viral DNA. Sus- or ts-mutants in any of these genes produce about ten times less DNA-free capsids than wild-type phage. These particles contain proteins HP1, HP3 and NP2.

Cistron O. Gene O controls the late expression of ϕ29 DNA (Anderson & Reilly 1974; McGuire *et al.* 1974). Under non-permissive conditions, mutants in cistron O do not induce the synthesis of late viral messenger RNA (unpublished results).

Cistrons J, G, A, E and H. Head morphogenesis is under the control of genes J, G, A, E and H.

Protein pJ (mol. mass 8000) is a non-structural late protein essential for head assembly because susJ mutants, under restrictive conditions, do not assemble capsids. Furthermore, a role of protein pJ in capsid assembly is indicated by the observation that tsJ mutants produce membrane-bound isometric particles. Although protein pJ is not found in infectious phage particles, it is present in DNA-free particles obtained after infection with mutants in several cistrons (figure 4, plate 5). Assuming that the mutant particles contain 5–6 copies of protein NP2 per particle (table 1), and integrating the density of the autoradiogram contributed by each band (figure 4), measured within the linear range of the film, the number of copies of protein pJ is estimated to be close to 70.

Sus mutants in gene G do not induce the synthesis of the major capsid protein (HP1) nor the capsid fibre protein (HP3) (Anderson & Reilly 1974; McGuire *et al.* 1974). As expected, susG mutants do not assemble capsids. On the other hand, tsG mutants produce, under restrictive conditions, membrane-bound isometric particles with neck and tail.

Gene A codes for the tail protein TP1 (Anderson & Reilly 1974; McGuire *et al.* 1974;

Carrascosa, Camacho, Viñuela & Salas, in preparation). Under restrictive conditions, susA- and tsA-mutants produce DNA-free abortive particles with a core. Morphologically, A-particles are prolate and more rounded than wild-type capsids. They are formed by proteins HP1, HP3, NP2 and the late proteins pN and pJ.

Gene E codes for the upper collar protein, NP2 (McGuire *et al.* 1974; Reilly *et al.* 1975; Carrascosa *et al.* in preparation). Sus-mutants produce, under restrictive conditions, DNA-free abortive particles. Morphologically, the E-particles are isometric and contain proteins HP1, HP3 and pJ. Therefore, the upper collar protein, NP2, is necessary for capsid elongation.

Gene H codes for the lower collar protein, NP3 (Reilly *et al.* 1975; Carrascosa *et al.* in preparation). SusH-mutants produce DNA-containing and DNA-free particles. The DNA-containing particles are formed by proteins HP1, HP3 and NP2. The DNA-free particles contain, besides, proteins pN and pJ. About half of the particles produced after infection with susH mutants are isometric suggesting that protein NP3 plays also a role in head elongation.

Cistron B. Gene B codes for protein pB (mol. mass 90000), that is cleaved before assembly to give protein NP1 (mol. mass 80000), the neck appendage protein (Anderson & Reilly 1974; Camacho *et al.* 1974; Carrascosa *et al.* 1974; Tosi, Reilly & Anderson, personal communication). Sus-mutants in gene B produce DNA-containing and DNA-free particles. The DNA-containing particles are formed by proteins HP1, HP3, NP2, NP3 and TP1. The DNA-free particles are formed by proteins HP1, HP3, NP2 and the late proteins pN and pJ. The capsid morphology of B-particles is similar to that of wild-type phage.

Cistrons I and L. Under restrictive conditions, sus- and ts-mutants in both cistrons produce DNA-free phage, morphologically similar to wild-type particles. I-particles contain, besides the structural proteins present in wild-type phage, about 70 copies of protein pJ per particle.

Cistron M. The product of gene M has not been identified. It is involved in cell lysis since susM-mutants do not lyse infected cells, but produce a normal burst-size after artificial lysis.

Cistron N. Gene N codes for a late protein, pN, of molecular mass 26000, which is synthesized in amounts comparable to those of the major capsid proteins (Anderson & Reilly 1974; Camacho *et al.* 1974). Nevertheless, in laboratory infected bacteria, protein pN is largely dispensable. Protein pN has a strong affinity for native and denatured DNA and it is associated to some extent with DNA-free particles produced by infection with several mutants.

Cistron D. Gene D codes for a late protein, pD, with a molecular mass of about 35000. Mutants in gene D produce, under restrictive conditions, DNA-free, morphologically normal capsids composed by the structural proteins HP1, HP3 and NP2 and the non-structural protein pJ (figure 4). The number of copies of protein pJ per particle, is about 70.

3. Discussion

Bacteriophage ϕ29 is one of the simplest viruses with a non-isometric capsid. The geometry of ϕ29 capsid may be similar to that of T4, since both phages have similar morphological variants, with different capsid lengths, that correlate very closely with a discrete increase in the number of equatorial capsomers. Furthermore, in both there is a good correlation between head structure models of normal capsids and the number of copies of head proteins per phage, found experimentally (Branton & Klug 1975).

A large number of sus- and ts-mutants have been distributed in 18 genes which have been ordered on a linear genetic map. As in the case of T4 (King & Laemmli 1971), P22 (King,

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Lenk & Botstein 1973) and λ (Casjens & Hendrix 1974), φ29 genes whose protein products interact more directly are closely linked.

Capsid morphogenesis is under the control of at least five linked genes, namely, J, G, A, E and H. Cistron G codes for the major capsid proteins HP1 and/or HP3, whereas cistrons A, E and H determine the tail protein, TP1, the upper collar protein, NP2, and the lower collar protein, NP3, respectively.

Proteins NP2, NP3 and TP1 play an important role in determining the shape of the capsid. NP2 determines capsid elongation since mutants in cistron E produce isometric capsids, probably lacking the equatorial belt of hexamers. NP3 is also involved in capsid elongation since mutants in cistron H produce about 50% of isometric particles. SusA-mutants produce prolate DNA-free, abortive capsids without the sharp corners characteristic of normal particles.

SusJ-mutants do not assemble capsids. Protein pJ is a non-structural protein which is present in DNA-free particles formed by mutants in several cistrons in a number of copies per particle close to 70. This result suggests that protein pJ could be a scaffolding protein with a role similar to protein p8 in phage P22 (King *et al.* 1973; King & Casjens 1974), although we do not know whether protein pJ is recycled or if the pJ-containing mutant particles are intermediate or abortive structures in φ29 morphogenesis. It may be significant that the genes coding for the major head protein and the scaffolding protein are adjacent in the genomes of P22 (King *et al.* 1973), T7 (Studier & Maizel 1969) and, perhaps, φ29.

The protein product of gene B seem to be assembled into phage particles in the late steps of the morphogenesis, because mutants in this gene form DNA-containing viral particles only lacking protein NP1.

The fact that DNA-negative mutants produce capsids suggests that DNA encapsulation takes place on a preformed head. Mutants in cistron D give also place to empty heads formed by proteins HP1, HP3, NP2 and pJ.

The site of action of proteins pI and pL is unknown; mutants in any of these two cistrons produce DNA-free normal particles.

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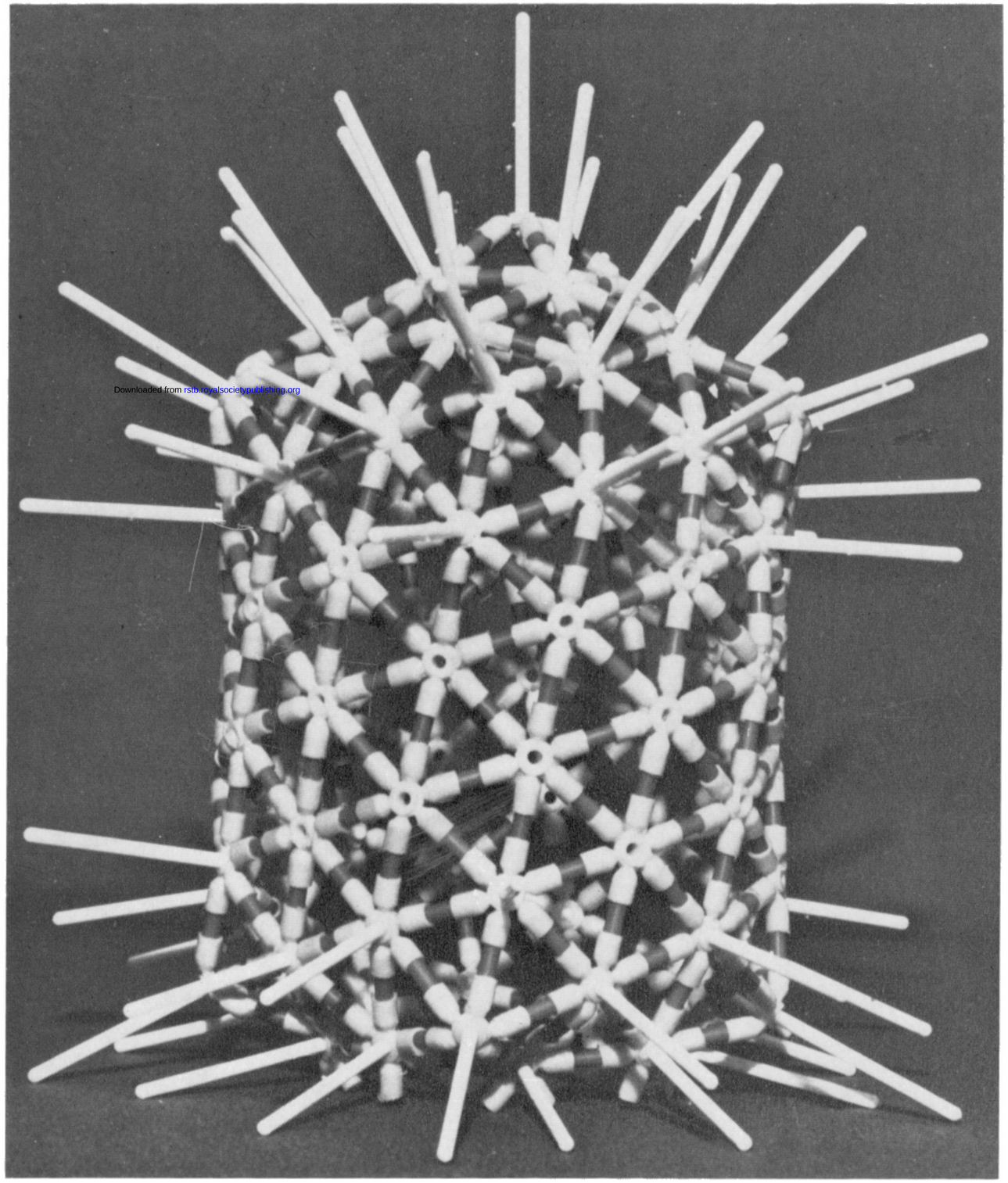

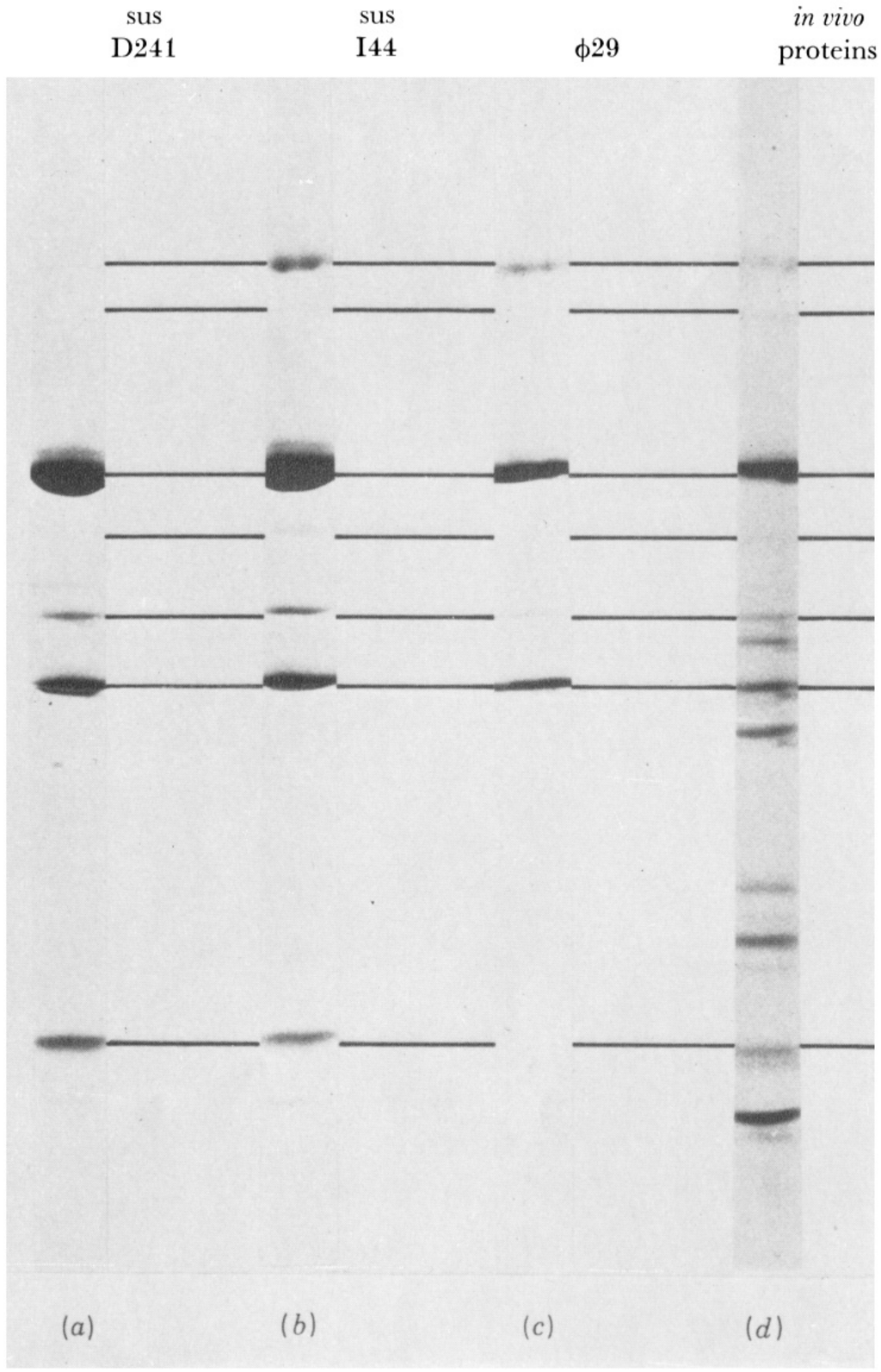

FIGURES 2 AND 4. For description see opposite.