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Studies on the maturation of the head of bacteriophage T4

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[Plates 3 and 4]

The presentation focuses on the structural rearrangements of the subunits and the processing of the various protein constituents which accompany the maturation events of the head of bacteriophage T4.

The major features of the maturation steps of the head are the following: (*a*) the viral DNA is pulled into an empty head in a series of events; (*b*) cleavage of two core proteins, P22 (mol. mass = 31000), to small fragments and the internal protein IPIII (mol. mass = 23000) to IPIII* (mol. mass = 21000) appears to be intimately linked to the DNA packaging event, whereas the cleavage of the major head protein of the viral coat, P23 (mol. mass = 55000), to P23* (mol. mass = 45000) precedes the DNA packaging event.

Recently, we have obtained information about the mechanism by which the viral DNA is pulled into a preformed empty head. Our evidence suggests that the DNA becomes attached to the inside of the empty head and is subsequently collapsed in the interior by the so-called internal peptides. These are highly acidic and derived from a large precursor protein by cleavage.

Introduction

The assembly of the head of bacteriophage T4 is controlled by many genes. The phenotypic expression of most of these genes has been investigated fairly well, and about half of the products coded by them have been identified (see Kellenberger, this volume, and Laemmli, Paulson & Hitchins (1974) for references and details). Progress has also been made in elucidating the pathway which leads to the mature T4 head. The major feature of this pathway is the finding that the DNA is pulled into an empty head in a series of events, during which several of the head proteins become cleaved.

The first particle in the pathway, called the prohead I, contains the major head protein P23 (product of gene 23), and (probably) the protein P22 and the internal protein IPIII (Laemmli & Favre 1973). The prohead I appears to contain no DNA, is very fragile in cell lysates, and is converted to a more stable particle, the prohead II, as a result of the cleavage of the major capsid protein P23 (molecular mass 55000) to P23* (molecular mass 45000) (Laemmli & Favre 1973). The prohead I and II contain no DNA and appear not to be attached to the replicative DNA complex.

DNA is packaged during the conversion of prohead II to prohead III, and, ultimately, during the conversion of prohead III to the mature head. The cleavage of the core protein P22 to small fragments accompanies this DNA packaging event (Laemmli & Favre 1973; Laemmli *et al.* 1974). The protein P22 is the major component of the internal core (Showe & Black 1973; Laemmli & Quittner 1974) seen in the precursor particles and the various aberrant head related structures (Simon 1972; Kellenberger, Eiserling & Boy de la Tour 1968).

The prohead I particles were found as a kinetic intermediate in pulse-labelled wild type infected cells (Laemmli & Favre 1973) but these particles are thought to be essentially identical to the so-called τ-particles (Laemmli & Johnson 1973; Luftig & Lundh 1973; see also Laemmli *et al.* 1974 for details). The τ-particles accumulate in cells infected with phage carrying a mutation in gene 21 or 24 (Epstein *et al.* 1963; Laemmli, Molbert, Showe & Kellenberger 1970*a*). Recent experiments showed that the τ-particles formed at non-permissive temperature in cells infected with a temperature sensitive mutant phage in gene 24 can be converted into active phage if the product of gene 24 is activated by temperature shift (Bijlenga, Scraba & Kellenberger 1973; Bijlenga, Broek & Kellenberger 1974). Thus, gene 24 appears to control the conversion of prohead I (τ-particle) to prohead II. These important experiments strongly support the pathway proposed. To further substantiate this pathway, we have directly measured the incorporation of DNA into preformed prohead I or prohead III particles. We found that a very substantial amount of DNA synthesized following assembly of these heads can be incorporated into preformed prohead I or prohead III particles (Laemmli, Teaff & D'Ambrosia 1974). Thus, we believe that most, if not all, of the DNA is packaged into empty preformed heads. This mode seems to be a general one. Phage P22 and phage λ for example, both package their DNA into preformed heads (see the articles by King and Hohn, this volume).

The major events of the maturation of the T4 heads have been outlined, and it becomes necessary to understand some of these steps in depth. This paper describes our approach and efforts to do so.

Results and discussion

(*a*) *Correlation between structural transformation and cleavage of the major head protein P23 to P23**

A striking feature of the pathway discussed in the Introduction is the finding that the τ-particles (prohead I) are about 10–20 % smaller in width and length than the mature head (Kellenberger *et al.* 1968). One would expect, therefore, that the prohead I particles have to be enlarged either by the addition of new subunits or by a rearrangement of the subunits into a surface lattice of expanded dimension during the maturation events. We have recently obtained direct evidence for such a structural rearrangement of the surface lattice induced by the cleavage of P23 to P23* (Laemmli, Amos & Klug 1976). The structural rearrangement of the subunit was studied with the help of the tubular polyheads which accumulate in cells infected with the phage containing a mutation in gene 20. This structure has many times the length of the normal phage head, contains no DNA and is very suitable for analysis by optical diffraction and image reconstruction (Klug & DeRosier 1966; DeRosier & Klug 1972). The surface structure of polyheads has been studied extensively and is based on a plane hexagonal lattice (symmetry p6) rolled into a cylindrical surface (Klug & Berger 1964; Kellenberger & Boy de La Tour 1965). Close analysis of the surface structure of polyheads showed that fresh lysates contained predominantly polyheads with a coarse appearance, whereas in aged lysates smoother structures predominated (DeRosier & Klug 1972). The smooth looking polyheads had an appearance in negatively stained preparations similar to that of the mature phage head. It was therefore suggested that the different appearance in the filtered images of polyheads might mimic the different maturation events of normal head maturation (Yanagida, DeRosier & Klug 1972).

The tubular polyheads contain the precursor proteins (P23, P22, IPIII) (Laemmli & Quittner 1974; Kellenberger & Kellenberger-van der Kamp 1970). We recently found

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conditions to induce the cleavage of the polyhead proteins *in vitro* (Laemmli & Quittner 1974) thus making it possible to follow the chemical and structural changes in parallel. Also, since the cleavage of the precursor proteins *in vitro* requires the presence of a functional gene 21 protein, the effect of the normal cleavage and possible non-specific changes due to incubation can be distinguished. In order to study the structural rearrangement of the surface subunits, we prepared fresh polyheads containing predominantly the uncleaved protein P23 (more than 95 %) and we prepared incubated polyheads containing predominantly the cleaved protein P23* (more than 95 %). These polyheads were negatively stained and examined in the electron microscope and their structure analysed by optical diffraction and image reconstruction.

Four different classes (I, II, III, IV) of structures were observed, (Laemmli *et al.* 1976). Fresh, uncleaved polyheads (class I) have a coarse structure which has been well described by earlier workers (see figure 1*a*, plate 3). As observed by Yanagida *et al.* (1972), the subunits of coarse polyheads lie on an ellipse rather than a circle. The polyheads have an average width when flattened of 75 nm. Freshly cleaved polyheads (containing more than 95 % of P23*) consist of an approximately one to one mixture of narrow (75 nm diameter) coarse polyheads (class II) and wider (average diameter 83 nm) smoother looking tubes (class III). The filtered images of the narrow incubated tubes (class II) are somewhat similar to those of the unincubated polyheads (class I) but not identical (see figure 1*a*, *b*). The subunits of the hexamer now lie on a circle rather than an ellipse. The pattern from the wider tubes (class III) in the same sample are strikingly different from those of class II polyheads. The hexamers are expanded in size relative to those of the coarse tubes and have rotated by a few degrees (figure 1*c*). The overall expansion of the unit cell is about 10–15 %, which is also reflected in the increased diameter of the class III polyheads over that of class I polyheads by about the same percentage. We have been able to show that the class II polyheads are the direct precursors of class III. The class II polyheads appear in a metastable state. They are completely converted to class III, either by freezing and thawing, or by storage at 4 °C for about a day.

The functional product of gene 21 is required for the cleavage of the precursor proteins of polyheads. In order to show that the structural rearrangement is indeed induced by the cleavage of the precursor proteins, we have carried out similar experiments with polyhead preparations in which the protein 21 was genetically eliminated. Such preparations only contained the coarse class I polyhead, whether or not they have been incubated, frozen and thawed, or stored at 4 °C. From these experiments it is clear that the cleavage of the head protein results in the conversion of the coarse class I polyheads into the expanded smoother looking class III polyheads as a result of the cleavage reaction. The class II polyheads are metastable intermediates in the scheme.

The question arises of whether the sequence of changes in the structure of the polyheads really parallels the maturation pathway of the normal phage head. At the gross level, the polyhead tubes are rather differently constructed than the tubular part of the normal capsid (Yanagida *et al.* 1972; Branton & Klug 1975). However, there is good evidence to suggest that, at the local level, the structures are very similar and undergo the same changes with time (Laemmli *et al.* 1976).

(1) We have studied the surface structure of τ-particles (prohead I) and found that they have essentially the same coarse structure as class I polyheads.

(2) The functional product of gene 21 (P21) and the cleavage of P23 to P23*, are required both for the maturation of the prohead I and for the structural changes of polyheads.

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(3) Both structures, the τ-particles and the polyheads, undergo a conservative expansion during maturation. The expansion of the unit cell following cleavage (as observed in polyheads) thus provides an explanation for the size difference between the τ-particle and the mature phage head.

(4) Polyheads containing the uncleaved P23 proteins are very unstable, considerably more so than mature heads. The uncleaved P23 protein is quantitatively extracted if these polyheads are treated with SDS at room temperature, while in order to quantitatively extract the cleaved P23* protein from mature heads, boiling in SDS is required (Laemmli 1970). We have shown that the latter property is also acquired by *in vitro* cleaved polyheads containing the cleaved P23* protein (Laemmli *et al.* 1976). Moreover, prohead I (τ-particles) and polyheads containing the uncleaved P23 protein dissociate in sodium iothalamate, while mature phage heads and *in vitro* cleaved polyheads are completely stable (Laemmli & Johnson 1973, and unpublished observations; Laemmli *et al.* 1976). Thus, the polyheads acquire as a result of the cleavage of P23 to P23*, a resistance to dissociation by SDS and sodium iothalamate which is similar to that of heads which contain P23*. For this reason it is likely that the subunit interaction in cleaved polyheads is very similar to that in mature heads.

A further type of polyheads (class IV) was occasionally observed in incubated lysates. These polyheads are very fine and have a very smooth looking appearance and their filtered images are variable and in most cases difficult to interpret. Figure 1 *d* shows an example. The capsomer appears as a small empty hexamer surrounded by an irregular ring of extra density. The nature of this extra density has been clarified by recent work of Ishii & Yanagida (1975). They have compared the surface structure of T2 phage heads to that of T4 phage heads and found that the T2 surface structure very much resembles the class III polyheads, while the T4 surface structure resembles more closely that of our class IV polyheads. Moreover, they showed that this difference in structure is due to a 10000 molecular mass protein which is present in T4 phage but missing in T2 phage (Forrest & Cummings 1971). Ishii & Yanagida have identified this protein as a major inessential component of T4 capsids, which they call *soc* (for small outer capsid protein). They showed that incubation of purified *soc* protein with purified T2 phage converts them to a smoother structure resembling T4. It seems likely therefore that traces of this component produced the small proportion of class IV polyheads that we found in phage T4 lysates.

(*b*) *Attachment of the phage head to the replicative DNA*

The replicative T4 DNA is a very compact and fast-sedimenting (about 300–900 S) structure (Frankel 1966*a*, *b*; Huberman 1968). We have found that the prohead III particle is attached to the replicative DNA (Laemmli & Favre 1973), while the prohead II is not. Attachment therefore has to occur during the prohead II to III conversion.

How do the precursor particles attach to the replicative DNA complex, do they attach to free ends or to an internal DNA section? Which proteins are involved?

We know that genes 16 and 17 must be involved in this reaction from the observations that the heads which accumulate in cells infected with an amber mutant phage in either of these two genes contain no DNA (King 1968) and are not associated with the replicative DNA (Luftig & Ganz 1972; Laemmli & Favre 1973). Interestingly, one of the temperature sensitive mutants in gene 17 (ts P22) produces heads (at restrictive conditions) which are attached to the replicative DNA (Wagner 1975). The temperature sensitive protein produced by this

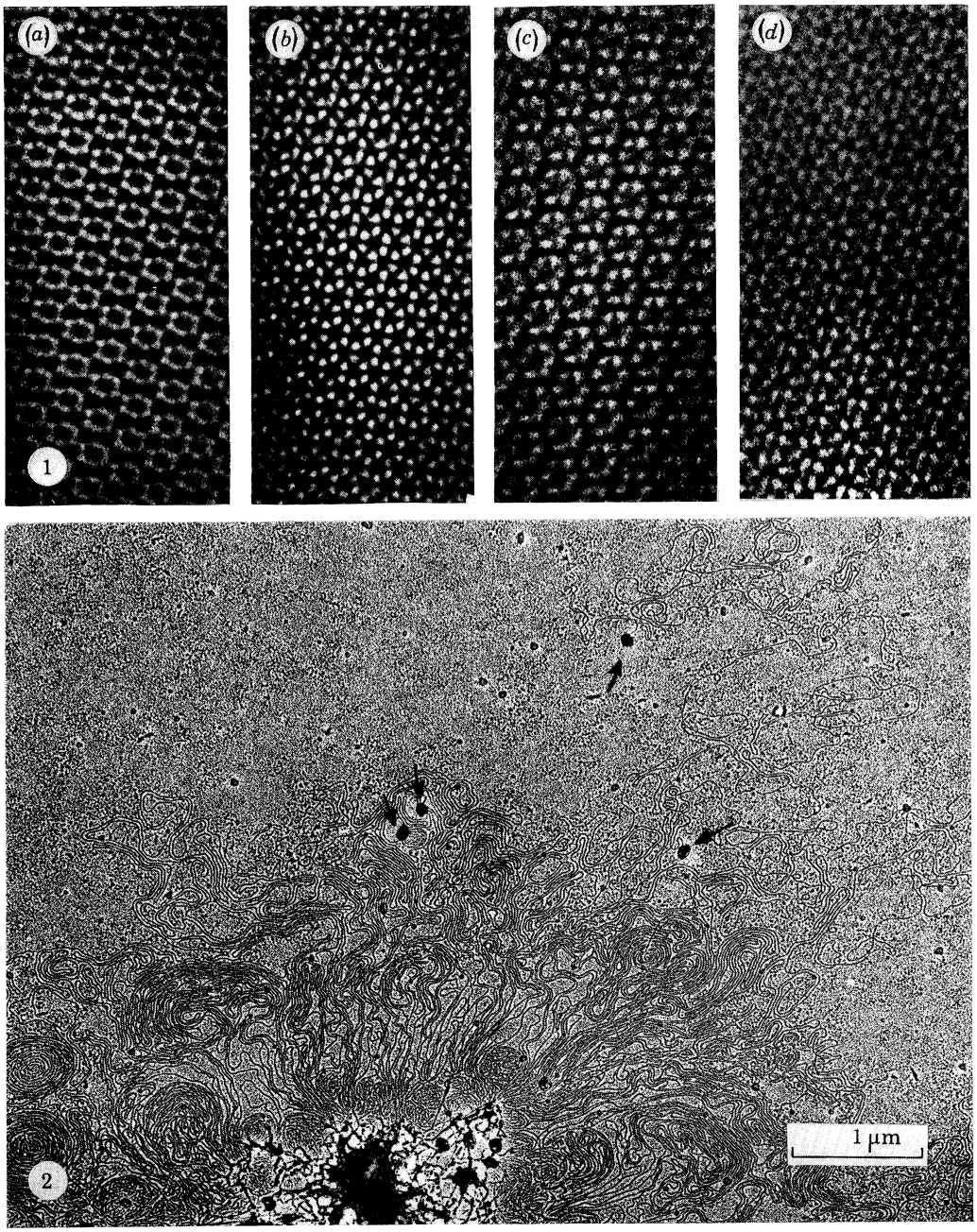

FIGURE 1. Filtered image of the surface structure of class I, II, III and IV polyheads. Negatively stained, uncleaved and cleaved polyheads were prepared and their surface structure revealed by image reconstruction (see Laemmli, Amos & Klug 1975). Panels (*a*, *b*, *c*, and *d*) are representative of class I, II, III and IV polyheads, respectively.

FIGURE 2. Electron micrograph of T4 replicative DNA complex containing attached heads. The replicative DNA complex was isolated from cells infected with a phage containing the mutation (ts P22) in gene 17 (Wagner 1975). The DNA complex was spread by the protein technique described by Inman & Shnös (1970). Arrows indicate attached phage heads.

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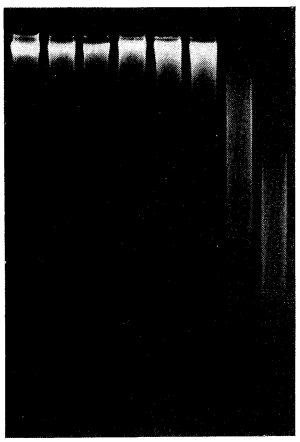

FIGURE 4. **Digestion** of calf thymus DNA exposed to increasing amount of poly(ethyleneoxide) with *Neurospora crassa* endonuclease. Calf thymus DNA in a buffer containing increasing amount of poly(ethyleneoxide) (PEO) were treated with the enzyme *Neurospora crassa* endonuclease for 15 min at 37 °C and analysed on a 6% polyacrylamide gel (Laemmli 1975). Sample *a* contained no PEO and was treated with endonuclease; sample *c*, *d*, *e*, *f*, *g* and *h* contained 10, 20, 40, 60, 80 and 100 mg/ml of PEO, respectively, and were treated with the enzyme.

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mutant phage is able to carry out part of its role (directly or indirectly), namely the attachment to the DNA, but completion of the DNA packaging process is blocked.

The mode of attachment of the prohead III particles to the replicative DNA complex was studied by electron microscopy. DNA complexes were isolated from cells infected with mutant phages which block the prohead III maturation. All known mutations in gene 49 and the mutation (ts P22) in gene 17 block phage head assembly at this point (Luftig, Wood & Okinaka 1971; Laemmli & Favre 1973; Wagner 1975). Prohead III maturation can also be blocked chemically by the addition of 9-aminoacridine to the medium (Wagner & Laemmli, in preparation). This observation is consistent with earlier data which indicated that 9-aminoacridine blocks a late step of head maturation (Piechowski & Susman 1966, 1967). The general structure of the replicative DNA seen in the electron microscope is similar to the one described by Huberman (1968). Each complex consists of a central core from which originate a very large number of DNA strands. The details of the attachment of the head to the DNA is most clearly seen at the periphery of the complex where the concentration of the DNA is low. Figure 2, plate 3, shows a section of this region. Some heads are clearly attached to a free end of a DNA molecule. The mode of attachment in regions containing many DNA strands is more difficult to see, but it is nevertheless possible to demonstrate that most of these heads are also attached to ends. We have counted the number of molecules that intersect a head by drawing a circle around the head and counting the number of DNA strands intersecting this circle. An odd number would indicate attachment to a free end, while an even number would not. Of 334 heads analysed, we found that 31 % were either attached to a free end or were intersected by an odd number of strands. Less than 0.6 % were intersected by an even number of strands, and 69 % were found in regions so densely packed with DNA that it was not possible to determine the number of strands intersecting the head. We conclude that most or, possibly, all of the prohead III particles are attached to a free end. The data presented were obtained from cells infected with a phage containing a mutation (ts P22) in gene 17. Similar data were obtained if the replicative DNA complexes studied were from cells blocked in gene 49 or from cells blocked with 9-aminoacridine.

Are the free ends generated as a result of head attachment or do free ends pre-exist? To answer this question we counted the number of free ends visible in each complex. In the complex isolated from cells blocked in gene 49, we found less than one free end per complex. We have also analysed the complexes from cells infected with a mutant phage in gene 23 in order to block the assembly of the head. In this case we counted an average of 2.2 free ends per complex. Since, under the conditions of this experiment, we find about 30–45 heads per DNA complex in cells blocked in gene 49, the considerably lower number of free ends suggests that free ends are *generated* by the attachment of the head. But we are hesitant to draw this conclusion, since it is not clear how efficiently ends can be detected. It would be possible that free ends exist which are buried in the centre of the complex and difficult to detect.

(*c*) *The problem of head length determination*

The head of bacteriophage T4 is not an isometric structure but is elongated in one direction. How is its length determined? This question is a general one in biology, since many subcellular structures have very precise dimensions and very little is known about how these are 'measured'. A perfect example is the tail tube of bacteriophage T4 which has a precise length of 100 nm (see King, this volume).

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A good approach to this problem is to select mutants which affect the size of the structure of interest in order to identify the gene products involved in length determination. Several mutations in gene 23 have been described which produce phage with altered head length (Eiserling *et al.* 1970; Doermann *et al.* 1973*a*, *b*). Five of these mutants produce smaller than normal phage (isometrics and intermediates) and three of them produce in addition some longer than normal phage (giants) (table 1). Gene 23 codes for the major capsid protein and might be expected to carry, by itself, sufficient morphogenetic information to form an icosahedral head, but one would expect that other gene products must be involved to form an *elongated* head. In particular, we hypothesized that mutants should be found in gene 22,

TABLE 1. MUTATIONS AFFECTING HEAD LENGTH

The table lists mutations in genes 23, 22, and 24 which effect the size of the phage head. The frequency of observance at a particular structure is given as a percentage of the total number of particles. + indicates significant occurrence of particles (> 1%), but exact figures have not been published.

		type of heads observed					
	gene	petite	inter-mediate	normal	giants	others	refs.
23	pt E920g	65%	.	35%	.	.	1
	pt 21–34	+	+	+	.	.	2
	ptg 19–2	+	+	+	+	.	2
	ptg 19–80	+	+	+	+	.	2
	ptg 191	.	+	+	+	.	2
22	tsA74 40 °C	85%	.	13%	.	2%	3
	30 °C	33%	.	59%	.	8% phage	3
	ts 22–2 40 °C	65%	.	34%	.	1% with	3
	30 °C	26%	.	68%	.	6% altered width	3
24	ts B86	.	.	+	+	.	4
T4D+		< 1%	.	> 99%	.	.	2, 3

1, Eiserling, Geiduschek, Epstein & Metler (1970). 2, Doermann, Eiserling & Boehner (1973*a*). 3, Paulson, Lazaroff & Laemmli (1976). 4, Aebi *et al.* (1974).

which codes for the major structural protein of the core seen in various precursor particles (see Laemmli *et al.* 1974). Luckily, two temperature sensitive mutants have been found in gene 22 (tsA74 and ts 22-2) which produce high frequencies (up to 85%) of isometric phage when grown at semi-restrictive temperatures (Paulson, Lazaroff & Laemmli 1976). The fraction of phage which are isometric is dependent on the temperature of the infected culture (table 1). One of the mutants (ts A74) produces 85% isometric phage when grown at 40° but only 34% isometric phage at 30°. These isometric phages are biologically active by virtue of their ability to complement a co-infecting amber helper phage (Paulson *et al.* 1976). Recently, Aebi *et al.* (1974) reported that temperature sensitive mutants in gene 24 produce at 36 °C a few phage with heads much longer than normal (giants) (table 1). From this, it is clear that at least 3 gene products are involved in the determination of the phage head length; these genes are 23, 22 and 24.

How might an interaction among the products of genes 24, 22 and 23 determine the head length? The protein P23 and most likely P24 are both structural components of the outer capsid shell. The protein P22, however, together with the internal proteins, forms the core seen in

the interior of the various particles. Thus, the length of the head is determined by an interaction between the core proteins (P22) and the outer shell (P23, P24). We would like to hypothesize that a Vernier mechanism (Anderson & Stephens 1964) may be involved. For such a mechanism one would need two cylindrical lattices, one inside the other, with different periodicities. Assuming that the 2 lattices are assembled together and start in register, the lattices would be out of phase as they grow but would get back into register after a certain distance of growth. Such a Vernier repeat may well be the signal to stop further polymerization. Our studies of polyheads reveal that the core consists of several helical chains wound about a hollow centre (Laemmli *et al.* 1974; Paulson & Laemmli, in preparation), with a structural repeat that is different from the repeat of the surface lattice. Thus, the requirement for two lattices with different periodicities is met. Further speculation at this point is unwarranted, but we hope that a study of the surface and core structure of particles containing either a mutationally altered P23 or P22 subunit will help to shed further light on this problem.

(*d*) *Study of the DNA packaging mechanism*

Role of DNA replication

What is the mechanism by which the DNA is pulled into a preformed head? In our attempts to answer this question, we have been thinking in terms of two different general models. In the first model the DNA is pushed into the interior by an apparatus which exists outside the head, while in the second model we propose that the DNA is packaged into the interior by the formation of a local environment inside the head which collapses the DNA. As the DNA collapses in the interior, it will exert a pulling action on the external part of the DNA and the rest of the head-sized piece will be drawn into the head (Laemmli *et al.* 1974). In both models, we have to assume that a DNA end somehow finds its way into the interior and becomes fixed to a protein there.

In the first model, DNA replication might be the active process which pushes the DNA into the interior of the head (Luftig & Lundh 1973). In order to test this possibility, we took advantage of the temperature sensitive mutants in gene 31. In cells infected with the phage containing a mutation in gene 31, no precursor heads accumulate, but instead, the major head protein does accumulate in what appears to be amorphous lumps which stick to the inner cell membrane (Laemmli, Beguin & Gujer-Kellenberger 1970*b*). This material can be in part rescued for normal phage assembly if the product of gene 31 is activated by temperature shift (Laemmli *et al.* 1970*b*; Coppo, Mauzi, Pulitzer & Takahashi 1973). With this system in hand, we ask the question whether active phages are produced if DNA synthesis is inhibited at the time of P31 activation (Wagner & Laemmli, in preparation). The experiment was carried out as follows: Cells were infected with phage (ts A70) in gene 31 at high temperature. The proteins synthesized during this period were labelled by the addition of [^{14}C]leucine to the culture. At 19 min after infection the culture was split in half, and the temperature of both cultures was lowered to 28 °C (permissive condition) in order to permit P31 activation. To one culture we added FUdR to block further synthesis, while the other remained untreated. [^{3}H]thymidine was added to both cultures at the time of the temperature shift to evaluate the inhibitory effect of FUdR. Following incubation of both cultures for 20 min, lysates were prepared and fractionated by sucrose gradient sedimentation in order to test for phage production. Figure 3*a*, shows the sucrose gradient profile of the lysate which has not been treated with FUdR. The gradient contains a peak of [^{14}C]leucine-labelled phage (750 S) which were formed following

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activation of gene 31. (Control experiments, not shown, have demonstrated that activated P31 is required for phage production.) The particles are heavily labelled with [^{3}H]thymidine indicating incorporation of DNA following activation of P31 (figure 3*a*). The number of heads matured in the presence of FUdR is roughly the same as evidenced by the fact that about the same amount of [^{14}C]leucine-labelled material sediments at the position of the phage (figure 3*b*). Moreover, only a very small amount of [^{3}H]thymidine labelled DNA is associated with these phage. From the [^{3}H]/[^{14}C] ratio we learn that heads matured in the presence of FUdR contain less than 1 % as much [^{3}H]thymidine as those matured in the

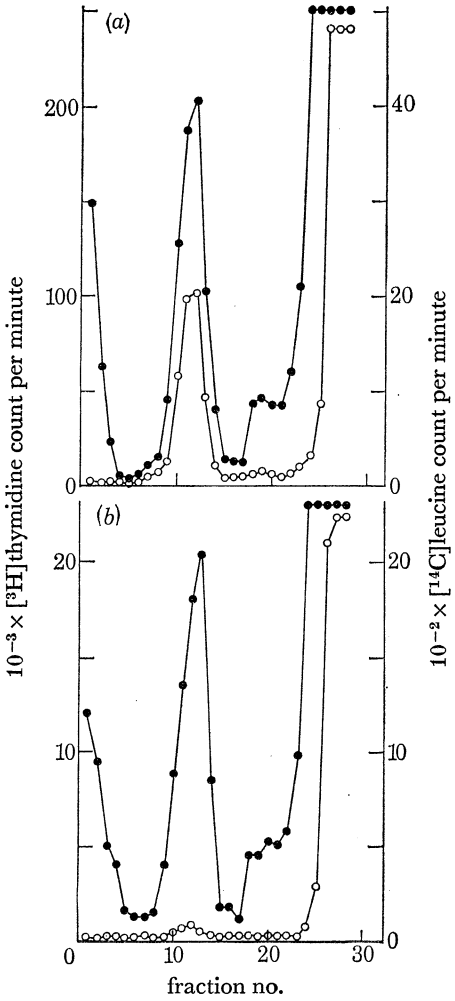

FIGURE 3. Maturation of phage heads in presence of an inhibitor of DNA synthesis. Cells were infected (at 41.5 °C) as usual (see, for example, Laemmli & Favre 1973) with phage containing a mutation (ts A70) in gene 31. The infected cells were labelled with [^{14}C]leucine (1 μCi/ml) beginning 13 min following infection. Incorporation of the label was halted by the addition of a 500-fold excess of cold leucine. At 19 min FUdR (50 μg/ml) and uracil (500 μg/ml) were added to part of the culture. Aliquots of the culture were then diluted (3-fold) into medium at 30 °C. The culture containing FUdR and uracil was diluted into medium containing FUdR and uracil, and [^{3}H]thymidine (6.3 μCi/ml), while the control culture was diluted in medium containing only [^{3}H]thymidine (6.3 μCi/ml). At 50 min after infection, all cultures were chilled, concentrated, lysed in poly(ethylene-oxide) and fractionated on a 10–30 % sucrose gradient (Laemmli & Favre 1973). Panel *a* shows the gradient profile of the lysate which was not exposed to FUdR; Panel *b* shows the sample treated with FUdR. ●——●, [^{14}C]leucine label, ○——○, [^{3}H]thymidine label. Note change of scale of the [^{3}H]thymidine count in panel *b*.

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absence of FUdR. From this we conclude that the material which accumulates at restrictive conditions in cells infected with the mutant phage in gene 31, converts to active phage with the same efficiency whether or not active DNA replication proceeds. This does not exclude the possibility that the small amount of DNA synthesis (about 1 %) in the presence of FUdR is of importance, but this experiment proves that DNA replication is *not* the major driving force of the DNA packaging process.

Role of internal peptides

We were able to show recently that the so-called internal peptides are involved in the DNA packaging mechanism (Laemmli *et al.* 1974; Laemmli 1975). One of the internal peptides (II) is a cleavage fragment of P22 (Goldstein & Champe 1974) and we know that P22 is cleaved in coordination with the DNA packaging process (Laemmli & Favre 1973). This peptide II and another (peptide VII, probably derived from a different precursor protein) are able to collapse DNA into a fast sedimenting structure above a critical peptide concentration (Laemmli *et al.* 1974). These peptides are highly acidic (Champe & Eddleman 1967). They do not bind to DNA (Hitchins & Laemmli unpublished) but are thought to collapse DNA by repulsive interaction as various polymers like poly(ethyleneoxide) (PEO) and polyacrylate do (Lerman 1971). We have also shown that either polyglutamic or polyaspartic acid can collapse DNA (Laemmli *et al.* 1974).

The idea that DNA might be collapsed by repulsive interaction into a highly ordered structure originated with the studies of Lerman and his collaborators (Lerman 1971; Jordan, Lerman & Venable 1972). They demonstrated that in a salt solution containing a sufficient concentration of a simple polymer, high molecular mass DNA undergoes a cooperative structural transition which results in a very compact configuration. Various methods demonstrate that the collapsed DNA is highly ordered and approaches the compactness of the DNA inside the phage head. Our electron micrographs of polyethyleneoxide collapsed DNA confirmed that particles of collapsed T4 or T7 DNA are only slightly larger than the corresponding phage head (Laemmli *et al.* 1974; Laemmli 1975). Some structure is seen on the surface of the negatively stained DNA particles, suggesting that the DNA is folded tangentially to the particle surface.

Experimental evidence suggests that the DNA inside the phage head is in a partially denatured conformation (Tikchonenko, Dobrov, Velikodvorskaya & Kisseleva 1966). For this reason, we have tested whether DNA collapsed with poly(ethyleneoxide) is sensitive to a single-strand specific nuclease. We found that the single-strand specific endonuclease from *Neurospora crassa* extensively breaks double-stranded DNA collapsed with PEO. Normal linear DNA is not measurably cut in the absence of the polymer and extensive digestion only occurs above the critical concentration of polymer required for the collapse (Laemmli 1975). Such an experiment is shown in figure 4, plate 4. Calf thymus DNA was added to a solution containing 0, 20, 40, 60, 80, and 100 mg/ml of PEO, treated with endonuclease and the samples analysed by gel electrophoresis. Figure 4 shows that untreated calf thymus DNA forms a band at the top of the gel. No fragmentation of the DNA is observed if the DNA is treated with endonuclease in the presence of up to 60 mg/ml of PEO (figure 4 *c*, *d*, *e*, and *f*). However, exposure of the DNA to the enzyme in the presence of 80 or 100 mg/ml PEO (figure 4 *g*, *h*) results in the conversion of the large molecular mass calf thymus DNA into a broad band of low molecular DNA

fragments. Under the ionic conditions used in this experiment, the DNA collapses between 60 and 100 mg/ml of PEO.

Extensive nuclease treatment produces a finite DNA fragment distribution. The distribution of the limit digest depends upon the PEO concentration in the range of the critical concentration and ranges between 200–400 base pairs in 100 mg/ml of PEO (6000) (Laemmli 1975). We take these results as evidence for the existence of enzyme-vulnerable sites, possibly unpaired or weakly hydrogen-bonded regions in PEO collapsed DNA. These regions are spaced about 200–400 base pairs apart, corresponding to a linear spacing of 70–140 nm. The enzyme-sensitive regions are probably the result of chain folding of the DNA into tight hairpin loops. The DNA in the PEO collapsed particles is probably not wound like a ball of string, since the DNA in such a structure would not contain tight 'kinks'. More likely, the PEO collapsed DNA contains tight 'kinks' laid down tangentially to the particle.

In support for an involvement of the internal peptides in the DNA packaging events are the following findings:

(*a*) The internal peptides are able to collapse DNA above a critical concentration.

(*b*) DNA collapsed by a repulsive interaction approaches the compactness of the DNA inside the T4 head.

(*c*) The electron micrographs of DNA collapsed with poly(ethyleneoxide) resembles those of '*in vivo*' DNA condensates for several coliphages (Richards, Williams & Calendar 1973).

(*d*) Collapsed DNA contains sites probably at its folds which are attacked by a single-strand specific endonuclease.

(*e*) The cleavage of P22, the precursor of one of the internal peptides (II), occurs concommitantly with the DNA packaging event.

How would a repulsive interaction between the internal peptides and the DNA package the DNA into the head? In our model, we propose that one end of the DNA becomes firmly fixed to a protein inside the precursor head which contains the uncleaved P22 protein. Cleavage of P22 then starts generating locally high concentration of acidic peptides which collapse the DNA. As the DNA collapses in the interior, it will exert a pulling action on the external part of the DNA since the end is firmly attached to the inside of the head. Thus, as more and more acidic peptides are generated the rest of the DNA (a head sized piece) is drawn into the head. We cannot prove this model at present. We feel that the model is thermodynamically plausible but by no means proven.

The major problem arises from the uncertainty of the concentration of the peptides inside the head. The concentration of internal peptides inside the head is roughly 10 to 20-fold below the concentration required to collapse the DNA in an *in vitro* system (Laemmli *et al.* 1974). But in order to calculate the concentration we assumed that the peptides have access to the total interior volume of the phage head. The volume taken up by the core as well as the volume taken up by the DNA as packaging proceeds, has not been deduced. Also, the critical peptide concentration for DNA collapse might be considerably lower if the correct counter ions (possibly polyamines) are used to shield the negative charges of the DNA. We used NaCl in our *in vitro* system.

The DNA can be collapsed by titration with a basic peptide like polylysine (Shapiro, Stannard & Felsenfeld 1969) or could be organized into a compact configuration by sequential interaction with DNA-binding protein (Laemmli 1970; Laemmli *et al.* 1974). The model discussed above required at least one site in the interior of the head to which the DNA binds

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firmly. The basic internal proteins (not to be confused with the acidic internal peptides) do bind to DNA (Black & Ahmad-Zadeh 1971). Recent experiments by Black (1974), however, show that the internal proteins are non-essential phage components, eliminating a primary role for them in the DNA packaging process. Other DNA binding proteins could exist, possibly as transient components and difficult to find for this reason. The involvement of basic DNA binding proteins is not excluded.

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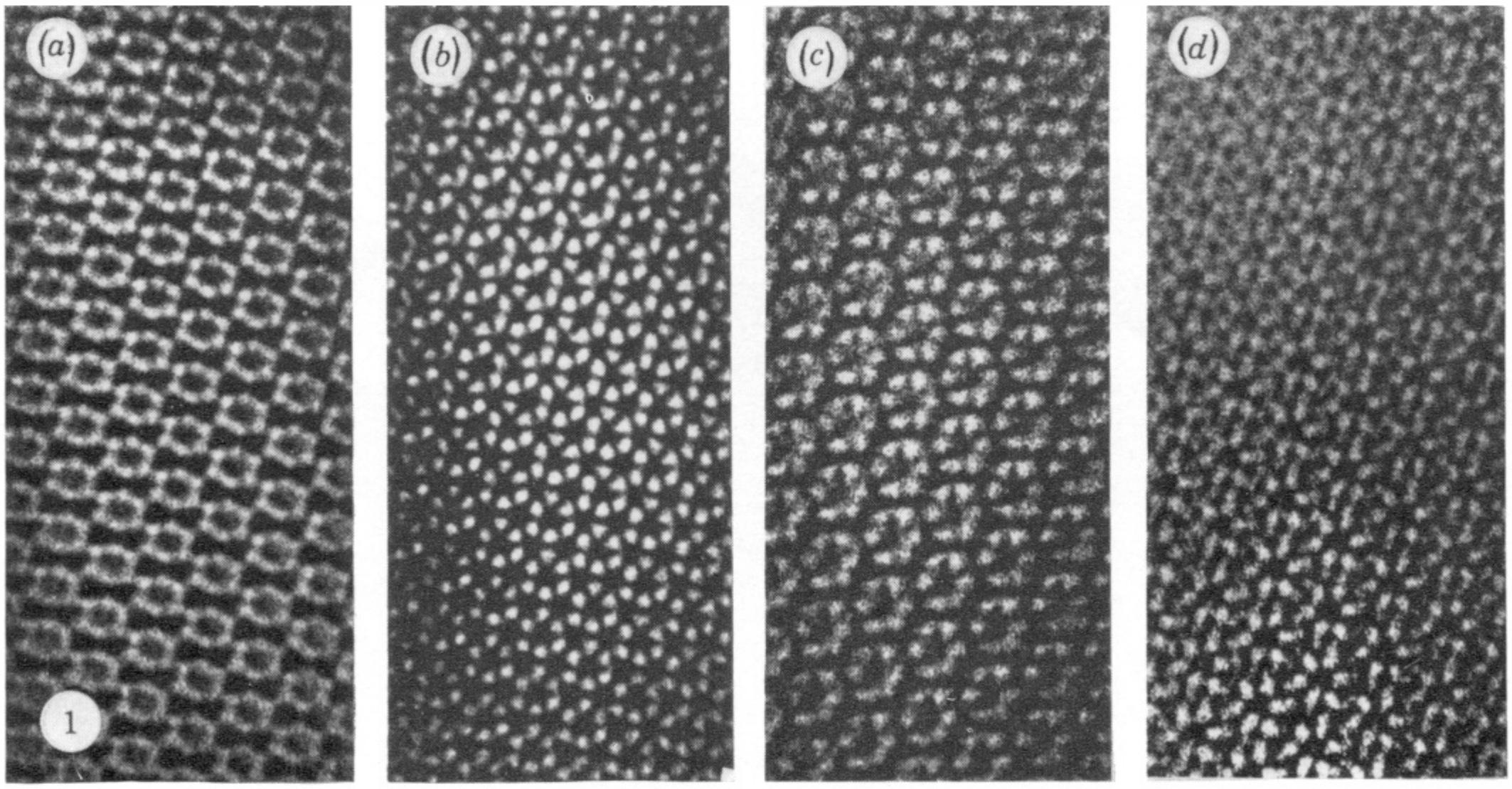

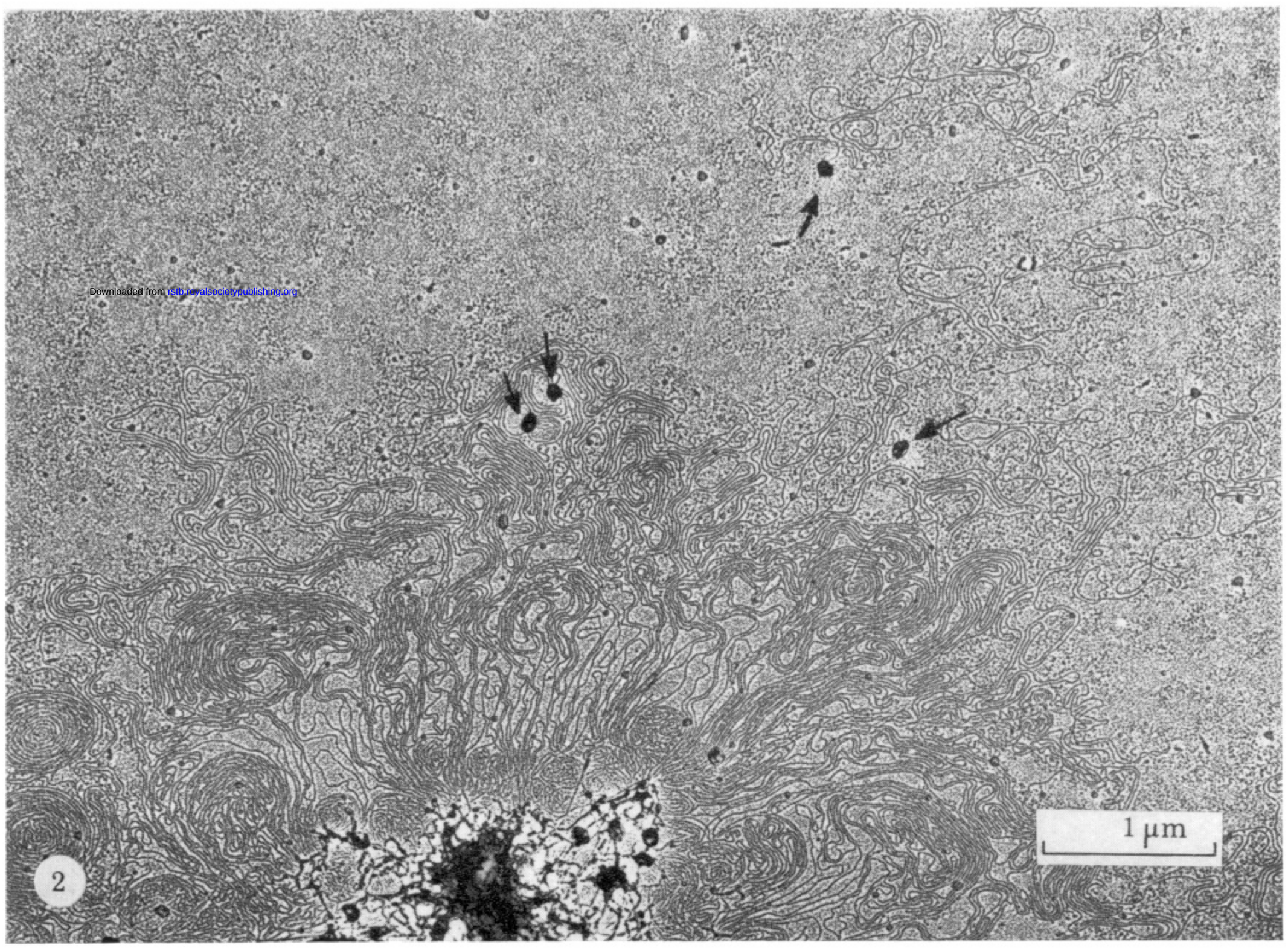

FIGURE 1. Filtered image of the surface structure of class I, II, III and IV polyheads. Negatively stained, uncleaved and cleaved polyheads were prepared and their surface structure revealed by image reconstruction (see Laemmli, Amos & Klug 1975). Panels (*a*, *b*, *c*, and *d*) are representative of class I, II, III and IV polyheads, respectively.

FIGURE 2. Electron micrograph of T4 replicative DNA complex containing attached heads. The replicative DNA complex was isolated from cells infected with a phage containing the mutation (ts P22) in gene 17 (Wagner 1975). The DNA complex was spread by the protein technique described by Inman & Shnös (1970). Arrows indicate attached phage heads.

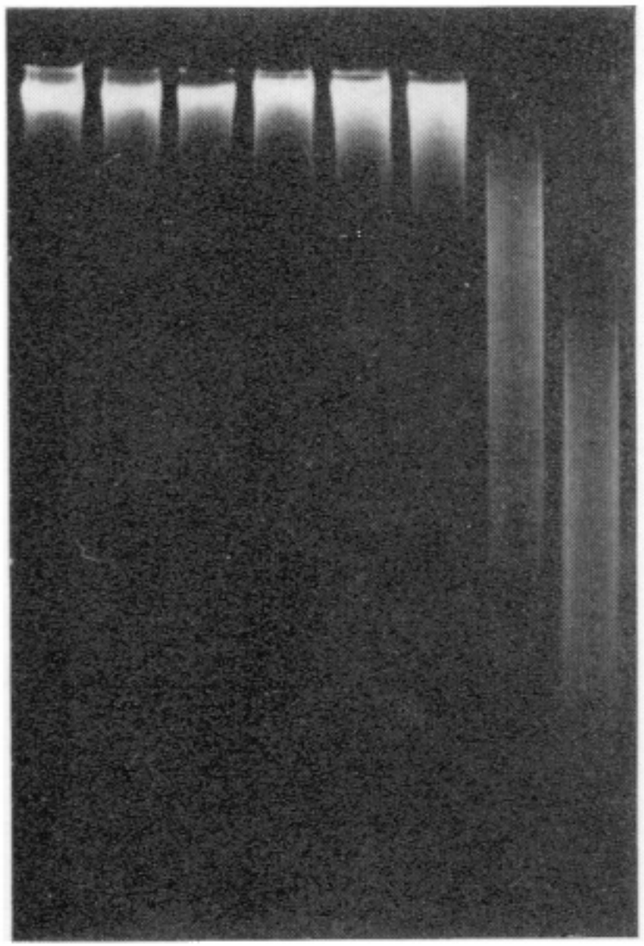

FIGURE 4. **Digestion** of calf thymus DNA exposed to increasing amount of poly(ethyleneoxide) with *Neurospora crassa* endonuclease. Calf thymus DNA in a buffer containing increasing amount of poly(ethyleneoxide) (PEO) were treated with the enzyme *Neurospora crassa* endonuclease for 15 min at 37 °C and analysed on a 6% polyacrylamide gel (Laemmli 1975). Sample *a* contained no PEO and was treated with endonuclease; sample *c*, *d*, *e*, *f*, *g* and *h* contained 10, 20, 40, 60, 80 and 100 mg/ml of PEO, respectively, and were treated with the enzyme.