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THE TRANSFORMATION OF τ PARTICLES INTO T4 HEADS

II. Transformations of the surface lattice and related observations on form determination*

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In part I of this paper (1) we give evidence that the P23-capsoid of τ-particles is transformed in situ into the P23*-capsid of normal phage. Using the polymorphism of phage T4, we have chosen polyheads as representative of P23 assemblies and giant phages as representative of P23* assemblies in order to study their surface crystals by optical filtration of micrographs. We found for polyheads a lattice constant of 112 Å with the typical hexameric, ringlike capsomer and for the giants a lattice constant of 124 Å with quite a different capsomer morphology, of the type (6+1). From the stoichiometry of the proteins composing the normal capsid we conclude that the protomer is a single P23* molecule and that the minor capsid-proteins must be in singular positions on the surface lattice or on the polyhedral head (center of capsomers, vertices, or basal part).

We extrapolate the findings on the giant head to the normal head and give a geometric model which is consistent with 1,100 molecules of P23* per capsid.

We discuss the part of form inheritance contributed by P23 and the other form-giving gene products and give evidence that morphologic characters are the result of pairs of a reaction chain of interacting gene products. The example we give is the giant head produced by a ts mutant in gene 24 at 36°C.

INTRODUCTION

In an earlier paper (1) experiments are described which indicate that the capsoids of τ-particles are transformed into the capsids of a mature T4 phage head in a conservative fashion. In other words, P23 of the capsoid is transformed in situ into P23* of the capsid. This is accomplished apparently without further addition of P23 although this transformation takes place with a clear increase of size, estimated to give about a 10–20% increase in length and width of the capsoid (2). At the same time the bonding between the capsid subunits increases very strongly as indicated by the threshold for dissolution into subunits (3). These changes must be accompanied by profound alterations in the arrangement of subunits on the surface lattice and probably also by marked changes of the tertiary

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structure of P23 [MW = 59,000 (4)] during its transformation into P23* [MW = 47,500 (4)].

Capsoids and capsids of τ-particles and phage heads are not adequate for precise crystallographic determinations of the positions of their protein subunits. T4, however, has the invaluable property of genetic polymorphism, as summarized in Fig. 1 (5, 6). From experimental observations accumulated through the years it has become clear that these variants fall into two distinct classes according to whether they are made of P23 or P23*. Each of the classes is characterized by several distinct properties with respect to stability towards dissociation, surface fine structure visible on negatively stained particles, serology, the thickness of the layer (90 Å) estimated from thin sections or determined precisely on multilayered polyheads (7), and the 40–60 Å thickness estimated on micrographs of capsids.

The τ-particles belong to the group of P23 assemblies, while normal phage heads belong to that of P23* assemblies. We have chosen the tubular forms – i.e., polyheads from mutants in genes 20, 22, 24, 40, and IPIII and capsids of giant phages (8) – as provisional representatives of the two groups for our studies of subunit arrangement by optical diffraction. Among these tubular forms are those arising simultaneously with τ-particles in mutants in gene 24; these polyheads seem to grow out of – or are capped by – a τ-particle (1). For reasons of continuity it is reasonable to assume that the surface crystal of this tubular form is an extension of that of the τ-particle. This hypothesis is further supported by the observation that cross sections of τ-particles cannot be distinguished from cross sections of the single-layered polyheads (1; Fig. 1).

Certain mutants in gene 23 lead to a small proportion (1–10%) of so-called giant T4 phages, which have been shown to contain P23* and the same minor proteins as normal phage (8).

We will show that the lattice constant of giant capsids is 124 Å and that the morphology of the repeating unit is also different when compared with that of polyheads. We then present data which show that in normal phage heads P23* is the main component of the lattice and therefore contains most of the specific bonding needed for the physical cohesion of the capsid.

Finally, we will present some preliminary data which suggest that the form of the morphological variants is not determined by the properties of the P23 or P23* lattices alone but is the consequence of interactions between P23 and minor proteins of the capsid or between capsid proteins and core proteins. Indeed, the same morphological variant can be the result of a specific gene product modification or of the absence of either one or several gene products.

These experimental observations will be discussed with regard to general considerations relating the form inheritance of the head of phage T4 to its pathway of maturation.

MATERIAL AND METHODS

Phage Techniques

To prepare concentrated lysates we followed the procedures given in Bijlenga et al. (1). The phage T4 mutants and the bacterial strains come from our collection unless another source is specifically mentioned. The mapping of ts mutants in gene 23 was done as follows: Cells of E. coli CR63 were grown in M9A to 4×10^8 cells/ml, centri-

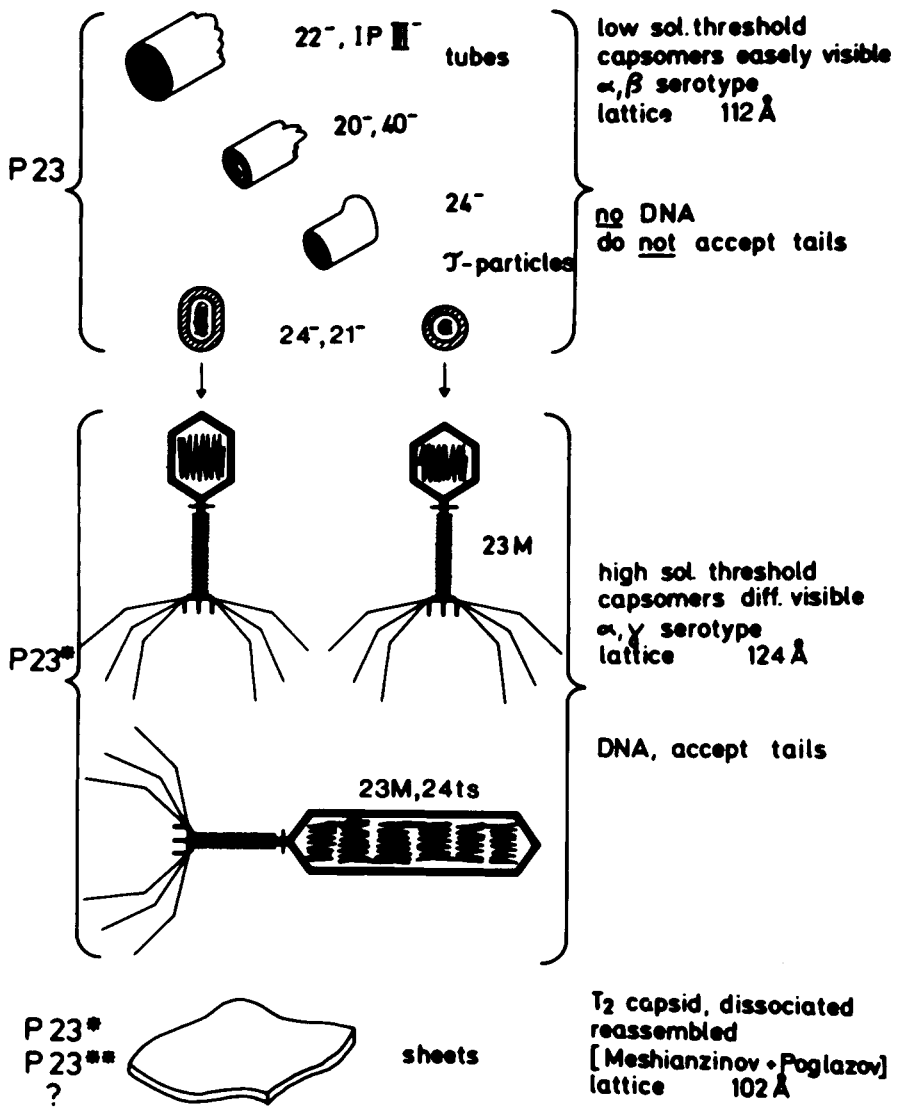

Fig. 1. Polymorphism in T-even phage. Illustrates the two groups of P23 assemblies and of P23* assemblies. Gene numbers with a (–) superscript indicate the results using amber mutants, where the fragment has shown no effect upon assembly. 23 M indicates mutant(s) in gene 23 which lead to a complete gene product, presumably with a single amino acid substitution. 24ts is a temperature-sensitive mutant which produces giants when grown at 35°C. P23** is a further modified P23*, which moves faster than P23* in electrophoresis.

fuged, and resuspended in phosphate buffer at a concentration of 2×10^8 cells/ml. Ten min prior to a cross, casamino acids (1%), glucose (0.5%), and NH_4Cl (0.1%) were added and the culture incubated at 30°C. At time 0 a mixture of both parents was added, each at a multiplicity of infection (m.o.i.) of 10. Ten min later the infected culture was diluted 1/200 in prewarmed (30°C) M9A; at 70 min the lysis-inhibited culture was lysed by chloroform, diluted further, and assayed for plaque-forming units.

This procedure is modified from that described in Sarabhai et al. (9) which we used initially. With that method we frequently experienced that the nongrowing cells in buffer showed lysis from without upon infection of those cells which statistically had the highest m.o.i.'s.

Electron Microscopy, Optical Diffraction, and Filtration

Electron microscopy. Specimens for electron microscopy were prepared by adsorbing a drop of sample to a copper grid with a carbon-coated Collodion film which was usually rendered hydrophilic by glow discharge in air under low pressure. The micrographs were recorded on Kodalith film and developed in Kodak DK60. In no case was use made of the beam-rocking (minimum exposure) technique of Williams and Fisher (10). An anticontamination device cooled by liquid nitrogen was used.

P23 lattices: polyheads. The polyhead lysates were partially purified by low-speed centrifugation to remove gross bacterial debris before being fixed in 2% formaldehyde and stored at 4°C until use. Unfixed reference specimens obtained immediately after lysis showed no difference under microscopic examination at the level of "biological resolution" (above 30 Å) achieved in these experiments. All preparations were used within one week. All micrographs of polyheads (negatively stained with 1.5% NaPT at neutral pH) were taken on an AEI 801 microscope operating at 80kV and fixed nominal magnification of 63,000 diameters.

P23* lattices: T4 giants. The specimen preparation and microscopy of the T4 giants was carried out by F. A. Eiserling, following the methods described in Doermann et al. (8).

T2 reassembled sheets. In this case, the best results were achieved by using 1% uranyl acetate as negative stain. The microscope was a Philips EM 300, working at 80kV in the magnification range of 35,000–45,000 diameters.

Calibration of magnification. Calibration was made relative to the separation of the first meridional reflections in the diffraction image of T4 tails which were also on a given micrograph. Tails were abundantly present in the polyhead lysates and, of course, were attached to the T4 giants. In the case of the reaggregated T2 sheets, tails from a lysate of a T4 headless mutant – 23(amHII) – were added prior to the preparation of specimen grids for microscopy. The distance from the diffraction plane origin of the first meridional reflections corresponds to the axial repetition distance of the sixfold annuli of protein subunits which make up the extended T4 tail. For this distance, we took the value of 38 Å (11). The reliability of this method of calibration was assessed statistically from measurements made on many tails observed on each of a number of different micrographs. The criterion of standard-deviation/mean estimates this calibration of magnification to be accurate to within 2%. Although the absolute values of the lattice-constant determinations is subject to the accuracy of the 38 Å figure assigned to the axial spacing of the subunits in the T4 tail, the relative values of the lattice constants are accurately known.

Optical processing of micrographs. Procedures to be followed for efficient optical diffraction and filtrations are now well established (12, 13, 14). In all such work reported here, the protocol and the equipment described by Aebi et al. (14) were used.

Thin sectioning. Thin sectioning of lysis-inhibited "pregnant" bacteria was performed as follows: Infected cells obtained by the methods outlined in Bijlenga et al. (1) were fixed in 3% formaldehyde and immediately centrifuged at 1,500 g. The total time for both operations was 5 min. The pellet was resuspended in a solution of 1% OsO_4 and 0.1% tryptone in Michaelis buffer (pH 6.2 and 0.1% $CaCl_2$). After standing at room temperature (22°C) for one hour the suspension was centrifuged for 3 min at 1,500 g and the pellet resuspended in agar. Small agar blocks (1 mm × 1 mm × 1mm) were cut and fixed in a solution of 1% OsO_4 in Michaelis buffer overnight at room temperature. Post-fixation was done in 1% uranyl acetate in Michaelis buffer for 2 hr, following which the blocks were dehydrated in acetone, embedded in Epon (Epicote 812), and sectioned (thicknesses of 250 ± 50 Å) on an LKB Ultratome Microtome. Sections were stained with 5% uranyl acetate for 1 hr and observed in a Zeiss EM10 electron microscope at 80 kV. Micrographs were recorded on Kodalith film.

Protein Composition of the Capsid

Three methods of labeling were used: (a) an appropriate dilution of ^{14}C glucose (NEC-042B from NEN) as a unique source of carbon was added to the medium just prior to inoculation with a 1:100 dilution of a saturated, overnight bacteria culture; (b) amino acid labeling with ^{14}C algal hydrolysate (CFB 104 Amersham) – (b1) constant label: four drops of an adequate dilution was added every minute to the culture, from 1 min after infection up to 60 min to reach a total of 250 μCi; (b2) a unique addition at 14 min after infection and harvest 5 min later.

We checked the differences in the labeling of phage heads using the algal hydrolysate or a mixture of equally labeled amino acids (CFB 152, Amersham). We performed amino acid analysis of the purified heads and found that the ratio of incorporation of ^{14}C into any given amino acid in the late proteins of the phage heads changes by no more than ± 30%. From this we estimate that the difference in amino acid ^{14}C-specific activities will not significantly affect the protein labeling and that an algal hydrolysate will be an adequate substitute for equally labeled amino acids. The differences between labeling with a mixture of amino acids and with glucose are visible in Table IV.

Preparation of phage heads for analyses. Heads and head ghosts were prepared from 10(amB255) • 18(amE18) double mutant-infected E. coli cultured in M9, infecting and superinfecting at an m.o.i. of 5 and harvested as described previously (1). At 90 min the infected cells were sedimented and resuspended in Tris buffer (0.05 M, pH 7.6). Chloroform and DNAse (treated with PMSF for inhibiting proteases) were added at 37°C to induce lysis. Bacterial debris was removed 15–30 min later by centrifugation. The supernatant was layered on a sucrose gradient (7% to 35%). This was all done rapidly, because 10^- heads very easily lose their DNA. Heads sediment at 1,200 S, head ghosts at 300 S. These two bands were harvested as two fractions and dialyzed against water.

In the case of glucose ^{14}C labeling, one aliquot of the head suspension was layered on a Cesium chloride "step" gradient with densities of 1.60 – 1.50 – 1.40. The heads were collected just above the 1.60 layer.

Determination of "true" capsid proteins by differential extraction. The two fractions ("heads" and "head ghosts") were treated in two ways: (1) aliquots of heads

were treated with DNAse with or without PMSF combined with 3 cycles of freezing and thawing, and (2) aliquots of heads and head ghosts were made in 8 M urea and dialyzed against 8 M urea at various pH's.

To separate residual capsids from the proteins dissociated by treatment the suspensions were centrifuged for 90 min at 72,000 g. The supernatant contains the differential extract with particles smaller than 170 S. The pellet contains the residual capsids. Both fractions were analyzed by SDS polyacrylamide gel electrophoresis.

SDS-polyacrylamide gels. Pellet and supernatant were analyzed on 10% acrylamide gels using discontinuous buffers as earlier and also using slab gels (15). The autoradiographs were quantitatively analyzed from optical density plots obtained with a Joyce-Loebl Chromoscan. The molecular weights were determined after calibration with the following proteins: catalase – 60,000; ovalbumin – 43,000; actase II – 33,500; actase I – 17,000; and lysozyme – 13,500.

Molecular weight of the capsid (Table I). Protein content per particle was determined: (a) by the method of Lowry et al. (16), using BSA as reference, and then divided by the particle concentration determined by electron microscopy counts with Polystyrene Latex as reference (17), and (b) by light scattering according to the procedure of Zimm et al. (18) using a program devised by Martin Zulauf and Roberto Marcoli (Department of Structure Biology, Biozentrum). The extrapolations needed to estimate the molecular weight, M, were made by weighted least squares fits, and the standard deviations of the extrapolated values were also calculated.

RESULTS

The Two-Dimensional Structure of P23 Capsoids as Compared with that of P23* Capsids

In order to get some insight into whether or not the lattice constant and the morphology of the capsomers are the same for all the P23 assemblies, we compared polyheads produced by mutants in five different T4 genes. Table II shows that to within experimental error the lattice constants all have the same value of 112 Å. Optical filtration analyses of 20^- and 22^- polyheads have already been published (12, 19). We have extended these studies to mutants in genes 40, 24, and IPIII and double mutants with other head genes. This work is being continued and a full account will be given at a later date (20). It should be emphasized that the figures in Table II refer exclusively to polyheads of the "coarse empty" type according to the classification of De Rosier and Klug (12) – that is, native polyheads from fresh, chloroformed lysates which either were used or were fixed immediately after lysis (see Material and Methods). Such polyheads show up on the microscope grid as, in effect, completely flattened and emptied of previously enclosed material. The power in their diffraction plane images lies mainly in the second-order spots. Figure 2 shows a typical micrograph of (22^-) polyheads, the diffraction pattern of a chosen length of polyhead, and the optically filtered image of one side.

Later we shall also discuss the other categories of polyheads which have been observed (19) and which probably arise through post-lysis modifications of the "coarse empty" type. It is noteworthy that the 24^- polyheads are the only tubular forms whose production is synchronous with that of τ-particles. Moreover, they are frequently closed at one end. These two observations suggest that their surface structure should have much

TABLE I. Molecular-Weight Determination of the Capsid

	Lowry* (gr/ml) $\times 10^{-4}$	Electron Microscopy (capsids/ml) $\times 10^{12}$	Lowry/EM (gr) $\times 10^{-16}$	MW $\times 10^{6}$	MW, Light Scattering, $\times 10^{6}$
Head	8.93	6.4	1.4	85	
Head	5.36	3.8	1.41	85	
Capsid	24.7	17	1.45	88	93† (87)

Molecular weight of capsid as determined by the two procedures given under Material and Methods.

*In control experiments with 10^{-} capsids we tested two other methods besides that of Lowry – the method of Biuret and the method of weighing dried capsids. We obtained approximately the same results and chose the method of Lowry for the main experiments because it is much more sensitive.

†The value of 93×10^{6} obtained by the method of light scattering was done on a 10^{-} capsid preparation known to be contaminated by polysheath. We therefore regard the second value of 87×10^{6} as more accurate (obtained as described in Material and Methods.).

TABLE II. Lattice Constants of the Different Native T4-Polyheads

Mutant	a_2 (Å)	a_1 (Å)	Number
20(amN50)	110.0 ± 3.4	112.2 ± 3.7	13
22(amB270)	115.0 ± 3.3	114.0 ± 3.9	6
24(amN65)	111.2 ± 3.1	110.4 ± 4.0	11
40(tsL84)	114.7 ± 2.3	115.1 ± 3.7	11
IPIII^{-}(amH9)	111.3 ± 3.5	110.9 ± 4.4	17

Lattice constants determined by diffraction on negatively stained polyheads. a_1 and a_2 are the mean lengths of the lattice vectors, as measured for the five different mutations in question. The notation is that of Yanagida et al. (19).

in common with that of 24^{-} τ-particles. Therefore, it seems significant to us that the lattice constant and capsomer morphology of the 24 polyheads do not seem to differ in any fundamental respect from those of the other tubular forms. A number of optical filtrations have been carried out of micrographs of the different polyheads; four typical examples are shown in Fig. 3. On comparing the filtered images we are led to the tentative conclusion that the "coarse empty" type of polyhead has a capsomer of characteristic morphology, positioned in a specific orientation relative to the lattice lines of the underlying hexagonal net. To within the resolution of biological information present in our electron micrographs, these features are independent of the mutation responsible for producing the polyhead and of the diameter of the polyhead itself.

In agreement with Yanagida et al. (7) we have found also that the pitch of the polyhead lattices varies among those produced by a given mutant. Our preliminary analysis suggests that there is a strong correlation between the pitch of the lattice and the diameter of the polyhead. Therefore, although a unique hexagonal net with a characteristic P23 capsomer morphology appears to be common to all P23 assemblies, the manner in which a three-dimensional cylinder may be formed from this net is not unique. In contrast, it seems likely that the wild-type head precursor corresponds to a unique folding of the hexagonal net into a closed capsoid structure with capsomers of specific morphology and orientation.

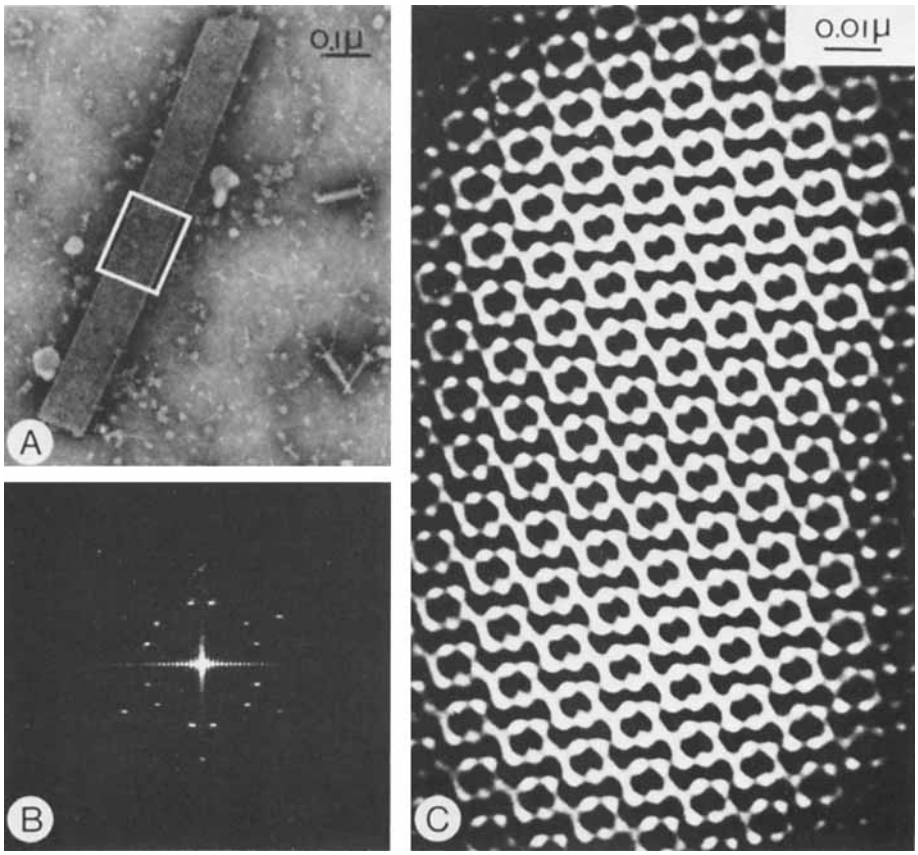

Fig. 2. Optical filtrations of a polyhead from T4 · 22(amB270). (A) shows a micrograph. The window encloses a portion in seemingly good state of preservation, whose optical diffraction pattern is shown in (B), printed in high contrast which suppresses the background noise and emphasizes its appearance as the superposition of two hexagonal patterns (one originating from each side of the polyheads). In this specimen, the pronounced "common" meridional spots lying outside the strong second order facilitate an indexation of u/v = 2/1 corresponding to a pitch angle of 19.1°. The optically filtered image of one side appears in (C), the protein being reproduced in white as in the original micrograph.

The giant phages which we have studied are the product of mutant (ptg191) in gene 23 (8, 39). These give diffraction patterns which can be indexed assuming a double layer of hexagonal structures (12, 13). The diffraction pattern shown in Fig. 4 exhibits no first- or second-order reflections but rather strong third- and fourth-order reflections. This is compatible with the smooth appearance of both the negatively stained giants and the normal heads.

We analyzed 18 micrographs with indexable diffraction patterns. In Table III we see that all patterns show a lattice constant of 124 ± 5 Å; in addition they all have the same u/v ratio (see footnote in Discussion), which is 3/1 and corresponds to a pitch of 13.9° for a perfect hexagonal lattice. This is in marked contrast to the wide variation in pitch found in polyheads (from 0° to 30°). Fig. 4c shows a filtration of the tubular part of an empty giant.

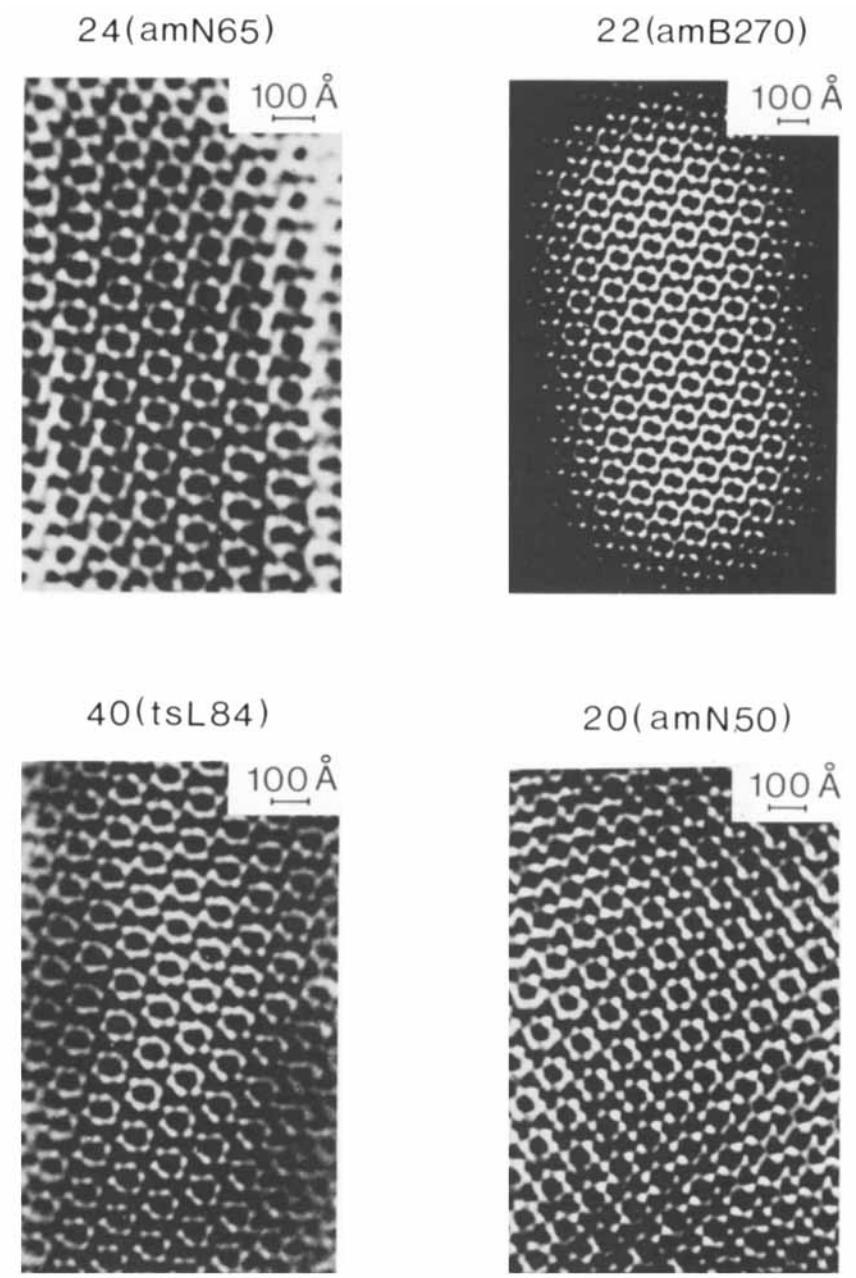

Fig. 3. Optical filtrations of polyheads. Here we give representative examples of polyheads stemming from lysates of mutants in different genes. To the available resolution (> 30 Å), the capsomers appear to have the same morphology and to lie in the same orientation relative to the lattice lines. The examples shown here were indexed as having the following u/v ratios (although this quantity was found to vary widely even for polyheads of a given mutant): 24^{-} (4/3), 22^{-} (2/1), 40^{-} (3/1), 20^{-} (5/2).

TABLE III. Lattice Constants of Polyheads, Giant Capsids, and Reassembled Sheets

Variant	Number of Measured Particles	Average Lattice Constant	Standard Deviation	u/v Ratio
T4 polyheads	58	112 Å	± 4 Å	heterogeneous: 2/1, 3/2, 4/3, . . .
T4 giants	18	124 Å	± 5 Å	most common 3/1
T2 lollipops	2	123 Å	± 2 Å	3/1
T2 sheets	4	102 Å	± 7 Å	—

Comparison of the lattice constants of some P23 and P23* assemblies: polyheads, giant capsids, T2 "lollipops," and reassembled sheets. For a definition of the "u/v ratio" see footnote on page 270.

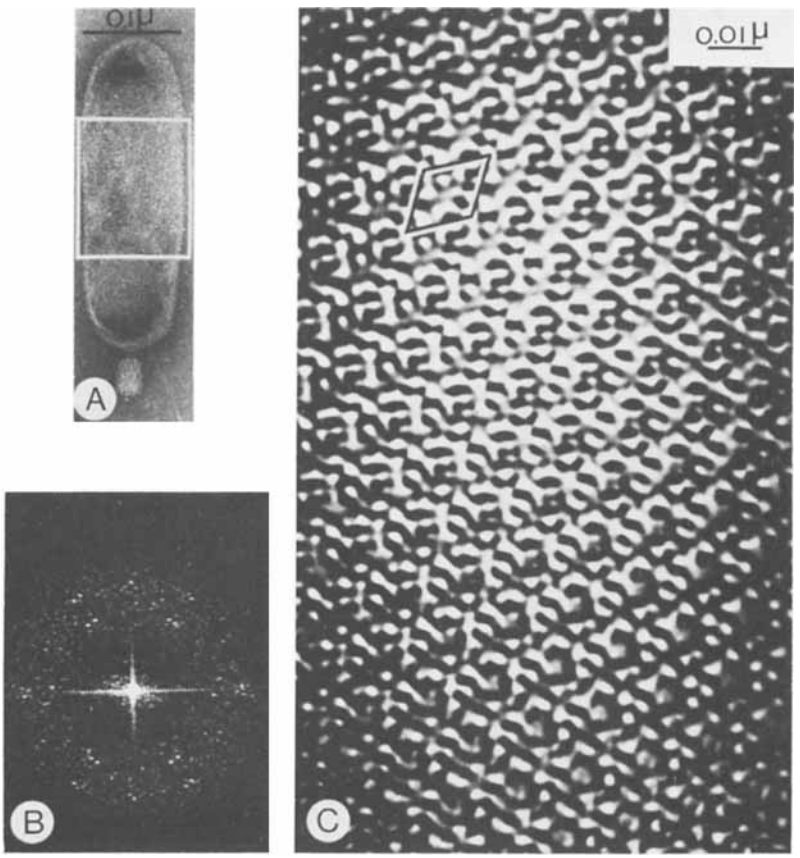

Fig. 4. Optical filtration of a ghosted giant phage T4 ptg191(23). (A) Electron micrograph. The negatively stained head is almost completely flat. The windowed area gives the diffraction pattern (B), which may be indexed according to a u/v ratio of 3/1 (pitch angle, 13.9°). As in the original micrograph (A), the protein of a filtration of one side (C) is depicted as white. A unit cell whose vertices are centered on four neighboring subunits is shown to aid identification of the features discussed in the text.

Comparing this filtration with Fig. 2c makes it clear that the P23* capsomers look different from the P23 capsomers: the protomers have a more elongated or armlike profile in projection rather than a round one and the centers of the capsomers are distinctly occupied by mass. The filtration in Fig. 4c is comparable to the ones shown by Yanagida et al. (19) of the "fine aged" and the "high temperature" polyheads which also show distinct (6+1) capsomers. We still have to establish the significant difference between these capsomer morphologies and those of giants. It is of great interest that the lattice constants measured by Yanagida et al. (19) for their "fine aged," "fine mottled," and "high temperature" categories of 22⁻ polyheads are all in the vicinity of 125 Å. In consequence their surface lattice would seem, in at least one important respect, to bear a closer resemblance to the lattices of the T4 giants (lattice constant 124 Å) than to that of the "coarse empty" polyhead (112 Å). This phenomenon is probably related to the "spontaneous" in vitro P23 cleavage observed in many laboratories when unfixed lysates containing P23 are stored without chloroform.

Further work on the implications of this observation is being carried out in this and other laboratories and will be reported in due course (Laemmli personal communication and 21).

The smooth appearance of the capsid surface in both direct and filtered micrographs of negatively stained preparations is in noteworthy contrast to the appearance of frozen-etched capsids, where capsomers are clearly visible (22). We have also studied the "lollipops" of phage T2 (23). These appear to be analogous to the giant phage of T4, but are produced by growing the T2-infected bacteria with canavanine, an amino acid analogue of arginine. We found that their lattice constant is 124 Å. the same as that of T4 giants (Table III).

Poglazov and Mesyanzhinov (24) and Poglazov et al. (25) have shown that material obtained by alkali dissociation of T2 capsids can be reassembled into sheets with dimensions of several microns. We repeated these experiments successfully, using T2L capsids, and attempted to extend them to T4, where they failed. The reason for the failure appears to lie in the higher pH required to dissociate T4 capsids – pH 12.8 for T4 and only 11.9 for T2 (26, 24). At pH 12.8 proteins are not only completely unfolded, but hydrolysis of peptide bonds in P23 also occurs. The optical filtration of the reassembled T2 sheets is shown in Fig. 5c. The lattice constant (Table III) is 102 Å, considerably smaller than that of polyheads. The capsomer morphology is also quite different from that of either T4 polyheads or giant phage. It appears to resemble more closely that of the in vitro transformed polyheads described by Laemmli (21). We have only a limited amount of information on the composition and characteristics of these sheets, which have not yet been purified. We cannot yet state definitively whether they are composed of P23*, some further modification of this protein (P23**), or even possibly of a minor capsid component. If, however, these sheets are shown to be made of pure P23* then comparison with its other assembly forms will be very interesting.

Protein Composition of the Final Phage Head and of Its Capsid

For the interpretation of the results reported above on the surface lattice of capsids, as well as for a better understanding of the mechanisms of form inheritance, a precise determination of the protein composition of the phage head and its capsid is indispensable. We have, therefore, analyzed the full head (produced by a mutant in gene 10 which fails to assemble tails), the "head ghost" obtained after release of the head content, and the

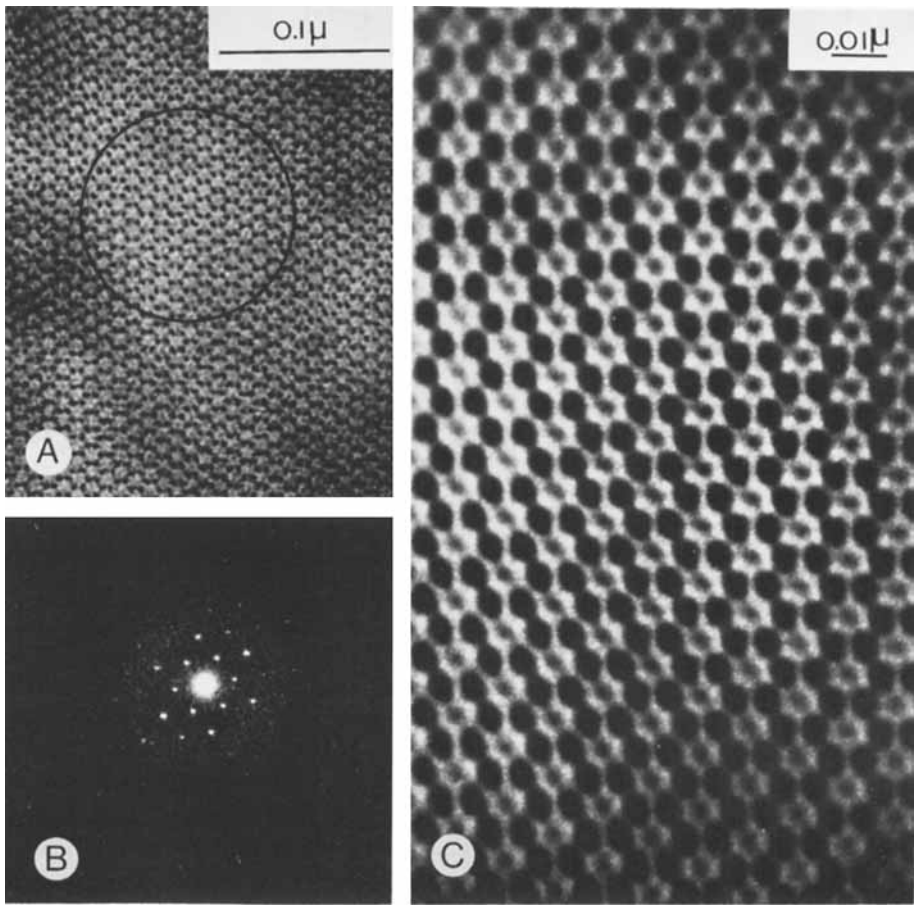

Fig. 5. Optical filtration of a reassembled T2 sheet. (A) shows the micrograph. The windowed area produces the diffraction pattern (B). Here, a single sheet is being observed and the hexagonal character of its diffraction pattern is not complicated by the superposition of patterns from successive layers. In the filtered image (C), the protein is shown as white, as in the original micrograph (A).

(residual) capsid after differential extraction by 8 M urea at pH 11. The results are given in Table IV. We can see that the head contains at least 11 proteins, while the residual capsid still contains at least six. P23* is represented in about 1,100 copies, and no other protein is found in comparable amounts.

It should be mentioned that one of the unidentified proteins which remains in the residual capsid even after differential extraction, is contributed by the host. We cannot yet give a reliable quantitative estimate for the number of these molecules. A protein contributed by the host was also found independently by L. Onorato (27).

The Contribution of P23 to Form Determination

Doermann et al. (39) have convincingly demonstrated by genetic means that ptE920g, a mutant producing 30–60% of phage with short isometric heads (36), is located

TABLE IV. Protein Composition of T4 Heads and Capsid

Protein	Band	Head†	Head‡	Head§	Head Ghost‡	Residual Capsid‡	Extractable§§
B1	12	55	110	60	80	0	+
P20	11	15	15	15	15	10	–
Whiskers	10	–	20	–	70	0	+
P23*	9	1,150	1,040	1,180	1,100	1,100	–
	8/6	–	60	–	60	60	?
P24*	7	150	170	150	170	170	–
	6/8	70	–	–	–	–	?
Host	5	nonlabeled		160	nonlabeled		–
	4	90	135	130	100	100	–?
IPIII*	3	165	175	180	150	0	+
	2	150	150		100	100	–?
IPII				800			
IPI	1	600	500		380	200	+/–
X							

In this table, composition of complete heads, of ghosted heads, and of the residual capsids remaining after differential extraction with 8 M urea at pH 11–12 are given. Band 12 is the one named B1 by Coppo et al. (40). It is not released by osmotic shock (41). With 20^- and 24^- we have confirmed the previous result (40) that this band is lacking in lysates of all X genes. Either band 8 or band 6 is found on the same gel; sometimes either band is superposed by band 9 or band 7. Bands 8 and 6 may be different forms of the same protein. We have found that all our samples of commercial DNAse contain proteases. They completely digest the proteins of bands 12 and 13. These appear only when the proteases are inhibited by pretreating the DNAse with PMSF. Band 1 is obviously a mixture of proteins which we have not further analyzed.

†^{14}C amino acid mixture labeling with a pulse at 14 min after infection without chase (method b2, page 000).

‡^{14}C amino acid mixture, constant labeling (method b1, page 000).

§^{14}C glucose labeling (method a, page 000).

§§A minus sign (–) denotes proteins which remain in the residual capsid when the capsid is differentially extracted at pH 12 in 8 M urea. These proteins (P20, P23*, P24*, the suspected host protein, and the proteins corresponding to bands 4 and 2 become soluble simultaneously when the entire capsid dissociates.

within gene 23. They were able to do this by using a newer amber mutant [E1236 (28)] which is near the C-terminal end of gene 23 (Fig. 8). The same authors isolated other form-mutants in gene 23 of which three are characterized and mapped (Fig. 8). All of them produce 0.1–10% of so-called giant-headed phages (8). Hence, no doubt remains that P23 has form-determining properties. This is corroborated by our earlier findings which showed that a ts mutant in gene 23 produces deformed or "crummy" heads (29). When comparing "crummy head" morphology with that of τ-particles (Figs. 6 and 7), it appears that the "crummy" heads are morphologically more closely related to τ-particles than to mature, empty heads. We submitted the lysates of three available ts mutants to gel electrophoresis and found that most of their P23 is not cleaved when the mutants are grown at the nonpermissive temperature of 40.5°C (Fig. 9). Preshift labeled P23 is not converted into P23* when the temperature shift-down is made in vivo (Fig. 9). These intracellular, "crummy" heads, therefore can not be converted into P23*-capsid related structures.† "Crummy" heads seem to be less firmly attached to the membrane than

†Preliminary results by L. Onorato and one of us (M. K. S.) have, however, shown that in vitro cleavage of P23 into P23* or P23** can occur under certain conditions. This also happens with polyheads. Related phenomena have been observed incidentally by several workers in the field (U. Laemmli, R. Bijlenga, H. Yanagida, I. Bendet, and C. Kellenberger) and now seem to have become experimentally controllable.

Fig. 6. "Crummy" particles produced by ts mutants in gene 23. E. coli B infected with T4 ·23 (tsA78) under conditions leading to lysis inhibition. In situ lysis in NaPT 2% on the specimen grid after induction with 0.01 OsO_4 (2).

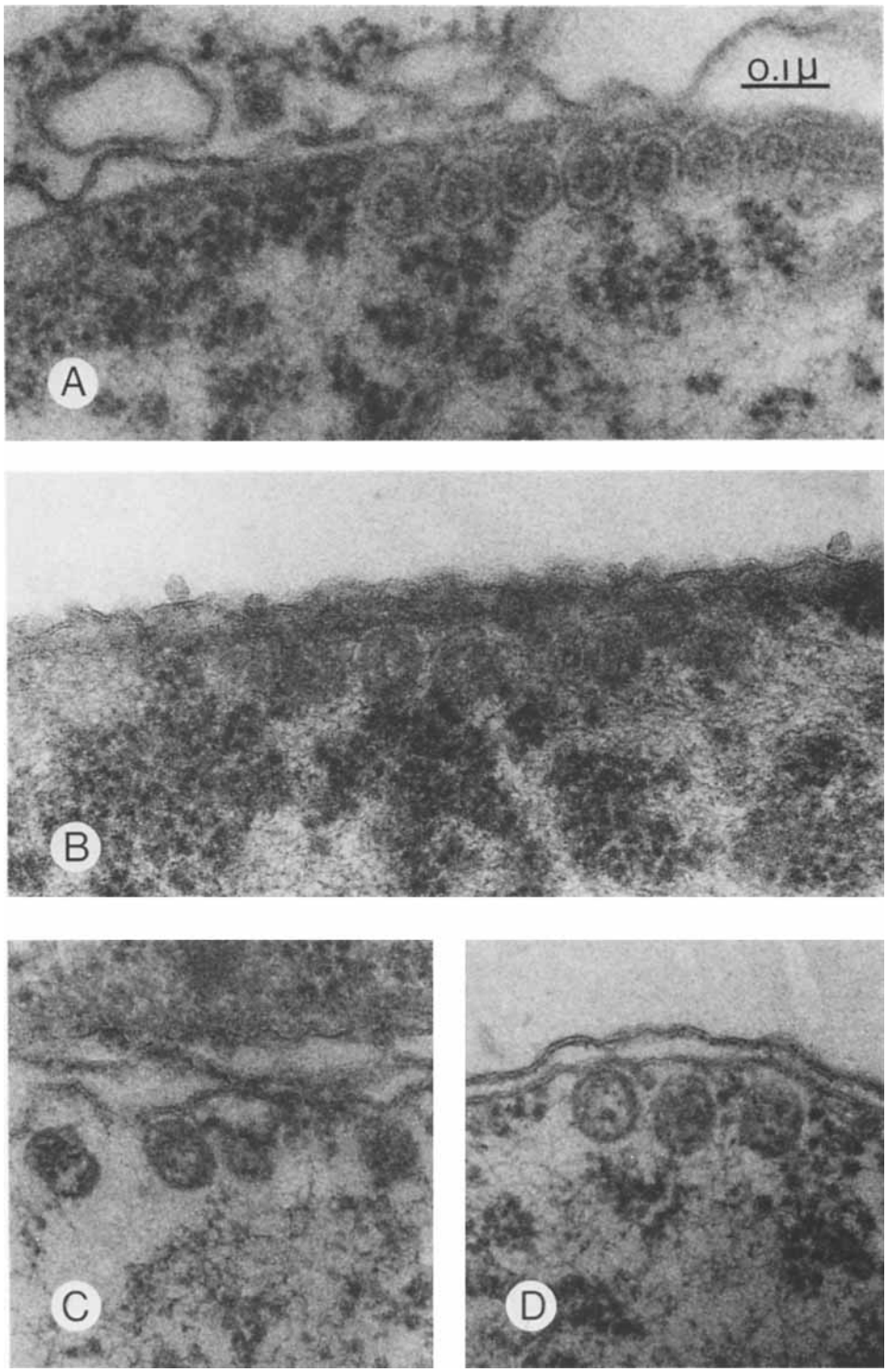

Fig. 7. τ-particles (A) and "crummies" (B, C, D) on thin sections. All infected cultures were made and processed for sectioning as described in Material and Methods. (A) τ-particles produced by T4 · 49(amE727) · 24(amN65). This double mutant gives exactly the same τ-particles as the mutant in 24 alone; it demonstrates that 24^- is epistatic over 49^-. (B) "Crummies" produced by T4 · 23 (tsA78). In well-preserved cells crummies are difficult to see. (C) "Crummies" produced by T4 · 23 (tsN37). In this partially lysed cell, the aspect and visibility of "crummies" is now somewhat different. (D) the same as (C), but with T4 · 23 (tsA78).

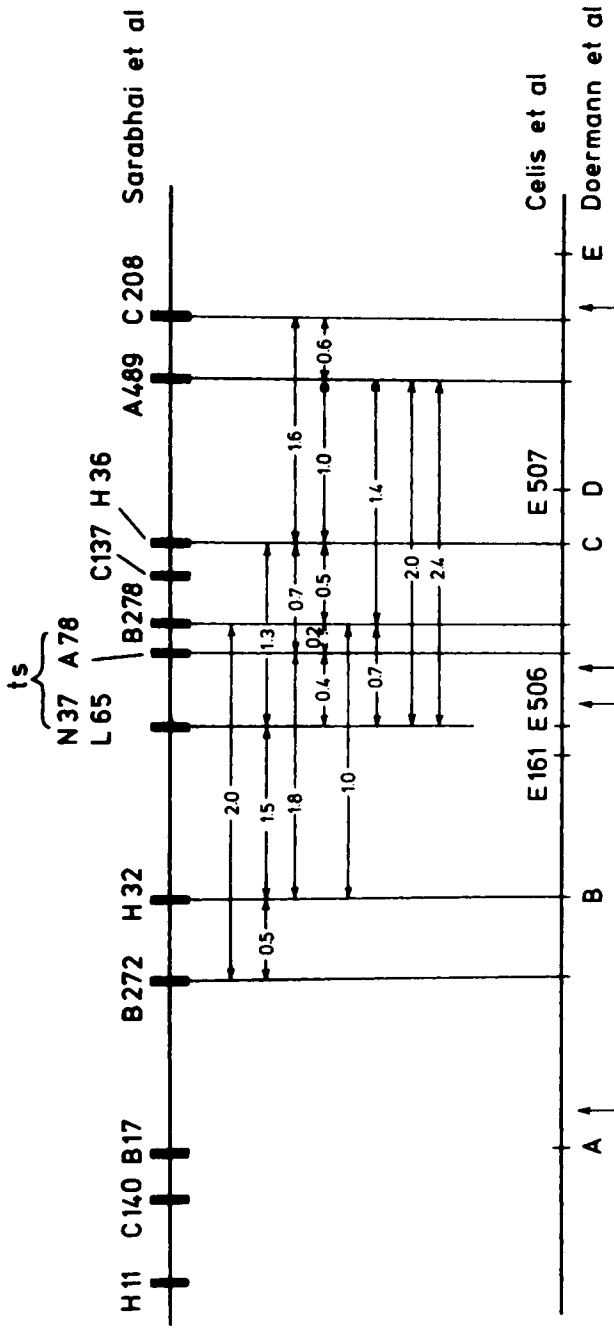

Fig. 8. Genetic map of gene 23. The combined genetic maps from Sarabhai et al. (9), Celis et al. (28), and Doermann et al. (39) are given, into which we have mapped the three ts mutants N37, L65, and A78. The other mutants are amber mutations with the exception of ptg19-2, ptg19-80, ptg191, and ptE920g (8, 39), which produce 1–10% of giant phages (indicated by ptg) or only the isometric variant (pt). The mapping of the ts mutants was made by two factor crosses, of which some of the results are indicated. The recombinations between N37 and L65 were not significantly enough above background to demonstrate that they are distinct. The detailed procedures are given under Material and Methods. To decide about the correct order, three factor crosses were made. The results are as follows:

Cross		Recombination Frequency	
B278 · H36	× C137	0.07	0.07
H36	× C137	0.33	0.5
B278	× C137	0.14	0.2
A78 × B278 · H36		0.15	0.15
A78 × B278		0.2	0.27
A78 × H36		0.67	0.8

C137 was incorporated because in some two-factor crosses this mutant behaved abnormally.

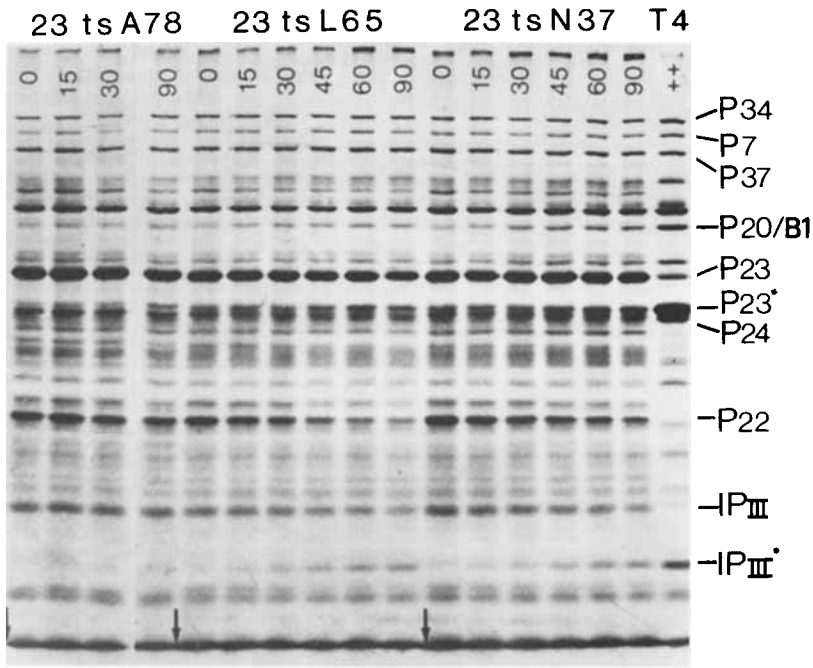

Fig. 9. Protein patterns of lysates obtained from ts mutants in gene 23. Autoradiographs of gels from concentrated, crude lysates, as described in Material and Methods and Bijlenga et al. (1). Infected cells were incubated at 40.5°C; the temperature shift-down to 30°C is indicated by ↓. As reference the pattern of pure T4 is given. Some P23* is produced at nonpermissive temperature, but there is no significant turnover of P23 into P23* after temperature shift.

τ-particles and can be isolated with much better recovery than the latter. Onorato and Showe found that the "crummies" contain P22 and IPIII, as do the 21⁻τ-particles (30). We made a double mutant 23(tsA78) · 21(tsN8) and found that the phenotype of this double mutant is nearer to that of "crummy" than to that of the τ-particle. However, the "crummy" τ-particles were more elongated than the normal "crummy heads" of the ts mutants of gene 23 alone. A double mutant, T4 · 23(tsL65) .20(amN50) was previously investigated and shown to produce rather unstable "crummy" polyheads (29).

Because these ts mutants are useful for futher studies, we mapped them within gene 23. The results are given in Fig. 9: tsN37 and tsL65 can be distinguished neither genetically nor by their physiological behavior (experimental results to be published.)

Despite the fact that intracellular cleavage of P23 is inhibited in these mutants they are distant from the site of cleavage, which is between amHll and amB272 (28).

These observations demonstrate that morphological variants occur as a consequence of mutations in gene 23 and that the major capsid protein contributes substantial amounts of information to the form. But we also know that many head-related morphological variants are under the control of other genes, the so-called form-giving (morphopoietic) head genes (31, 6, 5). The mechanism of form determination is therefore based on the interactions of P23 with other gene products. If such interactions are fundamental, then the same morphological variation could result from lack or modification of either of the two interacting products. This is consistent with our findings: 24(tsB86) grown between 34° and 37°C produces giant phages among normal ones. The ratio of

giants to normal phages is highest (by several percent) at about 35°C. At lower temperatures (down to 30°C), the absolute quantity of giant phages is undetectable by electron microscopy, and at permissive temperature the mutant behaves like the wild-type. A complete description of this type of giant is in preparation.

DISCUSSION

P23* Capsids

The lattice parameters of the capsids of giant T4 heads are apparently invariant: the lattice constant (124 Å), the pitch (13.90° as u/v = 3/1†), and the morphology of the major capsomer [(6+1) type] are the same for all 20 capsids studied. Since the width of giants appears identical with that of normal heads and since as far as is known, the qualitative protein composition of giants is comparable to that of normal, prolate capsids, it seems justified to infer that giants are related to normal heads in that only the cylindrical part of the head is more extended. On the basis of this assumption, we may then discuss some features of the normal head.

From our data on the protein composition of the capsid we know that P23* is the major component, represented by 1,100 molecules, followed by P24* with 160 molecules. We find all the other minor capsid proteins in still smaller numbers. Theoretically, the smallest asymmetric subunit sitting in a hexagonal lattice, the protomer, can be composed either of a single polypeptide or of two or more ("mixed" protomer). For T4 it is likely that the major protomer is composed solely of P23*, as we will see now. When estimating the number of capsomers from the size (712 Å × 1,100 Å completely unflattened) and thickness (50 Å) of the normal capsid and assuming a lattice constant of 124 Å, we arrive at 160 to 200 capsomers. The major capsomer must have sixfold symmetry. With six molecules of P23* per capsomer, we then obtain 960 to 1,200 P23* per capsid. These numbers agree very well with the number of 1,100 P23* molecules which we have determined from quantitative electrophoresis and the molecular weight of the capsid. This consideration also rules out the possibility that a protomer is composed of P23 and any of the minor proteins, as can easily be verified by direct calculation. The stoichiometry of capsid proteins requires that such a protomer would have to contain at least six copies of P23. This is clearly not among the possibilities which agree with our estimates, even when we associate them with broad safety limits. We conclude that the major portion of the capsid must be a surface crystal made with P23* protomers. Consequently, the minor capsid proteins (P20, P24*, and at least three genetically unidentified ones) must sit in particular singular positions on the polyhedral head or on the lattice – e.g., the vertices, the basal part of the capsid to which the tail attaches, or asymmetrically in the center of capsomers. This

†u and v are the coordinates of the circumference vector c of the tubular part of the particle with respect to the unit lattice vectors a_1 and a_2 of the considered cylindrical lattice (u and v have to be integers) (7). The u/v ratio can be determined from the diffraction pattern, especially where there are meridional reflections visible (13). There is a strict relation between the pitch angle α and the u/v ratio:

$$\alpha = \arctan \frac{a_2 \cdot \sin \gamma}{(u/v) \cdot a_1 + a_2 \cdot \cos \gamma}$$

where a_1 and a_2 are the moduli of the lattice vectors a_1 and a_2 and γ is the angle between them. Under isotropic lattice deformations the u/v ratio is an invariant, whereas the pitch angle is not.

last possibility is suggested by our observation that the capsomer is of the (6+1) type. The nature of the distinct mass in the center of the capsomer is presently under investigation in our laboratory to find out if it represents part of the six P23* molecules of the capsomer or if it is a built-in minor capsid protein. If the latter were true, P24* would be a good candidate since the amount of P24* corresponds to the estimated number of P23* capsomers (160–200). The manner in which an asymmetrical subunit is bound to a hexamer or pentamer is an interesting question raised already for the vertices of phage ϕ X 174 (32).

Our data can now be integrated with geometrical considerations about the construction of the T4 capsid. In accordance with previous calculations (33, 33a, 34) the number of asymmetric subunits (protomers) in a polyhedron produced by extending an icosahedron along one of its 5-fold symmetry axes is $S = 30 \cdot (T + Q)$†, T is of the form $m^2+m\cdot n+n^2$ where m and n are integers with the following constraints: $5m = u$ and $5n = v$, where u and v are the coordinates of the circumference vector *c* of the tubular part of the extended icosahedron with respect to the considered cylindrical lattice; and Q is any positive integer $\geqslant 1$. With $Q = T$ we describe the isometric case. For $Q > T$ we obtain the *prolate* forms (normal T4 head, giants) and for $Q < T$ the oblate forms.

Branton (personal communication) arrived from frozen-etched normal T-even heads to a u/v ratio of 3/1. We found the same from diffraction study of giant capsids. With this value the smallest possible T-number becomes equal to 13 ($m = 3$ and $n = 1$). For the corresponding isometric head, Q has to be 13 and thus we get 780 protomers and an unflattened diameter of about 712 Å, assuming the lattice constant to be 124 Å.

With $Q = 24$ for the normal prolate head and a lattice constant of 124 Å we obtain an axial length of 1,180 Å, an unflattened diameter of 712 Å, and 1,110 protomers. The possible increases or decreases of Q by 1 lead to ± 30 protomers and ± 30 Å in axial length. This allows us to predict the consequences of any hypothesis concerning the position of minor proteins. These positions are not yet known and are presently being investigated.

The calculated dimensions agree fairly well with actual measurements of the full head. A discrepancy is found between the actual and calculated values of the width of the head. It is generally agreed that using normal negative-staining methods, the full head has a width of 850 Å; if we calculate the diameter of the corresponding cylinder from the width of the completely flattened central part of empty giants, we obtain 712 Å. This is also in agreement with a further observation: if we compare the width of isometric, normal, and giant heads measured on the same preparation, we find an increase of width in the indicated order. This is easily explained by the decreasing resistance against flattening. For obvious reasons, a shell of a given elasticity is most resistant against flattening when nearly spherical in shape and least resistant when approaching a cylinder.

P23 Tubular Forms

The comparison of many polyheads clearly indicates that the lattice constant of

†T may also be interpreted as the surface of each of the ten equilateral triangles forming the two caps in units of the "basic triangle", which is half the surface of a unit cell of the underlying "basic" hexagonal lattice. In the same way, Q expresses the surface of each of the ten triangles which form the "cylindrical" part of the elongated polyhedron. Each of these ten triangles has to have one side in common with one of the ten equilaterial "cap triangles." In addition, the third vertex has to coincide with a point of the basic hexagonal net.

112 Å and the capsomer morphology are invariant features. We have confirmed the earlier observation of Yanagida et al. (7) that the pitch-related u, v numbers show a variation even within the class of polyheads arising from a mutation in a given gene.

A statistical examination of the distribution of diameters and u, v numbers for each class of polyheads mentioned above is currently being carried out in our laboratory (20). When fresh lysates are observed, the polyheads found in the populations produced as a consequence of conditional lethal mutations in different genes are apparently not fundamentally different.

In vitro aged or otherwise treated lysates contain new classes of polyheads (12, 19). These new types of polyheads are interesting in view of the lattice transformation which we discuss below.

Considerations on the In Situ Lattice Transformation

Comparing the surface crystal represented by the P23 capsoids of polyheads with that of P23* capsids, the difference is striking. Even when assuming the central part of the P23* capsomer to be occupied by a minor capsid protein, the aspect of the P23 protomer is quite different from that of the P23* protomer (Figs. 2 and 4). This difference, although possibly increased by effects of the preparation techniques and by the averaging procedures of the image processing, certainly involves strong differences in the positioning of the protomers and in their tertiary structure. The protomers of the two crystals can be related to each other by a 90° rotation around an axis lying in the lattice plane. With rigid protein molecules this is possible only when the sites of specific interactions are also quite different. The role of cleavage is then not easily understandable (42). We have also to take into account that this transformation apparently occurs in situ, probably without breakage of bonds and in a cooperative manner; through the transformation a much stronger bonding is achieved.

In Bijlenga et al. (1) we have given the evidence for a conservative, in situ transformation of the P23 capsoid of the τ-particle into the P23* capsid of mature phage. We have already said that it is not unreasonable to assume that the surface crystal of τ-capsoids is basically similar to that of polyheads and – with even stronger evidence – that the P23* capsid of giants is basically the same as the capsid of normal prolate heads. Accepting these hypotheses, then the conservative nature of the transformation of the P23 capsoid into the P23* capsid, involving no breakdown into or reuse of protomers, has very interesting consequences. First, the rearrangements can only be local – i.e., only affecting the positions of the protomers relative to their nearest neighbors – and must exclude large movements of protomers, as for example, a refolding of the closed hexagonal surface which would require the conversion of hexamers into pentamers and vice-versa. Second, since no breakdown should occur, bonding must be maintained or at least be reestablished within very short time intervals. When keeping these consequences in mind it is very difficult to imagine a rearrangement which does not involve a profound change in the tertiary structure of the protomer (42).

These considerations will gain in weight as soon as it is definitely established that the sheets obtained through reassembly of dissociated capsids are made of P23* and not of minor capsid proteins or further modified P23**. Sheets would then be a further form of assembly of P23* associated with a weaker bonding than that found in capsids.

Among the polyheads investigated by Yanagida et al. (19) the type consisting of (6+1) capsomers, with a lattice constant of 125 Å is obviously of particular interest, especially when it can be demonstrated that this type is chemically similar to the capsids of giants. As already mentioned, several laboratories are now able to modify polyheads in vitro (35). We have found that a P23 $\longrightarrow$ P23* transformation, even of empty polyheads, is possible as soon as chloroform is removed and as long as P21 is supplied by using or adding lysates containing it (4).

General Discussion of Form Inheritance

In Fig. 10 all our present knowledge about the early pathway of head maturation is summarized. From this and particularly from the observation that the combination of mutant ptE920g (in gene 23, prodcing 40–60% of isometric heads) with conditional, lethal mutant in gene 21 leads to an increased proportion of isometric τ-particles (36), we conclude that most of the form determination is already expressed at the level of τ-particles (or prehead I) (37). Only the size increase is left over for one or several of the ensuing steps.

Clearly a precise quarternary structure seems to be a prerequisite for the in vivo cleavage of P23. Only P23 of τ-particles of gene 24 ts mutants can later be cleaved in vivo, while in other polymorphic P23 variants this is not so. As we have shown in this paper, this is also true for the ts mutants of 23 which produce "crummies" when grown at nonpermissive temperature. The P23 of these structures is not cleaved in vivo after temperature shift-down. However, all these particles which in vivo are resistant to P23 cleavage can undergo it in vitro (4).

For polyheads, we have seen that the lattice constant and the morphology of capsomers are invariant, while u and v can be different. In giants u and v are strictly determined. In view of the conservative nature of the transformation – as discussed above – it is likely that the τ-capsoid has these parameters also fully determined. It seems therefore that the information contributed by P23 is by itself not fully determinative of these parameters. This agrees with further observations on form inheritance. Although P23 contributes a large part of the form-determining (morphopoietic) information – as is clearly indicated by the fact that mutants in 23 lead to increased amounts of isometric or giant heads or that ts mutants in 23 lead to abortive particles – other gene products contribute important information as well. Our finding that giant phages are produced when 24(tsB86) is grown at temperatures between 33° and 37°C in relative amounts which are of the order of those produced by mutants in gene 23 suggests that each form-giving effect is the result of an interaction of at least two gene products. Similar situations are found in the case of polyheads which are the consequence of the lack of either core proteins (P22, IPIII) or minor capsid proteins (P20, P24). For polyheads it is noteworthy also that native polyheads – as observed in thin sections – always contain a core occupying the entire length. This suggests that in vivo P23 alone, without the core, is not able to grow into tubes. The present state of our knowledge disallows any possibility of the reverse situation.

Successive, pairwise interactions of the different gene products are the basis of the theory of the regulation of assembly through sequentially induced conformational changes (31, 38). This theory explains also why two species of subunits – which will later have to interact – do not do so when they are still in a free subunit form. We believe that the conformational changes which our experiments suggest indicate new ways to check this theory experimentally.

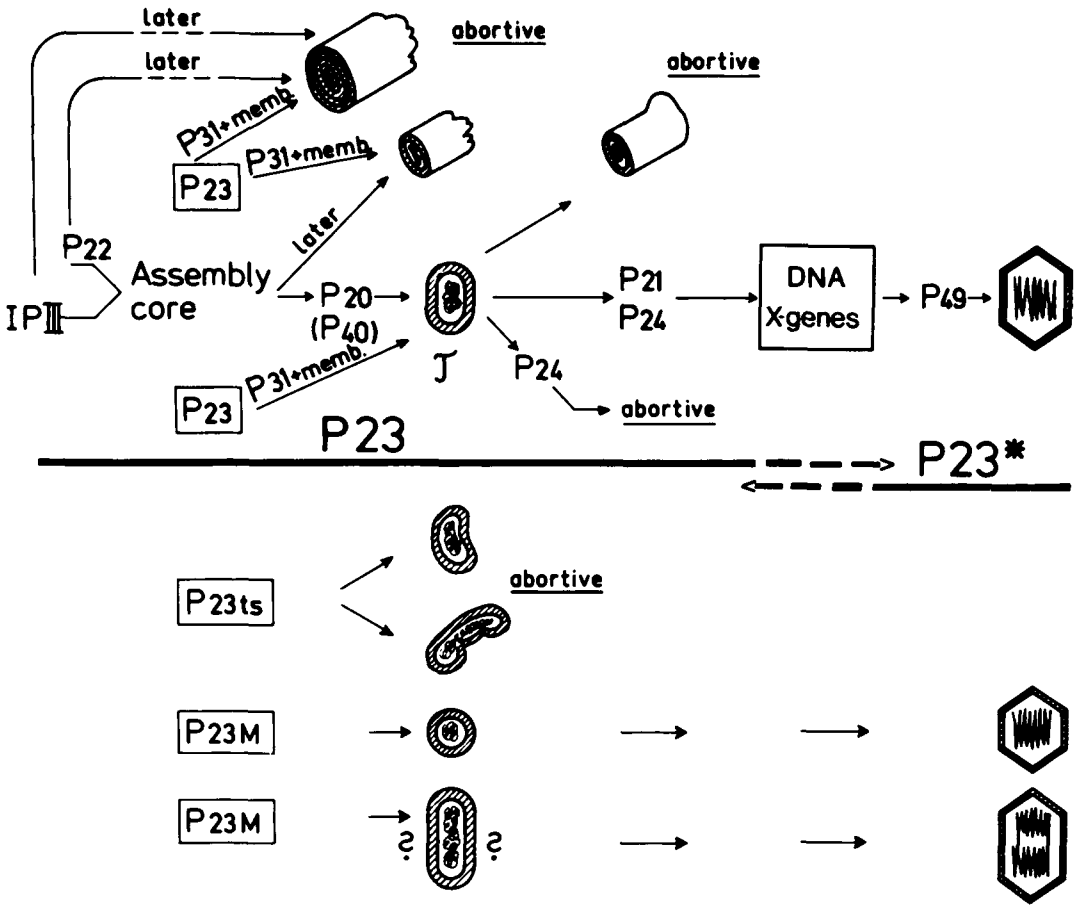

Fig. 10. Pathway of T4-head assembly, summarizing our own experiments and others' published work. The sidetracks leading to abortive polymorphic variants are also indicated. When "later" is indicated, this means that the considered structure appears with a delay of 10–15 min over the time corresponding to the branching off.

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