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THE TRANSFORMATION OF τ-PARTICLES INTO T4 HEADS

I. Evidence for the conservative mode of this transformation.*

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Asynchroneous T4 phage head maturation includes the step of P23 cleavage: P23 of head-related τ-particles is cleaved into P23* of capsids with a conservative mode of transformation as evidenced by "heavy" labeling in temperature shift-down experiments with mutant 24 (tsL90). Assuming a subunit pool, data indicate in situ cleavage on individual precursor particles. The interpretation becomes less interesting when assuming a compartmentation of the membrane surface; this hypothesis is not ruled out.

INTRODUCTION

Experimental results described in a previous paper (1) suggest that τ-particles produced by a phage T4 temperature-sensitive (ts) mutant in gene 24 can be matured into T4 phage heads after shift to permissive temperature (temperature shift-down). τ-particles produced in 21^- infected cells, which are morphologically indistinguishable, were found not to be maturable. When observed intracellularly by thin sections (Fig. 1), τ-particles are very characteristic (3). A core is surrounded by a capsoid; like the mature head, they are prolate but significantly less angular than the latter. In both length and width, τ-particles are about 20% smaller; the τ-capsoid is visibly thicker than the mature capsid (Fig. 1). τ-capsoids contain the uncleaved product of gene 23 (4), which has a molecular weight of 59,000 daltons, while the matured capsid is made with the cleaved P23 (P23*) (4–7) with a molecular weight of 47,500 (MW is determined by amino acid composition

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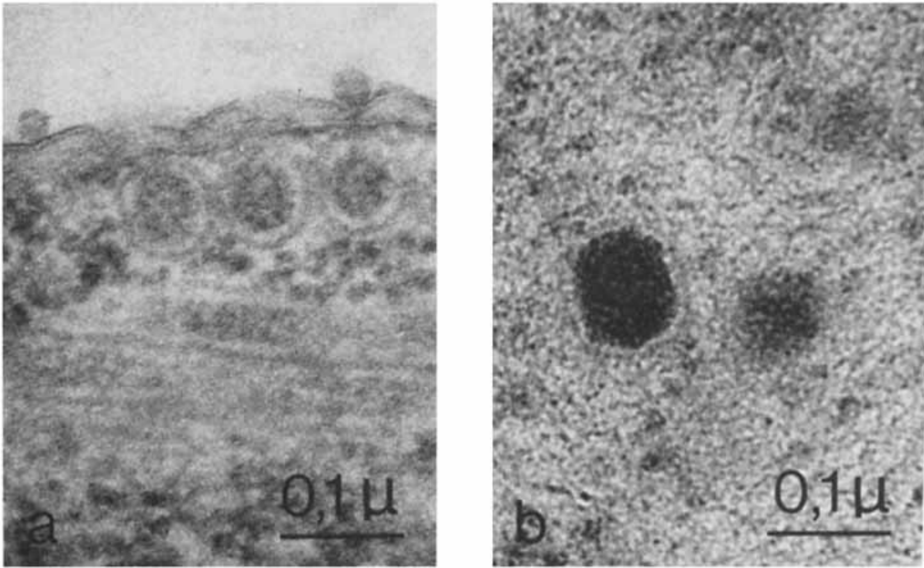

Fig. 1. Thin sections of τ-particles and phage head. A culture of *Escherichia coli* B^E was infected and superinfected with a multiplicity of 5 with T4.24 (am N65) or T4 wild type. Sixty min after infection the cells were prefixed with formaldehyde, then concentrated by centrifugation and osmium fixed under conventional R-K conditions (2) followed by embedding. τ-particles (a) characterized by their smaller dimensions (in length and width about 20%), by the presence of an internal core and by a thicker membrane when compared to the normal phage head (b). The τ-particles have their long axis perpendicular to the inner surface of the cell membrane. Polyheads (elongated structures) and ribosomes (dark dots) can also be seen (a).

Thickness measurements of the capsids and capsoids are not meaningful, as long as the stain distribution is not known and hence not interpretable (170,000 ×). Electron micrographs by J. van den Broek.

by A. Tsugita; to be published). For normal phage multiplication Laemmli (4) has shown that P23 is converted into P23* in roughly 2–3 min, which is about one-third to one-half of the total time needed for building a T-even head (8). In the present paper we will demonstrate that the uncleaved P23 is organized in the form of a precursor particle, in which, later, the conversion of P23 to P23* is conservative. Our experimental controls are mainly designed to check the possibility of a labile P23-particle, which after dissociation would replenish a P23 subunit pool. Experiments to test the compartmentation hypothesis are difficult to design, and so we limit ourselves to a discussion of some of its consequences.

Added to the previous observations (1, 12, 14) our results provide strong evidence that 24^- -τ-particles are maturable into phage heads and suggest that they are a normal precursor in the pathway of T4-head maturation.

MATERIALS AND METHODS

Bacterial Strains and Bacteriophages

Escherichia coli B^E was used as the nonpermissive strain for amber mutants and as the permissive strain for temperature-sensitive (ts) mutants. Phage stocks were grown in

M9A medium with *E. coli* CR63 at 37°C and with *E. coli* B^E at 30° for amber and ts mutants, respectively. General phage techniques were as described by Epstein et al. (1963). The double mutant phage T4.10 (amB255) .24 (tsL90) was constructed in our laboratory.

Media, Chemicals, and Buffer Solutions

Liquid cultures were grown and infected in M9 medium (see Ref. 1) or in "heavy" medium as described below. Concentrated crude lysates were resuspended in phosphate buffer with 10^{-3} M $MgSO_4$. The ^{14}C–amino acid mixture (CFB 104) and the ^{3}H-amino acid mixture (NET 250) were purchased from Radio Chemical Centre, Amersham and New England Nuclear, respectively. ^{13}C-glucose was from Isomet Corp. and $^{15}NH_4Cl$ for Merck, Sharp, and Dome (both from Canada). D_2O (97%) was from Würenlingen, Switzerland; Triton X-100, practical grade, was from Serva, and chloramphenicol (CAP) was a gift from Park-Davis.

Preparation of Concentrated "Heavy" Lysates

A culture of *Escherichia coli* B^E grown to a concentration of 4×10^8 cells/ml at 37°C was diluted (1:5) into prewarmed M9 medium (37°C) containing one part of D_2O and two parts of H_2O. After about two generations of growth, the culture was diluted 1:5 into M9 medium with one part of D_2O and one part of H_2O and allowed to grow for another two generations; then it was diluted 1:10 into completely "heavy" medium containing D_2O, $^{15}NH_4Cl$, and ^{13}C glucose and shifted to nonpermissive temperature (40.5°). At a concentration of 4×10^8 cells/ml the culture was infected and superinfected each at a multiplicity of 5 with either a 10^- mutant or a 10^- .24^- double mutant. Ten min after infection, ^{14}C labeled amino acids (1 μCi/ml) were added and at 25 min they were chased with 10 mg/ml casamino acids. At 30 min, the culture, infected with the double mutant T4.10 (amB255) .24 (tsL90), was filtered on a 0.45 μ membrane filter, washed twice with buffer and resuspended in normal M9A medium prewarmed at 30°. Sixty min after infection (unless otherwise specified) the pregnant bacteria were centrifuged for 10 min at 3500 g and crude 20–25 times concentrated lysates were prepared by resuspending the pellets in phosphate buffer with 10^{-3} M $MgSO_4$. Lysis was with chloroform in the presence of DNase.

Disc Electrophoresis with Sodium Dodecyl Sulfate (SDS) Containing Polyacrylamide Gels

0.5 ml of a sample of infected culture was centrifuged for 10 min at 3500 g and the pellets of pregnant bacteria were resuspended in 0.2 ml of sample buffer containing 2.3% SDS (4). This was then put in boiling water for 5 min and 20 μl of each sample was put on a 3% (upper) combined with a 10% (lower) SDS-polyacrylamide gel prepared as described by Laemmli (4). After fixation, staining and destaining, the gels were sliced and dried according to the method of Fairbanks et al. (9) and exposed on Kodirex X-ray film for about 24 hours.

Sucrose Gradients

Fifteen to forty percent sucrose gradients in phosphate buffer containing 10^{-3} M $MgSO_4$ were centrifuged at 30,000 rpm for 30 min, not including acceleration and de-

celeration times, in an IEC SB283 rotor. The gradients were fractionated (10 drop fractions) and to part of each fraction (20 μl), 10 ml of the following scintillation mixture was added: 7 parts of toluene containing 4 g of 2, 5 diphenyloxazole (PPO) and 100 mg of p-bis [2– (5-phenyloxazole)] benzene (POPOP) per liter with 6 parts of Triton X-100.

Caesium Chloride Gradients

The 300 S material (mainly emptied heads) of the sucrose gradient was centrifuged in CsCl with a starting density $\rho = 1.32$ for 15 hours at 50,000 rpm in an IEC SB 405 rotor. The fractions were collected in scintillation vials and before adding the scintillation mixture (see above), the refractive index of every fifth fraction was measured with a Bausch and Lomb refractometer.

RESULTS

τ-particles are fragile and attached to the inner surface of the cellular membrane. In spite of repeated attempts we were not yet successful in isolating 24^{-}-τ-particles in a quantitative and reproducible fashion. Hence, our experimental approach is somewhat indirect (1). With the ts mutant T4.24 (tsL90), τ-particles (and polyheads) accumulate when infected bacteria are grown at the nonpermissive temperature of 40.5°C. After temperature shift-down to 30°C (permissive temperature) active phage is produced. If we pulse label by radioactive or/and heavy isotopes in the preshift period, we can, after shift-down at 30 min, follow by sucrose gradient analysis the transfer of label into mature phage (or phage heads). By these types of experiments we have previously shown a) that about one-third of the preshift labeled material is transferred into mature phage after temperature shift-down. No significant transfer is observed with ts mutants in gene 21. b) Electron microscopy counts of paticles (by means of the in situ lysis method) had shown in the same experiments that the number of 24^{-} -τ-particles decreases after shift-down with a corresponding increase of the number of phage heads. This effect is particularly striking when protein synthesis is halted by adding chloramphenicol a few minutes before shift-down. In this case the number of phages produced corresponds closely to the decrease in the number of τ-particles. c) Polyacrylamide gel electrophoresis in sodium dodecyl sulfate (SDS) of preshift and postshift crude lysates showed that 30 min after temperature shift the amounts of P23, P22, and IP III have decreased while the amounts of P23* and IP III* have increased (Fig. 2). Not all of the uncleaved P23 is converted because polyheads, which contain mainly uncleaved P23, are also produced under nonpermissive conditions and were shown to be abortive. Therefore, the P23 in these particles is not cleaved in vivo. In the control experiment with T4.21 (ts N8) the number of τ-particles stayed approximately constant after shift-down, in agreement with the lack of transformation of the pulse labeled uncleaved P23 into P23*.

Transfer of Preshift Labeled Material into Postshift Produced Phage

In order to follow the fate of preshift labeled proteins produced in 24^{-} infected cells, we analyzed lysates by sucrose gradient velocity sedimentation. In a first type of experiment, the culture was pulse labeled with a mixture of ^{14}C amino acids between 15

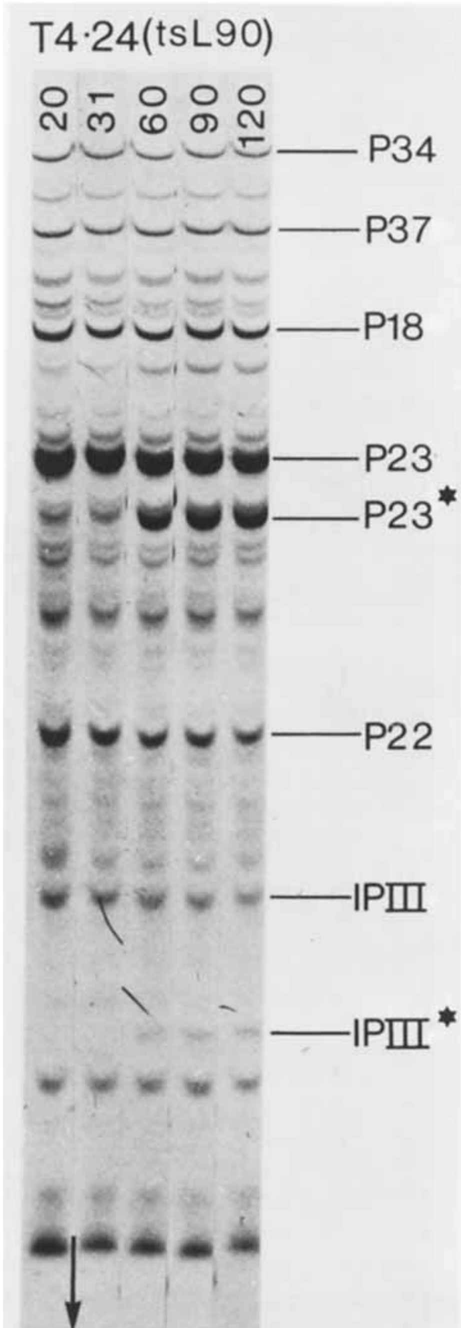

Fig. 2. Cleavage of products of genes 22, 23, and IP III after temperature shift-down of T4.24 (tsL90) infected cells; autoradiographs of gels of concentrated lysates.

An *E. coli* B^E culture grown in M9 (1) to a concentration of $2-4 \times 10^8$ cells/ml was infected and superinfected at a multiplicity of 5 with the ts mutant in gene 24. The infected cells, pulse labeled with a mixture of ^{14}C amino acids between 15 and 17 min, were shifted to the permissive temperature at 30 min after infection (arrow).

At the times indicated 20, 31, 60, 90, and 120 min after infection respectively), aliquots were prepared for gel electrophoresis as described in Materials and Methods section.

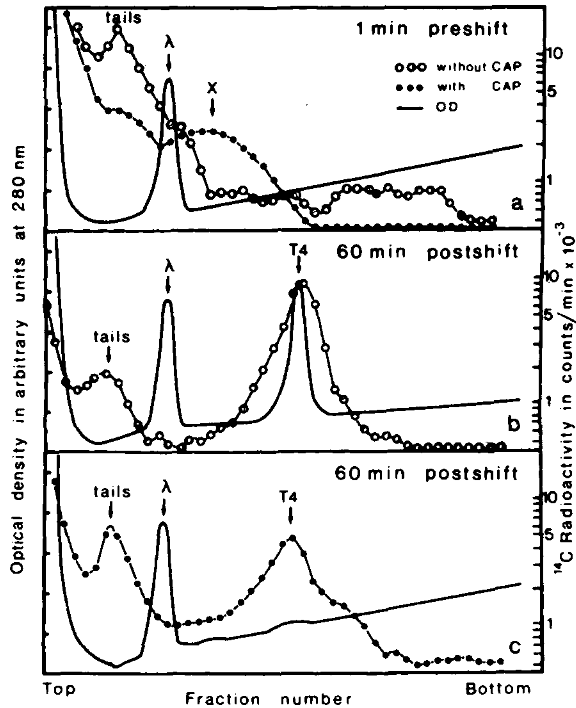

Fig. 3. Sucrose gradient profiles of preshift and postshift lysates from 24$^-$-infected cells. Infected bacteria were grown as described in Materials and Methods. Twenty to fifty times concentrated lysates were prepared by centrifugation for 10 min at 3500 g. Then the pellets were resuspended in phosphate buffer with 10^{-3} M $MgSO_4$ and lysed with chloroform in the presence of DNase. 100 μl of it were layered on top of a 15 to 40% sucrose gradient. Of the interesting fractions aliquots were prepared for gel electrophoresis (see Fig. 4).

In Fig. 3a, the profiles with open circles and filled circles, respectively, without and with chloramphenicol, represent the radioactivity distribution of preshift samples. The continuous line is that of optical density of the fractions determined at 280 nm in a flow-through quartz cuvette attached to a Gilford 240 spectrophotometer. Phage λ was added to each gradient as a migration marker (410 S). As described in the text, the peak indicated by "X" possibly contains τ-particles.

In Fig. 3b and 3c the open circles and filled circles, respectively, show the sedimentation profiles of the radioactivity from a sample taken 60 min after temperature shift either without (open circles) or with (filled circles) chloramphenicol added 6 min before temperature shift-down. In Fig. 3b a distinct peak is found at the position of phage T4 for both radioactivity and optical density. In Fig. 3c this peak is found for radioactivity, but not for optical density.

and 17 min after infection. Then, just before and 60 min after temperature shift-down, a sample was taken and prepared as described in Fig. 3. The velocity sedimentation properties of these samples are shown in Fig. 3 a and b. We can see that no defined peak except for phage tails is found before temperature shift (open circles in Fig. 3a). However, 60 min after temperature shift-down (Fig. 3b) we find an important peak in radioactivity (circles) as well as in optical density (continuous line) at the position of phage T4. In a second type of experiment, all conditions were identical to the previous experiment except that in this case chloramphenicol (CAP) was added 6 min before temperature shift-down. This allowed us to check if postshift synthesized proteins were needed during

growth at the permissive temperature. The results of this experiment are shown in Fig. 3a and c (filled circles). The sedimentation profile (Fig. 3a) of the sample taken before temperature shift shows a peak denoted by X; because of too low a concentration its content could not be identified by electron microscopy. Electrophoresis in SDS-polyacrylamide gels showed mainly one weak band of uncleaved P23, suggesting the presence of τ-particles. The sedimentation profile of a 60 min postshift sample (Fig. 3c) showed a radioactive peak at the position of phage T4, but no corresponding optical density peak. The optical density measures the total amount of phage in this peak. By comparing the phage peaks of Fig. 3b with that of Fig. 3c we can therefore conclude that with and without chloramphenicol about equal amounts (30–50%) of preshift pulse labeled proteins are transferred into phage after shift-down, but that the total amount of phage produced after shift is obviously much larger when chloramphenicol is absent. We conclude from these results that: a) preshift labeled material produced in 24^- infected cells can be transformed into phage after temperature shift-down, and b) this transformation is not dependent on postshift protein synthesis, indicating that the product of gene 24 recovers activity after shift-down. That the radioactivity transferred into phage is not primarily due to preshift produced phage tails has been ruled out by similar experiments in which the ts mutation in gene 24 was combined with an amber mutation in the base-plate-gene 10.

The top fractions of the sedimentation profiles shown in Fig. 3 from preshift and postshift samples, as well as the T4 phage peaks obtained after shift, were analyzed by SDS-polyacrylamide gel electrophoresis. The autoradiographs of these gels are shown in Fig. 4a, b, c. They demonstrate that P23* is found only in the fraction containing phage.

Evidence for a Conservative Mode of Transformation

Our previous results (1) as well as those reported above do not rule out the possibility that the phage capsids observed after temperature shift-down are built from subunits drawn from a pool containing subunits built before as well as after shift (in the following we shall distinguish them as "preshift subunits" and "postshift subunits"). A small, unobserved pool of preshift subunits could already exist independently of τ-particles; a pool could also be fed by the subunits resulting from the breakdown of preshift built τ-particles. In both cases, capsids of phages made after shift would be composed of a mixture of preshift and postshift subunits. The proportion of preshift subunits in the pool and consequently in the assembled capsids would decrease with time after shift. In these cases we would have a dispersive mode of transfer of preshift material into postshift capsids. The transfer would be conservative when the preshift subunits form already a particle which, after shift, is transformed as a whole into a capsid. A conservative mode could, however, be simulated in the case of breakdown and reuse, when the pool would simply not contain new, postshift subunits. Such a case would occur when protein synthesis is inhibited by chloramphenicol added shortly before temperature shift, or when postshift protein synthesis is strongly delayed by the adaptation to a different medium. With the following experiments (Figs. 5 and 6) we intend to demonstrate a true conservative transformation of a P23-capsoid into a P23*-capsid. The basic principle consists in density labeling the preshift subunits as "heavy" and the postshift subunits as "light." By supplementary radioisotope pulse labeling the fate of both types of subunits can be followed independently.

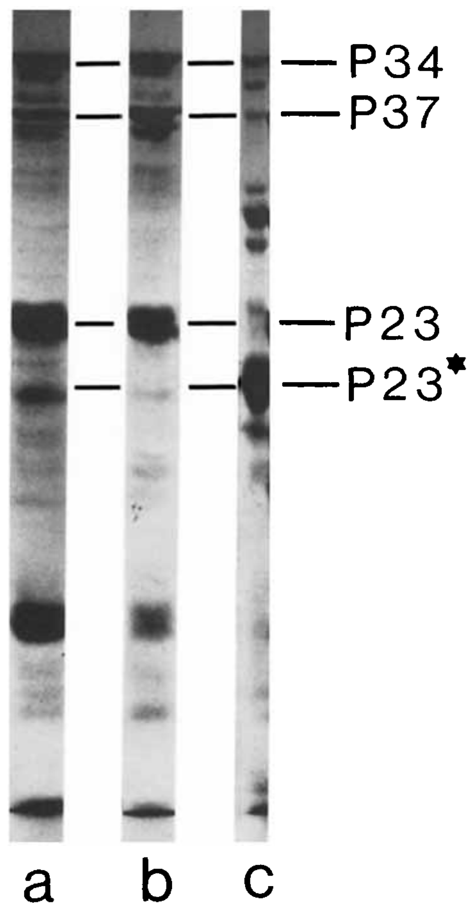

Fig. 4. Autoradiographs of gels from sucrose gradient fractions. From different fractions of surcrose gradients as those of Fig. 3 aliquots were taken and prepared for SDS-acrylamide gel electrophoresis as described in Fig. 2.

Autoradiographs (a) and (b) show the proteins found in the top fraction of a gradient from a 24° lysate prepared, respectively, 1 min before and 60 min after temperature shift-down; (c) shows the protein pattern of T4 phage obtained 60 min after temperature shift-down of a 24° infected culture.

Before infection, the bacterial culture was stepwise adapted to heavy medium containing D_2O, ^{13}C glucose and $^{15}NH_4Cl$. The culture in heavy medium was then infected, radioactively pulse labeled, and temperature shifted as described for the previous experiments; however at the time of shift-down the culture was also resuspended into light medium. For these experiments we used T4.10 (am B255) and T4.10 (am B255) .24 (ts L90). The tailless heads of mutants in gene 10 have the advantage of being easily converted into emptied heads by DNase treatment (Ref. 1 in Appendix). This allowed us to analyze the density of emptied heads without having to take into account the full DNA complement of a phage head and the density variability due to the mixing of preshift and postshift built phage tails. The emptied heads obtained by this procedure were partly purified by sucrose gradient centrifugation similar to that described in Fig. 3. A distinct peak appears now at the 300 S migration position. The fractions covering this peak showed empty

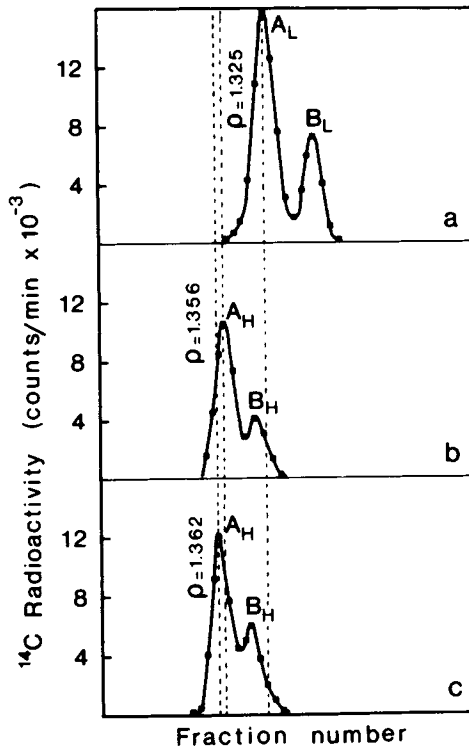

Fig. 5. Density distribution of DNase treated phage heads obtained in heavy medium alone, after transfer from heavy into light medium, and in light medium alone. DNase treated phage heads were purified by a sucrose gradient of which the emptied head-containing 300 S fractions were collected. These fractions were then analyzed by density equilibrium centrifugation. (a) Emptied heads obtained from 10⁻ infected cells grown in normal light medium. (b) Emptied heads obtained from 10⁻ · 24⁻ infected cells at 30 min after the culture was shifted from completely heavy medium at 40. 5°C into normal light medium at 30°C (permissive temperature). (c) Emptied heads obtained from 10⁻ infected cells grown in completely heavy medium.

The explanation for the existence of two peaks is discussed in the Appendix. Peaks A and B refer to partially emptied and completely emptied heads, respectively. The subscripts L and H refer to light and heavy medium.

heads or capsids by electron microscopy and P23*, together with other head proteins, by gel electrophoresis. These fractions were then submitted to equilibrium density centrifugation in CsCl. The density profiles for emptied heads obtained with 10⁻ infected cells in light medium is shown in Fig. 5a, and that of 10⁻ emptied heads obtained in heavy medium in Fig. 5c. In both cases we see two peaks, A and B, distinct by 0.021 g/ml. As we explain in more detail in the Appendix, these two peaks occur normally and are due to two sorts of emptied heads, those which are completely emptied (peak B_L ; ρ = 1.304 g/ml), and those (denser) still containing about 20% of DNA (peak A_L ; ρ = 1.325 g/ml). As discussed in the Appendix, the relative amounts of the two sorts of emptied heads are not reproducible and vary for every experiment.

From Fig. 5c we deduce that the two emptied head peaks A and B (double peak) are shifted by 0.037 g/ml when grown in heavy medium (see for comparison peaks A_L and A_H). The density profile of Fig. 5b is obtained from emptied heads which arose

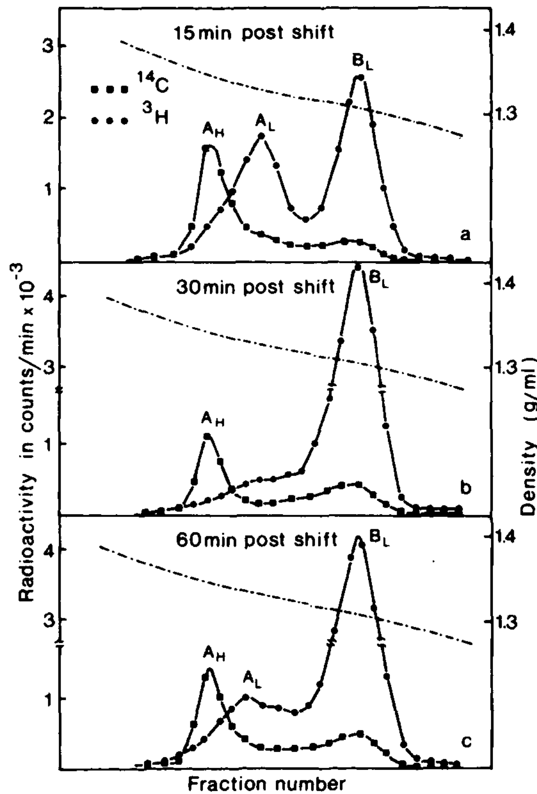

Fig. 6. Density distribution of pre- and postshift labeled emptied heads of 10⁻ · 24⁻ infected cells at different times after temperature shift-down. Heavy labeled heads were made as described in Materials and Methods for the double mutant 10⁻ · 24⁻ except for the following: The culture was resuspended after shift in M9 medium (without amino acids). 5 min after temperature shift-down, 10 μCi/ml of a mixture of ^{3}H labeled amino acids were added to the culture and 10 min after shift-down the label was chased with 10 mg/ml of casamino acids. Samples were taken at 15, 30, and 60 min after shift-down, run on sucrose gradients, and the 300 S material (emptied heads) was then put on CsCl for 11 hr at 50,000 rpm.
(a) Shows density distributions of preshift ^{14}C labeled empty heads (squares) and postshift ^{3}H labeled empty heads (circles) of a sample taken 15 min after temperature shift-down. (b) and (c) give similar density distributions, but of samples taken 30 and 60 min after temperature shift-down, respectively. Peaks A_L and B_L refer to partially emptied and completely emptied light heads. Peak A_H refers to partially emptied heavy heads.

after temperature shift-down and transfer to light medium with the double mutant 24 (ts L90). 10 (am B255). We see that this double peak is 0.031 g/ml denser than the light one. This is, however, 0.006 g/ml less than the heavy double peak. Such a small difference can be due to experimental variability or to the addition of light P24 after shift†), but can also be expected in the case of the "breakdown and reuse" hypothesis if, after transfer

†In a similar experiment but with chloramphenicol added 6 min before shift we found that the density was exactly that of the control experiment with 10 emptied heads in completely heavy medium.

after shift-down and transfer to light medium. The preshift material produced in infected bacteria in heavy medium was pulse labeled from 15 – 20 min with a mixture of ^{14}C amino acids. Immediately after temperature and medium shift (at 30 min), a 5 min pulse with a mixture of ^{3}H amino acids was given and samples were taken at 15, 30, and 60 min after shift. In Fig. 6 the density profiles for the emptied heads obtained in this experiment are shown. Only the postshift ^{3}H labeled emptied heads show the typical double peak (A_L, B_L), of which the peaks are again separated by the same density difference of 0.020 g/ml as before. For the preshift ^{14}C labeled emptied heads only peak A_H appears. The difference in density between pre- and postshift labeled emptied heads peaks A_H and A_L is 0.027 g/ml. Despite the slight complication through these double peaks, the profiles demonstrate that: a) nearly all the preshift ^{14}C label is confined to one sharp heavy peak, which is about the same when sampled at 15, 30, and 60 min after shift. b) only a small amount of preshift label appears in a light position. c) The postshift ^{3}H label collects in the light double peak. d) From this double label experiment we cannot estimate how much postshift protein is added to the preshift capsoids after shift. The small density difference of 0.006 g/ml[3] observed in the experiment of Fig. 5b would correspond to an addition of 16% of light material to the preshift heavy capsoid. For the three sampling times this light double peak is at the same density position but the relative amounts of A_L and B_L are different. This variability of the relative amounts is discussed in the Appendix. From these observations we conclude a) that shortly after shift the postshift-label enters the P23-subunit pool and P23 synthesis continues, and b) that virtually only two discrete density classes of emptied heads exist corresponding to either a homogeneous heavy or a homogeneous light labeling. Hence when assuming a subunit pool, the hypothesis of breakdown and reuse of a precursor particle, or of a hidden, but persisting preshift subunit pool, or, finally, of a precursor particle – not yet recognized besides the τ-particles – get very little support. We will discuss below that a possible lack of phenotypic mixing within P23 or P24 would lessen the importance of the experiments.

DISCUSSION

Our results show that in 24 infected bacteria, after shift to permissive temperature: a) up to 50% of the total preshift pulse labeled P23 is cleaved, b) the same proportion of preshift pulse label is transferred into capsids, c) the transformation of preshift labeled P23 into postshift P23*-capsids is conservative.

The reasons for only 50% cleavage of the major head protein P23 after shift-down has been discussed in detail in a previous paper (1). In short, after 24⁻ infection the preshift pulse labeled proteins are used under nonpermissive conditions to build τ-particles and polyheads in roughly equal amounts. We had shown that polyheads, which also contain uncleaved P23, are abortive products. Therefore, after temperature shift-down, only τ-particles or remaining protein subunits can contribute radioisotopic label to postshift capsids. The fact that radioactive phages are obtained even in the presence of chloramphenicol, added 6 min before temperature shift-down, indicates that the product of gene 24 can be reactivated after shift-down, and that only preshift built material is necessary for

further maturation (see Fig. 3c). The gel electrophoretic analysis of sucrose gradients showed that P23* is only found organized in the form of capsids, which are highly resistant to dissociation. Laemmli (4) previously found that P23* only exists in a dissociation resistant form. P23-capsoids, however, are very easily dissociated (10). In confirmation of Laemmli (4), who postulated that the uncleaved P23 is organized in a particulate precursor, our observations now give evidence that this is true and that the τ-particle is the most likely candidate for this role. Our data give good evidence that 24^--τ-particles can be transformed into phage heads in a conservative mode. The following experimental results suggest, but do not prove, that this type of τ-particle is also a precursor in the normal pathway of phage head maturation as proposed by Simon (11).

a) Laemmli and Favre (12) have given evidence by analyzing very short pulse-chase experiments in wild-type and several mutant phage-infected cells, that particles, which they called prohead I, II, and III, are successive precursors to the mature head. The prohead I particles contain predominantly uncleaved P23 and possibly P22 and IP III. These particles have similar properties (protein composition and S value) as the τ-particles.

b) Gene 24 is epistatic over all X genes (Wunderli et al., to be published), as evidenced by the fact that τ-particles are produced by all double mutants of X genes with 24^-.

c) Among the morphological variants built of P23, only the 24^--τ-particles can undergo further maturation. Studies by us (1) and by others (13, 14) show that 21^--τ-particles, which are morphologically indistinguishable from 24^--τ-particles, cannot be transformed. The same is true for the so-called "crummy heads" (11) produced by ts mutants of gene 24 for which we show in the second paper of this series (J. Supramol. Struct., in press) that in vivo cleavage is inhibited after temperature shift. To find out if the preshift label found after shift is derived from a pool of subunits or directly from τ-particles, we designed our experiments with heavy- and radioisotope label combined. Our results show that: a) $10^- \cdot 24^-$ infected cells grown in heavy medium and pulse labeled at nonpermissive temperature lead, after shift-down, to heavy and radioisotope labeled emptied heads; and b) these emptied heads are not built from a subunit pool which contains a mixture of preshift and postshift protein subunits. The difference in density (0.006 g/ml) between 10^- emptied heads and $10^- \cdot 24^-$ postshift emptied heads can be due to experimental error or would correspond to an addition of 16% of light material which could be postshift synthesized P24. A dispersive mode of transformation for the major head protein P23 is, however, ruled out.

Two different hypotheses are in agreement with a conservative mode of the capsoid-capsid (P23 – P23*) maturation: a) The "pool model" in which the protein subunits constitute a pool which has a continuous input of newly synthesized proteins and from which subunits are withdrawn for the building of phage precursor particles. Once such a precursor particle has been assembled, all further maturation steps would take place in situ on this particle, and this independently from all other maturation steps in its neighbourhood. b) The "compartment model" in which all the needed building blocks are from the beginning collected and maintained within a "cloistered compartment." All the steps of maturation would then take place within this compartment. Whatever the precise mechanism of assembly would be, it would behave in a "pseudo conservative" mode.

By extrapolating our knowledge on the phage DNA pool and the phenotypic mixing between DNA, capsid, tail, and tail fibers, it is generally believed that protein subunits also form pools. This is not yet demonstrated and some experiments on the phenotypic mixing of capsid proteins, as, e.g., the gene 24^- related osmotic shock resistance, are not in agreement (15). Experiments along this line are presently pursued in our laboratory.

The conservative mode of transformation of P23-capsoids into P23*-capsids is of less interest when assuming the compartment model, but it has very interesting consequences in the pool model. In this case, our experiments are compatible only with a conservation of the number of P23 subunits during transformation of τ-capsoids into phage capsids.

This conservation combined with a true transformation is particularly relevant for the interpretation of the observations reported and discussed in the second paper of this series (J. Supramol. Struct., in press) and which concerns the differences of the lattices and of the morphology of the capsomers observed on capsoids and capsids.

Electron microscopy only shows different particles; when these particles are the compartments themselves, all the interpretations are correct. Conventional electron microscopy, as used for these observations, is, however, not adequate to show compartments in the form of confined areas on the membrane surface to which the mobility, i.e., diffusibility of the subunits, would be restricted. Since the pathway up to τ-particles with certainty involves the membrane, further experiments are needed to clarify this point. The tentative pathway leading to τ-particles would be as follows (see also Fig. 9 of the second paper of this series, Aebi et al. [15a]). The proteins of the assembly core, made of (P22) $(IP\ I)_2$ $(IP\ II)_2$ $(IP\ III)_2$ (16) would lead to a nucleation center around which uncleaved P23 would assemble. P23 is likely to be associated with the membrane in some intercorrelation with P31 (17), as indicated by the work on mutants of the host (see Georgopoulos and Harvey [18]). The further steps of head maturation, in particular the role of the so-called X or head maturation genes, need further experiments as well as the DNA incorporation through the process of DNA condensation into one of the protein precursor particles (see Laemmli et al., [19]).

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APPENDIX

R. Bijlenga, R. v.d. Broek, and J. Hosoda

Phage heads from a 10⁻ lysate can be emptied of their DNA by DNase treatment (1). In sucrose gradient velocity sedimentation these emptied heads (capsids) band at one major 300 S peak. After CsCl density equilibrium sedimentation of the 300 S material we essentially find two peaks, as shown in Fig. 5. The relative proportion of each peak versus the total amount is, however, variable from one experiment to the other. Hosoda with collaborators (unpublished) have shown that the more dense of the two peaks contain some DNA, while the less dense has none. An explanation for this would be that in part of the phage heads some DNA "sticks" to the inner surface of the capsid and is consequently inaccessible to the nucleolytic activity.

We have redone an experiment to illustrate this hypothesis. Cultures *of E. coli* B^E and *E. coli* CR 63 nonpermissive and permissive hosts for amber mutants, respectively, grown in Tris minimal medium (TCG) supplemented with 20 μg/ml of phophorus, were infected with T4.10 (amB255) and pulse labeled with a mixture of ^{3}H amino acids and ^{32}P. The phage heads and phages were partially purified by velocity sedimentation on sucrose gradients and then certrifuged with CsCl for density equilibrium sedimentation. We then analyzed the unique phage peak (Fig. 1a) and the two emptied head peaks A and B (fig. 1b) for ^{3}H and ^{32}P counts. The results show that only one of the two emptied head peaks (peak A) contains DNA, as indicated by the ^{32}P counts. The DNA found in these partially emptied heads is about 20% of the DNA content of complete phage particles. (^{3}H phage counts were corrected for tail proteins, which were assumed to be about 10% of the total phage proteins.) This amount of DNA in the partially emptied

head is quite compatible with the difference in density between the partially emptied heads and the completely emptied heads (capsids). We have tried to get only emptied heads by prerunning the 10ˇ phage heads in a CsCl step gradient with EDTA added but, although, sometimes successful, this method was not reliable enough to be used in a series of more sophisticated experiments as those reported in the accompanying main paper.

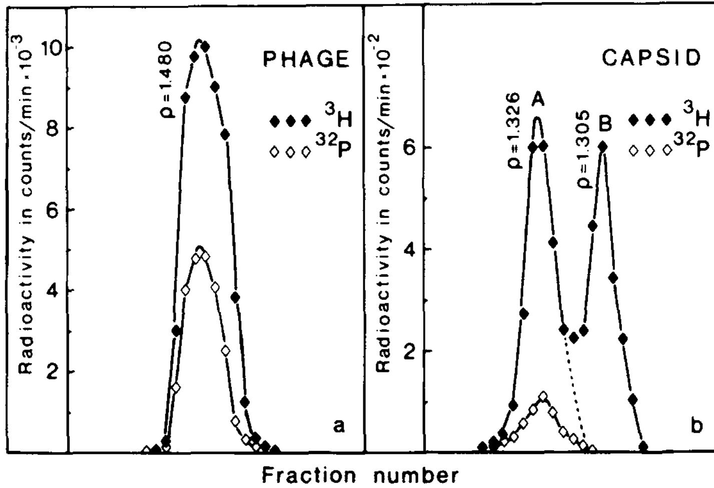

Fig. 1. DNA content of 10ˇ DNase treated phage heads as compared to the DNA content of wild-type phage. Cultures of *Escherichia coli* B^E and *Escherichia coli* CR63 were grown in Tris minimal medium (TCG) with 20 μg/ml of phosphorus. At a concentration of about 4 × 10^8 cells/ml the cultures were infected and superinfected at a multiplicity of 5 with T4.10 (am B255). Sixteen min after infection 16 μCi/ml of ^{32}P and 200 μCi/ml of ^3H amino acids (New England Nuclear) were added. At 90 min, the infected bacteria were pelleted and resuspended 50-fold concentrated in phosphate buffer with 10^{-3} M $MgSO_4$. The bacteria were lysed with chloroform in the presence of DNase. The crude lysates were layered on top of a 15 to 40% sucrose gradient with a 0.2 ml Angio-Conray cushion and centrifuged for 45 min at 30,000 rpm in an IEC SB 405 rotor. Ten drop fractions were collected and 10 μl of each fraction were counted with the Triton X-100–Toluene mixture (see Materials and Methods) to determine the 300 S peak corresponding to emptied heads obtained after *E. coli* B^E infection and the 1100 S peak corresponding to phage obtained after *E. coli* CR 63 infection. Part of these two peaks was then layered on CsCl with density $\rho = 1.32$ for emptied head and $\rho = 1.480$ for phage. Centrifugation was for 12 hrs at 50,000 rpm in an IEC SB 405 rotor. Five drop fractions were collected, the refractive indexes were read and 20 μl of each fraction was counted with the Triton – Toluene mixture in a scintillation counter. (a) Radioactivity distribution of ^3H labeled proteins, (filled diamond) and ^{32}P labeled DNA (open diamond) of phage. (b) Distribution of ^3H labeled proteins (filled diamond) and ^{32}P labeled DAN (open diamond) of emptied heads. The labeling conditions for phage and emptied heads were identical. Clearly, peak A contains DNA while peak B does not.

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