The Conversion of Viral Heads to T5 Phage in vivo

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The late stages of bacteriophage T4 maturation apparently involve the interaction of preformed components, since viable phage can be formed in vitro from viral parts such as heads, tails and tail fibres which accumulate in bacteria infected with mutant phage (Edgar & Wood, 1966). Similar conclusions have been reached for phages λ (Weigle, 1966) and P22 (Israel, Anderson & Levine, 1967). The experiments described here show that with wild-type phage T5 viral heads are precursors of mature phage in vivo.

Phage T5 and 32P-labelled phage were prepared by growth on Escherichia coli B/2 as described by Smith & Burton (1966). Carrier-free 32P-phosphate and [6-3H]thymidine, specific activity 17.7 c/m-mole, were obtained from the Radiochemical Centre, Amersham. In experiments involving artificial lysis, the host was E. coli C3000 since this behaved more reproducibly than strain B/2 in trial experiments.

Cultures of Escherichia coli C3000 were grown with aeration in low phosphate medium (Hershey, 1955) to a cell density of 5 x 10⁶/ml. and infected with T5 phage at a multiplicity of infection of 10. Radioactive phosphate or thymidine was added as indicated below. At intervals after infection, samples of the culture were chilled, and the bacteria collected by centrifuging for 5 min. at 3500 g; the bacteria were resuspended in 25% sucrose in 0.05 M-tris+HCl pH 8.1, and lysed with lysozyme and neutral detergents as described by Godson & Sinsheimer (1967), except that Mg²⁺ ions were omitted. The conditions did not affect the viability of the phage. Centrifuging the clear, or slightly opalescent, lysates at 10,000 g for 8 min. gave a gelatinous pellet which contained about 50% of the total cellular DNA. Samples (0.2 ml.) of the clear supernatant fluids were layered on to 5 ml. density gradients of 15 to 30% sucrose in 0.05 M-tris+HCl, pH 7.5, and centrifuged for 25 min. at 25,000 rev./min. in the SW 39 rotor of the Spinco model L centrifuge. Alternate single-drop fractions were collected from a hole punched in the bottom of the centrifuge tube. To measure radioactivity in DNA, each fraction was incubated for 17 hr with 0.5 ml. of 0.3 N-NaOH to hydrolyse RNA, then precipitated with 0.1 mg. carrier DNA and 7% trichloracetic acid, and washed on to a glass-fibre disc (2.5 cm., Whatman GF/A). The dried discs were placed in vials with 4 ml. of scintillation fluid (0.7% Butyl PBD [CIBA, Duxford, Cambs] in toluene), and the radioactivity measured in a Beckman scintillation counter, using the isosets for 3H, and 32P with 3H. The counts were corrected for background and the overlap between the two channels.

Figure 1 shows the result of zone centrifugation of 32P-labelled T5 phage with a sample of lysate from a culture which had incorporated [3H]thymidine (1 μc/ml.) from the 30th to 39th min. after infection. Besides 3H-labelled material sedimenting with intact phage (peak II), there was a faster-moving component (peak I). Preincubation of the lysate with 0.01 M-MgCl₂ and DNase (EC 3.1.4.5; Seravac Laboratories, grade 1) did not alter the sedimentation behaviour of these peaks. Using 543 S as the value for phage T5 (Cummings, 1964), the relative distances sedimented by...
components I and II gave an approximate sedimentation coefficient of 750 S for component I (Martin & Ames, 1961).

To characterize further the material in peak I, the lysate from 50 ml. of culture at 30 min. after infection was centrifuged at 10,000 g for 8 min. to remove debris, and for 1 hr at 18,000 g to concentrate components I and II. The pellet was resuspended in 0.5 ml. of 0.01 M-tris+HCl, pH 7.0 and layered on to a 26 ml. gradient of 15% to 30% sucrose made up as before. After centrifuging for 30 min. at 24,000 rev./min. in the SW 25 rotor, fractions of 0.6 ml. were collected from the bottom of the tube,

![Fig. 1. Sucrose density-gradient analysis of [3H]-thymidine labelled lysate and 32P-labelled T 5 phage. For details see text. •—•, 3H-counts; ▲—▲, 32P counts.](image)

and their extinction at 260 nm. measured. Besides material at the top of the gradient, two u.v.-absorbing components were present, the faster being present in about 25% the amount of the slower. Samples were taken from the peak tubes of each component, dialysed against a solution containing NaCl (0.1 N) and MgCl₂ (0.01 M) and examined in the electron microscope after negative staining with either uranyl acetate or oxalate. The slower component (Pl. I a) contained only whole T 5 particles, while the faster component (Pl. I b) contained mainly polyhedral particles indistinguishable from the heads of T 5 phage particles. A few tailed particles were observed among the heads, a small fraction of which had lost their DNA. The phage heads did not show any structure which might form the point of attachment of the tail. The particles of whole phage showed some details which have not been described before. The distal end of the tail carries some fibres, probably four in number, attached to a tail plate which is situated not at the tip of the tail but about 100 Å towards the head. The fibres, which are about 600 Å long, have a sharp kink at about 200 Å from their point of contact.
(a) Whole T5 particles found in the peak tube of component II. The particles show anatomical features described in the text. Negatively stained with uranyl acetate. (b) Particles found in the peak tube of component I. They are identical in shape and size with the heads of whole T5 particles. Negatively stained with uranyl oxalate.
with the base plate. The kinked arrangement is very similar to that found in the tail fibres of the T-even phages. It is not known whether the fibres have any function, but by analogy with the T-even phages they may be concerned with adsorption of the particle to its receptor.

In a further experiment, $^{32}$P-labelled material from the faster u.v.-absorbing component was shown to coincide with $[^{3}$H$]$$\text{thymidine}$-labelled peak I on a 5 ml. gradient. From its sedimentation behaviour, resistance to DNase, and the electron-microscopic evidence it was concluded that component I consisted of T 5 phage heads.

The mature phage and heads first appeared in lysates at 20 to 25 min. after infection; at later times the amounts of phage increased, while those of the heads remained almost constant. To investigate the kinetics of their formation, $^{32}$P-phosphate was used to label fully all cellular DNA and the fate of a $[^{3}$H$]$$\text{thymidine}$ pulse label of DNA followed by measuring the ratio $^{3}$H/$^{32}$P in the different components; in these as in other experiments, the cells were lysed at late times when host DNA is expected to have been broken down (Crawford, 1959). In one experiment a 30 ml. culture was grown to a bacterial density of $2.5 \times 10^9$/ml. when 50 $\mu$C of $^{32}$P-phosphate were added; when the culture reached $5 \times 10^9$/ml. it was infected with T 5 phage. At 28 min.
after infection, 30 µc of [3H]thymidine were added, followed by a large excess of unlabelled thymidine 30 sec. later. At intervals, 5 ml. samples of culture were removed, the corresponding lysates fractionated on 5 ml. sucrose gradients, and [3H] and [32P] in the gradient fractions measured as before. [32P] was counted with the same efficiency throughout the gradient, and it was assumed that [3H] was counted with the same efficiency in the head as in the phage peaks. DNA was measured in the original pellet and supernatant fractions after acid precipitation of the alkali-resistant material in the presence of 10 mg of carrier bovine serum albumin. The precipitates were washed 3 times with cold 0.2 N-HClO₄, and then heated at 70° for 20 min. with 0.5 ml. of 0.5 N-HClO₄; samples of these hydrolysates were used to measure deoxyribose (Burton, 1956) and [3H] and [32P]. The DNA deoxyribose present in the phage and head components was calculated from their respective contributions to the total [32P] counts on the gradients, and from the [32P] and deoxyribose contents of the alkali-resistant, acid-insoluble material in the supernatants, assuming that all the [32P] in the latter was present as DNA. This assumption appeared reasonable since virtually all phage phosphorus is in DNA, and after removing heads and phage, less than 2% of the acid-precipitable [32P] in the supernatants was resistant to successive treatments with DNase and NaOH.

[3H]thymidine was initially incorporated rapidly into the head component; the specific activity of this fraction then fell as [3H] accumulated in whole virus particles (Fig. 2). This labelling pattern is consistent with that to be expected if heads are precursors of mature phage.

The amounts of phage and head components and total DNA recovered in the supernatants, and the total DNA in the culture, are shown in Fig. 3. The lysis procedure was unsatisfactory since it only released about 50% of the cellular DNA in a non-pelletable form; but provided that the heads and phage were released with equal efficiency, the amounts of these in the supernatants must reflect their relative amounts in the infected cell. If this is so, then during the period of rapid DNA synthesis there was a fourfold increase in mature phage to a level of 53% of the DNA in the supernatant. By contrast the head component never exceeded 3% of the DNA-[32P] counts recovered in the supernatant. This is consistent with the behaviour of the [3H]thymidine pulse in indicating that heads are rapidly converted to whole virus once the structural proteins and enzymes required for maturation have been formed.

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REFERENCES


Short communications


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