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Structure and assembly of the capsid of bacteriophage P22

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[Plates 6 and 7]

Identification of the genes and proteins involved in phage P22 formation has permitted a detailed analysis of particle assembly, revealing some unexpected aspects. The polymerization of the major coat protein (gene 5 product) into an organized capsid is directed by a scaffolding protein (gene 8 product) which is absent from mature phage. The resulting capsid structure (prohead) is the precursor for DNA encapsidation. All of the scaffolding protein exits from the prohead in association with DNA packaging. These molecules then recycle, directing further rounds of prohead assembly.

The structure of the prohead has been studied by electron microscopy of thin sections of phage infected cells, and by low angle X-ray scattering of concentrated particles. The results show that the prohead is a double shell structure, or a ball within a shell. The inner ball or shell is composed of the scaffolding protein while the outer shell is composed of coat protein. The conversion from prohead to mature capsid is associated with an expansion of the coat protein shell. It is possible that the scaffolding protein molecules exit through the capsid lattice.

When DNA encapsidation within infected cells is blocked by mutation, scaffolding protein is trapped in proheads and cannot recycle. Under these conditions, the rate of synthesis of gp8 increases, so that normal proheads continue to form. These results suggest that free scaffolding protein negatively regulates its own further synthesis, providing a coupling between protein synthesis and protein assembly.

Introduction

The capsids of viruses have long been thought of as protective coats whose function it is to protect the nucleic acid from environmental hazard on the way from one host to the next. Over the last few years studies of the morphogenesis of double stranded DNA phages have made it abundantly clear that this is a very inadequate description of capsid function. In T4, P22, lambda, T3, T7, and other phages a shell is formed at first empty of DNA, which then encapsulates and condenses the viral chromosome (Simon 1972; Laemmli & Favre 1973; Kaiser, Syvanen & Masuda 1974; Botstein, Waddell & King 1973; Kerr & Sadowski 1974). Viral assembly becomes therefore not just a problem of constructing a passive shell, but of constructing a dynamic organelle.

This is not to imply that we understand how shell structures are constructed. Though the descriptive geometry of subunit packing in spherical shells is well understood, (Crowther & Klug 1975) we still know very little about how subunits come together to form shell structures. Do shells initiate with some special structure and then propagate, as in TMV assembly or do subassemblies such as vertices form and then assemble? What is the detailed reaction sequence

for the addition of repeated subunits forming an isometric shell? Are reactive sites limited to growing structures as in T4 tail assembly; if not, what prevents aberrant aggregation? In both T4 and P22 it is clear that proteins other than the major coat protein are essential for the proper assembly of the coat protein into a shell (Laemmli, Bequin & Kellenberger 1970; Showe & Black 1973; King, Lenk & Botstein 1973). We review below the rather surprising mechanism used by P22 in the construction of its precursor shell. We will also describe experiments on the relation between the regulation of virus assembly, and the regulation of the synthesis of proteins involved.

P22 is a lysogenic phage of *Salmonella typhimurium* (Levine 1972) which has been much used in bacterial genetics because of its ability to occasionally package and transfer segments of the host chromosome (Ebel-Tsipis, Botstein & Fox 1972). The particles are isometric, about 63 nm in diameter, with a small tailplate at one vertex (Israel, Anderson & Levine 1967). The DNA is about 27×10^6 mol. mass, and like phage T4, is terminally redundant. Electron micrographs of the phage are shown in figure 1, plate 6.

Morphogenetic genes

The experiments described below all depend upon the existence of phage carrying conditional lethal mutations in the genes for the late proteins (Epstein *et al.* 1963). Amber mutations defining the late genes of phage P22 have been isolated and mapped by Botstein, Chan & Waddell (1972). These defined eleven late cistrons, nine of which specify proteins involved in particle assembly (the other two control lysis). These genes are clustered according to their function in particle assembly, as shown in figure 2. Genes 5 and 8 specify proteins directly involved in capsid formation. Genes 1, 2 and 3 specify proteins involved in the packaging of the DNA into the precursor capsid. Genes 10 and 26 specify proteins needed for the stabilization of the newly filled (with DNA) capsid. Genes 16 and 20 specify proteins which are incorporated into the capsid, but are not needed for capsid formation; they function later in the injection of the DNA into the next host cell (B. Hoffman & M. Levine 1975; Ruth Griffin Shea, unpublished experiments).

Description of plate 6

Figure 1. P22 phage particles. The left hand panel shows negatively stained P22 phage. The right hand panel shows a thin section of P22 infected cells. Paracrystalline arrays of phage particles are common. All the phage strains used in the experiments described here carried the c_1^7 clear plaque allele to ensure lytic growth, and an amber mutation in gene 13, which prevents spontaneous lysis and prolongs phage production past the normal shut-off point. Details of the fixation and embedding procedures for this and subsequent figures are described in Lenk, Casjens, Weeks & King (1975).

Figure 3. Proheads from P22 mutant infected cells. The left hand panel shows negatively stained proheads, while the right hand panel shows a thin section of infected cells. In both cases the cells were infected with phage carrying an amber mutation in gene 2. The proheads do not form paracrystalline arrays, and are also not found along the cell membrane. Rather they appear to be dispersed through the nucleoplasm. The internal structure noticeable in negatively stained particles appears as an inner shell in favourable images of sectioned infected cells.

Figure 4. Empty and unstable heads from mutant infected cells. The left hand panel shows negatively stained empty capsids from 10^- infected cells. The right hand panel shows a thin section of the infected cells (80 min after infection, at 37 °C). Empty capsids can be seen among the paracrystalline arrays of DNA containing particles. These result from intracellular loss of the chromosome. After cell lysis all the particles lose their chromosomes. Within the cells the replicating DNA is cut to mature size, but at late times is then slowly degraded to smaller molecular mass material (Botstein *et al.* 1973).

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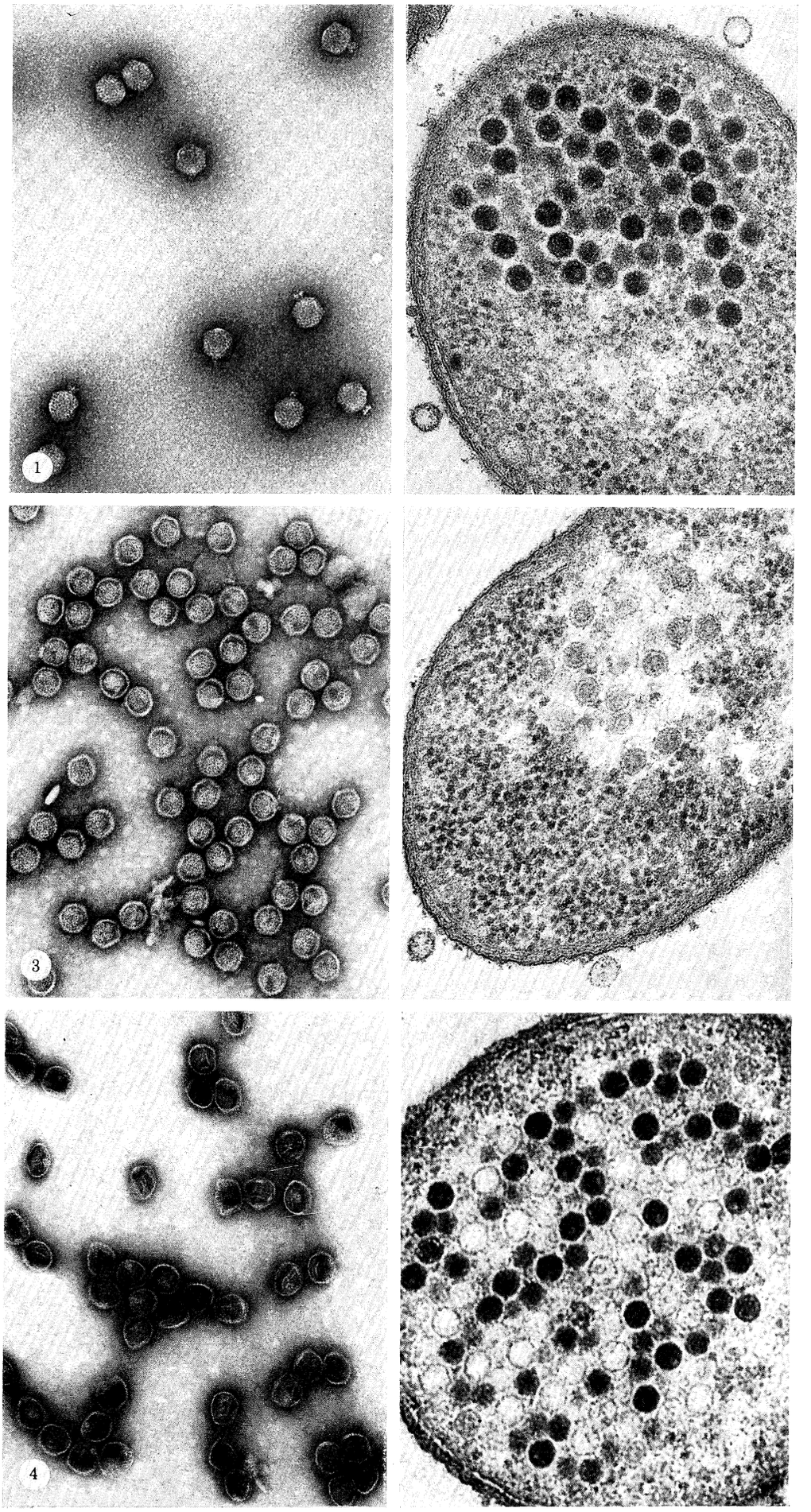

FIGURES 1, 3 AND 4. For description see opposite.

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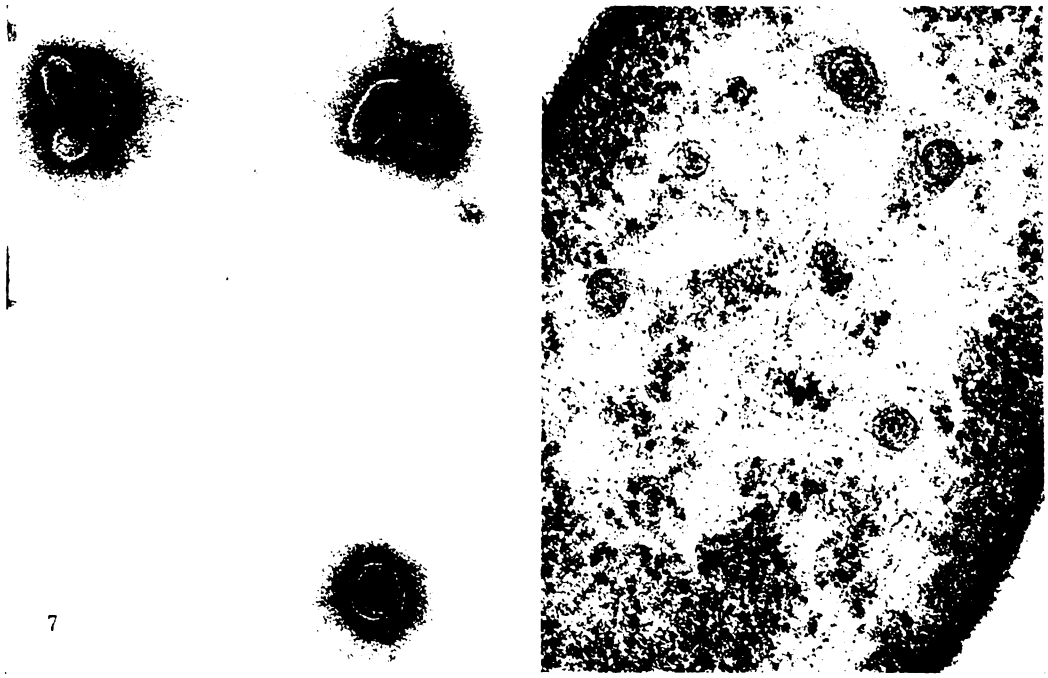

FIGURE 7. Aberrant structures from cells infected with an amber mutant defective in gene 8. Organized structures are rare in 8⁻ infected cells, and those that are seen are unusual. The left hand panel shows negatively stained particles from sucrose gradient fractions of 10 × concentrated 8⁻ infected cells. The right hand panel shows a thin section of such cells selected for showing a number of aberrant structures. In the absence of the scaffolding protein the coat protein assembles both inefficiently, and incorrectly.

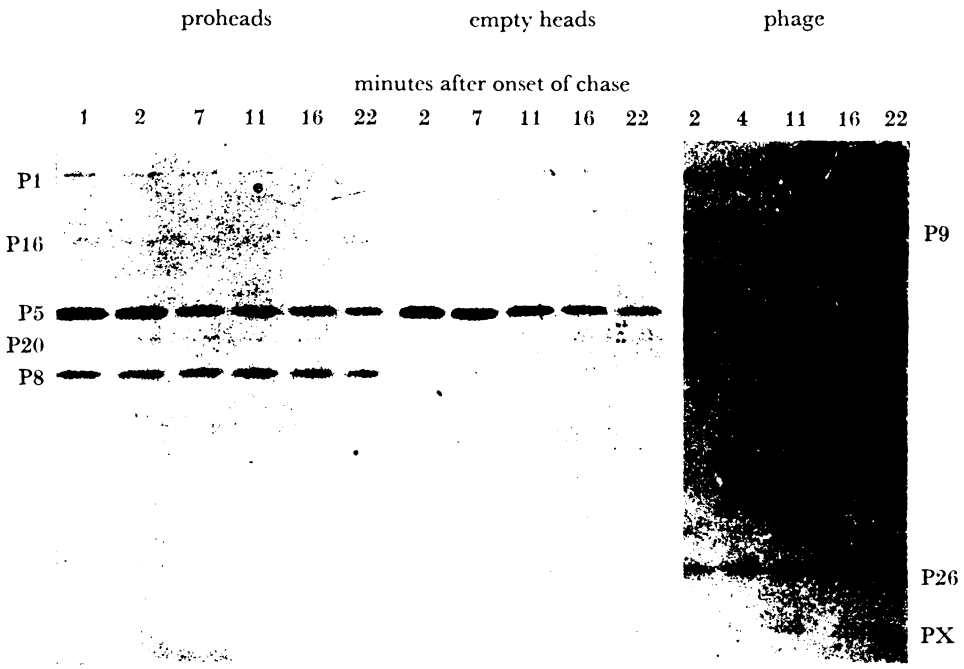

FIGURE 10. The scaffolding protein recycles. The figure shows an autoradiogram of particles isolated at various times after the chase of radioactivity which had been continuously present up to that time. As can be seen in the set of gels on the left, labelled coat protein chases out of proheads. It eventually appears in phage, though this is not apparent since the phage gels are overexposed with respect to coat protein. The scaffolding protein, however, does not chase out of proheads. The same amount of labelled p8 is present in 240S particles at the end of the experiment, as was present at the time of the chase. This labelled scaffolding protein must be associated with the newly synthesized coat protein present in the proheads formed at late times after the chase. Further details of this experiment are in Kings & Casjens (1974).

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The proteins specified by these nine genes have all been identified on SDS acrylamide gels (Botstein *et al.* 1973; Casjens & King 1974). Six of the proteins are in the mature particle; three are not. Mature particles contain two additional proteins, X and α. A. R. Poteete has recently isolated amber mutations identifying two new P22 genes (unpublished experiments). Cells infected with these mutants lack either the X or α bands. Thus the genes specifying all the known P22 morphogenetic proteins have been identified. Conversely the proteins of all the known P22 morphogenetic genes have been identified. This enables us to define precisely, though not understand, all the steps in the assembly of the particle which are specified by phage genes. We do not know, however, whether host functions are used in particle assembly, as in phages λ and T4.

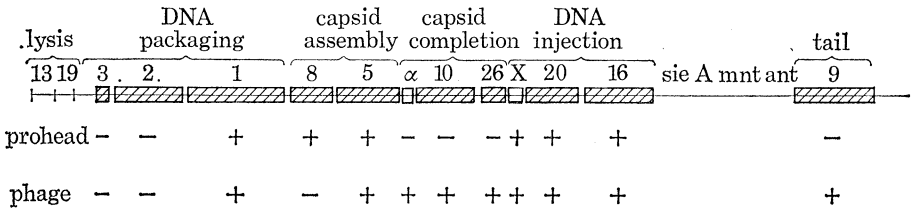

FIGURE 2. Genetic map of the morphogenetic region of the P22 chromosome. The lengths of the genes are proportional to the molecular masses of the corresponding polypeptide chains (Botstein *et al.* 1973; Casjens & King 1974). The signs below the map show the presence or absence of each gene product in proheads and in phage particles. The genetic data for this map are from Botstein, Chan & Waddell (1972), except for the positions of genes α and X which are from Poteete & King (manuscript in preparation).

ASSEMBLY INTERMEDIATES

Cells infected with wild type P22 phage display three classes of progeny particles; the predominant class are 500S DNA containing mature particles. Two minor classes of particles sediment around 240S and 170S. All three classes of particles are absent from both uninfected cells, and cells infected with phage carrying an amber mutation in the coat protein gene. If the slowly sedimenting classes of particles represent precursors to mature virus, they might be expected to accumulate in mutant infected cells. In fact cells infected with amber mutations in any one of the three DNA packaging genes accumulate only the 240S structure; phage and 170S particles are not formed. In the electron microscope these particles (proheads) appear round, with internal structure; they are the only particles seen in 1^-, 2^-, and 3^- infected cells (figure 3, plate 6). In contrast, cells infected with amber mutants of either of the two capsid stabilization genes, 10 and 26, display both 240S and 170S particles, with the largest part of the radioactivity in the 170S structure.

The 240S proheads from such cells look just like those described above. In contrast the 170S structures are fully penetrated by stain, are collapsed, and have corners, just like phage particles which have lost their DNA (figure 4*a*, plate 6). In fact, examination of thin sections of 10^- or 26^- infected cells (figure 4*b*), or of the freshly lysed cultures reveals large numbers of particles full of DNA. These are unstable and lose their DNA both within the cell and after lysis. Thus the 170S empty capsids from mutant infected cells appear to be derived from capsids which have packaged DNA, but have not been stabilized. These results suggest a simple pathway; 240S ⟶ (170S) ⟶ 500S virus. In this scheme the 240S prohead is the structure which encapsidates a headful of DNA. This newly filled capsid is unstable and can break down to a 170S empty capsid (Botstein *et al.* 1973).

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This pathway was demonstrated directly by pulse-chase experiments with wild type infected cells (figure 5). Radioactive amino acids appear first in the 240S (A) peak, very soon thereafter in the 170S (B) peak and then pass into mature virus. However, radioactivity passes sufficiently quickly into 170S shells that it is possible that they are not derived from the 240S class.

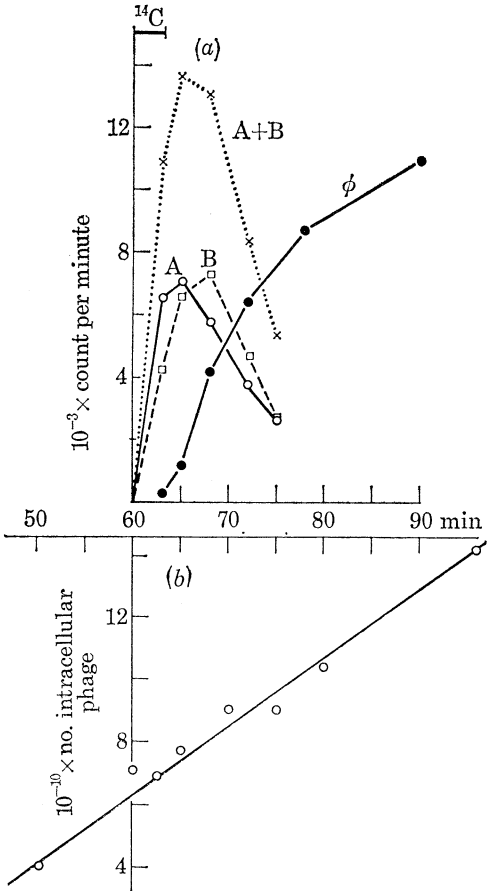

FIGURE 5. Pulse and chase experiment with 'wild type' infected cells. This figure shows the passage of labelled amino acids from 240S proheads (—O—O—) and 170S empty capsids (□---□) to 500S phage particles. The dotted line gives the sum of prohead and empty capsid activity. We presume the empty capsids derive from particles which have initiated DNA encapsulation prior to lysis, but have not completed it at the time the cells were broken open. However, it may be that there is an empty capsid which is a precursor in packaging, but which does not accumulate in any mutant infected cell. Panel (*b*) shows the formation of viable phage during the experiment, which was performed at 37 °C. Details of the experiment are given in King *et al.* (1973).

To verify more convincingly the sequence of events, we employed a temperature sensitive mutation in gene 3. At high temperature this mutant accumulates only 240S prohead shells. By exposing the infected cells to radioactive label at high temperature, then shifting to permissive temperature in the absence of label, we can identify intermediates between 240S proheads and phage. As can be seen in figure 6, at high temperature the ts3$^-$ infected cells accumulate only 240S proheads; after shifting to low temperature, 170S shells appear. Label from both structures eventually appears in mature, DNA containing virus particles.

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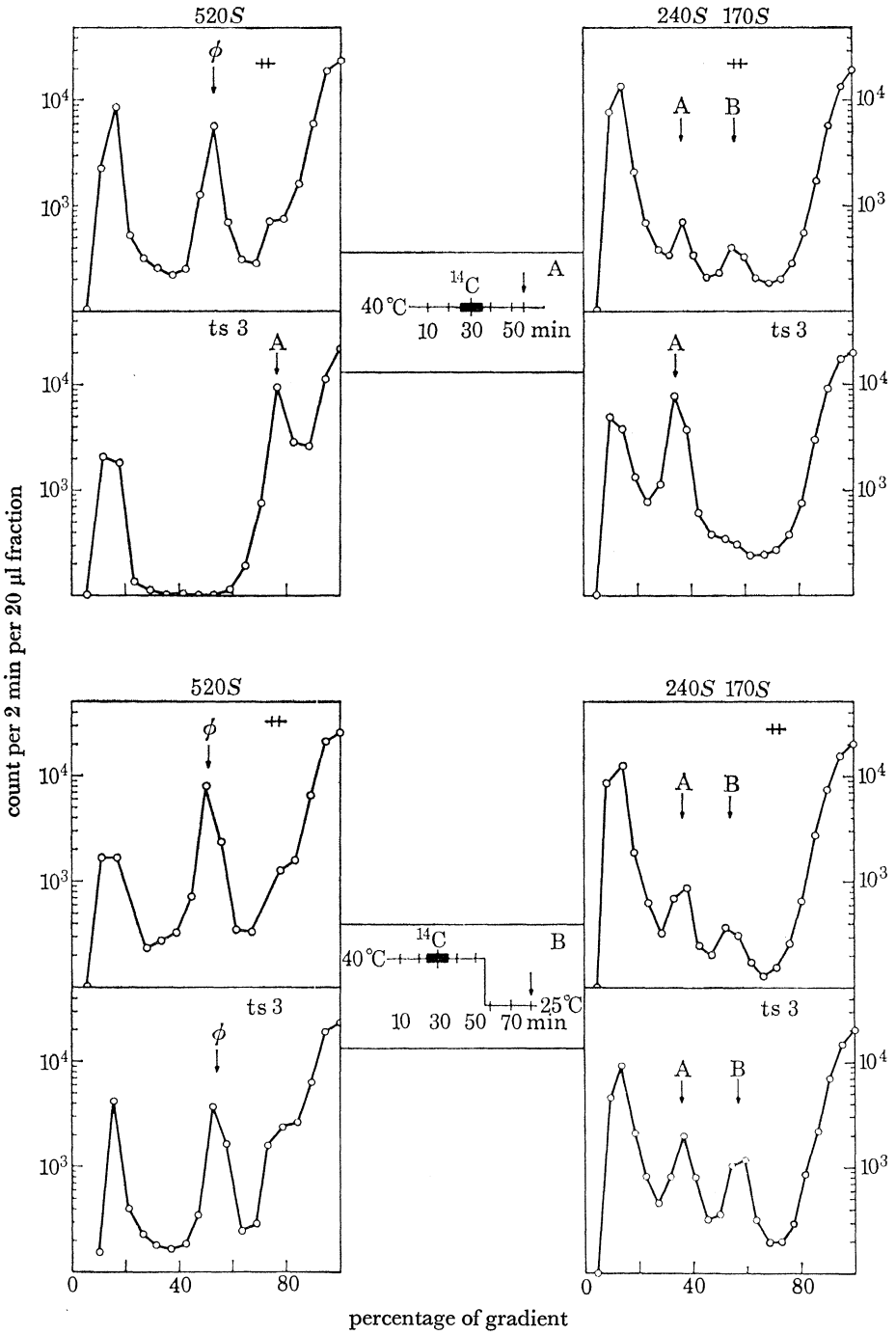

FIGURE 6. Pulse and chase temperature shift experiment. This experiment shows that the 170S empty capsid structure is a later intermediate than the prohead. Two cultures, one infected with 3⁻ temperature sensitive mutant and the other with reference phage (+ +), were exposed to a pulse of radioactive amino acids at high temperature, and then shifted to low temperature. The upper samples were taken before the temperature shift and the lower samples after the shift. The samples were lysed with chloroform and aliquots centrifuged through sucrose gradients. The left hand gradients have been centrifuged to separate phage particles; the right hand gradients have been centrifuged at higher speed to separate more slowly sedimenting precursor particles, proheads (A) and empty capsids (B). All gradients had a dense shelf at the bottom to catch any very rapidly sedimenting material (unlysed cells, absorbed phage, etc.).

The viable phage titres of the lysates were as follows:

	before shift	after shift
control; *am*13⁻c$_1$	2.3×10^{10}	5.4×10^{10}
*ts*3.1/*am*13⁻c$_1$	1.0×10^{6}	2.3×10^{10}

The *ts*3-infected culture left at high temperature for the duration of the experiment (no shift down) had 1.6×10^6 phage/ml. Data from King *et al.* (1973).

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Protein composition of precursor particles

The protein composition of 170S empty heads is quite similar to the protein composition of mature phage particles. Both are composed predominently of gp5 coat protein; they do not contain gp8. The empty heads lack only the tail protein, gp9, and the gene 26 product. The gene 10 product is presumably missing from 10^- empty particles, but this is difficult to observe directly because the gp10 band is obscured by the coat protein band.

The protein composition of the 240S prohead shells differs strikingly from that of empty shells and phage; proheads contain the 42000 mol. mass gene 8 product (figure 10). As noted below, in the absence of the gene 8 product very few organized structures are formed. Since the protein was not present in mature virions, but was needed for virion assembly, we termed it a scaffolding protein (King & Casjens 1974).

The increased sedimentation coefficient of proheads with respect to empty heads suggested that P8 might make up a considerable fraction of the mass of proheads. Results from labelling experiments were equivocal (King *et al.* 1973); pulse labelled proheads from wild type infected cells had only a small amount of radioactive P8 with respect to P5, whereas proheads accumulating in mutant infected cells blocked in DNA encapsidation (1^-, 2^-, 3^-) had ratios close to equimolar. To resolve this, proheads were isolated in high concentration from infected cells, and the P8/P5 ratio was determined by staining with Commassie Blue and Fast Green (Casjens & King 1974). At the time, we considered the possibility that the first round of particle assembly proceeded differently from the later rounds. To test this we isolated proheads from wild type infected cells at various times after infection. However, the results were similar for all samples tested. For every 420 gp5 molecules, proheads contain 250–300 scaffolding protein molecules. This accounts for the increased sedimentation coefficient, and perhaps the material within proheads.

As we show below, the differences between results from staining and results from labelling are due to the unusual life cycle of gp8.

Organization of the prohead

Cells infected with amber mutants of gene 8 synthesize almost normal amounts of coat protein (Botstein *et al.* 1973) but very little of this is assembled into particles. Figure 7, plate 7, is electron micrographs of the rare structures which do form in 8^- cells. These are aberrant aggregates of the coat protein (King *et al.* 1973), and look as if a shell had begun to form, but was unable to properly specify its curvature or dimensions. Apparently interactions between the coat and scaffolding proteins are necessary for prohead assembly.

Negatively stained images suggest that the prohead has material within the shell. This can be seen reasonably clearly in thin sections of the cells which accumulate proheads (figure 3). The material within proheads in thin sections is unlikely to be cell cytoplasm, or non-specific internal staining, since particles classified as empty in negative stain appear truly empty in the thin sections (figure 4).

To obtain more accurate information on the organisation of the proheads, concentrated solutions have been analysed by low angle X-ray scattering (Earnshaw, Casjens & Harrison 1976). Figure 8 shows radial density functions of phage, empty heads and proheads. The internal density of the phage sample is due primarily to DNA. Comparison with empty heads

shows that both have the same radius. However, the radial density function for proheads is quite different from that of empty heads. The radius of the particles is smaller, and there is substantial density at an inner radius. The simplest model to account for these observations is one in which the gp8 forms a thick shell within the outer coat protein shell. This is supported by experiments with purified proheads. Treatment with SDS results in the solubilization of gp8 – the resulting structures have lost the inner material and appear as single spherical shells.

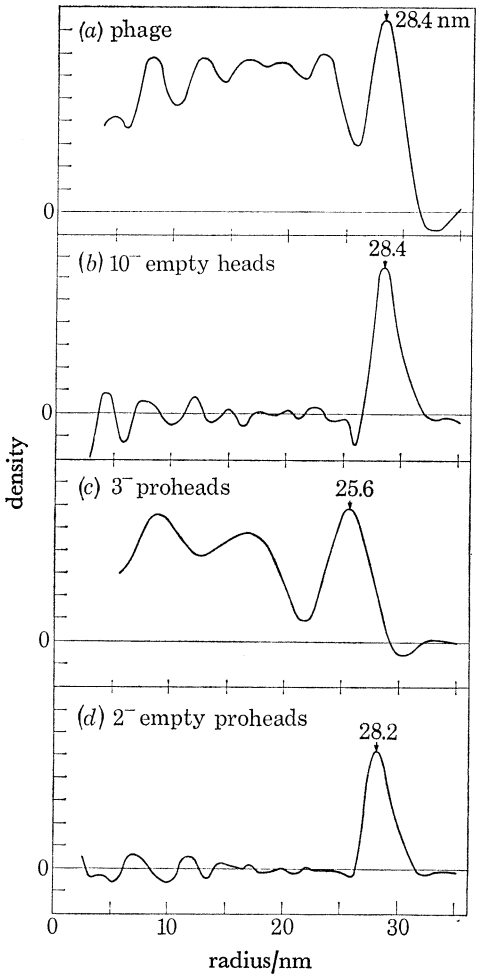

FIGURE 8. Organization of P22 particles as determined by low angle X-ray scattering experiments. The electron density of four classes of P22 particles is plotted (in arbitrary units) against their radii. The electron density within the outer shell of the phage sample is presumably due to DNA. The electron density within the outer shell of the prohead sample is presumably due to scaffolding protein. The 2⁻ empty proheads have been treated with SDS to remove the scaffolding protein. Data from Earnshaw, Casjens & Harrison (1976).

Acrylamide gel electrophoresis reveals that these are composed solely of coat protein (Casjens & King 1974). We imagine that the outer shell of the prohead is a somewhat open lattice, and that detergent treatment allows the scaffolding protein to exit through the coat lattice. This might mimic the *in vivo* mechanism for the departure of scaffolding protein.

The scattering experiments also yield information on the packing of the coat subunits in proheads and phage. There is no gross reorganization of the coat subunits; rather the whole lattice is expanded by about 10 %. This is consistent with the scaffolding molecules departing

through the coat protein lattice. This reaction does not proceed spontaneously at any substantial rate, since proheads are stable for weeks in solution. Inside the cell, the departure of gp8 appears to depend on the concerted action of the gene 1, 2 and 3 products. These might initiate DNA packaging which then destabilizes the gp5–gp8 complex, or they might act directly on the proteins.

Figure 9 shows schematic drawings of P22 capsids and capsid related structures. We do not know what the bonding relationships are between the coat protein and the scaffolding protein in the prohead.

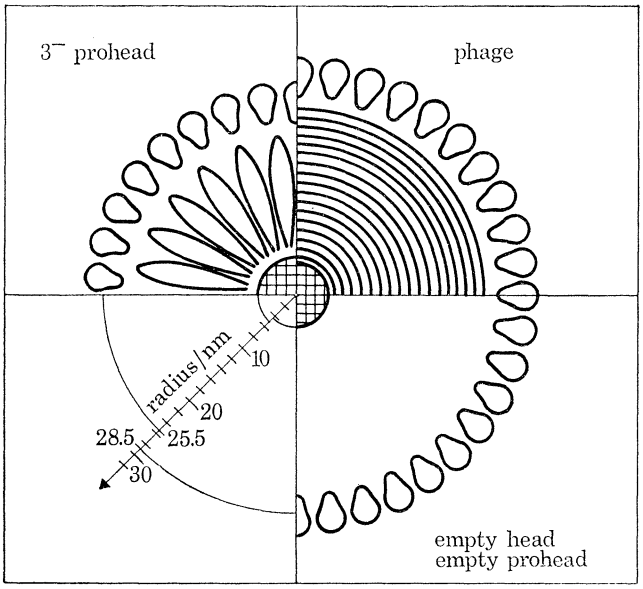

FIGURE 9. Structure of P22 particles. This diagram shows representations consistent with the density profiles shown in figure 8. The important feature is the presence of an inner shell or ball of protein subunits within proheads. The X-ray scattering experiments do not yield information about the innermost regions of the particles. The shape of the subunits and the packing of DNA in the phage particle are arbitrary.

DNA ENCAPSIDATION

At late times after infection P22 DNA is present in the form of concatemers, presumably the direct product of DNA replication (Botstein & Levine 1968). In cells infected with mutants defective in genes 5, 8, 1, 2, or 3 such concatemeric DNA accumulates, and mature chromosomes are not produced (Botstein *et al.* 1973). The concatemers are cut into mature size molecules only if a prohead can be assembled, and if the products of genes 1, 2 and 3 are present. These proteins are presumable directly involved in the packaging mechanism.

Since the precursor structure in packaging contains the scaffolding protein, while particles containing DNA do not, the scaffolding protein must be removed from the prohead before or during DNA packaging. In phage T4, the assembly core proteins are cleaved down to smaller fragments in concert with DNA packaging (Laemmli & Favre 1973). However, we have found no evidence for proteolytic cleavage during P22 morphogenesis (Botstein *et al.* 1973; Casjens & King 1974). The gp8 molecules must depart intact from the prohead in coupling with DNA packaging. The departure of *ca.* 250 molecules of gp8, and the resulting increased radius of the outer shell represents a major conformational change in the capsid. We do not know whether this is a prerequisite for DNA packaging, or is mechanistically coupled with it.

We know little of the actual packaging mechanisms. The products of genes 1, 2, and 3 are necessary for the process. The gene 2 and 3 proteins are not found in any particle; they may interact directly with the DNA. There is slow solubilization of DNA from concatemer, in 5^-, 8^-, 1^-, and 2^-, but not 3^- infections. This suggests that gp3 might be directly involved in DNA cleavage. In addition Raj *et al.* (1975) has found that phage giving high frequency of generalized transducing particles map in gene 3, supporting this idea.

The overall sequence of events has been defined by Tye, Huberman & Botstein (1974). By examining the distribution of the mature chromosome ends, they concluded that packaging initiated at one end of the replicating concatemer and proceeded sequentially down the concatemer. This suggests that double stranded ends might be the sites for initiation of packaging. The effect of deletions was not only to increase the length of the terminal redundancy, but also to increase the randomization of the distribution of chromosome ends around the genome. These findings confirm that Streisinger's headful packaging model is operating in P22 DNA packaging.

Recently V. Jarvik, S. Gottesman & Botstein (unpublished experiments) have developed an *in vitro* DNA packaging system for phage P22, similar to the λ packaging system described in this symposium by Hohn. It should soon be possible to compare the sequence specific cutting mechanism of phage lambda, with the non-sequence specific mechanism presumably operating in P22 packaging.

The scaffolding protein functions catalytically

Measurements of the rates of coat protein and scaffold protein synthesis indicate that only about one molecule of gp8 is being synthesized for every 10–20 molecules of gp5 at late times during infection. This is not enough scaffold protein to account for the number of virus particles which are being synthesized at late times, since each mature virus requires a prohead precursor containing about two gp8 molecules for every three gp5 molecules. The simplest solution to this paradox is to have gp8 recycle during assembly. gp8 is released from the prohead shell before or during DNA encapsidation. It is not proteolytically cleaved, and therefore should be available to go through the same cycle of complexing with coat protein to form further prohead shells. That this is the case was suggested by pulse chase experiments with wild type infected cells (King *et al.* 1973). The radioactive proteins of the prohead fraction were analysed at various times after a short pulse of labelled amino acids. Gel analysis showed that radioactive coat protein chased out of the prohead fraction into mature phage. However, the radioactivity in the gp8 band did not chase. Experiments with 5^- amber mutant infected cells showed that gp8 did not sediment rapidly in the absence of coat protein. Thus the radioactive gp8 sedimenting in the prohead fraction must have been in prohead structures, presumably complexed with newly synthesized non-radioactive coat protein.

The above experiments were not fully convincing, however, since the proheads formed after a short pulse had only low levels of radioactive scaffold protein. (This is clear with hindsight. Most of the gp8 in the proheads was recycled gp8 that had been synthesized before the short pulse. With such a regenerating pool of gp8, pulse labelled structures will be quite underrepresented in labelled gp8.)

To resolve the situation, an experiment was performed in which the cells were exposed to radioactive amino acids continuously from the onset of late protein synthesis (King & Casjens

1974). Thus all the gp8 in the cells should be labelled. The uptake was then stopped by addition of cold amino acids, and samples lysed at various times after the chase. These were fractionated by sucrose gradient centrifugation. The prohead, empty head, and phage peaks were then dissociated in hot SDS, and the proteins separated by SDS gel electrophoresis. The results are shown in figure 10, plate 7. Radioactivity in the coat protein chases out of proheads, and out of empty heads, into phage. However, the amount of radioactive scaffolding protein found in proheads does not decrease. Note that phage are being actively formed during this period. This is simply explained if the scaffolding protein, after exit from a precursor capsid rapidly complexes with newly synthesized coat protein, forming proheads with hot old scaffold protein and cold new coat protein.

Control of scaffolding protein synthesis

Proheads continue to accumulate in cells infected with mutants blocked in DNA packaging, even though the scaffolding protein is not recycling in such cells. This is due to overproduction of scaffolding protein in such cells (table 1). Cells infected with amber or temperature sensitive mutants in genes 1, 2 or 3 all overproduce scaffolding protein. If prohead assembly is blocked, for example in cells infected with mutants in the coat protein gene, gp8 is synthesized at the wild type rate. This is most simply accounted for if soluble gp8 depresses its own further synthesis. Normally gp8 is being released from proheads into a short lived soluble pool. In this form it depresses its own further synthesis. If DNA packaging is blocked, proheads accumulate. The gp8 within the prohead is unable to depress its synthesis and synthesis increases. The result is that coat protein and scaffolding protein are always present in the correct ratio, and aberrant capsids are not formed. This feedback system couples the synthesis of an important morphogenetic protein to the morphogenesis process itself.

Table 1. Scaffolding protein synthesis

	rate of protein synthesis (arbitrary units)† infected cells			
	$13^{-}c_1$	$3^{-}/13^{-}c_1$	$8^{-}/13^{-}c_1$	$5^{-}/13^{-}c_1$
scaffolding	10	32	—	7
coat	150	85	72	—

† The restrictive host was infected with the phage strains shown at 30 °C. At various times after the cells were exposed to a 2 min pulse of radioactive amino acids, lysed, and the proteins separated by electrophoresis through a 10% acrylamide SDS gel. After autoradiography the films were scanned with a microdensitometer and the peaks cut out and weighed. This was done for two or three different exposures of the gel to be sure that the band densities were in the linear range of the film. The data shown is for a pulse given at 50 min after infection, at which time the control cells, $13^{-}c_1$, contained 260 phage per cell. Phage continued to accumulate linearly for at least 30 minutes past this time. The data shown in this table are representative of data from many similar experiments, and also from experiments in which accumulation rather than rate was measured, with the proteins detected by staining rather than autoradiography (Casjens & King, in preparation).

We do not know whether the regulation of gp8 synthesis occurs at the level of transcription or translation. The synthesis of gp5 is always somewhat lower in 8^{-} infected cells than in control cells (table 1). We have tended to attribute this to polarity effects of the gene 8 amber mutation on gene 5. If this is true and the two genes are cotranscribed, the regulation is likely to be at the level of translation.

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Discussion

Prohead assembly

How does the scaffolding protein function in shell formation? It is appealing to think of the scaffolding protein as first complexing with itself to form a small shell of low T number, which then directs accurately the polymerization of the coat protein. However, we have no evidence to support this model. In the lysates lacking functional coat protein, gp8 does not form rapidly sedimenting complexes. In addition, we have not observed organized structures in thin sections of 5^- infected cells (Lenk *et al.* 1975). Unfortunately we do not know whether such gp8 structures would be detected by electron microscopy. Also, gp8 synthesis is fully depressed in 5^- infected cells, so such structures would be present only in small numbers.

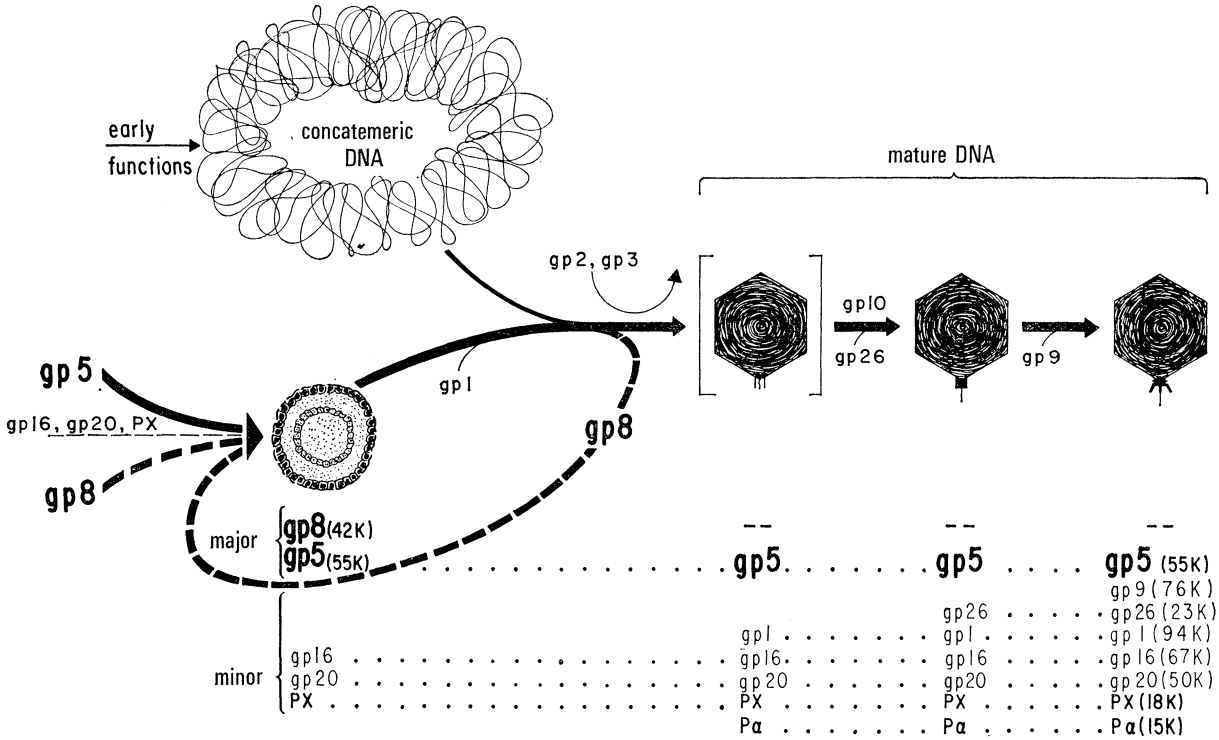

Figure 11. Pathway for P22 morphogenesis. This figure shows structural intermediates and gene controlled steps in P22 assembly. The proteins present in each structure are shown below it. Though the products of genes 16, 20 and X are found in the prohead, morphologically normal phage particles are formed in their absence. These are apparently blocked in DNA injection (Hoffman & Levine 1976; Shea, unpublished experiments). As noted in the text, the genes for proteins X and α have recently been identified. The structure in parenthesis is unstable and loses its DNA, both within the cell, and after lysis. Though gp1 is shown as acting after prohead assembly, it could be assembled into the prohead earlier. Gene products are labelled gp.

A mechanism more consistent with the observations would be the formation of gp5–gp8 complexes followed by their polymerization. In this case the mixed complex would have the additional specificity necessary to properly determine shell dimensions. In any of these models the scaffolding protein must be activated for self-bonding by interaction with the coat protein. However, after completion of the shell, it must be released. Whether this release is moderated directly by the gene 1, 2 and 3 proteins, or by interaction with the DNA directly, is unclear.

The scaffolding protein represents just one example of a larger class of morphogenetic

proteins involved in phage head assembly. The gene 22 protein probably performs a similar function for T4 (Showe & Black 1973), as does the gene 9 protein of T7, (Studier 1972). These proteins do not recycle, but they are necessary for head assembly, and are removed prior to DNA packaging. There may, however, be a recycling protein in phage T3 (Matsuokato & Fukisawa 1975).

Initiation of shell formation

Is there a unique site for the initiation of prohead assembly? The P22 prohead contains four minor protein species in addition to the coat and scaffolding proteins; the products of genes 1, 16, 20 and X. Any one of these four could be candidates for initiation proteins. Ruth Griffin Shea has constructed the triple mutant 1^-, 16^-, 20^-, and found that normal appearing proheads are still produced. A. R. Poteete has found that mutants unable to synthesize gpX also make normal appearing proheads. (In fact, morphologically normal though non-infectious phage particles are formed in the absence of gp16, gp20 or gpX). Thus none of these proteins appear to be candidates for an initiation protein. Of course, we do not know that shell formation initiates from a unique site. However, if it does not, such a site must be generated during assembly, for the later ejection of the chromosome.

Sequential assembly

The kind of rigorous data which exist for the sequential nature of phage tail assembly are not available in the case of head assembly. However, the general pattern of results indicates that head assembly is also stepwise. It seems likely to us that the same overall mechanisms may be operating; the proteins are synthesized in a form in which they are not reactive with each other. Rather a reactive site for them is generated by previous assembly steps; upon incorporation into this complex they themselves get activated to bind the next protein in the pathway. This might also be true for the major coat protein. The growing structure has the reactive sites; coat subunits are much more likely to bind to this site, than to each other in solution. Thus even capsid assembly might not be so different from flagellar assembly (Uratani *et al.* 1972).

Overview

The assembly of the large DNA phages described in this report – T4, lambda, P22, and $\phi 29$ – is basically quite similar. In all cases a precursor head is formed involving proteins not found in the mature phage. This undergoes complicated reactions and rearrangements in the process of packaging a chromosome. On the other hand the construction of the spherical plant viruses described at this symposium seems much simpler, at least at this stage of our knowledge. Some part of this is probably due to differences in life cycles. As pointed out by Laemmli, the phage chromosomes not only have to be condensed inside phage heads, they have to be able to get out. This must be built into the particles. For at least some of the plant viruses, however, the host cell provides the machinery for uncoating, which need not be built. (The relationship of assembly process to life cycle for animal viruses is discussed in Casjens & King's (1975) review.) Of course as Hohn points out, it is intrinsically easier to condense a single stranded RNA molecule than to condense a double stranded DNA molecule, and perhaps a much less complicated machine need be constructed to accomplish the task. Thus capsid formation in the ssRNA plant viruses may represent the formation of a protective shell, whereas capsid formation in the large DNA phages represents the formation of a DNA condensing (and de-condensing) organelle.

Perhaps some of the most interesting biology that will come out of the phage work, is the

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characterization of proteins that depend for their reactivity on the state of organization of a very large structure. Thus none of the phage DNA cutting enzymes cut DNA in solution. They only cut it after it is fully condensed into a phage head. Similarly the proteases in T4 only operate after the prohead is fully organized. Such proteins, whose reactivity depends on the prior achievement of a high order of organization, may be a key to understanding the regulation of cell function in higher organisms.

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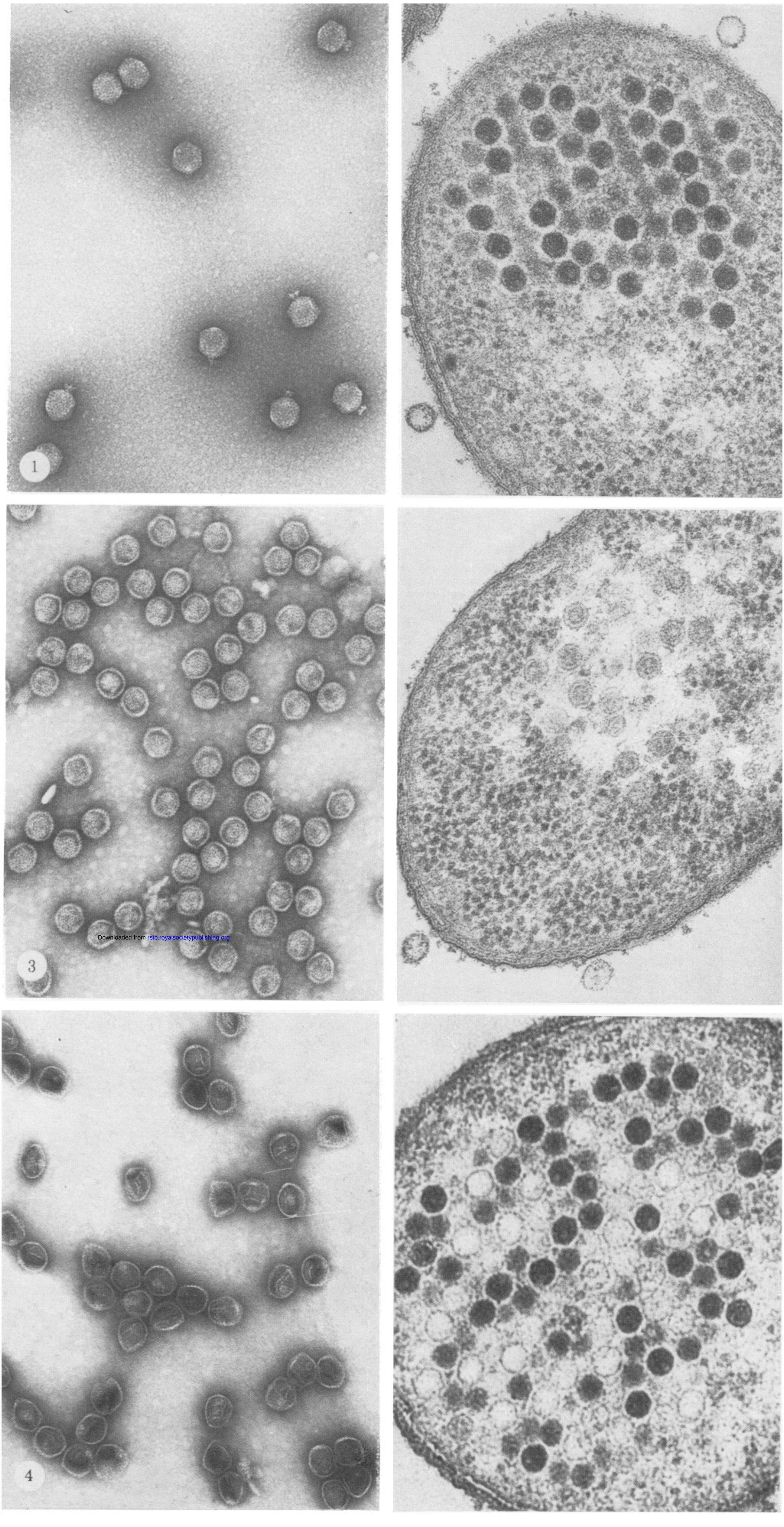

FIGURES 1, 3 AND 4. For description see opposite.

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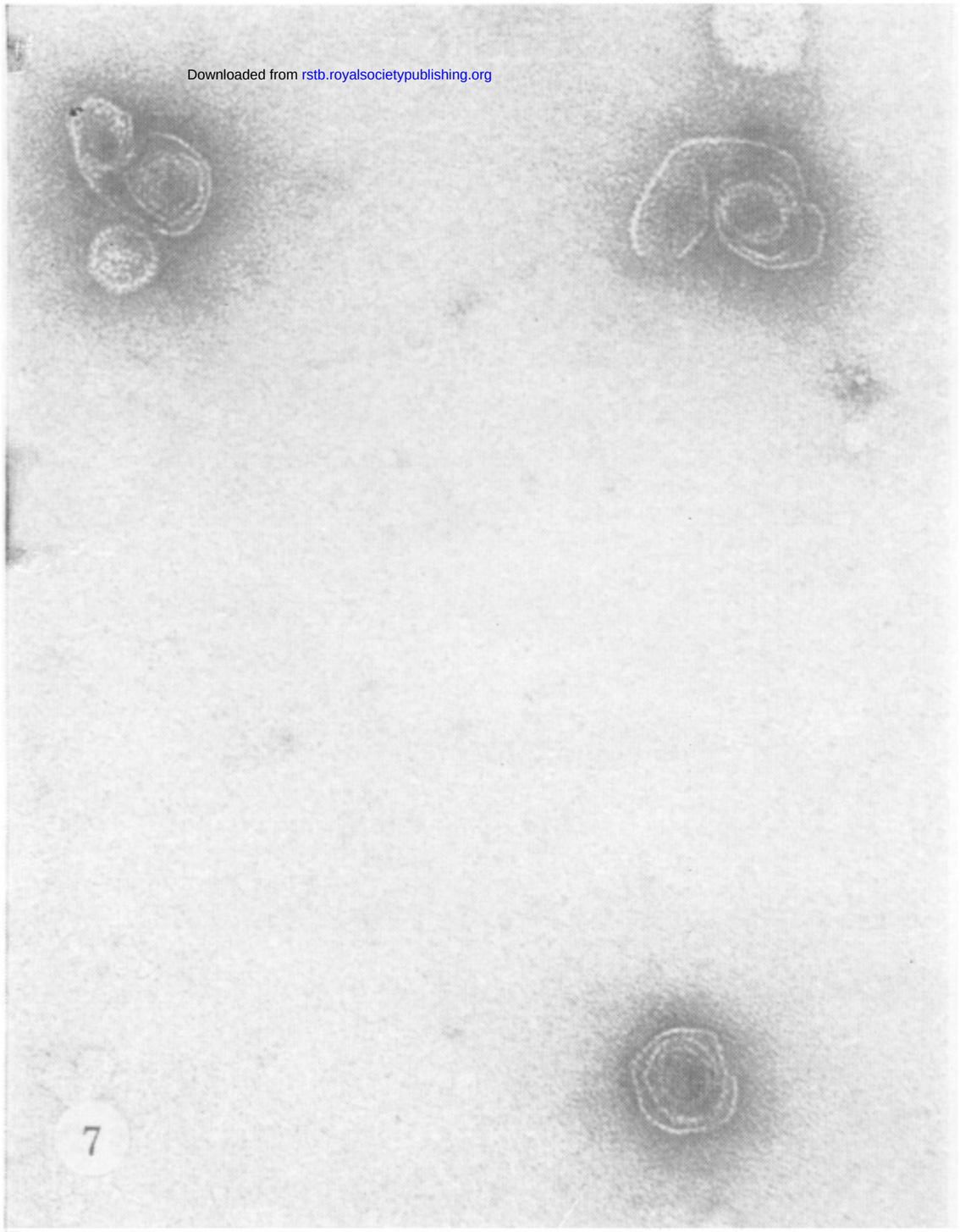

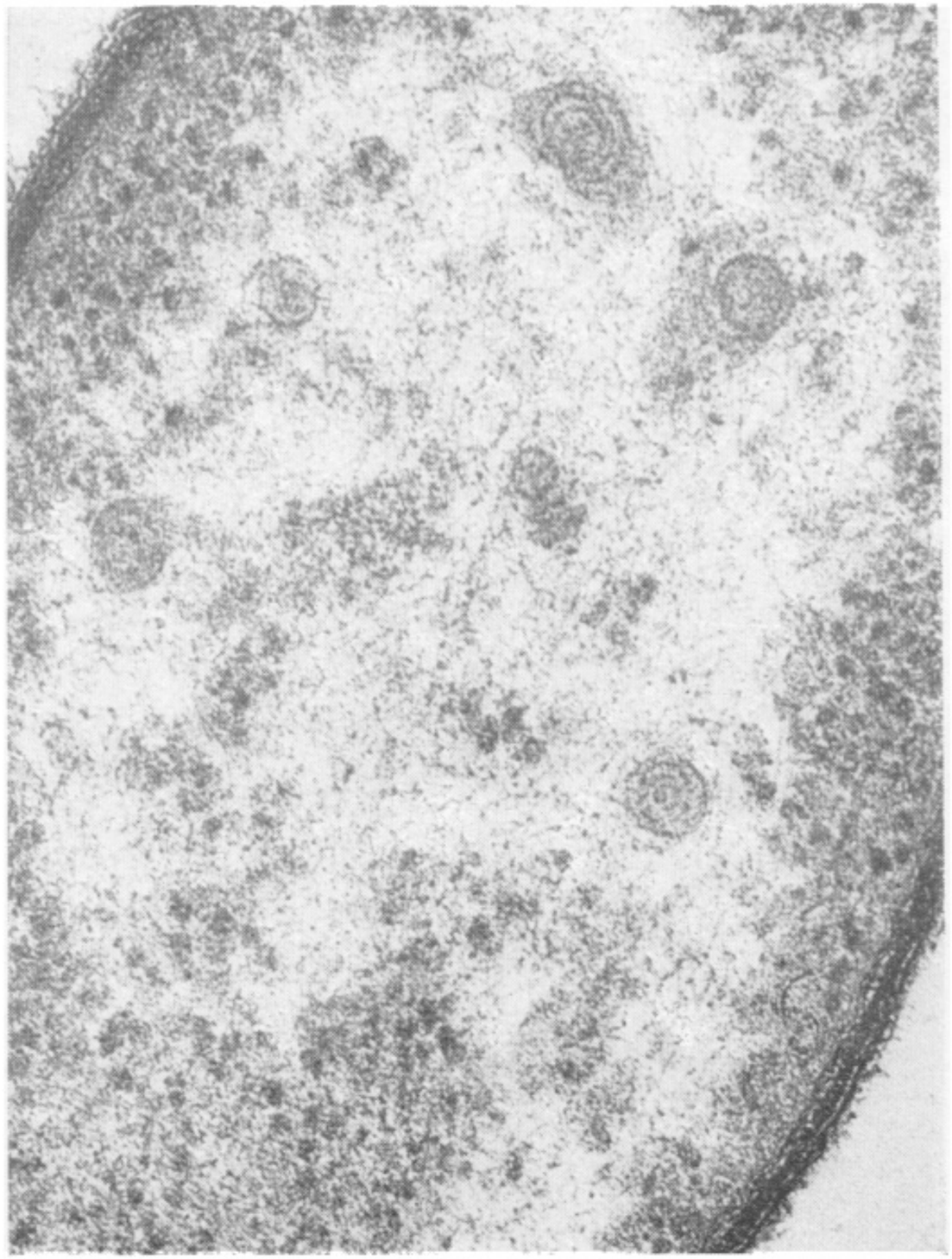

FIGURE 7. Aberrant structures from cells infected with an amber mutant defective in gene 8. Organized structures are rare in 8⁻ infected cells, and those that are seen are unusual. The left hand panel shows negatively stained particles from sucrose gradient fractions of 10× concentrated 8⁻ infected cells. The right hand panel shows a thin section of such cells selected for showing a number of aberrant structures. In the absence of the scaffolding protein the coat protein assembles both inefficiently, and incorrectly.

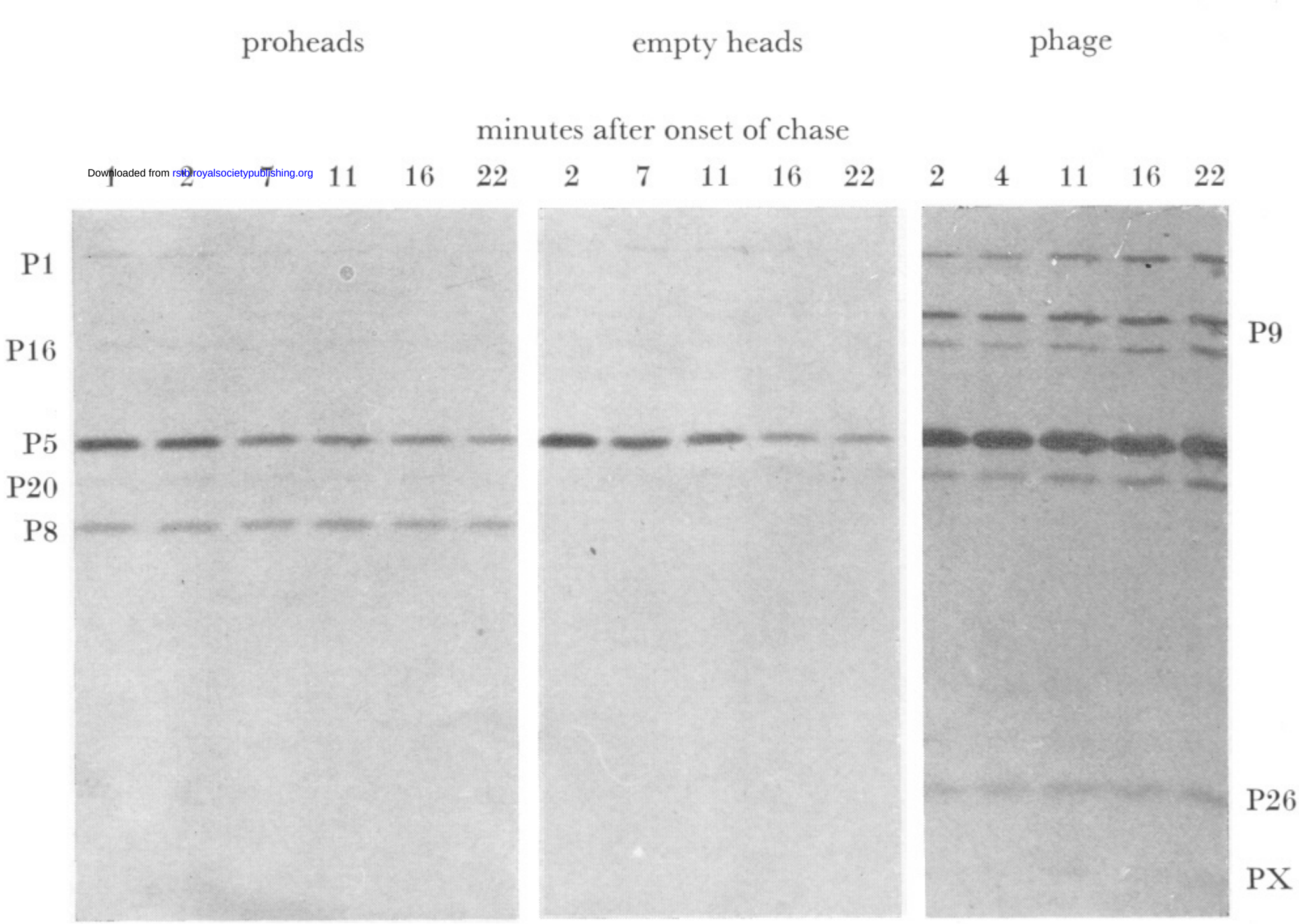

FIGURE 10. The scaffolding protein recycles. The figure shows an autoradiogram of particles isolated at various times after the chase of radioactivity which had been continuously present up to that time. As can be seen in the set of gels on the left, labelled coat protein chases out of proheads. It eventually appears in phage, though this is not apparent since the phage gels are overexposed with respect to coat protein. The scaffolding protein, however, does not chase out of proheads. The same amount of labelled p8 is present in 240S particles at the end of the experiment, as was present at the time of the chase. This labelled scaffolding protein must be associated with the newly synthesized coat protein present in the proheads formed at late times after the chase. Further details of this experiment are in Kings & Casjens (1974).