Electron Microscopy of the Morphogenesis of *Bacillus subtilis* Bacteriophage SP3

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**SUMMARY**

The capsid of *Bacillus subtilis* bacteriophage SP3 is assembled via a prohead intermediate which subsequently encases DNA and attaches a tail. The prohead contains a ring-like core structure. The spokes which extend from the core to the inner prohead surface are thought to form a scaffold for the polymerization of the prohead. Ninety percent of the proheads are assembled prior to the onset of DNA encapsulation. The first mature phage particles are observed at 45 min after infection; titres of intracellular phage demonstrate their infectivity. The core is visible in phage ghosts.

The process of capsid assembly has been elucidated in several bacteriophages (P22, T4, T3, T7, Φ29, λ and P2). It has been shown to proceed through an intermediate structure (prohead) which subsequently encases DNA (P22: Botstein *et al.* 1973; King *et al.* 1973; Lenk *et al.* 1975; T4: Kellenberger *et al.* 1968; Luftig *et al.* 1971; Simon, 1972; Laemmli & Favre, 1973; T3: Matsuo-Kato & Fujisawa, 1975; T7: Roeder & Sadowski, 1977; Φ29: Nelson *et al.* 1976; λ: Hohn & Hohn, 1974; Zachary *et al.* 1976; P2: Pruss *et al.* 1974; for review see Murialdo & Becker, 1978). Biochemical investigations of these phages have revealed a major protein component, 'scaffolding protein', which is present in proheads but absent in mature capsids. Mutants deficient in scaffolding protein cannot form heads (Luftig *et al.* 1971; King *et al.* 1973; Laemmli & Favre, 1973; Casjens & King, 1974; Matsuo-Kato & Fujisawa, 1975; Hagen *et al.* 1976). Electron microscopic observations reveal a core within the prohead which, with the exception of T7, is not retained in mature capsids (Kellenberger *et al.* 1968; Simon, 1972; Lenk *et al.* 1975; Nelson *et al.* 1976; Roeder & Sadowski, 1977; Serwer, 1978). It is now generally accepted that this core consists of scaffolding protein. The present study was conducted to ascertain whether SP3, originally isolated by Eiserling & Romig (1962), follows a similar pattern of capsid assembly.

An overnight culture of *B. subtilis* M47 (histidine and tryptophan deficient) in growth medium at 37 °C was diluted into fresh medium and grown to a density of 10⁸ cells/ml. Growth medium was composed of: nutrient broth, 8 g; yeast extract, 5 g; 10⁻³ M-MgSO₄; 2 x 10⁻⁵ M-MnCl₂; deionized H₂O, 1000 ml. At zero time, the culture was infected with SP3 at a multiplicity of 1. Samples (5 ml) were removed every 2.5 min for 80 min p.i. Glutaraldehyde was added to the samples to a final concn of 2% and the samples were stored at 3 °C overnight. Samples were washed in buffer, postfixed in 1% osmium tetroxide for 45 min and re-washed. Buffer was composed of: NaCl, 8.7 g; K₂HPO₄, 3.5 g; deionized H₂O, 1000 ml. They were then centrifuged at 7700 g for 10 min and the buffer decanted. Melted 4% agarose solution was added to the samples to a final concn of 2% and the samples were again centrifuged as the agar solidified. The samples were dehydrated overnight through an alcohol series followed by propylene oxide and a 1:1 mixture of propylene oxide and Epon. The samples were then placed in two successive Epon baths; final curing was for 72 h at 60 °C. Grey sections were cut with a diamond knife and stained with 2% aqueous uranyl acetate and lead citrate (Venable & Coggleshall, 1965) for 1 min each.
Fig. 1. (a) Prohead sectioned through the centre of the core. Spokes are indicated by arrows. (b) (c) Proheads in which the plane of section grazed the edge of the core. (d) Extracellular phage ghost. The core and spokes are present. All micrographs are at the same magnification.

Infective centres and intracellular phage titles were assayed concurrently with sampling for microscopy. Plating was performed using the soft agar overlay method.

The first phage-related structures observed were the proheads, which appeared at 37.5 min p.i. A small percentage of sectioned cells were observed to have burst at all time points during preparations; infected cells would thus have extruded their phage precursors into the surrounding medium. Sections through such extracellular proheads often afforded a more detailed observation of their fine structure than sections through intracellular proheads or negatively stained proheads. High magnification micrographs of proheads revealed a ring-like central core (Fig. 1 a, b, c). Spokes were observed spanning the distance between the core and inner prohead surface, similar to the core capsid 'radial arms' described for λ (Zachary et al. 1976). Sections through proheads which grazed the edge of the core (Fig. 1 b, c) permitted improved visualization of the spokes.

Table 1 lists the relative frequencies of proheads and full capsids from the time at which proheads first appear until lysis of the culture. Nearly all (90%) of the proheads were assembled prior to the initiation of DNA encapsulation at 45 min p.i., as evidenced by the appearance of dark-staining capsids. Tail attachment was first observed at 45 min p.i., coinciding with the appearance of the first mature phage progeny as measured by intracellular phage titles. The increase of newly assembled heads stopped at 52.5 min p.i. Lysis of the culture was complete at 70 min p.i., shown by assay of infective centres (data not shown, see Nishihara & Romig, 1964).

The core and spokes are not readily visible in full heads nor complete phage. Since they are present in page ghosts (Fig. 1 d) it is reasonable to suggest that they are retained in the phage head structure during DNA encapsulation. Virtually all of the ghosts, empty heads and proheads examined contained the core complex. However, the possibility that they are
Table 1. Relative frequencies of proheads and phage particles per cell*

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<th>Time p.i. (min)</th>
<th>Proheads</th>
<th>Full head</th>
<th>Total particles</th>
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* Ten perfect cross-sections of infected cells were examined for phage particle counts and scored for numbers of proheads and/or full heads, and the totals were averaged for each time point.

artefactual in origin has not been ruled out completely. The cores of T4, P22, T3, λ and φ29 exit prior to or concomitant with DNA encapsulation (Simon, 1972; King et al. 1973; Matsuo-Kato & Fujisawa, 1975; Nelson et al. 1976; Zachary et al. 1976). In the case of T7, a protein species has been shown to exit concomitant with DNA encapsulation, yet a second, which Roeder & Sadowski (1977) believe to function in the assembly complex, is retained. Electron microscopy of complete phages which have expelled or partially expelled their DNA reveals the persistence of the core structure (Serwer, 1978).

The probable function of the spokes is to form a scaffold to assist in the proper polymerization of the capsid. The retention of the core complex as a permanent capsid component leaves open the possibility that it functions in some capacity in the packaging and/or storing of DNA within the capsid.

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REFERENCES


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Short communications


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