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Bacteriophage SPO1 Structure and Morphogenesis
III. SPO1 Proteins and Synthesis

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The virion proteins of SPO1 have been determined by sodium dodecyl sulfate-polyacrylamide gel electrophoresis methods on purified phage components and on phage lysates. The phage head contains 16 proteins, and the connector or neck structure has an additional 3 proteins not found in the head. The proximal part of the tail, composed of sheath, tube and connecting components, contains six proteins. The distal baseplate is the most complex structure, with 28 proteins identifiable on sodium dodecyl sulfate gels. The maximum number of proteins found in phage subassemblies is 53, which would account for nearly half the coding capacity of the SPO1 genome.

The bacteriophage-infected cell provides a biochemically and genetically accessible system for studying morphogenesis and development, and regulatory processes have been described in detail at the transcriptional, translational, and gene product levels (20, 23, 36, 37). One requirement for such studies is the identification of phage-coded proteins, and the structural proteins in particular, for studies on morphogenesis. This has been accomplished for other well-studied phages such as λ, T4, P22, and T7, for example (6), but not for phage SPO1, although its pattern of gene expression has been well studied (9, 22). In this communication, we identify those SPO1 proteins which are structural components of the virion.

MATERIALS AND METHODS

Bacteria and phage. Bacteria and phage were obtained and grown as described by Parker and Eiserling (18).

Media, solutions, and chemicals. Low sulfate medium (LSM) contains, per liter, 6 g of KH_2PO_4, 14 g of K_2HPO_4, 1 g of sodium citrate, and 2 g of NH_4Cl. This is autoclaved, and the following sterile solutions are added per 50 ml: 0.05 ml of 3.6 mM $FeCl_3$, 1 ml of 40% glucose, 0.16 ml of 1 M $MgCl_2$, 0.04 ml of 0.5 M $CaCl_2$, 1.25 ml of 0.2% L-tryptophan, and 0.04 M Na_2SO_4 (0.25 ml for overnight cultures and 0.05 ml for labeling). The LSM used for labeling contains 40 μM Na_2SO_4 and 5 μg of casein hydrolysate per ml.

^{14}C labeling medium is CHT-50, described by Gage and Geiduschek (8), containing 5 μg of casein hydrolysate and 5 μg of L-tryptophan per ml.

Lysing buffer is prepared fresh from three stock solutions. Solution A contains 20 mg of chloramphenicol and 4 mg of phenylmethylsulfonyl fluoride (PMSF; Calbiochem, 52332) per ml in 50% isopropanol. Solution B contains 0.1 M Tris, pH 7.5, 0.1 M $MgSO_4$, 50 μg of Worthington RNase (R-653) per ml, 50 μg of Sigma DNase (DN-100) per ml, and 0.1 M sodium azide. Solution C contains 10 mg of freshly prepared Worthington lysozyme (LY71A) per ml in water. A 10× concentrated lysing buffer solution is made by mixing together 0.1 ml of A, 0.9 ml of B, and 0.1 ml of C. This solution is kept on ice for up to 1 h.

Base medium is made by the addition of 0.2 ml of 0.8 M $MgSO_4$, 0.2 ml of 0.5 M $CaCl_2$, 2.5 ml of 40% glucose, and 20.0 ml of SS10X (Spizizen's salts; see reference 8) to 170 ml of sterile distilled water. Medium NY contains, per liter, 8 g of Difco nutrient broth and 5 g of Difco yeast extract.

A stock solution of three protease inhibitors, *N*-α-*p*-tosyl-L-lysyl chloromethyl ketone (TLCK; Sigma T-7254), tosylamidophenylalanyl chloromethane (TPCK; Nutritional Biochemicals Corp., 8980), and phenylmethylsulfonyl fluoride, was prepared by dissolving all three inhibitors in 50% acetonitrile–50% water (vol/vol) to 3.7 mg/ml (10 mM), 8.8 mg/ml (25 mM), and 4.4 mg/ml (25mM), respectively. The solution was tightly stoppered and stored at 0°C until use.

Uniformly ^{14}C-labeled amino acids (NEC-445) and ^{35}S-labeled sodium sulfate (NEX-041) were obtained from New England Nuclear Corp.

Buffers for sodium dodecyl sulfate-polyacrylamide gel electrophoresis were those described by Laemmli (13).

Preparation of ^{14}C-labeled SPO1 lysates. A saturated overnight culture (15 to 18 h) of *Bacillus subtilis* 168M in CHT-50 was diluted 1:100 into an appropriate volume of ½ CHT-50. The culture was grown at 37°C in a shaker bath to an absorbance at 500 nm of 1.1 (2×10^8 cells per ml) and centrifuged for 6 min at 6,000 rpm at room temperature in an SS34 or GSA rotor. The cells were resuspended as quickly as possible to one-sixth of the original volume in labeling medium and transferred to a bubble tube. After an 8-min incubation

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at 37°C, the cells were infected at a multiplicity of infectin (MOI) of 5. Under these conditions, the latent period is 89 min, the cells lyse by about 120 min after infection, and the burst size is approximately 50 to 60 phage per infectious center. For continuous labeling, a mixture of ^{14}C-amino acids was added at 37 min (6 μCi), and 3 μCi was added at 47, 62, and 72 min after infection to a final concentration of 30 μCi/ml. Incorporation of label into trichloroacetic acid (TCA)-precipitable material was generally 25% for wild-type infections and 50% for mutant infections. At 100 to 120 min after infection, the cells were lysed by the addition of sodium azide to 10 mM, freshly prepared Worthington lysozyme to 100 μg/ml, Sigma DNase I to 10 μg/ml, PMSF to 44 μg/ml, TPCK to 88 μg/ml, and TLCK to 37 μg/ml.

Proteolytic processing of SPO1 proteins was followed by infecting sixfold-concentrated 168M cells in labeling medium at 37°C, at an MOI of 5 in a bubble tube. At the times after infection indicated in the figure legends, ^{14}C-amino acids were added to a 0.2-ml aliquot to a 33 μCi/ml final concentration. One or 2 min later, a 350-fold excess of cold amino acids was added to stop incorporation. At various times after this chase, 0.2-ml samples were rapidly transferred to an iced tube containing 10 μl of lysing buffer. This tube was incubated for 1 to 5 min on ice and then was transferred to 37°C until lysis was complete. The effect of protease inhibitors on proteolytic processing was measured by adding the appropriate inhibitor (PMSF, TLCK, or TPCK) 3 min before the label to final concentrations of 88, 74, and 176 μg/ml, respectively.

Preparation of ^{35}S-labeled lysates. A saturated overnight culture (15 to 18 h) of 168M was grown at 37°C in LSM and diluted 1:100 into LSM containing casein hydrolysate. The cells were grown to an optical density at 500 nm of 1.1 (2×10^8 cells per ml), centrifuged, and suspended in one-sixth the volume of LSM plus 5 μg of casein hydrolysate per ml. Cells were then incubated for 13 min at 37°C with aeration and infected at an MOI of 5. At 30 min after infection, ^{35}S-labeled sodium sulfate was added to a 19 μCi/ml final concentration. The culture was incubated until lysis, at which time the following were added: sodium azide to 10 mM, freshly prepared lysozyme to 100 μg/ml; DNase to 10 μg/ml; PMSF to 44 μg/ml; TPCK to 88 μg/ml; TLCK to 37 μg/ml; and unlabeled Na_2SO_4 to 0.05 M. Under these conditions, approximately 5% of the label was incorporated into TCA-precipitable material.

In vitro extract complementation. A saturated overnight culture of *B. subtilis* 168M grown in CHT-50 (15 to 18 h) was diluted into 500 ml of fresh CHT-50 in a 2.8-liter Fernbach flask and aerated on a rotary shaker at 37°C. At an absorbancy at 500 nm of 1.1, the culture was infected with 5 phage per bacterium. Five minutes later, 25 ml of 10× NY was added. At 37 min after infection, the infected bacteria were concentrated by centrifugation. The supernatants were quickly discarded, and a small metal spatula was used to remove each rubbery pellet. Each pellet was cut into thirds and dispensed into preweighed tubes which were frozen quickly in an ethanol-dry ice bath. The pellet weight was about 0.1 g per tube. The tubes were stored at −80°C for several months without detectable loss of complementation activity. The frozen pellets were thawed at room temperature for 5 min. Then 0.3 ml of Base medium containing 20 μg of DNase and 200 μg of freshly prepared lysozyme per ml was added. The tubes were incubated at 37°C and were repeatedly mixed on a Vortex machine until the pellets resuspended. This usually took 15 to 30 min. Freshly frozen pellets resuspended more easily than ones which had been stored for a longer period of time. If the pellet did not resuspend completely after 20 min, an additional aliquot containing 60 μg of freshly prepared lysozyme was added. The resuspended pellet ("extract") was kept on ice and used immediately.

Complementation assays were carried out by using 20 to 40 μl in wells of a microtiter plate. Samples were sealed with Parafilm, incubated for 2 h in a 30°C water bath, diluted in Base medium, and assayed for plaques on HA101-B indicator bacteria by standard methods. Each extract was also incubated separately and assayed.

In some experiments, "soluble" fractions of extracts were prepared by the addition of solid sucrose to a 3% final concentration (wt/vol) to a portion of the extract. A sample of 175 μl was centrifuged at top speed in a Beckman Air-Fuge for 45 min at room temperature. Using the Air-Fuge clearing factor (K) of 11, all material larger than 15S should sediment. The top 80 μl of each gradient fraction is referred to as the 15S supernatant. Examination of this supernatant by electron microscopy revealed no recognizable phage structures.

SDS-polyacrylamide gel electrophoresis (SDS-PAGE). Polyacrylamide gels were prepared as described in reference 2.

Purified bacteriophage T4D$^+$ (^{14}C-labeled) was used as a molecular weight standard. The T4 proteins used and their molecular weights were: gp23* (47,700) and IPIII* (18,300) from amino acid compositions (33); gp soc (9,700) from amino acid composition (1); gp18 (67,000) from amino acid composition (32); gp34 (150,000) and gp37 (123,000) from SDS-PAGE (35). Mobility plotted versus log of the molecular weight gave linear results between 67,000 and 9,700 molecular weight. Molecular weights about 67,000 were roughly linear but with greater slope. SPO1 protein molecular weights were obtained from extrapolation of the T4 standard molecular weights, using one or two T4 samples per gel.

TCA precipitation. Labeled proteins were precipitated onto filter paper pretreated with TCA by the method of Chung and Collier (4).

RESULTS

Isolation of phage structures. Phage structural components were isolated by a two-step centrifugation procedure described in an accompanying article (18). A sample of the lysate was layered on a preformed linear CsCl gradient of density range 1.20 to 1.65 g/cm^3. This allows separation of cell membrane, proteins, and protein nucleic acid complexes such as phage or phage heads. Fractions were collected and examined by electron microscopy (EM). Figure 1A shows a drawing of the separation pattern obtained. Bands 2, 3, and 4 were collected and pooled. Band 8, which contained mostly heads as seen by EM, was also collected.

Bands from the CsCl gradient were dialyzed and placed on a 5 to 45% linear sucrose gradient and centrifuged as described by us elsewhere (18). Contents of bands, as determined by EM, are shown in Fig. 1B and are complementary to those observed for phage T4 by Coombs and Eiserling (5). Band 2 was highly enriched in baseplates, band 3 contained mostly tails and some baseplates, bands 4 and 5 contained a mixture of apparently empty capsids as well as ghosted particles (empty-headed phage), and bands 6 and 7 contained mainly phage and full heads, respectively.

When heads are prepared directly from a CsCl gradient, rapid dilution of the CsCl by addition of water to the grid can cause some of the heads to lose their DNA. Thus, the microscope shows both heads and capsids. The position of the band in either CsCl or sucrose, however, suggests that the band contained only full heads, due to their high density and rapid sedimentation. When 1% phosphotungstic acid (pH 7.0) was used as a negative contrasting agent, it was found to cause tail contraction, head-tail disunion, and loss of DNA from SPO1 heads.

Determination of SPO1 structural proteins. We compared autoradiograms of wild-type whole phage, disrupted wild-type particles (tails, capsids, and ghosted phage), and intermediate or aberrant structures previously subjected to gradient centrifugation. The resultant bands on SDS-polyacrylamide gels should represent viral structural proteins and perhaps any labeled adherent bacterial proteins.

Micrographs of the isolated phage structures are shown in Fig. 2. Intact phage and heads are considered highly purified because of the CsCl equilibrium step, and *sus* 046-0 capsids were homogenous as derived from CsCl-purified heads and separated on sucrose gradients (Fig. 1B). The wild-type necked tails contain a minor capsid contaminant, as does the *sus* N49 tail preparation. All tail preparations contained some free baseplates as well as contracted and uncontracted tails and contracted sheath. The *sus* N49 baseplate preparation shown here contains baseplates as well as amorphous material which, judged by gel electrophoresis analysis (Fig. 3), is presumed to be dissociated baseplate substructures.

Figure 3 shows the electrophoretic peptide profiles of CsCl-purified phage and substructures labeled in vivo with ^{35}S and purified by the two-step centrifugation procedure (18). Wild type ^{35}S-labeled phage purified by CsCl banding were also included.

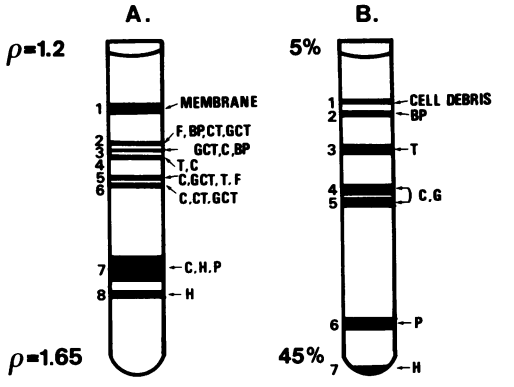
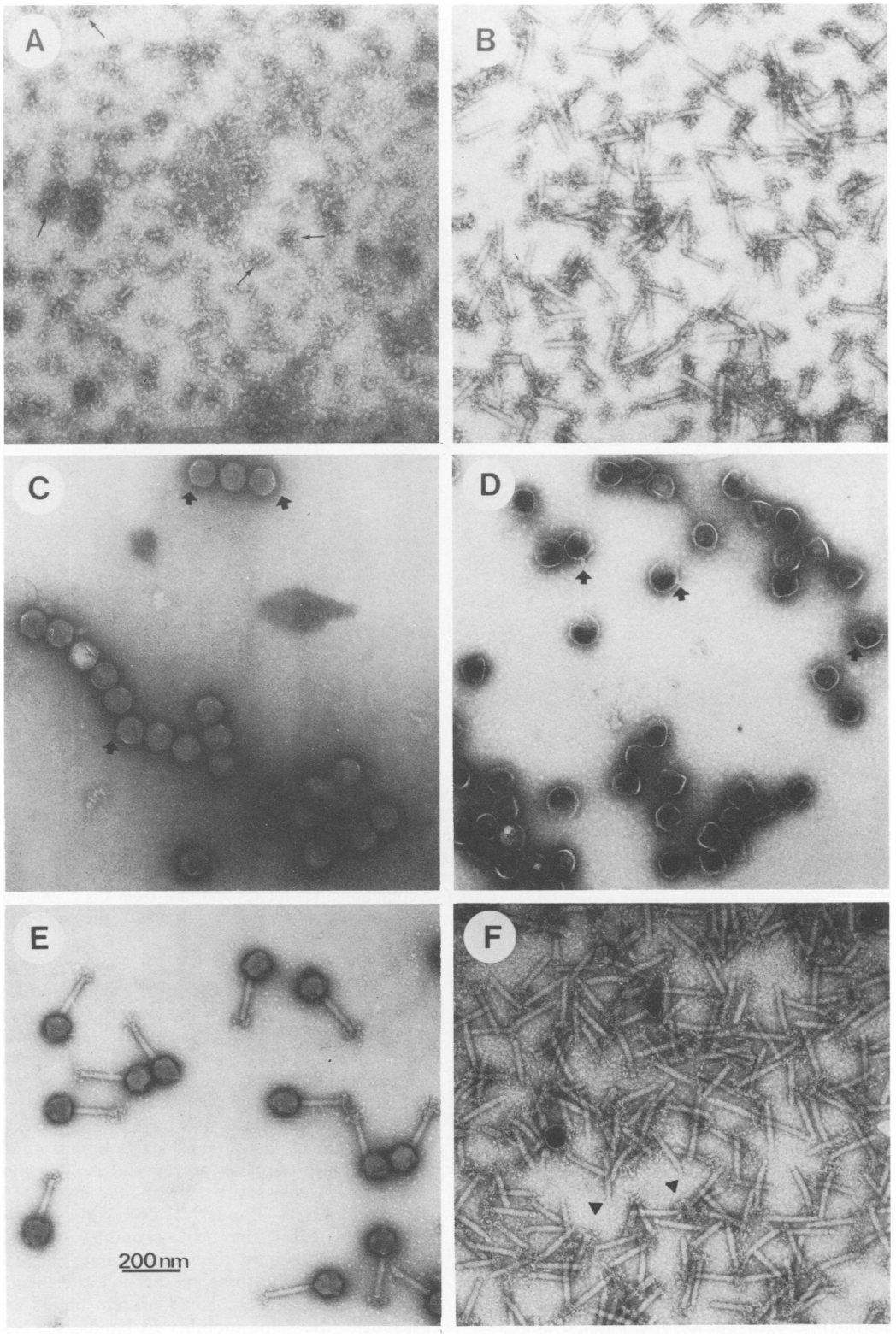


FIG. 1. Positions of phage components in gradients. A CsCl gradient is shown in A and a sucrose gradient in B. See reference 18 for details of preparation. Gradients are drawn to scale size in SW41 tubes. Abbreviations: F, flagella; BP, base plates, T, tails; C, capsids; G, ghosts; P, phage; H, heads; CT, contracted tails; GCT, ghost with contracted tails.

The wild-type phage profile in Fig. 3 has between 30 and 40 peptide bands. Baseplates isolated from *sus* N49 lysates reveal at least 28 bands, and 14 bands present in baseplate preparations are also easily seen in purified phage (Table 1). To test whether bacterial proteins adhered to the phage, we prepared a ^{14}C-labeled *B. subtilis* 168M lysate by adding lysozyme (100 μg/ml) and sodium azide (10 mM) to a suspension of 1.2×10^9 cells per ml previously labeled with ^{14}C-amino acids (3.1×10^6 cpm/ml, TCA precipitable). The cells were incubated at 37°C until lysis. Equal volumes of this lysate and unlabeled SPO1 wild-type phage (3.2×10^{12} PFU/ml) were mixed together and incubated at room temperature for 1 h. During this time period, none of the phage were biologically inactivated as determined by plaque assay. The mixture was subjected to CsCl equilibrium centrifugation, and the phage band was collected and dialyzed against Tris stabilization buffer. A small amount of radioactivity (undetectable by our autoradiographic conditions) was found associated with the phage (1.1×10^3 cpm/ml, about fourfold over background). Since most host protein synthesis is arrested late in infection (22), few labeled host proteins should be absorbed to phage structures.

FIG. 2. Micrographs of structures used for protein composition studies. Structures were isolated as detailed in the text. The *sus* N49 tails (B) and base plates (A) were isolated by the two-step centrifugation procedure. Wild-type phage (E) and *sus* 046-0 heads (C) were purified by CsCl equilibrium centrifugation. Wild-type necked tails (F) and *sus* 046-0 capsids (D) were prepared by osmotic shock and purified on a 5 to 45% sucrose gradient as described in reference 18. Note plug on *sus* 046-0 capsids and heads as indicated by large arrows. Small arrows (A) point to protein clusters which we interpret as baseplates.


A
B
C
D
E
200nm
F

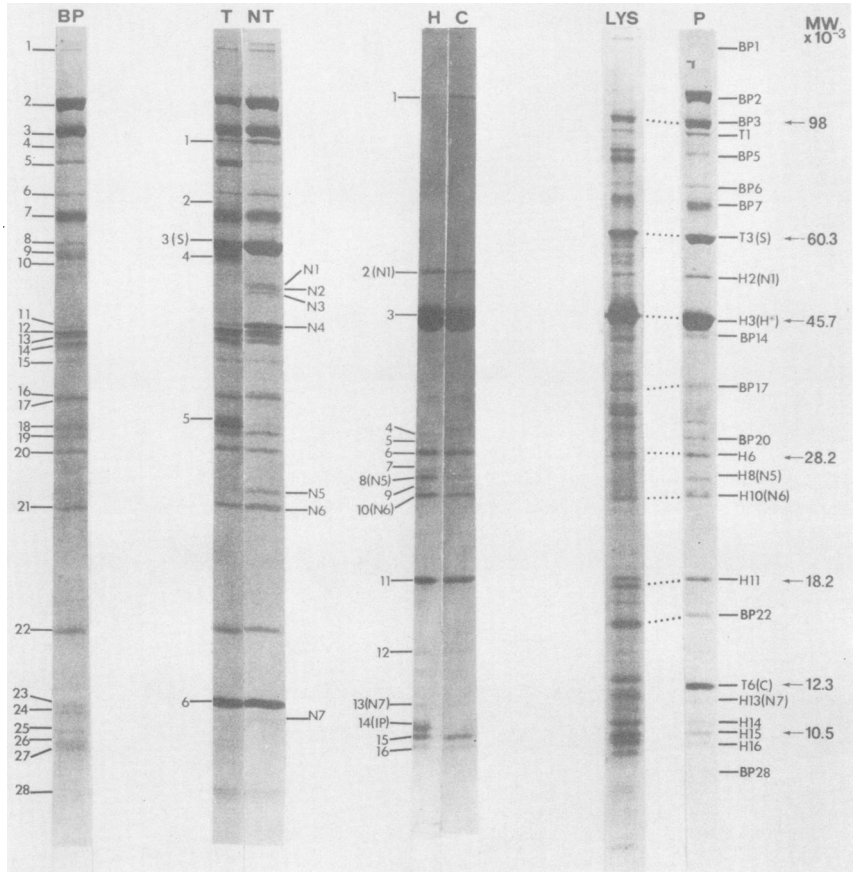


FIG. 3. Protein composition of SPO1 phage structures. ^{35}S-labeled structures were prepared as described in the text. Wild-type phage were separated by CsCl banding, and all other structures were separated by the two-step CsCl-sucrose procedure described in reference 18. Wells were loaded with 25,000 counts (TCA precipitable) in 8 to 50 μl. Autoradiograph exposure was 3 weeks. Bands are labeled sequentially from the top of the gel. In the text, bands are referred to by number, with the following prefix letter designations derived from the labels above the gel tracks: BP, baseplate; T, tail; H, head; S, tail sheath; C, tail core; and N, neck. Structures were isolated from the following mutant lysates grown under restrictive conditions: baseplates and tails from *sus* N49 (gene 2d); necked tails from osmotic shock of wild-type phage particles; heads and capsids from *sus* 046-0 (gene 8). Band T6 is also designated C for tail core.

Autoradiograms of tails isolated from a *sus* N49 lysate showed six bands not present in *sus* N49 baseplate (Fig. 3). Bands T3 and T6 are the sheath and core protein, respectively, since band T3 is the most abundant tail protein and is not present in isolated heads or baseplates, and band T6 is, by analogy with the tail tube of T-even phages, the most abundant small protein in tails and is also not found in head or baseplate preparations. Bands T1 and T5 were seen in wild-type phage, but bands T2 and T4 apparently were not. Also shown in Fig. 3 is an autoradiogram of necked tails produced by osmotic shock of wild-type phage particles (5). Comparisons between *sus* N49 tails and wild-type necked tails in Fig. 3 reveal an unexplained complication. Bands at the positions of BP5, BP18, and T5 are present in larger relative amounts in *sus* N49 tails than in either wild-type necked tails or whole phage. Whether these are proteins which are in fact modified or eliminated by using subsequent assembly of *sus* N49 tails was not investigated. Necked tails also differ from *sus* N49 tails in that they also have the connector region. The gel should show extra bands which correspond to the neck proteins. It is clear that there are at least seven extra bands in necked-tail preparations. Bands N1, N4, N5, N6, and N7 are probably the neck proteins, whereas bands N2 and N3 are difficult to explain because they do not appear to be present in either heads or phage (see Fig. 3). Head protein bands are present at the same mobility as bands N1, N5, N6, and N7. Their mobilities correspond to head

TABLE 1. Molecular weight of SPO1 structural proteins

Baseplate proteins				Tail proteins		Head proteins	
Protein	Mol wt ($\times 10^3$)	Protein	Mol wt ($\times 10^3$)	Protein	Mol wt ($\times 10^3$)	Protein	Mol wt ($\times 10^3$)
BP1	148.0	BP15	36.3	T1	95.5	H2 (N1)	53.7
BP2	112.0	BP16[a]	35.5	T2[b]	69.2	H3 (H*)	45.7
BP3	98.0	BP17	31.6	T3 (sheath)	60.3	H4[a]	30.2
BP4[a]	93.0	BP18[b]	30.2	T4[b]	58.9	H5[a]	29.5
BP5	74.0	BP19	29.5	T5	33.1	H6	28.2
BP6	67.5	BP20	24.0	T6 (core)	12.3	H7	26.9
BP7	60.2	BP21[a]	15.8			H8 (N5)	26.3
BP8[b]	58.8	BP22	15.5	Neck proteins		H9	25.1
BP9[a]	57.5	BP23[a]	12.3	Protein	Mol wt ($\times 10^3$)	H10 (N6)	24.5
BP10[a]	45.7	BP24[a]	11.7			H11	18.2
BP11[a]	44.6	BP25[b]	11.0	N1 (H2)	53.7	H12	14.1
BP12[a]	43.6	BP26[b]	10.6	N2[b]	53.0	H13 (N7)	11.7
BP13[a]	42.6	BP27[b]	10.2	N3[b]	53.5	H14	11.0
BP14	40.7	BP28	8.8	N4[a]	45.7	H15	10.5
				N5 (H8)	26.3	H16	10.2
				N6 (H10)	25.1	H1[a]	112.0
				N7 (H13)	12.0		

[a] Masked by other bands on some gels of intact phage.
[b] Not detected in intact phage.

protein bands H2, H8, H10, and H13, respectively. Band N4, if present in heads, would be obscured by the major head protein band.

Autoradiograms of CsCl-purified free heads from mutant *sus* 046-0 display at least 16 separate bands (see Fig. 3). If *sus* 046-0 heads are subjected to osmotic shock (5), the heads break open and release DNA and presumably any other material not firmly bound to the head membrane. The T4 internal proteins (26) and the *alt* gene product (12) are examples of such material. The only detectable difference between *sus* 046-0 heads and capsids is the absence of H14 in the capsid preparation, which is thus designated as an internal protein (Fig. 3). Band H14 was also missing from ghost particles (whole phage with empty heads).

Table 1 summarizes the composition of SPO1. The baseplate contains 28 proteins, the tail contains 6 more proteins, the head contains 16 proteins, and the neck contains 3 proteins not found in the head. The phage, therefore, contains at least 31 bands (Fig. 3) and possibly as many as 53 proteins (see below).

Proteolytic processing of phage proteins. To detect proteolytic processing of viral peptides, concentrated cells were infected; at various times after infection, samples were exposed to ^{14}C-amino acids for 2 min (pulse) and then were treated with a 350-fold excess of nonradioactive amino acids (chase). In the same experiment, an identical set of samples was treated with a mixture of the protease inhibitors PMSF, TPCK, and TLCK to inhibit cleavage. Figure 4 shows the results of such an experiment. At least two viral peptides decreased in intensity on gel autoradiographs after the chase (bands H and C3). Band H has a molecular weight of about 47,700 and is located just above the major head protein band (H*) found in mature phage or in lysates labeled late in infection. As this band decreases in intensity with time, the major head protein band concomitantly increases. This is presumptive evidence for a precursor-product relationship between bands H and H*. Further supportive evidence is shown in Fig. 5. This shows gel profiles of wild-type phage proteins and polyhead proteins derived from partially purified polyheads made by mutant *sus* 044 in gene 5 (see reference 34 for polyhead purification). The same molecular weights were observed for the uncleaved phage head protein and the major protein of gene 5 polyheads. Also shown in Fig. 5 are gel profiles of SPO1 wild-type and *sus* 044 lysates. There is no processing of the major head protein in the mutant lysate. We therefore conclude that the major head protein of SPO1 is cleaved from a peptide of molecular weight 47,700 to a final molecular weight of 45,700 in the finished head.

The other peptide, C3, with a molecular weight of about 28,000, is also processed. No smaller-molecular-weight products can be detected. This band comigrates with a band which appears to be a late protein (M. L. Parker, Ph.D. thesis, University of California, Los Angeles, 1979).

Addition of protease inhibitors before addition of label prevents the cleavage reaction of band C3 and blocks the conversion of H to H*.

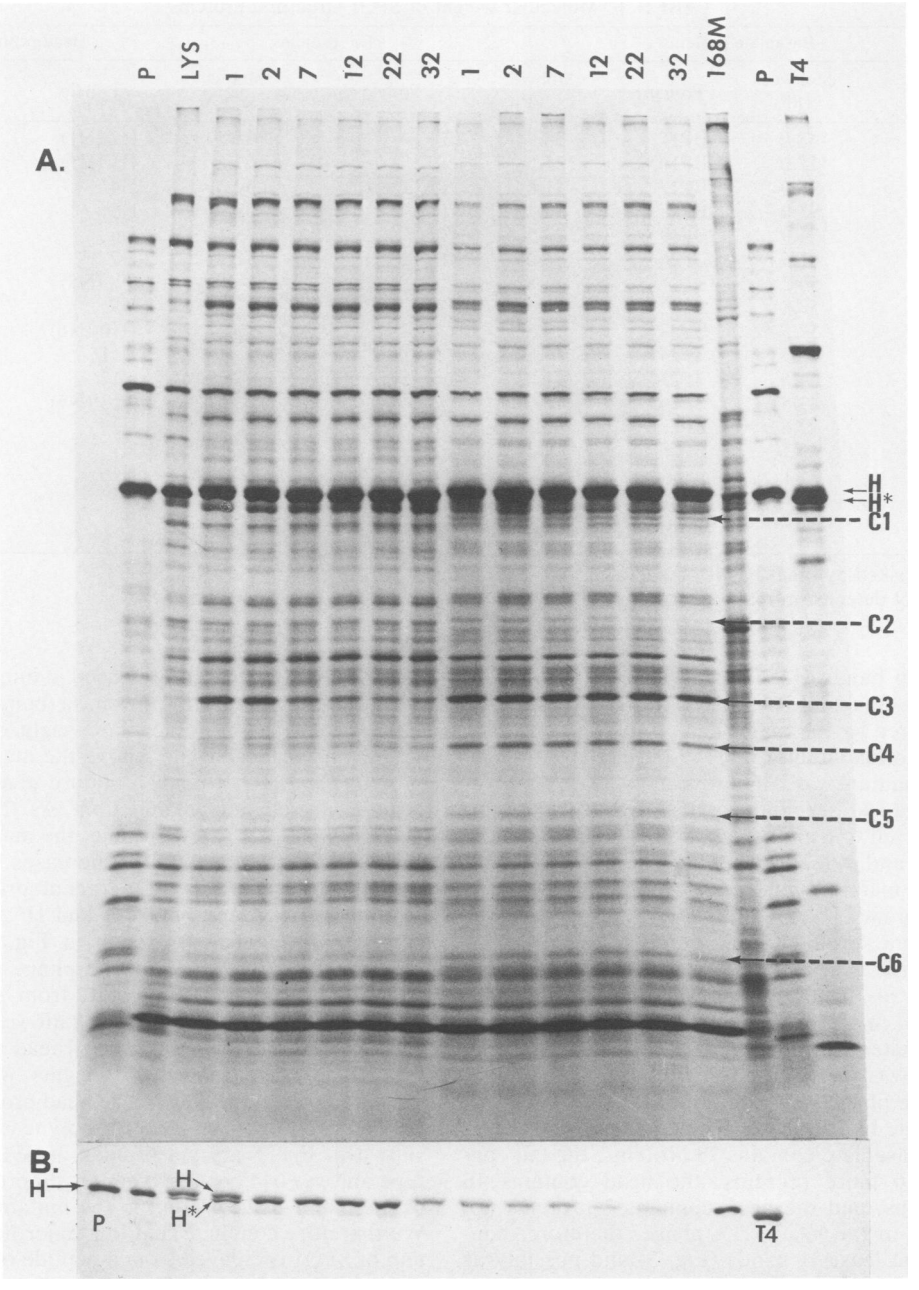


FIG. 4. (A) Autoradiograph of ^{14}C pulse-chase experiment with and without protease inhibitors. Details of sample preparations are in the text. The pulse was for 2 min at 62 min after infection, using concentrated cells. Each well on the polyacrylamide gel was loaded with 50,000 TCA-precipitable counts in 5 to 24 μl. Wells from left to right are: P (purified SPO1 wild-type phage); LYS (continuously labeled wild-type lysate [see the text for preparation]); 1, 2, 7, 12, 22, 32 (times after chase, no inhibitor); 1, 2, 7, 12, 22, 32 (times after chase, treated with mixture of protease inhibitors); 168M (labeled host cells); T4 (labeled T4 phage). H and H* are the unprocessed and processed major head proteins, respectively, and bands C1 through C6 identify differences in band intensities for several other proteins after protease inhibitor treatment. (B) Central region of the gel at shorter photographic exposure; the head protein cleavage step is more clearly visible.

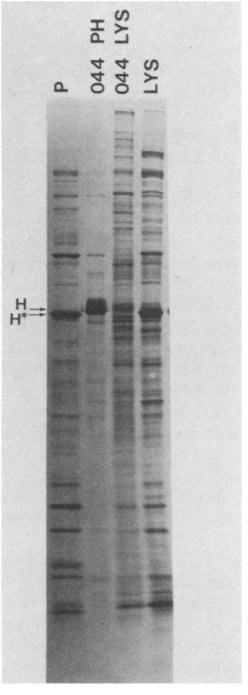


FIG. 5. Evidence for proteolytic cleavage of SPO1 head protein. Autoradiograph of ^{14}C-labeled proteins from purified SPO1 phage (P), partially purified polyheads from a *sus* 044 (gene 5) lysate (044 PH), a *sus* 044 lysate (044 LYS), and a wild-type lysate (LYS). H indicates unprocessed major head protein (M_r = 47,700), and H* is processed major head protein (M_r = 45,700). The polyheads were isolated from a ^{14}C-labeled *sus* 044 lysate by the method of R. van Driel (34), except that no $CHCl_3$ was used in the lysate.

Inhibition of processing was detected as a band which decreased with time without protease inhibitor treatment and which did not decrease at all or as rapidly after inhibitor treatment. This designation is necessary because there are bands with greater density present in samples from the inhibitor-treated culture (bands C1, C4, C5, and C6; Fig. 4). These bands may represent peptides which are cleaved but not detected as such in the untreated culture because of the long labeling interval. Band C2 is unique in that it does not appear in the untreated sample (see below).

DISCUSSION

Protein composition of SPO1 phage. Wild-type SPO1 phage appears to have at least 31 electrophoretically separable protein species which are visible in Fig. 3. There are likely to be other, undetected protein species since ^{35}S does not label all structural proteins efficiently (at least 35 are visible from purified phage in Fig. 5). There are five baseplate proteins, two tail proteins, and two neck proteins which have not been identified in electropherograms of purified whole phage. Eight more baseplate proteins are of uncertain assignment because they overlap bands of the head proteins.

Summation of the molecular weights of the maximum of 53 proteins (Table 1) which we find on gels indicates that 48% of the linear coding capacity of the SPO1 genome is required to code for these proteins, assuming one gene codes for one protein. Pero et al. (19) have mapped the regions of the SPO1 genome which hybridize to early, middle, and late transcripts. Approximately 80% of the SPO1 genome hybridizes to some late mRNA, with about 30% of the genome giving maximal amounts of hybridization. These data, however, are uncorrected for the different lengths of the restriction fragments used for hybridization. Another factor affecting the estimate is the low-level hybridization of some late transcripts (19), for example, the fragments including genes 16, 13b, 17, 6, and possibly 1, which we believe contain late genes based on structures we see in mutant lysates by microscopy (7; D. J. Fujita, Ph.D. thesis, University of Chicago, 1971; C. Beard and M. L. Parker, unpublished data) and on our identification of proteins in mutant lysates by gel electrophoresis. It therefore seems quite possible that nearly half the coding capacity of SPO1 is devoted to genes which code for structural proteins.

Synthesis of SPO1 proteins during development. Analyses of proteins synthesized in *B. subtilis* after infection by SPO1 have been previously carried out by Levinthal et al. (16), by Lawrie et al. (14), and by Reeve et al. (24). The gel systems used vary, and detailed band-by-band comparisons are difficult, if not impossible, to do. There are global similarities in that protein classes can be separated into early, middle, and late times of synthesis. One also finds that the synthesis of some proteins ceases later in infection, which is predicted by earlier DNA/RNA hybridization studies (8).

In general, the sequence of protein synthesis in SPO1-infected cells reflects the temporal sequence of the various classes of mRNA with a fair degree of fidelity based on pulse-chase labeling experiments (Parker, Ph.D. thesis). Patterns of peptide synthesis of SPO1-infected cells are known to be multiplicity dependent (J. D. Grau, Ph.D. thesis, University of Chicago, 1972) and mutant dependent (22), and they vary if UV irradiation is used before infection to decrease residual host protein synthesis (21). Thus, a detailed comparison of the timing of protein synthesis, using results from different laboratories, is of relatively little value.

Proteolytic processing of SPO1 proteins. Limited proteolysis occurs in the life cycle of many different animal and bacterial viruses (see refer-

ences 3, 11, 25, and 28 for reviews). In animal viruses, cleavage allows subdivision of genetic information into functional units after translation as well as stabilization of the virus particle. In bacteriophages, cleavage is part of the assembly pathway required for virus maturation. An exception is the *B. subtilis* phage ϕ29, in which the (nonessential) collar appendage may be cleaved before it is attached to the phage (31). Cleavages which lead to the maturation of large structural complexes occur during the assembly of coliphages lambda (17), P2 (15), and T4 (13, 29), and in tail assembly in lambda (10) and T5 (38). Host functions appear to be required for some cleavage-maturation steps (see reference 11 for a review).

We have identified at least two SPO1 proteins which are processed during infection. The major head protein is reduced in molecular mass by about 2,000 daltons. Band C3, another late protein, appears to be degraded into small fragments. Both of these cleavages are affected by specific serine protease inhibitors. This is in contrast to T4, in which the cleavage reactions are not sensitive to serine protease inhibitors or to inhibitors of sulfhydryl-group-dependent proteases (27). Summers et al. (30) were able to show that proteolysis of poliovirus proteins can be inhibited by 10^{-4} to 10^{-5} M serine protease inhibitor specific for chymotrypsin-like enzymes (TPCK), whereas trypsin-like enzyme inhibitor (TLCK) did not stop cleavage. This level of inhibitors also caused about a fourfold decrease in total protein synthesis in the HeLa cells. Chloromethyl ketones such as TPCK and TLCK are also potential nonspecific alkylating agents, particularly for disulfide bonds (30). These may be relevant points because of the following observations: (i) there is an overall decrease in incorporation of radioactive amino acids into protein at the concentrations of inhibitors used in our experiments (approximately 2×10^{-4} to 3×10^{-4} M); and (ii) changes in band patterns other than cleavage reactions may be a result of reactions which are ultimately a result of nonspecific protein modifications.

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