S Gene Product: Identification and Membrane Localization of a Lysis Control Protein

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The product of the bacteriophage *S* gene has been previously shown to be required for an essential step in triggering host cell lysis. By using two different protein labeling systems, maxicells and UV-irradiated infected cells, we identified the *S* gene product as an 8,500-molecular-weight polypeptide associated with the cell envelope. The apparent molecular weight is significantly less than the 11,500 predicted from the *S* gene sequence. We were unable to confirm two previous identifications of *S* gene products, an acidic 15,000-molecular-weight polypeptide found by two-dimensional gel electrophoresis of infected cells and a 5,500-molecular-weight polypeptide in purified phage particles.

Three bacteriophage genes are directly involved in the precisely scheduled cellular lysis that terminates the lambda infective cycle: *S*, *R*, and *Rz* (2, 6, 17, 25). These genes are the first three genes in the lambda late transcriptional unit (see Fig. 1). The cytoplasmic expression of these genes, whether from the solitary late gene promoter or from a heterologous promoter on a cloning plasmid, is both necessary and sufficient for the entire lysis phenomenon, including its correct scheduling (7). The *R* gene product is a transglycosylase, and *Rz* may encode the endopeptidase activity which was originally assigned to the *R* gene (2, 21); the natural substrate of these activities is the peptidoglycan, which requires both glycosidic and peptide bonds for its mechanical integrity. Since the peptidoglycan is located in the periplasm, *pR* and *pRz* are formally required to be secretory proteins. The activity of *pS* is unknown; however, *pS* is absolutely required for *pR* to attack the peptidoglycan (8, 17). In the absence of normal *pS* function, the bacteriolytic activity accumulates intracellularly, reaching very high levels without affecting cell growth or viability (7). Thus, *pS* appears to mediate transport of bacteriolytic activity to the periplasm. This is a distinctly different mode of membrane penetration from the mode described by Blobel and Dobberstein (4) and found applicable to many secretory systems, in which the secretory polypeptide has a "signal sequence" at its NH₂-terminal end. Furthermore, the signal sequence model of protein

secretion implies penetration of the membrane during polypeptide elongation. Not only is *pS* required for the bacteriolytic activities to penetrate the membrane, but also it performs this undefined action long after the synthesis of levels of the bacteriolytic activities adequate to achieve total lysis (5). The delayed-action timing of *pS* action can be subverted by the addition of a respiratory poison (e.g., cyanide or dinitrophenol) which causes premature lysis to occur under *S*⁺ *R*⁺ conditions; in *S*⁻ conditions, respiratory poisons have no such lysis-triggering effect (5, 7).

Despite its dramatic and unique action on the bacterial cell, the *S* gene product escaped identification for a long time. The *S* gene has been sequenced and shown to encode a polypeptide of 108 residues, with a predicted molecular weight of 11,600 (11.6K) and a net +3 charge (20). Recently, the identification of two *S* gene products with different molecular weights and different subcellular localizations has been reported (22, 23). One polypeptide, with an acidic isoelectric point and an apparent molecular weight of 15K, was identified in inner membrane fractions of λ -infected cells by two-dimensional gel electrophoresis (22). The other polypeptide, with an apparent molecular weight of 5.5K, was found in the purified virions prepared from λ^+ and λ Sam7 lysates but not in virions of two other λ *S* mutants (23). The surprisingly different molecular weights and apparent diverse functions of these alleged *S* gene products implied a complex and unprecedented maturation pathway for *pS* (22, 23).

We reported here a completely different identification of the *S* gene product. Our results were

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TABLE 1. Bacteria and bacteriophage

Strain	Genotype	Reference or source	Description
<i>Escherichia coli</i> K-12 CSH57	<i>ara leu lacY purE gal trp his argG malA rpsL xyl mtl ilv metA thi</i>	Cold Spring Harbor strain kit	
CSH57B	CSH57 <i>mal</i> ⁺	Cold Spring Harbor strain kit	
W3101	F ⁻ <i>pro phe</i>	(7)	
WFK	W3101 F' <i>lacI</i> ^Q <i>lacZ</i> ::Tn5	(7)	Carries <i>lacI</i> ^Q overproduc- tion allele
CFK	CSH57B F' <i>lacI</i> ^Q <i>lacZ</i> ::Tn5	Conjugation of CSH57B and WFK	Host <i>lacI</i> ^Q strain for lysis plasmids
S159	<i>uvr gal str ilv pro</i>	Laboratory strain	
MS3	W3101 <i>recA2463</i>	M. Syvanen	
MS3FK	W3101 <i>recA2463</i> F' <i>lacI</i> ^Q <i>lacZ</i> ::Tn5	Conjugation of MS3 with CFK	Maxicell <i>lacI</i> ^Q strain
W3101	W3101 <i>cI857</i>	Laboratory strain	
W3101 <i>Sam7</i>	W3101 <i>cI857 Sam7</i>	Laboratory strain	
Bacteriophage			
λ <i>bbn</i>	λ <i>b515 b519 cI857 nin5</i>	(7)	
λ363 <i>S</i> ⁺ <i>R</i> ⁺ <i>Rz</i> ⁺	λ <i>bbn</i> (Tn903) ₉₈	(7)	Tn903 inserted in extreme right end of λ genome at 0.98 on λ map
λ363 <i>S</i> ⁺ <i>R</i> ⁺ <i>Rz</i> ⁺	λ363 <i>Ram54 Ram60</i>	(7)	
λ363 <i>S</i> ⁻ <i>R</i> ⁺ <i>Rz</i> ⁺	λ363 <i>Sam7</i>	(7)	Constructed by recombina- tion with heteroimmune λ phages carrying <i>S</i> ⁺ <i>Ram54 Ram60</i> , <i>Sam7 R</i> ⁺ , and <i>Sam7 Ram54 Ram60</i> , respectively
λ363 <i>S</i> ⁻ <i>R</i> ⁻ <i>Rz</i> ⁺	λ363 <i>Sam7 Ram54 Ram60</i>	(7)	
λ <i>bbn S</i> ⁻ <i>R</i> ⁺ <i>Rz</i> ::Tn903	λ <i>b515 b519 cI857 nin5 Sam7</i> <i>Rz</i> ::Tn903	(25)	Tn903 inserted in the <i>Rz</i> gene
λ <i>S</i> ⁺	λ <i>cI857</i>	Laboratory stock	
λ <i>S1</i>	λ <i>cI857 S1</i>	M. Howe (9)	
λ <i>S2</i>	λ <i>cI857 S2</i>	M. Howe (9)	
λ <i>S3</i>	λ <i>cI857 S3</i>	M. Howe (9)	
λ <i>S4</i>	λ <i>cI857 S4</i>	M. Howe (9)	
λ <i>S5</i>	λ <i>cI857 Sam5</i>	M. Howe (9)	
λ <i>S6</i>	λ <i>cI857 S6</i>	M. Howe (9)	
λ <i>S7</i>	λ <i>cI857 Sam7</i>	M. Howe (9)	Original <i>S</i> mutants isolated by Goldberg and Howe (9)

obtained from λ-infected cells and also from induced plasmid-borne *S* gene clones. These data are incompatible with the previous identifications and suggest that *pS* function, and possibly maturation, may be much more orthodox.

MATERIALS AND METHODS

Materials. Acrylamide, *N,N'*-methylenebisacrylamide, and Nonidet P-40 were obtained from Bethesda Research Laboratories. Sodium dodecyl sulfate (SDS) was obtained from Gallard Schlesinger and recrystallized once. Ultrapure urea was obtained from Schwarz/Mann, ampholines were obtained from LKB Instruments, Inc., and electrophoresis-grade ammonium persulfate was obtained from Bio-Rad Laboratories. Silver nitrate was obtained from Apache Chemicals. All other chemicals were obtained from Sigma

Chemical Co. [³⁵S]methionine (1,057 Ci/mmol) was obtained from New England Nuclear Corp.

Strains and plasmids. Bacterial and bacteriophage strains used are listed in Table 1. The plasmids pRF26 (*S*⁺ *R*⁺), pJH2 (*Sam7 R*⁺), pJG1 (*S*⁺ *Ram54 Ram60*), and pJG3 (*Sam7 Ram54 Ram60*) carry the lysis gene region (see Fig. 1) inserted into the plasmid pBH20 so that the lysis genes are expressed under the control of the *lac* operator-promoter sequence. The plasmid pBH20 is identical to pBR322 except for the 208-base pair *lac PO* abutting the unique *EcoRI* site (11). All of the lysis gene plasmids carry the *EcoRI-HindIII* fragment of λ363, as shown in Fig. 1; thus, all contain the complete *S R Rz* lysis gene region. For a detailed description of the construction of these lysis gene clones, see references 7 and 8.

Media. Cultures were grown in M9 minimal salts medium (14) supplemented with 10 μg of thiamine per

FIG. 1. Lysis gene region of the lambda genome. The extreme right end (43 to 48.5 kilobase pairs from the left end) of the bacteriophage lambda genome is depicted. The approximate extents of the *Q*, *S*, *R*, and *Rz* reading frames are indicated by the boxes. The filled circle indicates the major late promoter p_L ; the open circle indicates the position of the terminator t_R . The derivative λ 363 carries a Tn903 insertion into the silent lambda DNA at 47.5 kilobase pairs; the insertion is drawn approximately to scale. e, b, and h, Cleavage sites for the restriction enzymes *Eco*RI, *Bcl*I, and *Hind*III, respectively.

ml, all amino acids except cysteine and methionine, and the appropriate antibiotics (ampicillin at 50 μ g/ml and kanamycin at 30 μ g/ml). Maxicell cultures were grown with 0.2% glycerol as a carbon source. Cells to be infected with λ were grown with 0.2% maltose as a carbon source.

SDS-polyacrylamide gradient gel electrophoresis. We have optimized the SDS-polyacrylamide gel technique of O'Farrell (16) for high resolution of low-molecular-weight polypeptides. Separating gels were poured in a Shadel model 2D slab gel apparatus (0.75-mm thickness) as an exponential gradient (total volume, 22.5 ml; mixing volume, 7.5 ml) of 10 to 20% acrylamide, with a ratio of 1:37.5 of *N,N'*-methylenebisacrylamide to acrylamide throughout. All samples were dissolved in sample buffer (5% [wt/vol] SDS, 2.5% [vol/vol] β -mercaptoethanol, 10% [vol/vol] glycerol, 0.0025% [wt/vol] bromophenol blue, 0.125 M Tris-chloride [pH 6.8]) and heated in a boiling water bath for 5 min before loading. Electrophoresis was at a constant 200 V and was terminated 5 min after the dye in a molecular-weight standard containing 0.0025% (wt/vol) Coomassie brilliant blue R-250 migrated from the end of the gel; the increased running time is essential for low-molecular-weight-range resolution.

Silver staining was done as described by Wray et al. (24), except that the ammoniacal silver solution was titrated during its preparation to ensure reproducibility. In all cases the gels were dried onto filter paper, and, where applicable, autoradiography was performed by exposure to Kodak XAR-5 X-ray film. Figure 2 shows the low-molecular-weight resolving power of this gel protocol.

Two-dimensional gel electrophoresis. The two-dimensional gel system of O'Farrell (16) and the sample preparation protocol of Ames and Nikaïdo (1) were employed.

Maxicells and UV-irradiated cell infection. We used a modified version of the technique of Sancar et al. (19). Each plasmid was transformed into MS3FK. Ten

milliliters of a culture at an absorbance at 550 nm of 0.4 were irradiated at 90 J/m² with a germicidal lamp and then transferred to red flasks. Cycloserine was added to a final concentration of 100 μ g/ml 30 min after irradiation, and aeration was continued overnight at 37°C (18). The cells were centrifuged, washed, and suspended in 1 ml of fresh medium lacking kanamycin. When indicated, the *lac* inducer isopropyl- β -D-thiogalactopyranoside (IPTG) was added to 1 mM, and the cells were incubated at 37°C for 5 min. [³⁵S]methionine was then added to 50 μ Ci/ml, and the incubation was continued for 30 min. Cells were then centrifuged and suspended in SDS sample buffer, and the labeled proteins were analyzed by SDS gel electrophoresis and autoradiography.

The phage infection protocol with UV-sensitive strain S159 was used exactly as described by Moore et al. (15).

Cellular fractionation. We used modifications of the procedures of MacGregor et al. (13) and Lutkenhaus (12). The labeled cells were suspended in 50 mM sodium phosphate (pH 7.1). The cells were lysed by one pass through an Aminco French pressure cell at 16,000 lb/in² in the cold. After removal of large debris by low-speed centrifugation, the lysate was added to 3.0 ml of buffer A (10 mM Tris, 5 mM disodium EDTA, 1 mM dithiothreitol [pH 7.8]) and centrifuged for 12

FIG. 2. High-resolution SDS gel electrophoresis of low-molecular-weight polypeptides. The mobilities of a set of low-molecular-weight standard proteins using the SDS gradient gel system (10 to 20% SDS gel) described in the text are plotted as a function of molecular weight. The molecular weight standards are: lysozyme, 14.3K; lysozyme cyanogen bromide fragment A, 13K; lysozyme cyanogen bromide fragment B, 11.5K; myoglobin cyanogen bromide fragment B, 10.7K; lysozyme cyanogen bromide standards C, 10.2K; myoglobin cyanogen bromide fragment C, 8.2K; myoglobin cyanogen bromide fragment D, 6.3K; insulin B, 3.3K; myoglobin cyanogen bromide fragment E, 2.5K.

FIG. 3. Identification of pS and pR in maxicells. Maxicells were prepared from strain MS3FK carrying the following lysis gene plasmids: lanes 1 and 2, pRF26 ($S^+ R^+$); lanes 3 and 4, pJG1 (S^+ Ram54 Ram60); lanes 5 and 6, pJG3 (Sam7 Ram54am60); lanes 7 and 8, pJH2 (Sam7 R^+). The maxicells were prepared, labeled with [35 S]methionine, and analyzed by gradient SDS gel electrophoresis as described in the text. The even-numbered lanes in the autoradiogram are samples labeled in the presence of 10^{-3} M IPTG, and the odd-numbered lanes are uninduced samples. Molecular weight standard mobilities are indicated on the right side of the autoradiogram.

min at 50,000 rpm in a Beckman Ti 70.1 rotor; 4 ml of the supernatant was removed for later analysis as the soluble fraction. The pellet was resuspended in 5 ml of buffer A and recentrifuged. The supernatant was discarded, and the pellet was dissolved in SDS sample buffer. Two milliliters of soluble fraction was supplemented with 50 μ l of 10% SDS and then evaporated to dryness with a Savant Speedvac concentrator. The pellet was resuspended in SDS sample buffer. These samples, constituting the membrane and soluble fractions of the labeled cells, were analyzed on SDS gels.

RESULTS

Identification of lysis gene products in maxicells. Many attempts to identify the *S* gene product in our laboratory and elsewhere by standard one-dimensional and two-dimensional SDS-polyacrylamide gel electrophoresis have failed. The small size and probable hydrophobic nature of the *S* protein as deduced from the DNA sequence (20) makes detection by the two-dimensional gel technique very difficult. To employ one-dimensional gels, we used the maxicell technique (19) to label the *S* protein without labeling host proteins. Figure 3 shows the results of a maxicell-labeling experiment with strains harboring lysis gene plasmids. The lysis gene plasmids were constructed by inserting the lysis gene region of $\lambda S^+ R^+$ and several different λ lysis mutants into the plasmid pBH20, so that the lysis genes were expressed from the *lac* promoter (7, 8) upon IPTG induction. Two bands dependent on IPTG are discernible: an 8.5K band in the S^+ but not Sam lanes and a 17.5K band in the R^+ but not Ram lanes. Both bands are more intense in the samples with inducer than in the samples without inducer, which indicates that they are encoded by genes

under *lacP* control; we conclude that the 8.5K band is pS and the 17.5K band is pR. The pR assignment agrees with the molecular size observed for purified pR (3) and the molecular weight predicted for pR from the DNA sequence (20).

Identification of pS and pR in infected cells. The maxicell results required us to concentrate our efforts on maximizing gel electrophoretic resolution in the molecular weight range below 12K. By using 10 to 20% gradient gels, we analyzed the proteins labeled during infection of UV-irradiated cells with bacteriophage λ lysis gene mutants. Figure 4 shows that an 8.5K band is observed in S^+ but not Sam7 infections, and a 17.5K band is observed R^+ but not Ram54am60 infections. Thus, maxicells in which the cloned *S* and *R* genes are being expressed from the *lac* promoter show the same gene products as infected cells in which the *S* and *R* genes are expressed from the homologous λ promoter. Since the *R* product assignment in these systems agrees with the molecular size obtained for the purified protein, we conclude that these two different methods demonstrate unambiguously that pS has an apparent molecular weight of 8.5K. Furthermore, although the UV-irradiated cells are physiologically perturbed, they do undergo lysis in $\lambda S^+ R^+$ but not λ Sam or λ Ram infections, albeit much more slowly than in unirradiated cells (data not shown). Since no other bands can be assigned to pS, the 8.5K band is probably the active form of the *S* gene

FIG. 4. Identification of pS and pR in infected cells. The UV-sensitive strain S159 was irradiated to suppress host incorporation, infected with bacteriophage, labeled with [35 S]methionine, and analyzed by high-resolution SDS gradient gel electrophoresis and autoradiography as described in the text. Lane A, uninfected; lane B, $\lambda 363 S^+ R^+$; lane C, $\lambda 363 S^+ R^-$; lane D, $\lambda 363 S^- R^+$; lane E, $\lambda 363 S^- R^-$; lane F, $\lambda bbn S^- R^+ Rz::Tn903$. Molecular weight standard mobilities are indicated on the left.

FIG. 5. Envelope localization of pS. Irradiated cells were infected and labeled as described in the legend to Fig. 4, using λ 363 $S^+ R^-$ (lanes 1, 3, 5, and 7) or λ 363 $S^- R^+$ (lanes 2, 4, 6, and 8). One-third of each sample was directly resuspended in SDS sample buffer and electrophoresed (lanes 1 and 2) as whole cell samples. The balance of the labeled, infected cells was lysed in a French pressure cell and fractionated by centrifugation, as described in the text, into fractions designated as large debris (lanes 3 and 4), membranes (lanes 5 and 6), and soluble (lanes 7 and 8).

product. In addition, neither method gives any indication of either the 15K pS or the 5.5K pS species reported elsewhere (22, 23).

Subcellular localization of pS and pR. We fractionated the labeled, UV-irradiated, infected cells analyzed in Fig. 4 by cell disruption in a French pressure cell. Most, if not all, of the 8.5K band identified as pS is associated with the envelope (Fig. 5, lanes 3 and 5). Up to 30% of the 8.5K band could be driven into the soluble sample if cell disruption was accomplished by sonication (data not shown). A significant fraction of labeled pR is also associated with the envelope fractions (Fig. 5, lanes 4 and 6); we assume that this is due to binding of the transglycosylase to its natural substrate, the peptidoglycan. Exact quantitation of the amount of pR in the soluble fraction is not possible due to the presence of a residual host band. However, enzymatic assays of lysates with large amounts of pR, due either to induction of an R^+ plasmid clone or infection by λR^+ without preirradiation by UV, show that very little endolysin activity is envelope associated under these conditions (data not shown). By comparison, very little pR is produced in the UV-irradiated, infected cell system (not detectable by *in vitro* endolysin assays; data not shown). We assume that this small amount of pR is bound to the large excess of cell wall material associated with the membrane function.

The finding that the 8.5K band assigned as pS is an envelope protein strengthens the conclusion that this molecular species is the active form of the *S* gene product, since the transport and permeability properties of the inner membrane are severely altered by pS function (8, 22).

Absence of pS in bacteriophage particles. We attempted to confirm a previous report that the bacteriophage particle contains a 5.5K *S*-encoded polypeptide in very high molar ratios to

other particle components (23). We obtained a number of λS mutants from the original collection in which Sam7 was isolated (9) and compared the polypeptide pattern visualized by silver staining of gels on which SDS-treated, purified bacteriophage particles were electrophoresed. No difference in the particle protein content was observed between S^+ and any *S* mutant (Fig. 6). We obtained samples of S2 and S4 mutant bacteriophage found in another laboratory to lack a major 5.5K band present in S^+ and Sam7 phage particles, when analyzed by phosphate-urea SDS gels and silver staining (see Fig. 3 of reference 23). In our hands, the lowest distinct silver-staining band is at 5.8K and is present in all phage particles, regardless of the *S* allele (Fig. 6). The S2 and S4 samples we obtained from the other laboratory did lack a diffuse, light grey-staining smear migrating in the 2K to 4K range in all of our samples, including our S2 and S4 samples (Fig. 6, compare lanes 5 and 8 to all other lanes). We have observed that this material increases with the age of the SDS sample and assume that it reflects breakdown products of the particle polypeptides; in any case, it is unlike any polypeptide band and is not specific for an *S* allele. Thus, we find no evidence for an *S*-encoded polypeptide in the bacteriophage λ particle.

Two-dimensional electrophoresis of λ late pro-

FIG. 6. Phage particle proteins are identical in λS mutants. Bacteriophage particles purified by CsCl equilibrium gradient centrifugation (14) were boiled in SDS sample buffer and analyzed by high-resolution SDS gradient gel electrophoresis and silver staining, as described in the text. MW, Molecular weight standards; S^+ , wild type; S1 through S7, *S* mutants with the indicated *S* allele; S2A and S4A, samples prepared directly from stocks of S2 and S4 mutants kindly provided by D. B. Wilson.

FIG. 7. Analysis of λS^+ and λS^- membrane proteins by two-dimensional electrophoresis. The lysogens W3103 λ and W3101 λ Sam7 were grown exponentially at 30°C in glucose minimal medium and thermally induced (20 min at 42°C, followed by aeration at 37°C). Under these conditions, lysis occurs at approximately 60 min after induction in the S^+ culture. Two-milliliter samples from each culture were taken at 50 min and labeled by the addition of 25 μ Ci of [35 S]methionine. After 2 min, the samples were pelleted by centrifugation for 30 s in an Eppendorf microfuge, suspended in 50 mM sodium phosphate buffer (pH 7.5), lysed, and fractionated into membrane and soluble components. The pelleted membrane or dried soluble samples were resuspended in the sample buffer of Ames and Mikaido (1) and analyzed by two-dimensional electrophoresis and autoradiography. Shown are the membrane components of the λS^+ induction (A) and the λ Sam7 induction (B). Molecular weight standards, in kilodaltons, are indicated on the right.

teins. We also attempted to confirm the previous report that a 15K to 18K polypeptide could be identified as pS by two-dimensional gel electrophoresis of membranes of infected cells (22). By using the O'Farrell (16) two-dimensional gel technique, we analyzed the proteins labeled by [35 S]methionine late during the vegetative growth phase of induced λS^+ and λ Sam7 lysogens; no polypeptides could be resolved that were missing under Sam7 conditions in the

membrane proteins (Fig. 7) or in the soluble fraction (not shown), although many attempts were made and we were able to identify hundreds of major and minor spots in identical positions. It seems likely that the 8.5K polypeptide that we resolved in one-dimensional SDS gels has a basic isoelectric point outside the focusing range of the two-dimensional gel ($pI > 7.5$ to 8), since the amino acid constitution predicted from the DNA sequence has a predicted net charge of +3 at a neutral pH. However, we were also unable to resolve pS in non-equilibrium two-dimensional gels designed to resolve basic proteins (data not shown); we do not give much weight to this negative result because, in our hands, the non-equilibrium gel technique does not give readily reproducible results for polypeptides with an apparent molecular weight of less than 15K. It is also possible that pS is lost from isoelectric focusing cylindrical gels during equilibration with SDS buffer before electrophoresis in the second dimension, since, in our hands, 30% of all acid-precipitable radioactivity is lost from these gels into the equilibration buffer (not shown). Given the hydrophobic nature of many membrane proteins, gentle (nonboiling) equilibration in SDS or direct electrophoresis without SDS equilibration is unlikely to permit denaturation and SDS binding adequate for obtaining a reasonable apparent molecular weight (10).

DISCUSSION

We have demonstrated that the λ gene *S* lysis protein can be observed as an 8.5K polypeptide associated with the cell envelope fraction. This assignment can be made by comparing the proteins synthesized in λS^- and λ Sam7-infected cells and also by comparing protein labeling patterns from maxicells harboring plasmids carrying the lysis gene region cloned from λS^+ and λ Sam7. Under $S^+ R^+$ conditions in both systems, lysis occurs, which indicates that the 8.5K polypeptide may be the active form of pS.

These results are incompatible with two recent reports identifying two different polypeptide species as *S* gene products. In the first case, pS was identified in the cytoplasmic membrane as a 15K to 18K polypeptide with an acidic isoelectric point (22). We were unable to confirm this report by using two-dimensional gel electrophoresis to analyze λ late proteins. Since simple inspection of the gels presented in the earlier report shows many spots present under S^+ but not S^- conditions (see Fig. 4 of reference 22), it is not clear to us how the assignment was made in the first place. Furthermore, several unprecedented covalent modifications must occur to convert pS as encoded in the *S* gene, an 11.6K polypeptide with a +3 charge, to an acidic

molecule with a molecular weight of 15K to 18K. The discrepancy between the predicted 11.6K size for pS and our observed 8.5K may reflect anomalous electrophoretic mobility or proteolytic processing; the highly hydrophobic amino-terminal region of the predicted S gene product could account for either effect. In any case, one-dimensional SDS gel electrophoresis should be much more reliable for the resolution of small, hydrophobic, positively charged polypeptides than two-dimensional gels, since the samples can be boiled in SDS sample buffer and applied directly and quantitatively to the gel.

The second reported S gene product was a 5.5K polypeptide found in purified bacteriophage particles (23); data were presented indicating that certain λ S mutants, namely, λ S2 and λ S4, lacked the 5.5K polypeptide and also caused partially abortive infections due to abnormally slow resealing of the infected cell (23). The presumed role of the pS molecule associated with the virion particle was thus to facilitate closure of membrane lesions caused by injection of the infecting DNA. We were unable to confirm this result; we can see no significant difference between the proteins present in λ S⁺ and any λ S mutants, including the original λ S2 and λ S4 obtained from M. Howe (9). It is possible that differences in the growth conditions used to prepare the λ S2 and λ S4 mutant bacteriophage in our laboratory and in the laboratory which reported the particle-associated pS might explain the apparent contradiction. Since λ S2 and λ S4 appear to be missense mutants (D. B. Wilson, personal communication; unpublished data) rather than nonsense mutants as originally reported (9), subtle differences in the intracellular environment might affect the incorporation of the defective pS into the virion. However, λ S2 and λ S4 bacteriophage samples obtained directly from the authors of the original report also failed to show any protein differences from λ S⁺ samples (Fig. 6). Thus, we believe that the earlier identification of a 5.5K pS molecule was incorrect. The differences reported in the recovery of the host cells from infection with λ S⁺ and λ S mutants may be due to significantly different titers in the infecting phage stocks; we have observed at least an order of magnitude difference in the plaque-forming efficiency (PFU per absorbance unit at 260 nm) of λ S⁺ and λ S2 or λ S4 mutants (not shown). In this case, "resealing" differences would simply derive from large differences in the number of infecting phage particles.

The pS molecule has now been positively identified. This small membrane protein somehow potentiates a complex and temporally regulated secretion event which is in itself lethal (8), even in the absence of the bacteriolytic enzymes

which are secreted. The elucidation of the mechanism of action of this polypeptide should reveal significant new information about the structure and maintenance of the bacterial envelope.

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