Subcellular Localization of Lethal Lysis Proteins of Bacteriophages λ and ϕ X174

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The gene products of the lethal lysis genes *S* and *E* of the bacteriophages λ and ϕ X174, respectively, were shown to be associated primarily with inner membrane material by isopycnic sucrose gradient centrifugation of lysates of infected cells. A small amount of each polypeptide appeared to be in the outer membrane fraction.

The bacteriophages ϕ X174 and λ have strikingly different lysis systems. ϕ X174 has only a single lysis gene, the *E* cistron (13); the expression of this gene is both necessary and sufficient for cellular death and lysis even in the absence of other ϕ X174 genes (12, 23). There are three lysis genes in λ : *S*, *R*, and *R₂* (6, 7, 18, 24). Expression of the *S* gene results in cell death and permeabilization of the inner membrane, regardless of the state of the other two lysis genes (9, 10). However, actual lysis of the cell requires, in addition, the expression of the *R* gene, which encodes a transglycosylase (4, 5). Moreover, the *R₂* gene product apparently facilitates lysis by acting on the outer membrane (J. Garrett, unpublished data); however, *R₂* function is strictly required only in medium containing elevated Mg^{2+} (24). The *S* and *E* genes are similar in size, and both genes encode an unusual amino terminus which more closely resembles a membrane-spanning sequence than a typical signal sequence (20, 21, 23). Moreover, bacterial mutants resistant to *S*-mediated killing are also resistant to *E*-induced lethality (D. Maratea, K. Young, J. Garrett, and R. Young, manuscript in preparation). We have shown previously that the *S* gene product is a polypeptide with an apparent molecular weight of 8,500 associated with the envelope fraction of λ -infected cells (2). Although it has been shown that expression of the *S* and *E* gene products results in lethal damage to the inner membrane (10, 14, 18, 23), there has been no direct localization of the proteins within the envelope.

We modified a membrane fractionation technique used by MacGregor et al. (15) in which cells are lysed in a French pressure cell without enzymatic pretreatment. This was necessary because the lysis proteins destroy the spheroplasting potential of cells (10, 18), thereby rendering impractical the classical inner-outer membrane separation method of Osborn et al. (16). Figure 1 shows a typical envelope fractionation. Displaying the entire gradient amounts to a two-dimensional analysis of the envelope; the membrane-bound polypeptides are separated in the first dimension by the density of the associated membrane material and then in the second dimension by the molecular weight of the polypeptide. Note that the distribution of different polypeptides peak in several different fractions, instead of forming two peaks,

an inner membrane and an outer membrane peak; this suggests a microheterogeneity in the structure of the inner and outer membranes. This microheterogeneity is lost if the membrane material is first pelleted and then resuspended before isopycnic centrifugation (data not shown). The molecular basis of this microheterogeneity is currently under investigation in our laboratory; in any case, the method gives a reproducible separation of inner and outer membrane markers (Fig. 1) and involves an absolute minimum of manipulation of the envelope material.

To determine the distribution of pS molecules in the envelope, cells were preirradiated with UV light, infected with λS^+ or λ Sam7, and labeled with [35 S]methionine after 40 min at 37°C; the labeled proteins were then analyzed by the method illustrated by Fig. 1. Figure 2A shows that the bulk of pS is found as an 8,500-molecular-weight species peaking in the fractions shown to have the maximum NADH oxidase activity (Fig. 1); this band is clearly missing in the Sam7 pattern (Fig. 2B).

This is the first direct demonstration for the inner membrane localization of pS, although indirect evidence has been provided by us and others (10, 22); its elusiveness can be attributed to its small size and low molar amounts. The ϕ X174 *E* gene product has been even more difficult to localize. Figure 3 shows that pE has an apparent molecular weight of 10,500 and is synthesized in very small quantities in infected cells, which confirms a previous report (17) and is consistent with the molecular weight predicted from the DNA sequence (3). Like pS (2), pE is associated with the membrane (data not shown) and has the same envelope distribution as pS (Fig. 4); this result extends the functional and structural similarities of pS and pE noted before (23).

In previous work, we have shown that the induction of cloned λ and ϕ X174 lysis genes leads to bacteriolytic phenomena identical to the lysis of phage-infected cells (9, 10, 23). The finding that the lethal lysis proteins of λ and ϕ X174 are associated with the inner membrane is consistent with the observed effects of *S* and *E* gene action on respiration, permeability, and viability (1, 10, 23). We have found that pS can be driven into the soluble fraction by sonication (E. Altman, unpublished data), which suggests that the entire molecule is not completely imbedded in the membrane. Both *S* and *E* genes encode 20- to 24-residue amino-terminal regions which are largely hydrophobic and end at a pair of positively charged residues (10); these amino-terminal primary structures resemble the canonical membrane-spanning sequences defined by von Heijne (19). However, most genes

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FIG. 1. Isopycnic fractionation of envelope material. A 1-ml sample of S159 (*Escherichia coli* K-12 *uvr gal str ilv pro*) cells at an A_{550} of 0.1 in M9 glucose medium supplemented with all amino acids except methionine and cysteine was labeled with 5 μCi of [^{35}S]methionine (1 $\mu\text{Ci}/\mu\text{l}$; New England Nuclear Corp., Boston, Mass.) for 5 min (2). The cells were pelleted, suspended in 50 mM sodium phosphate (pH 7.0), and ruptured by a single pass through a French pressure cell at 16,000 lb/in². After centrifugation at $2,000 \times g$ to remove unlysed cells, the lysate (total volume, 1 ml) was applied to a preformed sucrose step gradient consisting of 2.0-ml steps of 47, 44.5, and 40% sucrose, 1.5-ml steps of 35.6, 31, and 26.7% sucrose, and 0.75 ml of 22% sucrose (wt/vol). After centrifugation in a SW41 rotor for 16 h at 37,000 rpm and 5°C, nine aliquots were collected from the bottom of the tube. Half of each fraction was analyzed for NADH oxidase activity (11). The remainder of each fraction was diluted to 4 ml with 10 mM Tris (pH 8.0) containing 1 mM EDTA and 1 mM β -mercaptoethanol, and membrane material was collected by centrifugation in a 70.1Ti rotor at 50,000 rpm for 30 min at 5°C. Each membrane pellet was then suspended in sodium dodecyl sulfate-polyacrylamide gel electrophoresis sample buffer, heated at 100°C for 5 min, and analyzed by electrophoresis on 12 to 20% sodium dodecyl sulfate-acrylamide gradient gels and autoradiography as described previously (2). The quantitation of *omp* bands was done with a Joyce-Loebl densitometer. The refractive index was measured on an Abbe refractometer. The mass of standard polypeptides is indicated in kilodaltons.

encoding a membrane-spanning region also encode a signal sequence, presumably to direct the gene products to the membrane (19). It is not clear whether the amino-terminal structure of the *S* and *E* proteins also constitutes a signal sequence; no evidence for processing of *pS* or *pE* is available, except the rather low apparent molecular weight of *pS* (8,500), compared with the 11,500 molecular weight predicted from the gene sequence (7).

Interestingly, a small fraction of both *pS* and *pE* is found in the outer membrane fractions (Fig. 2A, lane 1, and Fig.

4B, lane 1). The molecular significance of this unexpected association is not understood; however, it may result from interaction of the lysis proteins with the cellular protein secretion apparatus. Preliminary results in this laboratory indicate that *prlA* mutations, which were selected for abnormal recognition of the *lamB* signal sequence (8), are also defective in *S*- and *E*-mediated cell lysis (K. Young, unpublished data). Experiments with *S*::*lacZ* and *E*::*lacZ* fusion genes, now in progress in this laboratory, should clarify the nature of the lysis protein signals.

FIG. 2. Distribution of pS in envelope. XK1890 cells were irradiated, infected, and labeled as described previously (2) and analyzed by the method described in the legend to Fig. 1. Panel A, Infection with λS^+ ; panel B, infection with $\lambda Sam7$. The first two lanes are whole-cell samples from *S*- and *Sam*-infected cells. The position of the bands corresponding to the *S* and *R* gene products as determined previously (2) are indicated (pR and pS). Fraction numbers are as indicated in Fig. 1, with the densest material in fraction 1. The mass of standard polypeptides is indicated in kilodaltons.

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FIG. 3. Identification of pE. Preirradiated cells were infected with $\phi X174$ (lane 3) or $\phi X174Eam3$ (lane 2), labeled, and analyzed by sodium dodecyl sulfate gel electrophoresis as described in the legend to Fig. 1, except that *E. coli C uvr* was used as the host. The positions of the $\phi X174$ *E* gene product is indicated (pE). The mass of standard polypeptides is indicated in kilodaltons. Lane 1 contains the uninfected control.

FIG. 4. Distribution of pE in the envelope. Procedure was identical to that described in the legend to Fig. 2 except that *E. coli* C uvr was used as the host and ϕ X174E⁺ (bottom) and Eam3 (top) were used for the infections. The mass of standard polypeptides is indicated in kilodaltons. The positions of the ϕ X174 pD and pE proteins is indicated.

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