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Lethal Action of Bacteriophage λ *S* Gene

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The functions of the bacteriophage λ lysis genes *S*, *R*, and *Rz* were investigated. Different combinations of wild-type and inactive alleles of all three lysis genes were cloned into the plasmid pBH20 and were expressed under the control of a *lac* operator-promoter. The involvement of the *Rz* gene in lysis was proposed in our previous work and was confirmed by the Mg^{2+}-dependent lysis defect of clones in which part of the *Rz* gene is deleted. Membrane vesicles prepared from induced S^+ cells were shown to have a severely reduced capacity for active transport of glucose; this defect was detectable at least 20 min before lysis. Cell viability was also shown to decrease very soon after induction, long before physiological death and lysis; this decrease in viability is absolutely dependent on *S* expression and independent of *R* and *Rz*. The nonviable fraction of cells at any time after induction was demonstrated to be equal to the fraction committed to eventual lysis. Induction of an *Sts* clone showed that the *S* gene product is stable and capable of inducing lysis long after the cessation of synthesis of *S* gene product. A model for *S* action is proposed.

The terminating event of the infective cycle of many viruses is lysis of the host cell with concomitant release of the progeny virus particles. Three genes have been directly implicated in lysis of the bacteriophage λ: *S*, *R*, and *Rz* (16, 19). The λ lysis gene region has been cloned under control of the *lac* promoter in the plasmid pBH20 (9). Induction of the lysis operon by the addition of the gratuitous *lac* inducer isopropyl-β-D-thiogalactopyranoside (IPTG) results in cell lysis in the $S^+R^+Rz^+$ clone. It is much easier to study the mechanism of action of the individual lysis proteins in the cloned system, since the lysis genes are removed from the multiple controls of bacteriophage development.

Two of the lysis proteins, those coded by genes *R* and *Rz*, have been implicated in the degradation of the rigid peptidoglycan cell wall (2–4). Together these activities constitute the bacteriolytic activity previously designated as endolysin (18). The *R* gene product (p*R*) has been purified and been shown to be a transglycosylase which degrades the peptidoglycan by attacking the glycosidic linkages (3). The *Rz* gene product (p*Rz*) has been tentatively identified as an endopeptidase which is believed to play a minor role in peptidoglycan degradation by digesting oligopeptide cross-links within the peptidoglycan (19).

The role of the *S* gene product (p*S*) is unknown, but there is evidence that it is required for the transport of bacteriolytic activity to the periplasm. Our previous work showed that induction of the $S^-R^+Rz^+$ clone results in extensive accumulation of intracellular endolysin (9); however, no lysis or reduction in the rate of cell growth was observed, indicating that endolysin was not reaching the vicinity of the peptidoglycan.

In early work on the mechanism of λ lysis, Reader and Siminovitch (17) suggested that p*S* attacks the cytoplasmic membrane to allow transport of endolysin to the periplasm. They observed that in the case of induced S^+R^- lysogens the cytoplasmic membrane becomes permeable to sucrose; that is, these cells can no longer be plasmolyzed by hypertonic sucrose. This suggests that the normal integrity of the membrane has been disrupted. However, electron microscopic examination of thin sections of induced λS^+R^- lysogens beyond the normal lysis time revealed no detectable change in the cytoplasmic membrane structure. There is no detectable phospholipase activity attributable to p*S*; in the absence of *S* gene function, cell lysis by mechanical methods results in a small amount of lipid degradation indistinguishable from that occurring in λ $S^{\pm}$-infected cells (14). Other workers have noted deleterious effects of p*S* action on membrane-dependent oxidative metabolism (1, 6), which also suggests an inner membrane lesion of some kind. However, no model for *S* action has been proposed.

In this work we have used a complete set of cloned $S^{\pm}R^{\pm}Rz^{\pm}$ lysis gene alleles to study the action of the *S* gene. We document the effect of induction of these cloned lysis genes on cell viability, membrane permeability and active

transport, and lysis kinetics. A general model for p*S* action is proposed.

MATERIALS AND METHODS

Bacterial strains, phages, and plasmids. The host bacterium for most experiments was the *Escherichia coli* strain WFK, which is W3101 *pro phe*/F′ *lacI*^{q} *lacZ*::Tn*5* (9). The methylation-deficient *E. coli* K-12 strain GM119 (F$^-$ *dcm dam-3 metB1 galK2 galT22 lacY1 tsx-18 supE44*) (obtained from J. A. Arraj) was used for the preparation of unmethylated phage and plasmid DNA. The bacteriophages and plasmids used are listed in Table 1.

Chemicals and enzymes. Ethidium bromide, EDTA, IPTG, phosphoenolpyruvate (PEP), glucose-6-phosphate dehydrogenase, lysozyme, and the restriction enzyme *Eco*RI were purchased from Sigma Chemical Co., St. Louis, Mo. T4 polynucleotide ligase was obtained from New England Biolabs, Beverly, Mass. All other restriction enzymes were obtained from Bethesda Research Laboratories, Gaithersburg, Md. [^{14}C]sucrose (477 mCi/mmol) and [^{14}C]glucose (500 mCi/mmol) were purchased from New England Nuclear Corp.

Media and growth conditions. Cultures for all experiments except membrane vesicle preparations were grown in minimal media (7) supplemented with amino acids and thiamine. Antibiotics used were ampicillin (50 μg/ml) and kanamycin (30 μg/ml).

For lysis kinetics and cell viability experiments, cultures were grown to a density of 10^8 cells per ml and were induced by the addition of 10^{-3} M IPTG. Cell viability was followed by plating dilutions of the culture on broth plates (1% tryptone, 0.5% yeast extract, 0.5% NaCl) at given times before and after induction.

Cultures for membrane vesicle preparations were grown in rich media (3.2% tryptone, 2.0% yeast extract, 0.5% NaCl) with vigorous aeration and were harvested at a concentration of approximately 5×10^9 cells per ml.

Plasmid construction. The $S^+R^-Rz^+$ (pJG1) and $S^-R^-Rz^+$ (pJG3) plasmids were constructed as described previously (9). The $S^{\pm}R^{\pm}Rz^-$ plasmids (pJG7, pJG8, and pJG9) were prepared by digestion of the unmethylated parent plasmid DNA (prepared from the *dam*$^-$ strain GM119) with *Bcl*I and *Bam*HI and religation. The $StsR^+Rz^-$ plasmid (pJG13) was constructed by digestion of unmethylated phage DNA with *Eco*RI and *Bcl*I and by ligation of the isolated lysis gene fragment into pBH20 DNA cleaved with *Eco*RI and *Bam*HI.

Transformation was performed with cryogenically stored competent cells by the method of Morrison (13). Transformants containing the lysis plasmids were identified as previously described (9).

Membrane vesicle experiments. Membrane vesicles were prepared from bacterial cultures by a modification of the lysozyme-EDTA method of Kaback (11). Cultures were harvested by centrifugation at 16,000 × *g* and were suspended at room temperature in 30 mM Tris-hydrochloride, pH 8.0, (1 g [wet weight] of cells per 80 ml) containing 20% sucrose. The cells were converted to spheroplasts by adding potassium EDTA, pH 7.0, and lysozyme to final concentrations of 10 mM and 50 μg/ml, respectively, and incubating for 1 h. The spheroplasts were collected by centrifugation at 16,000 × *g* for 20 min and were suspended with a glass homogenizer in the smallest possible volume of 0.1 M potassium phosphate buffer, pH 6.6, containing 20% sucrose, 20 mM magnesium sulfate, and 5 μg of DNase per ml. The suspension was poured into 300 volumes of 50 mM potassium phosphate buffer, pH 6.6, at 37°C and was incubated with vigorous swirling for 45 min. After 15 min of incubation, potassium EDTA, pH 7.0, was added to a final concentration of 10 mM, and after a further 15 min of incubation, magnesium sulfate was added to 15 mM final concentration. The suspension was then centrifuged at 45,000 × *g* for 1 h to collect the membrane vesicles. The vesicles were suspended by homogenization in 0.1 M potassium phosphate, pH 6.6, at a concentration of 7 mg of protein per ml, quick-frozen in ethanol-dry ice, and stored at −80°C. Vesicles were prepared from induced lysis clones at various times after the addition of IPTG.

Membrane vesicles were suspended at a concentration of 35 mg/ml in 40 μl of 0.1 M potassium phosphate, pH 6.6, containing 4 mM unlabeled sucrose and 10 μCi of [^{14}C]sucrose and were incubated overnight at 4°C. At time zero, the labeled vesicles were diluted

TABLE 1. Phages and plasmids

Designation	Genotype	Source or Reference
Phages		
λ363*R*$^-$	λ*b519b515*c*I857nin5R*Tn*903*.979	R. Young
λ363*Sam7R*$^-$	λ*b519b515*c*I857nin5Sam7R*Tn*903*.979	R. Young
λ*Sts*	λc*I857Sts9B*	D. B. Wilson
Plasmids		
pBH20	*Ap*^{r} *Tc*^{r}	Derived from pBR322 (10)
pRF26	*Ap*^{r} *S*$^+$ *R*$^+$ *Rz*$^+$	Derived from pBH20 (9)
pJH2	*Ap*^{r} *Sam7 R*$^+$ *Rz*$^+$	Derived from pBH20 (9)
pJG1	*Ap*^{r} *S*$^+$ *R*$^-$ *Rz*$^+$	Derived from pBH20 (this paper)
pJG3	*Ap*^{r} *Sam7 R*$^-$ *Rz*$^+$	Derived from pBH20 (this paper)
pJG7	*Ap*^{r} *S*$^+$ *R*$^+$ *Rz*$^-$	Derived from pBH20 (this paper)
pJG8	*Ap*^{r} *S*$^+$ *R*$^-$ *Rz*$^-$	Derived from pBH20 (this paper)
pJG9	*Ap*^{r} *Sam7 R*$^-$ *Rz*$^-$	Derived from pBH20 (this paper)
pJG13	*Ap*^{r} *Sts9B R*$^+$ *Rz*$^-$	Derived from pBH20 (this paper)
pKY2	*Ap*^{r} *Tc*^{s}	Derived from pBH20 (this paper)

200-fold into 0.1 M potassium phosphate (pH 6.6)–10 mM $MgCl_2$, and at various times 2-ml samples were removed, filtered through Millipore filters (pore size, 0.45 μm), and washed under suction with 5 × 1 ml of 0.1 M LiCl. The filters were dried and counted in a Tracor Analytic Delta 300 liquid scintillation counter.

Glucose-6-phosphate was assayed by the method of Olive and Levy (15), and the protein concentration was determined by the Bradford dye-binding assay (5).

RESULTS

Construction of hybrid lysis operons. In previous work (9), the λ lysis genes *S* (both S^+ and S^- alleles) and *R* were cloned into the plasmid pBH20 (Fig. 1). To complete the set of cloned genes, the S^+R^- and S^-R^- allelic combinations were cloned by identical methods; the resultant plasmids carrying S^+R^- and S^-R^- hybrid lysis operons were designated pJG1 and pJG3, respectively. Whereas induction of the S^+R^+ clone with 10^{-3} M IPTG results in a sharply defined lysis after 40 min (9; Fig. 2), induction of a culture of the S^+R^- clone results in an eventual halt in mass accumulation (Fig. 2), and the cells appear to lose motility under microscopic observation (data not shown). Predictably, induction of the S^-R^- clone has no detectable effect on culture growth.

Verification that no other genes promoter-distal to *Rz* (19; Fig. 1) are involved in cell killing or lysis was obtained by constructing another set of plasmids in which the region of λ DNA beyond the *BclI* site within the *Rz* gene (Fig. 1) was deleted by digestion with *BclI* and *Bam*HI and by subsequent religation. This series of clones, which all lack the intact *Rz* gene, resulted in lysis kinetics identical to those of the parental clones, showing that there are no other λ genes involved in lysis (data not shown). However, the $S^+R^+Rz^-$ clone did show aberrant lysis in the presence of 10 mM Mg^{2+} (Fig. 3). This aberrant lysis pattern is associated with the formation of mechanically fragile spheroidal cells and constitutes the Rz^- phenotype described for bacteriophages carrying the kanamycin resistance transposon Tn*903* inserted in the *Rz* gene (19).

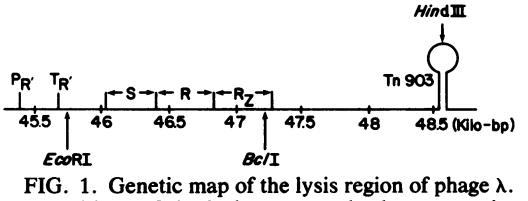

FIG. 1. Genetic map of the lysis region of phage λ. The positions of the lysis genes and relevant restriction enzyme sites are shown. The stem and loop structure represents the transposon Tn*903* inserted at 48.5 kilobase pairs.

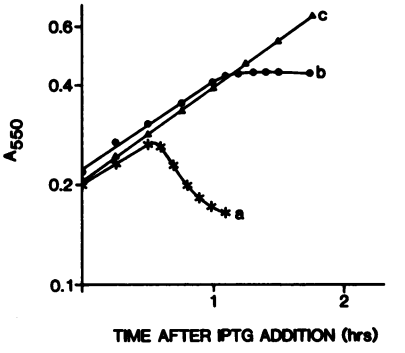

FIG. 2. Induction of lysis gene clones. Cultures were grown in glycerol-minimal media (7) at 37°C to a concentration of 10^8 cells per ml. The lysis genes were induced by adding 1 mM IPTG and culture mass accumulation followed. (a) $S^+R^+Rz^+$; (b) $S^+R^-Rz^+$; (c) $Sam7R^+Rz^+$; A_{550}, absorbance at 550 nm.

The $StsR^+Rz^-$ clone was constructed by digestion of λ*Sts* DNA with identical restriction digestions and ligation; the clones containing the lysis genes were screened for inducible loss of viability at the permissive temperature (30°C). Induction of the temperature-sensitive *S* gene at the restrictive temperature (42°C) had no effect on culture growth (Fig. 4). Subsequent shift of the culture to 30°C resulted in immediate lysis (Fig. 4).

The functional stability of the temperature-sensitive *S* protein was investigated. Cells that had been induced for 1 h at the restrictive temperature were diluted 25-fold into noninducer medium (Fig. 5). This dilution (5×10^{-4} M to 2×10^{-5} M) was shown to decrease β-galactosidase synthesis 50-fold from the induced level and to suppress gene *S* killing activity (Table 2) and should therefore turn off p*S* synthesis at the time of dilution. Temperature shift of the culture to 30°C after incubation in diluted medium for 1 h resulted in immediate lysis (Fig. 5). This indicates that p*Sts* is stable within the cell at the restrictive temperature.

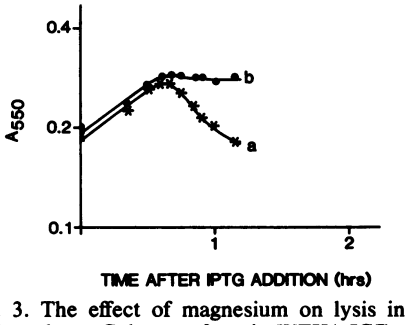

FIG. 3. The effect of magnesium on lysis in the $S^+R^+Rz^-$ clone. Cultures of strain WFK(pJG7) were grown and induced as described in the legend to Fig. 2 in glycerol-minimal medium without (a) and with (b) 10 mM magnesium chloride.

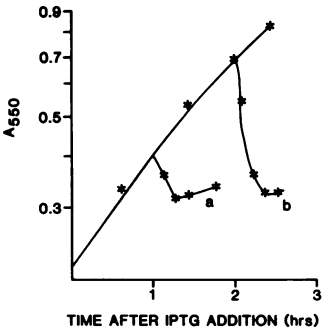

FIG. 4. Induction of the temperature-sensitive *S* protein. A culture of strain WFK(pJG13) (*StsR*$^+$*Rz*$^-$) was grown at 42°C in glycerol-minimal medium and was induced by adding 1 mM IPTG. Portions of the culture were shifted to 30°C after incubation in inducing conditions for 1 h (a) or 2 h (b).

Effect of gene *S* on membrane permeability. Since gene *S* is implicated in the transport of bacteriolytic activity to the periplasm and in the permeability of the inner membrane to sucrose (9, 17), it would be reasonable to expect that membranes isolated from induced *S*$^+$ lysis clones would have altered permeability properties. To characterize the action of p*S* on the membrane, we prepared membrane vesicles by the method of Kaback (11) from the induced *S*$^+$*R*$^-$ and *S*$^-$*R*$^-$ lysis clones as well as from cells containing no cloned lysis genes. Membrane vesicles prepared by this method have been shown to be whole cell vesicles, with the same permeability barriers as the intact cell (11). These vesicles were used in passive sucrose diffusion experiments. Vesicles from the *S*$^+$ clone were approximately fourfold more permeable to sucrose than were vesicles prepared from control bacteria (Table 3). Surprisingly, the vesicles affected by *Sam7* (*S*$^-$) were also found to have increased permeability to sucrose (Table 3). We believe this is an indication that the *Sam7* nonsense fragment retains some function; in fact, induction of the *Sam7* clone results in accumulation of large quantities of the nonsense fragment polypeptide (55 amino acid residues; 8; D. L. Daniels, Ph.D. thesis, University of Wisconsin, Madison, 1981) in the membrane (E. A. Altman, J. Garrett, R. Grimaila, R. Schultz, and R. Young, manuscript in preparation).

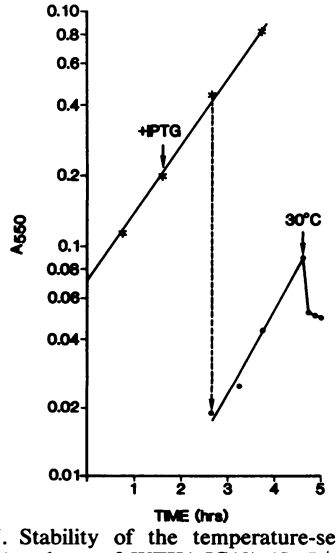

FIG. 5. Stability of the temperature-sensitive *S* protein. A culture of WFK(pJG13) (*StsR*$^+$*Rz*$^-$) was grown and induced as described in the legend to Fig. 4. After 1 h of incubation, a portion of the culture was diluted 25-fold into noninducer medium; 2 h later, the culture was shifted to 30°C.

TABLE 2. Effect of IPTG concentration on loss of cell viability[a]

IPTG concn (M)	% Survival at time after induction:	
	20 min	60 min
1×10^{-3}	27	3.6
5×10^{-4}	35.6	4.4
1×10^{-4}	75.7	7.6
2×10^{-5}	128.0	285.0

[a] Cultures of strain WFK(pRF26) (*S*$^+$*R*$^+$ *Rz*$^+$) were induced with various concentrations of IPTG. Cell viability was followed by plating dilutions of the culture on noninducer rich medium at various times after IPTG addition. Cell viability is expressed as the percentage of surviving cells at that time, with cells at time zero as 100%.

Effect of gene *S* on PTS-mediated active transport. In *E. coli* membrane vesicles, the uptake of several sugars (e.g., glucose, fructose) occurs by

TABLE 3. Passive diffusion of [^{14}C]sucrose from membrane vesicles[a]

Strain supplying vesicles	$t_{1/2}$ (min)[b]	Initial rate of efflux[c]
WFK(pBH20)	2	7.5
WFK(pJG) (*S*$^+$*R*$^-$)	0.46	32.6
WFK(pJG3) (*Sam7R*$^-$)	0.43	34.9

[a] Membrane vesicles were passively loaded with [^{14}C]sucrose overnight, washed quickly, diluted into label-free medium, and assayed for the retention of [^{14}C]sucrose at various times (14). Maximum capacity of the vesicle samples was estimated as 30 pmol of [^{14}C]sucrose per mg of protein.

[b] $t_{1/2}$, Time taken for one half of the maximum capacity of vesicle samples to diffuse out of the vesicles.

[c] Calculated from [^{14}C]sucrose lost in the first 30 s of incubation; expressed as picomoles of sucrose per minute per milligram of protein.

phosphorylation via the PEP-phosphotransferase system (PTS). We used the S^+-induced vesicles to determine the effect of p*S* PEP-driven [^{14}C]glucose uptake in these membrane vesicles. S^+-affected vesicles were completely deficient in PTS activity, in contrast to S^--affected and control vesicles (Fig. 6). This result was verified by assaying the glucose-6-phosphate produced by this system. Each experiment was allowed to continue for 2 h to allow all the glucose-6-phosphate to diffuse out of the vesicles before starting the assay. In vesicles made after only 15 min of S^+ induction, the membrane loses the ability to support PTS-mediated phosphorylation of glucose (Table 4). No effect is observed on glucose-6-phosphate production in the control and S^--affected vesicles. Thus, the loss of the ability to support PTS-mediated active transport of glucose occurs well before normal lysis time in S^+-affected membranes. Furthermore, the *Sam7*-affected membranes were essentially normal in their ability to support PTS-mediated active transport, despite their increased permeability to sucrose in vitro.

Kinetics of the loss of cell viability on induction of the *S* protein. The observed effect of the gene *S* protein on the PTS before normal lysis time led us to investigate the kinetics of the loss of cell viability in bacteria exposed to the action of the gene *S* protein. The sharply defined lysis curve observed at 40 min for the S^+R^+ clone is accompanied by the loss of colony-forming units beginning much earlier, about 8 min after induction (Fig. 7). Identical results are obtained with the S^+R^- clone (Fig. 7), so actual lysis is not required for loss of viability. The loss of viability is absolutely dependent on a functional *S* allele (Fig. 7); in fact, $S^-R^\pm$ clones can be subcultured indefinitely in the presence of inducer without detectable killing (9). It is clear from comparison of the results in Fig. 7 that the loss of viability function of the gene *S* protein takes effect much sooner than the observable effect on the accumulation of culture mass.

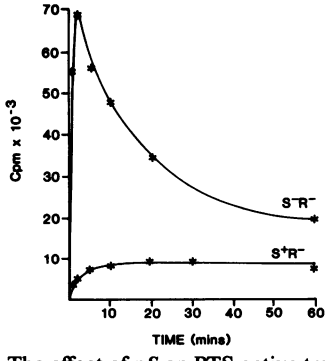

FIG. 6. The effect of p*S* on PTS-active transport in membrane vesicles. Membrane vesicles prepared from the S^+R^- and S^-R^- clones were suspended at a concentration of 20 mg of protein per ml in 50 mM potassium phosphate buffer (pH 6.6)–10 mM magnesium sulfate–0.3 M LiCl–0.1 M PEP (adjusted to pH 6.5 to 7.0 with sodium carbonate). The samples were incubated at 40°C. At various time points, 40-μl samples were removed, diluted into 2 ml of 0.5 M LiCl, rapidly filtered through a Millipore filter (pore size, 0.45 μm) under suction, and washed with another 2 ml of 0.5 M LiCl. The filters were dried and counted.

TABLE 4. Effect of gene *S* expression on PTS-mediated active transport[a]

Relevant genotype	Strain	Time of incubation (min)	Glucose PTS transport	
			Amt[b]	% of control
Host	WFK		16.2	81
Control	WFK(pKY2)		20.9	100
S^-R^-	WFK(pJG3)	0	14.9	74
		15	14.9	74
		30	14.3	71
		45	16.7	83
S^+R^-	WFK(pJG1)	0	10.4	52
		15	0.12	0.6
		30	0.09	0.4
		45	0.08	0.4

[a] Transport of glucose by the PEP-phosphotransferase system was measured by assaying the accumulation of glucose 6-phosphate. Purified membrane vesicles were preincubated with PEP and then were suspended in a reaction buffer containing glucose by the method of Kaback (12). Glucose 6-phosphate was assayed in a coupled system by using glucose-6-phosphate dehydrogenase and $NADP^+$. Strain WFK is the *lacI*^{q} host; plasmid pKY2 is a tetracycline-sensitive derivative of pBH20, and pJG1 and pJG3 are lysis gene clones (9).

[b] Expressed as nanomoles of glucose 6-phosphate per milligram of protein.

Loss of viability and commitment to lysis. To pursue the relationship between loss of cell viability and lysis, induced cells were diluted into noninducer medium at various times after induction (Fig. 8). The dilution was as described for the *Sts* dilution experiment (Fig. 5). Comparison of Fig. 7 and 8 shows that the percentage of cells no longer viable at a given time after induction will lyse at the normal lysis time. These results suggest that the function of the gene *S* protein consists of two distinct phases: initial attack on the cytoplasmic membrane, which is required for the destruction of PTS-mediated active transport and causes loss of cell viability, and subsequent lysis in gene R^+ conditions or physiological death in R^- conditions.

DISCUSSION

We have shown here and in previous work (9) that the expression of the bacteriophage λ *S* and

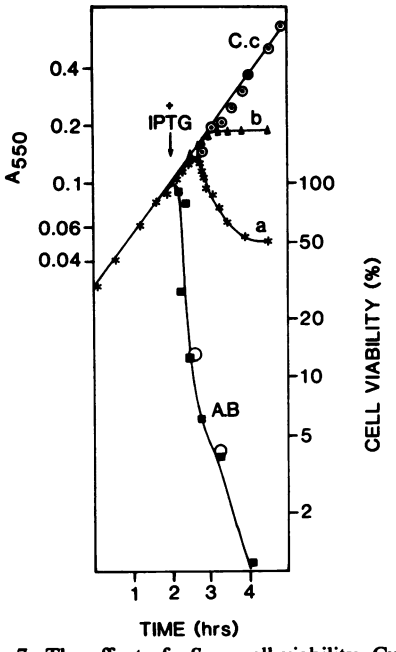

FIG. 7. The effect of p*S* on cell viability. Cultures of $S^+R^+Rz^+$ (a,*), $S^+R^-Rz^+$ (b,▲), and $S^-R^-Rz^+$ (c,⊙) were grown and induced as described in the legend to Fig. 2. Portions of the culture were removed, diluted, and plated on rich media to assay cell viability at times before and after dilution. Cell viability is plotted as percentage of survival with the time of induction as 100% for $S^+R^+Rz^+$ (A,■), $S^+R^-Rz^+$ (B,○), and $S^-R^-Rz^+$ (C,●).

R genes will result in a scheduled lysis under normal growth conditions. The expression of a third gene, *Rz*, is required in λ-infected cells (19) or in induced lysis gene clones (Fig. 3) in media containing a high Mg^{2+} concentration. No other phage proteins are required for lysis or for the timing of lysis; the expression of the lysis genes is necessary and sufficient for the complete phenomenon.

Neither the mechanism of lysis nor its timing is understood. The results presented here suggest that there is damage to the cytoplasmic membrane mediated by the gene *S* protein. The lesion is manifested as an increased passive permeability to sucrose and a destruction of the PTS-mediated active transport of glucose. The *Sam7* nonsense fragment polypeptide, which has a length of 55 amino acid residues and accumulates in the membrane as does p*S* (Altman et al., manuscript in preparation), also increases the passive sucrose permeability but does not destroy the PTS active transport of glucose and has no effect on cell viability.

Surprisingly, the membrane lesions are apparent in vesicles prepared very soon after induction of the bacteriophage lysis genes rather than just in vesicles prepared from the normal lysis time or later. This result led us to investigate the kinetics of cell viability after induction of the lysis genes since a membrane lesion leading to a collapse of active transport might be expected to be a lethal event. The results (Fig. 7) show that cell viability, measured as colony-forming potential, begins to decrease dramatically very soon after induction of the lysis genes, much earlier than the onset lysis in S^+R^+ organisms or cessation of mass accumulation in S^+R^-. The simplest interpretation of these results is that the early membrane lesion detected in the vesicle transport experiments and the early loss in viability are manifestations of an early membrane action of p*S*. Once a sufficient quantity of p*S* has been synthesized or has exerted its effect on the membrane, the cell is committed to lysis at a later time (Fig. 8), even though protein synthesis (and thus respiration) continue at exponentially increasing rates for at least 30 min after the lethal event.

What is the eventual lysis trigger, then, if the cell is committed to lysis at such an earlier time? Our results with the *Sts9B* allele suggest that p*S* is stable; furthermore, once a cell has accumulated enough p*S* no further synthesis is required for the eventual lysis to occur (Fig. 8). In previous studies, we have shown that the phenomenon of premature lysis is duplicated in the cloned lysis gene system (9). Premature lysis was first observed in cells infected with the bacteriophage T4, which has lysis genes *t* and *e*, apparently analogous to the phage λ genes *S* and

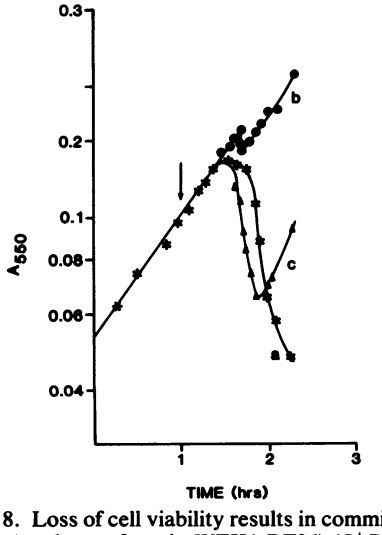

FIG. 8. Loss of cell viability results in commitment to lysis. A culture of strain WFK(pRF26) ($S^+R^+Rz^+$) was grown and induced as described in the legend to Fig. 2 (a). After 10 min (b) and 20 min (c) of induction, portions of the culture were removed and diluted into noninducer medium. The A_{550} was normalized to the undiluted culture in (b) and (c).

R; several laboratories have documented premature lysis in λ infections. The phenomenon is simple and dramatic; if cyanide or any respiratory poison is added to cells in which the *S* gene has been sufficiently expressed (but long before the normal lysis time), lysis occurs immediately (6). No lysis is observed if cyanide is added to S^--affected cells (6). Thus, poisoning respiration apparently acts to trigger lysis prematurely by subverting the normal scheduling mechanism.

Taken together with the results in this work, the premature lysis phenomenon suggests a general model for *S*-mediated scheduled lysis. Once a certain quantity of p*S* has accumulated in the membrane, the membrane is then susceptible to a lysis-triggering event. This triggering event is likely to be collapse of the chemiosmotic gradient across the cytoplasmic membrane since it can be emulated by respiratory poisons. Since no further p*S* accumulation is necessary for the trigger to occur, one possibility is that the triggering event occurs during the inception of cell division or septum formation, which is the only known singularity in membrane biogenesis. That is, the onset of septation in the presence of sufficient p*S* causes a catastrophic lesion in the membrane, resulting in the immediate collapse of the membrane potential. Alternatively, the accumulation of p*S* may result in a deterioration of the ability of the cell to maintain the chemiosmotic gradient. The cell can resist collapse of the gradient by compensatory hydrolysis of ATP in the cellular pools or by increased utilization of the oxidative metabolic pathways. The resistance would be overcome by further p*S* synthesis or by any substance which poisons respiration. This model predicts either decreasing ATP pool levels as the time of lysis approaches or more rapid oxygen consumption. Specific predictions of the septation trigger are less clear, except that some septation delay mutants should show severely retarded lysis scheduling. These predictions are currently being tested in this laboratory.

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