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Lytic Action of Cloned φX174 Gene *E*

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The φX174 lysis gene *E* was placed under control of the *lac* promoter by cloning into the multicopy plasmid pBH20. Other φX174 gene sequences were removed by nuclease digestion. Expression of gene *E* was shown to be necessary and sufficient to produce lysis phenomena exhibited by infection with intact phage. Lysis, its inhibition by $MgSO_4$ and spermine, its progression through a spheroplasting stage, and its dependence on an early chloramphenicol-sensitive step were reproduced in clones induced for expression of the *E* gene product. *Escherichia coli* clones carrying the *E* gene not under *lac* control, and clones under *lac* control but only minimally induced for gene *E* expression, exhibited morphological aberrations consistent with the view that the mechanism by which gene *E* mediates cell lysis is related to host cell division processes.

The mechanism by which bacteriophage φX174 lyses its host, *Escherichia coli*, is not known. In rich media, cells infected with φX174 show a sharp lysis after 20 to 25 min (12, 15, 16, 23). Lysis can be inhibited by $MgSO_4$ (13) and spermine (12) and depends on a chloramphenicol-sensitive step early in infection (23). The morphology of cells lysed in the presence of $MgSO_4$ suggests that lysis proceeds through a spheroplasting stage (5); such a stage has been observed for a similar bacteriophage, α3 (4).

It was established very early that an amber mutation in at least one viral gene, gene *E*, completely inhibited lysis of the host and so prevented release of mature phage (16). Other mutations displaying the same phenotype have since been identified within the DNA sequence coding for gene *E* (2). Even so, it remained unclear whether the lytic event involved a phage-specified lysozyme-like molecule, a novel method of phage release, or a cooperation of gene *E* with other viral genes (15). In bacteriophage lambda and T4 infections, lysis is accomplished by the combination of a protein which appears to act on the cytoplasmic membrane and one or more other proteins which act to degrade the peptidoglycan (11, 19). Lysis by φX174 could proceed in a similar fashion, since gene *E* codes for a protein having an amino acid sequence consistent with a membrane-associated function (2), membrane protein patterns in cells infected with wild-type φX174 show differences when compared with infection by *E*am phage (22), and at least two enzymatic activities appear to be responsible for cell wall degradation (21). Thus, gene *E* might cooperate with other viral genes to produce lysis as in lambda or T4. However, no bacteriolytic activity has been found in phage-infected lysates of cells (9, 10, 23); furthermore, the number, primary sequences, and essential functions of virtually all φX174 gene products are known (1, 28), yet no lysozyme-like activity has been identified. It remains possible, however, that a φX174 protein could play a dual role, or that some interaction with capsid structural proteins is required to accomplish complete lysis.

Knowledge of the complete sequence of the φX174 genome (28) has permitted us to clone gene *E* into a plasmid, where it can be expressed under control of the *lac* promoter. By eliminating all other φX174 gene sequences, we have observed that expression of gene *E* alone is necessary and sufficient to produce the observed lysis phenomena exhibited by phage-infected cells. We conclude that lysis is triggered by gene *E* alone among the bacteriophage genes and present some morphological observations which are consistent with the view that gene *E* acts via host cell division processes.

MATERIALS AND METHODS

Chemicals and enzymes. Isopropyl-β-D-thiogalactopyranoside (IPTG), ethidium bromide, dithiothreitol, EDTA, ATP, spermine tetrahydrochloride, chloramphenicol, and RNase A were purchased from Sigma Chemical Co., St. Louis, Mo. Restriction enzymes, T4 DNA ligase, T4 DNA polymerase, polynucleotide kinase, *Bal*-31 nuclease, bacterial alkaline phosphatase, *Hin*dIII linkers, and agarose were purchased from Bethesda Research Laboratories, Bethesda, Md. Absolute ethanol (EtOH) was from U.S. Industrial Chemicals Co., Tuscola, Ill.

Media and buffers. TYE broth is tryptone (1%)–yeast extract (0.5%)–NaCl (0.5%). Minimal medium

was that described by Clark and Maaloe (7), supplemented with amino acids and thiamine. Minimal plates had a carbon source plus the amino acids required by the strain. Restriction enzyme buffers were the "low-medium-high" buffers of Davis et al. (8). Polynucleotide kinase phosphorylation buffer was 70 mM Tris (pH 7.4)–10 mM $MgCl_2$–10 mM ATP–15 mM dithiothreitol. Ligase buffer was 70 mM Tris, (pH 7.4)–7 mM $MgCl_2$–0.7 mM ATP–1 mM dithiothreitol. Buffers for reactions with bacterial alkaline phosphatase, T4 DNA polymerase, and *Bal*-31 nuclease were those recommended by the supplier.

Phage and bacteria. φX174 was isolated as a wild-type revertant from a stock of φX174 *E*am3 supplied by T. Edlind (Texas A & M College of Medicine) and was prepared by polyethylene glycol precipitation of lysates of *E. coli* C990. φX174 *E*am3 was supplied by P. McCormack (Texas A & M College of Medicine), and high-titer stocks were prepared by infection of *E. coli* C990. All phage stocks were stored in 50 mM sodium tetraborate–50 mM EDTA (pH 9.5) and were tested on *E. coli* C990 (nonsupressing) and C991 (amber suppressor).

The following *E. coli* strains were used as plasmid hosts: RY1197 (*pro phe*/F'*lacI*^{q} *lacZ*::Tn*5*) (11); RY1109 (*supF thi*/F' *lacI*^{q} *lacZ*::Tn*5*); CQ (*ara leu lacI*^{q} *purE gal trp his argG rpsL xyl ilv metA* or *metB thi*). *E. coli* RY1197 was the original *lacI*^{q} cloning host and was used for early experiments. CQ was constructed by P1 transduction of *lacI*^{q} from LB109 (6) to CSH57B (25). Since the *lacI*^{q} allele for overproduction of the *lac* repressor is located on the chromosome in CQ instead of on an F' episome, it was used for many later experiments. The behavior of the two strains was qualitatively identical in the experiments described; however, CQ regularly required a longer period after induction before lysis occurred, for unknown reasons.

Cloning of the φX174 *E* gene. Replicative form I DNA of φX174 was prepared by adding phage at a multiplicity of infection of 0.5 to 1.0 to several 5-ml TYE cultures of *E. coli* C990. After 5 to 10 min of shaking at 37°C, chloramphenicol was added to give a final concentration of 20 μg/ml, and the incubation was continued for 3 h. Replicative form I DNA was extracted by the plasmid preparation method of Holmes and Quigley (14). Replicative form I DNA of φX174 *E*am3 was prepared by adding phage at a multiplicity of infection of 10 to a 100-ml TYE culture of C990, adding chloramphenicol to 50 μg/ml 18 min later, and incubating at 37°C for 4 h. Replicative form DNA was extracted as described above, treated with RNase, and ethanol precipitated.

Portions of the replicative form DNA preparations described above were digested completely with *Alu*I. The E^+ DNA was separated on a 1.0% agarose gel, and the largest *Alu*I fragment was electroeluted into dialysis tubing placed in a trough cut in front of the band. The *E*am3 DNA fragments were separated on a 0.7% agarose gel, and the largest *Alu*I fragment was cut from the gel and frozen at −20°C. The fragment was eluted from the frozen agarose plug by expressing the liquid into a Parafilm bag by squeezing between thumb and forefinger (the "freeze-squeeze" method) (32). The expressed liquid was extracted twice with an equal volume of buffer-saturated phenol and twice with an equal volume of $CHCl_3$ and ethanol precipitated.

*Hin*dIII linker oligonucleotides, previously phosphorylated with polynucleotide kinase, were added to each fragment at approximately a 10:1 molar ratio. T4 ligase was added to at least 1 U/5 μg of fragment, and the ligation reaction was incubated for 4 to 6 h at room temperature (1 U of ligase converts 1 nmol of $^{32}PP_i$ into [α,β-^{32}P]ATP at 37°C in 20 min). Ligase was inactivated at 65°C for 10 to 20 min, and NaCl was added to give a final concentration of 60 mM. *Hin*dIII was added to at least 5 U/μg of fragment and incubated at 37°C for 3 to 4 h. The fragments with *Hin*dIII ends were then separated from linker fragments by electrophoresis in a 0.7% agarose gel. The bands were isolated from the gel, and fragments were purified as described above.

These φX174 *Alu*I fragments (with *Hin*dIII ends) were cloned into the *Hin*dIII site of pBH20 (18). To decrease the number of plasmids not carrying insertions, the *Hin*dIII-cut vector had been previously 5'-dephosphorylated by incubation at 37°C for 30 min with 25 U of bacterial alkaline phosphatase per pmol of DNA ends. After ligation, the mixtures were transformed into RY1197 by the RbCl method of Kushner (20), and clones containing inserts were identified by the loss, or partial loss, of tetracycline resistance. Plasmid DNA from Tet^s candidates was extracted by the method of Holmes and Quigley (14) and screened with *Hin*dIII for the presence of inserted DNA. Orientation with respect to the *lac* PO region was determined by *Hpa*I-*Bam*HI double digest. Plasmid pKY67 carried an insert of the E^+ φX174 fragment, and pKY92 carried an equivalent insert of the *E*am3 DNA. Both were oriented so that gene *E* expression was under *lac* control.

Removal of *J* gene sequences. The plasmid pKY67 carrying the E^+ *Alu*I fragment was prepared in large quantity by the procedure of Davis et al. (8). This plasmid was cut at the unique *Hpa*I site located in the φX174 DNA insert, and the ends were digested at 30°C with *Bal*-31 nuclease (1 U/15 μg of DNA) for various times up to 110 s. The reaction was stopped by adding to the mixture an equal volume of 0.25 M EDTA, and the nuclease was removed by phenol-$CHCl_3$ extraction plus EtOH precipitation. The *Hpa*I site is 330 base pairs (bp) from the end of the *J* gene sequence and 451 bp from the end of the *E* gene. A *Bam*HI site is located 508 bp on the other side of *Hpa*I (Fig. 1). By choosing the appropriate conditions and incubation time, it was possible to digest into the *J* gene sequence without removing the *Bam*HI site.

The digested DNA was resuspended and cut with *Bam*HI, precipitated with ethanol, resuspended in appropriate buffer, and reacted with T4 DNA polymerase to produce blunt ends. At this point, each molecule in the population was presumed to have a filled-in *Bam*HI site at one end and a deletion of φX174 DNA of variable length at the other. This mixture was extracted with phenol-$CHCl_3$, precipitated with EtOH, and resuspended in ligase buffer containing approximately 1 U of T4 ligase per 8 to 10 μg of DNA. Dilute conditions (20 μg of plasmid per ml) were used to promote intramolecular ligation. The ligation mixture was transformed into RY1197 by the RbCl method (20), and transformants were replica plated to minimal glycerol agar with or without 1 mM IPTG and to TYE without IPTG. Those clones which failed to grow on IPTG-containing plates were processed for plasmid DNA by the procedure of Holmes and Quigley (14) and screened with a *Hin*dIII + *Bam*HI double diges-

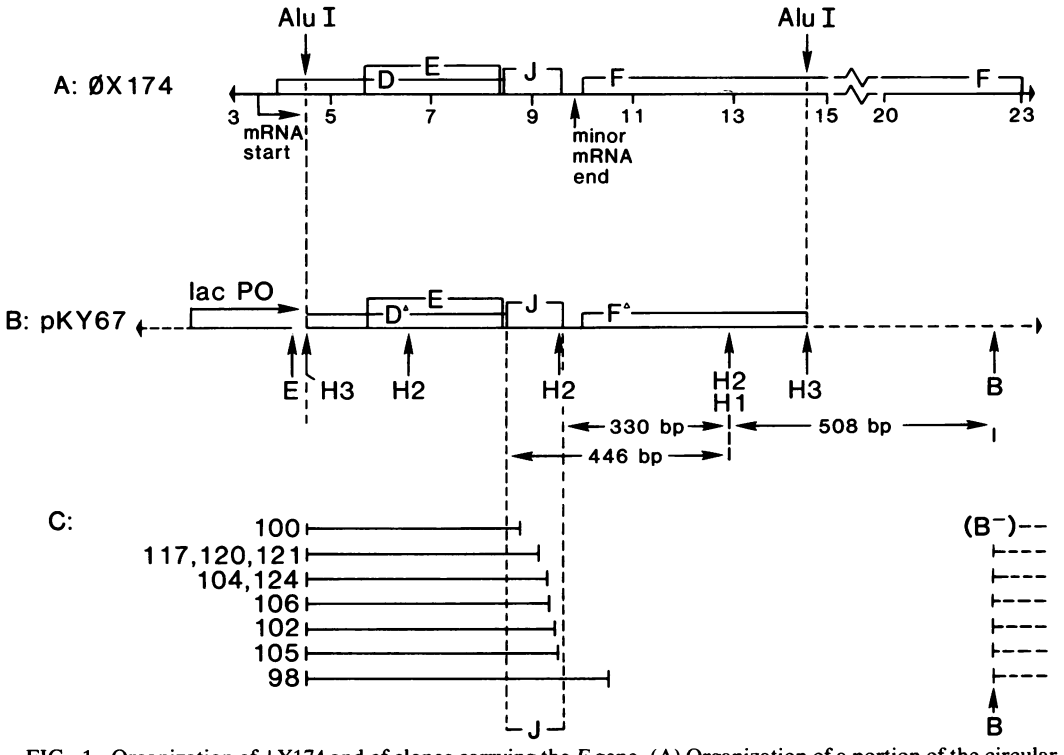

FIG. 1. Organization of φX174 and of clones carrying the *E* gene. (A) Organization of a portion of the circular DNA of φX174 (from reference 28). Gene sequences are represented by boxes; *E* overlaps *D* and is read in a different reading frame. Numbers below the line represent the bp numbering (in hundreds of bp) of Sanger et al. (28). The DNA extends beyond what is pictured to form a 5,386-bp circle. The φX174 promoter site nearest this sequence and a minor mRNA termination site are pictured. (B) Organization of pKY67. The *Alu*I-A fragment of φX174 was ligated to *Hin*dIII linkers, cut with *Hin*dIII, and inserted into the *Hin*dIII site of pBH20. φX174 genes *D* and *F* are truncated by the *Alu*I cleavages and are thus labeled D^{Δ} and F^{Δ}. Dotted lines represent pBR322 sequences of the vector plasmid. Distances from the *Hpa*I site to the *Bam*HI site and the beginning and end of gene *J* are denoted by arrows. (C) Various deletion plasmids derived from pKY67. The length of DNA deleted (±10 bp) is indicated by the gap between the solid and dotted lines. All plasmids except pKY100 have a reconstituted *Bam*HI site at the point of vector insert religation. Arrows denote locations of restriction enzyme cleavage sites: B, *Bam*HI; E, *Eco*RI; H1, *Hpa*I; H2, *Hin*cII; H3, *Hin*dIII.

tion to identify those which had a reconsituted *Bam*HI site (by ligation of a filled-in *Bam*HI site to a terminal guanine · cytosine bp) and those which had fragment lengths indicative of removal of the *J* gene sequence. Since a *Hin*cII site occurs in the sequence for *J* 10 bp from its termination codon, deletions into *J* beyond this point were confirmed by noting the disappearance of the relevant *Hin*cII fragment. The size of the deletion was measured by the length of the resulting fusion fragment. This general method for generating specific deletions with *Bal*-31 and simultaneously reconstituting restriction sites was taken from Panayotatos and Truong (26).

RESULTS

The DNA sequence of φX174 and the positions of the genes on its circular map are completely known (28). The nucleotide sequence for gene *E*, previously shown to be necessary for lysis, is located within the largest *Alu*I fragment (Fig. 1). All of gene *J*, which immediately follows gene *E*, is also included in this fragment, as are portions of genes *D* and *F*.

This 1,007-bp *Alu*I fragment was isolated, and *Hin*dIII linkers were ligated to its ends. Its insertion into the *Hin*dIII site of pBH20 is shown in Fig. 1. No known φX174 promoter sequences are included in this segment (Fig. 1; 28). Transcription of the fragment can therefore be controlled by the *lac* promoter when the plasmid is maintained in a host bacterium which overproduces the *lac* repressor (via the $lacI^{q}$ allele). The addition of IPTG allows transcription of the inserted DNA. If the φX174 fragment is in the opposite orientation to that shown in Fig. 1, transcription of sequences coding for genes *E* and *J* cannot be induced by IPTG but may still be transcribed at a low level from a constitutive promoter in the pBR322 portion of the vector (27, 30).

Expression of φX174 gene *E* necessary and

sufficient for lysis. Induction with IPTG of *E. coli* RY1197 carrying the E^+ plasmid pKY67 results in rapid lysis after 35 to 40 min. No lysis is observed in a nonsuppressing host when the *E*am plasmid pKY92 is induced, but the induction of this plasmid does result in lysis in a *sup*F host (Fig. 2). Another host, CQ, regularly requires 55 to 60 min after induction before lysis occurs (see Fig. 4 and 5).

The nucleotide sequence for gene *J* is present in the pKY67 and pKY92 plasmids. No mutation has yet been isolated in gene *J*, although it has been identified as a major capsid protein (1, 31). It is therefore conceivable that gene *J* could play a role in lysis.

To establish whether gene *E* alone is sufficient for producing lysis, deletions of *J* were constructed with plasmid pKY67. The plasmid was linearized by cleavage at the unique *Hpa*I site downstream from the end of gene *J* (Fig. 1). *Bal*-31 nuclease was added to delete as much of the *J* sequence as possible while leaving the *E* gene sequence intact. By using appropriate *Bal*-31 digestion conditions, the *Bam*HI site downstream from *E* was regenerated at the endpoint of the deletion into the ϕX174 sequence. Plasmids with deletions of various sizes in *J* were isolated and are shown in Fig. 1. That gene *E* is alone sufficient to trigger the lysis event is demonstrated by the response of these *J* deletion clones to induction by IPTG. Even in those clones in which there is very little *J* gene remaining (e.g., pKY100; Fig. 1), lysis still occurs to the same extent as in its presence (data not shown).

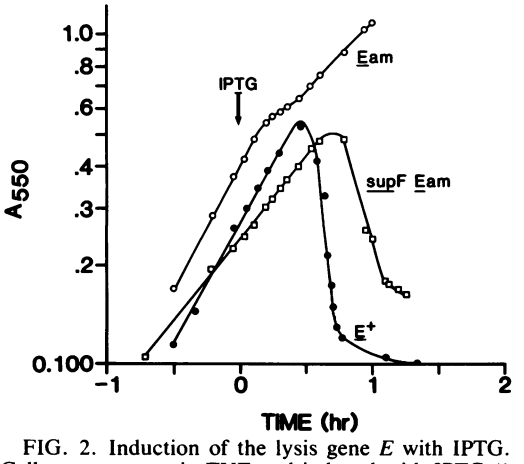

FIG. 2. Induction of the lysis gene *E* with IPTG. Cells were grown in TYE and induced with IPTG (1 mM final concentration) at the time indicated by the arrow. The host was either the suppressorless RY1197 or the *sup*F cell RY1109. The plasmids were pKY67 (E^+) or pKY92 (*E*am3). Symbols: ●, RY1197(pKY67); ○, RY1197(pKY92); □, RY1109(pKY92). A_{550}, absorbance at 550 nm.

Expression of ϕX174 gene *E* and phage-induced lysis. Several experiments verified that the observed characteristics of cell lysis by intact ϕX174 can be reproduced by the cloned *E* gene.

Lysis by infection with intact phage is inhibited by 0.2 M $MgSO_4$ (13). This concentration of $MgSO_4$ also inhibited lysis of E^+ clones induced with IPTG (Fig. 3A). Morphological changes over time indicated that lysis inhibition was accompanied by spheroplast formation (Fig. 3B). Control cells elongated when exposed to $MgSO_4$ but never formed spheroplasts. This cell-to-sphere transformation was exactly as described for phage-infected cells in the presence of $MgSO_4$ (13).

Spermine also inhibits lysis in phage-infected cells (12). The effects of spermine on lysis of an induced E^+ clone are shown in Fig. 4. Addition of spermine soon after induction (3 and 12 min) completely inhibited lysis. Addition of spermine at later times (37 and 57 min) stabilized the cells against complete lysis. These effects paralleled those observed after addition of spermine to infected cells (12).

A chloramphenicol-sensitive step has been described for lysis by intact phage (23). Figure 5 shows the results of the addition of chloramphenicol at various times to an induced E^+ clone. Up to at least 45 min after induction, addition of chloramphenicol completely abolished the lysis event. Near the normal time of lysis onset, however, addition of chloramphenicol was not inhibitory; in fact, it slightly accelerated lysis.

That lysis is preceded by spheroplast formation has been reported for ϕX174 and a similar phage, α3 (4, 5, 12). Lysis of an induced E^+ clone was also preceded by a spheroplast stage (Fig. 3B). The intermediate forms pictured are morphologically very similar to those of the α3-infected cells of Bradley et al. (4, 5).

Cell shape aberrations and gene *E*. The previous lysis results were obtained with plasmids in which gene *E* was inserted in the proper orientation for expression from the *lac* promoter of pBH20, as shown in Fig. 1. However, a different result was obtained with plasmids having gene *E* in the reverse orientation, which results in a constitutive low-level expression of *E* from minor counterclockwise promoters in the pBR322 sequence (27, 30). Bacteria with these plasmids were viable and were insensitive to induction by IPTG. Nevertheless, upon primary isolation, especially in minimal media, they displayed a high percentage of abnormal and misshapen cells (Fig. 6), including cells which have a bifurcated or "two-head" morphology. Although grossly deformed, most of these cells were still motile and elongating.

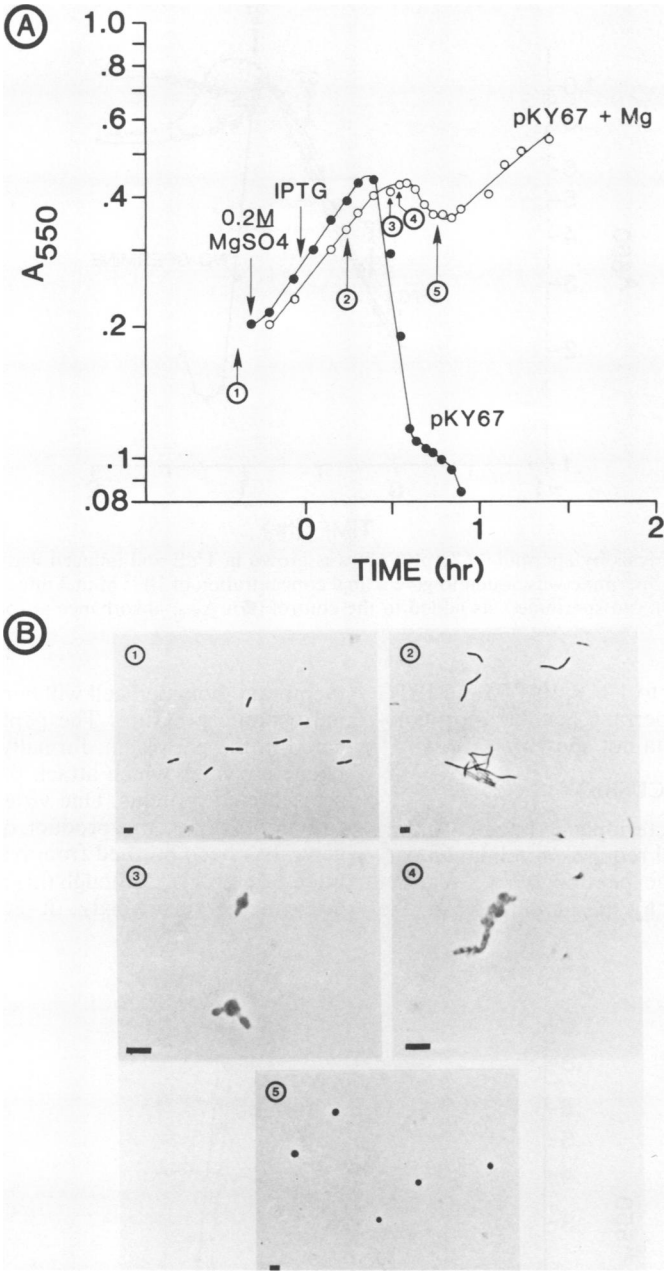

FIG. 3. Inhibition of lysis by $MgSO_4$. (A) RY1197(pKY67) was grown in TYE in the presence (○) or absence (●) of 0.2 M $MgSO_4$. IPTG was added at the time indicated. The circled numbers refer to the points at which photographs were taken. A_{550}, absorbance at 550 nm.(B) Photographs of RY1197(pKY67) + 0.2 M $MgSO_4$ at the time indicated on the graph in (A). Bar, 5 μm.

The dependence of these morphologies on expression of gene *E* was shown in two ways. First, equivalently reversed clones of the *E*am gene showed no such morphological effect. Second, an equivalent set of forms could be induced by low levels of IPTG in bacteria carrying plasmids with the properly oriented E^+ gene. The latter effect was very delicately balanced between IPTG concentrations having no effect at all and those resulting in complete lysis. No lysis occurred at 1.2×10^{-5} M IPTG, and total lysis was achieved with 1.8×10^{-5} M IPTG. Only in

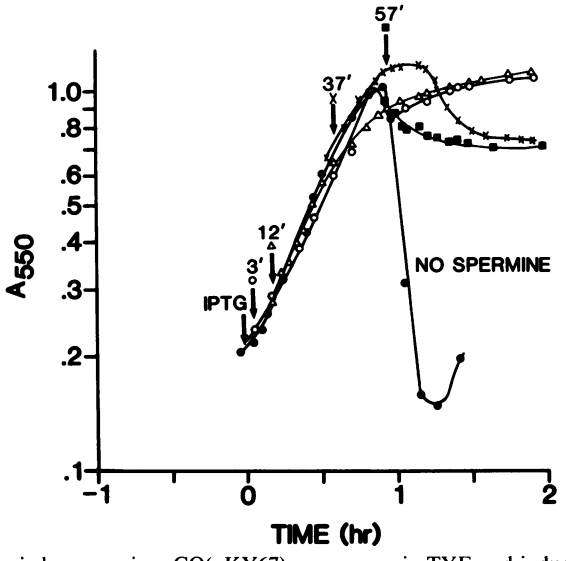

FIG. 4. Inhibition of lysis by spermine. CQ(pKY67) was grown in TYE and induced with IPTG at the time indicated by the arrow. Spermine was added to give a final concentration of 10^{-2} M at 3 min (○), 12 min (△), 37 min (X), and 57 min (■). No spermine was added to the control (●). A_{550}, absorbance at 550 nm.

the range 1.4×10^{-5} to 1.6×10^{-5} M did IPTG induce nonlysing, aberrant cellular morphologies in E^+ clones (data not shown).

DISCUSSION

The lysis of a bacteriophage-infected gram-negative bacterial cell requires at minimum partial destruction of the peptidoglycan. Without the rigidity provided by the peptidoglycan, the membrane-bounded cell will burst from its internal osmotic pressure. The peptidoglycan is located in the periplasm; formally then, bacteriophage enzymes which attack the peptidoglycan are secreted proteins. One viral-encoded bacteriolytic enzyme, the product of the lambda *R* gene, has been purified from lysates of lambda-infected cells (3). Although this must be a secreted protein, surprisingly, it is not detectably

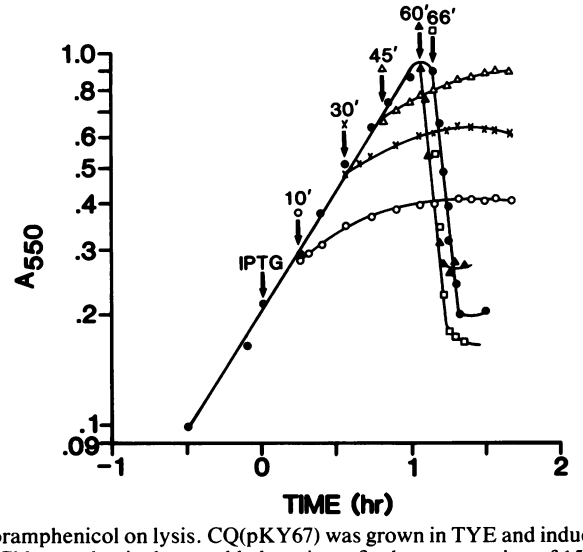

FIG. 5. Effect of chloramphenicol on lysis. CQ(pKY67) was grown in TYE and induced with IPTG at the time indicated by the arrow. Chloramphenicol was added to give a final concentration of 150 μg/ml at 10 min (○), 30 min (X), 45 min (△), 60 min (▲), and 66 min (□). No chloramphenicol was added to the control (●). A_{550}, absorbance at 550 nm.

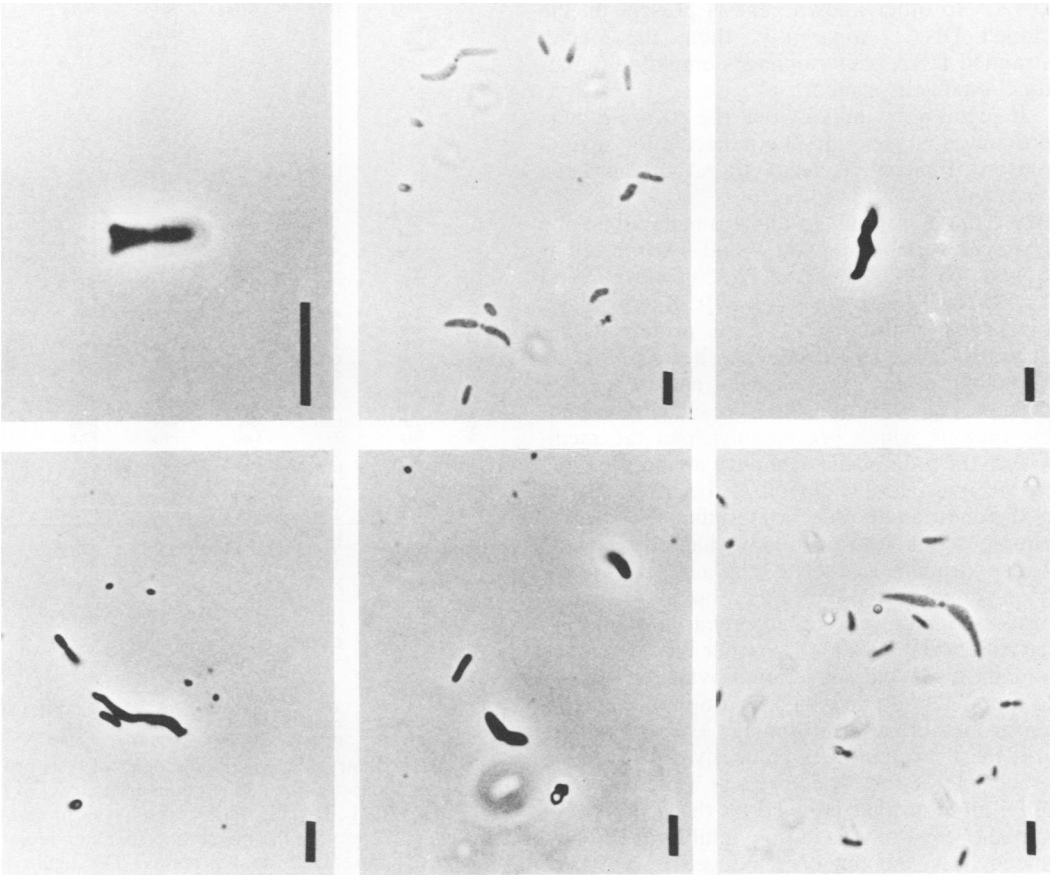

FIG. 6. Photographs of RY1197(pKY64) grown in minimal glycerol medium. pKY64 contains the same E^+ J^+ insert as does pKY67, but in the opposite orientation from that in Fig. 1. Bar, 5 μm.

processed; that is, its amino acid sequence is identical to that predicted from the DNA sequence of the *R* gene (17; D. L. Daniels, Ph.D. thesis, University of Wisconsin, Madison, 1981). p*R* gains access to the peptidoglycan through the action of a distinct bacteriophage-encoded protein, the product of the *S* gene. This appears to be a novel form of polypeptide transmembrane secretion and is under study in this laboratory. A similar system appears to function in bacteriophage T4, where the *t* and *e* genes fulfill the roles of λ *S* and λ *R* (19, 33). Thus, these two complex bacteriophages employ a "binary" lysis system elaborating different proteins to attack the cytoplasmic membrane and the peptidoglycan. However, the small single-stranded DNA bacteriophage φX174 appears to utilize a distinct, monofunctional mechanism for inducing host cell lysis.

The one gene directly implicated in lysis by φX174 is the *E* gene. Infection with an *E* mutant results in a complete absence of lysis and an extended period of bacteriophage particle accumulation (16); in contrast, E^+ infections result in rapid lysis of the infected cells after about 20 to 25 min in rich medium. Unfortunately, it has not been possible to exclude the participation of all other φX174 genes in lysis, mainly because mutants in some genes have not been obtained. Furthermore, some workers have suggested that capsid polypeptides may be involved in lysis (31). Since the complex, double-stranded DNA bacteriophages utilize a binary system and the complex gram-negative envelope would seem to require several different protein functions for its destruction, it would seem reasonable to suggest that some other φX174 gene was pleiotropic and possessed a lysis function in addition to its structural role.

The data presented here, however, demonstrate unequivocally that the *E* gene alone among the φX174 genes is required for host cell lysis. The *J* gene can be ruled out, since its nearly complete removal from φX174 DNA inserted into a cloning plasmid has no effect on lysis induced by the expression of that inserted

DNA. No other known gene is present on the cloned DNA. Apparently, then, the single-stranded DNA bacteriophages employ a monofunctional lysis system.

It seems very unlikely that the φX174 *E* gene product is a lysozyme-like molecule for several reasons. First, no bacteriolytic activity has been detected in φX174-infected cells (9, 10, 23). Also, the *E* protein is much smaller than the lysozymes elaborated by λ and T4 (molecular weight, ca. 10,000 versus 17,500 for λ p*R* and 18,635 for T4 lysozyme [17, 33]). Interestingly, the primary structure of the *E* protein as deduced from its DNA sequence bears a striking homology at the NH_2-terminal end to the λ *S* protein (Fig. 7). Both have 20-residue hydrophobic regions which presumably span the membrane, the amino acid sequence amino proximal to the hydrophobic region is the same size in both genes, and both genes terminate the hydrophobic region with positively charged residues. These primary sequence characteristics have been shown to be shared by a number of membrane-spanning proteins in procaryotic and eucaryotic organisms (34). Neither the T4 nor the lambda lysozymes have any obvious homology to *E* or *S*, nor do they have hydrophobic stretches for membrane insertion (17, 33).

If the *E* protein does induce lysis by a membrane interaction, it does so in a manner very different from that induced by the *S* protein of lambda. Spermine addition inhibits φX174-induced lysis, but not lysis induced by λ S^+ R^+ (12). Addition of chloramphenicol, sodium azide, or potassium cyanide to lambda-infected cells or to cells induced to express cloned lambda lysis genes results in an immediate "premature" lysis event (11). None of these produce early lysis in *E*-containing clones (Fig. 6 and unpublished observations). Sodium azide addition actually stops further lysis in the *E*-containing clones when added at times after lysis onset (unpublished observations). These observations suggest that cellular activity may temporarily repress lysis by lambda, but that some ongoing cellular activity is required for lysis by φX174.

If p*E* is not a lysozyme and resembles p*S* in its primary sequence and size, how can φX174 *E* gene product achieve more (i.e., lysis and concomitant release of bacteriophage particles) than λ S^+ R^- (which cannot lyse nonsuppressor host cells)? It has been suggested that φX174 activates host autolytic enzymes which are part of the peptidoglycan synthesis system (21). Since the cloned φX174 gene *E* produces morphological aberrations (Fig. 3B and 6), it is reasonable to postulate that p*E* interferes with normal cell shape control mechanisms, perhaps acting through cell division or peptidoglycan elongation processes.

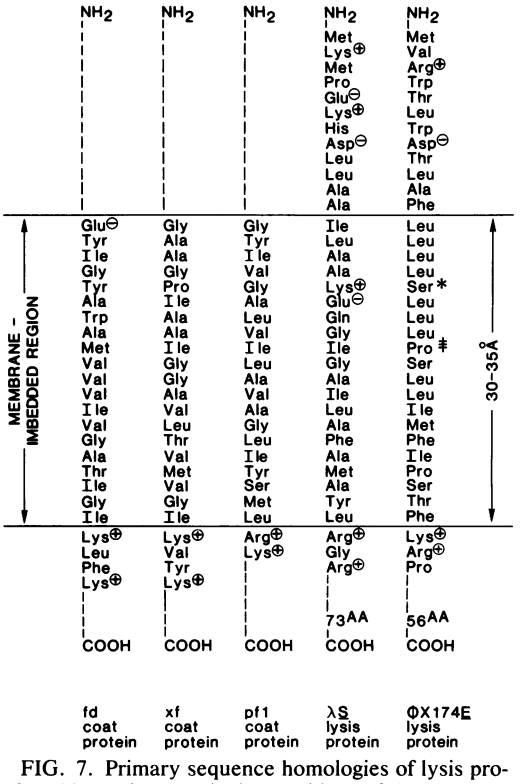

FIG. 7. Primary sequence homologies of lysis proteins. The amino terminal 35 residues of the φX174 *E* (28) and λ *S* (D. L. Daniels, Ph.D. thesis) lysis proteins are shown, along with the membrane-spanning region of three bacteriophage coat proteins. The figure is taken from von Heijne (34), with the λ *S* and φX174 *E* sequences added. The hypothetical membrane-spanning region of the *S* and *E* sequences are similar to each other and to the coat proteins in length and in the two positively charged residues at the cytoplasmic boundary of the region. Note other similarities between *S* and *E*: in both, there are 12 residues with a net neutral charge extending beyond the hypothetical membrane-spanning region; there are "helix-breaking" residues (at #) at the same site in both membrane regions (Pro in *E*; Gly-Leu-Gly in *S*); there are highly hydrophilic residues at the same site (*) in both membrane regions (Ser in *E* and the Lys^+-Glu^- salt-bridged residue pair in *S*).

Given that φX174 lyses host cells by a monofunctional mechanism which interferes with host control processes, why do the complex bacteriophages require a more complex binary system? We suggest that lambda, with a capsid diameter of 62 nm and an extended length of 235 nm (counting the tail and the tail fiber [24]), requires larger holes in the peptidoglycan layer than does φX174 to permit release of the progeny virions. The φX174 particles are isometric and much smaller, with a 32-nm diameter. The size of the lesion, however transitory in the peptidoglycan,

would thus have to be nearly an order of magnitude larger to allow release of lambda particles than needed for φX174. How the size of the lesion is determined is not known; however, it is reasonable to suggest that the binary lysis system, with its viral-encoded lysozyme activity, is more efficient at developing such lesions. In support of this possibility, electron microscopy of φX174-lysed cells reveals only very limited cell wall damage, whereas lysozyme action would be expected to extensively degrade the wall (23). Furthermore, there is evidence that lambda lysis is strictly regulated (11); this regulation causes an intracellular accumulation of bacteriolytic activity before lysis (11) and may permit a more violent or explosive lytic event and thus result in a larger lesion. In contrast, the φX174 *E*-dependent lysis is not as kinetically distinct, leaves much of the morphology of the cell intact (29), and is heavily dependent on the metabolic fitness of the cell. One prediction of this rationale is that *E*-mediated lysis will not result in efficient release of bacteriophage lambda particles, whereas *S R*-mediated lysis will be very efficient in releasing φX174 particles. This prediction is now being tested in our laboratory.

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ADDENDUM IN PROOF

While this manuscript was in preparation, B. Henrich, W. Lubitz, and R. Plapp reported cloning the same lysis region of φX174 (Mol. Gen. Genet. **185**:493–497, 1982).

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