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ϕX174 *E* Complements λ *S* and *R* Dysfunction for Host Cell Lysis

WILLIAM D. ROOF AND RY YOUNG*

Department of Biochemistry and Biophysics, Texas A&M University, College Station, Texas 77843-2128

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Hybrid lambda phages which have the *E* lysis gene of the bacteriophage ϕX174 in *cis* to defective nonsense and deletion alleles of the normal lambda lysis genes *S* and *R* have been constructed and shown to be fully competent for plaque-forming ability, which demonstrates that the single-gene, lysozyme-independent lysis system of ϕX174 and related phages can serve the lytic function for large complex phages. These hybrid phages are unable to form plaques on a *slyD* host. Moreover, plaque morphology indicates that in *E*-mediated lysis the soluble λ *R* endolysin can participate in lysis, indicating that the protein E-mediated lesions are not completely sealed off from the periplasm.

There appear to be at least two fundamentally different strategies by which bacteriophage accomplish lysis of the host cell (22). For most phages, including such disparate types as the lambdoid, T-even, and T-odd phages, two genes are required. One gene encodes an enzyme which degrades the peptidoglycan (e.g., the *e* lysozyme of T4 or the *R* transglycosylase of phage lambda). These enzymes appear during late protein synthesis as soluble cytoplasmic proteins, without a signal sequence to engage the *sec*-mediated secretory system. A second gene that encodes a protein that forms holes in the inner membrane, allowing the murein-degrading activity access to the periplasm, is required. These genes have been designated holin genes, the prototype for which is λ *S* (22). Holin function thus determines the termination of the vegetative cycle, and not surprisingly, holins have, at least in λ and T4, been shown to be under intricate functional regulation (1, 10). In contrast, the small single-stranded DNA phage ϕX174 has only a single lysis gene, *E*, which encodes a 91-residue polypeptide without murein-degrading activity (9, 17). A single host gene, *slyD*, is required for ϕX174-mediated lysis (12). Despite this genetic simplicity, the mechanism of ϕX174 lysis is obscure. No murein-degrading activity can be detected in ϕX174-infected cells (5). [^{3}H]diaminopimelate incorporation into murein stops at about the time when lysis is first detectable, and about half of the recently incorporated diaminopimelate is solubilized during lysis, indicating that cellular autolysins are activated (11). Wild-type cells, but not *slyD* mutants, infected with ϕX174 or related phages undergo dramatic morphological changes, notably the development of distended bulges near the cell midpoint (see Fig. 4) (3, 4). Electron micrographs of thin sections show clearly that the cell wall and outer membrane are disrupted, so that the complete cell sac is seen to emerge, leaving the broken but otherwise intact and empty sacculus (3, 4). This process terminates in saltatory lytic events in which almost all the progeny phage particles are released over a 1-min interval (8), presumably because of bursting of the unprotected membrane sac. Lysis can also be achieved if the *E* gene is cloned under the control of a heterologous promoter and induced, even in the absence of other phage genes, as long as the host *slyD* gene is functional (2, 7, 12, 21). However, the morphology of cells lysed by the cloned *E* gene is different. Light microscopy reveals that cells lysed as a result of an induction of a multicopy clone of *E* have the normal rod shape but appear to be empty of refractile contents. Moreover, Witte et al. (20) have presented scanning electron micrographs of *E*-lysed cells showing a single hole with a diameter of 50 to 100 nm, located either at the cell pole or at the midpoint. A transverse section of one "*E*-hole" indicated that the lesion spans the entire envelope. Also, *E*-mediated lysis has been shown to release cytoplasmic but not periplasmic enzyme activities (19), supporting the notion that the lesion is a *trans*-envelope "tunnel", sealed off from the periplasm.

One rationale for the existence of these different strategies is that the larger T4 and lambdoid phages might require total

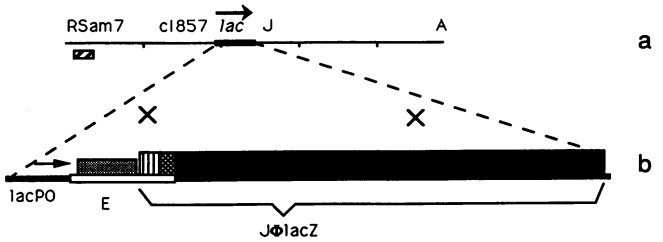

FIG. 1. Construction of hybrid lysis phages. The initial construct was plasmid pSM120, which has the ϕX174 *E* lysis gene (stippled region) cloned under *lacPO* control (b). Five base pairs downstream from the *E* gene is a reporter gene consisting of 20 codons of the ϕX174 *J* gene (vertically striped region) and 39 bp from the pBR322 *tet* gene fused (hatched region) in frame with codon 8 of *lacZ*. This plasmid can be induced to cause lysis, as reported for the parental plasmid pKY120 (21) from which pSM120 is derived by blunt-end filling of the unique *Bam*HI site (14). The entire *EJΦlacZ* construct was transferred to λp*lac5 Sam*7 by homologous recombination, as indicated by the large X denoting crossover events with the flanking *lac* homology (b). (The bar indicates *lac* sequences. In panel a, the normal λ gene order is reversed for clarity; the arrow denotes the direction of transcription of *lac*). The recombinant phage λ168 is λ$lacE_{\phi X}$ *c*I857 *Sam*7, where $lacE_{\phi X}$ denotes the ϕX174 *E* lysis gene, as distinguished from the λ *E* (capsid protein) gene, under *lacPO* control. The deletion of the *S* and *R* genes (indicated by the hatched box in panel a) was incoporated by crossing with λΔ*SR* (15), yielding λ172, or λp$lacE_{\phi X}$cI857 Δ(*SR*). Finally, this construct was crossed with a phage carrying *Sam*7 *R*am54*am*60, and a recombinant was selected with the genotype λ175, or λp$lacE_{\phi X}$cI857*Sam*7Ram54Ram60.

* Corresponding author. Electronic mail address: YOUNG@BIOCH.TAMU.EDU.

TABLE 1. $E_{\phi X}$ suppresses lysis gene mutations on wild-type but not *slyD* hosts[a]

Bacterial lawn	Efficiency of plating					
	λ168 ($E_{\phi X}^+$ *Sam* R$^+$)		λ172 [$E_{\phi X}^+$ Δ(SR)]		λ175 ($E_{\phi X}^+$ *Sam* Ram)	
	+IPTG	−IPTG	+IPTG	−IPTG	+IPTG	−IPTG
RY2131 (*slyD*$^+$)	0.49	$\approx 10^{-6}$	3.1	$<10^{-7}$	0.22	$<10^{-7}$
RY2047 (*slyD1*)	$\approx 10^{-6}$	ND[b]	$<10^{-7}$	ND	ND	ND
RY2347 (*slyD*$^+$ *supE*)	0.87	0.59	ND	ND	ND	ND
RY2441 (*supE supF*)	1.0	ND	1.0	ND	1.0	ND

[a] Bacteriophage suspensions were diluted in λ dilution buffer (0.01 M $MgCl_2$, 20 mM Tris HCl, pH 8.0) and plated by standard plating techniques (15) on lawns of bacteria with the indicated genotypes. Strain RY2131 is *E. coli* K-12 *ara leu lacI*^{q1} *lacZ*::Tn*5 purE gal his argG rpsL xyl*, a nonsuppressing derivative of the Cold Spring Harbor strain CSH57 (13). RY2047 and RY2347 are isogenic derivatives of RY2131. RY2441 is LE392 (16), a double-suppressor strain, not isogenic to the RY2131 derivatives. The efficiency of plating in this context is the plaque-forming titer relative to the titer on the double-suppressor RY2441. The absolute titers on RY2441 for λ168, λ172, and λ175 were 3.9×10^9, 4.8×10^8, and 1.9×10^9, respectively. The allelic state of *slyD* has no effect on the plating efficiency of λ S$^+$ R$^+$ (data not shown).

[b] ND, not determined.

cellular disruption to ensure efficient release of the progeny virions, whereas the smaller φX174 can escape readily through the *trans*-envelope *E*-holes. That is, a lytic strategy which does not involve extensive degradation of the peptidoglycan might put an upper limit on the size of released particles. To test this concept and, more generally, to assess whether the single-gene, autolysin-dependent lysis strategy of φX174 could serve as the lysis system for a larger phage, we constructed bacteriophage λ derivatives which carry the φX174 *E* lysis gene under *lacPO* control and defective alleles of the required lysis of genes *S* and *R* (Fig. 1). λ168 carries the *Sam7* allele, which is nonfunctional except in *supF* hosts. As expected, λ168 plates efficiently on an *supF* bacterial lawn, but λ168 also plates on a nonsuppressing bacterial lawn if the inducer isopropyl-β-D-thiogalactopyranoside (IPTG) is present (Table 1). The implication that *E* function is complementing the *Sam* defect is confirmed by the block imposed by the presence of the *slyD* mutation (Table 1). The rare plaque-forming phages which do arise on the *slyD* lawn were tested, and in eight of eight cases, the revertants plated on the nonsuppressing bacterial lawn in the absence of inducer, demonstrating that the *Sam7* allele had reverted (not shown). The plaque-forming ability of λ168 might not be a true reflection of *E* lytic competence, since the λ *R* gene is also functional in this phage. Incorporation of an *SR* deletion, however, did not change the complementation pattern (Table 1). Thus, *E* complements both *S* and *R* defects, demonstrating that the *E*-dependent lysis pathway is capable of causing the release of lambdoid phage particles. The plaques are smaller with λ175 (Fig. 2), which is isogenic to λ168 except for bearing nonsense mutations in both principal lambda lysis genes, *S* and *R* (see the legend to Fig. 1). Also, with the $E_{\phi X}$ *Sam* Ram phage, the lytic profile is much less sharply defined in liquid culture than with the R^+ phage (Fig. 3), indicating that in $E_{\phi X}^+ R^+$ conditions, the *R* transglycosylase does gain access to the murein contemporaneously with *E*-mediated lysis. This result implies that the putative transmembrane tunnel described by Witte et al. (20) does not completely seal off the periplasm. In any case, the cellular morphology characteristic of the cloned *E* gene is maintained: after lysis is detected by a dramatic fall in A_{550}, phase-contrast microscopy reveals empty cells with otherwise undisturbed morphology (Fig. 4). In contrast, cells lysed by the normal lambda lytic system are totally destroyed, reflecting the essential role of murein degradation in this event (Fig. 4). These findings thus eliminate the hypothesis that single-gene, lysozyme-independent lysis is inherently incapable of supporting the lysis required for plaque formation in large complex phage. Presumably, the more complex lysis strategy of T4 and λ reflects both the selective

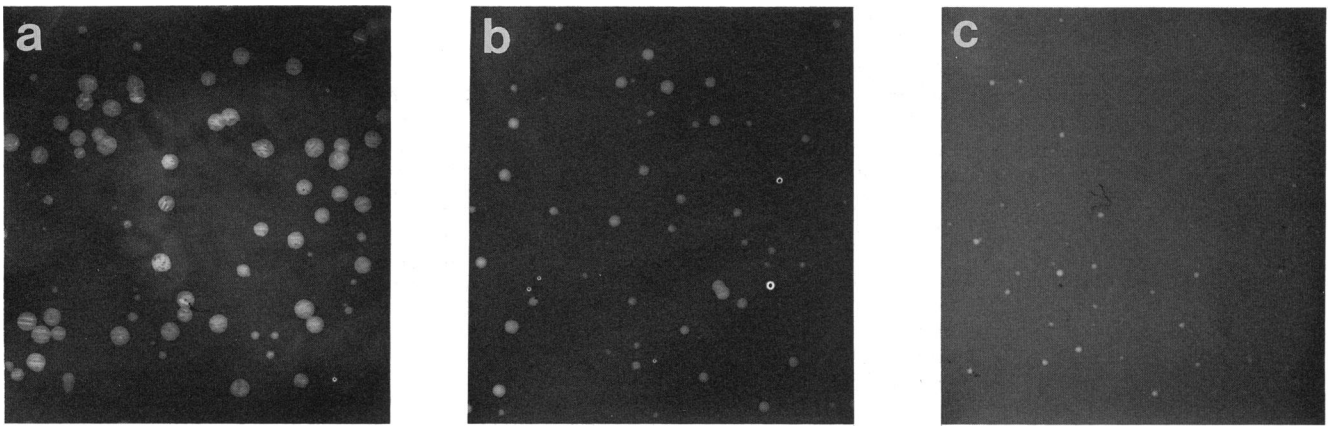

FIG. 2. Plaque morphology of λ carrying φX174 lysis gene *E*. Phages were diluted in λ dilution buffer (10 mM $MgCl_2$, 20 mM Tris, pH 8), and 0.1 ml was added to 0.1 ml of a freshly saturated culture of the nonsuppressing indicator bacteria MC4100 Δ*lac ara rpsL thi relA*. After preadsorbtion for 30 min at room temperature, the infected culture was mixed with 2.5 ml of soft agar, poured evenly on standard TB plates (13), and incubated overnight at 37°C. (a) λ S^+ R^+; (b) λ168 ($E_{\phi X}$ *Sam7* R^+); (c) λ172 [$E_{\phi X}$ Δ(*SR*)].

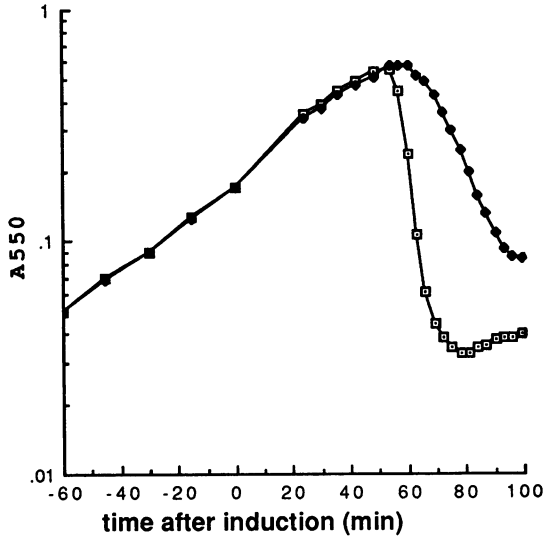

FIG. 3. Effect of endolysin gene function on *E*-mediated lysis. Lysogens of λ168 (R^+) (◻) or λ175 (R^-) (◆) were grown to an A_{550} of 0.02 in LB medium containing 1 mM IPTG (13) at 30°C, shifted to 42°C with aeration for 20 min to effect thermal induction, and then aerated at 37°C until completion of the lytic cycle. The lysogenic host was *E. coli* K-12 strain MQ, a $lacI^q$ lac^+ isogenic derivative of MC4100 (see the legend to Fig. 2).

advantage conferred by a carefully regulated lytic function and the availability of evolutionarily pliable genomic space. In contrast, *E* does not appear to be under active regulation of any kind and is expressed at a low level from all φX174 mRNA species (6).

It is interesting to note that constitutive expression of the *E* gene from *lacPO* in the uninduced prophage has no apparent effect on the cell, indicating that a substantial gene dosage is required for the lytic effect. Also, it is worth noting that the cellular morphology observed here during lysis matches that reported for the cloned *E* gene (18), but not that observed in infections of φX174 and related phages (3, 4). Whether this cellular morphology results from a qualitative difference in the lytic pathway (i.e., the auxiliary function of one of the nine other φX174 genes) or a quantitative difference in the level of *E* expression is under investigation in this laboratory.

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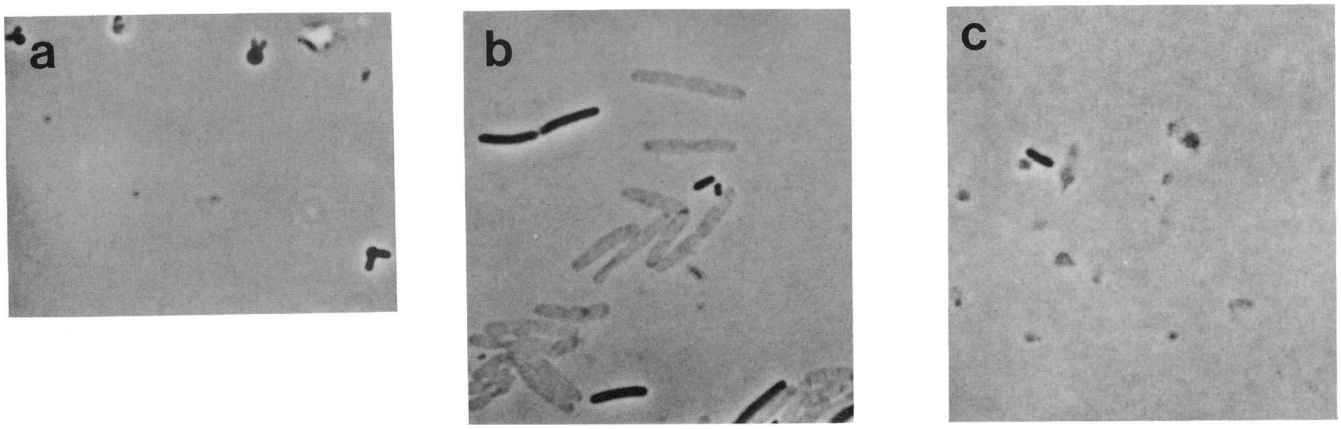

FIG. 4. Morphology of cell lysis by phase-contrast microscopy. (a) *E. coli* C strain C990 infected with φX174 at a multiplicity of infection of 5, sample taken after 30 min of infection; (b and c) *E. coli* K-12 strain MQ lysogens thermally induced as described in the legend to Fig. 3. Samples for phase-contrast microscopy were taken after allowing the A_{550} to decrease by approximately 50%. (b) Lysogen of λ172. No IPTG was added, since the host is Δ*lac*. (c) Lysogen with normal λ lysis genes (i.e., S^+ R^+). Magnifications, ×400.

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