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Lysogenization of *Escherichia coli him*$^+$, *himA*, and *himD* Hosts by Bacteriophage Mu

ROBERT B. BOURRET† AND MAURICE S. FOX*

Department of Biology, Massachusetts Institute of Technology, Cambridge, Massachusetts 02139

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The possible outcomes of infection of *Escherichia coli* by bacteriophage Mu include lytic growth, lysogen formation, nonlysogenic surviving cells, and perhaps simple killing of the host. The influence of various parameters, including host *himA* and *himD* mutations, on lysogeny and cell survival is described. Mu does not grow lytically in or kill *him* bacteria but can lysogenize such hosts. Mu c^+ lysogenizes about 8% of *him*$^+$ bacteria infected at low multiplicity at 37°C. The frequency of lysogens per infected *him*$^+$ cell diminishes with increasing multiplicity of infection or with increasing temperature over the range from 30 to 42°C. In *him* bacteria, the Mu lysogenization frequency increases from about 7% at low multiplicity of infection to approach a maximum where most but not all cells are lysogens at high multiplicity of infection. Lysogenization of *him* hosts by an assay phage marked with antibiotic resistance is enhanced by infection with unmarked auxiliary phage. This helping effect is possible for at least 1 h, suggesting that Mu infection results in formation of a stable intermediate. Mu immunity is not required for lysogenization of *him* hosts. We argue that in *him* bacteria, all Mu genomes which integrate into the host chromosome form lysogens.

The goal of our work is to try to understand what determines the outcome of a particular infection of *Escherichia coli* by the temperate bacteriophage Mu. The outcomes themselves vary in difficulty of assessment. Bacteria in which phage growth is lytic are easily assayed as PFU, even at a low multiplicity of infection (MOI).

Ignoring rare Mu^r mutants, the viable cells present after a high-multiplicity infection with Mu are lysogens or "nonlysogenic survivors." The latter comprise a substantial fraction of the total (22) and either survive infection without acquiring a Mu prophage or escape infection entirely.

Mu is capable of killing the host cell under special circumstances in which lytic growth cannot occur (50). For a normal infection, it is not known and indeed would be difficult to determine how frequently Mu kills the host without producing a phage burst. This class of events makes it difficult to know the fraction of biologically active Mu particles accounted for as PFU.

There are numerous regulatory factors which may influence the possible outcomes of a Mu infection. Integration of the Mu genome into the host bacterial chromosome occurs during both lytic growth (35) and lysogenization (25). The roles of integration and immunity in the events which fail to lead to either lytic growth or lysogenization are unknown.

Mu lytic growth proceeds by replicative transposition (reviewed in reference 7). The product of the *A* gene, transposase, is essential for integration and all of the DNA rearrangements catalyzed by Mu. In the absence of the Mu B function, these DNA transactions occur at 10- to 100-fold-reduced frequency.

The Mu repressor gene *c* is transcribed leftward (49) from the p_C promoter (20, 30), whereas the "early operon" (*ner*, *A*, *B*, and the semiessential early region) is transcribed rightward from p_E (20, 31). The ner repressor is required for lytic growth (18). The c and ner repressors each negatively regulate both p_C and p_E, although with different affinities (30, 48; N. Goosen, Ph.D. thesis, Rijksuniversiteit, Leiden, 1984), allowing Mu early gene expression to be controlled as a bistable switch (19). Consistent with the predominance of Mu lytic growth over lysogenization, p_E is a stronger promoter than p_C (30; Goosen, Ph.D. thesis).

Bacteriophage Mu does not grow lytically in or kill *himA* or *himD* hosts (27, 38). Reduced transcription from p_E (16, 30; R. K. Yoshida, Ph.D. thesis, University of Wisconsin, Madison, 1984) can account for these properties.

Mu lysogenization data have often been reported as lysogens per surviving cell, presumably for technical convenience. This formulation is the ratio of one outcome (lysogenization) to the sum of two outcomes (formation of lysogens and of nonlysogenic survivors) and ignores the common outcome of a Mu infection, i.e., lytic growth. In contrast, determination of the number of lysogens formed per infected cell normalizes one outcome against the total number of outcomes that can be calculated from PFU measurements. It has the additional advantages of being applicable at low MOI and in circumstances where Mu is incapable of killing its host.

We report here the effects of MOI, temperature, various phage and host mutations, and interactions between different phage genomes present in multiply infected bacteria on the frequency of Mu lysogens formed per infected cell.

MATERIALS AND METHODS

Media and chemicals. TCMG medium (51), Mu buffer (6), and SMO medium (26) have been described. LB medium was 10 g of tryptone (Difco Laboratories, Detroit, Mich.), 5 g of yeast extract (Difco), 5 g of NaCl, and 1 ml of 1 N NaOH per liter; 10 g of Bacto-Agar (Difco) was added for plates. Soft agar was 8 g of nutrient broth (Difco), 5 g of NaCl, and 6.5 g of Bacto-Agar per liter. SoftCaMg and LBCaMg signify soft agar and LB, respectively, supplemented with 1 mM $CaCl_2$ and 2.5 mM $MgSO_4$.

Stock solutions of 1 M ethylenebis(oxyethylenenitrilo) tetraacetic acid (EGTA; Eastman Kodak Co., Rochester, N.Y.) were adjusted to pH 8 with NaOH.

Titration of bacteria and bacteriophage. Titration of bacte-

* Corresponding author.
† Present address: Division of Biology, California Institute of Technology, Pasadena, CA 91125.

ria or phage was done on duplicate plates at dilutions intended to yield about 200 colonies or plaques per plate. Bacterial cultures were diluted in 0.85% NaCl; samples were mixed with 2 ml of soft agar–50 mM EGTA, poured onto LB plates, and incubated overnight at 37°C.

Samples to be titrated for Mu were diluted in Mu buffer and mixed with W3110 plating culture (~6×10^8 to 8×10^8 CFU/ml in LBCaMg). W3110*supF* was used for Mu *B*am phage. After 15 min of adsorption at 37°C, 2 ml of softCaMg was added, the mixture was plated on TCMG, and the plates were incubated overnight at 37°C.

Mu stocks. Mu *c*ts lysogens were induced by growing cells at 30°C in LB–10 mM $MgSO_4$ to ~2×10^8 CFU/ml, shifting the culture to 43°C for 30 min, and then shifting to 37°C until lysis. At 35 min postinduction, EGTA was added to 2 mM to selectively chelate Ca^{2+} (which is required for Mu adsorption [46]) and prevent readsorption to debris.

Stocks of Mu c^+ phage ($\geq 5 \times 10^{10}$ PFU/ml) were obtained from two cycles of liquid infection. W3110 was grown in LBCaMg at 37°C to ~10^8 CFU/ml, infected with Mu at a final concentration of 2×10^7 PFU/ml, and allowed to adsorb for 15 min before aeration was resumed. Higher concentrations of Mu *c*+pAp5 (1×10^8 PFU/ml) and Mu c^+pf7701 (2×10^8 PFU/ml) were used to compensate for poor growth. At 105 min postinfection, EGTA was added to a 2.5 mM final concentration, and incubation was continued until lysis was complete.

Mu lysates were treated with chloroform, centrifuged to remove debris, and concentrated and purified on gradients with steps of 1.3, 1.4, and 1.6 g of CsCl per ml in SMO–1 mM $CaCl_2$–10 mM $MgSO_4$–1 mM $CdCl_2$. After 2 h of centrifugation (Beckman SW28 rotor; 17°C; 87,000 × *g*), the phage band (47) was collected, dialyzed at 4°C against Mu buffer without gelatin–1 mM $CdCl_2$ twice for 1 to 4 h, and stored at 4°C.

Bacteria. The bacterial strains used are listed in Table 1. RBB67 was constructed by P1 transduction of *supF trp*::Tn*5* from NK5857 (N. Kleckner) into W3110. RBB116 was a spontaneous Trp^+ Kan^s revertant (frequency, ~10^{-9}) of RBB67. RBB189 and RBB193 were isolated after low-multiplicity infection and therefore are presumed to be monolysogens.

Y1100 is a λ *c*I857 *att*-defective transducing phage carrying an internal *Sma*I deletion in the *E. coli himA* gene (D. Friedman, personal communication). Y1100 can integrate by homologous recombination at the host *himA* locus to form a lysogen; excision of the prophage in some cases leaves *himA*Δ*Sma*I in the host chromosome. To construct RBB182, RBB63 was infected with Y1100 at a multiplicity of ~10 at 30°C and plated 4 h later for survivors (~10^{-3}) at 30°C. One of 40 survivors screened was a λ lysogen. Survivors (~10^{-2}) of RBB63(Y1100) at 42°C were screened by cross-streaking with λ *c*I60 and Mu *c*am7649. Among 100, all were λ $cI60^s$ nonlysogens, whereas 3 were Mu *c*am7649^r and Him^-; 1 was designated RBB182.

The Him phenotype of all strains was confirmed by spot tests. Mu fails to plate (27, 38, 39) and λ forms clear plaques (37) on *him* strains. Because Tn*10*-1230 is only ~70% linked to the *himD* locus (27), the *himD* genotype of RBB107 (*himA42 himD157* Tn*10*-1230) was confirmed by backcrossing Tn*10*-1230 out of RBB107 into W3110 (him^+) and examining the Him phenotype of the resulting Tet^r transductants. The *himA* genotype of RBB238 (*himA42 zdh-201*::Tn*10 himD*Δ*3*) was similarly confirmed by backcrossing out *zdh-201*::Tn*10*. Of 18 Tet^r transductants tested, 17 were Him^-, confirming tight linkage of *zdh-201*::Tn*10* to the *himA* locus

TABLE 1. Bacteria

Strain	Genotype or description	Source or reference
BNN45	*hsdR met gal lacY supE supF*	Fox laboratory collection
E529	N99 *himD*Δ*3*::*cat* (P1 Kan^r *clr-100*)	R. Weisberg; 13
K5242	K37 *himA*Δ*81*	H. Miller; 24
MH4386	K634 *zdh-201*::Tn*10*	M. Howe; 43
RBB63	F^- λ^- *galK2 rpsL200* IN(*rrnD-rrnE*)*1*	MH5775 = K37 = N99 = MM28; M. Howe; 2, 39
RBB64	K37 *himD157* Tn*10*-1230	MH5818; M. Howe; 43
RBB66	K37 *himA42*	MH5776 = K634; M. Howe; 39
RBB67	W3110 *supF trp*::Tn*5*	See text
RBB107	RBB66 *himD157* Tn*10*-1230	P1.RBB64 × RBB66[a], selecting for Tet^r
RBB110	W3110 $himD^+$ Tn*10*-1230	P1.RBB107 × W3110, selecting for Tet^r
RBB116	W3110 *supF*	See text
RBB143	RBB63(pRBB1)	
RBB144	RBB63(pRBB2)	
RBB145	RBB63(pRBB3)	
RBB182	RBB63 *himA*Δ*SmaI*	See text
RBB183	RBB63 *himD*Δ*3*::*cat*	P1.E529 × RBB63, selecting for Cam^r
RBB184	RBB182 *himD*Δ*3*::*cat*	P1.E529 × RBB182, selecting for Cam^r
RBB189	RBB63(Mu c^+pf7701)	See text
RBB193	RBB182(Mu c^+pf7701)	See text
RBB206	RBB182(pRBB1)	
RBB207	RBB182(pRBB2)	
RBB208	RBB182(pRBB3)	
RBB224	RBB63 *himD157* Tn*10*-1230	P1.RBB107 × RBB63, selecting for Tet^r
RBB225	RBB63 $himD^+$ Tn*10*-1230	P1.RBB107 × RBB63, selecting for Tet^r
RBB237	RBB63 *himA42 zdh-201*::Tn*10*	P1.MH4386 × RBB63, selecting for Tet^r
RBB238	RBB183 *himA42 zdh-201*::Tn*10*	P1.MH4386 × RBB183, selecting for Tet^r
SK1843	F^- *argE3 his-4 thi-1 aroD*$^+$ *rpsE rpsL*$^+$ *himA*$^+$ *zdh-201*::Tn*10* *lac*Δ*MS286* (ϕ80 d*lI lac*Δ*BK1*)	S. Kushner
W3110	F^- λ^- IN(*rrnD-rrnE*)*1*	C. Kenyon; 2

[a] Signifies infection of RBB66 with P1 grown on RBB64.

(43). The deletion strains RBB182, RBB183, and RBB184 were constructed without using linked Tn*10* insertions. Their *him* genotypes were confirmed by transducing in Tet^r from SK1843 (*himA*$^+$ *zdh-201*::Tn*10*) or RBB110 (*himD*$^+$ Tn*10*-1230) and scoring the Him and Cam phenotypes of the resulting transductants.

Bacteriophage. The bacteriophage strains used are listed in Table 2. The structures of the four Mu c^+ drug-resistant phage constructed were confirmed by restriction mapping with *Bam*HI and *Pst*I (data not shown). The locations of the *B*am mutations in the Mu *c*ts *B*ampAp1 phage were confirmed by marker rescue with whole plate assays as described by Howe et al. (23, 40).

Plasmids. The structures of all plasmids were confirmed by restriction mapping and by testing the ability of the plasmids to complement Mu *A*am and Mu *B*am phage for lytic growth (data not shown).

pRKY9 contains Mu *A B* (and no other intact Mu open reading frames) cloned under the control of the constitutive Kan^r promoter of pACYC177 (Yoshida, Ph.D. thesis). pRKY18 is a deletion derivative of pRKY9 producing only Mu B (Yoshida, Ph.D. thesis), whereas pLP103-32-2 produces only Mu A. pLP103-32-2 was constructed from the same pLP103 precursor (48) as pRKY9 and is effectively a deletion derivative of pRKY9.

A fragment bearing Cam^r from pBR325 was substituted for a nonessential vector portion of the Amp^r plasmids carrying Mu functions to make Cam^r Amp^s derivatives. The plasmids pACYC177 and pBR325 have identical Amp^r genes, which contain *Hin*cII and *Sca*I sites 61 base pairs apart. To make pRBB1, the 0.65-kilobase *Bam*HI-*Sca*I fragment of pLP103-32-2 was replaced by the 2.463-kilobase *Bam*HI-*Hin*cII fragment of pBR325. The 1.5-kilobase *Hin*dIII-*Sca*I fragments of pRKY18 and pRKY9 were replaced with the 2.117-kilobase *Hin*dIII-*Hin*cII fragment of pBR325 to make pRBB2 and pRBB3, respectively.

Overlay of colonies. To determine the number of lysogens formed by Mu c^+ drug-resistant phage, infected cells were plated in top agar. Lysogen formation after plating in the presence of as many as 10^9 Mu c^+ pAp1 phage was prevented by 50 mM EGTA in the top agar. At an empirically determined time sufficient for phenotypic expression of antibiotic resistance, the plates were overlaid with another layer of top agar containing the antibiotic.

Established Mu c^+pf7701 or Mu c^+pAp1 lysogens of W3110 (*him*$^+$), RBB63 (*him*$^+$), RBB182 (*himAΔSmaI*), RBB183 (*himDΔ3*), and RBB184 (*himAΔSmaI himDΔ3*) were resistant to 2-ml soft agar overlays containing up to at least 250 μg of kanamycin or ampicillin per ml, respectively, whereas 50 μg of antibiotic per ml was sufficient to kill RBB63 nonlysogens (data not shown). The yield of lysogens from low-multiplicity Mu c^+pf7701 or Mu c^+pAp1 infections of these five strains remained constant when overlaid with 250 μg of antibiotic per ml between 2 and 4 h after plating (data not shown). Therefore, plates were routinely overlaid with 2 ml of soft agar plus 250 μg of antibiotic per ml after 2 h of incubation at 37°C.

At 30°C, complete phenotypic expression of Amp^r from Mu c^+pAp1 in RBB63 required between 3 and 4 h, so plates were overlaid after 4 h of incubation.

Overlay of plaques. Plaques of Mu c^+ drug-resistant phage can be overlaid at an early stage of formation with a concentration of antibiotic sufficient to kill all the susceptible cells of the lawn but permit growth of lysogens present in a nascent plaque. Mu c^+pf7701 or Mu c^+pAp1 was plated for plaques as usual and overlaid at various times with 2 ml of soft agar plus 250 μg of kanamycin or ampicillin per ml, respectively. The yield of drug-resistant colonies was equal to the number of plaques formed in the absence of the overlay when the overlay was made after 3 h of plate incubation (data not shown). Overlaying at 2 h resulted in a reduced yield, whereas overlays at 4 to 6 h killed the lawn incompletely.

TABLE 2. Bacteriophage

Phage	Genotype	Source or reference
Mu c^+	Mu c^+	C. Kenyon; 46
Mu *c*am	Mu *c*am7649	M. Howe
Mu *c*ts *B*1979	Mu *c*ts62 *B*1979	M. Howe; 40
Mu *c*ts *B*1066	Mu *c*ts62 *B*1066	M. Howe; 21
Mu pf7701	Mu *c*ts62 Δ7701-1[a] Δ445-3[b]	M. Howe; 43
Mu pf7711	Mu *c*ts62 Δ7711-1 Δ445-3	M. Howe
Mu pAp1	Mu *c*ts62 pAp1	M. Howe; 32
Mu pAp5	Mu *c*ts62 pAp5[c]	M. Pato; 1
Mu c^+pf7701	Mu c^+ Δ7701-1 Δ445-3	Mu c^+ × Mu pf7701
Mu c^+pf7711	Mu c^+ Δ7711-1 Δ445-3	Mu c^+ × Mu pf7711
Mu c^+pAp1	Mu c^+ pAp1	Mu c^+ × Mu pAp1
Mu c^+pAp5	Mu c^+ pAp5	Mu c^+ × Mu pAp5
Mu *c*ts *B*1979pAp1	Mu *c*ts62 *B*1979 pAp1	Mu *c*ts *B*1979 × Mu c^+pAp1
Mu *c*ts *B*1066pAp1	Mu *c*ts62 *B*1066 pAp1	Mu *c*ts *B*1066 × Mu c^+pAp1
Mu *c*am pAp1	Mu *c*am7649 pAp1	Mu *c*am7649 × Mu c^+pAp1
λ	λ cI^+	λ++; M. Lichten
λ *c*I60	λ *c*I60	M. Lichten
λ *vir*	λ *vir*	S. Raposa
Y1100	λ *c*I857 (*E. coli himAΔSmaI*)	D. Friedman
P1	P1 c^+	M. Stahl

[a] Δ7701-1 is *kil gam* (43, 45).
[b] Δ445-3 was described previously (11).
[c] pAp5 is *gam* (1).

Measurement of lysogens per infected cell. A 500-fold dilution of an overnight LB culture was grown in LBCaMg at 37°C to ~2 × 10^8 CFU/ml, a 0-min sample was diluted into saline for bacterial titer, and 1-ml portions of the culture were distributed to flasks in a 37°C water bath. Phage were added to the cultures, mixed briefly, and allowed to adsorb for 15 min without aeration. Samples of each infection were then diluted 100-fold into LBCaMg at 37°C, to halt further phage adsorption, and also into Mu buffer containing chloroform, to assay unadsorbed phage. Aeration of the dilution of the infected cells was begun. At 30 min postinfection, samples from the dilutions of infected cells were titrated and later overlaid with antibiotic as described above. In some experiments, samples (30 min*) were overlaid without antibiotic to determine the titer of viable cells after infection.

The MOI was corrected for unadsorbed phage. The fraction of cells infected is given by the Poisson distribution as $P_{\geq 1} = 1 - e^{-MOI}$. The frequency of lysogens per infected cell was then defined as the titer of lysogens in the 30-min sample divided by the calculated titer of infected cells.

The sum of infected cells and unadsorbed phage could be measured in the *him*$^+$ case by plating the 15-min sample for total infective centers, and the number of infected cells could be derived as the difference between the chloroform-treated and untreated samples. The agreement between the two methods was good (data not shown). For consistency, the Poisson method was used for all calculations.

For lysogenization measurements with strains bearing the

TABLE 3. Lysogenization of *him*$^+$ bacteria at low MOI[a]

Strain	*him* genotype	Lysogens/infected cell (×100)[b]			
		Mu *c*$^+$pf7701	Mu *c*$^+$pf7711	Mu *c*$^+$pAp1	Mu *c*$^+$pAp5
W3110	*him*$^+$	19, 21	2.8, 3.6	7.6, 9.1	18, 20
RBB63	*him*$^+$	12 ± 1[c]	4.2, 5.8	7.7 ± 0.7	15, 15
RBB225	*him*$^+$ Tn*10*-1230	19			

[a] Measurements were made at an MOI of <0.10.
[b] Results of cases with one or two measurements are listed individually. Means ± standard deviations are listed for cases with four to six separate measurements.
[c] A fifth measurement gave 27; we cannot account for this observation.

Cam^r plasmids, overnight cultures were grown in LB–10 μg of chloramphenicol per ml, but chloramphenicol was absent during the actual experiments.

Mu adsorption. The receptor for Mu adsorption is a terminal portion of the cell wall lipopolysaccharide (44). It seems unlikely that there is a limited (in a practical sense) number of Mu adsorption sites per cell. For mixed infections with phage of two genotypes, the MOI of the minority phage was measured in an infection with it alone and was assumed to remain the same in parallel infections in the presence of the majority phage. This assumption is justified by the following observations. (i) In a series of mixed infections, titration of unadsorbed Mu *c*$^+$pf7701 and Mu *c*$^+$pAp1 phage with the plaque overlay assay demonstrated that adsorption of the minority phage was unaffected by the presence of the majority phage (data not shown). (ii) In all experiments with a single phage species, the fraction of unadsorbed phage was independent of MOI over an MOI range from 0.02 to 190 (data not shown).

As Mu stocks lose viability, they adsorb more poorly (data not shown). To accurately calculate MOI, the unadsorbed phage titer must be significantly smaller than the phage input. Stocks were discarded if unadsorbed phage exceeded 20% of the phage input in infections of *him*$^+$ cells or 10% for *him* infections.

Adsorption kinetics. The multiplicities achieved in the pulse-chase and variable-adsorption-rate experiments were calculated as MOI = $\phi_0(1 - e^{-kBt})/B$, where ϕ_0 is the initial phage concentration, B is bacterial concentration, and t is time (36). The adsorption constant of the Mu *c*$^+$pAp1 phage used was determined under standard infection conditions (MOI of ~5) by titrating unadsorbed phage at 2-min intervals. This yielded $k = 1.7 \times 10^{-9}$ ml/min for RBB63 (*him*$^+$) and $k = 1.8 \times 10^{-9}$ ml/min for RBB182 (*himA*Δ*SmaI*) (data not shown).

RESULTS

Lysogen formation by many bacteriophage species is influenced by various environmental conditions. We first determined Mu lysogenization frequency under standard conditions and investigated the effects of perturbing temperature and multiplicity. Next, we explored the properties of Mu lysogen formation in *him* bacteria, where competing lytic growth and killing did not occur. Finally, several experiments are reported that bear on the roles of the Mu A, B, and c functions in lysogenization.

Lysogenization of *him*$^+$ bacteria at low MOI. To examine the consequence of infection by a single phage, lysogen formation in *him*$^+$ hosts was measured at low MOI. Under these circumstances, Mu *c*$^+$pAp1 formed lysogens about 8% of the time (Table 3). This phage carries an Amp^r substitution in place of the right half of the G loop (sequences in this position are not expressed in wild-type Mu [17]) and is expected to behave like wild-type Mu with respect to lysogenization. In fact, when RBB63 (*him*$^+$) was infected with Mu *c*$^+$ at an MOI of 0.43 and the titer of lysogens was ascertained by screening 1,920 survivors for phage release, 8.4% of the infected cells became lysogens.

Mu *c*$^+$pf7701 and Mu *c*$^+$pAp5, bearing drug resistance substitutions in the semiessential early region, lysogenized *him*$^+$ cells at modestly higher frequencies than Mu *c*$^+$pAp1, suggesting that functions encoded in this region influence the likelihood of lysogenization.

Lysogenization of *him*$^+$ bacteria as a function of temperature. Decreasing temperature increases lysogenization by bacteriophage P1 (4) and λ (34). Limited evidence suggests temperature may similarly affect Mu lysogenization (M. M. Howe, Ph.D. thesis, Massachusetts Institute of Technology, Cambridge, Mass., 1972). In fact, the low-MOI lysogenization frequency for Mu *c*$^+$pAp1 in RBB63 (*him*$^+$) increased about threefold between 42 and 30°C (Table 4). As expected, lysogenization by Mu *c*ts62 pAp1 was strongly dependent on temperature. Even at 30°C, however, Mu *c*ts62 pAp1 formed lysogens less frequently than Mu *c*$^+$pAp1.

Lysogenization of *him*$^+$ bacteria as a function of MOI. Increasing MOI substantially increases the probability of lysogenization by bacteriophages P22 (33) and λ (14, 28, 29). Mu exhibits a different behavior: cell viability declines with increasing MOI (Howe, Ph.D. thesis). The fraction of RBB63 (*him*$^+$) cells surviving infection by Mu *c*$^+$pAp1 declined rapidly with increasing MOI and appeared to level off at ~1% (Fig. 1). The frequency of lysogens per infected cell in the same experiment declined with an apparently exponential dependence on MOI. Lysogenization frequencies in RBB63 were higher for Mu *c*$^+$pf7701 and Mu *c*$^+$pAp5,

TABLE 4. Lysogenization of *him*$^+$ bacteria as a function of temperature[a]

Temp (°C)	Lysogens/infected cell (×100) at low MOI[b]	
	Mu *c*$^+$pAp1	Mu *c*ts62 pAp1
42	4.3, 5.7*	0.16, 0.22*
37	8.5, 8.6	1.1
34	13	2.5
30	13, 17	7.1*, 9.3

[a] Growth of RBB63 cultures and the Mu infections (15 min of adsorption, 10^{-2} dilution, 15 min of further incubation) were at the indicated temperatures. The overlay assay to quantitate Amp^r lysogens was done at 30°C for all samples.
[b] One or two separate measurements are listed for each case. The three measurements indicated by an asterisk (*) were made at MOIs between 0.13 and 0.24 and do not appear to be systematically different from the remaining 10 measurements, which were all done at an MOI of <0.10.

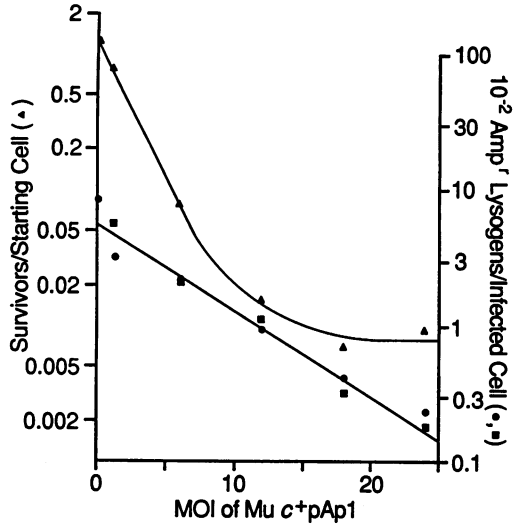

FIG. 1. Survival and lysogenization of RBB63 (*him*$^+$) bacteria as a function of multiplicity of Mu c^+pAp1. Survivors per starting cell (▲) = (30 min*)/(0 min). Lysogens per infected cell (●) were measured with the standard overlay assay. For an alternative determination of lysogenization frequency, colonies from the 30-min* plates were screened for Amp^r. Lysogens per infected cell (■) were then calculated as (30 min*) (fraction lysogens among survivors)/[($P_{\geq 1}$) (0 min)]. Note that screening survivors for Amp^r lysogens gave the same results as measuring lysogens by direct selection, further demonstrating the reliability of the overlay assay. See Materials and Methods for details of calculations.

consistent with the data in Table 3, but their decline with increasing MOI was similar in slope to that exhibited by Mu c^+pAp1 (data not shown).

Lysogenization of *him*$^+$ and *him* bacteria at high MOI. The frequency of Mu lysogens per surviving cell has been reported to be similar in *him*$^+$ and *himA* hosts at MOI ~5 (38; Yoshida, Ph.D. thesis). Since Mu kills *him*$^+$ cells but not *himA* cells, this observation implies that the actual frequency of lysogen formation per infected cell is higher in the *himA* case. To illustrate this point, we confirmed the original observations and extended them to include *himD* hosts.

him$^+$, *himA*, and *himD* bacteria were infected with sufficient Mu c^+pAp1 to infect >99% of cells. As expected, the *him* cells were not killed by Mu infection, whereas most of the *him*$^+$ cells were (Table 5). Colonies were tested for lysogeny both by Amp^r and by phage release. Approximately half the surviving *him*$^+$ colonies contained lysogens, as compared with one-quarter to one-half of the *him* colonies. The frequency of lysogens per infected cell was significantly greater in *himAΔSmaI* and *himDΔ3* bacteria than in *him*$^+$ bacteria at these multiplicities.

Lysogenization of *him* bacteria at low MOI. How does the lysogenic response compare in *him*$^+$ and *him* hosts after infection by a single Mu particle? The essentially wild-type phage Mu c^+pAp1 lysogenizes *him*$^+$ and *him* hosts at similar frequencies (Tables 3 and 6). This is a surprising result, given the known influence of integration host factor on early Mu transcription, and is considered in detail in the Discussion.

Each Mu c^+ drug-resistant phage exhibited similar low-MOI lysogenization frequencies in isogenic strains containing various combinations of the *himA42*, *himAΔSmaI*, *himD157*, and *himDΔ3* alleles (Table 6). By this criterion at least, the widely used *himA42* and *himD157* missense mutations resulted in a "lack of function" rather than an "altered function" phenotype. We chose to use RBB182 (*himAΔ SmaI*) as representative of *him* bacteria for further detailed examination of Mu lysogenization.

Lysogenization of *him* bacteria as a function of MOI. The data in Tables 5 and 6 suggest that Mu lysogenization frequencies increase with MOI in *him* hosts, in contrast to the decline observed in *him*$^+$ cells. In fact, the number of lysogens per infected cell increased roughly linearly with Mu c^+pAp1 MOI in RBB182 (*himAΔSmaI*) (Fig. 2), an observation confirmed in the *him* strains K5242, RBB183, RBB184, RBB224, and RBB237 (data not shown). At high multiplicities, lysogenization of *him* bacteria asymptotically approaches a saturation, such that most but not all of the infected cells become lysogens. Experiments with Mu c^+pAp1 in K5242 (*himAΔ81*), RBB224 (*himD157*), and RBB237 (*himA42*) included sufficient measurements at multiplicities above 10 to reveal this feature more clearly than in Fig. 2 (data not shown).

To investigate the frequency of cells that fail to become lysogens at high MOI, cells from three high-multiplicity infections of RBB182 (*himAΔSmaI*) with Mu c^+pAp1 were plated, and the colonies were screened for Amp^r. Even at a multiplicity of 43, 12% of colonies did not contain lysogens. A parallel experiment with Mu *c*am pAp1 gave similar results. Thus, the event which results in nonlysogenic survivors after infection of *him* bacteria does not appear to involve Mu immunity.

Helping effect in *him* bacteria. What is the basis for the increase in Mu lysogenization of *him* bacteria as the MOI increases? Changing the MOI alters both the number of phage capable of providing functions that play a role in lysogenization and the number of candidate prophage gen-

TABLE 5. Lysogenization of *him*$^+$ and *him* bacteria at high MOI

Strain	*him* genotype	Mu c^+pAp1 MOI	Survivors/IC[a] (×100)	Lysogens/survivor by:		Lysogens/IC[b] (×100)
				Amp^r	Phage release[c]	
RBB63	*him*$^+$	6.3	9.4	42/100	42/100	3.9
		12	2.2	54/100	54/100	1.2
RBB182	*himAΔSmaI*	4.3	150	21/100	4/100	32
		8.3	120	56/100	9/100	67
RBB183	*himDΔ3*	4.3	140	22/100	5/100	31
		8.2	110	51/100	12/100	56

[a] Survivors per infected cell (IC) = (30 min*)/[($P_{\geq 1}$)(0 min)]. See Materials and Methods for a discussion of calculations.

[b] In this table, lysogens per infected cell = (survivors/infected cell) (Amp^r lysogens/survivor). Note that when cells are not killed by Mu infection, i.e., in the *him* cases, the survivors per infected cell measurement becomes sensitive to titration error, which is reflected in the calculation of lysogens/infected cell.

[c] Colonies were streaked on a lawn of W3110 (*him*$^+$). All colonies which released phage were also Amp^r. Spontaneous phage release by *him* lysogens is so rare that this is not a useful test for lysogeny.

TABLE 6. Lysogenization of *him* bacteria at low MOI[a]

Strain	*him* genotype	Lysogens/infected cell (×100)[b]			
		Mu c^+pf7701	Mu c^+pf7711	Mu c^+pAp1	Mu c^+pAp5
RBB237	*himA42 zdh-201*::Tn*10*	10, 10		7.7 ± 1	
RBB182	*himAΔSmaI*	9.4 ± 2	5.2, 7.8	6.3 ± 1	5.1, 5.5
RBB224	*himD157* Tn*10*-1230	13 ± 2	7.2, 12	7.7 ± 1	8.9, 9.2
RBB183	*himDΔ3*	8.6 ± 0.7	6.4, 6.6	7.8 ± 2	5.4, 7.5
RBB238	*himA42 himDΔ3 zdh-201*::Tn*10*	9.3		6.5	
RBB184	*himAΔSmaI himDΔ3*	6.8, 7.6	5.5, 5.8	6.5 ± 0.6	6.4, 7.9

[a] Measurements were made at an MOI of ≤0.10.
[b] Results of cases with one or two measurements are listed individually. Mean ± standard deviations are listed for cases with three to eight separate measurements.

omes present within a cell. To analyze the effect of the presence of additional phage on lysogen formation by a particular phage, mixtures of phage with two different genotypes were utilized. Bacteria were infected with a Mu c^+ drug-resistant assay phage at a fixed low multiplicity, in the presence of increasing multiplicities of an unmarked auxiliary phage. The infected cells were then examined as usual for the formation of drug-resistant lysogens by the assay phage.

The probability of lysogen formation by Mu c^+pf7701 or Mu c^+pAp1 assay phage increased in the presence of increasing numbers of Mu c^+ auxiliary phage in RBB182 (*himAΔSmaI*) (Fig. 3). A helping effect of similar magnitude was observed in the *him* strains RBB183, RBB184, RBB224, RBB237, and RBB238 (data not shown). Assay phage lysogenization frequencies saturated, with Mu c^+pf7701 reaching a level two- or threefold greater than that of Mu c^+pAp1. A similar difference between Mu c^+pf7701 and Mu c^+pAp1 assay phage was observed in the other *him* strains for which a comparison was made (RBB237 and RBB238) (data not shown).

Kinetics of helping in *him* bacteria. A pulse-chase experiment permitted examination of the kinetics of the helping effect. A culture of RBB182 (*himAΔSmaI*) cells was pulsed with a low multiplicity of Mu c^+pAp1 assay phage, split into samples, and then at various intervals chased with a high multiplicity of Mu c^+ auxiliary phage. Assaying pairs of chase and no-chase samples for Amp^r lysogens gave the results shown in Fig. 4. The number of lysogens observed for the first no-chase sample corresponds to a lysogenization frequency of 0.053, in good agreement with the data in Table 6. Lysogenization was enhanced about threefold when the pulse was immediately followed by a chase of auxiliary phage. The extent of helping persisted even when the chase was delayed 60 min.

The ability of auxiliary phage to provide help long after the initial assay phage infection suggests that either immunity to high-multiplicity superinfection is not established during the experiment or the helping function is not under the control of immunity.

The failure of the lysogens formed in the no-chase infections to multiply over the course of the experiment suggests that host cell replication may be temporarily halted by Mu lysogenization. Such a lag is evident for lysogenization with bacteriophages P1 (4) and λ (15, 34).

To investigate the stability of helping activity, a pulse-chase experiment was carried out with auxiliary phage delivered as a pulse and the assay phage introduced as a chase (Fig. 5). Assay phage lysogenization was enhanced

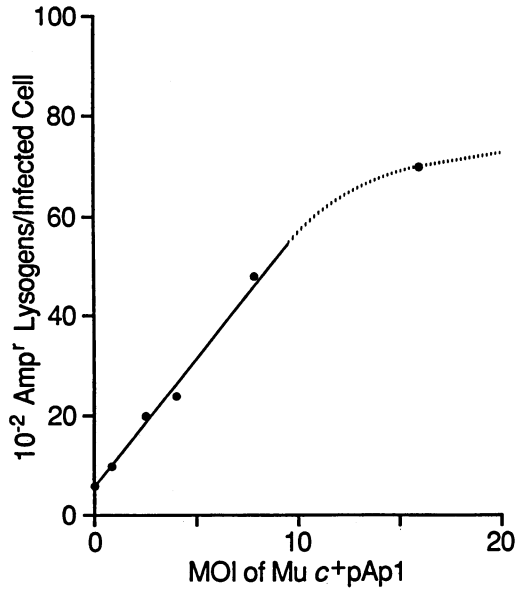

FIG. 2. Lysogenization of RBB182 (*himAΔSmaI*) as a function of multiplicity of Mu c^+pAp1.

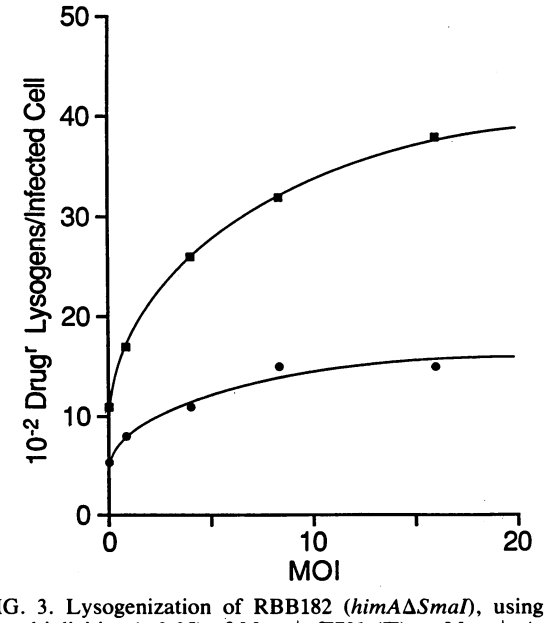

FIG. 3. Lysogenization of RBB182 (*himAΔSmaI*), using fixed low multiplicities (~0.05) of Mu c^+pf7701 (■) or Mu c^+pAp1 (●) assay phage and increasing multiplicities of Mu c^+ auxiliary phage.

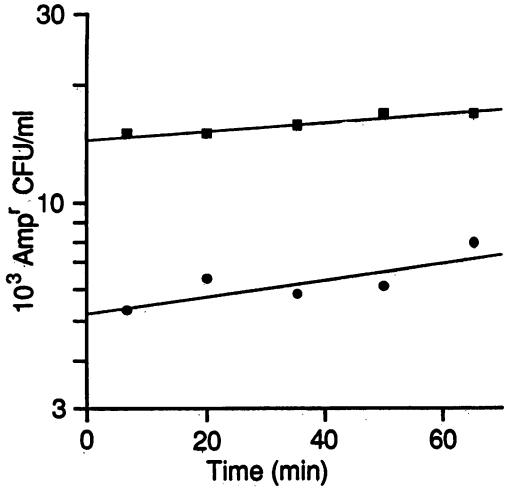

FIG. 4. Kinetics of helping in RBB182 (*himAΔSmaI*). Starting at 0 min, cells were infected with Mu c^+pAp1 assay phage for 5 min (MOI, 0.097) and then diluted 100-fold to terminate the pulse. At the indicated times, samples were infected with Mu c^+ (■) auxiliary phage for 5 min (MOI, 18). No-chase controls (●) were done in parallel. The chases were terminated by 10-fold dilution, followed 5 min later by the addition of 10 mM EGTA. Samples were plated for Amp^r lysogens by the standard overlay assay after 15 min more of incubation. The delay in EGTA addition was necessary to permit completion of infections begun before dilution (data not shown). Under standard infection conditions ($B = 1.4 \times 10^8$ RBB64 CFU per ml, $\phi_0 = 1.9 \times 10^9$ Mu c^+pf7701 PFU per ml), the presence of 10 mM EGTA eliminated measureable phage adsorption and reduced the yield of lysogens 25-fold (data not shown).

when infection immediately followed an auxiliary phage pulse. Delaying the chase reduced this helping effect, perhaps reflecting instability of the helping function(s) provided by the auxiliary phage as well as a delay in cell replication.

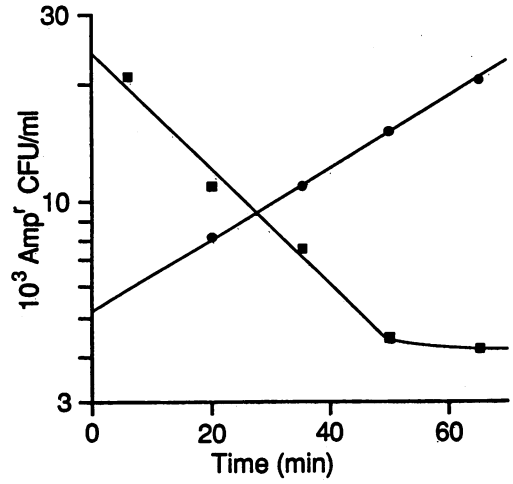

FIG. 5. Kinetics of helping in RBB182 (*himAΔSmaI*). Starting at 0 min, cells were infected with Mu c^+ (■) auxiliary phage for 5 min (MOI, 20), and then diluted 100-fold to terminate the pulse. A no-pulse control (●) was done in parallel. At the indicated times, samples were infected with Mu c^+pAp1 assay phage for 5 min (MOI, 0.99). The chases were terminated by 10-fold dilution, followed 5 min later by the addition of 10 mM EGTA. Samples were plated for Amp^r lysogens by the standard overlay assay after 15 min more of incubation.

Alternatively, establishment of immunity by the auxiliary (pulse) phage might reduce the expression of assay (chase) phage functions needed for lysogenization. Replication of uninfected cells before the addition of the assay phage accounts for the increase in lysogens in the no-pulse case.

Lysogenization of *him* bacteria in the presence of plasmids providing Mu A and/or B functions. The helping effect suggests that auxiliary phage can provide a function(s) which enhances lysogen formation by assay phage in the same *him* cell. Two obvious candidates are Mu A and B. Use of Mu *A*am or Mu *B*am auxiliary phage did not clarify the situation. Such phage actually interfered with assay phage lysogenization in the *him sup*$^+$ strains tested (RBB182 and RBB224) (data not shown). This interference could result from competition between assay and auxiliary phage genomes for limited A and/or B proteins in a *him* cell.

As an alternative approach, we measured low-MOI lysogenization frequencies in the presence of plasmids constitutively providing Mu A and/or B products (Table 7). In a *himAΔSmaI* strain background, provision of Mu A from a plasmid had little effect. The presence of pRBB2 (Mu B^+) resulted in a threefold enhancement of lysogenization, comparable to the maximal helping effect seen with auxiliary phage multiplicities of about 10 or more (Fig. 3). We suggest that the helping effect could be the result of provision of Mu B function by auxiliary phage. Supporting evidence is reported in the accompanying paper (5).

Providing both Mu A and B functions from a plasmid further enhanced lysogenization (Table 7). While the level of Mu B function is limiting for lysogen formation in *him* bacteria, the level of Mu A function may be close to limiting. The trivial alternative that more Mu B might be produced from pRBB3 (Mu A^+ B^+) than from pRBB2 (Mu B^+) has not been ruled out.

Lysogenization by Mu c^+pf7701 was about twofold greater than that by Mu c^+pAp1 in the *himAΔSmaI*(pRBB2) (Mu B^+) and pRBB3 (Mu A^+ B^+) strains (Table 7). A difference of the same magnitude was seen for helping provided by Mu c^+ auxiliary phage (Fig. 3).

Yoshida (Ph.D. thesis) found that providing Mu A and B functions constitutively from a plasmid enabled Mu to form plaques, although at reduced efficiency of plating (EOP), on *himA* strains. We infected RBB208 [*himAΔSmaI*(pRBB3)] with Mu c^+ at an MOI of 0.16 and plated the infected cells on a lawn of RBB63 (*him*$^+$) bacteria. The infected *himA* cells gave rise to infective centers with an efficiency of 9.7%. With only ~10% of low-multiplicity Mu c^+ infections of RBB208 resulting in lytic growth and ~25% resulting in lysogen formation (Table 7), it would appear that the levels of Mu A and B provided by the plasmid are not sufficient to cause all or even most infections of a *himAΔSmaI* host to result in either lytic growth or lysogen formation.

TABLE 7. Lysogenization of *him* bacteria in the presence of plasmids providing Mu A and/or B function

Strain	Plasmid	Lysogens/infected cell (×100) at low MOI[a]	
		Mu c^+pf7701	Mu c^+pAp1
RBB182	None	9.4 ± 2	6.3 ± 1
RBB206	pRBB1 (A^+)	4.3, 5.6	4.1, 4.8, 4.9
RBB207	pRBB2 (B^+)	26, 32	16, 19, 20
RBB208	pRBB3 (A^+ B^+)	43, 51	25, 26, 27

[a] Data for RBB182 are from Table 6. Individual measurements are listed for the plasmid-containing strains. Measurements were made at an MOI of ≤0.10.

Lysogenization of *him*$^+$ bacteria in the presence of plasmids providing Mu A and/or B functions. There is some evidence that the Mu A protein could have a regulatory role in addition to its transposase function. Footprinting experiments show that A binds specifically to the Mu operator sites but with lower affinity than c (12). Furthermore, Yoshida (Ph.D. thesis) reported that the EOP of Mu *c*ts62 is reduced to 8×10^{-4} on K37(pLP103-32-2) (*him*$^+$ Mu *A*$^+$) at 37°C. At 42°C, the EOP rose to 0.8. This observation is consistent with the suggestion of P. Van de Putte (personal communication) that the presence of an excess of A function might channel Mu development toward lysogenization, thus resulting in the reduced EOP. The presence of pRBB1 (a Cam^r Amp^s derivative of pLP103-32-2) does not enhance Mu lysogenization of RBB63 (K37) at low MOI (Table 8), however. A possible explanation of this paradoxical result is given in the Discussion.

The EOP of Mu is normal on K37 (*him*$^+$) bearing pRKY18 (Mu *B*$^+$) or pRKY9 (Mu *A*$^+$ *B*$^+$) (Yoshida, Ph.D. thesis). Lysogenization of RBB63 (K37) by Mu *c*$^+$pf7701 or Mu *c*$^+$pAp1 is little affected by the presence of pRBB2 or pRBB3, the Cam^r derivatives of these plasmids (Table 8). Presumably, neither A nor B function is limiting for lysogen formation in a permissive (*him*$^+$) host.

Lysogenization of *him*$^+$ *sup*$^+$ bacteria by Mu *B*am phage. Extensive integration of Mu DNA into the host chromosome occurs after infection (9) and presumably primarily reflects genomes initiating lytic replication. Whereas most Mu *B*am mutant phage exhibit little integration in a nonsuppressing host, Mu *B*1066 integrates normally but does not replicate (8, 53). Although the precise nature of the integration event leading to lysogen formation is unknown, it may occur by the same mechanism as integration leading to lytic growth. Does the apparently normal integration of Mu *c*ts62 *B*1066 observed in a nonsuppressing host result in an increase in the yield of lysogens?

Mu *B*am phage are reported to lysogenize *sup*$^+$ bacteria less frequently than Mu *B*$^+$ phage (41). Lysogen formation at 30°C by Mu *c*ts62 *B*1979pAp1 was in fact reduced about fivefold in a *sup*$^+$ host as compared with either the *supF* host or the *B*$^+$ phage (Table 9). Mu *c*ts62 *B*1066pAp1 lysogenized *sup*$^+$ bacteria at a frequency indistinguishable from that of Mu *c*ts62 *B*1979pAp1. The more than 10-fold greater integration of Mu *B*1066 than Mu *B*1979 reported under these conditions (8) is thus not reflected as an enhanced yield of lysogens. We conclude that at low MOI the presence of the Mu *B* function stimulates lysogenization about fivefold and that the *B*1066 amber protein is defective in this activity. The fate of *sup*$^+$ bacteria with an integrated Mu *B*1066 genome that is not detected as a lysogen is unknown.

Role of Mu immunity in formation of nonlysogenic survivors. Nonlysogenic survivors have been suggested to be the products of infections in which immunity was established without integration (Howe, Ph.D. thesis). If repression of p_E prevented synthesis of sufficient A and B proteins for integration, the infecting Mu genomes might either segregate away in daughter cells or suffer degradation.

To test this hypothesis, we infected RBB63 (*him*$^+$ *sup*$^+$) with Mu *c*$^+$pAp1 or Mu *c*am pAp1 at four multiplicities from ~5 to ~25 and plated without antibiotic. Colonies were screened for Amp^r to identify lysogens and nonlysogenic survivors. Nonlysogenic survivors were present at comparable titers in both sets of infections (data not shown). As expected, no lysogens were found among the products of infection with Mu *c*am pAp1 (data not shown). Immunity does not appear to play a role in the occurrence of nonlysogenic survivors after Mu infection.

Role of Mu immunity in lysogenization of *him* bacteria. Thermal induction of Mu *c*ts62 lysogens of *himA* or *himD* strains does not result in either transposition or killing (27, 38; Yoshida, Ph.D. thesis). The immunity function therefore does not appear to be required for the maintenance of lysogeny in *him* strains; the blocking of phage lytic growth as a consequence of the host mutation is sufficient. In fact, Mu *c*am pAp1 can lysogenize RBB182 (*himA*Δ*SmaI sup*$^+$) (data not shown), suggesting that *c* function is dispensable for establishment as well as maintenance of Mu lysogeny in *him* hosts. Mu *c*am pAp1 lysogens of those *him sup*$^+$ strains tested (RBB64 and RBB66) released viable phage, eliminating the possibility that Mu *c*am pAp1 can lysogenize a *him* host only by generating a defective Amp^r prophage.

Given that Mu immunity function is dispensable in a *him* background, is it actually expressed? Experiments with Mu p_E-*lacZ* fusions suggests that it is (42). We can add two pieces of evidence. First, low-multiplicity infection of the *himA*Δ*SmaI* (Mu *c*$^+$pf7701) lysogen RBB193 with Mu *c*$^+$pAp1 yielded 1.1×10^{-3} Amp^r lysogens per infected cell (average of two measurements), 60-fold lower than the yield with the nonlysogenic parental strain (Table 6). The simplest interpretation of this result is that Mu immunity is expressed in *him* cells, although a larger 500-fold reduction is observed in a *him*$^+$ lysogen (data not shown). Second, Mu *c*am pAp1 lysogens of *him sup*$^+$ bacteria grew poorly in comparison with Mu *c*$^+$pAp1 lysogens, a circumstance which prevented us from accurately measuring the lysogenization frequency for Mu *c*am pAp1. It could be that Mu functions expressed in the Mu *c*am pAp1 lysogen but repressed in the Mu *c*$^+$pAp1 lysogen account for the poor growth observed.

Lysogenization of *him*$^+$ bacteria as a function of phage adsorption rate. What is the basis for the multiplicity dependence of Mu lysogenization frequency? A critical parameter should be the number of phage (each of which statistically favors lytic growth) entering a cell before the establishment of immunity. Phage genomes entering a multiply infected cell later will be repressed and should not alter the lysis-lysogeny

TABLE 8. Lysogenization of *him*$^+$ bacteria in the presence of plasmids providing Mu A and/or B function

Strain	Plasmid	Lysogens/infected cell (×100) at low MOI[a]	
		Mu *c*$^+$pf7701	Mu *c*$^+$pAp1
RBB63	None	12 ± 1	7.7 ± 0.7
RBB143	pRBB1 (*A*$^+$)	5.1, 5.8	9.3, 12
RBB144	pRBB2 (*B*$^+$)	7.3, 8.9	3.0, 4.2
RBB145	pRBB3 (*A*$^+$ *B*$^+$)	11, 14, 15	7.2, 7.7, 9.6

[a] Data for RBB63 are from Table 3. Individual measurements are listed for the plasmid-containing strains. Measurements in plasmid-containing strains were made at an MOI of ≤0.12.

TABLE 9. Lysogenization of *him*$^+$ *sup*$^+$ bacteria by Mu *B*am phage

Phage	Lysogens/infected cell (×100) at low MOI[a]	
	W3110 (*sup*$^+$)	W3110 (*supF*)
Mu *c*$^+$pAp1	14	14
Mu *c*ts62pAp1	5.6	3.6
Mu *c*ts62 *B*1979pAp1	0.75	5.1
Mu *c*ts62 *B*1066pAp1	1.0	8.9

[a] The experiment was done at 30°C. MOIs were between 0.043 and 0.054.

decision. The kinetics of Mu mRNA synthesis (52) and DNA replication (53) suggest that to preserve the possibility of lysogen formation, repression of p_E must occur on the time scale of minutes (or faster) after infection.

We anticipated that an increased adsorption rate should result in diminished lysogen formation and could account for the decline in Mu lysogenization frequency with increasing MOI (Fig. 1). At low bacterial concentration, phage are adsorbed at a constant rate ($\phi_0 k$), and the MOI is simply $\phi_0 kt$ (see Materials and Methods). To discriminate between the effects of MOI and adsorption rate, we performed a series of infections in which the MOI was held constant by reducing phage concentration while increasing adsorption time proportionately. Changing the adsorption rate between 0.75 and 12 phage per min had little if any effect on the lysogenization frequency (Table 10). The observed frequency of between 0.005 and 0.01 is consistent with the value observed at an MOI of 12 in Fig. 1. Despite the inferred rapid establishment of immunity, the number of Mu genomes infecting a *him*$^+$ cell, rather than their entry rate, appears to directly or indirectly determine lysogenization frequency.

Aberrant *him* strains. Although it shares many features of lysogenization in the *him* hosts described above, Mu lysogenization in several *him* strains which we have studied (RBB64, RBB66, RBB107) is clearly different. In RBB64, lysogenization frequency was reduced 20-fold, facilitating observation of the intermediate formed in *him* cells. In RBB66 and RBB107, helping was not observed. These variations may reflect the presence of additional (unidentified) mutations. Some interesting properties of these strains are reported in the accompanying paper (5).

TABLE 10. Lysogenization of *him*$^+$ bacteria as a function of phage adsorption rate[a]

Adsorption time (min)[b]	Mu *c*$^+$pApl (PFU/ml)		Amp^r lysogens	
	Expected[c]	Observed[d]	CFU/ml	CFU/IC[e] (×100)
1	6.6×10^9	6.9×10^9	1.9×10^4	1.1
2	3.3×10^9	2.7×10^9	1.1×10^4	0.65
4	1.6×10^9	1.6×10^9	8.6×10^3	0.51
8	8.2×10^8	7.7×10^8	7.8×10^3	0.46
16	4.1×10^8	3.4×10^8	1.7×10^4	1.0

[a] Examination of the Fig. 1 experiment shows that the range of adsorption rates tested here (0.75 to 12 phage per min) is physiologically relevant. In Fig. 1, adsorption rates declined over the course of the infections as the free phage concentration diminished. Given that *B* was 1.8×10^8 CFU/ml and 2 to 3% unadsorbed phage after 15 min, and assuming standard phage adsorption kinetics (see Materials and Methods), the initial adsorption rates were 1 phage per min at an MOI of 5 and 4 phage per min at an MOI of 20. Note that a ninefold difference in lysogenization frequency was observed between these multiplicities. For the experiment described here, a control was done to confirm that infections were not disrupted by the handling involved in dilution after short periods of adsorption. RBB182 (*himAΔSmaI*) (2.2×10^6 CFU/ml) was exposed to 4.1×10^7 PFU of Mu *c*$^+$pApl per ml for 1, 2, or 4 min, yielding calculated MOIs of 0.070, 0.14, and 0.28. The frequencies of lysogens observed were 0.089 to 0.10, which is in agreement with the data of Table 6 and Fig. 2 within the limits of the technique.

[b] RBB63 was grown to 1.7×10^8 CFU/ml as usual and then diluted 10^{-2} in prewarmed LBCaMg, and samples were distributed to flasks at 37°C. Phage were added at different times to form a nested set of parallel infections, which were terminated by another 10^{-2} dilution in LBCaMg. Samples were plated for lysogens as usual 15 min after completion of the longest adsorption. The MOI was calculated to be 12 in all infections (see Materials and Methods).

[c] Based on titration of the input phage stock and the volumes used.

[d] Samples were taken in midexperiment and titrated for unadsorped phage as usual. Note that the expected and observed values all agree within 20% titration error.

[e] Lysogens per infected cell (IC).

DISCUSSION

Bacteriophage Mu development can result in lytic growth, lysogenization, formation of nonlysogenic products, and perhaps simple killing of the host. We have examined the influence of a variety of parameters on survival and lysogenization. The hypothesis that the likelihood of each possible outcome is influenced by the levels of expression of phage functions provides a framework for the coherent interpretation of most of our results.

Because multiple outcomes are possible, PFU underestimates the number of infectious Mu particles by an unknown amount. This introduces some uncertainty into the calculation of lysogenization frequency per infected cell. Nevertheless, we will proceed as if PFU constituted a good approximation of infectious particles.

Lysogenization of *him*$^+$ bacteria. After low-multiplicity infection by Mu *c*$^+$ at 37°C, about 10% of the infected *him*$^+$ cells form lysogens (Table 3). This frequency is about 10 times greater than is widely believed (9). The discrepancy may be attributable to the methods used to measure lysogeny and the common use of Mu *c*ts62.

Over the range from 30 to 42°C, Mu lysogenization of *him*$^+$ bacteria (i) decreases with increasing temperature and (ii) is more likely with Mu *c*$^+$ than with Mu *c*ts62 (Table 4).

The frequency of Mu lysogens formed per infected cell diminishes with increasing MOI in *him*$^+$ bacteria (Fig. 1) and is independent of phage adsorption rate (Table 10). The basis for this decline is unknown. Some infected cells, which might otherwise form lysogens, apparently fail to do so because at higher MOIs additional phage present follow a developmental path which is lethal to the host.

The hypothesis that levels of expression of phage functions are critical leads to a model of the Mu lysis-lysogeny decision after infection of a *him*$^+$ host. Most Mu genomes integrate into the host chromosome (10), because sufficient Mu A and B proteins are synthesized. This is a prerequisite for both lytic growth (35) and lysogen formation (25). Because p_E is a stronger promoter than p_C (30; Goosen, Ph.D. thesis), in most *him*$^+$ infections repression of p_C by ner commits the phage to lytic growth (48). Occasionally, repression of p_E by c occurs first, resulting in lysogen formation.

Lysogenization of *him* bacteria. Expression of Mu genes is altered in a *him* host (16, 20, 30, 42; Yoshida, Ph.D. thesis), with several consequences suggested by this model of the control of Mu development. The inability of a *him* host to support Mu lytic growth or killing, while retaining lysogeny as a possible outcome (27, 38), is attributed to reduced synthesis of early gene products. Since not all infections result in lysogeny, infecting phage genomes can enter another state. We argue that lysogeny is determined simply by whether integration occurs. Reduced transcription of Mu *A* and *B* in *him* hosts would result in diminished integration. Whereas in *him*$^+$ bacteria the determining factor in Mu lysogenization was proposed to be a regulatory decision, in a *him* host the switch is proposed to be the biochemical act of integration. Mu c function is not necessary for either the establishment (see Results) or maintenance (27, 38; Results) of lysogens of *him* bacteria, presumably because the host mutation blocks phage lytic development.

According to this view of Mu developmental control, the similarity of low-MOI lysogenization frequency in the *him*$^+$ and *him* strains (cf. Tables 3 and 6) is a coincidence. In *him*$^+$ bacteria, integration events are frequent, but relatively few lead to lysogeny. In *him* bacteria, integration efficiency is reduced, but all Mu genomes that integrate form lysogens.

The model is consistent with the observation that integration of ^{32}P-labeled Mu DNA is lower in a *himA* host than in a *him*$^+$ host (9).

Increasing multiplicity leads to an increased frequency of lysogens per infected cell in *him* hosts (Fig. 2). Both increasing the number of substrate genomes which can integrate and increasing the integration efficiency of each (Fig. 3) contribute to the increased lysogenization. Elevated levels of Mu functions mimic the helping effect (Table 7) and provide further support for the model.

A frequent outcome of low-multiplicity infection of *him* bacteria is formation of a structure which may be a normal intermediate in Mu lysogenization, trapped as a result of the block on Mu development imposed by the host mutation. The putative intermediate is stable and can be converted into a lysogen by the introduction of auxiliary phage even 60 min after assay phage infection (Fig. 4). The capacity of the auxiliary phage to provide help also persists for a limited period (Fig. 5). The gene product responsible for helping is tentatively identified as Mu B (cf. Fig. 3 and Table 7).

Formation of nonlysogenic survivors of Mu infection. Survivors of high-multiplicity infection that are nonlysogens do not require the presence of the Mu *c*$^+$ gene in either *him*$^+$ or *him* bacteria (see Results). The establishment of immunity in the absence of integration and subsequent segregation of unintegrated Mu genomes therefore cannot be the usual mechanism to account for such survivors. (If excess Mu A protein helped rapidly establish immunity, this mechanism could, however, account for the immunity-dependent reduction of EOP [Yoshida, Ph.D. thesis] and the absence of enhanced lysogen formation [Table 9] observed in the presence of a plasmid producing Mu A.) Furthermore, nonlysogenic products are so common after high-multiplicity infections of *him* bacteria (Fig. 2) that the possibility that they represent uninfected cells is unlikely. We propose a different mechanism, which accounts for survivors of both *him*$^+$ and *him* infections.

Nonlysogenic survivors can be viewed as an outcome of host, rather than phage, development. Even at high MOIs, the failure of Mu to integrate into every chromosome of a particular infected cell could result in the segregation of nonlysogenic progeny. This model predicts that the frequency of nonlysogenic survivors per infected cell should depend on the chance of any chromosomes escaping Mu integration. The data in Fig. 1 and 2 are consistent with this expectation. First, the frequency of nonlysogenic survivors declines with increasing MOI in both *him*$^+$ and *him* hosts. Second, at any given MOI, the yield of nonlysogenic products per infected cell is greater in a *him* host than a *him*$^+$ host. This could reflect diminished integration of parental phage genomes in *him* bacteria and the absence in *him* bacteria of replicative transposition linking daughter chromosomes into cointegrate structures.

A similar mechanism accounts for nonlysogenic survivors of infection by bacteriophages λ (34) and P22 (3, 33), since nonlysogenic cells are found clonally associated with lysogens. The establishment of immunity is necessary to prevent lytic growth and segregation of replicating λ or P22 genomes to daughter cells. The formation of nonlysogenic survivors after high-multiplicity infection of *him*$^+$ *sup*$^+$ cells with Mu *c*am pAp1 (see Results) suggests that a single Mu-infected *him*$^+$ cell may yield both an infective center and a nonlysogenic cell as progeny. This outcome is possible because Mu replication requires integration and thus segregates with the host chromosome(s). If cell division occurs before all host chromosomes contain lytically growing Mu, then a daughter cell could be formed that did not inherit any integrated Mu genomes, whereas the other daughter could produce a burst of phage.

Additional Mu functions involved in lysogenization. In addition to the Mu c, ner, A, and B functions, other phage functions may play a role in lysogenization. Phage bearing substitutions in the semiessential early region lysogenize both *him*$^+$ and *him* hosts at modestly but reproducibly elevated frequencies in comparison with Mu *c*$^+$pAp1 (Tables 3, 6, and 7; Fig. 3). We have argued that the determining factor for lysogenization in *him*$^+$ hosts is the establishment of immunity, whereas in *him* hosts it is integration. Elevation of lysogenization frequency presumably requires different alterations of phage function in the two hosts, suggesting that there may be as yet unidentified functions in the Mu semiessential early region which influence lysogenization.

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