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Intermediates in Bacteriophage Mu Lysogenization of *Escherichia coli him* Hosts

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Characterization of a putative intermediate in the Mu lysogenization pathway is possible in a variant *Escherichia coli himD* strain which exhibits greatly diminished lysogen formation. In this strain, most infecting Mu genomes form stable, transcribable, nonreplicating structures. Many of these genomes can be mobilized to form lysogens by a second Mu infection, which can be delayed by at least 100 min. This intermediate structure can be formed in the absence of Mu A or B function. We suggest that the inferred intermediate could be the previously reported protein-linked circular form of the Mu genome. Providing Mu B function from a plasmid enhances Mu lysogenization in this *him* strain, and the enhancement is much greater when both Mu A and B functions are provided.

In *Escherichia coli himA* or *himD* mutants, bacteriophage Mu cannot kill or grow lytically, but lysogenization occurs (16, 18). Integration is reduced compared with that in a *him*$^+$ host (5), probably due to reduced levels of the Mu A (transposase) and B functions. In the accompanying paper (3), some consequences for lysogen formation by Mu are described. The observation that establishment of Mu immunity is not required for lysogenization of *him* bacteria suggests that all phage genomes integrating into the host chromosome form lysogens. Upon infection of a *him* host, many Mu genomes form a stable intermediate structure, which is apparently trapped as a result of the reduced levels of Mu A and B. This intermediate can be mobilized to form a lysogen by the subsequent introduction of other phage genomes.

Investigation of a pair of independently constructed *himD* strains, presumed to be isogenic, revealed distinctly different phenotypes with respect to Mu lysogenization. We infer the presence of an as yet uncharacterized genetic difference. Although we have not identified the basis of the difference, the study of Mu lysogenization in the variant strain background has been informative and is reported here.

One of these *himD* strains displays a 20-fold reduction in the probability of lysogen formation per infected cell, compared with the other. This feature permits characterization of an intermediate in lysogen formation. We show that in this *him* host many, perhaps all, infecting Mu genomes form a stable structure(s) which is transcribed, does not replicate, does not require the Mu A or B function for formation, and requires a Mu-repressed function in addition to A and B for efficient conversion into a prophage.

MATERIALS AND METHODS

Materials. Media, chemicals, and preparation of Mu phage stocks were as described in the accompanying paper (3).

Bacteria. The bacterial strains used are listed in Table 1. Spot tests to confirm Him phenotype were as described in the accompanying paper (3). Note that *zdh-201*::Tn*10* is ≥95% linked to *himA* (3, 26) and that *himD157* and *himDΔ3* are 8 base pairs apart (11). Some strains (noted in Table 2) were constructed in duplicate independent transductions to ensure that a rare, undetectable recombination event involving these markers had not occurred.

Although somewhat arbitrary, for the purposes of describing this work, all *him* strains constructed in our laboratory from the *him*$^+$ parent RBB63 are referred to as standard *him* strains. Bacteria displaying Mu lysogenization behavior different from that of the standard strains are referred to as variant *him* strains.

Bacteriophage. Most bacteriophage strains used were described in the accompanying paper (3). In addition, Mu *c*ts (4) was obtained from C. Kenyon, Mu *c*ts62 *A*1093pAp1 (17) was from M. Pato, and Mu *c*ts62 *B*5175 (23) was from M. Howe. Mu *c*$^+$pf7701 and Mu *c*$^+$pf7711 carry Kan^r substitutions, whereas Mu *c*$^+$pAp1 and Mu *c*$^+$pAp5 carry Amp^r substitutions, in nonessential portions of their genomes.

Plasmids. The Cam^r plasmids pRBB1, pRBB2, and pRBB3, which respectively express the Mu *A*, *B*, and *A B* genes constitutively, were described in the accompanying paper (3). The Amp^r plasmid pRKY18 expresses Mu *B* constitutively (R. K. Yoshida, Ph.D. thesis, University of Wisconsin, Madison, 1984).

Determination of lysogenization frequency. Titration of phage and bacteria and measurement of the number of lysogens per infected cell were as described in the accompanying paper (3). Briefly, the infected cell titer was calculated assuming a Poisson distribution of the multiplicity of infecting phage (MOI). Infected cells were plated permissively and, after sufficient time for phenotypic expression of drug resistance, overlaid with top agar containing antibiotic to determine the titer of lysogens.

Conditions for titrating Amp^r lysogens of RBB64 and its derivatives were empirically determined to be overlaying with 2 ml of top agar containing 100 μg of ampicillin per ml 2 h after plating or 50 μg of ampicillin per ml 3 h after plating (depending on the source of ampicillin), rather than the standard conditions of 250 μg/ml at 2 h. Standard overlay conditions were used (3) for quantitation of all other lysogens.

Pulse-chase experiments. Adsorption constants for the phage stocks used in the kinetics of helping experiment (k = 1.3×10^{-9} ml/min for Mu *c*$^+$pf7701; $k = 1.7 \times 10^{-9}$ ml/min

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TABLE 1. Bacteria

Strain	Description	Source or reference
E529	N99 *himDΔ3*::*cat* (P1 Kan^r *clr-100*)	R. Weisberg; 11
MH4386	K634 *zdh-201*::Tn*10*	M. Howe; 26
RBB63	F$^-$ λ$^-$ *galK2 rpsL200* IN(*rrnD-rrnE*)*1*	MH5775 = K37 = N99 = MM28; M. Howe; 2, 19
RBB64	K37 *himD157* Tn*10*-1230	MH5818; M. Howe; 26
RBB66	K37 *himA42*	MH5776 = K634; M. Howe; 19
RBB69	K37 *himD115* Tn*10*-1230	MH5816; M. Howe; 26
RBB73	RBB64 (Mu *c*$^+$pAp1)	
RBB85	RBB64(pRKY18)	
RBB103	RBB64 (Mu *c*$^+$pf7711)	
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
RBB149	RBB64(pRBB1)	
RBB150	RBB64(pRBB2)	
RBB151	RBB64(pRBB3)	
RBB155	RBB73(pRBB2)	
RBB156	RBB73(pRBB1)	
RBB157	RBB73(pRBB3)	
RBB182	RBB63 *himAΔSmaI*	3
RBB183	RBB63 *himDΔ3*::*cat*	3
RBB224	RBB63 *himD157* Tn*10*-1230	P1.RBB107 × RBB63, selecting for Tet^r
RBB226	"RBB64"[b] *himDΔ3*	P1.E529 × RBB64, selecting for Cam^r
RBB228	"RBB64" *himDΔ3* Tn*10*-1230	P1.E529 × RBB64, selecting for Cam^r
RBB230	"RBB64" *him*$^+$ Tn*10*-1230	P1.RBB110 × RBB226, selecting for Tet^r
RBB237	RBB63 *himA42 zdh-201*::Tn*10*	P1.MH4386 × RBB63, selecting for Tet^r
RBB240	"RBB66" *him*$^+$ *zdh-201*::Tn*10*	P1.SK1843 × RBB66, selecting for Tet^r
RBB242	"RBB66" *himDΔ3 zdh-201*::Tn*10*	P1.E529 × RBB240, selecting for Cam^r
RBB243	"RBB66" *himA42 zdh-201*::Tn*10*	P1.MH4386 × RBB66, selecting for Tet^r
RBB245	"RBB64" *himDΔ3 zdh-201*::Tn*10*	P1.SK1843 × RBB226, selecting for Tet^r
SK1843	F$^-$ *argE3 his-4 thi-1 aroD*$^+$ *rpsE rpsL*$^+$ *himA*$^+$ *zdh-201*::Tn*10* *lacΔMS286* (φ80 d*II lacΔBK1*)	S. Kushner
W3110	F$^-$ λ$^-$ IN(*rrnD-rrnE*)*1*	C. Kenyon; 2

[a] Signifies infection of RBB66 with P1 grown on RBB64.
[b] For derivatives of the variant strains, "RBB64" or "RBB66" signifies the strain background, and the *him* and Tn*10* genotypes are given explicitly.

for Mu *c*$^+$) were estimated based on the fraction of phage remaining unadsorbed to RBB64 after 15 min under standard infection conditions (data not shown). The MOIs for the pulse and chase (MOIs, 2 and ca. 44, respectively) were estimated as described in the accompanying paper (3).

For the delayed lysogenization experiment, the pulse infections were by the normal protocol, i.e., 15 min of adsorption followed by 100-fold dilution. The times at which chases were begun are with respect to the start of the pulse. The 0-min auxiliary phage chase was simultaneous with the assay phage infection, with Mu *c*$^+$ MOIs of 18 and 17 for the *A*am and *B*am assay phage infections, respectively. Later chases were for 15 min, followed by the addition of 10 mM ethylenebis(oxyethylenenitrilo)tetraacetic acid and a further 15 min of incubation before plating. Based on unadsorbed phage in the 0-min chase infections, the adsorption constant (*k*) to RBB64 for this stock of Mu *c*$^+$ was 7.1×10^{-10} ml/min, which yields an estimated MOI of ca. 14 for the later chases.

Assay of transient kanamycin resistance. The appropriate dose of kanamycin was determined in reconstruction experiments with a mixture of 100 RBB64 to 1 RBB64(Mu *c*$^+$pf7711). Challenge with 25 μg of kanamycin per ml led to a survival curve which leveled off at about 1% (data not shown).

RESULTS

Reduced Mu lysogenization in the variant RBB64 (*him D157*) strain background was exploited to characterize intermediates in the Mu lysogenization pathway. We also briefly considered lysogenization in the variant RBB66 (*himA42*) strain background, in which infection by an auxiliary phage failed to enhance lysogenization of an assay phage.

Lysogenization of variant strain RBB64 and derivatives at low MOI. RBB224 (*himD157*) was constructed by transducing *himD157* from RBB64 (*himD157*) into RBB63 (*him*$^+$), the parent strain of RBB64. RBB64 and RBB224 should thus be isogenic, although their behavior challenges this expectation. Four different Mu *c*$^+$ drug-resistant phage each lysogenized RBB64 (*himD157*) at frequencies of ~0.003 lysogens per infected cell, which was 20-fold lower than that observed in RBB224 (*himD157*) (Table 2). Since RBB69 (*himD115*) displayed a phenotype similar to that of RBB64 and RBB64 and RBB69 were constructed at the same time, it seems possible that the genetic feature that distinguishes these variant strains was present in the parent from which they were constructed.

In a limited attempt to determine the basis for reduced lysogen formation in RBB64, the *himD* locus (RBB226, RBB228) or the *himA*$^+$ locus (RBB245) of RBB64 was replaced by P1 transduction. This did not alter the lysogenization frequency of Mu *c*$^+$pf7701 (Table 2), although lysogen formation was very frequent in the *him*$^+$ derivative of RBB64 (RBB230). Some characteristic of strain RBB64, unlinked to either *himA* or *himD*, endowed *himD* derivatives of the strain with a grossly reduced incidence of Mu lysogen formation.

As a working hypothesis, we suggest that Mu lysogenization is diminished in RBB64 due to reduction of early gene expression below even the level typical in a *him* host.

Helping effect in variant strain RBB64. Mixed infection of

TABLE 2. Lysogenization of variant strain RBB64 and derivatives at low MOI[a]

Strain	*him* genotype[b]	Lysogens/infected cell (×100)[c]			
		Mu c^+pf7701	Mu c^+pf7711	Mu c^+pAp1	Mu c^+pAp5
RBB183	*himDΔ3*	8.6 ± 0.7	6.4, 6.6	7.8 ± 2	5.4, 7.5
RBB224	*himD157* Tn*10*-1230	13 ± 2	7.2, 12	7.7 ± 1	8.9, 9.2
RBB69	*himD115* Tn*10*-1230	0.45			
RBB64	*himD157* Tn*10*-1230	0.46 ± 0.1	0.34 ± 0.09	0.21, 0.31	0.24, 0.30
RBB226*[d]	*himDΔ3*	0.49			
RBB228*	*himDΔ3* Tn*10*-1230	0.39			
RBB245*	*himDΔ3 zdh-201*::Tn*10*	0.19			
RBB230*	*him*$^+$ Tn*10*-1230	41			

[a] Measurements were made at an MOI of <0.10, except for RBB230 (MOI, 0.14) and RBB231 (MOI, 0.16). Unadsorbed phage was ≤14%.
[b] Data for the RBB63 derivatives RBB183 and RBB224 are from the accompanying paper (3). All other strains are derivatives of RBB64 except RBB69, which was made in parallel with RBB64.
[c] Results of cases with one or two measurements are listed individually. Means ± standard deviations are listed for cases with three to five separate measurements.
[d] Duplicates of the strains marked with an asterisk (*) were made in independent transductions and exhibited indistinguishable lysogenization frequencies (data not shown).

him bacteria with a fixed low multiplicity of an assay phage and increasing multiplicities of an unmarked auxiliary phage results in an increase in the frequency of lysogen formation by the assay phage (3).

Lysogen formation by Mu c^+pf7701 in variant strain RBB64 (*himD157*) was enhanced by the presence of Mu *c*ts B^+ auxiliary phage in a manner linearly dependent on the MOI (Fig. 1). Helping was more prominent in RBB64 than in standard *him* strains, due to the diminished background level of lysogen formation. The lysogenization frequency observed in Fig. 1 for Mu c^+pf7701 in the absence of auxiliary phage was higher than that in Table 2, because multiplicities of ~1.5 assay phage were used.

The presence of Mu *c*ts62 *B*1066 auxiliary phage had little impact on the assay phage lysogenization frequency (Fig. 1). Similar results were observed with Mu *c*ts62 *B*1979 and Mu *c*ts62 *B*5175 auxiliary phage (data not shown), which carry amber mutations mapping in different deletion intervals of the *B* gene (23). These observations suggest that helping either directly or indirectly involves the B function of the auxiliary phage.

Provision of Mu B function alone from pRKY18 enhanced assay phage lysogen formation in RBB64 by an amount equivalent to that provided by 20 to 25 Mu c^+ auxiliary phage (Fig. 2). Taken with the results described in Fig. 1, the helping effect can be accounted for as due to the B function provided by the auxiliary phage.

The presence of pRKY18 did not saturate the helping effect. Infection of RBB64(pRKY18) with Mu c^+ auxiliary phage in addition to the assay phage further enhanced lysogenization, with a dependence on MOI similar to that observed in the absence of the plasmid (Fig. 2). Infections of RBB64 with multiplicities of up to ~60 Mu c^+ auxiliary phage showed a linear increase in assay phage lysogenization, with no detected loss of host cell viability (data not shown). Either the level of Mu B provided by pRKY18 was not saturating, or auxiliary phage can provide an additional enhancing function (perhaps Mu A?) which the plasmid cannot.

Kinetics of helping in variant strain RBB64. Infection of the standard *him* strain RBB182 leads to formation of an intermediate structure, which can be mobilized to form a lysogen by subsequent auxiliary phage infection even 1 h after assay phage infection (3).

To determine the kinetics of helping in RBB64, a culture was infected with Mu c^+pf7701 assay phage, diluted 1,000-fold to terminate this pulse, and at various times chased with a high-multiplicity infection of Mu c^+ auxiliary phage. The number of Kan^r lysogens in the no-chase samples increased exponentially with time (Fig. 3), presumably due to replication of the lysogens formed by the pulse infection. An immediate chase with auxiliary phage resulted in a 10-fold increase in the number of assay phage lysogens formed. Delaying the chase infection for up to 100 min did not alter the number of assay phage lysogens formed. Thus, whereas lysogenization was an uncommon outcome of infection of RBB64, formation of an intermediate which can be mobilized to integrate by a helper phage was much more frequent. The intermediate failed to replicate and remained stable for 100 min or more.

Transient kanamycin resistance after infection of variant strain RBB64 by Mu c^+pf7711. The stability of the genomes subject to help provoked the question of whether sequences resident on these intermediates were transcribed. The experiment displayed in Fig. 3 can only assess the fate of those phage genomes which form lysogens. It might be possible to follow all of the assay phage genomes by their ability to express Kan^r. Cells should be Kan^r as long as they harbor a transcribed intermediate structure, but growth of a Kan^r

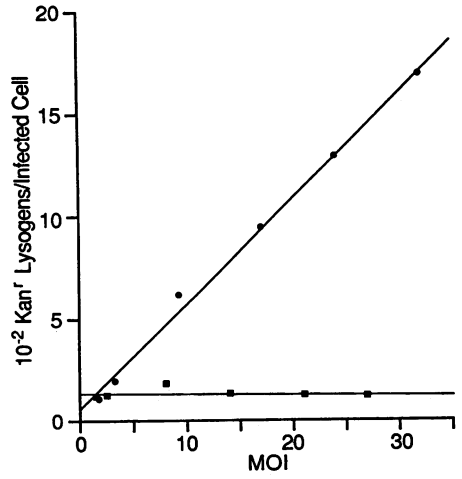

FIG. 1. Lysogenization of RBB64 (*himD157*) with fixed multiplicities (~1.5) of Mu c^+pf7701 assay phage and increasing multiplicities of Mu *c*ts B^+ (●) or Mu *c*ts62 *B*1066 (■) auxiliary phage.

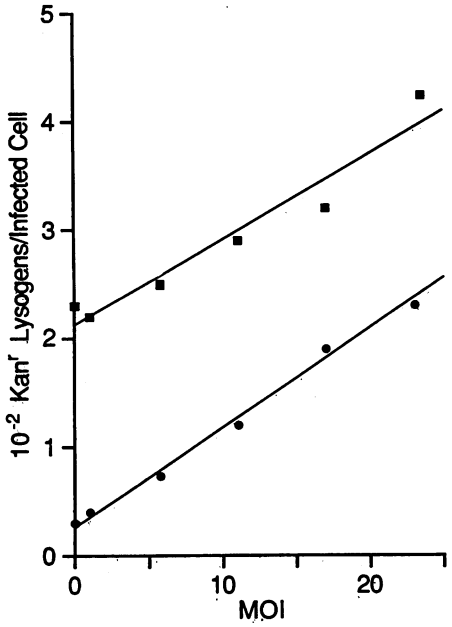

FIG. 2. Lysogenization of RBB64 (*himD157*) with a fixed low multiplicity (0.057) of Mu c^+pf7701 assay phage and increasing multiplicities of Mu c^+ auxiliary phage in the absence (●) or presence (■) of pRKY18 (Mu B^+).

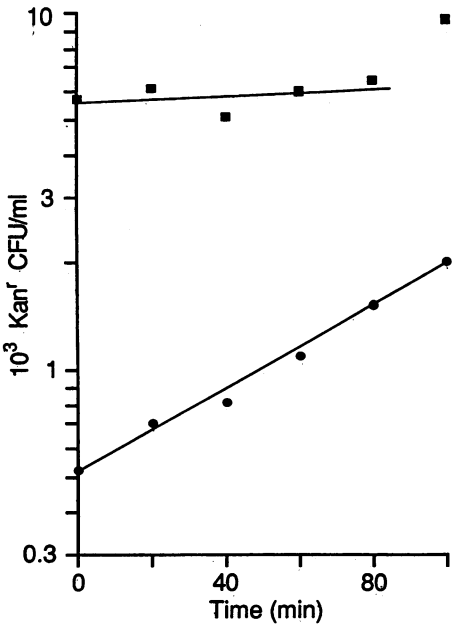

FIG. 3. Kinetics of helping in RBB64 (*himD157*). A standard LBCaMg culture was exposed to Mu c^+pf7701 assay phage for 45 s and then diluted 1,000-fold to terminate the pulse. At the indicated times, samples were infected with a high-multiplicity chase of Mu c^+ auxiliary phage (■) and allowed to adsorb for 15 min; 10 mM EGTA was added, and samples were plated as usual for Kan^r lysogens 15 min later. No-chase controls (●) were done in parallel. The 0-min timepoint, defined as the start of the first chase, was 2.6 min after the start of the pulse.

colony can only occur when the phage genome is transmitted to each daughter cell, i.e., a lysogen is formed. We therefore measured the yield of cells that were transiently resistant to a challenge with kanamycin.

A culture of RBB64 was split, and one half was infected with Mu c^+pf7711 at low multiplicity. At 45 min postinfection, kanamycin was added to both halves, and after various times of exposure samples were removed and titrated for viable cells. The uninfected cells were killed efficiently by kanamycin, with survivors declining more than 4 orders of magnitude over the course of the experiment (Fig. 4). There was evidence in the infected culture of a subpopulation of cells that were resistant to the kanamycin challenge. The size of this subpopulation approximated that of the infected cells. Only 0.0031 of infected cells actually formed lysogens, consistent with the data in Table 2.

A more ambitious experiment was done to characterize the products of infection in greater detail. An infected culture was divided into three portions, which were challenged with kanamycin at 45 or 105 min postinfection or remained unchallenged. The kanamycin treatment at 45 min after infection clearly revealed a population of cells that were transiently kanamycin resistant, i.e., survived the challenge but were not lysogens (Fig. 5). This population was comparable in size to that of infected cells and was still evident when the challenge was delayed until 105 min postinfection. It would appear that most of the Mu genomes infecting RBB64 assumed a form which was stable and transcribable. We acknowledge the unlikely possibility, however, that the act of infection rendered cells transiently Kan^r, independent of the presence of the Mu c^+pf7711 genome.

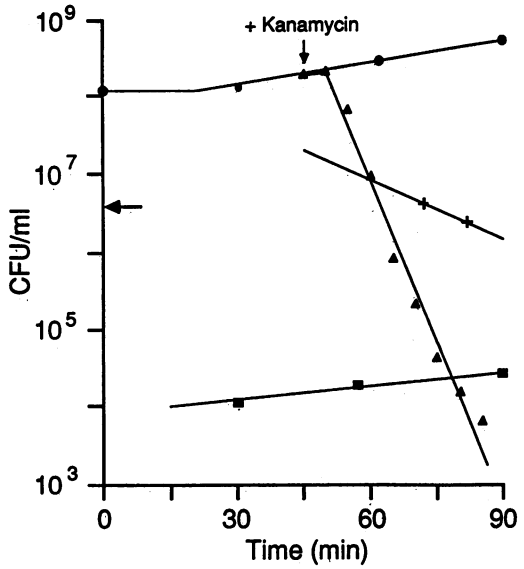

FIG. 4. Transient kanamycin resistance after infection of RBB64 (*himD157*) by Mu c^+pf7711. A culture of RBB64 was split, and one portion was infected with Mu c^+pf7711 (MOI, 0.033) under standard conditions. After adsorption and dilution, the infected and uninfected cultures were split, and one portion of each was challenged by the addition of 25 μg of kanamycin per ml at 45 min. At the indicated times, samples were diluted in saline and plated for surviving cells or Kan^r lysogens. Symbols: ←, titer of infected cells from infected culture; ●, total cells from uninfected, unchallenged culture; ■, Kan^r lysogens from infected, unchallenged culture; ▲, cells surviving kanamycin challenge from uninfected culture; +, cells surviving kanamycin challenge from infected culture.

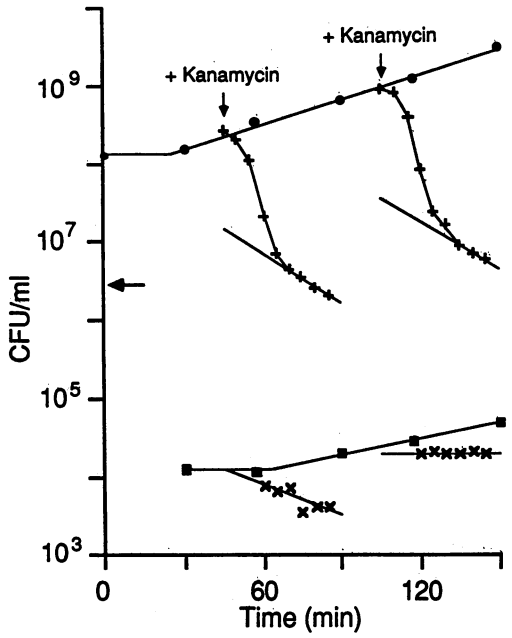

FIG. 5. Transient kanamycin resistance after infection of RBB64 (*himD157*) by Mu c^+pf7711. A culture of RBB64 was infected with Mu c^+pf7711 (MOI, 0.021) under standard conditions. After adsorption and dilution, the culture was divided into three portions, which were challenged by the addition of 25 μg of kanamycin per ml at 45 or 105 min or remained unchallenged. At the indicated times, samples were diluted in saline and plated for surviving cells or Kan^r lysogens. Symbols: ←, titer of infected cells; ●, total cells from unchallenged culture; ■, Kan^r lysogens from unchallenged culture; +, cells surviving kanamycin challenge; ×, Kan^r lysogens surviving kanamycin challenge.

Delayed lysogenization of variant strain RBB64. What phage functions are involved in the formation of the intermediate structure? In a different form of pulse-chase experiment, RBB64 (*sup*$^+$) was infected by Mu *A*am or *B*am assay phage. Lysogen formation by Mu *c*ts62 *A*1093pAp1 was not detectable, whereas that by Mu *c*ts62 *B*1979pAp1 was rare (Table 3). When cells were subsequently infected with a high multiplicity of Mu c^+ A^+ B^+ auxiliary phage, Amp^r lysogens were formed at a frequency similar to that observed with a Mu c^+ A^+ B^+pAp1 assay phage alone. The yield of assay phage lysogens was independent of the time at which complementing auxiliary phage were provided. This experiment suggests that neither the Mu A nor B function has to act immediately to preserve the potential for lysogen formation in RBB64.

Effect of Mu A, B, and c functions on lysogenization of variant strain RBB64. To further investigate the role of the Mu A, B, and c functions in helping and lysogenization of RBB64, a series of infections was carried out with Mu

TABLE 4. Effect of Mu A, B, and c functions on lysogenization of variant strain RBB64

Strain	Relevant description of host		Kan^r lysogens/infected cell (×100) after infection with[a]:	
	(Mu c^+pAp1)	Plasmid	Mu c^+pf7701 alone	Mu c^+ auxiliary
RBB64	−		0.71	3.6
RBB73	+		0.13	0.12
RBB150	−	pRBB2 (B^+)	3.9	6.2
RBB155	+	pRBB2 (B^+)	0.16	0.13
RBB149	−	pRBB1 (A^+)	0.73	1.3
RBB156	+	pRBB1 (A^+)	0.095	0.077
RBB151	−	pRBB3 (A^+ B^+)	48	21
RBB157	+	pRBB3 (A^+ B^+)	1.8	1.1

[a] The assay phage MOI ranged between 0.12 and 0.16. The auxiliary phage MOI ranged between 24 and 37.

c^+pf7701 assay phage, alone or in the presence of a high multiplicity of Mu c^+ auxiliary phage. In addition, Mu A or B function or both were provided by a plasmid, and Mu c function was provided by an established Mu c^+pAp1 prophage. The impact on the assay phage lysogenization frequency of all possible combinations of these additional Mu functions was tested (Table 4).

With additional Mu function provided only by an auxiliary phage, the helping effect was observed (Table 4); the presence of immunity reduced assay phage lysogenization and prevented helping. Immunity might prevent helping by repressing expression of phage functions either from the assay phage, necessary to form the intermediate, or from the auxiliary phage, necessary for helping. To help distinguish these possibilities, the Mu B function was provided from a plasmid. In the absence of immunity, assay phage lysogenization was enhanced but not saturated (Table 4), as expected from the data in Fig. 2. The effect of immunity, however, was not overcome (Table 4), suggesting that immunity blocked the expression of an assay phage function involved in either formation of the intermediate or its conversion into prophage.

The transposase, A, could be such a function. However, Mu A function provided by a plasmid did not alter Mu c^+pf7701 lysogenization frequency (Table 4), suggesting that the level of A is not limiting for lysogenization. In the presence of both immunity and the A-producing plasmid, lysogenization frequency remained reduced and helping was not observed (Table 4). Therefore, Mu A alone is not sufficient to circumvent the effects of immunity.

The presence of a plasmid producing Mu A seemed to reduce the helping effect (Table 4; data not shown). One possibility might be that A protein from the plasmid interacts with B protein from the auxiliary phage in such a manner as to prevent the B protein from assisting in assay phage lysogen formation. Alternatively, increased transcription of

TABLE 3. Delayed lysogenization in variant strain RBB64

Assay phage	MOI	Amp^r lysogens/infected cell (×100)					
		No chase[a]	High-MOI chase with Mu c^+ at time (min):				
			0	18	30	45	60
Mu *c*ts62 *A*1093pAp1	0.62	<0.00016	0.12	0.13	0.14	0.13	0.085
Mu *c*ts62 *B*1979pAp1	0.78	0.0019	0.42	0.19	0.25		0.15

[a] The comparable value for Mu c^+ A^+ B^+pAp1 at an MOI of ≤0.10 is ~0.26 (Table 2).

the Mu *A* gene is reported to result in a decrease in A activity (14).

When both Mu A and B functions were provided from a plasmid, the lysogenization frequency increased 70-fold (Table 4). The data in Table 4 suggest that Mu B function is limiting, and Mu A function is close to limiting, for lysogen formation in the *himD157* host RBB64. In the presence of auxiliary phage, the assay phage lysogenization frequency was somewhat reduced (Table 4). Since the presence of a plasmid providing Mu A and B functions permits formation of a few infective centers in RBB64 (data not shown), some loss in viability cannot be excluded.

Some lysogenization of immune bacteria occurred in the presence of the Mu A^+ B^+ plasmid, but very much less than occurred in the nonimmune bacteria (Table 4). A Mu function under the control of immunity, in addition to A and B, apparently plays a role in lysogen formation. Since Mu repressor is known to bind specifically to the Mu transposase-binding sites (8), it could be that the repressor can inhibit lysogenization by blocking access of transposase to the ends of Mu. The unidentified function necessary for lysogenization would then simply be the free ends of Mu DNA.

Lysogenization of variant strain RBB66 and derivatives. The helping effect was observed in all *himD* strains, and in all standard *him* strains, which we tested. There was no evidence of helping in RBB66 (data not shown), which is the widely used *himA42* strain K634, or in the RBB66 derivative RBB107 (*himA42 himD157*). This seems to be a feature of the strain in which the *him* mutation resides, rather than the allele itself. Transferring the *himA42* mutation from the K634 derivative MH4386 (*himA42 zdh-201*::Tn*10*) to RBB63 (*him*$^+$) resulted in a strain, RBB237, with a phenotype indistinguishable from any of the standard *him* strains, including RBB63 *himD157* and RBB63 *himAΔSmaI* (data not shown). Thus, there is evidence for an additional strain difference, distinct from that described for variant strain RBB64 (*himD157*), which has an impact on the consequences of Mu infection.

Mu lysogenization frequency was elevated two- to threefold in the variant strain RBB66 (*himA42*) compared with the standard strain RBB237 (*himA42 zdh-201*::Tn*10*) (data not shown). Replacement by P1 transduction of the RBB66 *himA* locus (RBB243), the *himD* locus (RBB107), or both (RBB242) did not greatly alter Mu lysogenization frequencies (data not shown), suggesting that a putative mutation responsible for the variant phenotype of RBB66 is not closely linked to either *himA* or *himD*.

DISCUSSION

Properties of intermediate(s) in RBB64. Evidence for an intermediate in Mu lysogenization has been described for standard *him* strains (3). An uncharacterized genetic feature of the strain background of RBB64 (*himD157*) results in a 20-fold reduction in the yield of lysogens per Mu-infected cell (Table 2). This feature provides a basis for a characterization of some of the properties of the intermediate(s).

Low-multiplicity infection of RBB64 with a drug resistant Mu assay phage results in a low frequency of lysogen formation per infected cell. Coinfection with Mu c^+ auxiliary phage results in an increase in the yield of drug-resistant lysogens (Fig. 1 and 2). The helping effect is observed even if infection by auxiliary phage is delayed. The assay phage genomes that can be mobilized to form lysogens by subsequent phage infection are referred to as intermediates. The presence of these intermediates provides the basis for the pulse-chase experiment displayed in Fig. 3.

The constant yield of assay phage lysogens, independent of the time of auxiliary phage infection (Fig. 3), demonstrates that the intermediate is stable and can be mobilized to form a lysogen for an indefinite period of time. Lysogens formed in the initial infection (no-chase samples) replicate, whereas the yield of lysogens formed when intermediates are mobilized (chased samples) fails to increase (Fig. 3). This contrast suggests that the intermediate does not replicate and thus is not integrated into the host chromosome. In a similar experiment with the standard strain RBB182 (*himAΔSmaI*), replication of the lysogens appeared delayed (3). It could be that the lag in replication of lysogens in RBB182 was caused by a phage gene product(s), whose expression is reduced in the variant RBB64.

Although not all intermediates can be experimentally mobilized to form drug-resistant lysogens, all of the infecting assay phage genomes carry the drug resistance marker. To test for expression of this marker, a culture of RBB64 infected at low multiplicity was exposed to a transient antibiotic challenge. The titer of viable cells remaining after the challenge revealed further properties of the intermediate. For more than 2 h, essentially all cells infected by Mu c^+pf7711 display transient resistance to kanamycin (Fig. 4 and 5), implying that upon infection of RBB64 most Mu genomes form the stable intermediate, which can be transcribed, but do not form lysogens. Inspection of the data in Fig. 5 reveals that the size of the transiently kanamycin resistant population may actually increase with time. This could be due to linear transmission of the intermediate and to cytoplamic inheritance in daughter cells of the enzyme responsible for kanamycin resistance. The population of transiently resistant cells seems to be more sensitive to the kanamycin challenge in 105 min than are the initially formed lysogens, which is consistent with this interpretation. The products of infection that are stable, transcribable, nonreplicating structures are reminiscent of the abortive transductants observed in generalized transduction mediated by bacteriophage P1 (1) or P22 (10, 24).

Functions and intermediates in Mu integration. A circular form of the Mu genome, closed by the Mu N protein (12) and capable of supercoiling, can be observed upon infection of lysogens (13) or minicells (25), conditions blocking further phage development. One simple interpretation of our results is that infection of *him* hosts traps Mu in the same intermediate structure. This view is consistent with the observations that neither Mu A nor B function is required to preserve the potential for lysogen formation (Table 3) and that the intermediate apparently does not replicate (Fig. 3). The biological role of the structure observed after Mu infection of lysogens or minicells has been the subject of discussion (12, 13, 15, 22, 25). The intermediate observed in *him* cells is a viable form of the Mu genome, since it can be efficiently converted into a prophage when Mu A and B functions are provided (Table 4).

Providing Mu B function alone is sufficient to convert some fraction of the intermediates into prophage (Fig. 2, Table 4) (3). A stable intermediate in Mu in vitro transposition formed in the presence of Mu A protein, *E. coli* HU protein, and donor Mu DNA has recently been demonstrated (7, 27). In the presence of Mu B protein and target DNA, this intermediate is converted in vitro into a complex in which Mu DNA is covalently attached to target DNA. Although any mechanistic relationship between Shapiro intermediate formation in vitro and lysogenic integration in vivo is un-

known, the comparison is provocative. Although we only have clear evidence for one intermediate, it could be that upon infection of *him* cells at least two intermediate structures can be distinguished. The first structure would require Mu N protein and would be converted by Mu A into the second intermediate form. Mu B would mobilize this second intermediate to form a lysogen. This model is speculative, since the order (or simultaneity) of action of Mu A and B during lysogenization is unknown.

We suggest the distribution of Mu genomes among intermediate structure(s) on the pathway from infecting to integrated DNA would reflect the levels of Mu A and B function available. In *him*$^+$ hosts, A and B are in excess and no intermediates are trapped. In standard *him* hosts, sufficient A and B are present to convert 5 to 10% of infecting genomes directly into lysogens, whereas two or three times as many genomes are in a form requiring only B to complete lysogen formation (3). In the variant *him* host RBB64, lysogens are rarely formed because levels of Mu A and B are so low, and most genomes are trapped in the initial form.

The only phage functions required for either replicative or nonreplicative transposition in vitro are Mu A and B and the Mu DNA ends (6, 21). The only additional function known to be required for efficient lysogen formation in vivo is repressor, which is required in *him*$^+$ but not *him* hosts. In RBB64 (*himD157*), immunity reduces Mu lysogenization 25-fold in the presence of a constitutive source of Mu A and B (Table 4). If Mu repressor is not directly interfering with the integration reaction (e.g., by occupying transposase-binding sites), this would suggest the existence of an additional unidentified phage function required for efficient Mu lysogenization. More specifically, because this experiment was done in a *him* host, where integration is presumably synonymous with lysogenization (3), the putative additional function would be involved in integration.

Variant *him* strains. The *himD157* and *himA42* mutations were transduced from RBB64 and MH4386, respectively, into RBB63 (*him*$^+$). This generates two new strains, RBB224 (*himD157* Tn*10*-1230) and RBB237 (*himA42 zdh-201*::Tn*10*), which should be essentially identical to the old strains RBB64 (*himD157* Tn*10*-1230) and RBB66 (*himA42*). Comparison of Mu lysogenization in the new and old strains, however, revealed two striking phenotypic differences. First, Mu lysogenization of the new *himA* and *himD* strains is indistinguishable (3). This is expected, because *himA* and *himD* encode subunits of the same protein, integration host factor (11, 20). If Mu lysogenization is influenced by integration host factor, then the phenotypes of *himA* and *himD* mutants should be similar. In contrast, lysogenization of the old *himA* strain is quite different than of the old *himD* strain (see Results). Furthermore, Mu lysogenization in the newly constructed *him* strains is unlike that in either old *him* strain (see Results). We presume that each of the old *him* strains harbors different mutational changes which modify the impact of disabling integration host factor. Although somewhat arbitrary, the simplest way to describe the set of phenotypes is to designate the new *him* strains as standard, and both old *him* strains as variant. The highly pleiotropic phenotype of *him* mutants (reviewed in reference 9) makes it possible that extragenic suppressor mutations might be favorably selected in *him* strains.

The unidentified alteration in the variants could affect the tightness of the *him* block on Mu lytic growth. Spontaneous phage release by Mu lysogens is rare in the variant *him* strain RBB64, more common in the standard *him* strain RBB182, and most frequent in the variant *him* strain RBB66 (data not shown). This trend correlates with the low-MOI Mu lysogenization frequencies in these strains and magnitude of helping effect (Table 2 and 4) (3). It could be that expression of the Mu *A* and *B* genes increases in the series of four host strain types which we have examined: variant *him* RBB64 < standard *him* < variant *him* RBB66 < *him*$^+$.

The variant strains manifest the primary phenotype characteristic of *him* bacteria, i.e., they do not support the lysogen formation by bacteriophage λ or lytic growth of Mu. However, the existence of supposedly isogenic strains of *him* bacteria with demonstrably different phenotypes suggests caution in the interpretation of results comparing various *him* phenotypes.

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