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Bacteriophage Lambda; Abortive Infection of Bacteria Lysogenic for Phage P2*

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Abstract. The efficiency of plating of wild-type λ on a host lysogenic for P2 is less than 10^{-6}, and only a small number of infected cells produce progeny phage. Lambda can adsorb and inject its DNA normally in such cells; the DNA can circularize and is not nicked or degraded, but replication is severely impaired. Mutants of P2, which as prophages no longer interfere with λ, have been isolated and found to be recessive to wild type, implying that P2 prophage codes for a diffusible product involved in λ interference. The P2 gene product responsible for preventing λ growth also kills recombination-deficient bacteria of the *recB* and *recC* classes under conditions where P2 does not normally kill the host. Mutants of λ that are resistant to interference are recessive to wild-type λ. Thus λ actively participates in its own interference. The λ-mutants that are resistant to interference are unable to synthesize at least two nonessential proteins. In addition, they are unable to grow on recombination-deficient bacteria of the *recA* class, but they can grow on *recA recB* double mutants.

Introduction. Lysogenic bacteria are immune to the type of phage that they carry as prophage. In addition to causing immunity, some prophages are also known to block the growth of one or several unrelated phages. This phenomenon has been called prophage interference.[1] We report here some genetic and biochemical features of one such case of prophage interference, the inability of λ-bacteriophage to multiply in a host lysogenic for P2.

Materials and Methods. The bacterial strains used in these studies are described in Table 1. The wild-type strain of λ used was λPaPa.[2] λ*vir* is λ-2 of Lederberg and Lederberg.[3]

Media: Minimal M9 contains: 7.0 gm Na_2HPO_4, 3.0 g KH_2PO_4, 1.0 g NH_4Cl, 2.0 ml NaCl (25%), 0.3 ml $FeCl_3$ (0.01 M) per liter of distilled water. Complete M9 contains the following additions per 100 ml of the basic salts media: 4 ml maltose (10%), 2.5 ml Norit treated casein amino acids (10%), and thymidine and uracil to 5 μg/ml. Minimal H-1 contains: 100 ml 1 M KH_2PO_4, 1 ml 1.8×10^{-3} M $FeSO_4$, 5 ml 3.0 M $(NH_4)_2SO_4$/liter of distilled H_2O. H-1 growth medium contains: 100 ml minimal

TABLE 1. *Strain list.*

Collection no.	Relevant genotype	Reference no.
C-1a	F^-, prototrophic	17
C-117	F^-, $(P2)_I$	18
C-1058	F′, (P2) (P2 *cai*)	19
C-1201	F^-, *arg-1*, *bio-1*	20
C-1760	F^-, *uvrA*, *thy* (P2)	21
JC2926	*recA13*	22
JC4693	F^-, *leu trpE his su*$^-$	22
JC4695	F^-, *leu trpE his su*$^-$ *recB21*	22
JC5495	*recB21*, *recA13*	23
AB2470	*recB21*	24
...	F^-, $(\lambda CI_{857})/\lambda$, *thy*	25
HF4704	F^-, *uvrA*, *thy*	5

H-1, 0.1 ml 1 M $MgSO_4$, 1 ml of a 1% threonine-leucine, 0.1% B_1 solution (sterilized by Millipore filtration), 0.5 ml 10% Norit treated casein amino acids, 0.5 ml 20% glucose, 1.5 ml 0.1% thymidine. H-1 induction medium contains: 0.1 ml 1 M $MgSO_4$, 1 ml of the threonine-leucine-B_1 solution, 6 ml 10% Norit-treated casein amino acids 1 ml 20% glucose, and either ^{3}H- or ^{14}C-thymidine to the desired specific activity.

Chemicals: ^{3}H- and ^{14}C-thymidine were purchased from Schwarz BioResearch Corp. ^{13}C-Glucose and $^{15}NH_4Cl$ were obtained from the Nuclear Equipment Co. Mitomycin C, lysozyme (Grade I), and pronase (Grade B) were purchased from Sigma Chemical Co. The pronase was self-digested for 2 hr at 37°C, followed by 2 min at 80°C, and then rapidly chilled.

Growth of radioactive bacteriophages: ^{3}H or ^{14}C λCI_{857} was prepared by temperature induction of a lysogenic strain derived from the thymine requiring CR34 (Table 1). An overnight culture, grown at 33°C in H-1 growth medium, is diluted $^1/_{50}$ into the same medium and grown with aeration at 33°C to a density of 3×10^8 cells/ml. The cells are resuspended in one half volume of H-1 induction medium containing 2 μCi/ml of either ^{3}H- or ^{14}C-TdR, and incubated for 10–12 min at 45°C, after which they are rapidly shifted to 37°C and grown with vigorous aeration. When lysis is complete, after 60–70 min, a few drops of chloroform and 0.1 ml/10 ml lysate of 1 M Tris pH 7.5 are added and the lysate rapidly chilled. The phage are purified by two cycles of differential centrifugation, followed by banding in CsCl. A typical preparation from 10 ml of lysate, gives a final phage stock of $\sim 10^{11}$ plaque-forming units/ml with a specific activity of 2×10^{-6} cpm/phage. $^{13}C^{15}N^3H$ λCI_{857} was prepared by the same procedure except that ^{13}C-glucose and $^{15}NH_4Cl$ were substituted in both the growth and induction media. This procedure is a modification of that used in the laboratory of D. Hogness.

Results. The effect of multiplicity of infection: The data in Table 2 present some characteristics of this abortive infection. Although the phage adsorbed well (>96%), only a small fraction of P2 lysogens produced progeny λ. However, the infected cells were killed even more efficiently than the similarly infected nonlysogen. The number of cells yielding phage increases at a rate roughly proportional to the increasing multiplicity, as shown by the ratio of yielding cells to adsorbed phage. This indicates that no multiplicity reactivation occurs, and thus each infecting λ-chromosome has a low probability of escaping the P2 interference independent of the presence of other λ-genomes in the same cell. The progeny phage produced by the small number of yielding cells was not modified, since they had the same low efficiency of plating on *E. coli* C (P2) as had the parental phage.

Circularization of injected DNA: To determine if λ-DNA is able to inject and form covalently closed circles in P2 lysogens, *E. coli* C and *E. coli* C (P2) were

TABLE 2. *Frequency of yielders λ as a function of multiplicity of infection in E. coli C and E. coli C(P2).*

Bacterial strain	Input multiplicity	Survivors (%)	Yielder frequency	Yielder frequency/ adsorbed phage	Burst size/ yielders
E. coli C	1.2	30	0.57	0.32	382
	5.0	48	0.49	0.10	157
	14.7	46	0.46	0.031	120
	29.0	51	0.50	0.017	93
E. coli C(P2)	2.1	45	0.008	0.0036	9.0
	8.5	10	0.010	0.0013	5.0
	25.0	9	0.023	0.0009	5.2
	50.0	9	0.046	0.0009	4.8

Frequency of yielders of λ^+ as a function of multiplicity of infection in *E. coli* C and *E. coli* C (P2). Exponentially growing cells were centrifuged, resuspended in 10^{-2} M $MgSO_4$, and infected with λ at various multiplicities. After 14-min incubation at 37°C the free phage was removed by λ-antiserum, the infected cells were diluted into prewarmed growth medium, and assayed on a P2-resistent *E. coli* C indicator. After 70-min incubation at 37°C with aeration, chloroform was added and the cultures were again assayed on the same indicator. The high proportion of *E. coli* C survivors is presumably due to lysogenization.

infected with tritiated phage and the DNA was examined by sedimentation through alkaline sucrose. It was found that in both the lysogen and the nonlysogen approximately 25% of parental λ-DNA is converted to a form which sediments 3.9 times more rapidly than linear DNA in alkaline sucrose (Fig. 1).

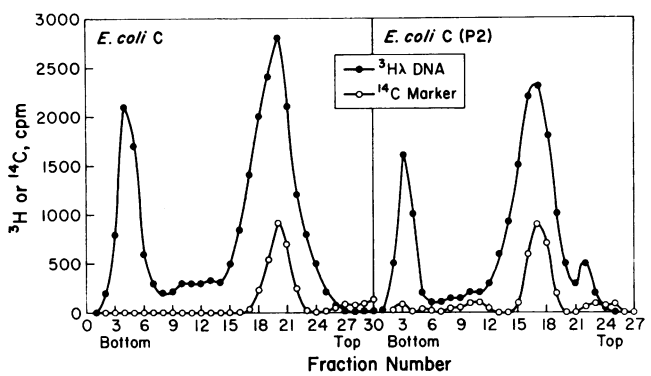

FIG. 1.—Intracellular forms of λ-DNA *E. coli* C-la and *E. coli* C (P2) = C-117, were grown at 37°C in complete M9 to a density of 2×10^8 cells/ml. 1×10^9 cells of each strain were infected at a multiplicity of infection of 5 with ^{3}H-λCI_{857} in 0.5 ml of 0.01 M Mg^{++} at 0°C for 15 min. Adsorption under these conditions is >95%. 5.0 ml of prewarmed complete M9 was then added and the cultures incubated at 37°C without aeration. After 15 min, the cells were rapidly chilled and centrifuged at 4°C. All further operations, unless otherwise specified, were performed at 0°C. The cell pellets were washed twice with cold minimal M9 and then resuspended in 0.2 ml of a solution of 0.5 M sucrose, 0.001 M EDTA, -0.01 M Tris, pH 10.5. 0.1 ml 1% lysozyme followed by 0.1 ml 0.2 M EDTA was added to each culture. After 15 min, 0.2 ml of the spheroplasted cells and 0.05 ml of ^{14}C-λ DNA marker was layered onto a 5 ml 5-20% linear sucrose gradient, pH 12.5, previously layered with 0.1 ml 1 M NaOH-0.1% EDTA, and centrifuged in an SW50 rotor for 60 min at 45,000 rpm. Approximately 30 fractions of two drops each were collected by puncturing the bottom of the tube. 50 μg of carrier salmon sperm DNA followed by 0.5 ml of 10% trichloroacetic acid was added to each fraction tube and, after 20 min, the contents were filtered through Whatman 2.4 cm GF/C glass fiber filters, washed five times with cold 10-ml aliquots of 10% trichloroacetic acid, and dried. The filters were placed in glass vials, a toluene-POP-POPOP solution added, and the vials counted on a dual-channel Nuclear Chicago liquid scintillation counter. Recovery of counts from the gradients was 70-80% for ^{3}H and 85-90% for ^{14}C.

These results are in agreement with the findings reported by Young and Sinsheimer,[4] and indicate that λ-DNA can inject and circularize to essentially the same extent in *E. coli* C (P2) as in *E. coli* C, and that little of the DNA is nicked or degraded.

DNA synthesis in infected cells: Since both injection and circularization of λ-DNA appears to be normal in P2 lysogenic cells, we next asked if DNA replication could proceed in these cells. To examine this we used the mitomycin technique of Lindqvist and Sinsheimer.[5] This technique utilizes the finding that mitomycin treated *hcr*$^-$ cells cannot replicate their own DNA but can support the growth of phage. Mitomycin treated cells were pulsed with ^{3}H-thymidine for 1-min periods every 5 min after infection with λ*vir*. The results (Fig. 2) indicate that λ-DNA synthesis is strongly inhibited in the P2 lysogen. The results of a similar "pulse-chase" experiment confirmed this interpretation and eliminated the possibility that λ-DNA is being synthesized and rapidly degraded, since the radioactivity incorporated into λ-DNA during the first 20 min after infection of HF4704 (P2) remains in an acid-insoluble form for more than 30 min after a "chase" of the label with a 1000-fold excess of cold thymidine. In order to examine more closely the residual DNA synthesis, a density transfer experiment was performed with $^3H^{15}N^{13}C$ λCI_{857} in the presence of ^{14}C-thymidine, and the DNA was examined by equilibrium sedimentation in CsCl. No transfer of radioactivity to a fully light position is seen (Fig. 3) and the transfer to material banding at a hybrid density indicates that no more than one round of λ-DNA replication occurs under these conditions.

Mutants of P2 which do not interfere with lambda development: Mutants of P2 which as prophages do not interfere with λ infection were isolated by mutagenizing strains lysogenic for P2 with *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine. Colonies formed by surviving cells were transferred to broth and grown up. These cultures were then used as plating indicators for λ. About 1% of the cultures allowed efficient plaque formation. When P2 produced by these strains was used to lysogenize sensitive cells, the new P2 lysogens also allowed λ-plaque formation. The efficiency of plating of λ on such strains is the same as on the non-lysogenic strain. While characterizing these mutants we found that they are identical to the previously described *old* mutants of P2 phage.[6] The *old* mutants differ from wild-type P2 in their behavior on a class of recombination-deficient strains of *E. coli* C: P2 wild type forms clear plaques on these strains and is unable to lysogenize them, but *old* mutants form turbid plaques and can establish lysogeny. We have isolated 17 different mutants of P2 which are unable to interfere with λ growth, and all of them behave like P2 *old*. Conversely, over 250 *old* mutants tested are unable to interfere as prophages with λ growth.

The inability of P2$^+$ to lysogenize certain Rec$^-$ strains of *E. coli* C was shown by Sironi[6] to be due to killing of the host and not to a defect in lysogenization. This interaction of P2$^+$ with Rec$^-$ *E. coli* C strains is similar to the interaction of P2$^+$ with the *recB*, *recA recB*, and *recC* strains of *E. coli* K listed in Table 1. The *E. coli* K *recA* strain, however, can be lysogenized by P2$^+$ and is not killed by the product of the *old* gene.

The killing of *recB* strains by the product of the P2 *old* gene can be demon-

strated most directly by using a P2 phage carrying a mutation in an early gene ($P2amB_{116}$), which does not normally kill a su^- host.[7] When such a mutant infects a su^- *recB* host, the viability of the culture drops 99-fold, and growth of the culture as measured by optical density reaches a plateau after approximately one generation. However, if a $P2amB_{116}old$ mutant is used to infect such a culture, growth is slowed only slightly and the culture remains viable.

P2 *old* mutations have been located at the left end of the P2 vegetative map,[8] removed from the immunity region, and the results are compatible with a single gene being affected. The *old* gene is the only known gene in this region of the map which is expressed from the prophage state.

When a strain of *E. coli* C, doubly lysogenic for P2 and a P2 *old* mutant, is

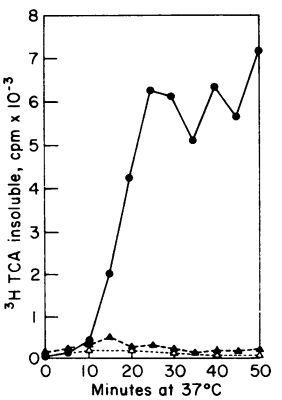

FIG. 2.

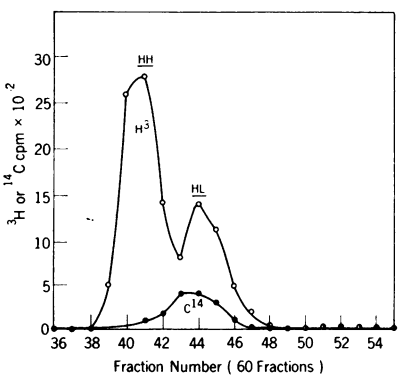

FIG. 3.

FIG. 2.—λ-DNA synthesis in a P2 lysogenic strain. HF4704 and HF4707(P2) were grown at 37°C in complete M9 to a density of 2×10^8 cells/ml. 10 ml of each culture were treated with mitomycin C (50 μg/ml) using the procedure of Lindqvist and Sinsheimer.[5] The treated cultures were resuspended in 1.0 ml of cold 0.01 M Mg^{++}, and 0.5 ml λ*vir* (2×10^{10} φ/ml) was added to 0.6 ml of each culture. After 20 min at 0°C, cold complete M9 was added to a final volume of 6 ml, the cells distributed in 0.5-ml aliquots to small tubes, and placed at 37°C. 5.0 μCi ^{3}H-TdR was added to a tube every 5 min and after 1 min the cells were killed with 0.5 ml of cold 10% trichloroacetic acid and the tubes placed on ice. The cells were centrifuged for 10 min at 10,000 rpm in an SS34 Sorvall rotor and the supernatants decanted. The pellets were dissolved in 0.5 ml 1 N NaOH and incubated overnight at 37°C, after which 0.5 ml 1 N HCl, followed by 1 ml of 20% trichloroacetic acid, was added to each tube. The precipitates were filtered and counted as previously described in the legend to Fig. 1. ●, HF4704 + λ*vir*; ▲, HF4704(P2) + λ*vir*; △, HF4704(P2) uninfected.

FIG. 3.—Density shift as a measure of λ-DNA synthesis in a P2-lysogenic strain. HF4704-(P2) was grown at 37°C to 2×10^8 cells/ml in complete M9 and 10 ml of cells treated with mitomycin C. After washing, the pellet of cells was resuspended in 0.2 ml *TM at 0°C and $^3H^{15}N^{13}C\lambda CI_{857}$ added to give a final multiplicity of 5. Adsorption was allowed for 20 min after which 2.0 ml of cold minimal M9 was added and the cells washed twice. The final washed pellet was resuspended in 2.0 ml of prewarmed (37°C) complete M9-TdR and 50 μCi of ^{14}C-TdR added. The infected cells were incubated at 37°C without aeration for 20 min. The culture was then rapidly chilled, 2.0 ml of cold minimal M9 added, and the cells pelleted. The final washed pellet was resuspended in 0.2 ml of a solution of 0.5 M sucrose, 0.1 M Tris, 0.001 M EDTA, pH 10.5, and lysed with lysozyme-sarkosyl, using the procedure of Young and Sinsheimer.[4] To the clear viscous lysate, 1.3 gm/ml of solid CsCl was added and the mixture centrifuged in an SW50 rotor for 48 hr at 40,000 rpm. Sixty 1-drop fractions were collected by puncturing the bottom of the tube; carrier DNA and 10% trichloroacetic acid were then added as before. The precipitates were filtered through GF/C filters, washed, and counted as described in the legend for Fig. 1. Sixty-five per cent of the ^{3}H counts were recovered.

used as a host for λ infection, it is found that λ does not multiply. This shows that the wild-type allele is dominant over the mutant one, and suggests an active role for the *old* gene in preventing λ-multiplication.

Lambda mutants resistant to P2 interference: When large amounts of ultraviolet induced λ are plated on *E. coli* C (P2), plaques are formed with a frequency of about 2×10^{-7}. After purification on a nonlysogenic strain, the phage from these rare plaques retain the ability to grow on strains lysogenic for P2. These mutants have been called *Spi*$^-$ (sensitive to P2 interference). Similar λ mutants have also been observed by L. E. Bertani (personal communication). These phages are unable to plate on a λ-resistant strain and are sensitive to λ immunity, confirming that they are in fact mutants of λ.

When P2-lysogenic cells are mixedly infected with λ$^+$ and a λ*Spi*$^-$ mutant, the number of cells yielding phage and the burst size drop to the level found when λ$^+$ alone is used (Table 3). This complementation test indicates that λ expresses one or more functions that play an active role in preventing growth in P2 lysogens.

TABLE 3. *Mixed infection of E. coli C and E. coli C(P2) with λ and λ Spi-12.*

Bacterial strain	Input Multiplicity λ	λ *Spi-12*	Survivors (%)	Yielder frequency	Burst size/ yielders
E. coli C	7.7	...	60	0.58	133
	...	4.6	23	1.10	150
	7.7	4.6	70	0.73	95
E. coli C(P2)	7.1	...	11	0.01	2.7
	...	4.3	21	0.70	42.0
	7.1	4.3	14	0.01	3.7

Mixed infection of *E. coli* C and *E. coli* (P2) with λ and λ *spi-12*. Exponentially growing cells were centrifuged, resuspended in 10^{-2} M $MgSO_4$, infected with phage, and adsorption allowed for 15 min at 37°C. The free phage was removed by centrifugation and, after resuspension, the cells were diluted into prewarmed growth medium and assayed on a P2-resistant *E. coli* C indicator. After 60-min incubation at 37°C with aeration, the cultures were again assayed on the same indicator.

All but one of the λ*Spi*$^-$ mutants we isolated are λ *p bio* transducing phage. This fact led us to test characterized λ *p bio* phage for ability to plate on P2-lysogenic strains.

Lambda *bio* 7-20, whose deletion does not include *exo*[9,10] (Fig. 4), behaves like λ wild type. Lambda *bio* 1 or 10, whose deletions extend beyond *β*[10,11] (Fig. 4),

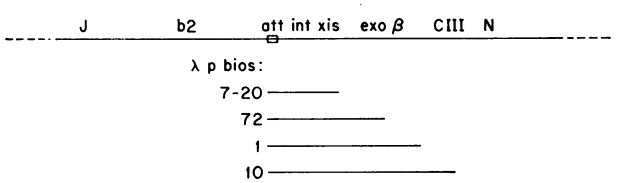

FIG. 4.—Simplified map of the middle region of the λ genome. The mutants are described in refs. 9–11. The deletion in λ *p boi* 10 extends into C_{III} according to Dr. G. Kayajanian (personal communication), and the deletion in λ *p bio* 1 extends beyond the *β*-gene according to James Zissler (personal communication). The physical length of each deletion was determined by Hradecna, Z., and W. Szybalski, *Virology*, **38**, 473 (1969).

behave like *Spi*$^-$ mutants. This result indicates that the portion of genome deleted in these mutants covers the genetic determinants necessary for full interference by P2 prophage to be expressed.

Lambda *bio* 72, whose deletion extends into *exo* but not into *β*, plates on *E. coli* C doubly lysogenic for P2 with higher efficiency (4×10^{-5}) than either λ or λ*bio* 7-20 (10^{-8}). Plaques of λ*bio* 72 on these strains, upon testing, appear to be produced by mutants of the *Spi*$^-$ type. The high ability of λ*bio* 72 to originate spontaneous *Spi*$^-$ mutants suggests that more than one mutation might be necessary in order for λ to plate on a P2 lysogen with an efficiency of one, and that at least one of these mutations might be in a gene deleted in λ*bio* 72, but intact in λ*bio* 7-20. However, this gene cannot be *exo* alone because an *exo*$^-$ mutant of λ, *red*$_3$,[12] does not give rise to *Spi*$^-$ mutants at the intermediate frequency displayed by λ*bio* 72.

Since Manly *et al.*[10] have reported that λ*bio* 1 fails to plate efficiently on the recombination-deficient *recA* strains of *E. coli*, we have tested the ability of *Spi*$^-$ mutants to plate on such hosts. All the λ*Spi*$^-$ mutants are unable to plate on strain JC2926 (*recA 13* but are able to plate with normal efficiency on AB2470 (*recB 21*) and JC5495 (*recA 13, recB 21*).

Discussion. We have found that the replication of λ-DNA is blocked in P2 lysogens, although the injected DNA can circularize and is not degraded. The genetic analysis of this phenomenon shows that both P2 and λ form diffusible products which are involved in the interference process. One of these products is formed by the *old* gene of P2 which is active in the prophage state.[6] The infecting λ itself forms at least two products which are required for full interference to occur. At least one of the genes coding for these products is deleted in λ*bio* 72, which plates with higher efficiency than λ wild type on P2 lysogens. The genes for both (or all) the lambda products are deleted in λ*bio* 1 which has the full *Spi*$^-$ phenotype, i.e., it plates with an efficiency of one on P2 lysogens.

The P2 *old* gene product kills *E. coli recB* and *recC* mutants. We feel that this killing is due to modification of host DNA. This modification may block normal replication. We expect that the modification caused by P2 *old* is usually removed from host DNA by a nuclease that is the product of the *recB* and *C* genes.[13-17] When λ infects a strain lysogenic for P2, the λ-DNA is probably modified in the same way as the bacterial chromosome, but in this case the modification cannot be repaired by the *recB,C* nuclease because the products of the *spi* genes interfere with the repair process.

λ*Spi*$^-$ mutants cannot form plaques on *recA* indicator but can do so on *recA recB* indicator. These findings suggest that the genes involved in the *Spi*$^-$ phenotype can substitute for the *recA* gene of the cell and protect the phage DNA from the action of the *recB,C* nucleases. Both the *spi* genes and the *recA* gene may protect λ-DNA from degradation by promoting recombination. If this model is correct, an examination of parental λ *Spi*$^-$ DNA after infection of a *recA* host should reveal a heterogeneous population of phage DNA molecules.

Most of our λ *Spi*$^-$ mutants are λ *p bio* phage. This fact indicates that we have discovered a powerful method for selecting λ-transducing phage of this type.

Note added in proof: We have been able to show degradation of λ *Spi*⁻ DNA in a *recA* host. Experiments to be presented elsewhere indicate that λ genes N, O, and *int* are expressed normally in a P2 lysogenic host.

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