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Host Influence on the Activity of Genes *c*1 and *c*3 in Regulating the Decision Between Lysis and Lysogeny in Bacteriophage P22

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A Polymyxin B-sensitive mutant of *Salmonella typhimurium* (Pox-1) channels all infecting wild-type P22 toward lysogenization. The efficiency of this channeling is sufficiently high that P22c^+ (wild type) cannot form plaques on Pox-1; phage mutants defective in repressor synthesis (P22*c*1, *c*2, *c*3) or refractory toward repressor (P22*vir* B) can form plaques. The lytic growth of all phages which have a functional *c*1 gene is retarded in Pox-1; this retardation is seen even in phages which cannot make repressor. We present experiments which are consistent with the explanation that the retardation is an exaggeration of a normal regulatory event. In a wild-type host, P22 genes *c*1 and *c*3 products, host RNA polymerase, and other host factors (?) interact at a promotor site (*c*27) in the phage DNA. This interaction promotes repressor synthesis and represses transcription of lytic genes. In the mutant Pox-1, a host product involved in viral DNA synthesis and transcription is altered. The altered host product results in stronger retardation of lytic gene transcription. The importance of this interaction in the decision between lysis and lysogeny is discussed. The mutant Pox-1 alters the expression or activity of another phage gene. Gene *c*3 product is absolutely required for lysogenization in this host, although it is not so required in wild-type *S. typhimurium*.

Bacteriophage P22 infection of *Salmonella typhimurium* can have either of two results: (i) the lytic response in which the infecting genome is transcribed and replicated to produce infectious progeny viruses; (ii) the lysogenic response in which the replication of the infecting viral genome is repressed and phage genome is integrated into the host chromosome to become a prophage (6, 12).

Lysogenization by temperate phage is known to be influenced by various environmental factors as well as the genetic constitution of the virus and host. Clear *c* mutants of phages P22 and λ were isolated and well characterized by Levine (11) and Kaiser (10). The *c*1 and *c*3 functions of phage P22 are required only for establishing lysogeny, whereas the *c*2 function is essential not only for establishing but also for maintaining lysogeny (11). Our previous work points out that functional and structural similarities exist in the regulatory mechanisms of repressor formation in phages P22 and λ (8).

In this paper, the diffusible product of a regulatory gene will be indicated by C and the number which identifies the gene, e.g., CII is the product of *c*II.

In repressor formation by phage λ, the CII -CIII complex (equivalent to P22 C1-C3) has bifunctional properties. The complex functions soon after infection, activates the promotor for repressor establishment (*pre*), and blocks the transcription of genes involved in lytic growth which are located on the opposite strand (6). Synthesis of λ repressor in lysogens is activated by repressor molecules at the promotor locus *prm* (7, 14). Phage P22 repressor formation after infection is initiated by an interaction between C1-C3 at a promotor defined by the *c*27 mutation which is analogous to λ *pre* (8). A possible bifunctional property of C1-C3 is discussed in this paper.

As compared with the large amount of information about environmental and viral control of lysogenization, little is known about host effects. A few studies utilizing hosts defective or abnormal in supporting lysogenization have been published. A colicin-tolerant mutant of *Escherichia coli* that has altered membrane proteins is lysogenized at high frequency by phage λ wild type (15). Belfort and Wulff isolated the *hfl*-1 mutant of *E. coli*, which causes λcIII to lysogenize with an increased frequency (2). Bacterial mutants which are defective in catabolic repression are known to channel temperate phages to lysis (9).

The polymyxine B-sensitive mutant used in

this study has some properties similar to the colicin-tolerant mutant of *E. coli* (15). The mutation in Pox-1 causes pleiotrophic effects: slow growth, temperature sensitivity, low level of cyclic AMP, as well as sensitivity to polymyxin B. Phage P22 wild type is unable to form plaques on Pox-1 because practically all infecting P22 wild type are channelled toward lysogenization. Mutants defective in producing repressor (*c*1, *c*2, and *c*3) or refractory to repressor action (*vir* B-3) are able to form plaques on Pox-1 with almost the same efficiencies as those observed on the parental strain MG 109 (19). Growth of various *c* mutants of phage P22 in Pox-1 are reported in this paper. Our results are interpreted to show that regulation of phage transcription, phage DNA synthesis, and the decision between lysis and lysogeny are partially dependent on host factors.

MATERIALS AND METHODS

Bacterial and phage strains. Strains used in this study are listed in Tables 1 and 2. MG 109 is wild type in supporting the growth of P22. The genetic locations of some of the phage genes mentioned in this paper are shown in Fig. 1.

Media. Nutrient broth contained 8 g of nutrient broth (Difco), 5 g of NaCl, and 1,000 ml of water. Nutrient agar and soft agar were made from nutrient broth by adding 12 g of agar (Difco) or 8 g of agar, respectively. Penassay broth contained 17.5 g of antibiotic medium 3 (Difco) and 1,000 ml of water.

TABLE 1. *S. typhimurium strains used*

Name	Genotype	Reference
MG 109	*ara* 9 *leu* 130; formerly called no. 387	Tokuno et al. (19)
Pox-1	*ara* 9 *leu* 130 pox*; mutant of MG 109	Tokuno et al. (19)

Test for lysogenization. Bacterial colonies were picked with a toothpick from agar plates and spotted onto a nutrient agar plate seeded with MG 109. Bacteria which liberated phages that caused lysis of the sensitive bacteria were scored as lysogens (19).

One-step growth curve. Bacteria in the exponential growth phase at 30 C and at a concentration of 2×10^8 cells/ml were infected with phage at a multiplicity of infection (MOI) of five. Ten minutes were allowed for adsorption, and then P22 antiserum was added to a *K* value of 2 or 3. Preparation, assay, and use of antiphage serum and platings for infective centers and free phage were carried out as described by Adams (1).

Assay of repressor. Phage P22 repressor was assayed by the procedure developed by Echols and Green (7) for phage λ. Protein concentrations in the extracts were estimated by the conversion factor: absorbance at 280 nm = 1 is equivalent to 0.3 mg/ml of protein. Radioactive ^{3}H-labeled P22c1 DNA for use in the assay was prepared according to Smith (16).

RESULTS

Growth of P22 regulatory mutants in Pox-1. Pox-1 channels P22c^+ toward lysogeny with such high efficiency that this phage forms no plaques on this host (19). All clear mutants of P22 and P22 *vir*B, which is refractory toward P22 repressor, form plaques on Pox-1.

Each of the P22 clear mutants produces a different pattern of growth when it infects Pox-1 in nutrient broth at 30 C (Fig. 2a). The *c*1 mutant has a latent period of 45 min and a sharp-rise period. Its burst size is 56. Phage P22*c*2 grows more slowly and has a latent period

TABLE 2. *Phage P22 strains used*

Genotype	Relevant properties	Reference or source
c^+	Wild type	Levine (11)
$c1^1$,	Unable to establish lysogeny	Levine (11)
$c2^5$, $c2^6$	Unable to establish or maintain lysogeny	Levine (11)
$c3^5$	Unable to establish lysogeny	Levine (11)
*c*27	Unable to establish lysogeny, defective in promotor for establishment of repressor synthesis	Bronson and Levine (3) Gough and Tokuno (8)
*K*5	Operator constitutive mutant, a component of the complex *vir* B-3 mutant	Bronson and Levine (3)
mnt	Unable to establish or maintain lysogeny, originally called *v1*	Zinder (20)
vir B-3	Virulent mutant	Bronson and Levine (3)
vir A-9	Virulent mutant	Bronson and Levine (3)

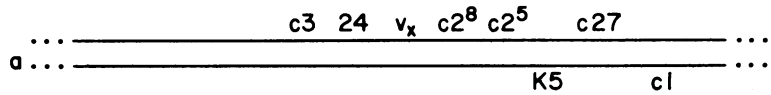

FIG. 1. *Map of the immC region of P22 (from Gough and Tokuno [8]).*

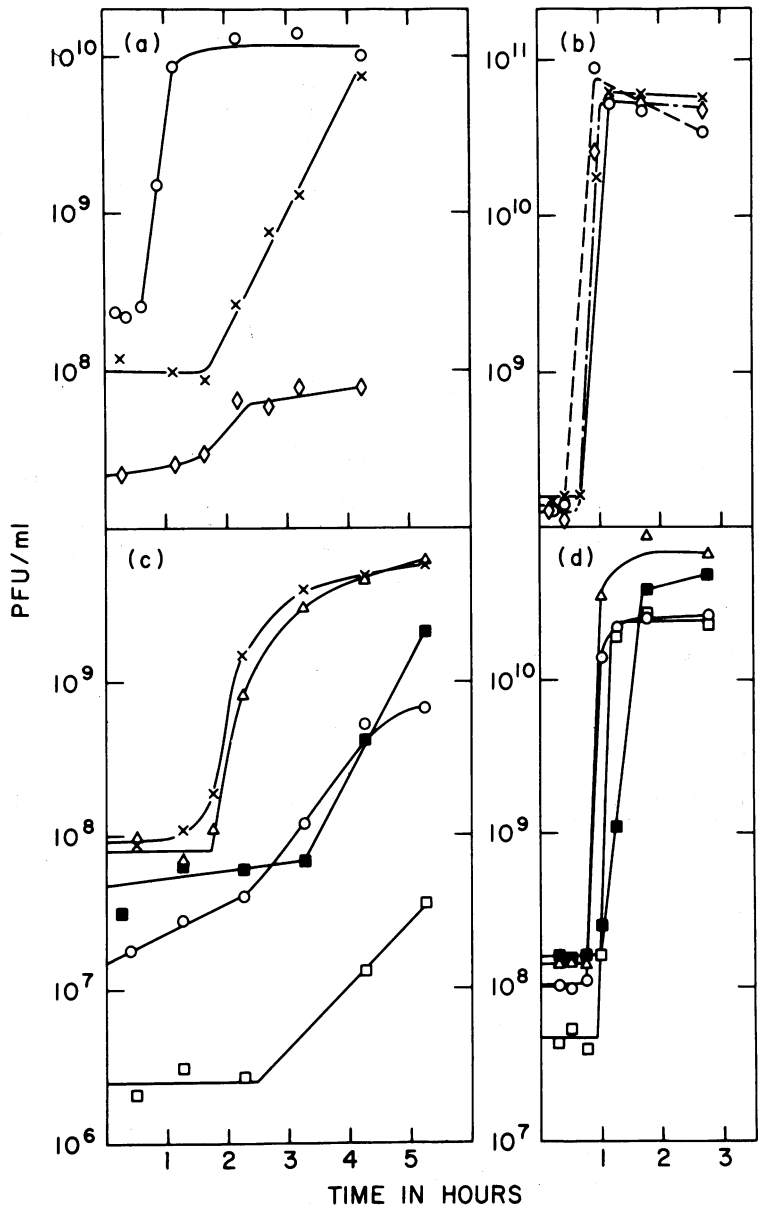

FIG. 2. *One-step growth curve of various P22 mutants.* (a) *P22c1*^{1} (○), *P22c2*^{6} (×), *P22c3*^{5} (◇), *in Pox-1. MOI = 5;* (b) *P22c1*^{1} (○), *P22c2*^{6} (×); *P22c3*^{5} (◇) *in MG 109. MOI = 5;* (c) *P22c*$^{+}$ (□); *P22c2*^{6} (×), *P22c27* (△), *P22K5* (○), *P22Vir B-3* (■) *in Pox-1. MOI = 5;* (d) *P22c*$^{+}$ (□), *P22c27* (△), *P22K5* (○), *P22Vir B-3* (■) *in MG 109. MOI = 5. MG 109 was used for indicator bacteria and plated on nutrient agar.*

of 110 min and a slow-rise period. Although it grows differently from P22c1, *c2* produces the same yield of phage, a burst of 60. P22*c3* grows most poorly, and it is difficult to define its latent period. Infections in the parental strain MG 109 (Fig. 2b) show that these three clear mutants grow essentially the same, in contrast to their different growth patterns in Pox-1. However, the *c1* mutant has a latent period 15 min shorter than *c2* and *c3* mutants in MG 109. MG 109 is wild type with respect to its influence on the growth of P22. The same patterns of viral growth were seen when these experiments were done in the richer Penassay medium containing glucose.

The plating efficiencies of less than 1 that are

observed when P22 is plated on Pox-1 do not result from some members of the Pox-1 population being unable to support any phage growth. The number of infective centers produced by a Pox-1 culture infected by c1 at an MOI of 5 is equal to the number of colony formers present before infection. These infective centers produced homogeneously large plaques on MG 109. This experiment shows that the productive fraction of a Pox-1 population can be 1.0. When this same experiment was done with P22c2, the productive fraction was 0.48, and the size of plaques formed on MG 109 was very heterogeneous. This difference between c1 and c2 is consistent with other observations in this paper that Pox-1 retards and restricts the growth of P22c2 but not c1.

The virulent mutant *vir* B-3 carries two operator mutations, *V*x and K5, which allow it to escape P22 repression and to grow in P22 lysogens. This mutant forms tiny plaques on Pox-1, much smaller than those made by PP22c2 (19). The results in Fig. 2c show that *vir* B-3 grows poorly in Pox-1 as compared to its growth in MG 109 (Fig. 2d) and that its growth curve resembles that of P22c^+. However, the productive fraction in *vir* B-3 infections of Pox-1 is about 10 times greater than in c^+ infections.

The wild-type allele of the c27 locus is necessary for the establishment of repressor synthesis (8). This mutant complements as a c2 (3, 11) and plates with the same efficiency as does c2 on Pox-1 (8, 19). c27 has a growth pattern identical to c2 (Fig. 2c). In strain MG 109, its growth pattern is also the same as that of c2 (Fig. 2d). The K5 mutation is a component of *vir* B-3. It results in constitutive expression of genes to the right of c2 (12) but it is sensitive to the Pox-1-imposed restriction of P22 growth (8). It grows very poorly in Pox-1 with no definite latent period (Fig. 2c). In MG 109, its growth is the same as that of c27 (Fig. 2d).

The severity of the restriction placed on P22 lytic growth by Pox-1 depends upon the phage genome. We have concluded (19) that all phages which produce repressor are restricted by Pox-1. Refining this general conclusion, the following pattern of restriction can be drawn. Wild-type phage with normal repressor and operators is most severely restricted. The K5 mutant which makes repressor (8; see Table 3) and has one wild-type operator grows poorly. Mutations of both operators in *vir* B-3 allow this phage to form tiny plaques on Pox-1 and to grow sluggishly in this strain. All c3 mutants so far isolated are leaky and are able to carry out all the functions necessary for lysogenization at low efficiency (11). This leakiness probably accounts for c3 growth resembling that of c^+. Finally, P22c2 carries out an early function necessary for lysogenization, the so-called "c1 repression" (17), and its growth is more retarded than P22c1, which does not carry out the early step. Thus, phages which manifest any of the processes leading to lysogeny are restricted by Pox-1 and, as the capability to lysogenize increases toward that of the wild type, the severity of the restriction increases.

TABLE 3. *DNA binding activity in P22-infected Pox-1*[a]

Infecting phages	MOI	Time after infection (min)	Counts/min per 100 μg of protein	Relative counts/min per protein
P22c^+	5	60	21,445	1.0
P22c1[1]	5	20	69	0.003
P22c2[6]	5	60	109	0.005
P22c3[5]	5	60	12,333	0.58
P22 *K*5	5	60	23,456	1.1
P22c27	5	60	772	0.03
P22 *Vir* B-3	5	60	44,030	2.05

[a] Pox-1 was grown to 3.2×10^8 cells/ml in 200 ml in a 1-liter flask at 30 C. Cells were centrifuged and suspended into 80 ml of fresh nutrient broth. A portion (20 ml) of each cell suspension was infected by each P22 phage mutant and incubated at 30 C for 5 min for adsorption. The infected cells were suspended in 100 ml of nutrient broth in a 500-ml flask and harvested at the indicated times. Assays were done in triplicate, and results were averaged. Pox-1 culture infected by P22c1 was harvested at 20 min because of the early lysis in this infection.

Two other regulatory mutants of P22 are known to produce antirepressor (13) which inactivates c2 repressor. Neither of these mutants, *mnt* nor *vir* A, grows lytically in Pox-1 (19).

P22 repressor formation in Pox-1. Amounts of P22 repressor after infection of Pox-1 were measured using the nitrocellulose filter binding technique (7). P22c1, c2 and c27 produce very little DNA binding activity, and phages P22K5 and *vir*-B produce more than wild-type levels (Table 3). Comparable results were obtained upon infection of a wild-type host by these phages (8). Phage P22c3, which is leaky, produces 58% of the amount of repressor produced by c^+.

The three phages which produce repressor c3, K5, and *vir*-B all grow poorly in Pox-1. This is so even for K5 and *vir*-B which have mutated operators that do not respond normally to repressor. These results support our conclusion that phages which make repressor are inhibited by Pox-1. Repressor synthesis, however, does not explain the retardation of growth of P22c2

and c27; neither makes repressor, but both grow slowly as compared to P22c1.

Phage P22c3 does not lysogenize Pox-1. The inability of the wild-type P22 to form phaques on Pox-1 is due to a high frequency of lysogenization. Phage P22c3 plates with about 0.1 efficiency on Pox-1 as compared to MG 109. Because P22c3 at a high MOI can lysogenize MG 109, we expected that P22c3 would also lysogenize Pox-1. However, no lysogenization of Pox-1 by P22c3 was found after infection with either high or low MOI (Table 4). These results indicate that the c3 function is essential for lysogenization in Pox-1.

DISCUSSION

Model to explain retardation of P22 growth in Pox-1. *S. typhimurium* Pox-1 channels P22 toward lysogeny. This channelling is associated with restrictions on lytic growth of the phage. These restrictions are imposed at two levels: (i) the growth of phages which carry out "c1 repression," an early event in the lysogenic pathway, is retarded in Pox-1 (and wild-type hosts); (ii) all phages which make repressor are also restricted. The well-characterized depression in P22 DNA synthesis rate at 6 min after infection is dependent on a functional c1 gene (17). We have explained c1 repression as resulting from sequential transcription of particular regions of the P22 genome (8). After infection, transcription from *Vx* and K5 (Fig. 1) results in the production of genes c1 and c3 products (C1-C3) and the products of genes *18* and *12*. Genes *18* and *12* are involved in making phage DNA (12), and their availability results in the DNA synthesis seen from 3 to 6 min after infection. During this time C1-C3 accumulates, and at 6 min it binds to the site defined by c27. Binding of RNA polymerase which is facilitated by C1-C3 at c27 results in transcription to the left to produce c2 mRNA and blockage of transcription to the right. Transcription across *18* and *12* is reduced, which reduces production of *18* and *12* products, and, if transcription of this region is required to prime DNA synthesis, primer also becomes unavailable. The absence of transcription and *18* and *12* products stops DNA synthesis and results in c1 repression.

We assume that the mutation in Pox-1 increases the c1 effect by increasing the ratio of c1 and c3 product to viral DNA. It may be that the mutation in Pox-1 reduces transcription of genes *18* and *12*. This would result in decreased phage DNA synthesis in Pox-1 as compared to the wild-type host and an increase in the efficiency of c1-repression. If the mutation does not interfere with expression of the c2 gene, the ratio of c2 product to phage DNA should be higher in Pox-1 than it is in a wild-type host. This result has been found (18). An alternative explanation is that the mutation in Pox-1 reduces the overall capacity of the cell to make DNA, and that *18* and *12* products are less efficiently utilized in this host.

The rightward transcriptional blockage is seen in wild-type hosts. Figure 2b shows that P22c2 has a longer latent period than does c1 in host MG 109. Because the c1-dependent retardation of lytic growth is seen in MG 109, we conclude that the growth retardation in Pox-1 is an exaggeration of a normal event.

Behavior of P22c27 in Pox-1. P22c27 is analogous to λ *pre* defined by the cy mutants (4). It is thought to define a promotor at which RNA polymerase, C1-C3, and other factors bind. In this scheme c1 and c27 are involved in the same complex event, and mutations in either c1 or c27 might be expected to produce

TABLE 4. *Efficiency of lysogenization by P22c3 in MG 109 and Pox-1 at 30 C*[a]

Host	MOI[b]	Fraction infected[c]	B/B_0[d]	Lysogens/ colonies[e]	Frequency of lysogenization[f]
MG 109	0.6	0.45	0.69	22/190	22/275 = 0.08
MG 109	6.0	1.0	0.69	134/224	134/314 = 0.43
Pox-1	0.6	0.45	0.66	0/158	0/239 = <0.01
Pox-1	6.0	1.0	0.73	1/150	1/205 = <0.01

[a] The infected cells were plated on nutrient agar with antiserum ($K = 2$) after 10 min of adsorption and 5 min of P22 antiserum treatment.

[b] Number of phage (PFU)/number of bacteria.

[c] $1-P_0$, where P_0 is the fraction of a population uninfected at MOI = m. $P_0 = e^{-m}$.

[d] Colony-forming units (CFU) before infection/CFU after infection.

[e] Determined as described in Materials and Methods.

[f] Lysogens/CFU × B_0/B. Multiplication by B_0/B corrects for infected bacteria that lysed and were not counted as CFU.

the same phenotype. This is not the case; PP22c27 grows as a P22c2 in Pox-1 (Fig. 2c). In these cases at 30 C, P22c27 behaves as a c2 mutant, and we conclude that the c27 mutation does not completely eliminate binding of C1-C3 and polymerase at *pre*. The binding is sufficient to hinder rightward transcription, but it is not sufficient to promote efficient leftward transcription and repressor synthesis (Table 2). Phage λ cy mutants, which define *pre*, behave differently from P22 *pre* mutants defined by c27 (4). This was shown by the results of measuring endolysin synthesis in λ-infected cells. Phage λ cI (analogous to P22c2) produces endolysin later than does λ cII (analogous to P22c1). Phage λ mutant in *pre* behaves as λ cII mutants. Thus a mutation in either the binding site, *pre*, or the structural gene for the diffusible cII product produces the same phenotypes; there is no leftward transcription from *pre* (no repressor is made by either cI, cII, or cy) and no blockage of rightward transcription. As suggested in this paper, in P22 the c27 mutation eliminates leftward transcription but not the blockage of rightward transcription. We think that this difference in *pre* reflects a difference in early regulation in the two phages. In P22 the wild-type interaction involving C1-C3 and c27 is sufficient to repress phage DNA synthesis (8, 17), whereas in λ no such repression is seen (6). Thus the wild-type interaction in P22 is more profoundly seen than in λ, and we interpret the residual regulatory activity of c27 as reflecting the difference in the wild-type interactions. Echols (6) has drawn attention to the differences in DNA regulation in the lytic growth of the two phages.

Growth of virulent mutants in Pox-1. Results in this paper show that Pox-1 retards lytic growth of all P22s except P22c1. This retardation results from suppression of lytic gene expression. Phages which can make repressor are then strongly channelled to lysogeny.

The virulent phage P22 *vir* A makes repressor in wild-type hosts and in Pox-1 (unpublished observations). P22 *vir* A does not form plaques on Pox-1, leading to the conclusion that the antirepressor constitutively produced by this strain (13) does not overcome repression. Two models can be advanced to explain this observation: (i) antirepressor is inactive in Pox-1 or (ii) phage P22 in Pox-1 overproduces repressor sufficiently to overcome antirepressor.

P22 *vir* B does form plaques on Pox-1, but they are very tiny. We interpret this finding to indicate that the mutant operators in *vir* B have some residual capacity to bind repressor (8), and that this interaction causes the small plaques.

Gene c3 product is essential in Pox-1. P22c3 mutants display the same phenotype as c1 with respect to DNA synthesis regulation (5) but, unlike c1, c3 is not absolutely required for the establishment of lysogeny in wild-type hosts (11). Furthermore c3 is known to be leaky in that it lysogenizes with a measurable frequency (11; Table 4), and it produces a substantial amount of repressor in Pox-1 (Table 3). It is surprising that P22c3 does not lysogenize in Pox-1 (Table 4). The mutation in Pox-1 interferes with host DNA synthesis (18), and c3 is involved in regulation of phage DNA synthesis (5). Perhaps c3 regulation is somehow essential in Pox-1. Alternatively, c3 may have a so-far undiscovered function that is involved in integration or some other aspect of lysogenization and that is absolutely required in Pox-1.

General conclusion. The interaction at c27 is probably an early critical step on the pathway to lysogeny. Our experiments suggest that host factors influence this interaction.

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