Interference in Bacteriophage Growth by a Resident Plasmid λ dv

II. Role of the Promoter-Operator

KENICHI MATSUBARA

Department of Biochemistry, Kyushu University School of Medicine, Fukuoka, 812, Japan

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The susceptibilities to interference by λ dv of a set of λ phages differing in the genetic structure of their right promoter-operator (pRoR) were compared. For this purpose mutant phages were added to λ dv-carrier cells at various multiplicities, and abilities to escape from interference, as represented by percentages of infected complexes to produce progeny phages, were compared. It was observed that phages that carry strongly constitutive pRoR were able to escape from interference at a low multiplicity of infection, whereas phages with weakly constitutive pRoR were able to escape only when a large number of these genomes entered into a cell. Several mutations in the pRoR were arranged in order of their constitutivity, or ability to escape from interference. Next, the abilities of a set of λ dv plasmids differing in their pRoR to cause interference were compared. The results showed that interference increased with increase in constitutivity of the pRoR in the plasmid genome. These observations are thought to reflect a regulatory system of the plasmid replicon, and a possible mechanism for the system is discussed.

In an accompanying paper, I report on a technique for isolation of new λ dv carrier cells (5). With this technique, it was possible to prepare several λ dv's with different promoter-operator (pRoR) that controls the expression and replication of the whole λ dv genome.

Plasmid λ dv interferes with growth of bacteriophage. Interference is specific to phages in which the immunity region is similar to that of the plasmid. The interference by λ dv acts on the right pRoR, among other places in the phage genome, possibly through a repressive action by a product from the gene *tof* (4, 7). Since the pRoR on the plasmid originates from a phage genome, a component(s) that acts negatively upon the phage should also act upon the plasmid genome itself.

In this investigation the effects of pRoR mutations in the phage on their susceptibilities to interference were compared. Several mutations were arranged in order of their constitutivity. Next, the effects of mutations in the pRoR of the plasmids on their abilities to cause interference were compared. It was found that plasmids that carry more constitutive pRoR's cause stronger interference.

To clarify the discussions below, it seems to be relevant here to state a hypothesis briefly.

The pRoR of the plasmid controls expression of gene *tof*, the product of which acts negatively at pRoR. Thus, the pRoR-*tof* system comprises a replicon-specific, negative-feedback regulatory circuit that acts to control the maintenance of plasmid state in a cell. If the pRoR is "strong," namely, if it allows constitutive expression of genes, then more *tof* products are produced, and more are being required to repress the transcription of λ dv genome. Then, at steady-state, the level of *tof* product would be high. If the pRoR is "weak," then the level of *tof* product would be low at steady state. Interference in phage growth is caused by the action of *tof* products on superinfecting phage genomes. Whether or not a λ -infected cell produces phage is determined by the level of the *tof* products in the cell, as well as the "strength" of the pRoR of the incoming phage genome and its multiplicity.

MATERIALS AND METHODS

The bacterial strains used are listed in Table 1. All the λ dv-carriers are derivatives of km723 and were prepared as described previously (5).

The bacteriophages used and the media were described in the preceding paper (5). Alleles which are wild type are not listed in the genotype. (All the phages, for which *cl* is listed in the table, such as λ cI90, have a mutation in gene *cl*. Other phages, e.g.,

TABLE 1. *Bacterial strains used*^a

Strain designation	λ dv	pRoR of the plasmid	Parental phage ^b
km723	None		
km605	λ dv1	<i>v1v3</i>	<i>λv2v1v3</i>
— ^c	λ dvA8	<i>virCa v3</i>	<i>λv2virCa v3</i>
—	λ dvBC2	<i>virC34</i>	<i>λcIsus34virC34</i>
—	λ dvBB1	Wild type	<i>λcIsus34</i>

^a The bacteria, all derived from strain km723, a derivative of *E. coli* K12, *F*[−] *str*^h*his*[−]*recA1 sup*[−]*gal*^{de1}, carry plasmid λ dv as shown in the second column. km605 carries an additional mutation, *thy*[−]. λ dv plasmids were prepared in the host bacteria from the parental phage as indicated.

^b For clarity, the counterparts of these parental phages, namely, h80 derivatives, have been omitted from the list.

^c A dash signifies no strain designation given.

λ v2v1v3 or *λ v2virCa v3*, have nonmutated *cI* alleles. The use of such phages with nonmutated *cI* alleles in this study is justified by observations that expression of this gene is limited in cells carrying λ dv [ref. 4; also D. Berg, private communication.] PB agar is the same as PBB agar except that Bonito extract is omitted.

Assay of the percentage of phage-producing λ dv-carrier bacteria as a function of multiplicity. Bacteria carrying λ dv were grown at 37 C in PBMM with aeration to a density of 5×10^8 cells per ml. The culture was chilled in an ice bath, and the number of colony formers was counted. To this was added phage at various multiplicities, and the mixtures were left for 30 to 60 min at 0 C to allow adsorption. The phage-bacteria mixtures were then incubated for 10 min at 37 C, and then for another 10 min at 37 C with anti- λ rabbit serum (final K value = 3). The cultures were then chilled in an ice bath, diluted, and immediately plated on PB agar to measure infective centers by using C600S (a ϕ 80-sensitive derivatives of C600) as an indicator bacterium. Under these conditions more than 90% of the added phage was adsorbed. The percentage of phage-producing bacteria in samples after multiple infection was calculated by dividing the number of infective centers by that of colony formers in the uninfected culture. The percentage of phage-producing bacteria in samples after single infection was measured by adding phage at a multiplicity of 0.1, and calculating the number of infective centers divided by the number of added phage.

RESULTS

Effect of the genetic structure of the pRoR of infecting phage on the susceptibility to interference. It was shown previously that a cell carrying λ dv becomes a phage producer only when multiplicities of λ infect a cell (4). This was confirmed with λ dv1, as shown in Fig. 1A. When the infecting phage was *λ v2v1v3*, and

hence the pRoR of the phage and the plasmid were identical (namely, *v1v3*), the fraction of phage-producing bacteria remained low up to a multiplicity of about 5. The number increased upon increasing the multiplicity above 5, and at a multiplicity of 30 almost all the cells became phage producers. The curve looks as if some repressive material(s) is titrated by increase in incoming phage genomes.

The susceptibility of interference by λ dv1 differs depending upon the genetic structure of the operators of the infecting phage. Thus, a family of titration curves was obtained when a set of λ with different pRoR's was employed. Figure 1A demonstrates that *λ v2virCc v3* is less susceptible to interference than *λ v2v1v3*, whereas *λ v2virCa v3* is more susceptible than *λ v2v1v3*. These phages are isogenic except for the constitutive mutations *virCc*, *v1*, or *virCa* in the pRoR, so the difference in susceptibility to interference (or the ability to escape from interference) must be determined by the structure of this genetic element. To illustrate this relationship, we shall call the pRoR *virCc v3* stronger than *v1v3*, and *virCa v3* weaker than *v1v3*.

Constitutive mutants *λ v2virCb v3* or *λ cIsus34 virL18virC34virR18* (2) behaved just like *λ v2v1v3* (data not shown). Therefore, *virCb v3* and *virC34virR18* must be the same strength as *v1v3*. Another constitutive pRoR mutation, *virC34*, which is roughly equivalent to *v1*, allows weak rightward expression (2). This phage escaped from interference by λ dv1 only when it was added at a multiplicity above 30. Apparently, the *virC34* is much weaker than *v1v3*. *λ cI90* in which the pRoR is wild type was more susceptible to interference than the others. *λ cIsus34* which also carries the wild type pRoR behaved just like *λ cI90* (data not shown). It could be argued that the leftward promoter-operator (pLoL) in the latter phages is wild type, and this might cause weakened ability to escape from interference. However, *λ v2cII1*, in which the pRoR is wild type but the pLoL is identical to that of *λ v2v1v3*, behaved like *λ cI90* or *λ cIsus34* (4).

These observations clearly demonstrate that the susceptibility to interference is at least partly controlled by the genetic structure of the pRoR of the infecting phage genome, as suggested previously (4). The order of susceptibility is: wild type > *virC34* > *virCa v3* > *v1v3*, *virCb v3*, *virC34 virR18* > *virCc v3*, which is inversely related to the order of constitutivity of the pRoR.

Effect of the genetic structure of pRoR of the plasmids on their ability to cause inter-

ference. Next, the abilities of different λ dv plasmids to cause interference were tested. To eliminate possible effects from the host, all subsequent experiments were done with isogenic host cells.

First, several isogenic, but independently isolated, λ dv plasmids all originating from λ 2v1v3 were compared. The family of titration curves obtained with these λ dv's was essentially the

same as that shown in Fig. 1A. Some minor variations were noted, but similar fluctuations were seen in different experiments with a single λ dv clone. Examination of eight different λ dv's showed that the abilities of plasmids to cause interference with phage growth were essentially the same, as long as the plasmids were isogenic. (Here, isogenic implies that the regions covering pRoR-O-P are identical, but it does not

FIG. 1. Fraction of phage-producing λ dv-carrier bacteria as a function of multiplicity. A set of isogenic bacteria carrying different λ dv's was prepared as described earlier (5). Exponentially growing λ dv-carrier cells were infected by various phages at different multiplicities. After eliminating unadsorbed phage, the number of phage-producing cells was assayed. The ordinate represents the percentage of phage-producers. A, B, C, and D show results with plasmids λ dv1, λ dvA8, λ dvBC2, and λ dvBB1, respectively. The pRoR's of the plasmids are represented in parentheses. The order of constitutivity of the pRoR's of both plasmids and phages is: $virCc v_3 > v_1v_3 > virCa v_3 > virC_{34} > \text{wild type}$. Symbols: $\bullet$, λ 2virCc v3; $\circ$, λ 2v1v3; $\odot$, λ 2virCa v3; Δ , λ cIsus34virC34; $\times$, λ cI90.

necessarily mean that the deletion end points of each plasmid are identical.)

Next, the abilities of several λ dv's carrying different pRoR's to cause interference were compared. Figure 1B demonstrates that λ dvA8, in which pRoR *virCa v3* is weaker than *v1v3* as carried by λ dv1, caused weaker interference as compared to λ dv1. No interference with the growth of λ v2*virCc v3* was observed; λ v2*v1v3* and λ v2*virCa v3* behaved as though they were λ v2*virCc v3* and λ v2*v1v3*, respectively, in cells carrying λ dv1 (see Fig. 1A); λ cI90 was able to convert 25% of the λ dvA8-carrier cells into phage producers at a multiplicity of 100, but none of the λ dv1-carrier cells at the same multiplicity.

Figure 1C and D demonstrates that the ability to cause interference is much weaker with λ dvBC2, carrying a weaker mutation *virC34* in the pRoR, and that it is lowered further with λ dvBB1 whose pRoR is wild type. In fact, in these samples the infecting phage λ v2*virCa v3* or λ cI*sus34virC34* that carry weak pRoR could completely "titrate" repressive substance at a multiplicity of infection of around 10. At a multiplicity of around eight, λ cI90 partially escaped from interference by plasmid λ dvBB1 that carries wild-type pRoR. However, the same phage could not give a progeny phage in a cell carrying λ dv1, even at a multiplicity of 100.

The experiments were repeated with two other sets of λ dv's isolated independently, and the results were essentially identical to those shown in Fig. 1. It is concluded from these results that the ability to cause interference is determined by the genetic structure of the pRoR of the plasmid.

DISCUSSION

Interference, the disadvantageous effect caused by λ dv for vegetative replication of the invading phage genome, represents the interaction of two related replicons. It acts only when replicons of the plasmid and phage genome are homologous, or very closely related (4).

In a somewhat similar situation known as "incompatibility," two plasmids having related replicons interact with each other, resulting in exclusion of one of them (for a review, see 6). Knowledge of the regulatory mechanism acting in incompatibility is limited but it is possible that the basic mechanism of this phenomenon is related to that of interference.

The susceptibility to interference by λ dv is determined by the pRoR in the phage replicon. The phage replicon is less or more susceptible depending upon whether the pRoR is strong

(constitutive) or weak. On the other hand, the pRoR in the plasmid replicon controls the ability to cause interference. If the pRoR of λ dv is weak, the plasmid's ability to cause interference is weak, and vice versa.

As discussed earlier (4), products from the gene *tof* may be important in interference through their negative action on promoter-operators. It is highly likely, then, that the difference in ability to cause interference is related to the cytoplasmic level of products of *tof*. If the curves of phage producers versus multiplicity in Fig. 1 are taken as simple representations of the titration of negatively acting *tof* products, and the 50% titration points of the "titration curves" of λ v2*virCa v3* are compared (this phage is chosen because it exhibits 50% titration points with all the plasmid-carrier cells), then the level of *tof* products for λ dvBB1 that carries the weakest wild-type pRoR is roughly $\frac{1}{2}$ and $\frac{1}{10}$, respectively, of those of λ dvBC2, that carries the stronger *virC34* pRoR, and for λ dv1, that carries the even stronger *v1v3* pRoR.

Undoubtedly, the *tof* products may also act negatively on the pRoR of the plasmid replicon. This pRoR, at the same time, controls transcription of the *tof* itself, along with genes *O* and *P*. It is proposed that the pRoR and *tof* constitute a negative-feedback control circuit: the plasmid replicon causes expression and replication until *tof* products accumulate to a critical level in the cytoplasm above which the pRoR is inactivated. If the pRoR of the plasmid is strongly constitutive, this critical level is high, whereas if it is weakly constitutive the level is low. As long as the level of *tof* product is higher than the critical level the plasmid replicon is unable to express gene functions or to replicate. The genomes and cytoplasmic levels of the gene products will be diluted upon increase in the mass of cytoplasm. Expression will be resumed when the level of products of *tof* decreases to below the critical level again and is no longer inhibitory to transcription from the pRoR. The steady state should thus be achieved around the critical level of *tof* products.

The interference in phage growth would simply be an extension of the effect of a regulatory system of the plasmid replicon to the incoming phage genome that shares the same replicon. There is evidence that the invading phage genome is forced to become an unstable plasmid (3).

Support for this hypothesis could be obtained by elucidation of the role of the gene *tof* and other indispensable structures in the plasmid genome, as well as from quantitative evaluation

of the level of products of *tof*. Further work along these lines is in progress.

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