Nature of *Escherichia coli* B(P1) Yielder Cells at the Time of Infection with Restricted T1

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In the infection of *Escherichia coli* B(P1) with restricted T1, it was shown that yielder cells consist of both special and nonspecial cells. Special or predetermined yielders occurred only among the earliest yielders. In most instances, yielder-cell formation was most easily explained by assuming that the first step was a chance escape of the restricted phage DNA from the degrading enzyme of the restricting cell.

In the host-controlled modification of the restricted bacteriophage T1 by restricting, P1-lysogenic bacteria, the chief block to productive infection by T1 is the degradation of the deoxyribonucleic acid (DNA) of T1 (7, 6). If yielder cells are merely restricting cells which have lost their ability to degrade restricted DNA (i.e., yielder cells are predetermined or special), then such cells should accept and allow replication of genetic variants of T1 in the same proportion with which such T1 variants adsorb to the cells. If, on the other hand, yielder cells represent cells in which the T1 DNA has fortuitously escaped degradation during injection, then, from the low (10^{-3} to 10^{-4}) efficiency of plating (EOP) of restricted T1 on restricting cells, it may be deduced that the chance of two phage genomes fortuitously escaping degradation in one cell is low. Accordingly most yielder cells should accept and produce only one genome.

Two types of experiments will be presented which attempt to answer the question as to whether or not yielder cells are predetermined. In one set of experiments the proportion of restricting cells to which two genetic variants of T1 have adsorbed is compared to the proportion of yielder cells which produce both phage types. In another set of experiments, restricting cells which are nonpermissive for the amber mutation are exposed to two complementing T1am mutants. Under these conditions, only cells receiving both amber types have a significant probability of becoming yielders.

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MATERIALS AND METHODS

Bacterial and phage strains. Strain KB-3 of *Escherichia coli* is permissive for T1am mutants; KB-3 and the T1am mutants employed were kindly supplied by Wolfgang Michalke and have been described by Michalke (8). A P1-lysogenic variant of KB-3 as well as high-level streptomycin-resistant (str-r) mutants of KB-3 strains were isolated in this laboratory. Both the B strains of *E. coli* and the Sh16 strains of *Shigella dysenteriae* are nonpermissive for the T1am mutants employed in these experiments. Sh16 strains are streptomycin resistant. The B and Sh16 strains and the phage strains T1hr and T1ge have been described elsewhere (4, 3).

Media and technique. The agar-overlay technique employed has been described elsewhere (3). The T1hr and T1ge produce distinctive plaque morphologies when plated on either Sh16 or Sh16(P1). The T1hr produces large plaques with small, clear centers and large, turbid halos. The T1ge produces smaller plaques with medium to large, clear centers and narrow, turbid halos. Plaques arising from infective centers which produce both T1hr and T1ge exhibit a clear center and a characteristic mottled, irregular halo. Absolute identification of the phage in a plaque can be made by plating some of the phage on a two dye medium (1, 5). In our laboratory, a comparison of the characterization of phage by use of a two dye medium with characterization by plaque morphology on the Sh16 or Sh16(P1) indicators has shown the latter technique to be more than 95% accurate.

RESULTS

In these experiments the fraction of restricting B(P1) cells to which both T1hr .B (i.e., T1hr grown on the nonrestricting strain B) and T1ge .B have been adsorbed may be compared to the fraction of cells which produce both phage variants at various periods of time after plating and incuba-

tion. After exposure to a mixture of T1hr.B and T1ge.B, the cells were centrifuged to remove free phage. The cells were resuspended and plated together with the restricting, str-r indicator strain Sh16(P1) by agar overlay and incubated at 37 C. At various periods of time after plating and incubation, streptomycin was added by agar overlay in order to kill the B(P1) cells. Table 1 gives cumulative data of 18 experiments. The data in column 4 were used to construct curve A, Fig. 1.

Data showing that the total number of yielder cells increased linearly over a 4- to 5-hr period were obtained but are not presented here. A discussion of this phenomenon may be found elsewhere (3). In these experiments about 40% of the total number of yielders were formed at 1-hr after plating and incubation, and the maximal number of yielders were formed at 4 or 5 hr after plating and incubation. Curves B and C are theoretical curves in which it was assumed that 40% of the total number of yielders were formed by $t = 1$ and 100% by $t = 5$. For ease of comparison to curve A, we arbitrarily set the percentage of mottled plaques in both curves B and C at 5.2% at $t = 1$ (Table 1). Curve B illustrates the percentage of mottled plaques if the fraction of cells yielding both phage types had remained constant at each sampling. Curve C illustrates the percentage of mottled plaques if no mottled plaques had been formed after the 1 hr.

It can be seen that the experimental data lead to a curve (curve A) which closely approximates curve C. It should be added that the fraction of mottled plaques formed as a fraction of the total yielders was nearly identical to curve C in each of the individual experiments.

These data may be interpreted in either of two ways. First, the data may indicate that a fraction

FIG. 1. Streptomycin-sensitive *Escherichia coli* B(P1) was exposed to T1hr.B and T1ge.B. The MOI was approximately 0.6 adsorbed virus particles of each type. About 40% of the total yielder cells are formed by 1 hr; the remainder are formed in a linear fashion between 1 and 4 (or 5) hr. Infective centers which produce both phage variants form mottled plaques on the streptomycin-resistant indicator *Shigella dysenteriae* Sh16 (P1). Curve A illustrates the formation of mottled plaques as observed experimentally (data from column 4, Table 1). Curves B and C are theoretical curves. Curve B illustrates the kinetics of mottled plaque formation if the fraction of infective centers which give rise to mottled plaques remains constant with time. Curve C illustrates the relative number of mottled plaques if no infective centers which give rise to mottled plaques are formed after 1 hr.

TABLE 1. Exposure of streptomycin-sensitive *Escherichia coli* B(P1) to T1hr.B and T1ge.B^a

Time of addition of streptomycin	No. of expts	Ratio of mottled plaques to total plaques ^b	Mottled plaques (%)
hr			
1	18	294/5,620	5.2
2	10	147/3,798	3.9
3	12	139/4,880	2.8
4	18	129/7,339	1.8
5	5	26/1,571	1.7
None added	16	39/5,997	0.7

^a MOI was approximately 0.6 adsorbed virus particles of each type.

^b Infective centers which produce both phage variants form mottled plaques on the streptomycin-resistant indicator *S. dysenteriae* Sh16(P1).

of the early yielder cells (i.e., those produced by 1 hour) can permit both injection and production of more than one phage genome, whereas late yielders (i.e., those produced after 1 hour) permit only the production of one phage genome. Second, possibly the cells which ultimately become yielder cells are special in that they permit injection by all phages which adsorb to them, but, subsequent to phage injection (but before the cells have an opportunity to produce more than one phage type), the phage genomes may often become separated due to cell division. The first hypothesis is considered to be the correct one and will be discussed further. The second hypothesis was considered and then rejected for the following reasons. (i) Under the experimental conditions employed, the generation time of B(P1) is 40 to 50 min (3). About 50% of the cells exposed to both phage types are exposed to at least three phage particles. Since 50% of the cells which con-

tain both phage types also contain an additional genome, segregation of *T1hr* and *T1ge* would take several generations to be relatively complete. A gradual decline in the relative number of mottled plaques over a period of several generations would lead to an experimental curve which was intermediate between curves B and C in Fig. 1. (ii) About 50% of the cells which are exposed to phage are exposed to more than one phage particle. If 50% of the cells which permit injection of restricted DNA are infected with at least two phage genomes and genomes are subsequently segregated by cell division, then the number of potential yielder cells should significantly increase during the course of the experiment. It has been shown (3) that, at a low (0.1) multiplicity of infection (MOI), yielders are formed in a linear fashion for several hours after plating and incubation. Under conditions which allow an increase in the number of potential yielder cells during the experiment, the number of yielder cells should increase more rapidly than an arithmetic progression; in fact, total yielder-cell formation occurred in a linear manner with time.

T1am16.KB-3 and T1am10.KB-3 on B(P1). B(P1) prevents the production of either T1am16.KB-3 or T1am10.KB-3 in two ways. In the first place, the B(P1) cells lack a suppressor for the amber mutation. Secondly, B(P1) cells, because they are lysogenic for P1, are restricting for T1 which has been grown on a host that is not lysogenic for P1. The EOP of either T1am16.KB-3 or T1am10.KB-3 on B(P1) is about 10^{-7} . An EOP of 10^{-7} is considerably lower than the EOP of either phage on the nonpermissive strain B (EOP = 10^{-5}) or on the permissive strain KB-3(P1) (EOP = 10^{-3} to 10^{-4}). The T1am16 and T1am10 are able to complement each other in strain B in such manner as to lead to productive infection (8). Multiple infection of P1-lysogenic cells by restricted T1 under the experimental conditions employed here does not lead to cooperation which overcomes the restriction caused by prophage P1 (5). Since T1am16.KB-3 and T1am10.KB-3 can cooperate to produce a productive infection in nonrestricting B strains, it is assumed that if a restricting B(P1) cell were infected simultaneously with both phages the only block to a productive infection would be the restriction caused by prophage P1. B(P1) cells infected with both T1am16.KB-3 and T1am10.KB-3 should have at least a 10^8 higher probability of producing phages than would B(P1) infected with either phage alone.

A mixture of T1am16.KB-3 and T1am10.KB-3 was adsorbed to B(P1) cells and plated under conditions similar to the preceding experiments. If restricting cells which permit the production of

FIG. 2. Plaque formation by streptomycin-sensitive *Escherichia coli* B(P1) exposed to T1am16.KB-3 and T1am10.KB-3 at an MOI of approximately 0.6 adsorbed virus particles of each type. The results of a typical experiment in which various streptomycin resistant indicators were employed is presented. Curve A illustrates plaque formation on KB-3; curve B illustrates plaque formation on KB-3(P1); and curve C illustrates plaque formation on Sh16(P1).

more than one restricted genome are early yielders (based on the data obtained in the preceding experiments), then under conditions where only cells which are infected with both amber types can become yielders, the formation of yielder cells should reach a maximum by 1 hr. Figure 2 illustrates the kinetics of total yielder-cell formation (curve A) with str-r KB-3 used as indicator, the formation of yielder cells which produce some unrestricted phage (curve B) with str-r KB-3(P1) used as indicator, and the kinetics of formation of those yielders which produce some unrestricted wild type T1 (curve C) with Sh16(P1) used as indicator. It can be seen from curves A and B that yielder-cell formation as measured by permissive indicator cells reached a maximum by 1 hr after plating and incubation. This confirms the results of the preceding experiments by showing that cells which yield both phage types do so early. Yields cell formation by those cells which give rise to some unrestricted wild-type phage did not reach a maximum by 1 hr. However, the production of wild-type phage involves an additional factor, namely genetic recombination.

DISCUSSION

When restricting cells are exposed to restricted T1 phage, some cells ultimately produce phage. Cells which yield phage do so in a linear manner over 4 to 5 hr (3). When two genetic variants of T1 are adsorbed to restricting cells, those cells yielding both phage types form a fraction of the total yielders which is smaller than that fraction

of cells to which both phage types are adsorbed. Cells yielding two phage types occur only among the earliest yielder cells. For example, when the MOI of T1_{hr}.B and T1_{ge}.B adsorbed to B(P1) is such (0.6 for each variant) that about 30% of the cells which receive phage receive both types, only about 5% of the yielders formed within 1 hr after plating and incubation liberate both phage types; this indicates that not more than 17% of the yielders which produce phage after 1 hr are special. Under conditions allowing only those restricting cells which have received each of two complementing amber mutants of restricted T1 to become yielders, all yielder cells are formed at 1 hr after plating and incubation. These results show that, after 1 hr, there is no appreciable increase in the number of cells which yield two types of phage. In the case of T1_{hr}.B and T1_{ge}.B in B(P1), the total number of yielders continues to increase up to 4 or 5 hr. Accordingly the relative number of cells which yield both phage types declines markedly after 1 hr (curve A, Fig. 1).

These results are interpreted as meaning that most yielder cells are not special or predetermined at the time of infection. Rather, it would seem that at the time of infection most yielders which were exposed to two phage particles were able to accept (permit injection of) one genome but rejected a second; an alternate explanation is that late yielder cells can accept more than one restricted genome but permit only one to reproduce. Those cells which do permit more than one phage genome to be injected and produced (i.e., those cells which appear to lack the ability to degrade restricted DNA) are different than the average yielder cell in that yielders which produce more than one phage type occur only among the earliest yielders.

Cells which contain P1 in the vegetative state do not restrict injection or growth of restricted T1 (2). Therefore a likely source of those yielders which are able to both accept and produce more than one variant of T1 is in that fraction of P1-lysogenic cells in which the prophage has shifted to the vegetative state prior to infection with T1.

Such cells would be expected to permit both injection of restricted DNA without degradation and normal development of the productive processes of T1.

The simplest explanation of the origin of those yielders which are not special at the time of infection is that with each cell-phage contact there is a small probability that the restricted DNA will escape degradation. Delay of phage production for up to 4 or 5 hr after infection could come about in a number of ways: (i) damage of restricted DNA during injection, (ii) the necessity of some unknown "activation event" which permits phage production, or (iii) interaction between the T1 genome and the P1 prophage (or products of the prophage) leading to prophage induction. Present knowledge is not sufficient for us to decide why a restricted T1 genome may delay as long as 5 hr in completing its life cycle in a restricting host.

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