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Loss of R Factors Promoted by Bacteriophage M13 and the M13 Carrier State

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The infection of different Hfr strains of *Escherichia coli* bearing derepressed R factors of the fi^+ or fi^- type can result in the loss of the R factor and the conversion of the infected cells to the R^- state. This extends earlier observations on the elimination of F′ factors by bacteriophage M13 infection. Variability in the efficiency of this conversion can arise because of genetic factors independent of the R factor being eliminated. A fraction of the infected but unconverted R^+ cells were M13 carrier strains. The carrier state had an intracellular basis, and single R^+ cells could maintain the carrier state.

When some F′ merodiploid strains of *Escherichia coli* are infected with the F-specific phage M13, they segregate F^- cells with high frequency and in the absence of any observable M13-induced lysis or killing (22, 23). Of relevance to the possible mechanism by which this occurs is the question as to whether this effect of M13 is specific for F′ factors, or extends to other autonomous bacterial plasmids such as R factors. There are two broad classes of R factors, Rfi^+ and Rfi^-, that are distinguishable on the basis of their ability to inhibit conjugal fertility conferred by the sex factor F (33). Wild-type Rfi^+ factors determine the production of a repressor that can repress some of the conjugal functions conferred by the presence of F. Wild-type Rfi^- factors determine the synthesis of a repressor that does not have such an effect on F function. In the present study, the effect of M13 infection on different strains harboring Rfi^+ or Rfi^- factors has been examined with respect to the loss of the antibiotic resistance or transfer factor (RTF) associated with each R factor. One may infer that, when all of the known phenotypic properties associated with an R factor are lost from a cell, the autonomous R factor has been physically eliminated. The study has indicated that the previously observed ability of phage M13 to eliminate an autonomous F′ factor (22, 23) extends to R factors of the fi^+ and fi^- type. All strains bearing R factors are not equally susceptible to this effect. The basis of variability in the efficiency of elimination was examined by using different strains, some of which bore the same R factor.

A remarkable feature of the infectious cycle of M13 and similar phages is the absence of lysis or death of cells that propagate the virus (3, 11, 27). On infection, susceptible strains of bacteria frequently give rise to cell lines that are carriers of the phage (12, 13). The question as to whether in such cases the carrier state is sustained only by repeated cycles of virus release and reinfection or whether in fact such cells also retain a capacity for intracellular virus production and release has not always been satisfactorily resolved. The system under use provided us with the opportunity to investigate the nature of the M13 carrier state, and evidence was obtained which indicates that the carrier state could be maintained under conditions when new cycles of reinfection were prevented.

MATERIALS AND METHODS

Bacterial strains and R factors. The bacterial strains and R factors used, their properties, and derivation are indicated in Table 1. Both of the R factors used were derepressed mutants of the original R factors R64 (16) and KR9 (14). The former was obtained from J. Urban and conferred transferrable resistance to streptomycin (Sm), tetracycline (Tc), and sulfonamide (Su). The latter was isolated in this laboratory by the technique described by Meynell and Datta (19). It conferred transferrable resistance to Sm, Tc, chloramphenicol (Cm), kanamycin (Km), and neomycin (Nm). KR9-1 and KR9-2 are two segregants of the derepressed KR9 factor, obtained during conjugational transfer of the derepressed mutant of KR9. They confer resistance to Cm, Nm, Km and Cm, Tc, respectively.

Nomenclature. The nomenclature for genotypes and phenotypes and the symbols used generally

TABLE 1. *Bacterial strains and R factors used*[a]

Strain	Chromosomal markers	F or R mating types	R factor (when present)	Drug resistance markers on R factor	Reference to strain or method of construction or selection
C03	*pro thi*	Hfr			
C03/KR9-1	*pro thi*	Hfr Rfi$^+$	KR9-1	*cml nem kan*	Isolated as a segregant following the conjugational transfer of a derepressed mutant of the R factor KR9 to C03.
C03/KR9-2	*pro thi*	Hfr Rfi$^+$	KR9-2	*cml tcl*	As above.
C046	*thr leu thy lac*	F$^-$			This laboratory.
C046/KR9-1	*thr leu thy lac*	Rfi$^+$	KR9-1	*cml nem kan*	Constructed by conjugating C03/KR9-1 with C046.
C046/KR9-2	*thr leu thy lac*	Rfi$^+$	KR9-2	*cml tcl*	Constructed by conjugating C03/KR9-2 with C046.
C018	*met his leu arg recA*	F$^-$			Isolated as a spontaneously cured derivative of strain K41 (22).
C018/R64-11	*met his leu arg rec A*	Rfi$^-$	R64-11	*str tcl sul*	Constructed by conjugating an auxotroph bearing R64-11 with C018.
C036	*thi*	Hfr			Strain K37 (23, 25).
C036/R64-11	*thi*	Hfr Rfi$^-$	R64-11	*str tcl sul*	Constructed by conjugating C018/R64-11 with C036.
C03-1/KR9-1*	*pro thi*	Hfr Rfi$^+$	KR9-1*	*cml nem kan*	Selected as a strain that resisted the M13-induced effect (after a single exposure to M13).
C046/KR9-1*	*thr leu thy lac*	F$^-$	KR9-1*	*cml nem kan*	Constructed by conjugating C03-1/KR9-1* with C046.

[a] Strain numbers are designatives of this laboratory. The symbols for the drug resistance markers are as follows: chloramphenicol (*cml*), neomycin (*nem*), kanamycin (*kan*), tetracycline (*tcl*), streptomycin (*str*) and sulphonamide (*sul*). For the origin of KR9-1*, see footnote *b* to Table 2.

follow Demerec et al. (8) and Taylor (31). In all cases a strain carrying a particular R factor is indicated as X/Y, where X denotes the designation of the strain and Y that of the R factor. Phenotype symbols are indicated where appropriate.

Media, solutions, and chemicals. All media and media constituents were purchased from Difco Laboratories Ltd., Michigan. The minimal broth (MB) was that of Davis and Mingioli (7). The *p*H of all organic media was adjusted to 7.0 ± 0.2. Certified reagent grade chemicals were used. Crystallized, lyophilized Nagarase was purchased from Enzyme Development Corporation, New York. Antibody against M13 was prepared by using rabbits and as described by Salivar, Tzagaloff, and Pratt (29). The antibiotics were carrier-free and gifts from the pharmaceutical companies manufacturing them. Amino acid supplements where needed were sterilized separately by filtration through sterile cellulose nitrate filters (0.45-μm porosity, Millipore Filter Corp., Bedford, Mass.) and added to yield a concentration of 10 μg per ml.

Phage M13. The original stock of this phage was obtained from D. Pratt and has since been propagated on strain C036. Phages were harvested by the confluent lysis procedure (1) using media described by Salivar, Tzagaloff, and Pratt (29). Phages were harvested, concentrated, and purified as described by Brown and Dowell (3) and stored at 4 C.

Use of M13 in the R factor elimination experiments. R$^+$ cultures were grown overnight at 37 C in MB containing required growth supplements and one antibiotic to which the strain was resistant. Overnight cultures were diluted 20-fold into nutrient broth (NB) and incubated for about 2 hr at 37 C with shaking to permit them to reach exponential phase. The cultures were centrifuged, and the cells were resuspended in an equal volume of a starvation buffer (9). After 90 min of incubation at 37 C, the cells were centrifuged and the pellet was resuspended in NB to yield approximately 10^8 to 10^9 cells per ml. This cell suspension was then divided into two equal portions, one of which was infected with phage M13 at a multiplicity of infection of 20 plaque-froming units. An equal volume of the phage dilution buffer (DB) of Salivar, Tzagaloff, and Pratt (29) was added to the second tube and both tubes were incubated with aeration at 37 C. At various intervals between 0 and 6 hr, 0.1-ml amounts were removed, suitably diluted, and spread on nutrient agar (NA) or NA containing an antibiotic to give the counts of total (R$^+$ and R$^-$) and R$^+$ colonies, from which the frequency of R$^+$ to R$^-$ conversion was estimated. This conversion frequency was confirmed by replica-plating colonies from nutrient agar to nutrient agar containing each of the antibiotics.

Conjugation experiments. To examine for the transfer of R factors from an R$^+$ donor to an established or presumptive R$^-$ recipient, the donor culture was in the exponential phase of growth and the

recipient culture in the stationary phase. The two cultures were mixed in the proportion of 1 donor to 10 recipients and incubated for 30 min at 37 C in a shallow layer with very gentle shaking. Suitable dilutions were then plated on media selective for recipients and containing antibiotics to which the donor alone was resistant. Control platings of donors and recipients were carried out concurrently.

RESULTS

Loss of derepressed Rfi$^+$ and Rfi$^-$ factors promoted by M13 infection. It had been observed initially (22, 23) that a particular F′ factor, KLF41, was eliminated from both Rec$^-$ and Rec$^+$ merodiploid strains of *E. coli* when these strains were infected with M13. Following this study, several other F′ merodiploid strains of bacteria have been tested (Iyer and Messmer, *unpublished data*). Some F′ strains, including those that are resistant to curing by acridine, are efficiently converted by M13. Under the same conditions, other F′ strains are not. It is possible that under different conditions of growth and infection such F′ strains will also be converted by M13, but the basis of this resistance to conversion has not been investigated.

In the case of R factors, it is known (21, 33) that most wild-type factors are repressed in their fertility function and that there is a coordinate repression of conjugal fertility, specific pilus synthesis, and sensitivity to pilus-specific phages (6, 19). There are two broad groups of R factors, Rfi$^+$ and Rfi$^-$, both of which may exist naturally as repressed factors (21, 33). Rfi$^+$ factors determine the synthesis of a pilus similar to the F pilus determined by the sex factor F (6, 15, 19). Furthermore, when a wild-type (repressed) Rfi$^+$ factor is resident in an Hfr strain, the synthesis of the F pilus (and therefore of M13-attachment sites) is repressed (4, 6). To ensure that such attachment sites were available, we have used derepressed mutants of the R factors rather than wild-type factors.

Figure 1 shows the effect of M13 infection on the ability of a strain carrying the derepressed Rfi$^-$ factor to yield colonies on NA supplemented with Tc, an antibiotic to which resistance is conferred by the presence of the R factor. M13 infection resulted in an initial reduction in total viable counts. Subsequently, growth and cell division proceeded at a rate lower than in the uninfected population. Such a marked effect of M13 infection on the growth of the cells was not observed with the second strain (Fig. 2). Since the same wild-type phage M13 was used in both cases, the effect observed in Fig. 1 must be strain- or R factor-

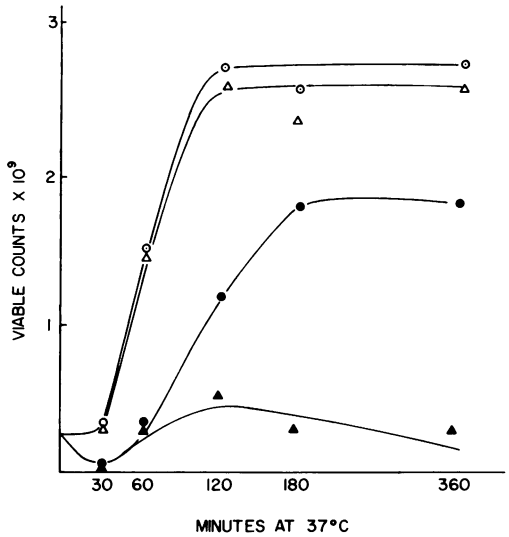

FIG. 1. *Effect of M13 infection of strain C036/R64-11 on the total number and on the R$^+$ colonies (Tc-resistant) developing after infection. Symbols: O, colonies on nutrient agar (NA) without infection; Δ, colonies on NA + 50 μg of Tc per ml without infection; ●, colonies on NA after M13 infection; ▲, colonies on NA + 50 μg of Tc per ml after infection.*

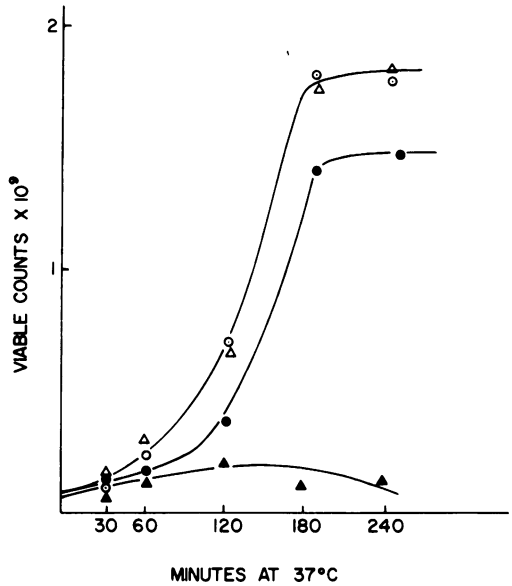

FIG. 2. *Effect of M13 infection of strain C03/KR9-1 on the total number and on the R$^+$ colonies (Km-resistant) developing after infection. O, colonies on nutrient agar (NA) without infection; Δ, colonies on NA + 50 μg of Km per ml without infection; ●, colonies on NA after M13 infection; ▲, colonies on NA + 50 μg of Km per ml after infection.*

specific. This kind ot specificity is not surprising as it is known that the colony-forming ability of an infected strain is reduced under a variety of different conditions (25, 26).

At the start of these experiments (0 min in Fig. 1 and Fig. 2), Tc-sensitive or Km-sensitive segregants were undetectable (<0.05%). It should be recalled (see Materials and Methods) that usually, before infection, the strains were grown in the presence of Tc or Km so that spontaneous R^- segregants were selected against. In the experiment of Fig. 1, within 3 hr of infection, 90% of the population had become Km-sensitive. This indicates that the mechanism of conversion cannot merely involve the selective growth or survival of rare spontaneous R^- segregants since at the start of the experiment such segregants occurred at a frequency of <0.05% and in 3 hr an average of not more than four cell divisions have occurred. Furthermore, in the experiment of Fig. 2 with a different strain and R factor, it is clear that a high frequency of M13-induced conversion can be secured in the absence of inhibition of colony-forming ability. The assumption that Tc-sensitive or Km-sensitive colonies represent R^- cells was confirmed by two observations. (i) All of 50 to 100 Tc-sensitive or Km-sensitive colonies tested had concurrently lost all the resistance markers associated with the respective R factor. (ii) In the experiment of Fig. 2, when some of the presumptive R^- segregants were used as recipients in a conjugal cross involving a different R factor (KR9-2) of the same compatibility type, the frequency of transfer of the R factor was comparable to normal R^- recipients (note: in subsequent experiments, it is shown that this frequency can be reduced, but only by about 50% if the recipient is a carrier of phage M13). A strain that harbors an R factor is expected to exert superinfection immunity against a second R factor of the same compatibility type, and the factors KR9-1 and KR9-2 derive from the same original factor KR-9. The absence of R factor-induced superinfection immunity in the segregants converted to antibiotic sensitivity confirms that these strains have lost their respective R factors. It is concluded that derepressed R factors of both the fi^+ and fi^- type may be eliminated by phage M13 from strains bearing them.

Variability in the efficiency of M13-induced R factor loss. Table 2 shows the results of additional experiments similar to those of Fig. 1 and 2 in which the same R factors were introduced by conjugation into different strains and the efficiency of M13-induced R^+ to R^- conversion in these constructed strains was examined. These experiments were done as described in Materials and Methods except that the antibiotic was omitted in the growth medium prior to addition of the phage. Thus, for each strain, M13-induced conversion was studied in the background of spontaneous conversion which was monitored separately.

The results of Table 2 confirm the conclusions reached from Fig. 1 and Fig. 2 and also show (experiment 5) that, when a derepressed Rfi^+ factor is harbored by an F^- strain, such a strain is also subject to the M13-induced effect. When an Rfi^+ factor is harbored by an F^- strain, M13-binding sites become available on the pili determined by the Rfi^+ factor, and this ensures infection and the subsequent effects. In all experiments, a fraction of cells resist curing. As is shown in subsequently described experiments, some cells that have resisted curing produce M13. Therefore, the resistance exhibited by these cells to the M13-induced effect must have an intracellular basis. When a resistant strain derived from a first round of M13 infection (experiment 2, Table 2) was regrown and reinfected again with M13 (experiment 7, Table 2), the conversion to Km sensitivity was only 2.7% as compared to 86% with the primary strain. From this observation, it can be concluded not only that the resistance to the M13-induced effect has an intracellular basis, but that this resistance is also inherited. To determine whether this conversion efficiency was host- or R factor-controlled, the R factor from the strain showing resistance to curing (strain C03-1/KR9-1*, Table 2) was conjugally transferred to the F^- strain C046 to yield strain C046/KR9-1*. To serve as a control, a strain C046/KR9-1 was also constructed by conjugating C03/KR9-1 with strain C046 (see Table 1). From the results of experiments 5 and 6 of Table 2, it can be seen that, when C046/KR9-1 and C046/KR9-1* were infected with M13, they showed an induced conversion frequency of 48.9 and 38.9%, respectively, figures that were well above the frequency of 2.7% seen with the resistant C03-1/KR9-1* (experiment 7, Table 2). This indicated that in this instance, the efficiency of conversion was determined by the host cells harboring the R factor rather than the R factor itself. Since C03-1/KR9-1* was an M13 carrier strain that continued to produce M13, it was possible that the carried M13 was affecting the efficiency of conversion. This M13 carrier, like others, engendered progeny strains that were no longer

TABLE 2. *Effect of M13 infection on the viability and conversion to R⁻ phenotype of strains bearing different R factors*

Expt	Strain and R factor borne by it	Antibiotic resistance marker scored	Per cent spontaneous conversion in 4 hr[a]	Per cent viability relative to uninfected control	Per cent induced conversion (corrected for spontaneous conversion)
1	C036/R64-11	Tc	4.5	65	85.5
2	C03/KR9-1	Km	7	100	86
3	C03/KR9-2	Tc	1	100	86.0
4	C03/KR9-2	Tc	2.5	100	72.5
5	C046/KR9-1	Km	8.3	100	48.9
6	C046/KR9-1*[b]	Km	6.1	100	38.8
7	C03-1/KR9-1*	Km	4.3	100	2.7

[a] During this 4-hr period the number of generations as estimated from viable counts ranged from 3 to 6 (average 5.2).

[b] KR9-1* identifies the R factor which was derived by selecting a strain from experiment 2 that failed to show loss of the R factor. This resistant strain was purified and used in experiment 7. It is identified as C03/KR9-1*. The R factor from this strain was transferred to C046 to yield C046/KR9-1*, the strain used in experiment 6.

carriers but were still R^+, indicating that resistance to conversion is not directly correlated to the carrier state.

M13 carrier state of infected R^+ cells. When susceptible Hfr, F^+, or F′ strains of *E. coli* are infected with M13 or related filamentous phages, a variable fraction of the derived strains continues to propagate the phage (12, 13), and this property has been referred to as the "carrier" state. Since M13 does not cause a marked loss in the viability of its propagating host, the carrier state of a strain could simply be maintained by repeated cycles of phage infection. On the other hand, the carrier state could have an intracellular basis, and some cells in an infected population could retain an intracellular potentiality to produce the phage without secondary extracellular infection. In preliminary experiments, we observed that a large fraction (50–75%) of colonies derived from strain C03/KR9-1 infected with M13 produced M13, and furthermore, in 66% of such strains the M13-producing capacity was retained on subculturing under conditions which minimized reinfection [i.e., in the presence of 400 μg of Nagarase/ml, a proteolytic agent known to inactivate M13 (29)]. This situation prompted us to examine this question with a view to discriminating between the two possibilities indicated above.

Inactivation and elimination of M13 by treatment with Nagarase or M13 antiserum and extensive washing. If a strain will produce M13 under conditions when extracellular reinfection is prevented, this would indicate that the carrier state has an intracellular basis. Salivar, Tzagaloff, and Pratt (29) have shown that free M13 virus particles can be effectively inactivated by treatment with M13 antiserum or the proteolytic enzyme Nagarase, and this has been confirmed in this laboratory. In the present study, this observation was first extended by showing that such an inactivation also occurs when M13 particles are attached to free pili. Fifty millilters of a broth culture of strain C03/KR9-2 containing approximately 10^{12} cells was centrifuged, and the supernatant fluid was mixed with a suspension containing 10^{12} plaque-forming units of M13. The mixture was allowed to stand at 28 C for 2 hr and the M13-pili suspension was then filtered through a cellulose nitrate filter (Millipore, 0.45-μm porosity). The filter was then removed and gently washed with 5 ml of DB. Seven per cent of the input phage was recovered in this manner from the filters. In contrast, when a preparation of free phage particles was passed through a separate filter as a control, only 0.001% of them was recovered. The DB washing was then divided into two equal portions, one of which was treated with 400 μg of Nagarase per ml for 60 min at 37 C and the other left untreated at 37 C. At the end of this period, the suspensions were blended in a blendor constructed as described by Low and Wood (17). The blended suspensions were then assayed for plaque-forming units with strain C036; 99.30% of the pili-bound phages were found to be inactivated. Although this inactivation is substantial, it was noted that under similar conditions 99.99% of free phages were inactivated. The procedure that was eventually

adopted to free culture suspensions of extracellular phages involved the following steps: washing the cells three times by centrifugal pelleting and resuspension in chilled DB, exposure to 400 μg of Nagarase per ml for 1 hr at 37 C, and repeating the three cycles of washing again. In reconstruction experiments with mixture of cells and phages, we have found that this procedure reduces the titer of free phages to 10^{-8} to 10^{-9} of those initially present.

M13 production by colonies of a previously infected strain. Single colonies derived from independent M13-infected cultures were purified once by restreaking, inoculated into 5 ml of NB, and grown overnight at 37 C with aeration. When such suspensions were pelleted and the supernatant fluids assayed for phages, their number varied from 10^3 to 10^6 for different cultures. The pellets were washed, exposed to Nagarase as described above and resuspended in DB, and a suitable dilution spread on NA or NA plus Km to yield discrete colonies on subsequent incubation of the plates at 37 C. These colonies were then tested by replication for Km resistance and for the presence of M13 by centrifuging suspensions of the colonies and assaying supernatant fluids by the agar overlay technique (1) using strain C036 as the host. Of the colonies tested, 67.5% were still Km-resistant, and of these 50% still produced M13. This observation provided the first clear suggestion that M13-producing ability could be maintained by strains under conditions when infection by extracellular phages was rendered unlikely.

Propagation of the M13 carrier state by single cells. The above conclusion was confirmed as follows. A purified single colony of an R^+ carrier strain was treated as described above, except that one part of the cell suspension was exposed to Nagarase and another to M13 antiserum. Following the subsequent washing procedure, each suspension was extensively diluted so that a 0.1-ml sample of each suspension could carry a viable cell with only a very low probability. A 0.1-ml amount of each of the diluted suspensions was then used to inoculate 60 tubes of NB. Only six and five of the tubes inoculated with the two respective suspensions yielded growth. Thus, the probability that any one tube was inoculated with more than one viable cell was very low. Nevertheless, on subsequent examination, five of the six broth cultures arising from the Nagarase-wash treatment and all of the broth cultures arising from the antibody-wash treatment showed the presence of M13 in supernatant fluids. It was concluded that single cells of a carrier strain could propagate the M13 carrier state and that this carrier state could be sustained by a cell without reinfection.

Carrier state and conjugal recipient ability. When an Hfr strain bearing an R factor (strain C03/KR9-1) is infected with M13, R^- strains segregate. A fraction of these R^- segregants are M13 carrier strains and another is not. We have used a representative of each of these two types of strains to determine whether the carrier state affects the ability of the strain to function as a recipient. The identical strain that had never been exposed to M13 was used as control. These experiments were done in duplicate. In one of each conjugation mixture, the recipients had been exposed to 400 μg of Nagarase per ml for 60 min prior to conjugation and during conjugation. The results are presented in Table 3 and indicate that the carrier strain has suffered a 50% reduction in its ability to function as a recipient. The possibility that it is the local but extracellular concentration of phage M13 that is responsible for this effect cannot be rigorously ruled out in this experiment. However, the observation that Nagarase treatment does not relieve this reduction to any significant extent suggests that this effect is not due to the presence of any extracellular phage interfering with the conjugation events. Other studies in this laboratory (Iyer and Messmer, *unpublished data*) involving the analysis of segregants of an F′ merodiploid infected with M13 indicate that infection can result in an irreversible loss of phage adsorbtion sites from the surface layers of bacterial cells. If similar changes have occured in the present case, it is

TABLE 3. *Relative conjugal recipient ability of strains that carry or do not carry M13*[a]

Origin and relevant phenotype of recipient (Hfr R^-)	M13-producing ability of strain	Frequency (per 10^6 donors) of recipients acquiring Tc^r from the R factor of donor	
		Nagarase absent	Nagarase present
Strain C03	–	4.73×10^{-2}	4.67×10^{-2}
Strain C03/KR9-1 infected with M13 and selected for absence of KR9-1 and M13.	–	5.20×10^{-2}	4.86×10^{-2}
Strain C03/KR9-1 infected with M13 and selected for absence of KR9-1 but presence of M13.	+	2.52×10^{-2}	2.61×10^{-2}

[a] Conjugal donor strain, C046/KR9-2; donor to recipient ratio, 1:10; selection medium, minimal agar + proline + thiamine + Tc.

conceivable that this could affect the mating efficiency of recipients.

DISCUSSION

When it was initially observed (22, 23) that an autonomous F′ factor was eliminated on M13 infection, one possibility that was considered was that the locus of the F pilus at the cell surface is also a locus for F′ factor maintenance. An event or events specifically involving this locus (e.g., initiation of M13 infection) could therefore also result in a perturbation of the locus sufficient to result in the loss of the F factor. Rfi^+ and F factors specify the synthesis of pili which are very similar to one another and at which M13 may initiate infection (4, 6, 15). However, an Rfi^+ and an F factor can be stably maintained together in the same cell (33), and the present observations indicate that both can be eliminated by M13 at least in a situation where they are present separately in cells. Thus, if the events promoted by M13 infection involve intracellular maintenance sites for these factors, more than one such site must be affected. This is supported also by the observation that a factor which does not specify an F or F-like pilus (the Rfi^- factor) is also eliminated upon M13 infection. It is still possible that elimination is triggered specifically by events promoted by M13 either at the F pilus or during the subsequent intracellular development of the phage. It is clear however that the effect of such events must extend either directly or indirectly to several bacterial sex factors that can coexist as separate elements in the same cell. Events induced at one locus on a bacterial cell surface can have effects at loci that are spatially removed, and the theoretical feasibility of such events has been considered (5, 34). Changes in the morphology of the cell membrane (2, 11, 30) and in membrane-associated physiological properties (10, 28) have been reported following infection by this type of phage. However, it is not known that any of these changes are causally related to the elimination of the plasmids. The question of mechanism of elimination is an open one except that it should account for the elimination of more than one type of plasmid that can cohabit the same cell.

Marvin and Hohn (18) have pointed out that the fundamental question as to whether a cell infected with a filamentous virus can retain an intracellular capacity to produce phage has not been satisfactorily answered. The major technical problem in answering this question unambiguously has been the difficulty in eliminating all extracellular phages and preventing infection from without. Frequently (see reference 32 for a review), it has been assumed that if the carrier state cannot be sustained for prolonged periods in the presence of specific virus antibody, this implies that the carrier state must only have an extracellular basis. However, both in this laboratory with phage M13 and elsewhere (18) it has been observed that phage antiserum will prevent or reduce the division of infected cells while having no such effect on uninfected cells. The antiserum, besides having an effect on extracellular phage, would therefore encourage the selective outgrowth of cells that do not carry the phage and render the detection of such carrier cells difficult under these circumstances. In the present experiments, the carrier strain was exposed to either M13 antiserum or to Nagarase for a limited period, being also extensively washed both before and after this treatment. We know that at the start of the experiment the strain would have 10^5 to 10^6 extracellular phages associated with it. The reconstruction experiments indicated that 10^8 to 10^9 of these are eliminated by the treatment. It is therefore unlikely that clones emerging from single cells of such a treated suspension would have free phage associated with them. Nevertheless, most of the clones emerging from such single cells were found to be M13 carriers. The carrier state must therefore have been sustained by single cells. These experiments do not enable us to decide whether the carrier state was also replicated.

From the experiments presented here and from others, we infer that the efficiency of detectable M13-induced elimination of an R factor will be affected by whether the R factor is derepressed, the degree of synchrony of infection, and probably other ill-defined factors associated with the particular R factor or the host that harbors it. Nevertheless, the present observations indicating that R factors *can* be eliminated by this means have some practical implications. They should encourage further studies aimed at evolving procedures for the elimination of such factors from natural populations. If an agent such as M13 is to be exploited in natural populations for such a purpose, it will probably be necessary to find and use agents that will potentiate its action or agents that will prevent a cell "cured" of its R factor from reacquiring an R factor from cells in the population that initially did not succumb to the M13 effect, or both. The grave implications of R factors for the epidemiology of bacterial diseases warrant such approaches.

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