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# Bacteriophage SP82G Inhibition of an Intracellular Deoxyribonucleic Acid Inactivation Process in *Bacillus subtilis*[1]

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The stability of SP82G bacteriophage deoxyribonucleic acid (DNA) after its uptake by competent *Bacillus subtilis* was examined by determining the ability of superinfecting phage particles to rescue genetic markers carried by the infective DNA. These experiments show that a DNA inactivation process within the cell is inhibited after infection of the cell by intact phage particles. The inhibition is maximally expressed 6 min after phage infection and is completely prevented by the addition of chloramphenicol at the time of infection. The protective effect of this function extends even to infective DNA which was present in the cell before the addition of intact phage. Continued protein synthesis does not appear to be a requirement for the maintenance of the inhibition. In an analogous situation, if infectious centers resulting from singly infecting phage particles are exposed to chloramphenicol shortly after the time of infection, an exponential decrease in the survival of infectious centers with time held in chloramphenicol is observed. If the addition of chloramphenicol is delayed until 6 min after infection, the infectious centers are resistant to chloramphenicol. The sensitivity of infectious centers treated with chloramphenicol at early times after infection is strongly dependent upon the multiplicity of infection and is consistent with a model of multiplicity reactivation. These results indicate that injected DNA is also susceptible to the intracellular inactivation process and suggest that the inhibition of this system is necessary for the successful establishment of an infectious center.

Genetic studies on the infectivity of deoxyribonucleic acid (DNA) isolated from *Bacillus subtilis* bacteriophages SP82G, SP01, SPP1, and SP50 have shown that the participation of several molecules of DNA is required to establish an infectious center (4, 17, 23). In agreement with this, the infectivity of DNA isolated from these and a number of other (though not all) *B. subtilis* phages exhibits a dependence on DNA concentration that is greater than first order (3, 4, 17, 18, 19, 20). By investigating the ability of superinfecting phage particles to rescue genetic markers from DNA-infected cells, Green (5) has shown that in the case of bacteriophage SP82G this multimolecular requirement is the result of an intracellular inactivation mechanism that exponentially inactivates and unlinks genetic markers carried by the infective DNA.

Further investigations have revealed that this inactivation process can be modified by appropriate treatment of the cells. Infection of competent cells by marker rescue phage particles prior to the addition of infective DNA results in an inhibition of intracellular inactivation (5). This modification, termed preinfection protection, has a maximal effect when the infective DNA is added 6 min after phage infection. Epstein has found that the inactivation may also be inhibited by adding ultraviolet (UV)-irradiated homologous or heterologous DNA to the competent cells (2) or by irradiating the competent cells themselves (1). The maximal protective effect afforded by this treatment is obtained when infective DNA is added 20 min after the UV-irradiated DNA (or after whole-cell irradiation) and is presumed to be the result of an entrapment of host nuclease(s) by the irradiated DNA. The relationship between these two protective

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mechanisms is unclear. However, the different times at which UV-irradiated DNA and preinfecting phages give a maximal effect suggest that two different protective mechanisms may be operative. In normal infection of *B. subtilis* by bacteriophage SP82G particles, there is no evidence to indicate that the phage DNA is inactivated (2, 5); either injected DNA is not susceptible to intracellular inactivation or the phage manages to directly overcome the inactivation process.

We conclude that the modification of intracellular inactivation by preinfecting phage is the result of a function which inhibits the inactivation process. This function is maximally expressed by 6 min after phage infection and is completely prevented by the addition of chloramphenicol (CM) at the time of infection. We have also observed that, if the infecting phage particle is prevented from carrying out the early protective function, its own (injected) DNA appears to be susceptible to an inactivation process similar to that observed during infection of cells by isolated phage DNA.

## MATERIALS AND METHODS

**Bacterial strains, growth conditions, and phage preparations.** *B. subtilis* strain SB-1 (14) was the host cell for all experiments. Techniques for the growth and detection of bacteriophage SP82G are identical to those of Green (4). Temperature-sensitive (*ts*) mutants of SP82G which will grow at 33 C but not at 47 C, and the techniques for scoring wild-type (WT) recombinants have been described previously (5, 8).

The primary media for the growth of all phage preparations was Nomura salts (NM) (16) supplemented with 0.5% glucose, 0.2% casein hydrolysate, $2.5 \times 10^{-3}$ M $MgCl_2$, 0.1% yeast extract, 0.05 mg of DL-tryptophan per ml, 5 mg of arginine per ml, and 0.2 mg of L-histidine per ml.

Phage lysates grown at 33 C were concentrated by two cycles of high (10,000 × *g*)-low (6,500 × *g*)-speed centrifugation and stored in 1 × NM salts plus $10^{-3}$ M $MgCl_2$. Phage DNA was prepared by phenol extraction (12) and stored after dialysis against 1 × SSC (0.15 M NaCl, 0.015 M sodium citrate). The concentration of DNA preparations was determined from their absorbance at 260 nm.

Competent cells were prepared by a modification of the technique of Spizizen (24). Slants of SB-1 grown overnight on Tryptose blood agar (Difco, Detroit, Mich.) at 37 C were transferred to Spizizen's high tryptophan-high casein hydrolysate (HS) media supplemented with 8 mg of arginine per ml and 400 μg of histidine per ml and grown for 4.5 hr at 37 C. At that time, the media was made 7.5% in dimethyl sulfoxide and stored at −97 C. On the day of use, samples were thawed at 47 C, diluted 1:10 into low tryptophan-low casein hydrolysate (LS) media supplemented with $5 \times 10^{-4}$ M spermine tetrahydrochloride, $2.5 \times 10^{-3}$ M $MgCl_2$, and $5 \times 10^{-4}$ M $CaCl_2$ and grown at 37 C for 90 min.

**Marker rescue experiments.** Details of each marker rescue experiment are described in the text and figures. A typical marker rescue experiment not involving preinfected cells was carried out as follows. Competent cells (grown in LS for 90 min as above) were exposed to DNA isolated from a *ts* mutant phage at a concentration of 2 to 3 μg of DNA/ml. After 3 min at 33 C, the cells were diluted 1:10 in fresh LS medium, held for an additional 10 min, and treated with deoxyribonuclease (beef pancreas, 10 μg/ml; Miles Laboratories, Elkhart, Ind.). Genetic markers were rescued from the intracellular phage DNA by infecting the cells at intervals with phage particles having another *ts* mutation and plating at 47 C where only WT recombinants will give rise to plaques. A modification of this technique in which the cell was also infected with a helper phage having a genotype such that it could not contribute the genetic information necessary for the formation of a WT recombinant was also used.

**Survival of infectious centers and bacteria in CM.** Protein synthesis in *B. subtilis* is rapidly inhibited by the addition of CM (10). The effect of CM treatment on the survival of infectious centers was examined by briefly exposing bacteria to phage and then adding sufficient antisera to inactivate 99.9% of the unadsorbed phage. These rapidly infected complexes were diluted into NM containing CM (500 μg/ml; Sigma, St. Louis, Mo.) and held for various lengths of time until plating. Incubation of uninfected bacteria in the inhibitor did not affect their survival.

## RESULTS

**Effect of CM on preinfection protection.** A single molecule of SP82G DNA incorporated into the cell does not result in the production of phage in that cell (4). The genetic potential of that molecule can be measured, however, by superinfecting the DNA-infected cell with phage particles of a different genotype. Genetic markers carried by the infective DNA are rescued by the superinfecting phage and appear in the progeny of the superinfected cells as recombinant particles which may be detected under suitable conditions.

One result of the intracellular inactivation process is that the simultaneous rescue of two genetic markers from the infective DNA decreases exponentially as a function of the genetic map distance between the markers (5). Under conditions in which the inactivation mechanism has been modified (i.e., when marker rescue phage have been added to the cells 6 min prior to the addition of the infective DNA) the rescue of marker pairs no longer exhibits the severe dependence upon intermarker map distance (5).

If protein synthesis is necessary for the reduction in intracellular inactivation caused by preinfecting phage, then no modification of intracellular inactivation should occur when cells

are preinfected in the presence of CM. An experiment was performed in which genetic markers carried by infective DNA were rescued under three conditions: (i) by preinfecting phage added 6 min prior to the DNA, (ii) by preinfecting phage in the presence of CM, and (iii) by superinfecting phage added after the infective DNA. The rescue of marker pairs in each group was expressed as the percentage of the single marker H362+ rescued under the same conditions and plotted as a function of the map distance subtended by the markers.

When marker rescue phage were added prior to the infective DNA, the rescue of double markers did not exhibit the high degree of dependence on intermarker map distance that is characteristic of superinfection marker rescue (Fig. 1). However, if preinfection was carried out in the presence of CM, the rescue of marker pairs still showed a strict dependence upon intermarker map distance. We conclude that preinfecting phage cannot modify the intracellular inactivation process in the presence of CM.

**Time course of preinfection protection.** If infective DNA is added to cells at intervals after their infection by marker rescue phage, the maximal number of WT recombinants is obtained when the infective DNA is added 6 min after phage infection. This observation defined the concept that by 6 min preinfection protection has reached a maximum and that at this time the intracellular inactivation process is substantially inhibited (5). If the attainment of preinfection protection is the result of the synthesis and accumulation of a necessary protein within the cell, then it should be possible to duplicate this effect by arresting protein synthesis in the cells at various intervals after phage infection and adding the infective DNA at some later, fixed time. To test this hypothesis, cells were preinfected at 33 C with a helper phage carrying three *ts* mutations (H20-H362-H15). At intervals, protein synthesis in the infected cells was arrested by the addition of CM. Nine minutes after infection, all cells were permitted to take up DNA isolated from the *ts* mutant phage H15. The linkage integrity of markers H20+ and H362+, introduced by the infective DNA, was then examined by superinfecting the complexes with marker rescue phage (H20-H362) and plating at 47 C. (The preinfecting helper phage has a genotype such that it is unable to contribute information necessary for the formation of a WT recombinant infective center [IC].) The maximal rescue of markers H20+ and H362+ was obtained when protein synthesis was interrupted 6 min after preinfection (Fig. 2). These observations agree with the previous results (5) obtained

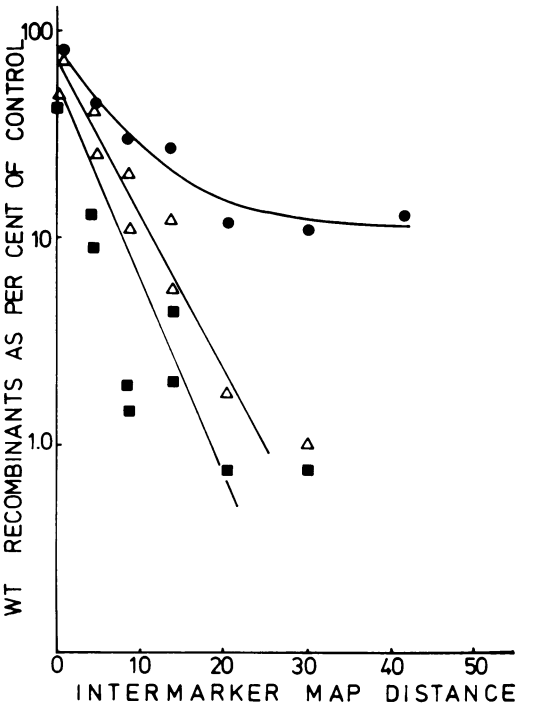


FIG. 1. *Effect of chloramphenicol (CM) on the modification of intracellular inactivation. The ability of a number of double mutant phages to simultaneously rescue two genetic markers from infective DNA was measured under three conditions: superinfection marker rescue (■), preinfection marker rescue (●), and preinfection marker rescue in the presence of CM (△). Competent cells were preinfected by exposure to marker rescue phage at a multiplicity of infection of 10.0 for 2 min and diluted into either LS or LS containing CM (100 μg/ml) and held for an additional 6 min. These cells, and another sample which had not been preinfected, were exposed to WT phage DNA at 0.1 μg/ml for 15 min and treated with deoxyribonuclease. Samples from the third group were then superinfected with marker rescue phage. Scoring for WT recombinant infective centers was accomplished by plating dilutions of all samples at a nonpermissive temperature (47 C). The rescue of marker pairs within each group is expressed as the percentage of the single marker H362+ rescued under the same conditions and plotted as a function of the map distance subtended by the markers.*

when infective DNA was added to cells at intervals after preinfection and in addition indicate that the appearance of preinfection protection with time reflects the synthesis of a protein in the phage-infected cell.

**Stability of genetic markers in preinfected cells.** The peak of recovery of WT recombinant IC observed in Fig. 2 is presumably the result of the increasing inhibition of intracellular inactivation in the preinfected cell. This interpretation

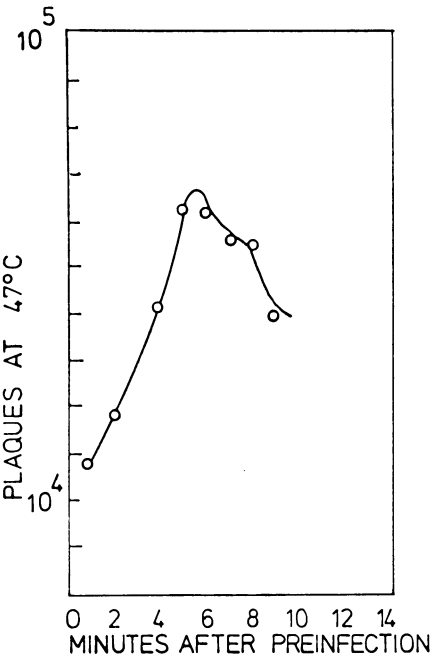


FIG. 2. *Time course of preinfection protection. Competent cells were preinfected at a multiplicity of infection of 10.0 with helper phage carrying three ts mutations (H20-H362-H15). At intervals after phage infection, CM was added to samples to a concentration of 100 μg/ml. Nine minutes after infection, all samples were exposed to phage DNA (H15) at 1 μg/ml for 3 min, diluted 1:10 into LS containing CM, and held for an additional 10 min. Deoxyribonuclease was added, and the cells were exposed to superinfection marker rescue phage (H20-H362), diluted, and plated at 47 C.*

predicts that the stability of markers in the preinfected cell would be greater if protein synthesis were arrested 6 min after the addition of helper phage than if protein synthesis were arrested at the time of phage infection.

To examine this, the integrity of genetic markers introduced into preinfected cells by infective DNA was measured at intervals by superinfecting the complexes with marker rescue phage. The yield of WT recombinant IC as a function of the time of rescue reflects the stability of the markers in the preinfected cell. Protein synthesis in the cells was arrested either at the time of preinfection or 6 min thereafter, corresponding to the times at which the lowest and highest yields of WT recombinants were obtained under the conditions described in Fig. 2. The stability of markers with the time of residence in non-preinfected cells was examined by omitting the preinfection step but otherwise following the same procedure. Protein synthesis in these non-preinfected cells was arrested either at the time of addition of the infective DNA or was not arrested at all.

The results of this experiment are shown in Fig. 3. When non-preinfected cells were used, a sharp decline in the number of WT recombinant IC with time was observed. This is characteristic of intracellular inactivation. Neither the rate of decline nor the yield of WT recombinants was affected by the addition of CM to non-preinfected cells at the beginning of the experiment. One can conclude from this that the intracellular inactivation phenomenon itself is not dependent

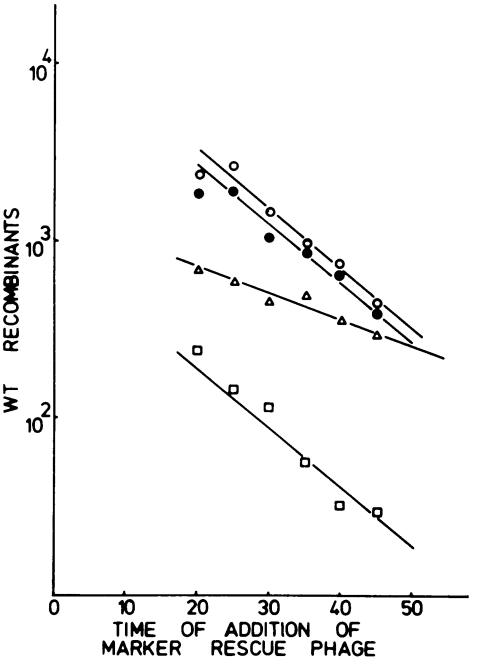


FIG. 3. *The effect of arresting protein synthesis on the stability of genetic markers introduced into preinfected or non-preinfected cells. At time zero, competent cells were exposed to ts mutant phage (H20-H362-H15) at a multiplicity of infection (MOI) of 10. After 6 min, these preinfected cells and cells which had not been preinfected were exposed to DNA isolated from the ts mutant phage H15 at a concentration of 3 μg/ml and held for 3 min. The cells were diluted 1:10 into LS and after an additional 10 min were treated with deoxyribonuclease. At intervals thereafter, samples were withdrawn, superinfected with the ts mutant phage H20-H362 at a MOI = 10 for 4 min, and dilutions were plated at 47 C. The number of WT recombinant IC is plotted as a function of the time of addition of the superinfecting phage. Upper curves are for non-preinfected cells which were not exposed to CM (○) or which were exposed to CM (100 μg/ml) from the time zero (●). Lower curves are for results obtained with preinfected cells which were exposed to CM from time zero (□) or exposed to CM from the sixth minute (△).*

on new protein synthesis. Similarly, it follows that the uptake of DNA by competent cells does not require induced enzyme synthesis.

In contrast, with preinfected cells the cessation of protein synthesis had a large effect on the stability of markers subsequently introduced by the phage DNA. If CM was added at the same time as the preinfecting phage, the rate of loss of WT recombinant IC was identical to that observed in cells which were not preinfected. However, if the addition of CM was postponed until 6 min after the addition of the preinfecting phage, a marked improvement in the stability of markers introduced by transfecting DNA was observed. The results were even more striking when the stability of single genetic markers was examined under the same conditions (Fig. 4).

In a similar experiment (not shown) in which the addition of CM was delayed until 9 min after preinfection, the rate of loss of recombinants was greater than if protein synthesis was interrupted 6 min after preinfection. Clearly, the peak of preinfection protection observed in Fig. 2 reflects the stability of genetic markers in the preinfected cells. The addition of CM at the time of preinfection completely prevents modification of intracellular inactivation, whereas the maximum stability is achieved when protein synthesis is arrested 6 min after phage infection.

The absolute recovery of WT recombinant IC from preinfected cells is less than that obtained from non-preinfected cells. Two aspects of the preinfection technique are possibly responsible for this lowered recombinant production. First, the multiplicity of infection of the *ts* helper phage in the preinfection experiments is twice that of the superinfecting marker rescue phage. The increased number of defective genes in the gene pool would decrease the probability of rescue of the WT genes from the infecting DNA by the superinfecting phage. Secondly, analysis of preinfection and superinfection rescue of markers in the *cis* and *trans* configuration (Green, *unpublished data*) suggests that, whereas nearly all the markers issuing from superinfection are recombinant, less than 25% of those issuing from preinfection conditions are recombinant. Thus, the nonrecombinant progeny of the DNA infecting under preinfection conditions would further lower the frequency of WT recombinants. We have considered the alternative that preinfecting phage render some portion of the cell population refractory to attack by the superinfecting phage. Two arguments mediate against this. Superinfection exclusion does not occur in SP82G (8), and the observed decrease occurs even when protein synthesis is inhibited at the time of addition of the preinfecting phage.

**Stabilization of markers after inactivation has begun.** These experiments demonstrate that the infection of a competent cell by intact phage particles results in increased stability of genetic markers which are subsequently introduced by infective DNA. We now ask whether infective DNA present in the cell before the time of phage infection can also be protected by the inhibition of intracellular inactivation. That is, once the inactivation of DNA has started, can it be stopped?

Competent cells were permitted to take up H15 phage DNA and were then infected with a triply mutant helper phage (H20-H362-H15). The complexes were treated with CM either immediately or 6 min after the addition of the phage. The linkage integrity of markers $H20^+$ and

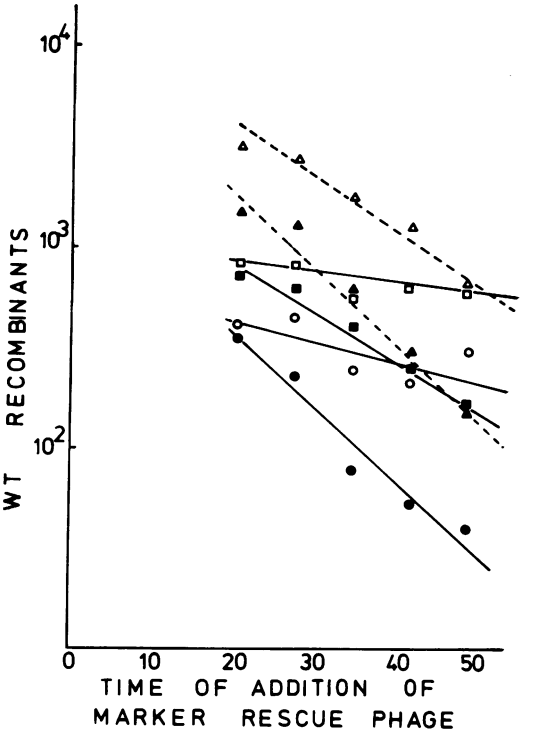


FIG. 4. *Stability of double and single markers in preinfected cells. Competent cells were preinfected with the ts mutant phage H20-H362-H15 and exposed to H15 phage DNA as described in Fig. 3. Protein synthesis in the cells was arrested either at the time of phage infection (filled symbols) or 6 min thereafter (open symbols). At intervals, the cells were superinfected with the ts mutant phage H20 (squares) or H20-H362 (circles) and plated at 47 C. The number of WT recombinant IC is plotted as a function of the time of addition of the superinfecting phage. Dashed lines represent the survival of markers in non-preinfected cells (△, H20; ▲, H20-H362).*

$H362^+$ introduced by the infective DNA was examined as before by superinfecting the cells with the double mutant H20-H362 and plating at 47 C. The results (Fig. 5) show that when protein synthesis was arrested at the time of addition of the helper phage there was a loss of rescuability of markers with time. The rate of loss was identical to that observed in cells in which intracellular inactivation had not been modified. If the arrest of protein synthesis was delayed until 6 min after the addition of helper phage, however, a marked improvement in the stability of markers $H20^+$ and $H362^+$ was observed. These results indicate that the addition of phage to competent cells which have already taken up infective DNA results in the inhibition of intracellular inactivation in the cells. Thus, intracellular inactivation which is in progress may be modified by the infection of the cell with a phage particle. This process may be called superinfection protection.

**Sensitivity of infectious centers to CM.** After the infection of *B. subtilis* by intact SP82G phage particles, the injected phage DNA does not appear to be susceptible to intracellular inactivation (2, 5). The preceding experiments demonstrate that protein synthesis must take place for an infecting phage particle to reduce the intracellular inactivation of infectious DNA. Thus, the possibility arises that the arrest of protein synthesis shortly after phage infection might lead to the inactivation of injected DNA as well. If this were the case, the survival of infectious centers established by phage infection alone should be sensitive to the effects of CM until the inactivation mechanism has been modified. To examine this possibility, bacteria were synchronously infected by a brief exposure to a low multiplicity of phage particles followed by the addition of an excess of phage-specific antisera. At intervals after infection, samples were diluted into media containing CM. After 30 min in the inhibitor, samples were withdrawn and assayed for surviving infectious centers. The results (Fig. 6) indicate that at early times infectious centers established by SP82G are sensitive to CM, but that by 5 to 6 min after infection they become resistant to the effects of inhibition of protein synthesis.

To examine the sensitivity of infectious centers more closely, the survival of infected and uninfected bacteria as a function of the time spent in the presence of CM was monitored. As seen in Fig. 7, maintenance of uninfected cells in CM had no effect on their survival or on their ability to subsequently support phage growth. Under the same conditions, however, the number of infectious centers decreased exponentially with time

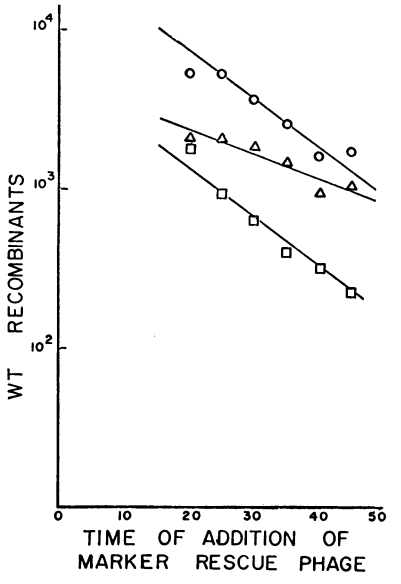


FIG. 5. *Stability of genetic markers in superinfected cells. At time zero, competent cells were exposed to H15 phage DNA at a concentration of 3 μg/ml. After 10 min, DNA uptake was terminated by the addition of deoxyribonuclease. The cells were then infected with the ts mutant phage H20-H362-H15 and were treated with CM either at the time of infection (□) or 6 min thereafter (△). At intervals, samples were withdrawn and infected with the ts mutant phage H20-H362 and plated at 47 C. The number of WT recombinants is plotted as a function of the time of addition of the second (marker rescue) phage. The survival of markers H20-H362 in non-preinfected cells is also shown (○).*

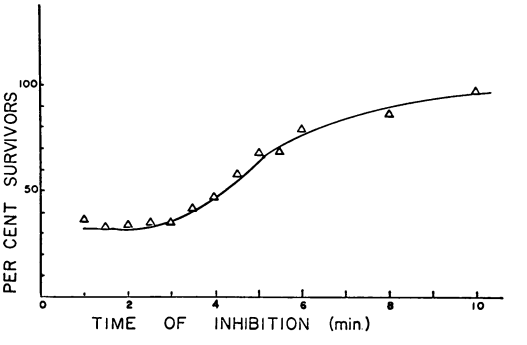


FIG. 6. *Sensitivity of infectious centers to chloramphenicol. Bacteria at $10^8$ cells/ml were exposed to phage at a multiplicity of infection of 0.1 for 15 sec, and the adsorption was terminated by the addition of phage-specific antisera. At intervals, samples were diluted into NM containing chloramphenicol (500 μg/ml) and held for 30 min at 33 C. The surviving infectious centers are expressed as the percentage of a control in which protein synthesis was not arrested.*

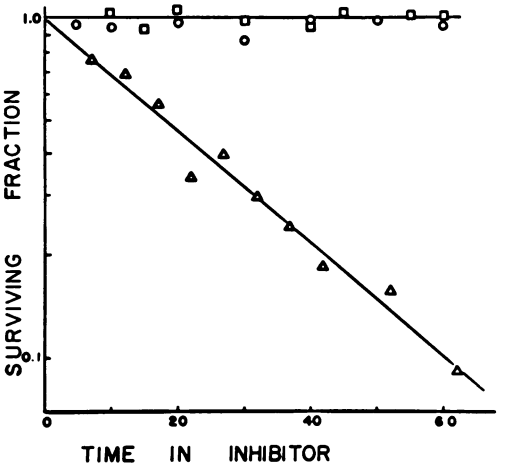


FIG. 7. *Effect of incubation in chloramphenicol on survival of infectious centers, uninfected bacteria, and the ability of uninfected bacteria to support phage growth. Uninfected bacteria, and bacteria which had been infected by a brief exposure to phage particles as in Fig. 6, were diluted into media containing CM (500 μg/ml) at 33 C. At intervals, samples were withdrawn and assayed for surviving colony formers (○) and infectious centers (△). At the same time, samples of the uninfected bacteria were exposed to phage at a multiplicity of infection of 2.0 for 5 min. Unadsorbed phage particles were inactivated by a 3-min exposure to antisera (K = 1.35), and the samples were assayed for plaque-forming units (□).*

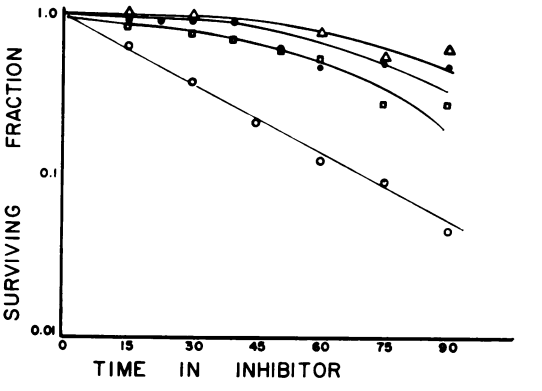


FIG. 8. *Effect of the multiplicity of infection on the survival of infectious centers in chloramphenicol. Bacteria at $10^8$ cells/ml were briefly exposed to phage at various multiplicities. Phage adsorption was terminated by the addition of phage-specific antisera. After 2 min, the samples were diluted into media containing chloramphenicol (500 μg/ml). At intervals, the survival of the infectious centers was determined. The measured multiplicities of infection were: 5.1 (△), 3.0 (●), 1.7 (□), and 0.13 (○).*

and reached an $e^{-1}$ survival in 32 min. Identical results were obtained with bacteria that had been grown in a competence regime. Kahan (9) has shown that prevention of phage DNA synthesis in SP82G-infected cells for periods of up to 2 hr does not affect the subsequent development of infectious centers. It is thus unlikely that these results are due to interference with DNA synthesis.

Although these results are consistent with the interpretation that the inability to inhibit intracellular inactivation results in the inactivation of injected DNA, the sensitivity of infectious centers to CM might also be due to the inability of the infected cell to repair damages caused by the phage during infection. If this were the case, then increasing the multiplicity of infection should increase the number of damages sustained by each cell and lead to a decreased survival in CM. On the other hand, if the intracellular inactivation of injected DNA were responsible for the loss of infectious centers in CM, then the presence of more than one phage genome in the cell should increase the chance of survival of an infectious center. For, under these conditions, undamaged portions of the phage genome could cooperate in the successful establishment of an infectious center.

To test these possibilities, bacteria, which had been multiply infected by a brief exposure to varying concentrations of phage, were diluted into media containing CM, and at intervals the survival of infectious centers was determined. As the multiplicity of infection was increased, the survival of the infected bacteria in CM improved (Fig. 8). The increased survival in the presence of CM with increasing multiplicity may be considered formally analogous to multiplicity reactivation (McAllister, Ph.D. thesis, Univ. of New Hampshire, 1970). A trivial explanation for this phenomenon—that in the absence of protein synthesis phage particles cannot inject their DNA and slowly elute from the cell—can be excluded since CM has no effect on the transfer of the SP82G genome (11).

## DISCUSSION

These experiments demonstrate that the observed decrease in intracellular inactivation after phage infection requires the synthesis of protein in the infected cell; however, this paper presents no evidence on whether the synthesis is host- or phage-specific.

The maximal stability of infective DNA in cells infected with helper phage was observed when protein synthesis was arrested 6 min after infection by helper phage. Even under these conditions, complete stability is not achieved (there is still a slight loss in the ability of the infective DNA to contribute genetic markers to marker rescue phage with time of residence in

the cell). The fact that this is greater for double markers than for single markers (Fig. 4) indicates that this is not due to the loss of the entire genetic input of the infective DNA (as would result from the death of the cell), but rather from a partial inactivation of the genome—as in intracellular inactivation. This observation raises the possibility that continuous protein synthesis might be a requirement for the complete inhibition of the inactivation process. However, under conditions in which protein synthesis is not arrested, the rescue of closely linked double markers by preinfecting phage is still lower than that of single markers (Fig. 1 and reference 5). This suggests that even under these conditions intracellular inactivation may not be completely turned off.

The mechanism by which the phage inhibits intracellular inactivation is not known. The onset of DNA synthesis in SP82G-infected cells under these conditions (33 C) is not observed until 13 min after infection (Green, *unpublished data*), well past the time of maximal preinfection protection. Thus, the accumulation of SP82G DNA within the phage-infected cell does not appear to be the basis of the inhibition.

It has been found that after infection of *B. subtilis* with bacteriophage SP50 there is a marked stimulation in the uptake of infective SP50 DNA (N. Biswal and T. A. Trautner, *personal communication*). Such a process is probably not involved in the establishment of preinfection protection since superinfection of DNA-infected cells in which DNA uptake has been terminated by the addition of deoxyribonuclease results in a similar inhibition of intracellular inactivation (Fig. 5). One possibility is that the modification of the inactivation process involves the recombination of damaged infective DNA molecules into an intact (injected) genome. However, it has recently been shown that for the unrelated bacteriophages SP50 and SP02 the process which is necessary to construct an intact replicon from inactivated infective DNA molecules is mediated by the host recombination system and is not effected by the phage recombination system (23). Since intracellular inactivation proceeds unabated in cells which have been preinfected with bacteriophage SP82G in the presence of CM, it is apparent that the host recombination system by itself is unable to prevent inactivation events, even in the presence of an intact genome. For these reasons, and since injected DNA is also susceptible to an inactivation process, it seems more likely that the phage inhibits the inactivation system in some other manner. The inhibition of cellular nucleolytic activities has been observed in *E. coli* after infection with bacteriophages T3 and T4 (21, 24, 26). In the former case, the mode of action of the phage function is to cleave a cofactor required for enzymatic activity (13). The mechanism of inhibition in the latter case remains unknown. It is possible that the inhibition of DNA-inactivating events by SP82G phage particles is a result of the generalized changes induced in the cell physiology during phage infection and is not the result of a single, specifically directed function.

If infecting phage particles are prevented from carrying out the modification function, their DNA appears to be susceptible to a similar type of inactivation phenomenon. The sensitivity of injected DNA, however, is less than that of infective DNA. Green (5) has estimated that the rate of inactivation of an infective DNA molecule is about 0.3 lethal event per min per $10^8$ daltons. This would result in an $e^{-1}$ survival of the phage genome in about 3 min. In contrast, infectious centers resulting from singly infected bacteria attain an $e^{-1}$ survival in about 32 min when held in CM. Although a marked disparity exists in the sensitivities for the two types of DNA, the similar times at which complete phage achieve protection suggests that the processes are related (Fig. 2 and 6). Only a minor portion of the cells in a competent cell population are able to take up DNA (7, 15, 22). Since phage may infect cells which are not in a state of competency, these very different sensitivities might reflect a much lower activity of the inactivation enzyme(s) in noncompetent cells. Alternatively, if the activity of the enzyme(s) was the same in both types of cells, the different sensitivities might be related to differences in the DNA substrates as a result of their mode of entry, or might reflect differences in the mode of entry itself (e.g., the physical location of the DNA after its introduction into the cell).

The question as to why infecting phage particles are able to overcome the inactivation mechanism under normal conditions while infective DNA is unable to do so is not answered by this study. One possibility is that during phage infection DNA is introduced into the cell in such a way as to ensure the proper programming of the required phage function and that during DNA infection these conditions are not met. The DNA of bacteriophage SP82G has a linear, nonpermuted structure which is colinear with the genetic map (6). Blendor experiments have shown that markers contributed by phage injection are transferred rapidly (within 1.4 min at 33 C) in a linear, polar fashion consistent with the genetic and physical maps, and that markers concerned with early functions are transferred first (11). In contrast, G. L. Williams and D. M. Green (*in press*) find that, although the actual time of entry of infecting DNA is similar to that of

phage injection, the order of entry is exactly the reverse of that observed during phage injection. An alternative explanation is that damages which the infective DNA has sustained as a result of its mode of entry may prevent the early expression of phage functions (regardless of the polarity or speed of entry).


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