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Mechanism of Transfection with Deoxyribonucleic Acid from the Temperate *Bacillus* Bacteriophage ϕ105

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Bacteriophage ϕ105 is a temperate phage for the transformable *Bacillus subtilis* 168. The infectivity of deoxyribonucleic acid (DNA) extracted from mature ϕ105 phage particles, from bacteria lysogenic for ϕ105 (prophage DNA), and from induced lysogenic bacteria (vegetative DNA) was examined in the *B. subtilis* transformation system. About one infectious center was formed per 10^8 mature DNA molecules added to competent cells, but single markers could be rescued from mature DNA by a superinfecting phage at a 10^3- to 10^4-fold higher frequency. Single markers in mature DNA were inactivated at an exponential rate after uptake by a competent cell. Prophage and vegetative DNA gave about one infectious center per 10^3 molecules added to competent cells. Infectious prophage DNA entered competent cells as a single molecule; it gave a majority of lytic responses. Single markers in sheared prophage DNA were inactivated at the same rate as markers in mature DNA. Prophage DNA was dependent on the bacterial *rec-1* function for its infectivity, whereas vegetative DNA was not. The mechanism of transfection of *B. subtilis* with viral DNA is discussed, and a model for transfection with ϕ105 DNA is proposed.

The introduction of a viral nucleic acid into a sensitive cell is sufficient to establish a productive infection as first shown in the classical experiment of Hershey and Chase (7). Nucleic acids from a large number of viruses have since been shown to be infective (6). Of particular usefulness for a study of infectious nucleic acids are the bacterial transformation systems, which are characterized by their ability to take up isolated deoxyribonucleic acid (DNA) during some particular state of growth (competence; 16). Thus, DNA from several *Bacillus subtilis* phages can transfect competent bacteria but do so at very different efficiencies (16). The infectivity of a viral nucleic acid is clearly dependent on factors determined both by the host and the virus, but very little as yet is known about specific factors which influence the infectivity of free viral nucleic acids.

Temperate phage ϕ105 can lysogenize the transformable *B. subtilis* 168 (14). The DNA of a temperate phage can exist in three, at least functionally, easily distinguished states, prophage, vegetative, and mature DNA. The aim of the present study is to determine the infectivity of these three classes of DNA from ϕ105 in the *B. subtilis* transformation system. The experiments to be presented show that prophage, vegetative, and mature ϕ105 DNA all exhibit different properties when used to transfect competent *B. subtilis*. A further study of these DNA classes should prove fruitful for understanding some factors of basic importance for the infectivity of viral nucleic acids.

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MATERIALS AND METHODS

Phage and bacteria. The bacterial strains used are listed in Table 1. Phage ϕ105 and various mutants of this phage have been described (14).

Media and growth of bacteria and phage. All media employed as well as the conditions for assay of bacteria and phage have recently been described (14).

Extraction of DNA from bacteria and phage. The methods for extraction of DNA from bacteria have been described (14).

Phage stocks for DNA preparation were prepared in the following manner. The ϕ105 wt, W168 (ϕ105) was grown in 500 ml of tryptone broth [tryptone (Difco), 10 g; NaCl, 5 g; distilled water, 1,000 ml] to a density of about 5×10^7 bacteria per ml with shaking at 37 C; 0.5 μg of mitomycin C was added per ml (Sigma Chemical Co., St. Louis, Mo.) and, after 10 min in the presence of the drug, the bacteria were centrifuged, suspended in fresh broth, and further incubated until lysis (about 2 hr). The lysates had titers of 10^{10} to 5×10^{10} plaque formers per ml. Fifty μg of lysozyme (Sigma) and 5 μg of deoxyribonuclease

TABLE 1. *Bacterial strains employed*

Strain	Genotype	Origin
B. subtilis W168	Prototrophic	Our collection
B. subtilis BR95	*trp-2, phe-1, ilvA1*	Our collection
B. subtilis GSY 1025	*trp-2, met-4, rec-1*	Hoch et al. (8)
B. subtilis GSY 1026	*trp-2, met-4*	Hoch et al. (8)

(Worthington pancreatic) were added per ml and after 30 min at 37 C the lysate was centrifuged at 12,000 × *g* for 20 min in the SS-34 rotor in a Sorvall RC-2 centrifuge. The supernatant was then decanted and centrifuged 90 min at 43,500 × *g* in the same rotor, and the pellet was suspended in 10 to 20 ml of 0.05 M $NaPO_4$, 0.1 M NaCl, 5 × 10^{-3} M $MgSO_4$, *p*H 7.5. Lysozyme and deoxyribonuclease were added again, and the process of high and low speed centrifugation was repeated twice. Phage was finally suspended in 0.2 M NaCl, 0.01 M ethylenediaminetetraacetic acid (EDTA; *p*H 8) or 2 × SSC (SSC = 0.15 M NaCl, 0.015 M sodium citrate). The recovery of plaque formers in the final suspension was 50 to 75%. Stocks of φ105 ccl and c4 were prepared by seeding plates with 10^4 to 10^5 plaque formers mixed with 5 × 10^7 to 10^8 W168 indicator bacteria. After 16 to 18 hr of incubation at 37 C, about 5 ml of buffer was added to the plates together with a few drops of chloroform, and the top layer was scraped off; 30 min later, all liquid was removed from the plates, and agar and bacterial debris were removed by low speed centrifugation. The phage suspensions had titers of 5 × 10^{10} to 20 × 10^{10} per ml. The phage was further purified as described above.

DNA was extracted by gently shaking by hand a phage suspension (5 × 10^{11} to 10^{12} phage per ml) mixed with an equal volume of phenol (Mallinckrodt Chemical Works, St. Louis, Mo.; analytical grade) saturated with phage buffer. The phases were separated by centrifugation, and the aqueous phase and interphase were re-extracted once. The aqueous phases were dialyzed against two changes of 1,000 volumes of buffer at 4 C to remove residual phenol. The concentration of DNA was assayed by Burton's modification of the diphenylamine method or by reading the absorbancy at 260 nm, taking one unit to correspond to 50 μg.

DNA extracted from mature φ105 particles will be referred to as mature DNA, DNA from φ105 lysogenic bacteria as prophage DNA, and DNA from induced lysogenic bacteria as vegetative DNA.

The φ1 phage and its DNA were prepared as described by Reilly and Spizizen (13).

Transformation and transfection procedures. The procedures for transformation and selection of transformants have been described (14). In transfection, bacteria brought to competence were exposed to viral DNA, and uptake in general was terminated by the addition of deoxyribonuclease. Infectious centers were scored by plating suitable dilutions together with W168 indicator bacteria as described (14). The procedure for rescue of single markers from φ105 DNA is described in connection with the individual experiments.

Induction experiment. W168 (φ105) was grown in tryptone broth with shaking at 37 C to about 5 × 10^7 bacteria per ml. Mitomycin C was added to 0.5 μg/ml, and, after 10 min in the presence of the drug, the bacteria were centrifuged and resuspended in about 0.25 volume of fresh broth and incubated at 37 C with forced aeration. Infectious centers were measured as described (14); intracellular mature phage was measured by assaying chloroform-shaken samples of the culture for plaque formers. The rate of DNA synthesis was measured by diluting a small volume of the culture into broth containing an excess of ^{3}H-thymidine (New England Nuclear Corp., Boston, Mass.). After 1 min, uptake of the isotope was terminated by addition of 1 volume cold 10% trichloroacetic acid. The samples were then filtered through HA membrane filters (Millipore Corp., Bedford, Mass.), washed with cold water, and dried. Acid-insoluble counts were determined in a Tri Carb liquid scintillation spectrometer [Packard Instrument Co., Inc., Downers Grove, Ill.; (scintillation liquid: toluene, 1 liter; 2,5-diphenyl oxazole, 4 g; 1,4-bis-2-(4-methyl-5-phenyl-oxazolyl)-benzene, 100 mg)].

For isolation of DNA from induced cells, samples were removed from the culture and poured over frozen broth containing 10^{-3} M NaN_3. The bacteria were centrifuged, washed once with 0.2 M NaCl and 0.01 M EDTA (*p*H 8.1), and suspended in buffer. Lysozyme was added at 200 μg per ml, and, after 30 min at 37 C, sodium dodecyl sulfate was added to a concentration of 0.5%. To the lysed bacteria was added an equal volume of buffer-saturated phenol, and the mixture was shaken at 4 C for 30 min; the two phases were separated by centrifugation. The aqueous phase and interphase were re-extracted once. The aqueous phase was collected and dialyzed against two changes of 1,000 volumes of buffer to remove residual phenol. The infectivity of each preparation was assayed as described.

RESULTS

Infectivity of mature DNA. For a viral nucleic acid to productively infect a sensitive cell, a full complement of viral genes must enter the cell and be expressed in an orderly fashion. DNA extracted from φ105 phage particle sediments as a single species of a molecular weight of 25 × 10^6 to 28 × 10^6 in the ultracentrifuge, roughly twice the molecular weight of alkali-denatured DNA (Birdsell, Hathaway, and Rutberg, *in preparation*), indicating that the phage genome can be extracted without fragmentation from φ105 particles. When mature DNA is added to competent bacteria, about one infectious center is formed per 10^8 phage genomes added (Fig. 1). This is similar to the infectivity of DNA from the temperate phage SPO2 (12).

If the poor infectivity of mature DNA is due to an inability of the DNA to establish an in-

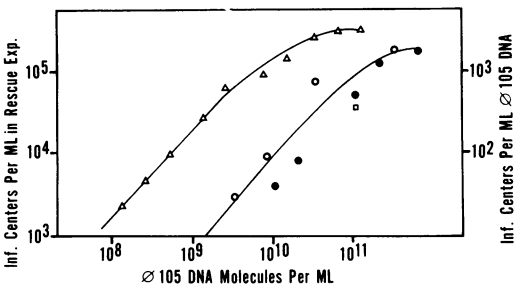

FIG. 1. *Infectivity of mature DNA from φ105 particles. φ105 DNA was added to competent BR95 at the concentrations indicated. After 30 min of incubation 50 μg of deoxyribonuclease was added. Cells were (i) plated for infectious centers at 37 C or (ii) superinfected with φ105 tsN15 at a multiplicity of 5 to 10; after 10 min of adsorption infectious centers were scored at 40 C (rescue experiment). Symbols:* ○ = *infectious centers φ105 wt DNA,* ● = *φ105 cc1 DNA,* □ = *φ105 c4 DNA,* △ = *infectious centers at 40 C.*

fection, rather than poor uptake by competent cells, it might be expected that single markers can be rescued from such DNA by a superinfecting phage at a considerably higher frequency. To test this possibility, competent bacteria were exposed to mature DNA and uptake was terminated by addition of deoxyribonuclease. Samples of the bacteria were then superinfected with *ts* mutants of φ105, and the complexes were plated at a temperature nonpermissive for the growth of the *ts* mutant. From these experiments (Fig. 1 and 2), it is calculated that at least one mature DNA molecule in 10^4 to 10^5 can contribute any of the markers employed to the progeny resulting from phage superinfection. Once a mature DNA molecule has entered a competent cell, it is rapidly inactivated, however, as evidenced by the decrease in infectious centers formed at the nonpermissive temperature as the time of superinfection is successively delayed (Fig. 2). The rate of inactivation of the markers tested corresponds to one lethal hit every 15 to 30 min. This rate is similar to that reported for single markers in phage SP82 by Green (4).

We do not know why markers in mature DNA are inactivated at different rates, although several likely explanations can be entertained, such as the function of the gene, the size of the gene, etc. All mutants employed revert to wild type at a measurable frequency, and the genetic data (14) give no indication that they carry more than one *ts* mutation. If we assume the number of cistrons in φ105 to be 30 to 60 (1), the half-life of a mature DNA molecule inside a competent cell is considerably less than 1 min.

Infectivity of prophage DNA. When competent bacteria are exposed to DNA extracted from φ105-lysogenic bacteria, infectious centers are formed (Fig. 3). The infectivity of the DNA is destroyed by deoxyribonuclease but not by φ105 antiserum. The molecular weight of the *B. subtilis* chromosome is about 4×10^9 (2) and that of the φ105 chromosome about 25×10^6. Each chromosome of a lysogenic bacterium is assumed to carry only one prophage. From these data and those of Fig. 3, the infectivity of prophage DNA is calculated to be one infectious center per 10^3 to 10^4 prophage genomes. Prophage DNA is thus more infectious than mature DNA by several orders of magnitude. The shape of the curve for infectious centers shown in Fig. 3 suggests that the infective DNA enters the competent cell as a single molecule. This suggestion is confirmed by an experiment where DNA from lysogenic bacteria was added to competent bacteria and uptake was terminated at intervals by addition of deoxyribonuclease. Trp^+ transformants and infectious

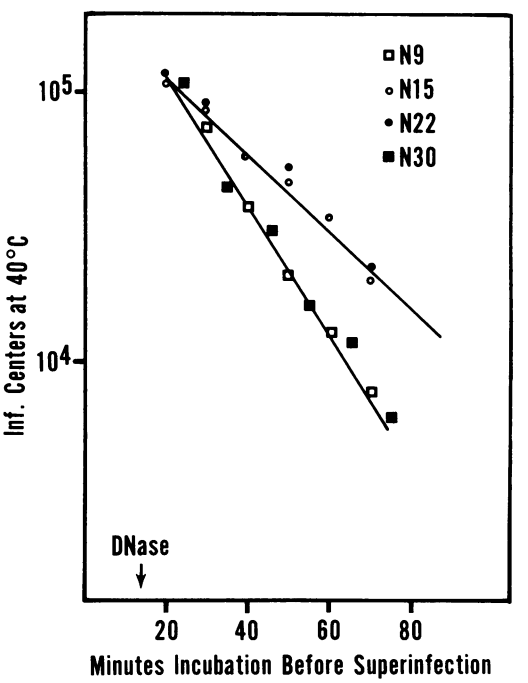

FIG. 2. *Effect of delayed superinfection on infectivity of φ105 wt DNA at the nonpermissive temperature. φ105 wt DNA (3 μg; ca. 7×10^{10} molecules) was added to competent BR95. After 15 min, 50 μg of deoxyribonuclease was added. At intervals samples were withdrawn and superinfected with either of φ105 ts-mutants N9, N15, N22, or N30 at a multiplicity of 5 to 10. After 10 min of adsorption at 37 C, infectious centers were assayed at 40 C.*

centers were scored, and the data were plotted in a manner introduced by Goodgal (3; Fig. 4). The rate of formation of deoxyribonuclease-resistant Trp+ transformants and infectious centers is identical.

The different infectivity of mature DNA and prophage DNA might reflect a different sensitivity to inactivation after uptake by a competent cell, i.e., due to a different superprinting pattern

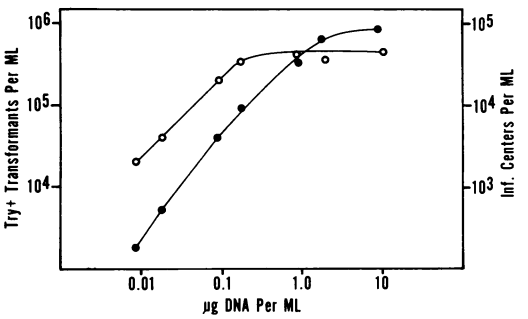

FIG. 3. *Transformation and infectivity from W168 (φ105). Phenol-extracted DNA from W168 (φ105) was added to competent BR95 at the concentrations indicated. After 25 min, trp+ transformants (○) and infectious centers (●) were assayed.*

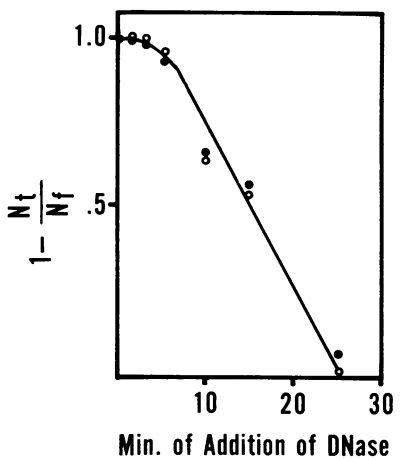

FIG. 4. *Rate of formation of deoxyribonuclease-resistant transformants in BR95 infected with phenol-extracted DNA from W168 (φ105). Phenol-extracted DNA (0.5 μg) from W168 (φ105) was added to competent BR95. At the times indicated, a sample was diluted into transformation medium containing 50 μg of deoxyribonuclease, and, about 10 min later, the samples were assayed for trp+ transformants and infectious centers. N_f = transformants or infectious centers at times; N_t = final number of transformants or infectious centers. Symbols: ○ = trp+ transformants, ● = infectious centers.*

in the two types of DNA (1). If this suggestion was correct, the inactivation of a marker might occur at a much faster rate in mature DNA compared to prophage DNA. To test this prediction, DNA from W168 (φ105) was sheared to reduce the infectivity of the DNA. Sheared and unsheared DNA were added to competent cells, and uptake was terminated with deoxyribonuclease. Samples were then superinfected at intervals with φ105*ts*N9 and infectious centers were scored at the nonpermissive temperature. The rate of inactivation of the N9 marker in sheared prophage DNA was found to be the same as in mature DNA (cf. Fig. 5 to 2). Little destruction

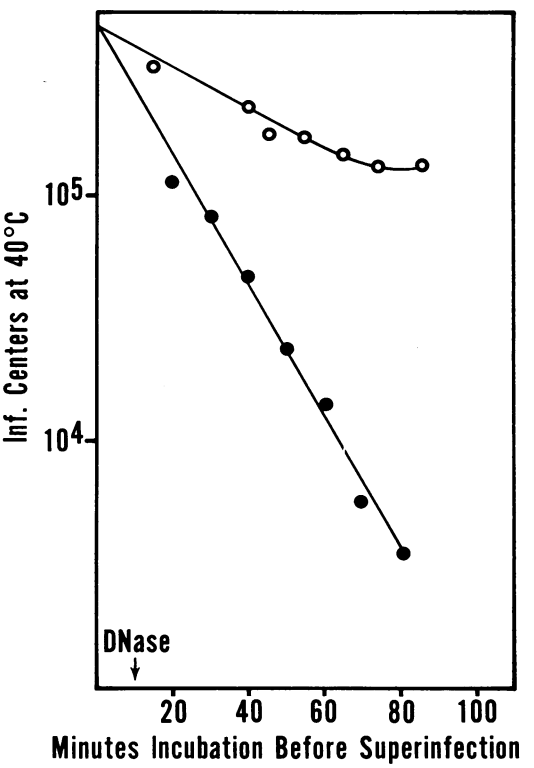

FIG. 5. *Infectivity of sheared and nonsheared DNA from W168 (φ105). DNA was extracted with isoamylalcohol-chloroform from W168 (φ105). Part of the DNA was sheared in a Virtis homogenizer equipped with a horizontal blade at setting 5 for 30 min. One μg sheared or nonsheared DNA respectively was added to competent BR95. Deoxyribonuclease was added at the time indicated and samples were superinfected at intervals with φ105tsN9 at a multiplicity of 5 to 10 and plated for infectious centers at 40 C. The nonsheared DNA gave 7 × 10⁴ infectious centers without superinfection, whereas less than 10 were given by the sheared DNA. Symbols: ○ = nonsheared DNA, ● = sheared DNA.*

of the marker occurred due to shearing, since the curves for sheared and unsheared DNA extrapolate to the same value. The results of this experiment thus do not support the idea that mature and prophage DNA have a different sensitivity to the inactivating enzymes of the host. The rate of inactivation of the N9 marker is comparatively very slow in unsheared DNA and soon reaches a plateau; the possible reasons for this will be discussed later.

Most of the infectious DNA molecules extracted from ϕ105 lysogenic bacteria will contain not only prophage DNA but, indeed, bacterial DNA. The hypothesis was next considered that bacterial DNA attached to prophage DNA is essential for the infectivity of prophage DNA, e.g., by providing a region homologous to the bacterial chromosome, making integration of the prophage possible. One prediction of this suggestion is that the infectivity of prophage DNA should be dependent on the bacterial recombination system.

To test this prediction, prophage DNA was added to competent cultures of *rec-1* and *rec*$^+$ bacteria (8), and infectious centers and *trp*$^+$ transformants were measured. As a control, the infectivity of DNA from the virulent phage ϕ1 was also measured in the two strains. The results of this experiment show (Table 2) that the infectivity of prophage DNA is reduced in the *rec-1* strain in proportion to the decrease in the number of transformants; ϕ1 DNA is equally infectious in the two strains. This result thus lends support to the above hypothesis.

TABLE 2. *Transformation and transfection of rec*$^-$ *and rec*$^+$ *strains*[a]

Transformation and transfection	Strain	
	GSY 1025 (*rec-1*)	GSY 1026 (*rec*$^+$)
Total colony formers	1.2×10^8	1.4×10^8
Trp$^+$ transformants (i)	3.4×10^4	2.2×10^5
ϕ 105 Infectious centers (i)	1.2×10^3	1.4×10^4
ϕ 1 Infectious centers (ii)	2.5×10^4	2.4×10^4
Infectious centers at 40 C in rescue experiment (iii)	6.7×10^4	7.9×10^4

[a] To competent bacteria, was added (i) 1 μg of phenol-extracted DNA from W168 (ϕ 105); (ii) 10 μg of ϕ 1 DNA; or (iii) 3 μg of ϕ 105 c4 DNA. After 30 min the first culture (i) was assayed for trp$^+$ transformants and infectious centers. After 45 min, the second culture (ii) was assayed for infectious centers. After 25 min, 50 μg of deoxyribonuclease per ml was added to the third culture (iii), and 10 minutes later the bacteria were superinfected with ϕ 105*ts*N9 at a multiplicity of 5 to 10; after an additional 10 min for adsorption, infectious centers at 40 C were assayed.

The recombination values observed in crosses with vegetative ϕ105 are the same irrespective of whether the cross is performed in a *rec-1* or *rec*$^+$ strain (Rutberg, *unpublished experiments*). Rescue of a phage marker from mature DNA by a superinfecting phage is equally efficient in *rec-1* and *rec*$^+$ bacteria (Table 2). Marker rescue is thus (partly) dependent on phage-specific enzymes. The data of Table 2 also demonstrate that the different infectivity of prophage DNA in *rec-1* and *rec*$^+$ bacteria is not due to less efficient uptake of DNA by *rec-1* cells.

An experiment was next performed to determine whether prophage DNA gives a lytic or lysogenic response when added to competent bacteria. Prophage DNA was added to competent cells and uptake terminated by addition of deoxyribonuclease. The bacteria were divided into two parts; one part was diluted into broth, the other one into broth with ϕ105 antiserum. Plaque-formers and colonies were assayed over an extended period of time. The results of this experiment (Fig. 6) show that about 80% of the infected cells give a lytic response. We interpret this to mean that, although the prophage is originally integrated, the cell is nonimmune and induction of the prophage will rapidly occur, a situation formally analogous to zygotic induction (17).

Infectivity of vegetative DNA. Lytic development of prophage ϕ105 can be induced in more than 90% of a lysogenic culture by exposure of the cells to mitomycin C. Logarithmically growing W168 (ϕ105) were induced, and samples were taken at intervals for extraction of DNA. The rate of synthesis of DNA and the appearance of intracellular mature phage were also measured. The infectivity of the DNA preparations was then measured. The number of infectious centers formed per unit weight of DNA remains roughly constant during the first 30 min after induction. Concommitant with an increase in the rate of DNA synthesis, the infectivity of the DNA preparations increased about 50-fold and reached a plateau at the time when maximal rate of DNA synthesis is attained (Fig. 7), whereas mature phage continued to increase until lysis occurs. Further experiments are needed to quantitate the efficiency with which vegetative DNA can infect competent bacteria, but it can be estimated to be of the same order of magnitude as prophage DNA. The infectivity of vegetative DNA is not dependent on the bacterial *rec-1* function and can thus be distinguished from prophage DNA by that criterion (Table 3). Preliminary experiments

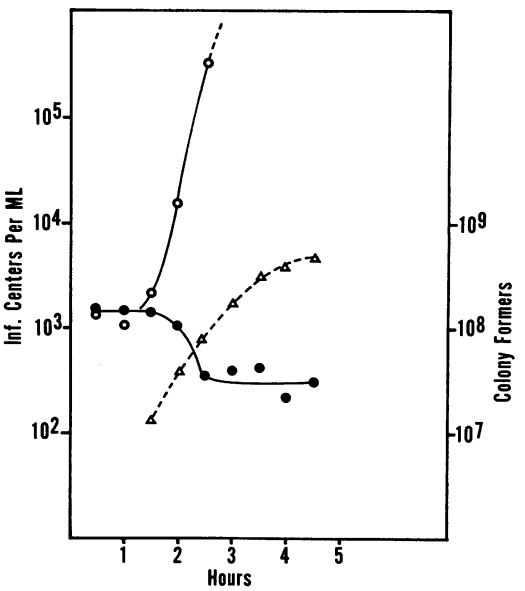

FIG. 6. *Colony formation and infectivity of prophage DNA in BR95 with or without 105 antiserum. To competent BR95, was added 1 μg of phenol-extracted DNA from W168 (φ105). At 15 min later, 50 μg of deoxyribonuclease was added, and, after an additional 10 min, the bacteria were diluted 1:20 into (i) broth or (ii) broth with φ105 antiserum (K = 3), put at 37 C, and aerated. At intervals the cultures were assayed for plaque formers at 37 C, and colony formers were also assayed in the serum-containing culture. Symbols: ○ = plaque formers in broth without serum, ● = plaque formers in broth with serum, △ = colony formers in serum-containing culture.*

also indicate that genetic linkage between bacterial and prophage markers is selectively destroyed during prophage induction.

DISCUSSION

Our experiments demonstrate that prophage, vegetative, and mature DNA from phage φ105 show different properties when infecting competent *Bacillus subtilis* 168. At least 1 prophage or vegetative-phage DNA molecule in 10^3 to 10^4 is infectious, whereas about 10^8 mature DNA molecules are needed to form one infectious center. Single markers can be rescued from mature DNA at a 10^3- to 10^4-fold higher frequency, thus demonstrating that the low infectivity of mature DNA is not due to poor uptake of such DNA by competent cells. Sedimentation studies and electron micrographs of DNA molecules extracted from φ105 particles show that the molecules are not fragmented during extraction (Birdsell, Hathaway, and Rutberg, *in preparation*), but the hypothesis cannot be excluded that the low infectivity of mature DNA is due to breakage of the molecule during its entry into a competent cell. Once taken up by a competent cell, however, mature DNA is rapidly inactivated, as evidenced by the decay of rescuable markers as the time between addition of DNA and addition of a superinfecting phage is prolonged. Mature DNA does not seem to be per se more sensitive than prophage DNA to the inactivating enzymes of the host.

It must be assumed that the minimum size of

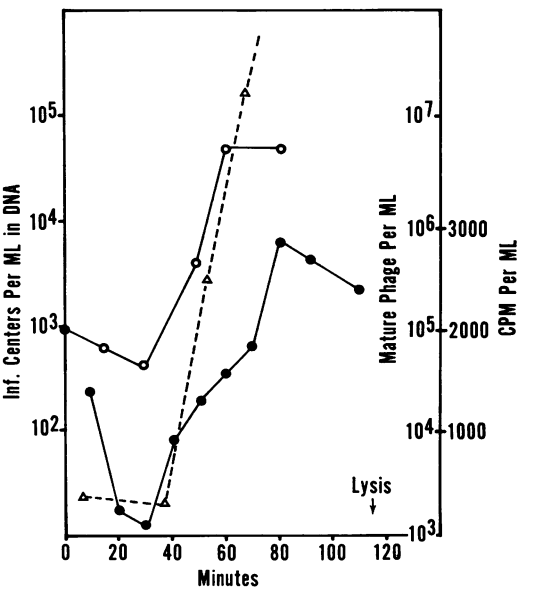

FIG. 7. *Relationship of infectivity to DNA synthesis. About 0.1 μg of DNA was added from the different DNA samples to competent BR95, and assays were made for infectious centers at 37 C. Symbols: ● = acid-insoluble radioactivity, △ = mature phage, ○ = infectious centers in DNA preparations.*

TABLE 3. *Infectivity of DNA extracted at various times from induced W168 (φ 105) in strains GSY 1025 and GSY 1026*

DNA sample at[a]	Infectious centers (per ml)	
	GSY 1025 (*rec-1*)	GSY 1026 (*rec*$^+$)
min		
0	2.0×10^2	1.2×10^3
60	1.4×10^4	2.1×10^4
80	4.0×10^4	3.0×10^4

[a] DNA samples from the experiment of Fig. 7 were added at about 0.2 μg per ml to competent cultures of 1025 and 1026. After 30 min infectious centers were assayed at 37 C.

an infectious molecule is the same in prophage, vegetative, and mature DNA. This suggests that the structure of the DNA molecule, rather than its size, determines its infectivity. In the lambda system only linear molecules with single-stranded ends are "infectious" in the helper assay, whereas both linear and circular molecules are infectious in the spheroplast assay (11, 19). The difference between these two assay systems is most easily explained, however, by assuming that circular molecules can only enter spheroplasts (19).

Mature DNA from several *Bacillus* phages is infectious for competent bacteria, but the infectivity varies considerably between different phages. The size of the DNA molecule does not seem to determine the infectivity although a more complex curve for the dependence of the infectivity on DNA concentration is generally seen with larger phages (13). For phage SP82, Green (4) has shown that after entry into a competent cell the phage DNA is rapidly fragmented, and whole phage is later reconstituted from these fragments. The ϕ105 mature DNA is also fragmented after entry (Rutberg, *unpublished experiments*), but reconstitution of an infectious molecule, if it occurs, must be very inefficient compared to SP82. The vegetative genetic map of SP82 is linear (10), whereas that of ϕ105 is thought to be circular (14). If the genetic maps of these two phages correspond to a unique (SP82) and permuted (ϕ105) sequence of nucleotides in the respective DNA molecules, an attractive hypothesis can be entertained to explain the different infectivity of SP82 and ϕ105 mature DNA. After fragmentation of a DNA molecule in the competent cell, a complete molecule can easily be reconstituted from two or more molecules of a unique sequence providing only that they are cut at different places. Reconstitution of a complete molecule from two or more randomly cut permuted molecules puts severe restraints on where the cuts may occur and which fragments may combine with each other. The data of Green (5) are compatible with a unique order on the SP82 DNA molecule of the *ts*-mutations employed. The anatomy of the mature ϕ105 DNA molecule is currently being studied.

To account for the properties of ϕ105 prophage, vegetative, and mature DNA when infecting competent *B. subtilis*, we have adopted the following working model. When taken up by a competent cell a ϕ105 DNA molecule is initially restricted to a small compartment in the cell, probably bound to a site in the cell membrane (9, 18). The genetic potential of the DNA cannot be expressed while bound at this site. DNA can be moved from this site by recombination (integration) or by replication. If it remains bound at the site it will soon be degraded. To replicate or to integrate, mature DNA is assumed to require expression of one or several genes (15), it will thus remain bound and only rarely escape fragmentation.

Prophage DNA will often be extracted from lysogenic cells bound to bacterial DNA. The bacterial DNA, bound to prophage DNA, provides a region of strict base homology with the chromosome of the competent cell, making integration of the prophage by the bacterial recombination system possible. The fact that the infectivity of prophage DNA is dependent on the bacterial *rec-1* function lends support to this suggestion. Whether prophage DNA will ultimately give a lytic or lysogenic response depends on events occurring after its removal from the binding site. The slow rate of inactivation of *ts* markers in unsheared prophage DNA is thought to reflect removal of incomplete prophage genomes from the binding site. Vegetative DNA, finally, enters the cell in a form which can be replicated without prior expression of any phage function, assuming that the host replication enzymes can also replicate ϕ105 DNA. Vegetative DNA may also exist in a form less susceptible to the inactivating enzymes of the host.

Isolation and characterization of various DNA forms associated with the vegetative development of ϕ105, combined with studies of the anatomy of the mature phage DNA molecule, seem to offer one way by which we can reach an increased understanding of the correlation between the structure and infectivity of a viral nucleic acid molecule.

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LITERATURE CITED

1. Campbell, A. 1967. Regulation in viruses, p. 323-382. *In* J. H. Taylor (ed.), Molecular Genetics, part II, Academic Press, Inc., New York.
2. Dubnau, D., C. Goldthwaite, I. Smith, and J. Marmur. 1967. Genetic mapping in *Bacillus subtilis*. J. Mol. Biol. **27**:163-185.
3. Goodgal, S. H. 1961. Studies on transformation of *Haemophilus influenzae*. IV. Linked and unlinked transformations. J. Gen. Physiol. **45**:205-228.
4. Green, D. M. 1966. Intracellular inactivation of infective SP82 bacteriophage DNA. J. Mol. Biol. **22**:1-13.
5. Green, D. M. 1966. Physical and genetic characterizaiton of sheared infective SP82 DNA. J. Mol. Biol. **22**:15-22.
6. Harm, W., and C. S. Rupert. 1963. Infection of transformable cells of *Haemophilus influenzae* by bacteriophage and bacteriophage DNA. Z. Vererbungsl. **94**:336-348.
7. Hershey, A. D., and M. Chase. 1952. Independent functions

of viral protein and nucleic acid in growth of bacteriophage. J. Gen. Physiol. **36**:39–51.

8. Hoch, J. A., M. Barat, and C. Anagnostopoulos. 1967. Transformation and transduction in recombination-defective mutants of *Bacillus subtilis*. J. Bacteriol. **93**:1925–1937.
9. Javor, G. J., and A. Tomasz. 1968. An autoradiographic study of genetic transformation. Proc. Nat. Acad. Sci. U.S.A. **60**:1216–1222.
10. Kahan, E. 1966. A genetic study of temperature-sensitive mutants of the subtilis phage SP82. Virology **30**:650–660.
11. Kaiser, A. D., and R. B. Inman. 1965. Cohesion and the biological activity of bacteriophage lambda DNA. J. Mol. Biol. **13**:78–91.
12. Okubo, S., and W. R. Romig. 1965. Comparison of ultraviolet sensitivity of *Bacillus subtilis* bacteriophage SPO2 and its infectious DNA. J. Mol. Biol. **14**:130–142.
13. Reilly, B. E., and J. Spizizen. 1965. Bacteriophage deoxyribonucleate infection of competent *Bacillus subtilis*. J. Bacteriol. **89**:782–790.
14. Rutberg, L. 1969. Mapping of a temperate bacteriophage active on *Bacillus subtilis* 168. J. Virol. **3**:38–44.
15. Signer, E. R. 1968. Lysogeny: the integration problem. Ann. Rev. Microbiol. **22**:451–488.
16. Spizizen, J., B. E. Reilly, and A. H. Evans. 1966. Microbial transformation and transfection. Annu. Rev. Microbiol. **20**:371–400.
17. Wollman, E. L., and F. Jacob. 1957. Sur le processus de conjugation et de recombination chez *Escherichia coli*. II. La localization chromosomique du prophage et les consequences de l'induction zygotique. Ann. Inst. Pastuer (Paris) **93**:323–337.
18. Wolstenholme, D. R., C. A. Vermeulen, and G. Venema. 1966. Evidence for the involvement of membraneous bodies in the processes leading to genetic transformation in *Bacillus subtilis*. J. Bacteriol. **92**:1111–1121.
19. Young, E. T., II, and R. L. Sinsheimer. 1967. Vegetative bacteriophage lambda DNA. I. Infectivity in a spheroplas assay. J. Mol. Biol. **30**:147–164.