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Bacteriophage Infection: Which End of the SP82G Genome Goes in First?

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The transfer of deoxyribonucleic acid (DNA) from bacteriophage SP82G to its host may be halted by chilling but is affected little by chloramphenicol, actinomycin D, or cyanide. The order of entry of markers on the phage genome was determined by halting the transfer of DNA at intervals, removing the untransferred DNA by blending, and assaying for the presence of markers in the blended complexes. Markers on the phage genome are transferred in a linear, polar fashion consistent with the previously determined genetic and physical maps. Those markers concerned with early functions enter first, and the rate of transfer is temperature dependent.

The fact that the deoxyribonucleic acid (DNA) molecule of SP82G has a linear, nonpermuted structure which is co-linear with the genetic map (2) and the fact that there is no superinfection-exclusion phenomenon with this phage (5) permit one to ask the question: what is the order of entry of markers along the phage genome in normal SP82G bacteriophage infection? The transfer of DNA from phage to host was first examined by Hershey and Chase (4). Lanni (6, 7) showed that in bacteriophage T5 infection, markers carried on a certain portion of the genome [the first-step-transfer (FST)-DNA] must be transferred first since these markers control functions necessary for the transfer of the remainder of the genome.

In experiments described in this paper, the order of entry of markers along the entire phage genome was determined by interrupting the transfer of DNA at intervals, removing the untransferred DNA by blending, and assaying for the presence of the markers in the blended complexes. The results clearly show that markers on the phage genome are transferred in a linear, polar fashion consistent with the genetic (5) and physical (2) maps. Those markers concerned with early functions enter first, and the rate of transfer is temperature dependent.

MATERIALS AND METHODS

Bacterial strains, growth conditions, and phage preparations. *Bacillus subtilis* strain SB-1 was the host cell for all experiments. Techniques for the isolation, propagation, and assay of bacteriophage SP82G are identical to those of Green (1). Nomura salts media (NM) (9) supplemented with 0.5% glucose, 0.2% casein hydrolysate, 2.5×10^{-3} M MgCl, 0.1% yeast extract, 0.05 mg/ml of DL-tryptophan, 4 mg/ml of arginine, and 0.2 mg/ml L-histidine was used for growth of phage.

Radioactively labeled phage was obtained by high-low centrifugation of appropriate lysates. For ^{32}P-labeled phage, $H_3{}^{32}PO_4$ was added to the growth media at 5 μc/ml along with the phage inoculum. For ^{14}C-labeled cultures, lysates were grown on a minimal medium similar to NM but having no casein hydrolysate and only one-quarter the usual amounts of DL-tryptophan, L-histidine, and yeast extract. ^{14}C-lysine was added to the cultures 25 min after the addition of phage to a final concentration of 0.01 μc/ml. Lysates were further purified on a preformed CsCl gradient extending from 1.2 to 1.7 g/cm^3. Under these conditions, only 10% of the label appeared in the DNA of the phage as measured by phenol extraction (8).

Blendor experiments. Bacteriophages were rapidly adsorbed to bacteria concentrated to 4×10^9/ml. The measured multiplicity of infection (MOI) was 7 to 8 for radioisotope experiments, and 0.1 for marker entry studies. Adsorption was terminated either by dilution and centrifugation away from unadsorbed phage (radioisotope experiments) or by a 45-sec exposure to phage-specific antisera sufficient to inactivate 99.9% of the unadsorbed phage (marker entry studies). After adsorption the infected bacteria were diluted in NM at the appropriate temperature to 10^8/ml. After a suitable holding period in the warm growth media, samples were removed and rapidly chilled by dilution into equal amounts of cold NM in an ice bath. Samples of 20 ml were blended in an ice-jacketed Omni-Mixer (Ivan Sorvall, Inc., Norwalk, Conn.) at 11,500 rev/min. The duration of blending was 1.5 min unless otherwise specified. The blended samples were assayed for surviving infectious centers, and then spun at 7,000 $\times$ g. Bacterial pellets were resuspended in water, transferred to planchets, and dried, and their radioactivity was assayed in a gas-flow detector (Nuclear-Chicago Corp., Des Plaines, Ill.).

Marker rescue studies. Temperature-sensitive (*ts*) mutants of SP82G which will grow at 33 C but not at 47 C were originally isolated and described by Kahan (5), and other *ts* mutants were subsequently isolated by D. M. Green (*personal communication*). In marker rescue experiments, a sample of a temperature-sensitive mutant phage was rapidly adsorbed to bacteria for 45 sec, and adsorption was terminated by antisera. The cells were diluted into growth media at the appropriate temperature, held for various lengths of time, and exposed to the chilling and blending regimen described above. Subsequently, 0.5-ml portions of blended samples and nonblended controls were superinfected with a different *ts* mutant at a MOI of 15 to 20 for 4 min. Suitable dilutions were plated at 47 C to determine the frequency of wild-type recombinants.

RESULTS

Effect of blending. The separation of phage protein from DNA and infected cells was effected by a blendor experiment similar to that of Hershey and Chase (4). Radioactively labeled phage were rapidly adsorbed to bacteria. The bacteria were then spun free of unadsorbed phage and resuspended in growth media (NM). After 12 min at 33 C the mixture was chilled and then blended for various lengths of time. Samples were then assayed for surviving infectious centers and for radioisotope still associated with the bacterial pellet (Fig. 1). The results indicate that, whereas blending has no effect on the survival of infected bacteria, it removes 50% of the ^{14}C (approximately 90% of the ^{14}C label is in the protein of the phage). No more than 22% of the ^{32}P can be removed from the bacteria by blending. This demonstrates the transfer of DNA from phage to host during infection.

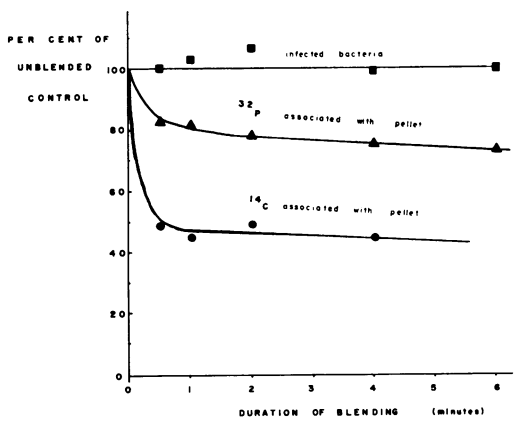

FIG. 1. *Effect of blending. Bacteria at 4 × 10⁹/ml were exposed to radioactively labeled phage for 2 min and diluted 1:100. Unadsorbed phage was removed by centrifugation, and the infected bacteria were resuspended in NM at 33 C at 10⁸/ml. After 12 min, the suspension was chilled, and samples were blended for the lengths of time indicated. Samples were assayed for surviving infectious centers, and the amount of radioactivity still associated with the bacterial pellet after spinning at 7,000 × g for 10 min.*

Effect of chilling on DNA transfer. The effect of chilling on blendor sensitivity was examined by chilling the phage-host complexes at early and late times after adsorption (Table 1). Chilling at an early time after adsorption resulted in a phage-host complex of low blendor resistance, but complexes chilled at later times were resistant to blending. When the sensitive complexes chilled at an early time were reheated for a further 10 min, the high blendor resistance characteristic of complexes chilled at later times was achieved.

These results indicate that chilling causes an interruption in the transfer of DNA and that any DNA which has not been transferred by the time of chilling is removed during the blending operation. The transfer of DNA in the chilled unblended complexes is resumed when they are warmed to 33 C. This interpretation would predict that the process of DNA transfer could be interrupted at any time by chilling, and the untransferred DNA removed by blending. This was tested by monitoring the transfer of ^{32}P from the phage to host at various times after adsorption, and by assaying for the viability of the blended complexes as infectious centers (Fig. 2).

The shapes of the curves for ^{32}P transfer and blendor resistance are different, but reach saturation levels at the same time. The ^{32}P transfer curve is consistent with the prediction that, by chilling and blending the complexes at various times after adsorption, the transfer of DNA may be interrupted at any point. The delayed ap-

TABLE 1. *Effect of chilling on blendor sensitivity*[a]

Time of chilling after phage adsorption[b]	Per cent of infectious centers resistant to blending[c]	
	Treatment 1[d]	Treatment 2[d]
min		
2	2	100
10	85	80

[a] Bacteria were rapidly infected with phage at a MOI = 0.1 and diluted into NM at 33 C, as described in Fig. 2.

[b] At 2 and 10 min after the addition of phage, samples were removed and rapidly chilled.

[c] One portion of each sample was reheated to 33 C for 10 min and rechilled. All samples were blended and assayed for surviving infectious centers.

[d] Treatment 1: chilled to 15 C and blended; treatment 2: reheated to 33 C for 10 min, chilled, and blended.

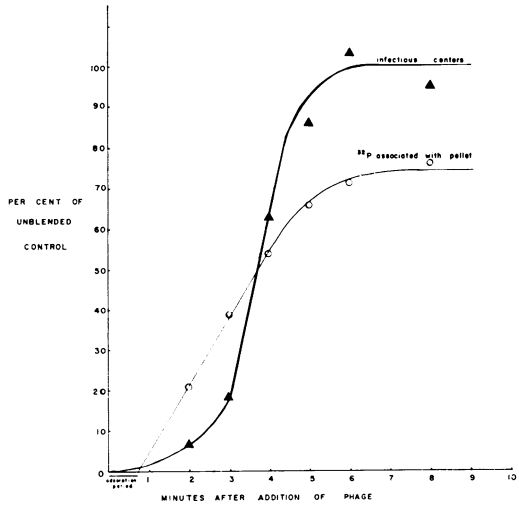

FIG. 2. *Transfer of DNA and blendor resistance. Concentrated bacteria were exposed to radioactively labeled phage for 45 sec. Adsorption was terminated by a 45-sec exposure to antisera, and the bacteria were diluted to 10^8/ml in NM at 33 C. At the times indicated samples were removed, chilled, and blended. Samples were assayed for surviving infectious centers, and the amount of radioactivity still associated with the bacterial pellet.*

pearance of blendor-resistant infectious centers relative to the ^{32}P uptake is expected. For, although transfer starts at an early time after adsorption and proceeds towards saturation, the first appearance of blendor-resistant infectious centers in the population can occur only when transfer is complete in any given complex. The transfer of DNA in the population is complete by about 6 min, which coincides with the complete blendor resistance of the population. Identical experiments conducted at 28 C show that complete blendor resistance requires 10 to 12 min at this temperature.

Effect of metabolic inhibitors. Since Lanni (6) has demonstrated the necessity for protein synthesis in achieving blendor resistance in T5, the effect of various metabolic inhibitors on SP82G blendor resistance was examined (Table 2). Chloramphenicol, actinomycin D, and cyanide had little effect on the achievement of blendor resistance whether added shortly after adsorption or prior to adsorption. Thus, the transfer of DNA from SP82G to its host does not require synthesis of a phage protein.

Marker entry studies. The ability to interrupt DNA transfer in a stepwise fashion by the methods described above and the absence of a superinfection-exclusion phenomenon in SP82G (5) permit a study of the transfer of various remarks on the phage genome. Temperature-sensitive mutant phages which will not form plaques at 47 C but which will grow normally at 33 C were used in this study. A sample of a temperature-sensitive mutant phage was rapidly adsorbed to bacteria, and the transfer of DNA from phage to host was interrupted at intervals by blending. Blended complexes were then superinfected with a different mutant phage and plated at 47 C. At this selective temperature only wild-type recombinants are able to form plaques, and these could only be formed when the superinfecting phage was able to "rescue" the necessary genes from the blended complex. Clearly, only genes transferred at the time of blending can be rescued from such blended complexes. The time at which a marker is able to be rescued from a blended complex reflects the time of transfer of that marker from phage to host.

One such marker rescue experiment is shown in Fig. 3. The *ts* mutant phage H167-H362 was rapidly adsorbed to bacteria, and the mixture was blended at intervals as described above. Separate samples of the blended complexes were exposed to three different superinfecting phage: H177, E119, and the double mutant H177-E119. They were subsequently plated at 47 C to determine recombinants. The number of complexes able to give rise to recombinants at any time was expressed as the per cent of an unblended control. Also shown is the appearance of blendor-resistant infectious centers detected by plating the blended complexes alone, without exposure to superinfecting phage, at the permissive (33 C) temperature. This indicates the transfer of all information necessary for plaque formation.

The results indicate that marker H177 is trans-

TABLE 2. *Effect of metabolic inhibitors on blendor sensitivity*[a]

Inhibitor	Per cent of infectious centers resistant to blending	
	Treatment 1[b]	Treatment 2[b]
Chilled to 15 C		5.3
Chloramphenicol (200 μg/ml)	77.0	73.4
NaCN (0.0025 M)	92.5	87.0
Actinomycin D (10 μg/ml)	100.0	100.0

[a] Bacteria were rapidly infected with phage at a MOI = 0.1 as described in Table 1.

[b] Inhibitors were added either 5 min prior to phage adsorption (treatment 1) or at 2 min after adsorption (treatment 2). After 10 min at 10^8/ml., samples were blended and assayed for surviving infectious centers.

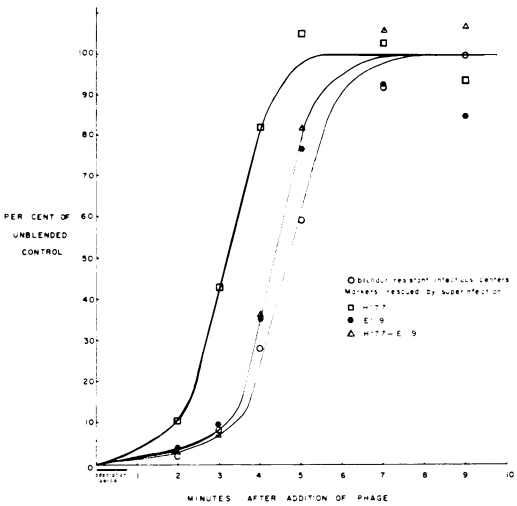

FIG. 3. *Rescue of markers from blended complexes. Bacteria were rapidly infected with a temperature-sensitive mutant phage at MOI = 0.1. The transfer of DNA was interrupted at intervals by chilling and blending. Blended complexes were superinfected with the ts mutants indicated and plated for recombinants at 47 C. Blendor-resistant infectious centers were assayed by plating blended complexes alone at 33 C.*

ferred at an early time, and E119 at a later time. This polarity of entry was verified by the use of the double mutant H177-E119. In this case both markers must be rescued and the appearance of blendor resistance coincides with the entry of the latest marker to be transferred—E119. The complete transfer of all information necessary for successful infection follows shortly thereafter.

Another marker rescue experiment showing entry time for closely linked markers at three different locations on the phage genome is presented in Fig. 4. Closely linked double mutants were used in this study for the purpose of lowering the "leakiness" and reversion rate sometimes associated with single markers. The map positions of the mutants used in this study are shown in Fig. 5 (3).

The results of this experiment indicate a polar entry of markers, that is, markers on the left end of the map (H177-G55) enter first, followed by markers in the middle of the map (H167-A4) and, subsequently, those on the right end of the map (E14-H24). The same polarity of entry and the same times of entry were observed in all experiments regardless of the position of the marker carried by the preinfecting phage. (Three different preinfection phages were tested in various experiments: H20, H362-H180, and H27-H326.)

By plotting the mean time of entry for the markers tested (the time at which a 50% level of blendor resistance is achieved) against the map distance from the left-hand origin of the map, the relationship between genetic map distance and entry time can be seen (Fig. 6). The transfer of markers on the phage genome to the recipient bacterium proceeds in a linear, polar fashion con-

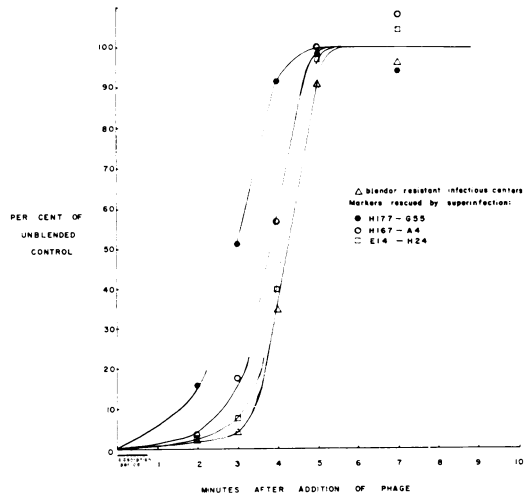

FIG. 4. *Rescue of markers from blended complexes. Bacteria were rapidly infected with a ts mutant phage (H362-H180) at a MOI = 0.1, and a blendor experiment was carried out as described in Fig. 2. Blended complexes were superinfected with the ts mutants indicated and plated for recombinants at 47 C. Blendor-resistant infectious centers were assayed by plating blended complexes alone at 33 C.*

FIG. 5. *Genetic map of temperature-sensitive mutants used in this study. The known terminal markers (NG14 and H201) are also shown; from Green (3).*

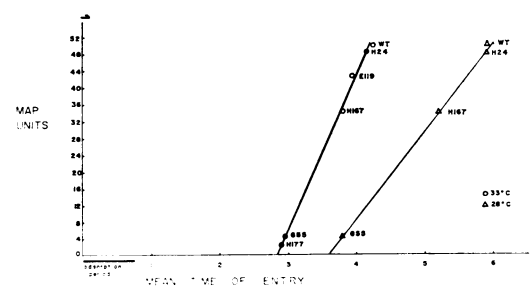

FIG. 6. *Map position and time of entry of markers. The mean time of entry of markers, calculated from Fig. 3, 4, and 5 is plotted against the cumulative map distance from the left end of the map. Values obtained from similar experiments performed at 28 C are also shown.*

sistent with the genetic and physical maps of SP82G. Markers on the left end of the map enter first, and the rate of entry is temperature dependent. It can also be seen that the time of initiation of transfer is also temperature dependent.

DISCUSSION

These experiments indicate that the transfer of DNA from phage to host in SP82G infection proceeds in an orderly, programmed fashion. The known markers on the left end of the genetic map are largely concerned with early phage functions (those occurring prior to phage DNA synthesis) (5; Laman and Green, *personal communication*), whereas those at the right end are concerned with late functions [e.g., structural and assembly proteins; the time of action of these markers was determined by temperature pulse experiments (Laman and Green, *personal communication*)]. Thus, those markers which code for early functions are transferred first, whereas those concerned with late functions are transferred later.

Some indication of the rate of transfer of the DNA can be obtained from these experiments. The time at which ^{32}P transfer begins and the first appearance of blendor-insensitive infectious centers can be calculated from Fig. 2 by extrapolating the linear portions of the curves to the base line. The difference between these values (1.7 min) is the time required for the complete transfer of the genome. Similarly, the mean time required for the entry of the phage genome from origin (map unit = 0) to terminus (map unit = wild type) is 1.4 min at 33 C (Fig. 6). The contour length of the SP82G DNA, measured by electron microscopy (D. M. Green, *personal communication*) is 52.9 μm. By using the value of 1.4 min, this is a rate of transfer of 1,852 base pairs/sec at 33 C.

The rates of transfer of DNA at other temperatures may also be calculated from the mean times required for the transfer of the complete genome (2.3 min at 28 C, 4.0 min at 25 C). The rates of transfer thus obtained may be fitted to an Arrhenius plot which is linear in this range (25 to 33 C) and which gives a value for the energy of activation of 18.76 kcal/mole of base pairs, and for a pre-exponential factor of 3,277.

The rate of transfer of DNA at 15 C extrapolated from this plot, if we assume linearity to this temperature, would be 191 base pairs/sec. This is about 10% of the rate observed at 33 C. Since all complexes were initially chilled to 15 C and subsequently held at 4 C, the transfer of DNA occurring after chilling may be considered negligible.

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