Physiological and Genetic Aspects of Abortive Infection of a *Shigella sonnei* Strain by Coliphage T7

R. HAUSMANN, BEATRIZ GOMEZ, AND BEVERLY MOODY

Division of Biology, Southwest Center for Advanced Studies, Dallas, Texas 75230

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Phage T7 adsorbed to and lysed cells of *Shigella sonnei* D₂ 371-48, although the average burst size was only 0.1 phage per cell (abortive infection). No mechanism of host-controlled modification was involved. Upon infection, T7 rapidly degraded host deoxyribonucleic acid (DNA) to acid-soluble material. Phage-directed DNA synthesis was initiated normally, but after a few minutes the pool of phage DNA, including the parental DNA, was degraded. Addition of chloramphenicol, at the time of phage infection, prevented both the initiation of phage-directed DNA synthesis and the degradation of parental phage DNA. Addition of chloramphenicol 4.5 min after phage was added permitted the onset of phage-directed DNA synthesis but prevented breakdown of phage DNA. Mutants of T7 (*ss*⁻ mutants) have been isolated which show normal growth in strain D₂ 371-48. Upon mixed infection of this strain with T7 wild type and an *ss*⁻ mutant, infection was abortive; no complementation occurred. The DNA of the *ss*⁻ mutants was degraded in mixed infection like that of the wild type. Revertant mutants which have lost their ability to grow on D₂ 371-48 were isolated from *ss*⁻ mutants; they are, in essence, phenotypically like T7 wild type. Independently isolated revertants of *ss*⁻ mutants did not produce *ss*⁻ recombinants when they were crossed among themselves. When independently isolated *ss*⁻ mutants were crossed with each other, wild-type recombinants were found; *ss*⁻ mutants could then be mapped in a cluster compatible with the length of one cistron. We concluded that T7 codes for an active, chloramphenicol-sensitive function [*ss*⁺ function (for suicide in *Shigella*)] which leads to the breakdown of phage DNA in the *Shigella* host.

The term "abortive infection" refers to instances where the invasion of a bacterial host by a bacteriophage results in the ultimate loss of the phage genome; the host may or may not be killed in this process. The molecular bases for abortive infection, although not known in detail, fall into broad classes, depending on whether, for example, abortive infection is due to repression of a phage genome by immunity substances coded for by a similar genome of a resident prophage (15), to the presence of conditional lethal mutations susceptible to functional complementation (3, 7), or to "host restriction" of improperly "host-modified" phage (2).

A case of abortive infection unrelated to these causes is presented in this paper. It specifically affects phage T7, whose genome appears to code for a conditional self-destroying function. The unique features of this T7-specific restriction may be helpful in studying certain aspects of host-virus interaction.

MATERIALS AND METHODS

Bacteria. The host which was abortively infected by T7 was the *Shigella sonnei* strain D₂ 371-48 from the collection of I. Suassuna, Instituto de Microbiologia, Rio de Janeiro, Brazil. It was singled out in the course of a systematic screening of about 300 enterobacterial strains from that collection for hosts of phages T3 and T7. The stock used for all our tests was obtained from a single colony, isolated from the original culture; this strain is subsequently referred to as strain D. Other bacterial strains used were *Escherichia coli* BBw/1, subsequently called BB, whose origin was described earlier (14); *E. coli* B/1, subsequently called B, from C. Bresch's collection; and *E. coli* B208, isolated in W. Arber's laboratory and kindly supplied by M. Geftter. *E. coli* B208 was used only as a host for λ_{vir} and is also called B since it has the B-specific restriction and modification properties. Strains B and D were nonpermissive for amber mutants; strain BB was permissive for amber mutants.

Phage. Stocks of T3 and T7 were those described

in an earlier report (14). Other phages of the T-series were from C. Bresch's collection. Phages P1 and λ_{vir} were given to us by R. C. Clowes. When pertinent, the designation of the last host on which a phage grew, preceded by a point, follows the designation of the phage (viz., P1·B denotes phage P1 last grown on host B).

Media. As a liquid medium, 1% Nutrient Broth (Difco), pH 7, was used throughout. Bresch's color agar (5) and tryptone-agar supplemented with CaCl_2 ($2.5 \times 10^{-3}\text{M}$) were used as solid media. The latter was used only for plating phages P1 and λ by the method of agar overlay. Otherwise, spreading on predried color-agar plates was used.

Isolation of phage mutants. T7 mutants that could grow on D were isolated by plating T7 wild-type lysates on D, at various concentrations, and picking the few plaques which developed. From these mutants, revertants unable to grow on D were isolated in the same manner as that described for amber mutants (14), except that B was used as a permissive host and D as a nonpermissive host. The ratio of permissive to nonpermissive host cells in the indicator mixture was, in this case, 1:5 (not 1:1, as it was for amber mutants).

Experimental conditions. Overnight broth cultures were diluted 1:100 and incubated with aeration until cell concentrations of 10^8 to 3×10^8 cells per ml were reached. Phage was generally added directly to 10- to 30-ml samples of these exponentially growing cultures, placed on a rotatory shaker at 37°C. However, when labeled phage was used, the cells were concentrated by centrifugation before being exposed to phage. After 2 min at 37°C to allow adsorption, the infected cultures were diluted 20-fold with cold (10°C) medium and were recentrifuged for 2 min at $10,000 \times g$. The pelleted cells, free from unadsorbed phage, were then resuspended in a small amount of cold medium, diluted to the original volume of medium at 37°C, and further incubated. Phage was always added at a multiplicity of infection (MOI) of at least 10.

Where indicated, ^3H -thymidine (0.5 to 1 $\mu\text{C}/\text{ml}$ of culture) was added to culture samples as specified in the description of individual experiments. Samples (1 ml) were then withdrawn at various times and chilled in equal volumes of ice-cold 10% trichloroacetic acid. Acid-precipitable material was collected on membrane filters. Acid-soluble material was assayed in the supernatant fluid of centrifuged trichloroacetic acid-treated samples to which carrier deoxyribonucleic acid (DNA) had been added (10 optical density units of calf-thymus DNA) before centrifugation. A sample (0.5 ml) of supernatant liquid was added to 10 ml of Bray's solution that contained 0.04 ml of 2M ammonium hydroxide. All samples were counted in a liquid scintillation spectrometer.

Phage crosses were done as described earlier (14). For burst-size measurements, the same procedures were used as for phage crosses; a sample of infected cells was also plated before lysis to assay their precise titer. The presence of cyanide, as described in the crossing procedure (14), had no influence on the burst sizes.

Labeled phage was prepared as follows. Samples of 20 ml of medium, to which ^3H -thymidine (20 $\mu\text{C}/\text{ml}$) and deoxyadenosine (200 $\mu\text{g}/\text{ml}$) had been added, were inoculated with BB and were grown overnight on a shaker. The cells were then pelleted, resuspended in nonradioactive medium at a concentration of 2×10^8 cells/ml, and incubated for about 15 min. Phage at an MOI of 0.1 was then added, and the cells were further incubated until lysis occurred. The labeled phage were partially purified by one cycle of low- and high-speed centrifugation and were then banded in CsCl . Finally, they were dialyzed for a few hours against growth medium.

Spot tests to check complementation, ultraviolet irradiation, and other procedures, were as described in a previous publication (14). Enzyme assays were as described by Geffer et al. (9).

RESULTS

Restriction spectrum of D. Phages P1, λ_{vir} , and all phages of the T-series, except T1 which did not adsorb to D, were tested for their ability to undergo restriction and host-controlled modification (2, 17) when changing from the B host to the D host or vice versa. This was performed by determining the relative efficiencies of plating (EOP) on these hosts, checked by adsorption measurements when indicated (Table 1). Phages T2, T3, and T6 had the same EOP on B and on D, regardless of the previous host. Adsorption of T4 and T5 to D was very poor, which is probably the only reason for their low EOP on this host. Phage P1·B was not restricted by D, but P1·D was restricted by B. Phage λ_{vir} ·B was restricted by D and, reciprocally, λ_{vir} ·D was restricted by

TABLE 1. Restriction and host-controlled modification in the B-D system

Phage	Efficiency of plating on	
	B	D
P1·B	1	1
P1·D	$\sim 3 \times 10^{-2}$	1
λ_{vir} ·B	1	$\sim 10^{-3}$
λ_{vir} ·D	$\sim 10^{-3}$	1
T2·B	1	1
T2·D	1	1
T3·B	1	1
T3·D	1	1
T4·B	1	$\sim 5 \times 10^{-2a}$
T4·D	1	$\sim 5 \times 10^{-2a}$
T5·B	1	$\sim 0.2^a$
T5·D	1	$\sim 0.2^a$
T6·B	1	1
T6·D	1	1
T7·B	1	$\sim 10^{-7}$
T7·D	1	$\sim 10^{-7}$

^a Low efficiency of plating related to inefficient absorption.

B. In both P1 and λ_{vir} , the few particles which escaped restriction produced host-modified progenies; their behavior agreed with the picture of host restriction and host-controlled modification in the classical sense (2, 20). This was found not to be the case with the restriction process suffered by T7.

Restriction of T7 by D. Phage T7-B when plated on D produced plaques of normal size (ca. 3 mm in diameter) at a frequency of about 10^{-7} relative to the number of plated phage. Very small "pinpoint"-type plaques were sometimes found at a frequency of about 10^{-4} . The low EOP could not be attributed to inefficient adsorption. Indeed, T7 consistently adsorbed better to D than to B; the average adsorption velocity constant (1) to D was 2.5×10^{-10} ml/min and that to B was 1.6×10^{-10} ml/min. When concentrated lysates of T7 were spot tested on a D lawn, the spotted areas remained perfectly clear; this occurrence denoted killing of the host. Exposure to T7 of an exponentially growing broth culture of D (3×10^8 cells/ml) at a MOI high enough to allow most cells to be infected (about 5 particles per cell) brought about visible lysis of the culture after 30 min. Infection of D cultures at lower multiplicities was also carried out, and the infected cultures were plated for surviving colony formers. The results were the same as those of the control experiments with B cells: if a Poisson distribution of adsorbed phage particles was assumed, then the data indicated that adsorption of one particle to a host cell was sufficient to destroy its colony-forming ability.

When phage from "pinpoint" plaques of T7 grown on D were replated on D, again only "pinpoint" plaques developed, and the EOP relative to that on B was low; on B, phage from "pinpoint" plaques produced plaques of normal size. Phage from normal-sized plaques on D had the same EOP on B and on D, and in both instances the plaques were of normal size. These phages could be subcultured on either B or D without affecting their plating characteristics. Thus, no system of host-controlled modification was operative. The genetic stability and host independence of the new plating characteristics led us to conclude that particles able to form plaques on D were mutants. On B, T7 wild type and these mutants were undistinguishable, and they gave bursts of 80 to 100 particles per cell. T7 wild type had an average burst size of 0.1 on D (which is too small to support plaque formation). When grown on D for a second cycle, the burst size of T7 wild type remained the same. T7 wild type is termed ss^+ (suicide in *Shigella*). Mutants forming "pinpoint" plaques on D gave bursts of 2 to 4 phages per cell on this host; they were not investigated

further in this study. Mutants forming normal plaques on D gave bursts of about 100 particles on B and on D; they are termed ss^- mutants. Eleven independently isolated ss^- mutants were used in this study; they were numbered consecutively $ss1^-$, $ss2^-$, and so forth.

Lysates of $ss1^-$, $ss3^-$, and $ss10^-$ were treated either with nitrous acid or with hydroxylamine. Phage of the mutagen-treated lysates were pre-adsorbed to B and were then plated on an indicator mixture of B plus D. On this indicator, particles unable to grow on D form characteristic "starry" plaques. Altogether, 50 independent phenotypic revertants, or partial revertants, which were either unable to grow or which grew poorly on D, were then isolated. All of these revertants (generically termed $ss^- rev$) were able to lyse D cells, but the burst sizes varied from 0.2 to about 15, as exemplified in Table 2. It is not yet clear why most of the revertants gave burst sizes somewhat higher than T7 wild type.

No striking temperature effect on the burst sizes of ss^+ or $ss^- rev$ mutants was found within the tested range of 25 to 40 C. The latent periods on D of all tested $ss^- rev$ mutants, regardless of their burst sizes, were the same, namely, about 25 to 30 min at 37 C. The latent period of ss^- mutants was a few minutes shorter. Other physiological aspects of infection of D by ss^+ and $ss^- rev$ mutants are discussed in the next paragraphs.

Fate of host DNA. The acid solubility of 3H -thymidine previously incorporated into DNA of the D host was monitored after infection by ss^+ or by various $ss^- rev$ stocks. Cells labeled for various generations with 3H -thymidine (0.5 μ C/ml) were transferred into nonradioactive medium; phage was added at an MOI of about 10, and the incubation was continued. At various intervals, samples were withdrawn and chilled in

TABLE 2. Burst sizes of some T7 ss -types on B and D

Phage type	Origin	Burst size on	
		B	D
ss^+ (T7 wild-type)	—	85	0.1
$ss1^-$	ss^+	81	89
$ss3^-$	ss^+	103	93
$ss10^-$	ss^+	98	107
$ss1^- rev1$	$ss1^-$	89	2.0
$ss1^- rev9$	$ss1^-$	94	3.2
$ss1^- rev20$	$ss1^-$	86	0.4
$ss1^- rev37$	$ss1^-$	79	2.5
$ss1^- rev43$	$ss1^-$	92	13.3
$ss3^- rev61$	$ss3^-$	107	3.0
$ss10^- rev5$	$ss10^-$	97	0.2

5% trichloroacetic acid. The acid-precipitable radioactivity that remained in these samples was plotted in Fig. 1. This figure shows that after infection with ss^+ or with $ss^- rev$ mutants a sharp decrease in acid-precipitable counts was observed early during infection. Breakdown of host DNA started abruptly 4 to 5 min after the addition of phage, and at 10 to 15 min it leveled off at different levels, depending on the phage stock. By the time of lysis (30 min) more than 90% of the host DNA was broken down to acid-soluble material in cells infected with ss^+ or $ss1^- rev37$. Phage $ss1^- rev43$, which of all revertants produced the highest burst size on D (13 phages per cell), solubilized about 60% of the host DNA. In contrast, infection by $ss1^-$ brought about only a slight drop in acid-precipitable counts. This is very similar to what we observed with infection of B by T7 wild type (14), which was interpreted as reflecting the simultaneous breakdown of host DNA and reincorporation of its subunits into the pool of phage DNA. [It is known that most of the subunits of T7 DNA are derived from host DNA (18).] Consequently, we thought that ss^+ and $ss^- rev$ mutants yielded low bursts in D because of a failure to initiate an efficient phage-directed DNA synthesis, similar to what we described earlier (14) for amber mutants of the DO-B type. To test this hypothesis, phage-

directed DNA synthesis was evaluated in D cells infected with ss^+ or $ss^- rev$ mutants.

Synthesis of T7-directed DNA. Exponentially growing D cells were irradiated with ultraviolet light (ca. 10,000 ergs/mm²) to minimize host DNA synthesis (14). ³H-thymidine (1 μ Ci/ml) and phage were then added, about 3 and 6 min after irradiation, respectively. Samples of the infected cultures were withdrawn after various times of incubation and chilled in 5% trichloroacetic acid. The amount of radioactivity incorporated into acid-precipitable material is plotted in Fig. 2. As shown in this figure, infection by $ss1^-$ stimulated thymidine incorporation in a manner similar to that described and discussed (14) for T7 wild-type infection of B. Infection of D by T7 wild type or by $ss^- rev$ mutants led to a completely different pattern (Fig. 2). Thymidine incorporation started normally at 4 min after infection and proceeded until about 8 min, reaching levels of incorporation of about 50% of the maximal level of incorporation by $ss1^-$. A rapid resolubilization of incorporated thymidine in cultures infected with ss^+ or $ss^- rev$ mutants followed, whereas cells infected with $ss1^-$ continued net thymidine

FIG. 1. Breakdown of bacterial DNA after infection of the D host with ss -mutants. Cells were grown in the presence of ³H-thymidine for several generations and then transferred to unlabeled medium. A few minutes later, phage (multiplicity of infection, 10) was added. The cells were further incubated and, at intervals, samples were chilled in equal volumes of 10% trichloroacetic acid. Acid-precipitable radioactivity was plotted. The black arrow indicates the time of visible lysis.

FIG. 2. Phage-directed thymidine incorporation after infection of ultraviolet-irradiated D cultures with ss -mutants. Exponentially growing cells were given a dose of about 10,000 ergs/mm². After a few minutes, ³H-thymidine was added. Again after a few minutes, phage (multiplicity of infection, 10) was added. At intervals, samples were chilled in equal volumes of 10% trichloroacetic acid. Acid-precipitable radioactivity was plotted. The open circles cover points referring to all curves.

incorporation. Again, mutant *ssl*⁻ *rev*43, which gave the highest burst size among revertants, showed less solubilization. The pattern of phage-directed DNA synthesis during abortive infection of D was thus quite different from that of abortive infection of B by DO-B amber mutants because these mutants were unable to initiate phage-directed thymidine incorporation (14).

Addition of chloramphenicol (80 μ g/ml) at times up to 2.5 min after adding the phage completely inhibited phage-directed DNA synthesis. When chloramphenicol was added 4 min after the phage, thymidine incorporation proceeded at about half the normal rate, and no breakdown was detected in any instance. When chloramphenicol was added 4.5 min after the phage, the initial rate of thymidine incorporation was nearly normal (Fig. 3); the breakdown of phage-directed DNA in *ssl*⁺-infected D cells, however, could still be inhibited. When chloramphenicol was added 5 min after the phage, *ssl*⁺-directed breakdown continued at roughly one-third the normal rate, and, when added 7 min after infection, it was virtually without effect on DNA breakdown (Fig. 3). Thus, it seemed that *ssl*⁺-directed breakdown of DNA required phage-directed protein synthesis at a later stage than it was required for phage-directed DNA synthesis to occur.

Fate of parental phage DNA. The question

FIG. 3. Chloramphenicol sensitivity of breakdown of phage-directed DNA in ultraviolet-irradiated D cultures infected with T7 *ssl*⁺. Experimental conditions were the same as described in Fig. 2, but, at 4.5, at 5, or at 7 min after infection, chloramphenicol (80 μ g/ml) was added to different samples of infected cells. For comparison, the effect of chloramphenicol on samples infected with T7 *ssl*⁻ was also checked.

arose as to whether only newly synthesized phage DNA was broken down or whether the parental genomes were also destroyed. Evidence for destruction of parental phage DNA was found as a result of single- or mixed-infection experiments with *ssl*⁻ and *ssl*⁺ phage, in which the DNA of one of the parental types was labeled with ³H-thymidine. Exponentially growing cells were concentrated 50-fold and then either infected with *ssl*⁺ and *ssl*⁻ simultaneously or infected with *ssl*⁺ or *ssl*⁻ only. After 2 min at 37 C, to allow adsorption, the mixture was diluted with cold Nutrient Broth and centrifuged to remove unadsorbed phages (MOI about 10; 5 of each type, in mixed infection). The pellets were resuspended in a small volume of cold medium and diluted into Nutrient Broth at 37 C to give a final concentration of 2×10^8 cells per ml (time zero). Samples were withdrawn at intervals, and acid-soluble counts were monitored. Figure 4 shows that upon infection of D cells by labeled *ssl*⁻ alone, only a minute amount of the adsorbed radioactivity appeared in an acid-soluble form. In all other cases, i.e., infection by labeled *ssl*⁺ alone, infection by labeled *ssl*⁺ and unlabeled *ssl*⁻, or infection by unlabeled *ssl*⁺ and labeled *ssl*⁻, acid-soluble material originating from the infecting phage genomes was clearly detected about 7 min after infection, and about 25 min after infection a maximal of 40 to 60% of the adsorbed counts was rendered acid-soluble. This solubilization process was almost totally inhibited by the addition of 30 μ g/ml of chloramphenicol at the same time as the phage inoculum. The breakdown of both *ssl*⁺ and *ssl*⁻ genomes following mixed infection of D is in contrast to that observed in an experiment made for comparative purposes—the mixed infection of B with the DO-B amber mutant *amH102* and T7 *am*⁺ *ssl*⁻ (Fig. 4). Although single infection of B by the amber mutant was also followed by a chloramphenicol-sensitive breakdown of parental phage DNA, this breakdown was prevented by co-infection with T7 *am*⁺ *ssl*⁻.

Functional complementation. The nature of the apparently dominant function involved in the breakdown of phage DNA was examined further by studying the patterns of functional complementation between *ssl*⁻ mutants and either *ssl*⁺ or *ssl*⁻ *rev* mutants. Burst-size measurements and analysis of the progeny phage of D cells mixedly infected with *ssl*⁻ and *ssl*⁺ were undertaken to check whether *ssl*⁻ (able to grow on D) could provide a function to be utilized by co-infecting *ssl*⁺ genomes. Table 3 gives the results of crosses in D, where one parental phage was *ssl*⁻ and the other was a conditional-lethal mutant. Four dif-

FIG. 4. Breakdown of parental phage DNA after single or mixed infection with phage stocks ss^+ , ssl^- , or $amH102$. Exponentially growing host cells were concentrated and exposed for 2 min at 37 C either to concentrated suspensions of purified 3H -thymidine-labeled phage or to mixtures of labeled and unlabeled phage. The adsorption mixtures were then diluted into cold medium and were centrifuged. The supernatant liquid with the unadsorbed phages was discarded, and the pellets resuspended in warm medium (37 C), at the original cell concentration (time-zero). At intervals, samples were chilled with equal volumes of 10% trichloroacetic acid and were later assayed for acid-soluble radioactivity. At the left, the results of infection of D (nonpermissive for ss^+) are reproduced. To the right, infection of B (nonpermissive for amber mutants) is represented. CM indicates addition of chloramphenicol (30 $\mu g/ml$), together with the phage inoculum. Except for $amH102$, which is a DO-B amber mutant of ss^+ , all stocks are am^+ . Black arrows indicate the times of visible lysis.

TABLE 3. Comparison of complementation patterns of ss^- with ss^+ and with $ss^- rev$ or with amber mutants^a

Parental phages	Burst sizes on D					Burst sizes on B				
	Total	Per allele				Total	Per allele			
		ss^-	ss^+ or $ssl^- rev$	am^+	am		ss^-	ss^+ or $ssl^- rev$	am^+	am
$ss^- \times ss^+$	3	2.5	0.5	—	—	91	37	54	—	—
$ss^- \times ss^+ amH131$	0.8	0.8	<0.1	0.8	<0.1	49	29	20	28	21
$ss^- \times ssl^- rev1$	14	12	2	—	—	104	40	64	—	—
$ss^- \times ssl^- rev9$	13	9	4	—	—	96	47	49	—	—
$ss^- \times ssl^- am668$	56	36	20	35	21	48	32	16	29	19

^a Crosses (MOI 5, of each type) were performed on D and on B. After lysis, phage were plated on BB, on D, and on B, for countings of total progeny, ss^- types, and am^+ types, respectively. As a control, the burst sizes upon infection with only one parental type were also determined in this experiment. They were as follows, for infection of D and B, respectively: ss^- , 102, 90; ss^+ , 0.1, 111; $ss^+ amH131$, <0.05, <0.05; $ss^- am668$, 0.15, 0.25; $ssl^- rev1$, 2.8, 93; and $ssl^- rev9$, 4.2, 105. Unless otherwise indicated, the parental phages were am^+ .

ferent conditional-lethal parental types were used, namely, ss^+ , a DO-B amber mutant (14) of ss^+ , $ss^- rev1$, and $ssl^- rev9$. As a control, a DO-B amber mutant of ss^- was also crossed with ss^- .

As a further control, the same crosses were also performed on the B host. After lysis, progeny phage were plated on BB, on D, and on B to evaluate the total progeny and the percentage of

ss^- or am^+ types, respectively. Not only was $ss1^-$ unable to complement functionally ss^+ or $ss^- rev$ genomes, but also their presence actually prevented $ss1^-$ genomes from growing on D (Table 3). Under conditions of equal input (MOI 5 of each type), mixed infection with $ss1^-$ and ss^+ reduced the average burst size of D cells to 3 to 5% of the values corresponding to the mixed infection of B or to the single infection of D by $ss1^-$. Mixed infection with $ss1^-$ and $ss^+ amH131$ gave even smaller bursts, whereas a control cross with $ss1^- am668$ showed complementation of the amber mutant in both D and B. Phages $ss1^- rev1$ and $ss1^- rev9$ (which on D gave somewhat higher bursts than ss^+) depressed the growth of $ss1^-$ to a lesser extent, but no functional complementation occurred.

Furthermore, ss^+ or revertants of $ss1^-$ not only were unable to be complemented by $ss1^-$, but they were also incapable of contributing the functions lacking in amber mutants of $ss1^-$. Amber mutants isolated from $ss1^-$, representing seven different complementation groups, did not yield measurably increased bursts upon co-infection with $ss^+ am^+$ or $ss1^- rev9 am^+$.

Genetic crosses with ss mutants. We first thought that T7 wild type might have a naturally occurring mutation in a cistron required only for growth on D and that T7 ss^- essentially represented a back mutation to an ancestral type which had a functional cistron. Crosses between independently arisen $ss^- rev$ mutants would then permit fine-structure genetic mapping of the cistron in question. With this in mind, random pairs among 50 independently isolated phenotypic revertants of ss^- mutants (and also ss^+) were crossed in B, and the progeny were plated on B (permissive for ss^+) and on D (nonpermissive for ss^+). More than 100 different crosses were made. No recombinants with the ss^- phenotype could be detected in any combination of conditionally lethal parental stocks. The resolution power in these crosses was of the order of a recombinant frequency of 10^{-4} . In contrast, we have found that an average cistron of T7 extended over more than five recombination units, as judged by crosses with amber mutants (Hausmann and Gomez, unpublished data). Thus, in randomly distributed mutations within a cistron of average size, recombination percentages would be expected to be, in some instances, about 100 times greater than the mutational background. We concluded that $ss^- rev$ mutants were indistinguishable among themselves and indistinguishable from ss^+ by genetic recombination, and formally mapped at the same site.

The results of these crosses corroborated the notion arrived at by the study of the physiology of infection and by complementation experi-

ments, namely, that the active function was coded for by the conditionally lethal genome. A defect in this function would allow growth on D, and a reversion of this defect would restore the conditional-lethal phenotype. If this premise were true, by crossing independent mutants of ss^- among themselves, recombinants with the conditionally lethal ss^+ phenotype should be found. These were indeed visible after the 11 ss^- mutants were crossed in every possible combination. After the crosses, progeny phage were preadsorbed to B (permissive) and plated before lysis on an indicator mixture of B plus D, where phage unable to grow on D would form "starry" plaques. (The method was the same as that used for identifying $ss^- rev$ mutants.) Reconstruction experiments showed that, under our conditions, 90 to 100% of the conditional-lethal particles formed plaques which could be recognized by their characteristic morphology. The percentages of conditional-lethal plaque formers were doubled, to account for the reciprocal types, and were plotted on the linkage map reproduced in the upper part of Fig. 5. This figure demonstrates that ss^- mutants map over a segment of about 2.5 recombination units. [The ss^- mutants placed above mapped markers showed very little (less than about 0.2%) or no recombination with these markers and among themselves.] Self-crosses of ss^- mutants were made as controls; 4,000 to 6,000 progeny plaques were examined for each cross, and in every case no plaques corresponding to the conditional-lethal phenotype were found.

Location of the $ss1$ site relative to the DO-B complementation group of amber mutants. An attempt was made to relate the site of one ss^- mutant to amber markers. The procedure was first to isolate a series of about 100 amber mutants of T7 $ss1^-$ which were then spot tested against representatives of already identified spot-test groups (14) of amber mutants of T7 ss^+ . From each complementation group, one representative amber mutant of T7 $ss1^-$ was then crossed against ss^+ or against revertants of $ss1^-$, by use of BB as a host (permissive for am and ss^+). After lysis, the progeny were plated on BB and on D. Plaques that formed on the D indicator (nonpermissive for am and ss^+) were considered $am^+ ss1^-$ recombinants. The recombination percentages were doubled to account for the reciprocal types ($am ss^+$), and the pertinent distances were plotted on the map shown in the lower part of Fig. 5. The $ss1$ site mapped adjacent to the far-right markers of the DO-B complementation group (14) of T7. Recombination percentages of revertants of $ss1^-$ with amber mutants of distantly situated groups (for which no continuous linkage map is as yet available) were consistent with the

FIG. 5. Upper part is tentative map showing linkage relationships between different *ss*⁻ mutants. Independently isolated *ss*⁻ mutants were crossed among themselves. The progeny were preadsorbed on B (permissive) and then plated on an indicator mixture of B plus D. Distances are given as twice the percentage of plaques formed by particles with a conditional-lethal phenotype; for each distance plotted, 15 to 80 such plaques were counted. The mutants placed above the mapped markers showed no, or less than about 0.2% recombination with these markers or among themselves. Lower part is map position of the *ss1* site relative to the DO-B complementation group. The amber mutants with letter prefixes were isolated from T7*ss*⁺; they are reproduced in the sequence published earlier (14). Mutants am764 and am668 are the outermost DO-B type amber mutants isolated from T7*ss1*⁻. Distances are averages of recombination percentages of three to five crosses. Independently isolated revertants of *ss1*⁻ when crossed among themselves gave no recombinants that were able to grow on D, and they thus mapped at the same site. Two values are given for the distance between am764 and *ss1*. The first was obtained as an average of three crosses of am764 *ss1*⁻ × am⁺ *ss*⁺; the second one corresponds to an average of twelve crosses in which one parental type was always am764 *ss1*⁻ and the other one always a different revertant of *ss1*⁻. The standard errors are given.

corresponding percentages in crosses with amH131 or am668. Also with this type of genetic crosses, the behavior of phage *ss*⁺ was indistinguishable from that of *ss*⁻ *rev* mutants.

Synthesis of S-adenosylmethionine-cleaving enzyme (SAMase). A striking biochemical difference between the related phages T3 and T7 is that the synthesis of a phage-directed "early enzyme"

(SAMase) which cleaved S-adenosylmethionine into homoserine and thiomethyladenosine was evoked by T3 but not by T7 (9). To establish whether this property of T3 was related to its ability to grow on D, two mutants of T3 unable to induce SAMase [*sam*⁻ mutants (12)] were plated on D. Both produced normal plaques on D. Furthermore, *ss*⁻ mutants of T7, able to grow

on D, were tested for SAMase production in both B and D, as described by Gefter et al. (9). No detectable enzyme activity was observed in either case. Therefore, we concluded that the absence of SAMase was not involved in abortive infection of D.

DISCUSSION

Although strains B and D have different specificities of host restriction and host-controlled modification (2, 17) vis-à-vis certain phages such as P1 or λ , abortive infection of D by T7 does not have the general characteristics of restriction of improperly host-modified phage in the sense originally described by Bertani and Weigle (4) and Luria and Human (20), and recently reviewed by Arber (2) and by Klein (17). Systems of host restriction in this classical sense generally affect broad classes of related phages (8, 11), whereas in the present case T3, which is closely related to T7, does not undergo any restriction. Another difference is the absence of host modification of T7 after one growth cycle in the new host. The ability of T7 to grow on D is acquired by mutations which are genetically stable (ss^- mutations). Such mutants behave like T3 insofar as they grow equally well on B and on D, regardless of their previous host. Host restriction without modification has also been reported by Glover et al. (10) and by Eskridge et al. (8) for phage T3, infecting cells lysogenic for phage P1, and in more detail by Kerszman et al. (16) for λ , infecting cells lysogenic for phage $w\phi$. As shown here for T7, the latter authors were able to show for λ that susceptibility to modification-independent restriction was subject to mutational alterations in the phage genome. However, the result of mixed infection of D with T7 wild type and a mutant able to grow on D is in contrast with the analogous situation described for infection of *E. coli* ($w\phi$) by λ wild type and λw . In this case, λ wild type is restricted, and λw is not restricted by *E. coli* ($w\phi$), regardless of whether single or mixed infection occurs. Kerszman et al. (16) have therefore termed the difference between λ wild type and λw as "passive"; they also postulated hypothetical models of "active negative" and "active positive" roles of the restricted phage vis-à-vis the nonrestricted, in which, respectively, the nonrestricted phage would be destroyed by, or would protect, a co-infecting restricted genome. To our knowledge, mixed infection of D with T7 wild type and ss^- mutants constitutes the first known example where such a model of an "active negative" effect applies. A possible explanation for this type of restriction is the synthesis by the restricted phage of a product which modifies the phage genomes, rendering them recognizable to a re-

stricting host factor, present in D and absent in B.

In agreement with a phage-induced breakdown function is the fact that no appreciable breakdown of parental phage DNA occurs if chloramphenicol is added together with the phage inoculum. The notion of a phage-induced autodigestion is further corroborated by the observation that no breakdown of phage DNA occurs during the early steps of infection, in contrast to what was observed with host-restricted λ (6, 19) and with nonglucosylated T-even phages (11). In the T7 system, breakdown of phage DNA, both parental and progeny DNA, starts only about 8 min after phage infection, at a point where phage-directed DNA synthesis has proceeded normally or nearly normally for about 4 min, and about at the time where the first progeny phage particles should start to mature. Addition of chloramphenicol to D cultures at 4 or 4.5 min after infection with T7 does not interfere with the initiation of phage-directed DNA synthesis, but the subsequent breakdown of phage-directed DNA is blocked. Breakdown can thus be characterized as a phage-directed, conditional-lethal function, which is expressed later than those functions required for phage-directed DNA synthesis. In contrast to the T7-directed buildup and breakdown of a DNA pool described here, Hattman (11) found that non-glucosylated T-even phages were incapable of initiating phage-directed DNA synthesis in restrictive conditions.

Genetic crosses of two kinds also favor the idea of a phage-directed suicide function. First, crosses between mutants unable to grow on D, which were independently isolated from those able to grow, failed to give recombinants able to grow on D. If T7 were defective in a function needed for growth in D [by analogy, for example, with the *rII* function needed for growth of T-even phages in *E. coli* K12(λ)], and if mutants of T7 that were able to grow on D were essentially back mutants to a hypothetical ancestral type of T7 which had that type of function, then crosses between newly isolated defectives should have allowed fine-structure mapping of the cistron in question.

Second, recombinants unable to grow on D were found in crosses between mutants that were able to grow. They mapped within one genetic segment of about 2.5 units of recombinations, which is compatible with the dimension of a single cistron. This situation is formally analogous to the findings of Streisinger and Franklin (21) with host-range mutants of phage T2: when different h^+ mutants were isolated from a T2 h stock, crosses between h^+ mutants yielded h recombinants. However, when h mutants isolated

from a T2h⁺ stock were crossed, no recombinants were found. The interpretation is that the functional integrity of a cistron can be destroyed by mutations at various sites, but, in general, it can be repaired only by mutation at one site, namely, the site of the original defect. Thus, in our case, the functional cistron would indeed be carried by the conditional-lethal genome.

The significance of the genetic location of this suicide function adjacent to the right end of the DO-B complementation group cannot yet be evaluated. Infection of D by *ss*⁺ or revertants of *ss*⁻ mutants, on the one hand, and infection of B by DO-B amber mutants on the other hand, bear some superficial similarities insofar as in both cases the phenotype of abortive infection is characterized by an efficient breakdown of host DNA, followed by lysis of the cells. Besides, in both cases, no phage-specific DNA (or very little) is made as an end result; although upon DO-B amber infection no net synthesis of phage-specific DNA occurs at any time (14), whereas after *ss*⁺ infection an initially normal DNA synthesis is followed by a rapid breakdown. A further similarity between DO-B amber mutants and *ss*⁺ is that in both cases, under nonpermissive conditions, infection is followed by a chloramphenicol-sensitive autodigestion of the invading phage genomes. However, the fundamental difference is that mixed infection of B with T7 wild type and a DO-B amber mutant prevents this autodigestion, whereas in the case of mixed infection of D with *ss*⁺ and *ss*⁻ mutants, the *ss*⁺ genome brings about breakdown of both genomes. In view of this very basic difference, it may now be wise, to consider the map proximity of DO-B mutants and *ss* mutants as incidental.

The very existence in T7 of a phage-directed function, apparently superfluous in one host and lethal in another, invites speculation. Phage-directed functions which seem to be superfluous in all known hosts have been found in T3, and in T2 and T4. In T3, the *sam* gene, coding for SAMase (9), can be eliminated without measurable consequences for the efficiency of phage growth (12). In T2 and T4, the phage-specific DNA-methylating enzymes (13) exert no known advantageous function, and by comparison with the closely related T6, which does not direct the synthesis of such an enzyme (13), it can be concluded that they are indeed superfluous, at least in the usual laboratory hosts. However, more bewildering than "superfluous" functions is the conditional-lethal function described here, because it implies an interaction with the host to bring about lethality. Thus, the additional enigma of the nature of the host-specific factor arises. More data need be accumulated before useful speculation

can be undertaken. The identification of a host in which this function would be advantageous or even essential may be helpful. Similarly, the isolation of permissive mutants of D would also be of value.

In general, a more detailed investigation of the physiology of abortive infection will be needed to pinpoint its molecular mechanism. In particular, the phage-directed DNA synthesized during abortive infection needs to be analyzed with regard to its physico-chemical properties, such as molecular weight or the possible existence of single-strand breaks. The possible correlation of mutual exclusion between related phages such as T3 and T7 (14) and the present case of self-exclusion of T7 must also be investigated. Self-exclusion could be brought about if a specific exclusion mechanism of T7, directed normally toward related but nonidentical genomes, fails to discriminate between "own" and "foreign" in the specific host environment provided by the D host.

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