Evidence for Heterogeneity in Populations of T5 Bacteriophage

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Each T5 stock contains a population of particular phages that, just after adsorption onto the host bacteria, release their entire chromosome outside the bacterial membrane in a place where it is sensitive to bacterial enzymes. This release takes place before the sensitization step to deprivation of calcium and before the transfer of the first-step DNA fragment. Secondly, this released DNA is degraded by bacterial enzymes, mainly by the endonuclease I; the products of degradation are spontaneously released in the surrounding medium. Thus, in each T5 phage stock it seems that there is a minor population that is deficient for the mechanism of controlled DNA injection into the bacteria.

When bacteriophage T5, labeled in its DNA, is adsorbed onto its host cell, one observes a particular phenomenon as described earlier by Lanni (15, 17) and by Luria and Steiner (24): the supernatant of pelleted T5-infected bacteria contains only about 2% of the infectious particles present before adsorption, but as much as 30% of the total input radioactivity present before adsorption. To explain this discrepancy, it was earlier proposed (15, 17, 24) that the T5 stocks contained about 30% of phages that had lost their attachment sites. Recently, we obtained evidence showing that this hypothesis seemed, at least in part, wrong. (i) B. Labedan et al. (13) observed that the radioactivity found in the adsorption supernatant was mainly attributable to free, partially degraded, phage DNA and not to virions, because it was in part trichloroacetic acid nonprecipitable. (ii) By using electron microscopy and the Kleinschmidt technique, B. Labedan and B. Stevens (unpublished data) observed small DNA fragments in the medium surrounding the *Escherichia coli*-T5 complexes arrested at the FST stage (13, 17). These two preliminary observations suggested to us that, in fact, part of the adsorbed (radioactive) DNA was released in a fragmented state outside the infected bacteria. A study of this phenomenon was then undertaken, and we show in this paper that each purified T5 stock contains at least three classes of particles: (i) infectious phages irreversibly adsorbable; (ii) nonadsorbable particles; and (iii) noninfectious particles capable of adsorption but releasing all of their DNA outside the bacteria, thus containing "reversibly adsorbable" radioactivity. The existence of these last aberrant particles is discussed with respect to

the mechanism of injection of T5 DNA into the host cell.

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MATERIALS AND METHODS

Bacteriophage strains. The heat-stable strain T5stO (18) obtained from Y. T. Lanni was used in all the experiments. The T7 phage was obtained from R. Devoret.

Bacteria strains. *E. coli* F is a fast-adsorbing strain for T5 (16). *E. coli* B41, an endonuclease I mutant (7), was obtained from H. Hoffmann-Berling. *E. coli* BB, host strain of T7, was obtained from F. Gros.

Media and buffers. Precultures of different bacterial strains were made on T Broth (10 g of tryptone [Difco] and 5 g of NaCl per liter). Two synthetic media were used: for T5stO, the MGM medium (18) was supplemented with 0.5% Casamino Acids (Difco); for T7, the TCG medium (1) was used. For experiments involving adsorption, the MGM buffer, which is the MGM medium minus glucose, NH_4Cl , and Casamino Acids, was used. The phage stocks were stored in phage buffer composed of 10^{-2} M tris(hydroxymethyl)aminomethane (Tris)-hydrochloride, pH 7.4, 0.1 M NaCl- 10^{-3} M MgSO_4 - 0.01% gelatin. All the media and buffers were supplemented with 10^{-3} M CaCl_2 for T5stO (17).

Growth of phage stocks. To prepare T7, the method described by Abelson and Thomas (1) was used. *E. coli* BB grown to 5×10^8 cells/ml in TCG medium was infected at a multiplicity of infection (MOI) of 0.25; lysis occurred after 30 min.

The optimum conditions for preparing high titers of T5stO were as follows. The infection was made in Erlenmeyer flasks (2 liters) containing 200 ml of MGM at 37 C, with a very fast agitation on a gyratory mechanical shaker. In these conditions, the aeration, which is an essential factor for T5 growth (16), was good. A preculture of the preceding day arrested in

exponential phase was inoculated in MGM medium; thus, there is no lag. When the bacterial concentration attained 3×10^8 cells/ml, the phage was added at an MOI of about 0.01. The optimum multiplicity is variable according to the ratio of lytic phages to killer phages (17) in the infecting suspension and must be determined in a pilot experiment for each stock of phage. The best yields were obtained when lysis occurred after 80 min, the optical density dropping from about 0.9 to 0.18 in 20 min. No chloroform was added because we observed that this addition increased the proportion of incomplete phage particles liberated with respect to spontaneous lysis (unpublished data).

To obtain labeled phages, [2- 14 C]uridine (0.4 μ Ci/ml) or [6- 3 H]uridine (0.8 μ Ci/ml) was added at the time of infection. Due to the existence of a viral thymidylate synthetase, labeled uridine is actively incorporated into the phage DNA. The labeled precursors were purchased from the CEA, Saclay, France.

Purification of phage stocks. After a low-speed centrifugation (15 min at $5,000 \times g$) to eliminate the bacterial debris, the lysate was treated by 10 μ g each of DNase and RNase per ml for 30 min at 37 C. It was then purified by filtration under vacuum through Hyflo Supercel layered on Whatman filter paper in a Buchner funnel. The phages contained in the filtrate were precipitated by addition of NaCl and of polyethylene glycol 6000 to concentrations of 0.5 M and 10%, respectively (33). In some cases the phages were also sedimented by differential centrifugation cycles (29). After one night at 4 C, the PEG-precipitated virions were collected by low-speed centrifugation, and then the phages concentrated by either method were then centrifuged through a preformed CsCl step gradient. For T7, the Crothers and Zimm technique (6) was used without modification; for T5stO, a layer of phages was deposited over three layers of CsCl (1.7, 1.5, and 1.3 g/cm 3) and centrifuged for 1 h at 39,000 rpm in the Spinco 50 Ti rotor at 4 C. Under these conditions, two bands of phages were obtained. The lower and thinner one that contained tailless particles (12) was discarded, and the upper band was diluted 100 times in the phage buffer and reconcentrated by sedimentation for 45 min at 25,000 rpm in the Spinco 30 rotor to eliminate the CsCl. We used this simple method instead of dialysis because T5stO is resistant to osmotic shock (33). We observed that one CsCl gradient was sufficient to give a highly purified phage stock. The phage stocks were stored in the phage buffer at 4 C at concentrations varying between 2.10^{12} and 5.10^{13} infectious particles per ml.

Extraction of phage DNA. To obtain internal sedimentation markers, T7 and T5stO DNAs were extracted by 7.5 M sodium perchlorate, pH 7, as described by Freifelder (9). Phenol-extracted DNA was obtained by the methods of Mandell and Hershey (25) or Thomas and Abelson (29).

Preparation and purification of the adsorption supernatant. Standard conditions were as follows. A fresh preculture of *E. coli* F was grown in the MGM medium until a bacterial concentration of 5×10^8 cells/ml was attained, and the bacteria were immediately centrifuged for 15 min at $5,000 \times g$ at 4 C and

resuspended at 5×10^8 cells/ml in the MGM buffer. After 15 min of incubation at 37 C, the phages were added at an MOI of about 20, and the incubation was continued for 10 min at 37 C. Then, these *E. coli*-T5 complexes arrested at the FST stage (20) were chilled in ice and centrifuged for 10 min at $6,000 \times g$ in the Sorvall SS 34 rotor. ("FST stage" signifies that the FST-DNA is inside the bacterial membrane, and the rest of the T5 chromosome is outside this membrane [20].) The supernatant was carefully removed and immediately centrifuged for 45 min at 25,000 rpm in the Spinco 30 rotor at 4 C to eliminate some unadsorbed phages (about 1% of the total input phages) and some *E. coli*-T5 complexes not centrifuged at $6,000 \times g$. The high-speed supernatant was immediately frozen and kept at -18 C until use. Some modifications were occasionally introduced in these standard conditions and will be described in detail in the Results section.

Chromatography on methylated serum albumin-kieselguhr. A MAK column was prepared essentially as described by Mandell and Hershey (25) but in a 5.10^{-2} M phosphate buffer, pH 6.7, without NaCl. The sample was adsorbed, and the column was washed by about 100 ml of phosphate buffer. The elution was then performed by applying a linear gradient (25 ml/h) of 0.4 to 0.8 M NaCl in the phosphate buffer. After the gradient was ended, 10 ml of 1 M NaCl was applied. All the operations were made at room temperature.

Chromatography on Dowex 1 formate resin. The Sinsheimer technique as described by Privat de Garilhe (28) was used on a microsize scale: a 1-ml column of AG 1 X 2 (Bio-Rad 200 to 400 mesh) made in a 2-ml pipette was equilibrated in the 0.1 M ammonium formate buffer, pH 4.5. By using this technique, only the nucleotides were adsorbed on the column; by raising the buffer concentration stepwise to 0.25, 0.5, 1, 2, and 3 M, the adsorbed oligonucleotides elute according to their sizes. All of the operations were made at room temperature.

Linear sucrose gradients. To analyze MAK samples and NaClO $_4$ -extracted DNA, 10 to 25% sucrose (wt/vol) gradients were prepared in 1 M NaCl and 0.05 M phosphate buffer, pH 6.7, using the Britten and Roberts apparatus (4). The samples were carefully layered with a mechanically driven wide-bore pipette. The runs were carried out at 20 C in the SW 39 rotor. After centrifugation, the gradient was fractionated by piercing the bottom of the tube with a stainless-steel needle, giving drops of an average volume of 40 μ liters. Fractions of 4 or 5 drops were directly collected on Whatman GF/B 25-mm disks.

Radioactivity determinations. The samples were layered on membrane HA disks (Millipore Corp., Bedford, Mass.) or on GF/B disks for the analysis of column fractions or gradient fractions. After infrared drying, these membranes were covered with 5 ml of toluene-0.4% 2,5-diphenyloxazole-0.01% 1,4-bis-2-(5-phenyloxazole)benzene mixture. The radioactivity was counted in a Beckman spectrometer. When both isotopes, 3 H and 14 C, were present, the cross-contamination of 14 C into the 3 H channel was uniformly 12% and the reverse contamination was zero.

RESULTS

T5 stocks obtained independently with several methods of purification were used to prepare *E. coli*-T5 complexes arrested at the FST stage as described by Lanni (17) and by us (13). In all cases, although the adsorption supernatant of these complexes contained only about 1% of the total infectious phage present in the inoculum, the radioactivity found free in the surrounding medium was much more than 1% of the total input radioactivity.

Importance of the purification procedure.

The following facts may be noted about the influence of the purification on this percentage of radioactivity freed in the surrounding medium of T5-*E. coli* complexes.

(i) This percentage was the same when a lysate was divided and purified either by differential centrifugation cycles (29) or by precipitation with polyethylene glycol 6000 (33).

(ii) After CsCl gradient and elimination of all the tailless particles (lower band), the amount of liberated radioactivity during the adsorption process was lowered only by about 50%. Thus, at least part of the released radioactivity proceeded from entire particles (upper band of the CsCl gradient). This fact does not support the hypothesis proposed by Lanni (17) and Luria and Steiner (24).

(iii) If, after a first round of purification (differential centrifugation or PEG-precipitation followed by a CsCl gradient) a second round was carried out on the once-purified T5 stock, the same percentage of released radioactivity was obtained before and after this second round. Thus, it seems that the purification method was not responsible for the appearance of releasing radioactivity by damaging some previous normally infectious phages.

(iv) When a purified phage stock was treated by DNase, all of the radioactivity was trichloroacetic acid precipitable. When the DNA was extracted from a labeled phage stock by use of the NaClO₄ technique (9) and immediately carefully layered on a neutral sucrose gradient, about 95% of the total radioactivity sedimented in one peak at 48S (sedimentation constant of T5stO DNA, [23]; another peak of radioactivity sedimenting at a different rate was never observed. Thus, the radioactivity released from T5-*E. coli* complexes did not proceed from intact or degraded free DNA already present in purified phage stock.

First features of the released radioactivity.

Phage purified through the PEG and CsCl steps were used in the experiments described thereafter because we were only interested in the radioactivity originating from entire phages.

We observed that the percentage of released

radioactivity in the adsorption supernatant varied from one phage stock to another. With eight labeled stocks prepared and purified separately in the same way, this percentage varied as 12, 14, 16, 16, 18, 19, 19, and 26%. But this percentage was constant for a given stock from one experiment to the next and did not vary during the storage after the entire purification. For example, the percentage for one stock remained constant for 6 months after its purification.

We observed that this percentage, by using the same phage stock, was the same (i) when we used different bacterial strains (B, B41, or F); (ii) regardless the duration of adsorption (see below); (iii) regardless the adsorption taking place at 37 or at 4 C; and (iv) regardless the MOI, including MOI lower than 0.1; therefore, the observed phenomenon is not due to a superinfection-exclusion mechanism (2, 8).

Thus, it seems that the percentage of radioactivity released in the medium surrounding the T5-infected bacteria is a characteristic of the phage population.

We tried to understand the problem of this variation from one phage stock to another. The following facts may be stated. When several stocks were made by infecting a series of cultures of the same bacterial strain in the same growth conditions (see Materials and Methods) with the same initial T5 stock, there was still a variation from one purified stock to another (figures given above for the eight stocks), and the variation was statistically the same with different bacterial strains (B, F, or B41). Moreover, when lysis occurred under unsatisfactory conditions (as defined in Materials and Methods), the percentage of DNA released into the medium was significantly increased (about 25 to 35%); in the same way the addition of chloroform, even after an optimum spontaneous lysis, increased this percentage by noticeably increasing the proportion of heads in the lysate (unpublished data).

Thus it seems that all T5 stocks released some radioactivity after adsorption, but the percentage of this released radioactivity is a feature of each well-defined purified stock that is the result of possible events happening during the intracellular growth and that is not appreciably modified by the purification of the lysate.

State of the released DNA. As already stated in the introduction, we found some degraded DNA in the adsorption supernatant. We have undertaken to study more precisely this degraded DNA.

After 10 min of adsorption at 37 C, the *E. coli*-T5 complexes were centrifuged, and the supernatant was purified as usual and then

chromatographed on a methylated albumin-kieselguhr column as described in Materials and Methods. Figure 1 shows the profile obtained. Of the total radioactivity, 57% was not retained on the column; with a NaCl gradient, 10% was eluted at 0.55 M NaCl and 33% was eluted at 0.64 M NaCl. In these conditions of chromatography, the intact T5 DNA (48S) elutes at 0.67 M NaCl and the FST-DNA (20S) at 0.64 M NaCl (13). The three classes of labeled compounds were then analyzed.

The first class, nonadsorbable on MAK column, was chromatographed on Dowex AG X 2 column made in 0.1 M ammonium formate, pH 4.5, and was eluted by increasing molarities of this formate buffer. Figure 2 shows the profile obtained: 21% was not retained on the column, 26.5% was eluted at 1 M formate, and 43.5% was eluted at 2 M formate. Thus, according to the data of Sinsheimer (28), this first class appears to be composed of about 20% of degradation products of nucleotides and of about 80% of large oligonucleotides.

The second and third classes were analyzed through neutral sucrose gradients. Figure 3 shows the sedimentation profiles with T7 DNA as an internal marker of sedimentation (32.6S according to Leighton and Rubenstein [23]). The species eluting at 0.55 M NaCl gives one peak sedimenting at about 10S. Thus, this species contains small double-stranded DNA fragments measuring about 1% of the T5 chromosome (5). The species eluting at 0.64 M NaCl gives a

FIG. 1. MAK column chromatography of an adsorption supernatant. A supernatant of *E. coli*- ^{14}C -labeled T5 complexes incubated 10 min at 37°C in MGM buffer supplemented with 10^{-3} M CaCl_2 was chromatographed on a 5-g MAK column prepared in $5 \cdot 10^{-2}$ M phosphate buffer, pH 6.7. After elution of the nonadsorbable compounds (class 1), a linear gradient of NaCl molarity (---) was applied to obtain the two other classes (2 and 3). The different classes were indicated by arrows. We have verified that after elution of class 1 compounds, nothing was eluted between 0 and 0.4 M NaCl.

FIG. 2. Analysis on Dowex of the compounds non-adsorbable on MAK column (class 1). The Sinsheimer technique as described by Privat de Garilhe (28) was used to determine the nature of the compounds non-adsorbable on MAK column. The class 1 sample was diluted to the initial salt concentration of the Dowex column before adsorption on the resin. The different steps of elution by increased molarities of formate buffer are indicated by arrows.

FIG. 3. Analysis of degradation products (classes 2 and 3 on MAK column) through sucrose gradients. The species 2 (○) and 3 (●) eluting in the NaCl gradient (Fig. 1) were each cosedimented with the NaClO_4 -extracted ^3H -labeled T7 DNA (□) through a 10 to 25% sucrose gradient made in 1 M NaCl, $5 \cdot 10^{-2}$ M phosphate buffer, pH 6.7. After 3 h at 35,000 rpm at 20°C in the SW 39 Spinco rotor, 33 fractions were collected and their radioactivity was determined as described in Materials and Methods.

broad peak sedimenting between 20 and 30S being composed of fragments measuring from 8 to 25% of the T5 chromosome.

Thus, the mechanism of degradation of the released DNA seems essentially an endonucleolytic process yielding large double-stranded fragments of T5 DNA, large oligonucleotides, and some degradation products of nucleotides.

Is the adsorption necessary? To test if the presence of bacteria is necessary to obtain the release of some phage DNA, the following exper-

iment was undertaken. A suspension of labeled T5 was added to a supernatant after the centrifugation of uninfected bacteria concentrated to $5 \cdot 10^9$ cells/ml in the MGM buffer. After 30 min at 37 C, 100 μ g of pancreatic DNase per ml were added, and the incubation was continued for 30 min. Then the trichloroacetic acid-precipitable compounds were measured. At variance with what happens when bacteria are present, all the radioactivity was precipitable. Therefore, the medium surrounding the bacteria does not contain any compound capable of triggering the release of phage DNA under a DNase-sensitive form. The observed phenomenon of release needs the presence of bacteria.

When does the process of release take place? Lanni (17, 18, 21) recognized several early steps necessary to produce the bacteria-phage complexes arrested at the FST stage. In particular, the adsorption can be separated from a step involving sensitization to calcium starvation, and also from the first-step transfer of DNA, by utilizing the fact that only adsorption occurs at 4 C. Therefore, to determine at what early step the process of release takes place, we carried out the following experiment. After concentration in the MGM buffer at $5 \cdot 10^9$ cells/ml, the bacteria were divided into two samples. In the first one, T5 was adsorbed at 4 C; in the second one it was adsorbed at 37 C. After 10 min, the two samples were centrifuged and the percentage of radioactivity released in each supernatant, purified as usual, was measured. In both cases, the same percentage (13%) was obtained. Thus, the phenomenon of release takes place before the sensitization to calcium deprivation (18) and before the first-step transfer of DNA.

Kinetics of degradation. When the time of adsorption was prolonged beyond 10 min, the other conditions being kept the same, we observed that the same percentage of released radioactivity was always obtained for the same phage stock, whatever the duration of adsorption, as far as 100 min. However, the proportions of the different classes of degradation products changed qualitatively. Table 1 shows that the longer the duration of adsorption, the greater the proportion of oligonucleotides and degraded nucleotides.

Thus, it seems that the process of fragmentation may continue after DNA release is completed.

Nature and location of the responsible enzyme. As shown above, part of the endonucleolytic action seems to take place in the medium surrounding the bacteria. The following experiments were performed to verify the

TABLE 1. *Kinetics of degradation*^a

Time of incubation (min)	Oligonucleotides and degraded nucleotides (%)	Small double-stranded fragments (%)	Great double-stranded fragments (%)
10	57	10	33
20	68	32	0
30	90	10	0

^a The adsorption of T5 onto *E. coli* F at $5 \cdot 10^9$ cells/ml in MGM buffer supplemented with 10^{-3} M CaCl₂ was made during variable times at 37 C before the centrifugation. The different species present in the adsorption supernatants were separated on a MAK column and analyzed on Dowex and sucrose gradient as already described.

presence of a nuclease in the supernatant. After concentration to $5 \cdot 10^9$ cells/ml, the bacteria were divided into two samples; unlabeled phages were added to one sample under standard conditions (10 min at 37 C); the other sample was uninfected and kept for 10 min at 37 C. Then, after centrifugation, phenol-extracted ¹⁴C-labeled T5 DNA was added to each supernatant, and the mixtures were incubated a long time (80 min) to allow a noticeable degradation by the supposed supernatant nuclease. After the incubation was completed, the two mixtures were analyzed on a MAK column as usual. In both cases all of the radioactivity eluted in one peak at 0.58 M, and on neutral sucrose gradient this species sedimented from 12 to 20S, being composed of fragments measuring from 1 to 8% of T5 DNA.

Two conclusions evolve from this experiment. (i) There is only a small amount of nuclease in the medium surrounding the bacteria, because even after 80 min we do not obtain nucleotides but only double-stranded DNA, unlike the results obtained with bacteria (Table 1). (ii) The nuclease is present regardless of whether the bacteria are infected or not: therefore, it is a bacterial enzyme.

It is noticeable that, in all the experiments described here, the products of degradation were obtained without any treatment to lyse the bacteria. Moreover, when the infected bacteria were treated to give spheroplasts, we observed (i) that there was no increase of released radioactivity, and thus the cell wall did not retain the DNA released from the adsorbed phages; (ii) but the degradation was accelerated (data not shown). Thus, the supernatant of these spheroplasts contained more enzyme than that of the intact bacteria.

Together these different results suggest that the endonucleolytic action takes place essentially between the phage receptors and the cell

membrane, and also in the medium surrounding the bacteria.

Is the *E. coli* endonuclease I the responsible enzyme? It is well recognized that in a precise location of the cell wall, the periplasmic space, (see reference 26), there are some degradation enzymes, in particular the endonuclease I and some degradation enzymes of nucleotides (see reference 10 for a review). In addition, Nossal and Heppel (27) have reported that part of the endonuclease I, which is normally in the periplasmic space, is spontaneously released in the surrounding medium by washing exponentially growing cells, exactly as we do to concentrate *E. coli* F from 5.10^8 cells/ml in the MGM medium to 5.10^9 cells/ml in the MGM buffer. This last fact may well explain the results of the degradation kinetics as discussed above, because the released DNA is always submitted to degradation by the enzyme liberated in the supernatant, and not only at the time of release. We have verified that, with thrice-washed bacteria (elimination of most of the supernatant enzyme), the percentage of released radioactivity is the same as usual, but the degradation rate is lowered with respect to once-washed bacteria (standard conditions).

These facts allow us to suppose that the endonuclease I may be the enzyme responsible for the degradation of the released DNA.

If this hypothesis is right, an endonuclease I-deficient strain would yield the same release, but the kinetics of degradation would be different. We used the B 41 strain, which contains only 0.2% of the endonuclease I of the wild strain (7). With this strain the following results were obtained. (i) Quantitatively, the degradation was very poor. After 60 min of adsorption at 37 C, only 9% of the radioactivity was not retained on a MAK column and the rest eluted between 0.64 and 0.67 M NaCl. It is a very different result from the one that was obtained with *E. coli* F (Table 1). (ii) Qualitatively, the degradation products were the same with respect to the F strain. The 9% first class of compounds contained about the same quantities of degradation products of nucleotides (20%) and of large oligonucleotides (80%). The species eluting in the NaCl gradient were large fragments of T5 DNA measuring about 40 to 50% of the total DNA (Fig. 4). Then, the degradation is also an endonucleolytic one and possibly is performed by the remaining 0.2% endonuclease activity (7).

Origin of the degraded DNA. Is the degraded DNA proceeded from released total chromosome? To try to obtain entire T5 DNA in the release products, we used several conditions

FIG. 4. Analysis of degradation products in the *E. coli* B 41-T5 system. The species (●) eluting in the NaCl gradient of a MAK column of an adsorption supernatant of B41-T5 system were cosedimented with NaClO_4 -extracted ^3H -labeled T5 DNA (□) in the same conditions as described in Fig. 3. We collected 26 fractions.

delaying the degradation. When ^{14}C -labeled T5 was adsorbed for 2 min at 4 C onto thrice-washed *E. coli* B 41, the released DNA analyzed on sucrose gradients gave large fragments, most of which were entire chromosomes.

Therefore, we may conclude that the observed phenomenon is caused by the release of the entire chromosome of a part of the phage population and not by the loss of part of the chromosome by all the phages. For the releasing population it is an all-or-nothing phenomenon.

DISCUSSION

We know little about the first steps (adsorption, DNA injection) of the infectious process of a bacterium by a phage. For example, the mechanism of spatial and temporal regulation of the injection of a viral chromosome into its host cell is not clearly demonstrated. However, in the case of the T5 phage, several facts are well known: the bacterial receptors have been isolated and characterized (31), and the interactions between these isolated receptors and phages have been directly studied by electron microscopy (30, 34). However, Lanni (18) distinguished several steps after the adsorption itself, namely the sensitization to calcium deprivation and the desensitization. Lanni furthermore has also shown that the injection of T5 DNA is a two-step process (see reference 20 for review).

In this paper, we described a particular phenomenon. After adsorption onto the host cell, some phages release their DNA under a de-

graded form into the medium surrounding the infected bacteria. This phenomenon can be decomposed in two independent processes.

(i) The release itself appears to be a property of the phage population and does not depend on the bacterial strain because, whatever the conditions, the same phage stock gives the same percentage of released radioactivity. This release takes place before the sensitization step (18), occurs only after adsorption onto entire bacteria, and may be completed even at 4 C.

(ii) On the contrary, the degradation of the released DNA seems to be carried out only by bacterial enzymes, essentially by the endonuclease I. To support this suggestion, some evidence has been provided by comparing the results of the present work with the known features of the endonuclease I. The endonucleolytic action takes place in the cell wall and also in the medium surrounding the bacteria as described for the endonuclease I (10, 26, 27). The large oligonucleotides obtained on Dowex column may correspond to the degradation products of an average length of seven nucleotides made by the endonuclease I (22), and an endonuclease I⁻ mutant strain gives a very slow kinetics of degradation with respect to the wild strain. In fact, this degradation appears only as a consequence of the release and is not the essential phenomenon.

Therefore, the observed phenomenon may be described in the following way. After the process of adsorption was completed, a part of the adsorbed phages spontaneously release their DNA through the tail of the capsid outside the bacterial membrane, and this DNA is immediately attacked by the degrading bacterial enzymes (10). The degradation products are then eluted in the surrounding medium where they continue to be degraded by the endonuclease I released from washed bacteria (27). The rest of the adsorbed phages never release their DNA during that step.

Then each T5 stock contains phages aberrant for DNA injection into the host cell. That the occurrence of such particles is not due to purification method was shown by an indirect way. If a highly purified stock is again submitted to all the steps of the purification procedure, we found exactly the same percentage of aberrant phages after this second round of purification as after the first one. Thus, it seems that the aberrant phages are formed before the purification begins, i.e., during phage growth or lysis, or both. A challenging problem is the variation of the proportion of these aberrant phages from one stock to another even when all the conditions of growth and lysis seem the

same. Now it appears very difficult to understand exactly what physiological defect is responsible for the production of these peculiar phages, but we may suppose that it is an assembly defect, the frequency of which is linked to some unmastered conditions of the phage growth.

Recently Zarybnicky, Zarybnicka, and Frank (34) claimed that upon adsorption onto purified free T5 receptors, 70% of the T5 virions released their DNA into the medium. This *in vitro* result seems to be in contradiction to several previous *in vivo* observations that demonstrated that in FST complexes, most of the phage chromosome was located inside the adsorbed capsids. Lanni (17) showed that mechanical blending, as described by Hershey and Chase (11), destroyed the plaque-forming ability of the complexes while removing the capsids. Labedan et al. (13) observed that the heads of phages arrested at the FST stage were "full" and that the gentle removal of the capsids resulted in unrolling of the chromosome into the surrounding medium, the FST section being inside the bacteria. Bayer (3) also observed that a large proportion of T5 virions exhibited "full" heads after 3 min of adsorption, although no attempt was made to characterize the FST stage. A tentative explanation of the apparent contradiction between the *in vivo* (3, 13, 17) and *in vitro* (34) results is the following model. One may assume that, during their purification, the isolated receptors (34) have been deprived of some element responsible for the *in vivo* arrest of the injection of the viral chromosome after the entry of the FST section, the rest of the DNA waiting *in situ* for the synthesis of the protein(s) responsible of its active transport inside the bacteria (14). Then the aberrant phages, described in this paper, must have such a defect that they do not efficiently interact with this hypothetical element responsible for the two-step transfer mechanism. Thus, these phages must eject their DNA outside the bacterial membrane in the same way as all the T5 phages do in the presence of purified T5 receptors. Experiments are under way to test this model.

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