Penetration into Host Cells of Naked, Partially Injected (Post-FST) DNA of Bacteriophage T5

B. LABEDAN AND J. LEGAULT-DEMARE

Laboratoire de Biochimie, Institut du Radium-Fondation Curie, Université Paris-XI, Bâtiment 110, 91405-Orsay, France

Received for publication 21 February 1973

The experiments presented here suggest that the naked post-FST DNA, attached to the bacteria after a centrifugation at $6,000 \times g$ of *Escherichia coli*-T5 complexes arrested at the FST stage, can be injected into the host cell if protein synthesis is allowed. This results from the observation that the production of infectious centers by this naked DNA is sensitive to DNase or moderate shearing treatments. The process of injection seems to be the same as that for DNA normally rolled in an adsorbed capsid.

Phage T5 DNA is injected into its host cell by a two-step process (6). First there is the injection of the so-called FST DNA, a fragment which amounts to 8% of the total chromosome (3, 9). Secondly, there is an obligatory pause during which proteins are synthesized, one of these being required for the transfer of the rest of the DNA molecule (1, 5, 10). If protein synthesis is repressed, the *E. coli*-T5 complexes are arrested at the FST stage (5). We have shown (2a) that, when these complexes are centrifuged at $6,000 \times g$, most of the adsorbed capsids are cut at the level of their tails and released into the surrounding medium. However, the uninjected DNA which they contained remains attached to the bacteria via the previously injected FST-DNA and is entirely uncoiled. This is a challenging state for the naked post-FST DNA, and whether or not it could be injected if protein synthesis was allowed is the outstanding question. In this paper we present results suggesting a positive answer.

MATERIALS AND METHODS

Strains. T5stO and its host cell *Escherichia coli* F are commonly used (4); T5 am15 is an FST amber mutant which grows on *E. coli* HR34 (*su*⁺) but not on *E. coli* F (*su*⁻). These different strains of bacteria and phages were kindly supplied by Y. T. Lanni.

Media and buffers. Precultures of both bacterial strains were prepared on T broth (10 g of tryptone [Difco] and 5 g of NaCl per liter). For the study of the injection of T5 DNA, synthetic medium (MGM) (4) supplemented with 0.50% Casamino Acids (Difco) and MGM buffer (MGM medium minus glucose, NH₄Cl, and Casamino Acids) were used. To determine the number of infectious centers, 0.05 to 0.2 ml

of an appropriate dilution made in MGM buffer was mixed with 0.1 ml of plating bacteria and 2.5 ml of top agar (7 g of agar [Difco] per liter of T broth), and the mixture was poured onto an agar plate (15 g of agar per liter of T broth). The plating bacteria were always freshly grown daily and used in early log phase, the plates usually being incubated overnight at 37 C. All the media and buffers were always supplemented with CaCl₂ at 10^{-3} M (3).

Phage stocks. The preparation of phage stocks has been previously described (2a).

Preparation of naked post-FST DNA attached to bacteria. Routinely a preculture of the preceding day, arrested in late log phase and kept at 4 C, was diluted to about 5×10^7 bacteria/ml in MGM medium and incubated at 37 C on a gyratory shaker. When a concentration of 5×10^8 cells/ml was attained, the culture was centrifuged for 15 min at $5,000 \times g$ at 4 C and resuspended at 5×10^9 cells/ml in MGM-Ca²⁺ buffer. After a 15-min period of incubation at 37 C in a centrifuge tube without aeration, these bacteria, in which protein synthesis stopped (5), were infected by T5stO or T5 am15 at low multiplicity (MOI = 0.05). The incubation at 37 C was continued for 10 min to allow the injection of the FST fragment to take place (3, 4). After this procedure, the *E. coli*-T5 complexes were chilled in ice and a 0.1-ml sample was removed to determine the number of infectious centers; this number was taken as the 100% value in the estimation of infectious centers after centrifugation. At this time the *E. coli*-T5 complexes arrested at the FST stage were centrifuged at 4 C for 5 min at $6,000 \times g$ in a fixed-angle rotor (Sorvall SS34). The supernatant fluid was carefully decanted and discarded, and the pellet was very gently resuspended at 4 C according to the following method. The pellet was covered with 0.5 to 1.0 ml of MGM-Ca²⁺ buffer, and the centrifuge tube was slowly rolled in a slightly tilted position. After about 30 min, the pellet was found entirely resuspended; at that time, it was diluted to the initial

concentration of 5×10^8 bacteria/ml with MGM- Ca^{2+} buffer. This resuspended pellet contained a mixture of uninfected bacteria, T5-*E. coli* complexes with unbroken adsorbed capsids, and T5-*E. coli* complexes carrying a naked uncoiled DNA molecule (2a).

Study of the process of transfer of post-FST DNA. The resuspended pellet was divided into three samples, without pipetting, by use of microsize graduated plastic cylinders. The first one was incubated with 200 μg of pancreatic DNase per ml for 30 min at 37 C; the second one was sheared by 20 repeated passages through the capillary part of a Pasteur pipette (230-mm size, Wu Mainz); the third one was not treated but kept in ice. Each sample was then diluted into 9 vol of prewarmed MGM medium and incubated at 37 C in a water bath without agitation, but in a large flask so that the surface-thickness ratio of the liquid was very high, thus allowing a relatively good aeration. From the different diluted samples 0.1-ml samples were removed at different times to measure the number of infectious centers. We have verified that, in these conditions of dilution without forced aeration, a one-step growth experiment gives results virtually indistinguishable from those of a homologous experiment in an agitated flask (results not shown).

Kinetics of transfer of naked post-FST DNA. T5-*E. coli* complexes arrested at the FST stage were prepared as described above. A sample was removed and kept in ice as control A. The rest was centrifuged and gently resuspended in buffer to obtain naked post-FST DNA-bacteria complexes. This suspension was again divided in two parts: one of them was either pipetted or treated with DNase and constituted a second control B; the second one was kept in ice and provided the experimental sample C. At time zero, the three samples were carefully diluted in complete medium; samples were removed at different times from each dilution and plated. Thus the centrifuged, untreated sample C was in fact sheared at the times of dilution for plating, whereas the centrifuged, treated control B had been already sheared at time zero. The plot of the infectious titer versus incubation time in the centrifuged, untreated sample should then reflect the fact that the naked chromosomes become progressively protected against shear while entering the cells.

RESULTS

Evidence for the injection of naked post-FST DNA. When *E. coli*-T5 complexes arrested at the FST stage are centrifuged and resuspended under the carefully controlled conditions described in Materials and Methods (see also reference 2a) the portion of the viral DNA not yet injected may be found either normally wrapped in undamaged capsids or naked when the phage tail has been ruptured. In the first case this DNA must be insensitive to DNase or moderate shear, whereas in the second case both treatments will degrade it. It was of interest to determine whether the conditions allowing the injection of

the DNA of intact virions, i.e., the restoration of protein synthesis, would also permit the entry of the naked chromosomes. To test this hypothesis, there must be a one-to-one relation between the number of injected chromosomes and the number of observed infectious centers, a situation encountered at low MOI, so that there is less than 1 phage per bacterium. Therefore, if the number of plaques produced by a sample treated by DNase or pipetting (as described in Materials and Methods) is significantly different to the number of plaques produced by an untreated sample, this difference will be convincing evidence of an effective injection of naked T5 DNA and even a measure of the number of naked DNA molecules entirely injected.

Table 1 gives the results of such a comparison, each percentage being the mean from 12 independent and identical experiments. Considering these results, the salient features appear to be the following. (i) DNase or shearing treatments give fairly reproducible percentages of resistant capsids from one experiment to another. Hence the yield of naked DNA molecules during one centrifugation, in the well-defined conditions described in Materials and Methods, is about 60%. (ii) The untreated samples show a significant increase of 35% of infectious centers; there is almost a doubling with regard to the samples treated to eliminate the naked DNA. Therefore, there is a transfer of naked DNA into the host cell. It may be seen that the efficiency

TABLE 1. *Injection of naked post-FST DNA of T5stO in host cells^a*

Treatment before plating	Infectious centers ^b (%)	Confidence interval ^c (%)
200 μg of DNase per ml	40	4
Pipetting	41	4
No treatment	76	15

^a The different samples were prepared as described in Materials and Methods and analyzed for their capacity to produce infectious centers. Each percentage is the mean of 12 independent experiments made in exactly the same conditions with the same suspension of T5stO.

^b Measured as percentage of number present before centrifugation.

^c The confidence interval at 95% on 12 experiments was calculated by the formula: confidence interval = $1.96 \times \sigma / \sqrt{n - 1}$, with σ = the standard deviation and n = the number of independent experiments. The confidence interval reflects the homogeneity of the results obtained with the treated samples, by contrast with the relatively higher variability of the figures obtained with untreated samples.

of this transfer varies from one experiment to another in relatively great proportions. This is because it is impossible to master all the conditions allowing a perfect integrity of all the unrolled DNA molecules. Taking into account the mean values listed in Table 1, the mean yield of actual injection is about 60% for all the naked post-FST DNA molecules.

We have verified that the *E. coli*-T5 complexes blocked at the FST stage and not centrifuged are insensitive to highly concentrated pancreatic DNase (500 μ g/ml) and to repeated pipetting for the production of infectious centers.

Study of the kinetics of transfer of naked post-FST DNA. The same test of the influence of DNase or shearing treatments on the yield of infectious centers was used to study the kinetics of the transfer of post-FST DNA. From the results shown in Fig. 1 different points may be underlined. (i) The naked post-FST DNA is injected relatively quickly, the transfer lasting about 10 min and being nearly maximum after

FIG. 1. Kinetics of transfer of naked post-FST DNA. The number of infectious centers produced by untreated and centrifuged samples (—●—) was compared with the number obtained by DNase-treated or sheared centrifuged samples (—△—) and that obtained with noncentrifuged samples (—○—). For more details, see Materials and Methods. Zero time is defined as the time at which the dilution is made in the medium allowing protein synthesis. The other times are the times at which a sample is removed from the samples kept at 37°C. The number of infectious centers present in the noncentrifuged sample is taken as 100%.

only 5 min. All the DNA molecules which were not entirely transferred at 10 min are definitively lost. (ii) The particular increase of infectious centers corresponding to the transfer of naked post-FST DNA takes place a long time before the general increase in the number of plaques from 40 min, due to the lysis of early infected bacteria. (iii) This lysis happens at the same moment (40 min) in the different samples. Therefore, the sample in which the injection of naked DNA took place has not taken delay with regard to the other samples which have all the capsids undamaged.

We have verified that the duration of the protein synthesis block before adding the phage did not influence either the kinetics of transfer of post-FST DNA or the time of intracellular growth leading to the lysis of infected bacteria (data not shown).

Transfer of the naked T5 am15 DNA. The T5 am15 mutant is unable to make the transfer protein necessary for the injection of post-FST DNA and to degrade the bacterial DNA (7). It was isolated by Lanni et al. (8) and normally grows on *E. coli* HR34 (*su*⁺) but not in the F (*su*⁻) strain (7). This mutant was used in the same type of experiments we have already described to verify that the transfer of naked post-FST DNA requires the presence of the transfer protein. We have compared the efficiency of transfer of the naked DNA to produce infectious centers in the T5 am15/HR34 and T5 am15/F systems. In the first one, virtually the same results were encountered as in the T5stO/F system (Table 1). On the contrary, in the second one no appearance of infectious centers by possible injection of post-FST DNA occurred. Therefore, the transfer protein seems to be responsible for the entry of the post-FST DNA when this part of the T5 chromosome is naked and uncoiled, as well as when it is normally rolled in its capsid.

DISCUSSION

We have already described (2a) the obtainment of naked T5 DNA attached to the bacteria, the floating post-FST part being retained by the FST fragment previously injected. We have shown that, in many cases, this floating naked DNA can be transferred into the host cell if protein synthesis is restored. This statement is based on a simple experimental test devised to measure the extent of this transfer by the production of infectious centers, taking into account that this production is very sensitive to DNase treatment or repeated pipetting. We found by use of this test that about 60% of the intact naked DNA molecules can be transferred into

the host cell and produce infectious centers. On the contrary, we did not observe the entry of intact free T5 chromosome extracted by phenol into the centrifuged *E. coli*-T5 complexes arrested at the FST stage when protein synthesis is restored (data not shown). The described phenomenon is then limited to T5 DNA attached to the bacteria by the intermediary of its injected FST end.

The process of transfer of this entirely unrolled and freely floating DNA seems identical to the injection of the DNA rolled in its capsid. Indeed, the kinetics of transfer we obtained are nearly the same as were described by Lanni (3) for the rolled DNA. In both cases the injection is essentially finished about 5 min after the time of protein synthesis recovery. Another common requirement is the presence of the transfer protein (10), as is suggested by the experiment with the T5 am15 mutant.

Thus, the naked DNA can enter the host cells without additional requirements to those of the DNA rolled in its capsid. This fact seems to imply that no phage protein is necessary for the injection of post-FST part, without taking in account the internal proteins possibly injected with the FST fragment.

On the contrary, we have seen that the transfer protein is absolutely essential and this protein would seem to participate in a mechanism capable of drawing into the bacteria a DNA molecule about 15 times longer than the bacteria itself, even when this molecule is entirely unrolled. This fact raises questions about the accepted opinion on the mechanics of transfer of a viral DNA into its host cell. Until now the process of injection was generally conceived as only a passive phenomenon such as the contraction of the tail sheath of the T4 capsid (11). However, this example of the transfer of partially injected, naked DNA shows that, in some cases, it is rather active, without prejudging the actual mechanism of action of the transfer protein. To understand more fully this particular process of injection we must now isolate this transfer protein and study its mechanism of action in vitro.

We have shown a possible injection of total naked T5 chromosome. We did not measure the transfer of broken naked DNA molecules because it would appear that a test demonstrating such a transfer is more difficult than the simple measure of infectious centers for unbroken DNA molecules. However, such a system of injection of precisely sized fragments of T5 DNA prepared as already described (2a) could allow one to obtain direct information on the location of T5 functions. Such a mapping could then be compared with the results obtained by genetic methods (2, 7).

LITERATURE CITED

1. Beckman, L. D., M. S. Hoffman, and D. J. McCorquodale. 1971. Pre-early proteins of bacteriophage T5: structure and function. *J. Mol. Biol.* **62**:551-564.
2. Hendrickson, H. E., and D. J. McCorquodale. 1971. Genetic and physiological studies of bacteriophage T5. I. An expanded genetic map of T5. *J. Virol.* **7**:612-618.
- 2a. Labedan, B., Crochet, J. Legault-Demare, and Stevens. 1973. *J. Mol. Biol.* **75**:213-234.
3. Lanni, Y. T. 1960. Invasion by bacteriophage T5. II. Dissociation of calcium-independent and calcium-dependent processes. *Virology* **15**:514-529.
4. Lanni, Y. T. 1961. Invasion by bacteriophage T5. III. Stages revealed by changes in susceptibility of early complexes to abortive infection. *Virology* **15**:127-135.
5. Lanni, Y. T. 1965. DNA transfer from phage T5 to host cells: dependence on intercurrent protein synthesis. *Proc. Nat. Acad. Sci. U.S.A.* **53**:969-973.
6. Lanni, Y. T. 1968. First-step-transfer deoxyribonucleic acid of bacteriophage T5. *Bacteriol. Rev.* **32**:227-242.
7. Lanni, Y. T. 1969. Function of two genes in the first-step-transfer DNA of bacteriophage T5. *J. Mol. Biol.* **44**:173-183.
8. Lanni, Y. T., F. Lanni, and M. J. Tevethia. 1966. Bacteriophage T5 chromosome fractionation: genetic specificity of a DNA fragment. *Science* **152**:208-210.
9. McCorquodale, D. J., and Y. T. Lanni. 1964. Molecular aspects of DNA transfer from phage T5 to host cells. I. Characterisation of first step transfer material. *J. Mol. Biol.* **10**:10-18.
10. McCorquodale, D. J., and Y. T. Lanni. 1970. Patterns of protein synthesis in *Escherichia coli* infected by amber mutants in the first-step-transfer DNA of T5. *J. Mol. Biol.* **48**:133-143.
11. Simon, L. D., and T. F. Anderson. 1967. The infection of *E. coli* by T2 and T4 bacteriophages as seen in the electron microscope. I. Attachment and penetration. *Virology* **23**:279-297.