Evidence for the coupling of T7 DNA injection with its transcription during infection

S.K. Zavriev and S.M. Vorob'ev*

Institute of Applied Molecular Biology and Genetics, 125206 Moscow, and
**Institute of Medical Enzymology, 123242 Moscow, USSR*

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The kinetics of T7 phage DNA transfer from the virion into *E.coli* during infection in the presence of chloramphenicol was compared under the same conditions with the kinetics of its in vivo transcription. The results obtained allow us to conclude that these two processes are tightly coupled.

T7 phage *RNA polymerase* *DNA injection* *Transcription*

1. INTRODUCTION

In previous work we began investigating the mechanism of the virus DNA transport for the case of T7 phage infection [1–3]. The virion of this phage contains a linear double-stranded DNA molecule which consists of 3 temporal gene classes (see fig.1) and penetrates the cell wall in a polar fashion starting with the left end of the T7 chromosome [5]. Transcription of T7 DNA is initiated by the bacterial RNA polymerase on 3 strong A-promoters, located at the DNA left terminus [6]. Under normal infection conditions the host enzyme reads the class I genes only, while the class II and III genes are transcribed by the phage-induced RNA polymerase encoded in the T7 gene 1 [6]. It

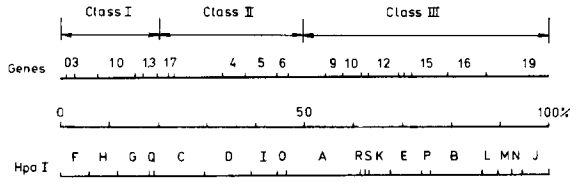

Fig.1. Maps of bacteriophage T7. (Top) The T7 genes are identified by the gene number and grouped according to their function and observed time of expression (see [3]). The *Hpa*I restriction map of the T7 DNA molecule is from [4].

was shown that during infection T7 DNA transfer is stepwise and depends on the *E.coli* RNA polymerase activity [2,3]. Some probable consequences of the proposed T7 DNA transport model as well as its agreement with the functional structure of T7 chromosome and with T7 development were discussed [3].

We continue here the study of the mechanism of T7 DNA transfer into the host cell. Our results show that the kinetics of the phage DNA entrance into the cells correlates with that of its transcription and both are independent of the presence of active restriction-modification cell systems.

2. MATERIALS AND METHODS

E.coli B or *E.coli* C culture was grown at 37°C in LB or low phosphate medium to 5×10^8 cell/ml. [^{3}H]Thymidine-labeled T7 phage suspension and T7 DNA were prepared as in [1]. Chloramphenicol was added to final concentration of 200 μg/ml 20 min before infection. T7 DNA penetration kinetics during infection was followed as in [1].

The in vitro synthesis of [^{14}C]uridine-labeled T7 RNA by *E.coli* RNA polymerase holoenzyme was carried out in 100 μl of a reaction mixture containing 0.15 M NaCl for 30 min at 37°C as in [7]. The ^{32}P labeling and isolation of T7 mRNA were done by a modification [7] of the method in [8].

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The *Hpa*I restriction fragments of T7 DNA were electrophoresed in 1.4% agarose gel [4], denaturated in situ, transferred on nitrocellulose filters, and hybridized with T7 RNA as in [9].

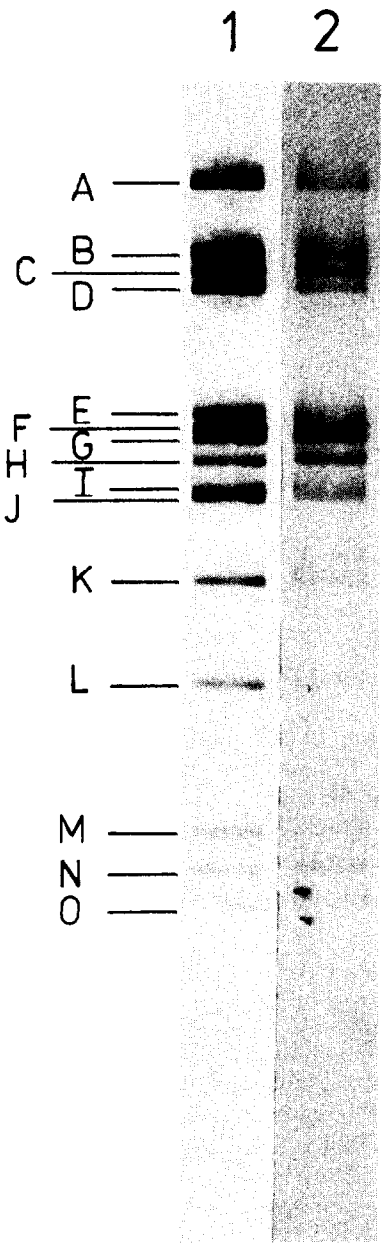

Fig.2. The *Hpa*I restriction fragments of T7 DNA hybridization with [^{14}C]RNA synthesized in vitro by *E.coli* RNA polymerase (lane 2); (lane 1) autoradiogram of *Hpa*I restriction fragments of [^{3}H]T7 DNA transferred on the filter strip: the strip was treated with 1 M sodium salicylate, dried, and autoradiographed.

3. RESULTS AND DISCUSSION

During infection in the presence of chloramphenicol the T7 DNA can be transcribed only by the host RNA polymerase. The termination of transcription by this enzyme takes place at the early gene transcription terminator located at 18.8% of the phage chromosome [6]. With some probability this terminator can be read through and the transcription of the template can continue. To examine how far can the T7 DNA be transcribed in this case we used the hybridization technique in [9] because the electrophoretic analysis of the RNA does not reveal from what parts of the DNA the analysed RNA was transcribed.

The results of the hybridization of the *Hpa*I restriction fragments of T7 DNA with [^{14}C]RNA synthesized by the *E.coli* RNA polymerase in vitro are shown in fig.2. Naturally the most intensive hybridization takes place with the early gene region – F, H and G fragments, corresponding to the first 20% of the T7 chromosome (see fig.1). It is also seen that the whole late gene region is transcribed, although with a lesser intensity. The presence of RNA corresponding to the right end of the tem-

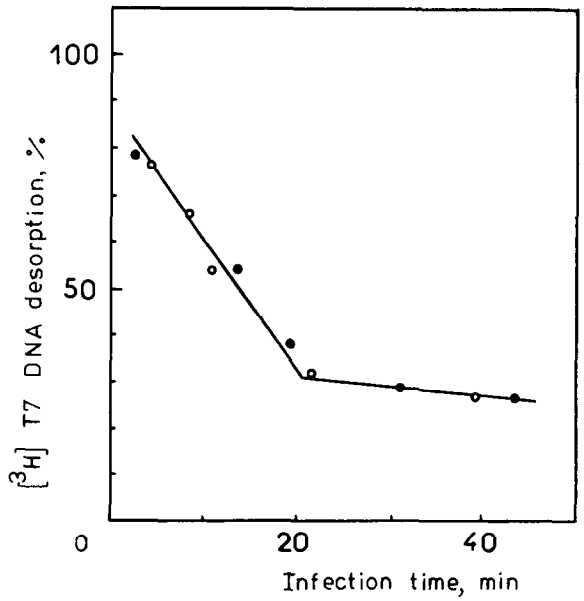

Fig.3. The kinetics of T7 DNA entrance into *E.coli* B (●) and *E.coli* C (○); 200 μg/ml chloramphenicol was added to the cell culture 20 min before the phage infection.

plate (bands M, N and J) cannot be explained by the transcription initiated from the E-promoter because at the ionic strength used (0.15 M NaCl) the initiation from this promoter does not occur [10]. Thus, in vitro the bacterial RNA polymerase is able to transcribe the whole T7 chromosome. It would appear that the observed [2,3] stop of the T7 DNA entrance during infection in the presence of chloramphenicol at the region of the late gene transcription terminator at 61% is provided as suggested in [3] by the specific interaction between the nucleotide sequence of this region with component(s) of the virion and/or cell wall, but not by the inability of the host enzyme to read through the late gene transcription terminator.

If during the T7 infection the 0.3 gene is not expressed, as in the case of the infection by the 0.3 mutant or in the presence of a translation inhibitor, the injected part of the phage DNA is cleaved by the host restriction system [6,7]. We investigated how the presence of an active restriction-modification cell system influences the T7 DNA entrance kinetics.

We compared the kinetics of phage DNA transfer during infection in the presence of chloramphenicol of two strains: *E.coli* B carrying the restriction-modification system, and *E.coli* C which lacks this system.

From the data presented in fig.3 one can see that the kinetics of phage DNA transfer into *E.coli* B and *E.coli* C cell fractions are identical. It means that the restriction of the transferring DNA has no

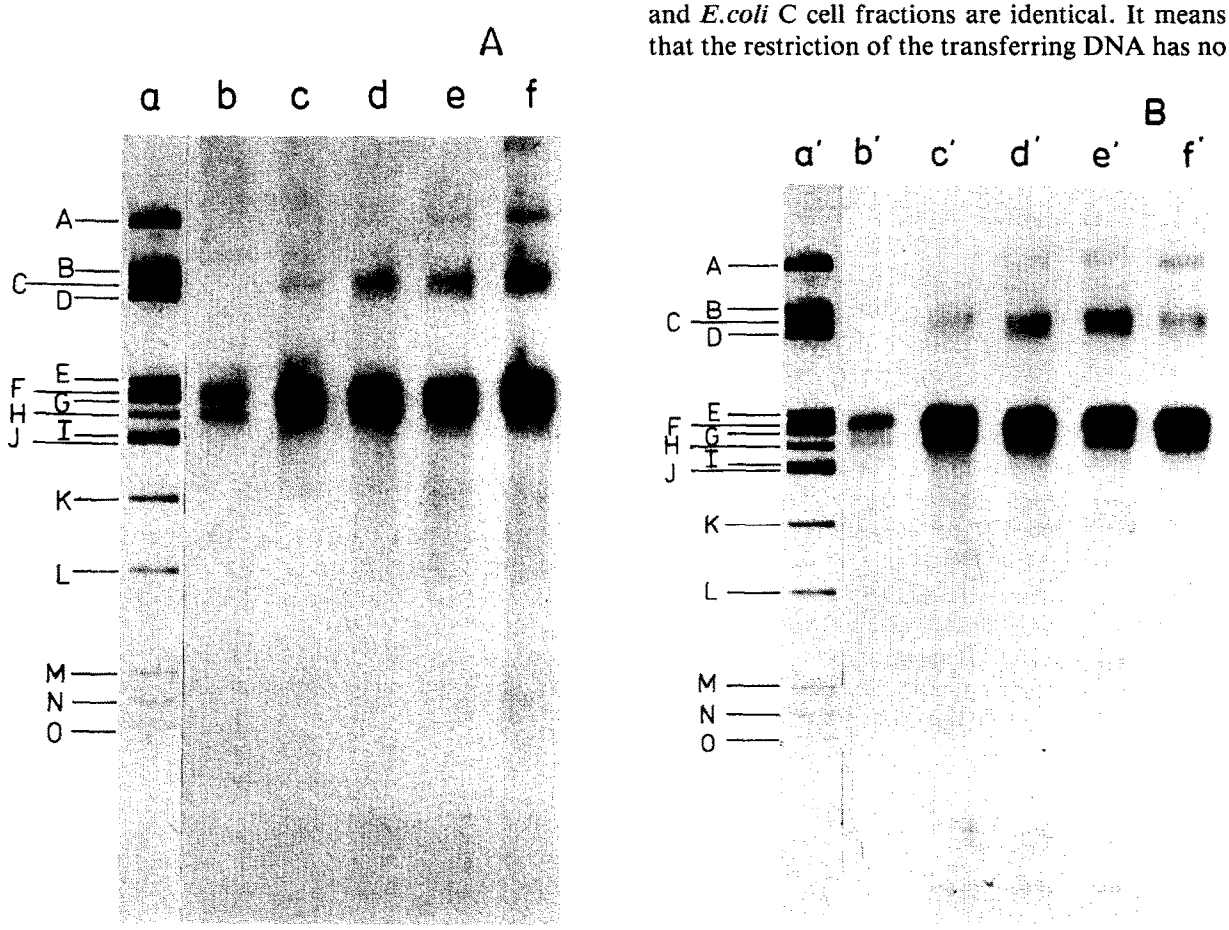

Fig.4. The kinetics of transcription of the phage DNA in *E.coli* B (A) and *E.coli* C (B) cells T7-infected in the presence of chloroamphenicol. Each lane in the figure is the autoradiogram of one filter strip; lanes a and a', autoradiogram of sodium salicylate-treated strip with transferred DNA fragments. The intervals during which incorporation of label took place in the infected cells were: lanes b and b', 0–5 min after infection; lanes c and c', 5–10 min; lanes d and d', 10–15 min; lanes e and e', 15–20 min; lanes f and f', 20–25 min.

effect on the transport process. If the suggestion is right that the T7 DNA transfer during infection is coupled with its transcription, the restriction can take place only after the DNA has been transcribed at least once. Such a situation can occur only when molecules of the host RNA polymerase, which participate in the T7 DNA penetration, are close to the inner surface of the cytoplasmic membrane, and only the transcribed parts of the T7 DNA are available for the restriction enzymes.

To determine whether the T7 DNA injection is coupled with its transcription by the bacterial RNA polymerase, we compared the kinetics of these two processes. Identification of the transcripts was done by the hybridization technique of the in vivo-synthesized [^{32}P]RNAs with *Hpa*I restriction fragments of T7 DNA. The results of this hybridization with pulse-labeled RNAs isolated from *E.coli* B and *E.coli* C cells infected in the presence of chloramphenicol are presented in fig.4. Firstly it must be pointed out that both hybridization patterns are almost identical. This observation additionally indicates that the presence of host restriction-modification systems does not directly influence the T7 DNA transport process.

From fig.4 it is seen that in the first minutes of infection only the transcripts corresponding to the left end of the T7 chromosome (F and H fragments, see fig.1) appear. Within 10 min of infection the RNAs corresponding to G and C fragments appear, by 15 min the D-band is clearly pronounced and the A-band appears whose intensity reaches its maximum by 20 min of infection. This indicates that the gradual entrance of the left ~60% of T7 DNA into the cell fraction takes place simultaneously with its transcription. It should be noted that under the infection conditions used even within the interval of infection from 20 to 25 min in both infected strains the whole left 60% of T7 chromosome is transcribed. This indicates that at the late periods of infection in the presence of chloramphenicol the early gene transcription is re-initiated.

Thus, from the present results and data in [1–3] we can conclude that within 20 min of infection in the presence of chloramphenicol the first ~60% of the T7 chromosome are gradually transferred into the cells and simultaneously transcribed by the *E.coli* RNA polymerase, i.e. these two processes are tightly coupled.

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