INFLUENCE OF THE DELETIONS OF A_2–A_3 PROMOTERS OR A TERMINATOR OF EARLY GENES UPON THE RATE OF T7 DNA ENTRANCE INTO *ESCHERICHIA COLI* CELL

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1. Introduction

The process of T7 DNA transfer from the virion into *Escherichia coli* cell depends on the host RNA polymerase activity [1,2]. Indeed, only 10% of T7 genome, i.e., ~50% of the early gene region penetrates into the *E. coli* cells in presence of rifampicin. In contrast, with chloramphenicol almost all DNA enters gradually the cell fraction. It takes ~20 min at 37°C. These data testify to the direct involvement of *E. coli* RNA polymerase in the process of T7 phage DNA transfer into the cell. Specifically, the phage DNA transfer could be directly coupled with its transcription by the host RNA polymerase (early region) and possibly by T7 RNA polymerase in the absence of antibiotics.

Here, the transfer rates of wild-type T7 phage DNA and its mutants, D111, (deletion of the early promoters A_2, A_3 and B region) and D53 (deletion of the early transcription termination region) [3] into *E. coli* cells are studied. In the presence of chloramphenicol the D111 deletion decreases and D53 deletion increases the rate of this process. These data support the direct involvement of *E. coli* RNA polymerase in the process of T7 DNA transfer into *E. coli*. It can be also suggested that T7 DNA has specific sequences responsible for a stepwise injection of the phage DNA into the cell.

2. Materials and methods

T7 phage mutants were from Dr F. W. Studier (Brookhaven National Laboratory). [^{3}H]Thymidyne-labelled T7 phage suspensions were prepared as in [1].

T7 DNA penetration into the host cell was traced in the following way. *Escherichia coli* C culture was grown at 37°C in LB medium to 5×10^8 cells/ml, then the antibiotic was added to final concentration of 200 μg/ml in the case of rifampicin or chloramphenicol and of 50 μg/ml in the case of tetracycline. After inhibition of the culture growth by the antibiotic (30–40 min) the labelled phage was added with a multiplicity of infection 0.5–1.0. Aliquots of 10 ml were removed at the indicated time intervals, quickly chilled in ice bath, and harvested by centrifugation to remove the unadsorbed phage. The cells were resuspended in 5 ml 10^{-3} M $MgCl_2$, 10^{-3} M $CaCl_2$ and the adsorbed phage was desorbed from the cell surface using the ultrasonic treatment to split off virus DNA which did not enter the cells [1,2]. Percent of T7 DNA which did not enter the cell fraction was determined by the radioactivity of the split off DNA fragments which remined in supernatant after centrifugation of the ultrasonic-treated cell suspension.

The in vitro T7 RNA synthesis and its electrophoretical analysis in 2% polyacrylamide–0.5% agarose gel was done as in [4]. The reaction mixture (50 μl) containing 1.25 μg RNA polymerase holoenzyme (~100% active molecules according to [5]) and 5 μg T7 DNA was preincubated 8 min at 37°C to form the stable promoter complexes. The RNA synthesis was started by adding 4 NTPs plus rifampicin (final conc. 5 μg/ml). After 10 min incubation at 37°C the reaction was stopped by EDTA.

3. Results and discussion

Fig.1a shows the dependence of the wild-type T7 [^{3}H]DNA % desorbed by ultrasound from the time

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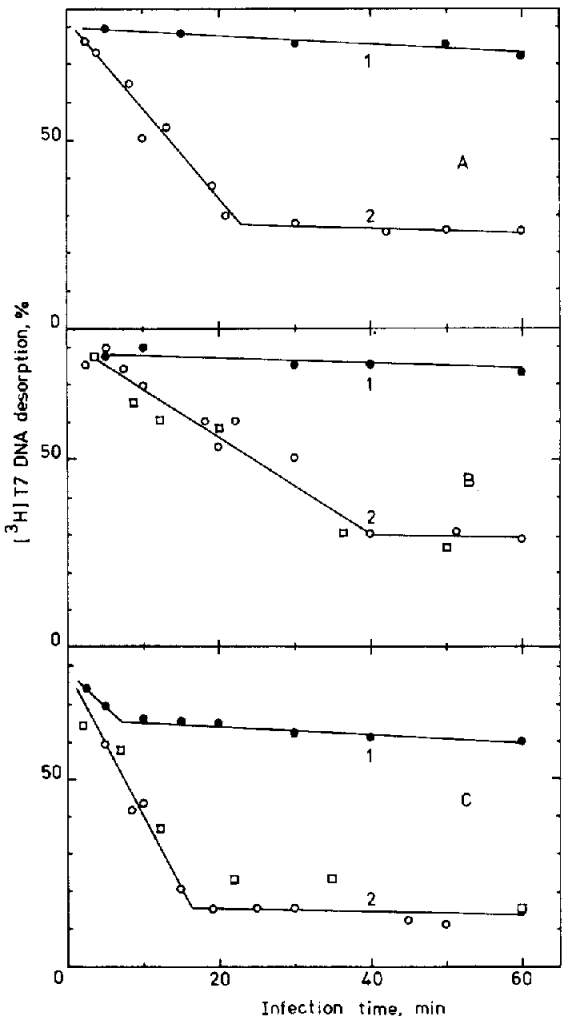

Fig.1. The kinetics of T7 DNA entrance into *E. coli* cell fraction: (A) wild-type; (B) D111 mutant; and (C) D53 mutant; (○) 200 μg chloramphenicol/ml; or (●) 200 μg rifampicin/ml; or (■) 50 μg tetracycline/ml; were added to the cell culture 30 min before the phage infection. See section 2 for details.

after infection. It can be seen that in the presence of rifampicin just after infection ~20% of the virus DNA remains in the cell fraction, and its further transfer is stopped (curve 1). A part of T7 DNA, irreversibly bound to the cells after phage adsorbtion–desorbtion in presence of rifampicin, is in the form of intact molecules while the remaining part is present as the left end fragment of the T7 chromosome, which corresponds to nearly a half of the early gene region [2]. The presence of intact T7 DNA in the cell fraction appears to be a result of incomplete desorbtion of the phage from the cell walls due to the short tail may hamper the desorbtion.

Further entering T7 DNA into the cell is blocked by rifampicin and can be observed in the absence of this antibiotic. Fig.1a (curve 2) shows that further T7 DNA transfer into the cell can be observed in the presence of chloramphenicol over 20–25 min. Unfortunately it was impossible to detect the T7 DNA transfer under normal conditions in the absence of antibiotics due to the virus-induced cell wall destabilisation immediately after the infection which causes the cell destruction during the procedure of phage desorbtion by ultrasound.

However the phage DNA entering the cell was not blocked when the protein synthesis was inhibited by chloramphenicol or tetracycline (fig.1a–c, curves 2). Thus the observed rifampicin-dependent block does not result from the phage early gene expression. It means that the early gene products may not be necessary for the T7 DNA injection into the cell. Similar and other experimental results provided the basis for the supposition that the process of the T7 DNA transport from the virion into the *E. coli* cell is tightly coupled with its transcription [1,2].

In a further experiment we attempted to obtain additional evidence for such a mechanism. We proceeded from the assumption that, if a transcription-dependent mechanism of T7 DNA transport really exists, the deletions of phage DNA regulatory sequences must affect the kinetics of its transfer from the virion into the cell. For this purpose the kinetics of DNA transport for two deletion mutants of T7, D111 and D53 phages [3], was investigated. The deletion D111 spares the early promoters A_2, A_3 and B region (1.3–4.1% region of the T7 chromosome) while the deletion D53 comprises the early genes transcription terminator (18.3–21.15% region).

As it can be seen on the fig.1b (curve 2) the D111 mutant DNA enters the cell ~2-times more slowly (35–40 min) than the T7 wild-type DNA (20–25 min). It means that the rate of T7 DNA transfer into the cell depends on the intensity of its transcription by the *E. coli* RNA polymerase. Essentially, the transcription of D111 DNA starts at one major A_1 promoter. This strongly decreases the number of RNA polymerase molecules which simultaneously transcribe the early gene region. Perhaps the reason of the observed difference in the rates of the DNAs transfer is a result of more rapid read-through of the terminator due to more extensive transcription of wild-type T7 DNA

because it initiates at the 3 strong A promotors.

On the other side, the T7 DNA enters the cell faster (15–20 min) if the transcription terminator deletion mutant D53 instead of wild-type phage is used (fig.1c, curve 2). It is clear, that this effect is a direct consequence of the absence of the readthrough mechanism during the transcription of the whole D53 mutant phage DNA molecule. Thus the delay of T7 DNA entrance from virion into the cell in the case of D111 mutant and the acceleration in the case of D53 mutant give a new evidence of involvement of the *E. coli* RNA polymerase in the process of T7 DNA transfer into the host cell.

Another interesting fact emerges from the results presented in fig.1. In the presence of rifampicin D53 phage mutant introduced into the cell fraction more DNA than the wild-type or D111 phages. Moreover, the extra D53 DNA entered into the bacterium not at once, but over 5–7 min. This result suggests that the transcription terminator or some other neighboring nucleotide sequence, including in the D53 deletion, is an important element of a mechanism which stops T7 DNA molecule transport before the class II genes early in the infection. Interaction between this sequence and some component of the virion or of the bacterial cell wall could provide a basis of this mechanism. Meanwhile, only first 35% but not the total DNA molecule of D53 mutant phage entered the cell fraction in the presence of rifampicin (fig.1c, curve 1). This fact suggests the existance of another region in T7 DNA responsible for temporal retardation of the DNA transport into the cell. It is necessary to remember here that a second transcription terminator at 30.1% from the left end of T7 DNA molecule was found [6]. The position of this terminator is in a good correlation with D53 DNA portion which enters the cells fraction in the presence of rifampicin. Thus at least two sites appear to be on T7 DNA molecule which are responsible for its stepwise entrance into the host cell.

Fig.2 shows an electrophoretical distribution of the labelled RNA, synthesized by purified *E. coli* RNA polymerase holoenzyme on T7 DNAs of wild-

Fig.2. Electrophoresis of RNAs synthesized by *E. coli* RNA polymerase holoenzyme on T7 DNA templates of wild-type (a), D111 mutant (b) and D53 mutant (c) in 2% polyacrylamide–0.5% agarose gel. See section 2 for details. (A,A',C,D,E) transcripts corresponding to respective promoters; (r-t) readthrough transcript.

type and above-mentioned mutants. In accordance with data of [4] several RNAs which are initiated at A_{1-3}, B, C, D, and E promoters of the wild-type T7 DNA and terminated at the early gene transcription terminator, can be seen there (fig.2a). Slowest RNA is originated from the terminator read-through case. Deletion D111 gives rise to a more moderate transcription of the T7 DNA and to slightly shorter RNA molecules which are initiated at the A_1 promoter (fig.2b). Majority of the D53 DNA-directed RNA synthesized consists of the very large RNA molecules forming due to the terminator deletion (fig.2c). Therefore the transcriptional properties of DNAs of the used mutants do serve the purpose of the above experiments.

Thus the results obtained gave supporting evidence for our hypothesis [1,2] about the direct involvement of the host RNA polymerase in the process of T7 DNA transfer from the virion into *E. coli* cell. At the same time they imply a rather complicated mechanism of the phage DNA transport process including in particular elements which give rise to the stepwise DNA penetration. Such a mechanism for the transfer of T7 DNA appears to be closely connected to the temporal control of the T7 gene expression. For example, this mechanism can explain the consequent transcription of the II and III class genes by T7 RNA polymerase.

In view of this mechanism for T7 DNA transport previously incomprehensible facts about interrelations between T7 phage and *E. coli* restriction systems become clear. T7 DNA has a number of recognition sites for *E. coli* restriction nucleases but is not cleavaged by these enzymes in spite of the sites being unprotected by the methyl groups [7]. This is because the early 0.3 gene product is an effective inhibitor of the *E. coli* restriction-modification system. This discovery gives a good explanation why the parental T7 DNA is not attacked by restriction enzymes and the progenic one is not methylated after the 0.3 gene expression. Still it was not clear why the restriction enzymes had no possibility to cleave the unprotected parental T7 DNA before 0.3 protein appears. The proposed mechanism of a gradual T7 DNA transfer allows us to clear the point: 0.3 gene has time to be completely expressed and inhibit the host restriction system before the first restriction site of the T7 DNA enters the host cell.

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