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RNA polymerase-dependent mechanism for the stepwise T7 phage DNA transport from the virion into E. coli

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ABSTRACT

The influence of rifampicin, streptolydigin, tetracycline,and chloramphenicol on phage DNA transport from the T7 virion into the *E. coli* cell was studied. It has been found that the DNA transport proceeds in at least three stages. During the initial stage the phage injects into the host cell the left ~10 per cent of its DNA molecule. The entrance of the next 50 per cent of T7 DNA molecule is blocked by inhibitors which block transcription but not translation. Moreover, the entrance time of this part of the T7 DNA increases in the case of the T7 mutant D111 (which contains a deletion of the A_2 and A_3 promoters) and decreases in the case of the D53 mutant (which contains a deletion in the region of the early gene transcription terminator). It would appear, that the second stage of the phage DNA transport is tightly coupled with its transcription and that a mechanical function is carried out by RNA polymerase. The translation inhibitors completely block the entrance of the remaining 40 per cent of the T7 DNA molecule (class III genes) into the host cell. It would appear that some class I and (or) II gene product(s) are obligatory components of the final stage of T7 DNA transport. Some probable consequences of this virus DNA transport model as well as its agreement with the functional structure of T7 chromosome and with T7 development are discussed.

INTRODUCTION

DNA transfer from the virion into the host cell is a key event in the development of all DNA-containing bacteriophage. However this problem has been studied in detail only for the T-even phages.The tail of these phages has a special contractile apparatus which allows for the rapid injection of its DNA into the host cell after adsorption to the cell-wall |1|. It is probable that the mechanism by which DNA is transferred from the viral particle to the host cell will differ in the case of other unrelated bacteriophage.

We have attempted to study the mechanism of virus DNA transport in case of T7 phage infection. The virion of this phage contains a linear double-stranded and terminally-redundant DNA molecule |2,3|. During infection this DNA penetrates through the cell-wall in a polar fashion starting with the left end of the T7 chromosome |4|. The phage chromosome consists of three temporal gene

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classes whose distance from the left terminus and expression time during infection show a good correlation with each other |2,3|. Transcription of T7 DNA is initiated by the *E. coli* RNA polymerase on three strong A-promoters disposed at a distance ~1.5% from the DNA left terminus |2| (see Fig. 1). The bacterial enzyme reads the class I genes only, while the class II and III genes are transcribed by the virus-induced RNA polymerase encoded by the T7 gene 1 |5|. The first promoters for T7 enzyme lie at a distance ~15% from the left end of the T7 DNA |6|.

All of these facts have led us to propose that T7 phage has an unique mechanism for its DNA transport into the host cell with RNA polymerase as an important participant. We assumed that the RNA polymerase property of directional movement along the template may enable it to carry out a mechanical function during the DNA transport process.

The results obtained suggest the direct involvement of the *E. coli* RNA polymerase in the process of T7 DNA transport from the virion into the host cell and reveal some details of the transport process. Preliminary reports of this investigation have been published |7-9|.

MATERIALS AND METHODS

Bacteria, Phage, and Culture Media. *E. coli* 18-3 ($T7^s$, rif^r, stl^r) carrying a rifampicin and streptolydigin-resistant RNA polymerase |10|, its isogenic

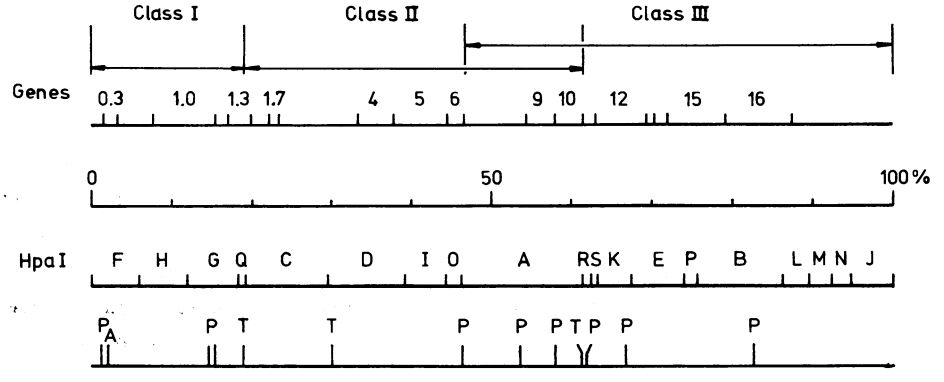


Fig. 1. Maps of bacteriophage T7. At the top, the T7 genes are identified by gene number and grouped according to their function and observed time of expression |22|; the alignment of physical and genetic maps has been derived from various sources (see |3|). P_A - A_1, A_2, and A_3 promoters; P - T7 RNA polymerase-specific promoters; T - terminators. The Hpa I restriction map of the T7 DNA molecule is from McDonell et al. |13|.

precursor *E. coli* RTS 522 ($T7^S$, rif^S, stl^S), and *E. coli* C were obtained from Dr. S.Z.Mindlin.The deletion mutants of bacteriophage T7 - D111 and D53 |11| were generously provided by Dr. F.W.Studier. $|^3H|$ thymidine- or $|^{32}P|$-labelled phage suspensions were prepared by a procedure described earlier |12|.

In all experiments bacteria were propagated at 37^o to $5 \cdot 10^8$ cells/ml in medium containing per liter: 10 g Bacto-tryptone, 5 g Bacto-yeast extract (Difco), and 10 g NaCl. The cells were infected at a multiplicity of one phage particle per cell.

Antibiotics. The following antibiotics were used: tetracycline (Medprom, USSR), chloramphenicol (Boehringer), rifampicin (Sigma), and streptolydigin (Lepetit). The antibiotic was added to the cell culture at a final concentration of 200 μg/ml except tetracycline which was presented at 50 μg/ml.

Quantitative determination of phage DNA which had not entered the cell. Following antibiotic inhibition of culture growth (20-40 min after its addition) the cells were infected with the $|^3H|$ DNA-labelled phage and at intervals after infection, 10 ml portions of the culture were withdrawn, quickly chilled in an ice bath, and harvested by centrifugation to remove unadsorbed phage. The cells were resuspended in 5 ml of 10^{-3} M $MgCl_2$, 10^{-3} M $CaCl_2$, 10^{-2} M NaCl (solution A) and the adsorbed phage particles were desorbed from the cell surface using sonication to split off virus capsids containing the DNA which had not entered the cells |7|. The ultrasound disintegrator UZDN-1 (USSR) was used at 22 kHz for phage desorption. The efficiency of desorption was examined using $|^3H|$ protein-labelled T7 phage. Cell integrity was determined by assaying for turbidity. The ultrasonic conditions which provided maximal phage desorption without cell destruction were experimentally selected in the frequency region close to but not equal to the resonance region.

The per cent of T7 DNA which did not enter the cells was determined by the radioactivity of the DNA fragments which remained in the supernatant after centrifugation of the sonicated cell suspension. In all experiments the supernatant radioactivity of cells which had not been sonicated was counted and subtracted; it did not exceed 10% of the total infected cell suspension radioactivity.

Sedimentation analysis of intracellular phage DNA fragments. To an *E. coli* C cell culture (200 ml) inhibited by rifampicin or chloramphenicol $|^{32}P|$ T7 phage was added. At intervals following infection 50 ml portions of the culture were withdrawn, quickly chilled, harvested by centrifugation to remove the unadsorbed phage and resuspended in 5 ml of solution A. The phage particles were desorbed by sonication. The cells were collected by centrifugation, resuspend-

ed in 2 ml of 25% sucrose, 0.01 M tris-HCl pH 8, 0.1 M NaCl, 10^{-2} M EDTA and treated with 2 mg/ml lysozyme (Boehringer) for 15 min at 4^{o}. The spheroplasts were lysed with 0.2% SDS and the *E. coli* DNA was removed by centrifugation for 40 min at 20000 rpm at 4^{o}. The supernatant was treated for 20 min at 30^{o} by 100 μg/ml RNase (previously heated at 100^{o} for 10 min). DNA was extracted with phenol and then precipitated with 2 vol. 95% ethanol. The DNA pellet was dissolved in 150 μl of 0.02 M tris-HCl pH 8, 0.01 M NaCl, 1 mM EDTA, and centrifuged in a 5 to 20% sucrose gradient in the Spinco SW-50 rotor for 4 hours at 40000 rpm at 5^{o}. Fractions of 5 drops each were collected and their radioactivity was determined.

Restriction analysis of the phage DNA which did not enter the cells. An *E.coli* C cell culture (50 or 200 ml) inhibited by rifampicin or chloramphenicol was infected with |^{3}H| DNA-labelled phage. At intervals after infection 40-50 ml portions of the culture were withdrawn, quickly chilled, and harvested by centrifugation to remove the unadsorbed phage. The cells were resuspended in 20 ml of solution A and recentrifuged . To completely remove the unadsorbed or weakly adsorbed phage particles the cells were washed in solution A twice. The cells were then resuspended in 5 ml of solution A and the phage were desorbed as described above. In the experiments with cells infected in the presence of chloramphenicol, the washing and desorption was carried out in solution A containing 100 μg/ml chloramphenicol.

After the desorption procedure the cells were centrifuged (30 min and 5000 rpm), and 4 ml of supernatant containing the desorbed phage particles and the T7 DNA fragments which were not transferred into the cell fraction were carefully removed. The DNA was extracted with phenol, precipitated with 2 vol. 95% ethanol, and collected by centrifugation in the Spinco SW-40 rotor for 40 min at 30000 rpm at 2^{o}. The pellet was resuspended in 200 μl of 20 mM tris-HCl pH 7.4, 10 mM $MgCl_2$, 6 mM KCl, and 1 mM DTT (buffer B). Then 25 μl of 3 M sodium-acetate, pH 5 and 0.8 ml of ethanol was added. After chilling in a dry ice-ethanol bath the DNA was precipitated again, centrifuged (15000 rpm at 4^{o}), and the precipitate was washed with 1 ml of 70% ethanol. The pellets were dissolved in 25 μl of buffer B and incubated for 2 hours at 37^{o} with HpaI restriction endonuclease. The reaction was stopped by addition of 3.5 μl of a mixture containing 0.1 M EDTA, 0.1% SDS, 60% glycerol, and 0.05% bromphenol blue.

The HpaI restriction endonuclease was used because it cuts T7 DNA into rather small fragments (Fig. 1) which are easily separated by electrophoresis in a 1.4% agarose gel |13|. Electrophoresis was carried out as described in |13|. The gel slabs were washed with distilled water for 20 min, treated with

1 M sodium salicylate |14|, dried, and autoradiographed with a preflashed |15| HS 11 ORWO film.

The autoradiographs were scanned on a PMQ II Opton densitometer.

RESULTS

Participation of *E. coli* RNA polymerase in transport of T7 phage DNA. If *E.coli* RNA polymerase directly participates in T7 DNA transport from the virion into the *E. coli* cell, specific inhibitors of the enzyme will block the transport but translation inhibitors will not. In Fig. 2 it can be seen that the penetration of labelled phage DNA in the presence of chloramphenicol (open circles) starts immediately after infection and finishes at 20-25 min. By this time about 2/3 of T7 DNA is in the cell fraction while another 1/3 can be desorbed from cells up to 110 min after infection. The entrance of T7 DNA into the cells is inhibited by rifampicin both in the absence (open squares) and in presence (dark squares) of chloramphenicol. This inhibition is observed only with the host strain *E. coli* RTS 522 carrying a normal rifampicin-sensitive RNA poly-

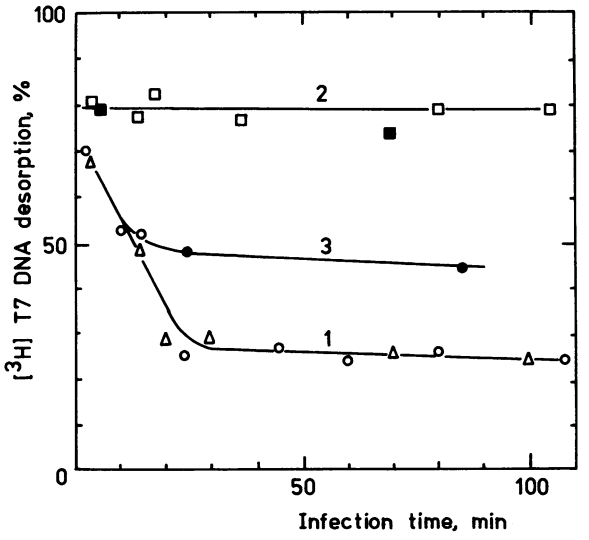


Fig. 2. Dependence of |^{3}H| thymidine-labelled T7 phage DNA uptake into cell fraction upon its incubation time with *E. coli* RTS 522 cells infected in presence of chloramphenicol (o - o), rifampicin (□ - □), and with the both antibiotics together (■ - ■) as well as infected in presence of chloramphenicol with following addition of streptolydigin after 8 min of infection (● - ●), or with *E. coli* 18-3 cells infected in presence of both chloramphenicol and rifampicin (Δ - Δ).

merase. However, rifampicin does not prevent T7 DNA transfer into cells of an isogenic strain mutant *E. coli* 18-3 containing a rifampicin-resistant RNA polymerase (triangles, Fig. 2). Moreover, the previously started process of T7 DNA penetration into the cells can be blocked by streptolydigin (dark circles). It is known that this antibiotic inhibits the host RNA polymerase during elongation as well as at initiation of transcription |16|.These results suggest a direct dependence for T7 DNA transfer on the presence of active host RNA polymerase. The possibility that the rifampicin blockage is a consequence of the expression of some early gene(s) inhibition whose product(s) is(are) necessary for DNA transfer is excluded because transfer is not suppressed by chloramphenicol. Similarly DNA transfer is not inhibited by tetracycline (see Fig. 3) which is known to be a more effective inhibitor of translation.

Thus, T7 DNA transport depends on the host RNA polymerase itself but not on phage gene expression. It seems most probable that RNA polymerase pulls the T7 DNA into the cell simultaneously with its transcription. However, it can not be excluded that RNA polymerase molecule or at least its β subunit participates in some other way in the DNA transport mechanism independent of transcription. To support or to refute this possibility we investigated the in-

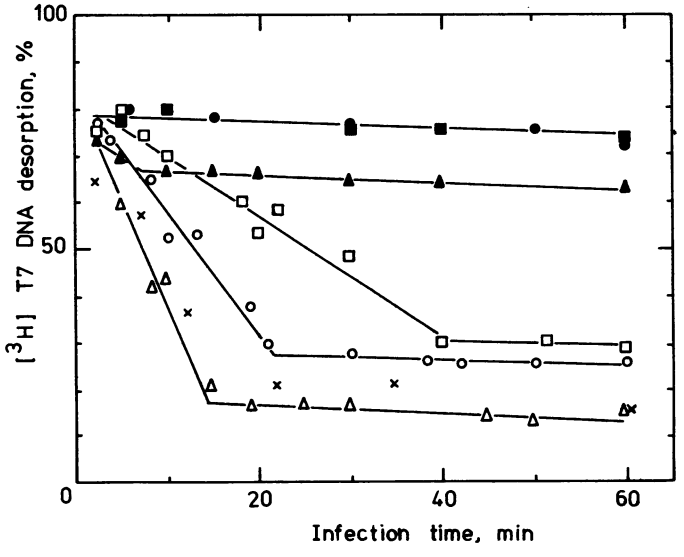


Fig. 3. Entrance kinetics of T7 DNA of wild type phage (circles), D111 mutant (squares), and D53 mutant (triangles and crosses) into *E. coli* cells infected in the presence of chloramphenicol (open figures), tetracycline (x), or rifampicin (dark figures).

fluence on phage DNA transport of mutations affecting the transcription control elements in T7 DNA. With this aim we determined the kinetics of DNA entrance for two deletion mutants of T7 phage: (i) D111 deleted in the A_2 and A_3 promoters, and (ii) D53 containing a deletion of the early gene transcription terminator |11|.

In Fig. 3 it can be seen that the DNA entrance time of the D111 mutant is approximately twice longer when compared with the wild type phage. In contrast the DNA entrance time of the D53 mutant is about 1.5 times shorter than in the case of the wild type. The delay of DNA transfer in the absence of the A_2 and A_3 promoters seems to be a result of reduction of the number of RNA polymerase molecules simultaneously transcribing the same T7 DNA molecule, and its acceleration in the case of the D53 mutant is probably a result of a free passage of RNA polymerase from class I to class II genes during transcription in the absence of early gene terminator at 18.8% |17|. It would appear that deletions of transcription control elements in a T7 DNA molecule affecting the general rate of its transcription simultaneously have definite effects on the rate of its transport. These data exclude the possibility of a T7 DNA transport mechanism where RNA polymerase participates in a non-transcriptional mode. Thus T7 DNA transport from the virion into the cell depends on the functional activity of both transcriptional components: RNA polymerase and T7 DNA itself and the data support a model for the tight coupling of the injection process with transcription.

It can be seen in Fig. 2 and 3 that some of the T7 DNA still enters the cell in the presence of rifampicin; ~20% in the case of wild type T7 DNA or the D111 mutant and ~30% in the case of the D53 mutant. At the same time in the presence of chloramphenicol ~1/3 of T7 DNA does not enter the cell even after prolonged incubation after infection. Providing that the data obtained thus far are not merely a consequence of the conditions used for desorption of the phage, the results suggest that the entrance of the T7 DNA into the cell is stepwise.

Stepwise penetration of T7 DNA into *E. coli* cell. In order to further elucidate the mechanism of DNA transport it was necessary to establish what parts of T7 DNA molecule enter the cell under different conditions. Initially we attempted to analyse the |^{32}P| T7 DNA isolated after phage desorption from the T7-infected *E. coli* C cells in the presence of rifampicin or chloramphenicol. Fig. 4 shows that after sucrose gradient centrifugation each preparation of intracellular labelled DNA is separated into two peaks, one of which coincides with intact T7 DNA. The presence of the full-size T7 DNA could result from

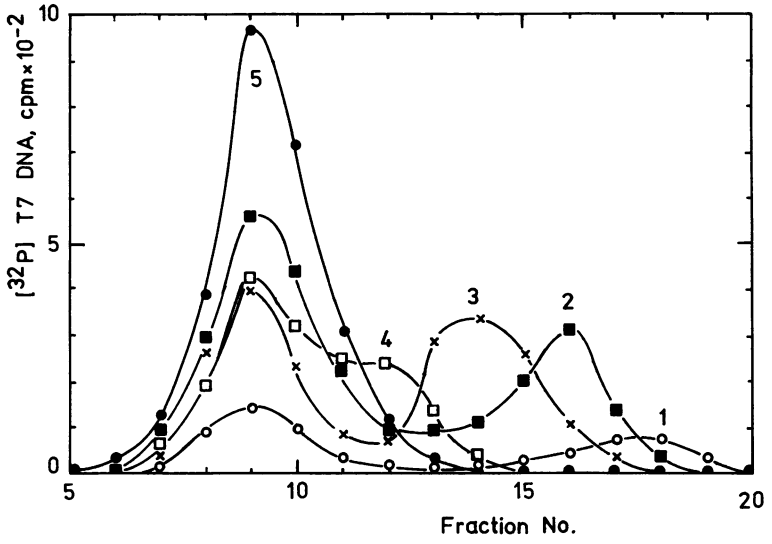


Fig. 4. Sucrose gradient centrifugation of phage DNA isolated from |^{32}P| T7-infected *E. coli* C cells after phage desorption at different times of infection: 1 - 20 min of infection in presence of rifampicin; 2 - 2 min, 3 - 6 min, and 4 - 20 min of infection in presence of chloramphenicol; 5 - intact T7 DNA.

spurious binding of incompletely desorbed phage. The position of the second peak depends on the conditions of infection. This variable fraction consists only of short T7 DNA fragments when DNA is isolated from cells infected in presence of rifampicin. When the cells were infected in presence of chloramphenicol the molecular size of the variable DNA fraction was dependent on the infection time. The shortest fragments were observed early in infection and the longest after 20 min of infection.

HpaI restriction analysis of the variable DNA fraction revealed that in presence of rifampicin the phage introduces into the cell only a short left-end piece of its DNA |8|. In the presence of chloramphenicol a progressive increase in the size of the intracellular leftward end of the T7 DNA was observed. However the presence of intact T7 DNA in the cell fraction created considerable difficulties in determining the maximum size of that part of the T7 DNA molecule which could enter the cell in the presence of chloramphenicol. To avoid these difficulties we decided to analyze the T7 DNA remaining out of the cellular fraction after sonication of the infected cells.

Before this analysis it was necessary to establish the extent of T7 DNA

destruction during ultrasonic treatment of the infected cells. To determine this T7 DNA in solution A (10 μg of DNA per ml) was sonicated under the conditions used for phage desorption. At intervals 1 ml aliquots were taken and the DNA was precipitated with ethanol. The precipitate was dissolved in 40 μl of buffer B and divided into two equal portions, one of which was incubated with HpaI. Electrophoretic analysis of these samples shows (Fig. 5) that sonication fragments the DNA and that the degree of this fragmentation is proportional to the time of sonication. However fragmentation is not very extensive and even sonication for 20 min did not change the HpaI-specific electrophoretic distribution of the T7 DNA fragments. Therefore we were certain that partial fragmentation of non-entered T7 DNA due to sonication will not distort the results (12-14 min ultrasonic treatment is quite enough for complete phage desorption |7|) and restriction analysis of desorbed DNA will allow one to follow penetration of T7 DNA into the host cell.

The electrophoretic distribution of HpaI fragments of that part of T7 DNA molecule which was desorbed from *E. coli* C cells infected with wild type T7 phage in the presence of chloramphenicol is shown in Fig. 6. One can see that soon after infection at first bands F and H lessen in intensity and disappear

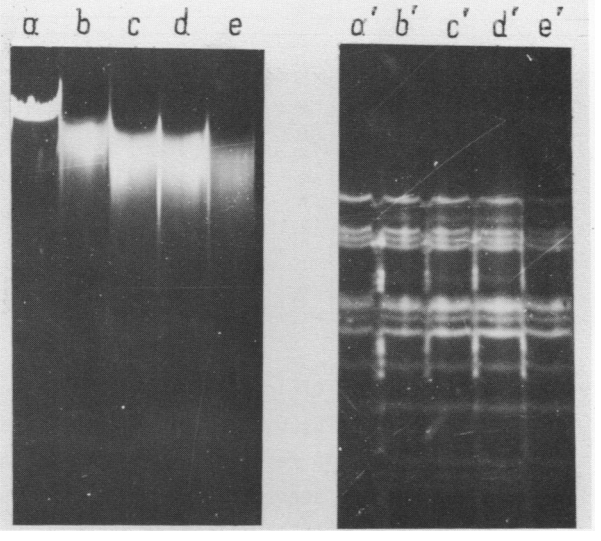


Fig. 5. 1% agarose gel electrophoresis of T7 DNA sonicated for: a - 0 min, b - 5 min, c - 10 min, d - 15 min, and e - 20 min; a'-e' - the same samples digested with HpaI . The gel was stained with ethidium bromide and visualised under UV light.

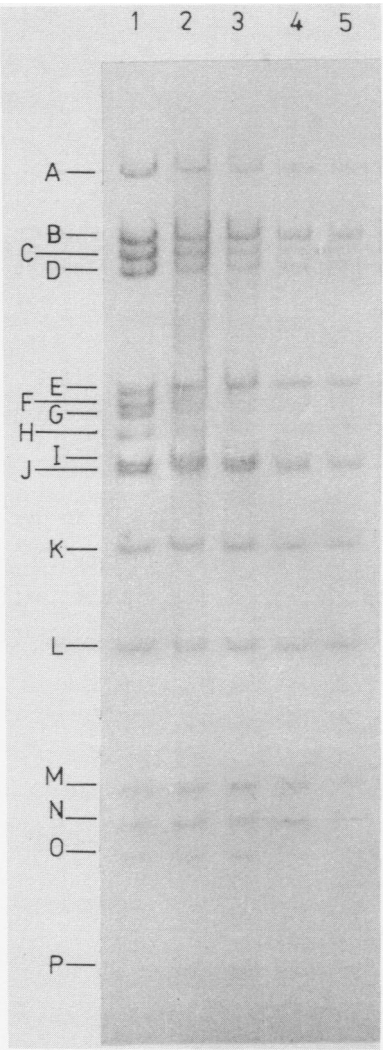


Fig. 6. 1.4% agarose gel electrophoresis of $|^{3}H|$ T7 wild type DNA fragments obtained after digestion with HpaI of native DNA (lane 1), and DNA remaining in the supernatant after phage desorption at 5 min (lane 2), 10 min (lane 3), 15 min (lane 4), and 20 min (lane 5) of infection in the presence of chloramphenicol.

(~5 min of infection). After 10 min of infection band G disappears and bands C and D lessen. Within 15 min bands C, D, I and O disappear and band A lessens. Finally by 20 min in the desorbed DNA restriction digests the A band also disappears and only those bands can be seen which correspond to HpaI fragments of the rightward 40% of the T7 DNA molecule (bands K, E, P, B, L, M, N, and J; see also Fig. 1). This result was more clearly seen when lanes 1 and 5 shown in Fig. 6 were scanned (Fig. 7): the peaks corresponding to the rightward 40% of the chromosome fragments are very pronounced but all peaks corres-

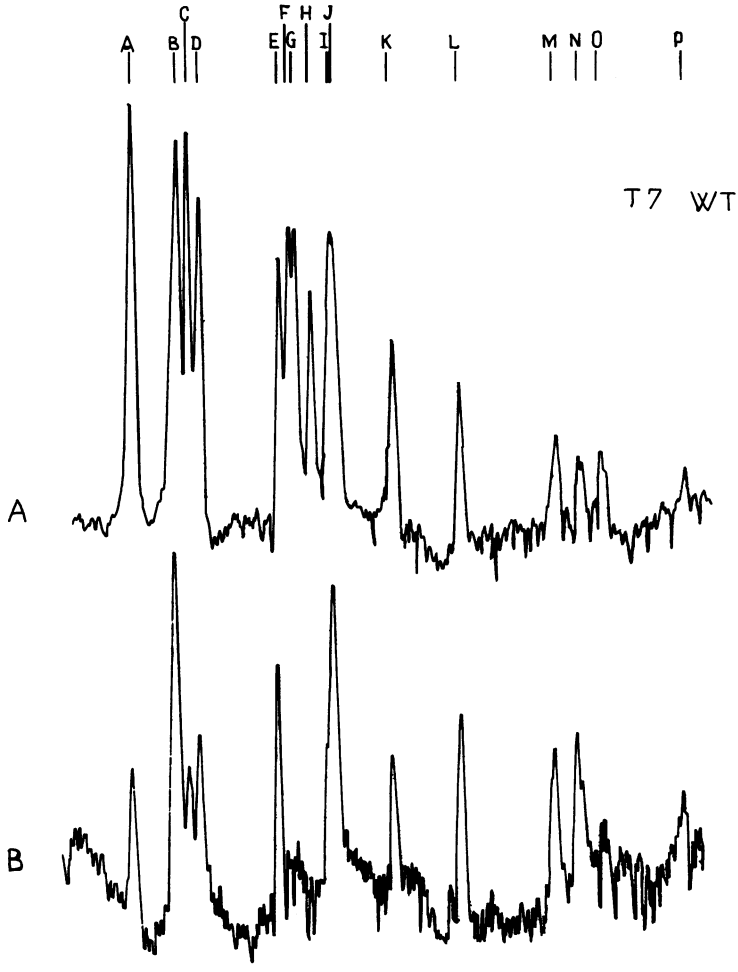


Fig. 7. Densitometric scans of lane 1 (A) and 5 (B) of the radioautograph shown in Fig. 6.

ponding to fragments of the leftward 60% of the phage chromosome have background values after 20 min of infection. The pattern (Fig. 6, lane 5) was not changed until 60 min of infection.

Similar results were obtained for the deletion mutants D111 and D53 with only one difference: the penetration time of the leftward 60% of the T7 DNA took 30-35 min in the case of the D111 mutant and 15 min in case of the D53 mutant instead of 20 min as seen with the wild type phage. This is in good agreement with the results presented in the preceeding section (see

Fig. 3).

Thus, the results presented in Fig. 6 and 7 show that only the leftward 60% of the T7 DNA molecule can enter the host cell when translation is inhibited. This takes about 20 min and after that the DNA transport process stops. The stop point sequence lies somewhere in the region of HpaI R and S fragments (see Fig. 1), i.e. at 61-63% of the chromosome because the A fragment does and the K fragment does not enter the cell at the stop point of DNA transfer. It is of interest to note that in this region of the T7 DNA there is a terminator (61%) which terminates class II gene transcription |18|.

A completely different picture was observed when T7 phage infection of *E. coli* was carried out in the presence of rifampicin. In this case it was established that even after 30 min of infection almost the entire T7 DNA molecule was removed after phage desorption. On the electrophorogram of the desorbed and HpaI-digested wild type T7 DNA (Fig. 8a) one can see all of the bands with a normal intensity and only the F band with a lowered intensity. The same result was obtained with the D111 mutant but in the case of D53 mutant both F and H bands lessened in intensity or disappeared from the HpaI digest of the extra-cellular T7 DNA (Fig. 8b).

Thus, the results of these experiments indicate that in the presence of rifampicin the T7 phage introduces into the cell only a small piece of the left end of its DNA molecule which is not greather than the HpaI F fragment. At the same time the size of the introduced end probably increases in the case of the D53 mutant since the H fragment also disappears.

DISCUSSION

The results obtained reveal that T7 DNA transfer from the virion into the host cell is a rather complicated process consisting of at least three major stages each having a different mechanism. This conclusion is based on the following facts: (i) the initial injection of the small piece of leftward arm of the T7 chromosome (~ 10% of the molecule) is independent on transcription and translation, (ii) the ensuing transfer of the next 50% of the phage DNA depends on transcription , and (iii) the final 40% of the molecule enters the cell in a manner dependent on translation, i.e. probably on complete expression of some gene(s) of class I and(or) II.

The data are consonant with a direct coupling of phage DNA transport with its transcription at the second stage of the process. This tight coupling derives from the demonstration that this stage depends on the transcriptional activity of both the *E. coli* RNA polymerase and the transferred T7 DNA. This

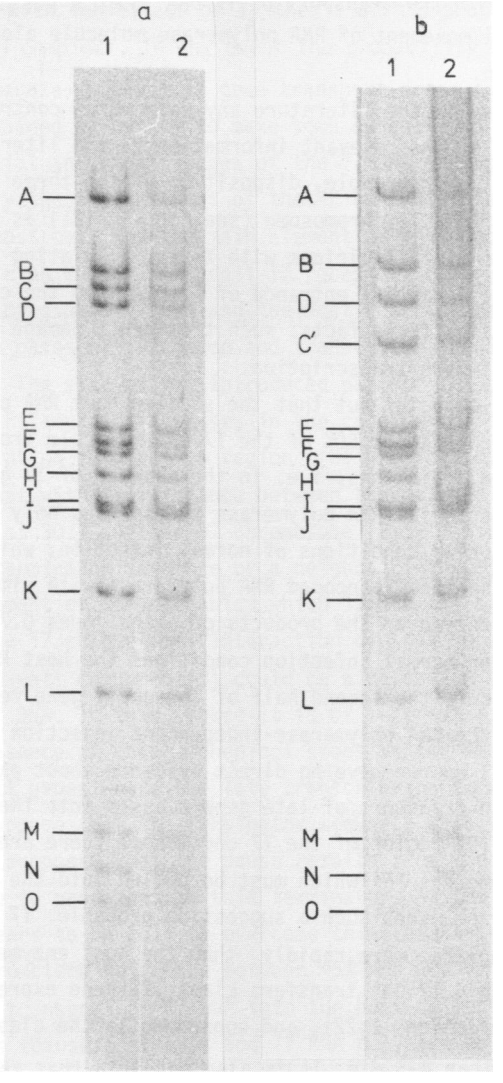


Fig. 8. 1.4% agarose gel electrophoresis of [^{3}H] T7 wild type DNA (a) and D53 mutant DNA (b) obtained after digestion with HpaI of native DNA (1) and DNA remaining in the supernatant after phage desorption at 20 min of infection in the presence of rifampicin (2).

was more apparent after HpaI restriction analysis of the T7 DNA which was desorbed from *E. coli* cells infected under different conditions. Based on these data we would propose that RNA polymerase must be capable of carrying out a

mechanical function in the transport of T7 DNA. Such a possibility follows from the directional movement of RNA polymerase molecule along the template during transcription.

We could not find in the literature any data which contradict such a model. In fact much of the relevant information in the literature tend to agree with our model. For example, disposition of the three strong A-promoters at the left end of the T7 chromosome (see Fig. 1, |2|) as well as data that the moderate treatment of T7 virions with X-ray, alkylating or depurinating reagents causes only a partial entrance of T7 DNA into the cell |19-21|. The proposed model explains these facts: such treatments damage the DNA structure preventing its continuous transcription.

It is necessary to point out that the studied host RNA polymerase-dependent transfer of the leftward 60% of the T7 DNA molecule proceeded in the presence of translation inhibitors, i.e. in the absence of T7 gene expression. It is known that the bacterial RNA polymerase transcribes only the first 20% of the T7 chromosome during conditions of normal infection, while the remaining 80% is transcribed by the T7-encoded RNA polymerase with simultaneous inactivation of the host enzyme by the products of phage genes 0.7 and 2 |3|. Hence it follows that under normal infection conditions the host RNA polymerase can pull into the cell only the second half of the early gene region while taking into consideration the RNA polymerase-independent injection of the first half of this region. Till now we have no direct evidence about participation of the T7 RNA polymerase in transport of late gene classes into the cell. However the fact that near the 15% region of the T7 chromosome there are a few T7 RNA polymerase-specific promoters |6| which must be pulled into the cell by the *E. coli* RNA polymerase makes such a suggestion probable. T7 RNA polymerase transcribes the template more rapidly than the host enzyme |2,3|. Therefore it can provide a rapid T7 DNA transfer: class III gene expression starts from the 8 min after infection |2,22|, and consequently the class II gene transfer must take less than 4-5 min. It is also possible that the coupling of transcription-translation may cause a quick entrance of T7 DNA under normal infection conditions.

It cannot be excluded that chloramphenicol blockage of the final 40% of the T7 DNA molecule transfer is connected not with T7 RNA polymerase but with some other virus-encoded proteins necessary for the RNA polymerase-independent DNA transport. It is possible that the penetration of the final part of the T7 DNA molecule is coupled to its replication which has been noted during the process of bacterial conjugation |23|. In this case the chloramphenicol block

would be connected with inhibition of synthesis of the protein of the virus-encoded replication complex . In any event the probability of the participation of the T7 RNA polymerase in class II gene transport seems to be rather high.

The results obtained allows us to make some suggestions regarding the details of the initial and final stages of the T7 DNA transport mechanism. It is evident that only the initial part of the T7 DNA molecule is actively introduced into the host cell and the main element of this mechanism is a component of the T7 virion and(or) the cell T7 receptor. Otherwise the interrelations between the introduced T7 DNA and the host restriction systems would be not so different in cases of infection and transfection as it was found by Erlich et al. |24|. The size of the introduced part of the phage DNA molecule in the presence of rifampicin increases in the case of the deletion of the early gene transcription termination region (|8| and Fig. 8b). Moreover this increase is consonant with the distance between the deleted terminator and the second one located at 30.1% of the T7 chromosome |2,3|. This suggests that the transcription terminator sequence or a neighbouring nucleotide sequence is an important element of the mechanism which limits the size of initially injected part of the T7 chromosome. Interaction of this sequence with some virion and(or) cell wall component(s) could be a basis for the observed stepwise transfer of T7 DNA. In this regard it is of interest to point out that DNA entrance in presence of chloramphenicol stops at the boundary region of the class II and III genes where the late transcription terminator is located. Therefore we can suppose that the terminator sequence also takes part in this pausing. It can be supposed that the pause results from the inability of the host RNA polymerase to pass through this terminator. At the same time the T7 RNA polymerase appears to be able to overcome this terminator when it starts transcription at promoters which are between 45% and 60% of T7 DNA |3,25,26|. Thus, the stepwise transport of T7 DNA into the host cell could be provided with temporary stops (pauses) of host and T7 RNA polymerases at the phage DNA transcription terminators. These stops could become stronger or more effective owing to interactions of the terminators with virion or cell-wall components.

It is important to note that the proposed stepwise transcription-dependent mechanism of T7 DNA transfer provides a good model for the temporal control of T7 gene expression during phage development. In particular it can explain the mechanism of the switch over of class II to class III gene transcription which was not understood previously |27|. Unfortunately we were not able to study the penetration of T7 DNA into the cell in absence of antibiotics because of the destabilization of *E. coli* cell-wall caused by some early gene

expression soon after infection |7|. Nevertheless the data obtained are sufficient to believe that the prolonged entrance of the phage DNA takes place during normal infection also thus providing the basis for a new principle of temporal control mechanism of gene expression.

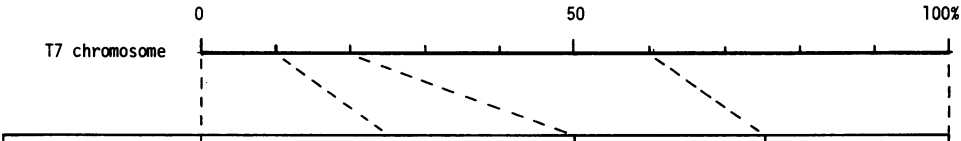
From the standpoint of the model proposed for T7 DNA transport previously unexplained facts about the interrelation between T7 phage and *E. coli* restriction systems are clarified. It is known that T7 DNA has a number of recognition sites for *E. coli* restriction endonucleases |3,28|. However this DNA is not cleaved by these enzymes *in vivo* even though the sites are not protected by methyl groups. This is because some early gene products, in particular the gene 0.3 product are effective inhibitors of a number of host restriction-modification systems |29,30|. This discovery provided a good explanation why the parental T7 DNA is not attacked and the progeny DNA is not modified by this system after 0.3 gene expression. However it was not clear why the restriction enzymes do not digest the unprotected parental T7 DNA before the 0.3 protein appears. The proposed mechanism allows a resolution of this question: the 0.3 gene has time to be completely expressed and to inhibit the host restriction system before the first restriction site of T7 DNA enters the cell. In this regard it is interesting to note that when a phage has a special mechanism for providing a rapid injection of the whole DNA molecule (T-even phages) the DNA is protected from the nuclease action before injection.

The initial injection of a small piece of the T7 DNA molecule left-end acquires a special significance because this part of the phage genome contains the 0.3 gene, and first restriction sites for a number of T7-permissive *E. coli* strain-specific endonucleases are beyond this part of the T7 DNA (see |3|). This provides for subdividing class I genes into two subclasses: immediate early and delayed early (to conform with current terminology). The immediate early genes are injected actively while delayed early genes are pulled into the cell through the participation of the *E. coli* RNA polymerase.

The results obtained and discussed are presented diagramatically in Fig. 9.

The proposed mechanism for T7 DNA transport into the host cell during infection is compatible with the data presented and differs radically from other known mechanisms for virus DNA transfer. We propose that the same or similar mechanisms are utilized by other phages in which chromosomes have a functional structure similar to T7, such as T3 and other related phages |2,3,31,32|.

It can not be excluded that the known delay of T5 DNA entrance after the 8% of the left end of the DNA molecule is introduced into the cell during T5 phage infection |2| has a close functional relation with the case examined



Genes	Class I		Class II	Class III
	immediately early	delayed early	late	
Transport mechanism:				
with rifampicin	injection	no transport	no transport	no transport
with chloramphenicol	injection	*E.coli* RNA pol.-dependent	*E.coli* RNA pol.-dependent	no transport
without antibiotics (supposed)	injection	*E.coli* RNA pol.-dependent	T7 RNA pol.-dependent (?)	unknown
Transport dependence on T7 gene products	independent	independent	dependent on gene 1.0 (?)	dependent

Fig. 9. Summary of the data regarding the mechanism of the uptake of T7 DNA during infection of *E. coli*.

for the T7 phage.

We are continuing to investigate the mechanism of DNA transfer to host in other phage systems as well as extending our studies on T7 phage.

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