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The Terminal Redundant Regions of Bacteriophage T7 DNA

Their Necessity for Phage Production Studied by the Infectivity of T7 DNA after Modification by Various Exonucleases

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Summary. Some aspects of the involvment of the terminal redundant regions of T7 DNA on phage production have been studied by transfection experiments with T7 DNA after treatment of the molecules with λ exonuclease or λ exonuclease plus exonuclease I. It was found that terminal 5′gaps between 0.08 and 6.4% of the total length did not decrease the infectivity of the molecules although such gaps cannot be filled directly by DNA polymerases. Rather, compared to fully native DNA the infectivity of gapped DNA increased up to 20 fold in *rec*$^+$ spheroplasts and up to 4 fold in *recB* spheroplasts. This indicates a protective function of the single-stranded termini against the *recBC* enzyme in *rec*$^+$ and possibly another unidentified exonuclease present also in *recB*. The possibility that spontaneous circularization of the gapped molecules in vivo provides protection against exonucleolytic degradation was tested by transfection with T7 DNA circularization in vitro by thermal annealing. Such molecules were separated from linear molecules by neutral sucrose gradient centrifugation. They displayed a 3 to 6 fold higher infectivity in *rec*$^+$ and *recB* compared to linear gapped molecules, which shows that T7 phage production may effectively start from circular DNA.

When the 3′single-stranded ends from gapped molecules were degraded by treatment with exonuclease I the infectivity of the molecules was largely abolished in *rec*$^+$ and *recB* as soon as 40 to 80 base pairs had been removed per end. It is concluded that the terminal regions of T7 DNA molecules are essential for phage production and that the redundancy comprises probably considerably less than 260 base pairs. The results are discussed with respect to the mode of T7 DNA replication.

Introduction

The main components of the DNA replicating machinery in T7 infected cells of *Escherichia coli* are coded by the viral DNA. Extensive studies on T7 DNA replication in vivo and in vitro have revealed many if not all of the gene products required for replication and have identified their specific roles in this process (for references see Hausmann, 1976). Moreover, electronmicroscopy as well as studies on DNA sedimentation have provided fundamental information on the structural aspects of T7 DNA replication. It appears that, in contrast to the replication of many other bacterial viruses which proceeds via circular intermediates, T7 DNA is replicated as a linear monomer during the first round of replication (Wolfson et al., 1972; Dressler et al., 1972) and also as a linear concatemer during late stages of the intracellular phage development (for references see Hausmann, 1976). The role of these concatemers in replication, particularly their formation and their sizing during phage maturation are not yet understood. Studies on the structure of the concatemers indicated that they are formed by end to end annealing of T7 DNA by means of the terminal redundant regions (Ritchie et al., 1967; Schlegel and Thomas, 1972). On the basis of these results and the fact that DNA replication generally proceeds discontinuously with RNA primers to provide the 3′OH start points for DNA synthesis (Okazaki et al., 1968) Watson (1972) proposed a scheme on T7 DNA replication which postulated that concatemers are essential intermediates in T7 phage production. The scheme considers that the replication of a linear duplex DNA will produce daughter molecules each having one terminal 5′gap in the newly synthesized strand in the position where the most terminal RNA primer was removed (removal of internal primers will leave gaps which can be easily repaired by DNA polymerase and ligase). These terminal gaps cannot be filled by any known DNA polymerase due to the general dependence of these enzymes on 3′OH primers. Upon further replication of the daughter molecules a successive loss of genetic material from the ends would result. However, alignment of the daughter molecules by their exposed com-

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plementary base sequences at the terminal redundant regions will produce one dimeric molecule which may, after further replication, form aggregates such as tetramers, octamers etc. With respect to the sizing of concatemers into unit length molecules during phage maturation, Watson (1972) postulated a specific endonucleolytic activity. This is supposed to introduce staggered nicks at the ends of an "internal" redundant region. Now a DNA polymerase may synthesize with strand displacement (Masamune and Richardson, 1971) at the nicks and thereby will physically separate the molecules while completing native terminal redundant regions.

We have studied some of the structural requirements for T7 DNA replication and phage production using the following approach. The ends of T7 DNA were made single stranded or were completely removed by treatment with specific exonucleases and the biological activity of the resulting structures was assayed by transfection of *Escherichia coli* spheroplasts. The results show that the terminal redundant regions are essential for phage production. Terminal 5'gaps do not impair the infectivity but rather improve the infectivity in cells with an active *recBC*-enzyme. Evidence will be presented that phage production will effectively start from in vitro circularized T7 DNA. The results will by discussed with respect to the mode of T7 DNA replication.

Materials and Methods

Bacterial and Phage Strains. Spheroplasts for transfection were prepared from *E. coli* AB1157 *rec*$^+$ and its derivative AB2470 *recB21* (Howard-Flanders and Boyce, 1966). AB1157 was the plating indicator throughout. Transfecting DNA was isolated from T7$^+$ obtained from Dr. F.W. Studier.

T7 DNA. ^{3}H-thymidine-labeled T7 DNA was isolated as previously described (Seroka and Wackernagel, 1977). The specific radioactivities of the preparations were 4.3×10^6 cpm/μmol nucleotide for the experiment in Figure 1 and 2.9×10^6 cpm/μmol for the other experiments.

λ Exonuclease. The enzyme was isolated according to Little et al. (1967). The preparation had a specific activity of 31,000 units/mg protein (one unit degrades 10 nmol DNA at 37° in 30 min under the conditions described below) and did not contain β-protein (Radding and Shreffler, 1966) as judged by polyacrylamide gel electrophoresis in the presence of sodium-dodecylsulfate (Radding et al., 1971). Saturation of linear double-stranded DNA with λ exonuclease required 9.5 polypeptides per DNA end, a result which is in accord with published data (Little, 1967; Radding and Carter, 1971).

Exonuclease I. Isolation of this enzyme from *E. coli* 1100 was as reported by Lehman and Nussbaum (1964). For this work the hydroxylapatite fraction was used which had a specific activity of 10,000 units/mg protein when assayed with heat denatured DNA of phage P22. One unit degrades 1 nmol DNA at 37° in 30 min under the conditions described below.

Partial Degradation of T7 DNA with Exonucleases. ^{3}H-thymidine labeled T7 DNA was degraded by λ exonuclease in a mixture consisting of 67 mM glycine-KOH, pH 9.6, 1 mM EDTA, 5 mM $MgCl_2$, 3 mM β-mercaptoethanol and between 8 and 90 μg/ml T7 DNA. λ exonuclease was added at a 5 fold excess over the amount required for saturation which was determined as being 7 units for 10 nmol T7 DNA. Time and temperature were adjusted to obtain the desired degradation (e.g. 1% degradation was usually obtained after 13 min at 6° C). After the reaction EDTA was added in an equimolar amount to complex the Mg^{++}, and the mixture was heated for 3 min at 75° to inactivate the enzyme. A sample was removed for measuring the amount of nucleotides soluble in 5% trichloroacetic acid. Determination of radioactivity was as described (Wackernagel and Hermanns, 1974). The sample size was adjusted to the expected amount of acid soluble radioactivity in order to obtain a reliable number of counts in the scintillation counter (generally between 200 and 500 cpm).

In some experiments the DNA was subsequently treated with exonuclease I. For this purpose the reaction mixture was adjusted to 6 mM $MgCl_2$ and exonuclease I was added at 0.05 units per 10 nmol of T7 DNA. The reaction proceeded for 30 min at 37° and was stopped by addition of an excess of EDTA over the $MgCl_2$ followed by heating for 3 min at 75°. Acid soluble radioactivity was again determined to control that the additional amount of degraded DNA matched that produced by λ exonuclease in the first reaction. DNA preparations were stored at +4°. They were used directly for transfection which involved an at least 40 fold dilution.

Circularization of T7 DNA by Thermal Annealing. T7 DNA was partially degraded by λ exonuclease, then diluted to 2 μg/ml with H_2O and adjusted to 2 × SSC (standard saline-citrate: 0.3 M NaCl, 0.03 M sodiumcitrate). The solution was heated for 45 min at 65°, then for 45 min at 55° and finally cooled down to 40° over a period of two hours. After dialysis against 10 mM Tris-HCl, pH 7.5, with 1 mM EDTA the solution was concentrated 10 fold by blowing nitrogen over the surface.

Neutral Sucrose-Gradient Centrifugation. Linear gradients were prepared from 5 to 20% sucrose in 10 mM Tris-HCl, pH 7.5 and 1 mM EDTA. Centrifugation was in an SW 56 rotor of a Beckman L2-65B ultracentrifuge at +4°. The sample size did not exceed 200 μl. Centrifugation was for 2.5 h at 56,000 rpm. The tubes were punctured at the bottom. About 40 fractions were collected per gradient.

Transfection of Spheroplasts. Preparation of spheroplasts and transfection were performed as reported (Wackernagel, 1972) with the modifications applied for T7 DNA (Seroka and Wackernagel, 1977). Samples from sucrose gradients were used directly for transfection, since it has been shown that the presence of sucrose does not inhibit the infectivity of DNA in this bioassay (Wackernagel, 1972). The efficiency of transfection is defined as the number of infected spheroplasts per DNA molecule. Each experiment with exonuclease treated DNA included a control transfection with native T7 DNA as a standard for the determination of relative infectivities.

Results

1. Effect of Terminal 5'Gaps on the Infectivity of T7 DNA

Terminal 5'gaps in DNA molecules cannot be filled directly by any known DNA polymerase. Repair of

such gaps would require one or several RNA primers laid down on the template to provide the 3'OH groups necessary for the gap-filling DNA synthesis. It is generally assumed that in vivo RNA primers are subsequently removed by specific enzymes (DNA polymerase I and/or RNase H in *Escherichia coli*) and that the final covalent closure of the DNA poses no enzymatic problem. However, after removal of the most terminal primer a 5'gap would still remain at the end of the otherwise duplex DNA molecule. According to Watson's hypothesis on the replication of T7 DNA, replication of a molecule with 5'terminal gaps larger than 50% of the terminal redundancy should not produce concatemers containing complete molecules, which, in turn, would prevent phage production.

We introduced terminal 5'gaps of various length into T7 DNA molecules by limited digestion with a saturating amount of λ exonuclease. The DNA molecules were then transfected into *E. coli* spheroplasts and the formation of plaques was scored. For these experiments spheroplasts of two different *E. coli* strains were used as recipients: a rec^+ strain (AB1157) and a *recB* derivative of this strain (AB2470) lacking a functional *recBC*-enzyme (Wright, Buttin and Hurwitz, 1971; Goldmark and Linn, 1972). It has been shown earlier that the transfection efficiency of T7 DNA increases as much as about 100 fold in spheroplasts lacking the *recBC*-enzyme (Benzinger et al., 1975; Seroka and Wackernagel, 1977; Wackernagel et al., 1977). The reason for this effect is probably the following: the transfecting molecules are inactivated in the spheroplasts by the *recBC*-enzyme before a T7 specific function is expressed which normally inhibits the *recBC*-enzyme a few minutes after T7 infection (Wackernagel and Hermanns, 1974).

Figure 1 shows the infectivity of λ exonuclease treated T7 DNA relative to untreated DNA plotted against the length of the terminal 5'gaps (between 0.08 and 6.4% of the total length). Clearly, terminal 5'gaps in no case reduced the infectivity of T7 DNA molecules, a result which is consistent with earlier observations (Ehrlich et al., 1976; Wackernagel et al., 1977). In rec^+ the infectivity is increased 20 fold at 1% digestion with λ exonuclease and drops to only 3–5 fold stimulation of infectivity between 1 and 2% digestion. Beyond 2% digestion about 20 fold stimulation is again observed, which is maintained even up to more than 11% digestion (data not shown). In *recB* significantly lower stimulation is observed with a maximum of about 3 fold at 1% digestion. Terminal gaps larger than 1.5% do not stimulate infectivity in this strain. The differential increase of infectivity in rec^+ and *recB* argues against the simple interpretation of the stimulatory effect as being

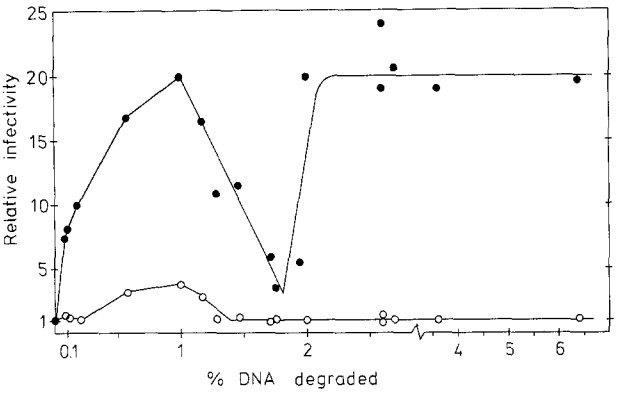

Fig. 1. Infectivity of T7 DNA with terminal 5'gaps. T7 DNA was degraded by an excess of λ exonuclease to various extents as described in Materials and Methods. The percentage of DNA made acid soluble reflects the average length of the 5'terminal gaps at both ends of the molecules. The infectivity of the DNA preparations was assayed in spheroplasts of AB1157 (rec^+; ●) and AB2470 (*recB*; ○). The relative infectivity is that of exonuclease treated DNA to untreated DNA (control). The control infectivity ranged in the various experiment from 2.5×10^{-9} to 1.6×10^{-8} in AB1157 and from 1×10^{-7} to 2.2×10^{-6} in AB2470

merely produced through facilitated uptake of λ exonuclease treated molecules by the spheroplasts. In addition, it was observed that the infectivity of the various DNA preparations depended linearly on the DNA concentration (data not shown). This indicates that one terminally gapped molecule suffices for the production of an infective center.

The increased infectivity of terminally gapped T7 DNA in rec^+ may be caused in two different ways. Either the terminal single strands per se provide a protection of the molecules, since single-stranded DNA is a poor substrate for the *recBC*-enzyme (Goldmark and Linn, 1972). On the other hand it is also conceivable that the single-stranded terminal redundant regions allow rapid circularization of the molecules which would make them resistant against exonucleolytic attack. This latter possibility was tested by determining the infectivity of in vitro circularized terminally gapped T7 DNA.

2. Infectivity of Hydrogen-Bonded Circles of T7 DNA

T7 DNA was degraded with λ exonuclease to 1.8% acid soluble nucleotides. Part of this DNA was subjected to thermal annealing after dilution to a concentration of 2 μg/ml. These two DNA preparations, together with native DNA were assayed for infectivity (Fig. 2). Apparently, the thermal annealing increased the infectivity of the molecules over that of linear terminally gapped DNA. However, since in this ex-

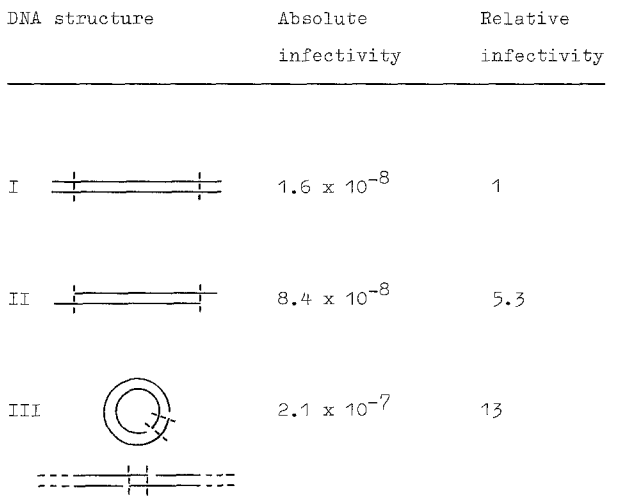

Fig.2. Infectivity of various structures of T7 DNA. Native DNA (I) was degraded by an excess of λ exonuclease to 1.8% acid soluble nucleotides (II). Part of this DNA was thermally annealed at 2 µg/ml as described in Materials and Methods to yield preparation III which probably consisted of linear and circular monomers and also aggregates of several molecules. Dotted lines schematically represent the ends of the terminal redundant regions. Transfection of *rec*$^+$ spheroplasts was performed with all three preparations

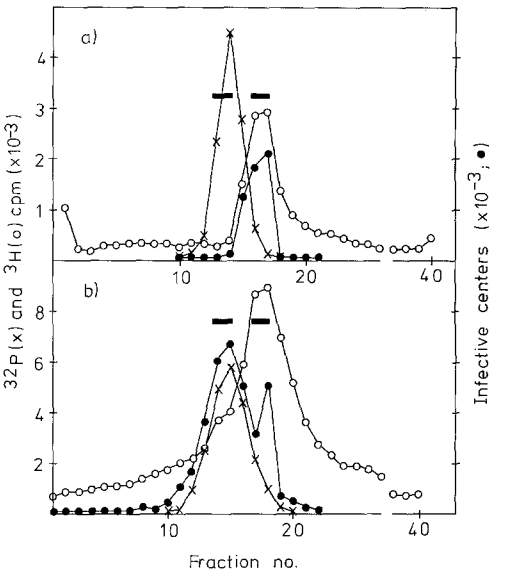

Fig. 3a and b. Infectivity of gapped linear and in vitro circularized T7 DNA. ^{3}H-labeled T7 DNA was degraded with λ exonuclease to 1.8% acid soluble material. This preparation was centrifuged on a neutral sucrose gradient either directly (**a**) or after thermal annealing (**b**) at 2 µg/ml (see Materials and Methods). ^{32}P-labeled DNA from phage λ was added as a marker. Fractions of about 0.1 ml were collected from the bottom of the tubes. From these 20 µl were used for the determination of the amount of ^{3}H and ^{32}P radioactivity and the remainder was assayed for infectivity by transfection of AB1157 (*rec*$^+$) spheroplasts. ^{3}H-T7 DNA/fraction (•); ^{32}P-λ DNA/fraction (×); infective centers/fraction (○)

periment transfection was performed with a mixture of linear and circular monomers and possibly also aggregates of several molecules, the increased infectivity could not be attributed to any of them specifically. Therefore, terminally gapped DNA before (control) and after thermal annealing was sedimented through neutral sucrose gradients in order to separate the molecular species. As a marker for sedimentation velocity ^{32}P-labeled DNA from phage $\lambda cI857Sam7$ was centrifuged in the same gradients. This phage does not form plaques on the strains employed for transfection. The DNA sediments almost at the same rate (34.4 S; Freifelder, 1970) as circular T7 DNA (1.13 × 32 S; Freifelder, 1970; Wang and Davidson, 1966). After centrifugation the gradients were fractionated and the infectivity in the fractions containing DNA were assayed. Figure 3 shows the results of experiments with *rec*$^+$ spheroplasts used for transfection. In the control gradient with linear terminally gapped DNA (Fig. 3a) the infectivity resides under the peak of ^{3}H-labeled DNA sedimenting at 32 S, a value indistinguishable from the sedimentation rate of native T7 DNA. Thus, the infectivity in this DNA preparation does not come from spontaneously formed circles or polymers. After thermal annealing

Table 1. Infectivity of linear and circular gapped T7 DNA after centrifugation in neutral sucrose gradients

Gradients from Figure 3	Specific infectivity of T7 DNA in the position of	
	linear DNA	circular DNA
Gradient a (before annealing)	1 (6.9×10^{-7})	0.3 (2.2×10^{-7})
Gradient b (after annealing)	1 (4.5×10^{-7})	3.8 (1.7×10^{-6})

(Fig. 3b) most of the infectivity resides under a shoulder of T7 DNA which sediments at a similar rate as the λ DNA marker and therefore consists of circular T7 DNA molecules. The specific infectivities calculated for the DNA in the respective peak fractions (indicated in Figure 3 by horizontal bars) are listed in Table 1. The comparison of the infectivity of linear and circular molecules within the same gradient shows that the circles are about 4 times as infective as the linears in *rec*$^+$ (in a similar independent experiment the factor was 3). When this type of experiment was repeated with *recB* spheroplasts, again 3 fold and

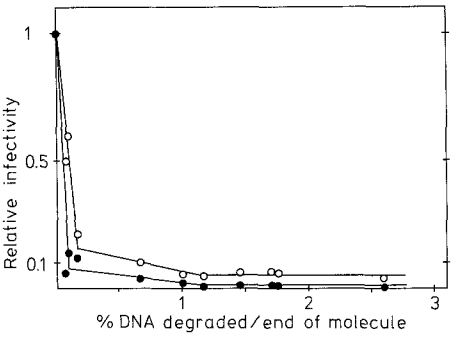

Fig. 4. Infectivity of T7 DNA from the ends of which double-stranded portions have been removed. T7 DNA preparations which had been partially degraded with λ exonuclease (see Fig. 1) were subsequently treated with exonuclease I as described in Materials and Methods to remove the 3′single-stranded ends. The percentage of DNA made acid soluble reflects the length of duplex DNA removed per end of molecule. Transfection with this DNA was done with AB1157 (*rec*$^+$; ●) and AB2470 (*recB*; ○). The infectivity of the DNA preparations is expressed relative to the infectivity before treatment with exonuclease I. For absolute infectivities see legend to Figure 1

6 fold increased infectivity of circular T7 DNA over linear terminally gapped DNA was observed (data not shown). These results indicate that in vitro formed "Hershey circles" of T7 DNA are able to trigger phage production in vivo. Moreover, the increased infectivity of circular T7 DNA in *rec*$^+$ as well as in *recB* implies that circularization provides an advantage to transfecting molecules which derives not only from the inavailability to the degradative action of the *recBC*-enzyme. This point will be considered in more detail in the discussion.

3. Infectivity of T7 DNA without Terminal Redundancies

In another series of experiments it was examined how the successive removal of both DNA strands from the ends of T7 DNA molecules would affect their biological activity. In particular we wanted to test whether or not the terminal redundant regions would be required for phage production, even in a biological environment which does not contain *recBC*-enzyme. Various DNA preparations which had been degraded with λ exonuclease to different extents (Fig. 1) were additionally treated with exonuclease I to remove the terminal 3′single strands. Completion of this reaction was controlled by monitoring the production of the same amount of acid soluble nucleotides as in the primary reaction with λ exonuclease. Transfection was then performed with the resulting DNA preparations in *rec*$^+$ and *recB* spheroplasts.

In Figure 4 the infectivity relative to that before exonuclease I treatment is plotted against the percentage of total length removed per end of molecule. In *rec*$^+$ about 90% of the molecules lost their infectivity as soon as 0.1 to 0.2% of both DNA strands (corresponding to 40 to 80 base pairs) are removed per end. In *recB* a similar result was obtained[1]. These results show that the ends of the molecules are essential for infectivity. If the terminal redundant regions were required for the formation of concatemers which in turn are a prerequisite for phage production then the loss of $\geqq$50% of the terminal redundant regions should prevent concatemerization and thereby block infectivity of a molecule. Assuming that the terminal reduncancy comprises 260 base pairs (Ritchie et al., 1967) then the loss of infectivity seen in Figure 4 occurs too early. This discrepancy could be explained by the additional assumption that the original ends of the T7 DNA molecule are required for an attachment to specific regions of the membrane. This attachment might be necessary to provide a protection against the *recBC*-enzyme until this enzyme is inhibited by a T7-specified inhibitor 6 min after infection of the host cell (Wackernagel and Hermanns, 1974). In *recB*, however, such a protection would not be necessary.

On the other hand more recent data obtained by the fragmentation of T7 DNA by restriction endonucleases suggest that the terminal redundance consists of only about 70 base pairs, corresponding to about 0.2% of the total length (Ludwig and Summers, 1975). In this case the infectivity of molecules should be lost as soon as 0.1% of the DNA are removed per end if formation of concatemers is a crucial event. Our data are more consistent with a terminal redundancy of about 70 base pairs.

Discussion

One important result of the experiments presented above is the fact that the complete ends of T7 DNA are required for the infectivity of T7 DNA molecules. As soon as 0.1 to 0.2% (corresponding to 40–80 base pairs) of DNA are removed per end by the successive action of λ exonuclease and exonuclease I a dramatic frop in infectivity is observed. If it is the terminal redundant region of the DNA which is necessary for

[1] The background of infectivity in *recB* in Figure 4 (about 5%) after removal of even more than 2% of DNA per end of molecule is probably due to a fraction of about 5% of the molecules which escaped the primary treatment with λ exonuclease (those molecules also remain resistant against exonuclease I). These fully native molecules have in *recB* essentially the same infectivity as the terminally gapped molecules. In *rec*$^+$ fully native molecules are reduced about 20 fold in infectivity as compared to terminally molecules and therefore do not show up in the plot

its biological activity then our results are more consistent with a length of the terminal redundancy of 70 base pairs (Ludwig and Summers, 1975) than with 260 base pairs (Ritchie et al., 1967). Even in *recB* spheroplasts the DNA ends are essential for infectivity indicating that the function of the terminal redundancies is not simply to provide some "mechanical" protection against exonucleolytic degradation, e.g. by the *recBC*-enzyme.

Replication of T7 DNA involves the formation of concatemers. Certain genetic defects, such as mutations in gene 2 or 6, block the formation of concatemers and simultaneously prevent phage production (Center, 1975; Hausmann and LaRue, 1969; Miller et al., 1976). In these cases the role of the genetic defect is not clear. Either it prevents phage production because the respective gene product is required for the formation of concatemers which in turn are a prerequisite for phage maturation or the defect inhibits phage production by a process that is not directly linked to concatemer formation. Our results support the first interpretation. The transfecting DNA molecules in our experiment did not carry mutations in structural genes nor were vital promoter regions missing (e.g. the early promoters between 1 and 2% from the left genetic end; Portmann et al., 1974; Bordier and Dubochet, 1974; Darlix and Dausse, 1975). The loss of the ability of these molecules to promote phage production must be caused by the absence of the terminal redundancies (or a significant part of them). This implies that concatemers per se (which cannot be formed by molecules without terminal redundancies) are essential precursors for mature particles.

Terminal 5′gaps did not reduce the infectivity of DNA molecules. Circularization in vitro of such molecules by thermal annealing further improved their infectivity over that of linear gapped DNA. On the basis of the high infectivity of these "Hershey-circles" it must be considered that T7 DNA replication may start under particular experimental conditions from circular structures. The melting of the aligned ends in circular T7 DNA has been measured in vitro by a test system that will be described elsewhere (Dreiseikelmann and Wackernagel, in preparation). It was shown to require 92° at 0.3 M Na^+, which makes spontaneous linearization of the circles in the cell rather unlikely. Consequently, soon after infection a covalently closed circle may be produced by ligation after the gaps flanking the aligned region have been filled by a DNA polymerase. [Such a process appears to be reasonable efficient as shown with similar DNA structures of phages which require a circular DNA for replication, such as λ DNA (Wackernagel and Radding, 1973) and P22 DNA (Sgaramella et al., 1976).] The resulting circle will contain an "internal" terminal redundant region as in T7 concatemers. Opening of the circle by the same enzymatic mechanism that splits concatemers into unit length molecules during T7 maturation would produce a complete linear DNA molecule which could start replication in the fashion characteristic of T7. This process might involve the steps outligned by Watson (1972). Before the circle is opened transcription has to occur in order to provide the necessary gene products required to linearize the DNA and to create the environment for the replication of linear DNA. After normal phage infection the sizing of concatemers occurs late during T7 development, when maturation proceeds after synthesis of the phage structural proteins. In the case of circular T7 DNA, phage development would have to wait until the necessary gene products are made, possibly as a consequence of some kind of disregulation. It should be mentioned that the described pathway of T7 development provides a simple means for the repair of terminal 5′gaps. This may account for the increased infectivity of circular T7 DNA in rec^+ as well as in *recB* over the infectivity of terminally gapped linears. Circularization, covalent closure and subsequently linearization will restore a complete molecule and will bypass the need for RNA-primed successive repair synthesis at the terminal 3′single strands. On the other hand, RNA-primed repair synthesis with primers as short as only four nucleotides (Scherzinger et al., 1977) would also restore an almost fully native molecule. The absence of four terminal bases would not be an obstacle to the formation of concatemers, since sufficient overlap of replicated molecules would remain even if the redundant regions comprise only 70 base pairs (Ludwig and Summers, 1975).

Terminal 5′gaps affect the infectivity of T7 DNA differentially in dependence on the phenotype of the recipient cell and the length of the gap. The fate of transfecting gapped molecules may be interpreted on the basis of the data in Figure 1. Gaps up to 1% in length may allow efficient circularization in vivo which leaves the molecules insensitive against exonucleolytic attack by enzymes which require native DNA as substrate. Such a DNase is the *recBC*-enzyme and possibly another unidentified DNase X present in rec^+ and *recB* strains which may be less aggressive than the *recBC*-enzyme. [DNase X is probably not the K restriction endonuclease, since this enzyme does not discriminate between linear and circular DNA. It has been shown recently that T7 is subject to restriction during transfection (Ehrlich et al., 1976) when a T7 specific anti-restriction function is not sufficiently expressed (Studier, 1975; Krüger et al., 1977).] Consequently, the infectivity of gapped mole-

cules rises strongly in rec^+ strains, but also in *recB*, although to a lesser extent (see Fig. 1). In rec^+ the protecting influence against DNase X may be masked by the strong protection against the *recBC*-enzyme. The circular intermediate also allows the repair of the 5′gaps as outlined above. Gaps longer than 1.5% on the other hand may make circularization less efficient because coil formation of the single-stranded ends may impede the hybridization step. Completion of these molecules may then proceed by conventional RNA-primed repair synthesis. As long as the terminal primers are significantly shorter than 50% of the redundant region replication of the repaired molecules will produce structures that can form concatemers from which normal replication can proceed. This type of repair will maintain the molecules in a linear conformation throughout. Thereby they will remain sensitive to DNase X. The *recBC*-enzyme, however, will be blocked by the long single stranded ends and will be inhibited later by a T7 specific anti *recBC* function (Wackernagel and Hermanns, 1974) when transcription has started and the repair is being completed. Thus, when gaps are longer than 1.5% the infectivity will remain in *recB* at the same level as fully native molecules (which are sensitive against DNase X) but will stay in rec^+ at the increased level characteristic for molecules which escaped the *recBC*-enzyme. No explanation can be presented for the drop of infectivity in rec^+ of molecules with gaps of 1–2%. By coincidence, the three promoters of *E. coli* RNA polymerase fall into this area on the left genetic end of the T7 DNA.

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