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 involvement 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, electron microscopy 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-
plimentary 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 concatamers 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 be 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.** 3H-thymidine-labeled T7 DNA was isolated as previously described (Seroka and Wackernagel, 1977). The specific radioactivities of the preparations were 4.3 x 10⁶ cpm/μmol nucleotide for the experiment in Figure 1 and 2.9 x 10⁶ 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 hydroxyapatite 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.** 3H-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 MgCl₂, 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 MgCl₂ 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 MgCl₂ 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 H₂O and adjusted to 2 x 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 activities.

**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