ASSYMETRIC ANNEALING OF AN RNA LINKED DNA MOLECULE ISOLATED DURING THE INITIATION OF BACTERIOPHAGE T7 DNA REPLICATION.*

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ABSTRACT

An RNA linked DNA molecule has been isolated from T7 infected cells after pulse labeling with ^{3}H-Tdr. This molecule binds almost exclusively to the R strand of T7 DNA.

Recent results (1,2,3) have indicated that an RNA molecule may act as a primer to initiate DNA replication in bacteria and bacteriophage systems. Thus, at the initiation of DNA replication in bacteriophage T7 infected cells, one ought to be able to isolate a ribopolynucleotide covalently attached to a growing deoxypolynucleotide chain. In this communication, we report the isolation and partial characterization of such a molecule.

Thomas, Kelley and Rhoades (4) have reported that the initiation of T7 DNA replication occurs at approximately seven minutes after infection at 30°C. We have confirmed their results by hybridizing T7 DNA with extracted DNA pulse labeled with ^{3}H-thymidine (^{3}H-Tdr) at various times after infection. Therefore, to isolate the initial progeny fragment of T7 replicative DNA, ^{3}H-Tdr was pulsed from 6 min and 45 sec after infection to 7 min and 15 sec after infection. E. coli

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B23 was grown at 30°C to 3 x 10^8 in 10 x PO_4TCG medium (5) and incubated for 10 min with 5-fluorodeoxyuridine at 5 µg/ml and uracil at 25 µg/ml. The bacteria were infected with T7 bacteriophage at multiplicity of infection = 10. At 6 min and 45 sec after infection ^{3}H-Tdr was added at 200 µc/ml and at 7 min and 15 sec, the bacteria were ice-chilled in 20 mM Tris-150 mM NaCl - 10 mM EDTA pH 7.6 and lysed with 1% sodium laurel sulfate. The replicative DNA was extracted by the pronase-phenol method (6) and banded in a Cs_2SO_4 density gradient (7). The gradient was collected in vials and the absorbance at 260 nm was determined to define the location of the extracted RNA. The main peak of absorbance (260_{nm}) at the bottom of this Cs_2SO_4 gradient is RNA. Aliquots of the fractions were mixed with bovine serum albumin and precipitated with 5% trichloroacetic acid (TCA). The precipitates were washed with 5% TCA, dissolved in 1 M NH_4OH and transferred to glass fiber filters for counting in a Nuclear Chicago ISOCAP 300 scintiallation spectrometer. The results of the experiment are presented in Figure 1A. Two main peaks of ^{3}H-Tdr labeled material are found in the gradient, one banding at the top with ^{32}P labeled reference DNA and one banding very close to the bulk of RNA. The material in the two peaks was dialyzed against 20 mM Tris - 1 mM EDTA pH 7.6 and analyzed as follows.

To determine if the ^{3}H-Tdr had in fact been incorporated into a deoxypolynucleotide, the material in the two peaks was incubated with pancreatic RNase (100 µg/ml) and pancreatic DNase (100 µg/ml + 6 mM $MgCl_2$) and precipitated with 5% TCA as before. The results presented in Table 1 show that the material in peaks I and II are almost completely resistant to RNase and sensitive to DNase.

To test whether the material in peak I is composed of an RNA molecule covalently attached to a DNA polynucleotide, the material

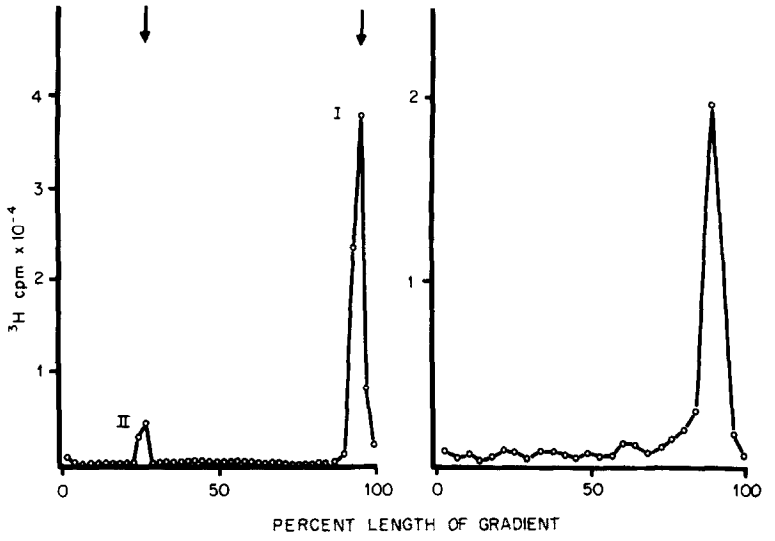

Figure 1(A) Cs_2SO_4 density gradient analysis of replicative T7 DNA pulse labeled with ^{3}H-Tdr. The arrow at the top of the gradient indicates the position of ^{32}P labeled T7 reference DNA. The arrow at the bottom indicates the major peak of absorbance (260 nm).

1(B) Cs_2SO_4 density gradient analysis of material in peak II (Fig. 1A) after treatment with RNase.

Enzyme	RNase		DNase
peak I	before	89721	146678
	after	83273	6121
peak II	before	2810	2239
	after	2476	270

Table I. Nuclease sensitivity of material in peaks I and II of Figure 1A. Numbers represent total radioactivity (^{3}H counts/10 minutes) which is TCA precipitable before and after treatment with RNase or DNase.

was re-analyzed by Cs_2SO_4 density gradient analysis after treatment with ribonuclease. If the moiety is rebanded prior to treatment

DNA	E. coli	T7L	T7R
peak I	813	6791	10455
peak II	26	62	965

Table II. Hybridization of material in peaks I and II with E. coli DNA and the separated strands of T7 DNA (R and L). Numbers represent total radioactivity annealed (^{3}H counts/10 min).

with RNase, the overwhelming majority of the label bands at the original "RNA density" position, as expected. After treatment with RNase, the material bands at the DNA location in the gradient (Figure 1B). Thus the density of the ^{3}H-Tdr labeled DNA molecule depends on the integrity of an RNase sensitive moiety. Similar results have been obtained with bacteriophage T4 infected cells by Buckley, Kosturko and Kozinski (personal communication).

Since all T7 messenger RNA is transcribed from one strand of the T7 duplex (8), we might expect the RNA linked DNA to complement the same strand as the messenger RNA. To test this hypothesis, the material in peaks I and II were hybridized against the individual strands of T7 DNA separated by standard procedures (8,9). As a control ^{32}P labeled separated strands were hybridized against the same unlabeled strands bound to the nitrocellulase filters. This calibrates the relative binding efficiencies of the two strands with this particular batch of filters. The results in Table II show that though the material in peak I binds to the separated strands of T7 with almost equal efficiency (and the same as the reference cross-hybridizations), the material in peak II binds almost exclusively to the r-strand of the phage; this is the same strand which codes for the m-RNA (8). In the case of T4, the RNA linked DNA anneals with the same strand as does the early messenger RNA (Buckley, Kosturko and Kozinski, personal communication).

Our interpretation of these results is that during initiation of DNA replication, an existing RNA molecule is used to prime a growing deoxypolynucleotide chain. This RNA primer is supplied by an RNA polymerase which binds to the strand of the duplex containing the promoters for RNA transcription.

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