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Initiation sites for discontinuous DNA synthesis of bacteriophage T7*

(RNA priming/DNA sequence/mapping of RNA–DNA junctions/RNA-linked DNA pieces/signal for initiation)

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ABSTRACT **We have previously shown that the discontinuous replication of bacteriophage T7 DNA is primed by tetraribonucleotides (major component) or pentaribonucleotides. Both tetramers and pentamers start with pppA-C and are rich in A and C at the third and fourth nucleotides. In this study, the sites of transition from primer RNA to DNA *in vivo* have been located on a 340-nucleotide segment of the H strand of the T7 genome by ^{32}P-labeling *in vitro* of the 5′-hydroxyl ends of DNA resulting from alkaline hydrolysis of RNA-linked T7 DNA fragments. Five strong transition sites were detected with a common sequence 5′-G-A-C-N1-N2-N3-N4-3′, in which N1 was either C or A, N2 was A, C, or G, and either N3 or N4 was the nucleotide for the switchover to DNA synthesis. We conclude that the complementary sequence 3′-C-T-G-G/T-N′2-(N′3)-5′ in the template strand is the most frequently used signal for synthesis of primer RNA. Whereas primer-RNA synthesis starts at a precisely defined nucleotide, the transition to DNA synthesis varies within two nucleotides. Because the observed signal sequence would be present on a statistical basis once per 128 nucleotides, only about 10% of the existing signals are used for primer synthesis in each round of replication so that nascent fragments 1000–2000 long result. This provides an unexpected flexibility for RNA priming of DNA synthesis.**

DNA replication of T7 phage proceeds discontinuously via short-chain intermediates that are primed by RNA (1, 2). When the activities of the exonucleases coded for by gene 6 of phage T7 and *polA* of *Escherichia coli* are inhibited, short DNA chains covalently linked to RNA accumulate (1). Thus, both exonucleases remove T7 phage primer RNA. The accumulated RNA-linked DNA chains have been a rich source for analysis of the structure of T7 phage primer RNA. The T7 phage primer RNA is mostly a tetraribonucleotide of sequence pppA-C-N1-N2, in which N1 and N2 are rich in C and A (N1, C > A >> U > G; N2, C ≅ A >> U > G) (3, 4). A small amount of pentaribonucleotide primer that starts with pppA-C has also been detected (3, 4). The primers appear to be synthesized *de novo* and to be used without processing at the 3′ termini; primers of the same chain length and nucleotide sequence are synthesized *in vitro* by T7 gene 4 protein (T primase) and extended with deoxyribonucleotides by T7 DNA polymerase in a reaction that is stimulated by DNA-binding protein (5, 6). *In situ* synthesis of the primer RNA is suggested by the diversity of the third (N1) and fourth (N2) nucleotides of the primer sequence. Thus, the sequence signaling primer RNA synthesis should be 5′ to the transition sites for DNA synthesis.

The signal sequence was unraveled in this study by mapping the sites of transition from primer RNA to DNA in a 340-nucleotide segment of the T7 genome. The deoxyribonucleotide at the point of switchover from primer RNA to DNA was detected by labeling, with [γ-^{32}P]ATP and T4 phage polynucleotide kinase, the 5′-hydroxyl ends of DNA uncovered by alkaline treatment of accumulated RNA-linked short DNA chains (7). Five strong transition sites were found with the common sequence 5′-G-A-C-N1-N2-N3-N4-3′, in which N1 was either A or C, N2 was A, C, or G, and either N3 or N4 was the transition point for DNA synthesis. The major features of the initiation of discontinuous DNA synthesis of T7 are now known.

MATERIALS AND METHODS

Bacteriophage Growth. *E. coli* C-N3 (*his, polA480*) and bacteriophage T7 *ts136* (temperature-sensitive mutant of gene 6) have been described (1). Cells were grown in M9 medium (8) supplemented with 0.5% Casamino acids.

Chemicals and Enzymes. [γ-^{32}P]ATP (0.3–1 Ci/μmol; 1 Ci = 3.7×10^{10} becquerels) was prepared as described (9). [^{3}H]Thymidine (51.4 Ci/mmol) was from New England Nuclear. Deoxydinucleoside diphosphates were from Collaborative Research (Waltham, MA). *Hpa* II and *Hin*fI were from Boehringer Mannheim and Bethesda Research Laboratories (Rockville, MD), respectively: *Hha* I was a gift of M. Takanami (Kyoto University); and *Hae* III was purified as described (10). Nuclease SW was from Seikagaku Kogyo (Tokyo); nuclease S1 (781 units/mg) (11) and nuclease P1 were provided by T. Ando (Institute of Physical and Chemical Research) and A. Kuninaka (Yamasa Shoyu, Choshi, Japan), respectively. Snake venom phosphodiesterase, T4 DNA polymerase, and T4 polynucleotide kinase have been described (12).

Preparation of Complementary Strands of T7 Phage DNA and Restriction Fragment *Hpa* II-H3. The H and L strands of T7 phage DNA were prepared as described for P2 phage DNA (13). A *Hpa* II digest of T7 *ts136* phage DNA was fractionated by electrophoresis in an 8% (wt/vol) polyacrylamide gel, and fragment H3 (14) was eluted with 10 mM Tris·HCl, pH 8.0/0.1 mM EDTA. The strands of the fragment were then separated partially by banding in a CsCl density gradient containing poly(U,G) (13). Fractions rich in H and L strands were then self-hybridized in a hybridization buffer (10 mM Tris·HCl, pH 7.3/0.5 M NaCl/0.05% $NaDodSO_4$) at 65°C for 2 hr and the portions that remained single-stranded were isolated by hydroxyapatite column chromatography (15). The resulting H- and L-strand fractions were more than 95% pure as judged by hybridization to the appropriate strands of T7 DNA.

Purification of Nascent DNA Chains and Labeling of Transition Sites. Nascent DNA chains shorter than 1400 nucleotides were purified up to the nitrocellulose step, as previously described, from a 200-ml culture of *E. coli* C-N3 cells infected with T7 *ts136* (1). After heat denaturation (90°C, 2 min), the nitro-

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cellulose fraction was banded in a Cs_2SO_4 density gradient. DNA fractions were pooled and phosphorylated with T4 polynucleotide kinase and ATP and were then hydrolyzed in 0.15 M NaOH for 18 hr at 37°C. The 5′-hydroxyl ends of DNA uncovered by the alkaline hydrolysis were phosphorylated with [γ-^{32}P]ATP and T4 polynucleotide kinase at 0°C for 60 min (7) in a reaction mixture (3.3 ml) containing 67 mM Tris·HCl (pH 8.0), 17 mM 2-mercaptoethanol, 10 mM $MgCl_2$, 6 μM [γ-^{32}P]ATP, $\approx$ 13 nM alkali-treated DNA molecules, and 33 units of enzyme per ml. Seventeen picomoles of 5′-^{32}P-labeled DNA chains was obtained; 98% was derived from the T7 genome, as judged by hybridization to T7 and *E. coli* DNAs.

Purification of 5′-^{32}P-Labeled Short Chains of H Strands. The 5′-^{32}P-labeled DNA chains containing $\approx$15 μg of T7 DNA were incubated with 30 μg of the H strand of T7 phage DNA in 750 μl of hybridization buffer at 64.5°C for 40 min and then sedimented through a 5–20% neutral sucrose gradient in 15 mM NaCl/1.5 mM sodium citrate/1 mM EDTA/0.1% $NaDodSO_4$. Slowly sedimenting labeled material, composed mainly (90%) of H strand, was self-hybridized in 200 μl of the hybridization buffer at 64.5°C for 60 min. DNA remaining in single-stranded form was then isolated by hydroxyapatite chromatography (15). 5′-^{32}P-labeled short chains from the H strand were obtained in 99% purity.

Hybridization of 5′-^{32}P-Labeled Short Chains from H Strands to L Strand of H3 Restriction Fragment and Nuclease Digestion of Unhybridized DNA. About 6 μg of the ^{32}P-labeled short chains from the H strand were hybridized to the L strand of restriction fragment *Hpa* II H3 (0.38 μg) in 200 μl of hybridization buffer at 64.5°C for 10 hr. To the hybridization reaction mixture, an equal volume of a solution containing 80 mM sodium acetate (pH 4.6), 1 mM $ZnCl_2$, and 20 μg of nuclease S1 per ml was added, and the mixture was incubated at 37°C for 30 min. During the digestion, less than 10% of the 5′-^{32}P label of double-stranded DNA was made acid soluble. The S1 digest was treated with phenol, passed through a Sephadex G-100 column, and then digested with the 3′-to-5′ exonuclease of T4 DNA polymerase. The reaction mixture contained 67 mM Tris·HCl (pH 8.0), 6.7 mM $MgCl_2$, 10 mM 2-mercaptoethanol, 0.13 mM dTTP, and 16 polymerase units of T4 DNA polymerase and was incubated at 37°C for 60 min. The T4 DNA polymerase digest was treated with phenol and passed through a Sephadex G-100 column.

Determination of Chain Length of 5′-^{32}P-Labeled DNA. The T4 DNA polymerase digest was denatured in 70–100 μl of 50 mM NaOH/12 mM EDTA/5 M urea at 90°C for 30 sec and subjected to electrophoresis on 10% polyacrylamide/7 M urea gels with external size-marker DNAs (16). The gel was subjected to autoradiography at −80°C in the presence of an intensifying screen (3). DNA chain length was calculated from the mobility relative to the marker DNAs.

***Hae* III Digestion.** *Hae* III was used to cleave single-stranded DNA at a fixed position (17). DNA molecules extracted from the gel were precipitated with ethanol and incubated in 40-μl reaction mixtures containing 10 mM Tris·HCl (pH 8.0), 10 mM 2-mercaptoethanol, 10 mM $MgCl_2$, 4 μg of gelatin, and 72 units of *Hae* III at 37°C for 12 hr.

Analyses of 5′-Terminal Mono- and Dinucleotides. 5′-Terminal dinucleotides of 5′-^{32}P-labeled DNA were obtained by complete digestion with nuclease SW in a 20-μl reaction mixture containing 10 mM sodium carbonate buffer (pH 10), 1 mM magnesium acetate, and 20 units of enzyme. Dinucleotides were separated by two-dimensional chromatography on polyethyleneimine–cellulose plates (Polygram Cell 300 PEI, Macherey-Nagel, Germany) with 0.6–1.2 M lithium formate (pH 3.1) in the first dimension and 0.5 M LiCl/7 M urea in the second dimension. ^{32}P-Labeled dinucleotides were located on the chromatogram by autoradiography (3). 5′-Terminal mononucleotides of the 5′-^{32}P-labeled DNA were released either by successive digestions with nuclease SW and snake venom

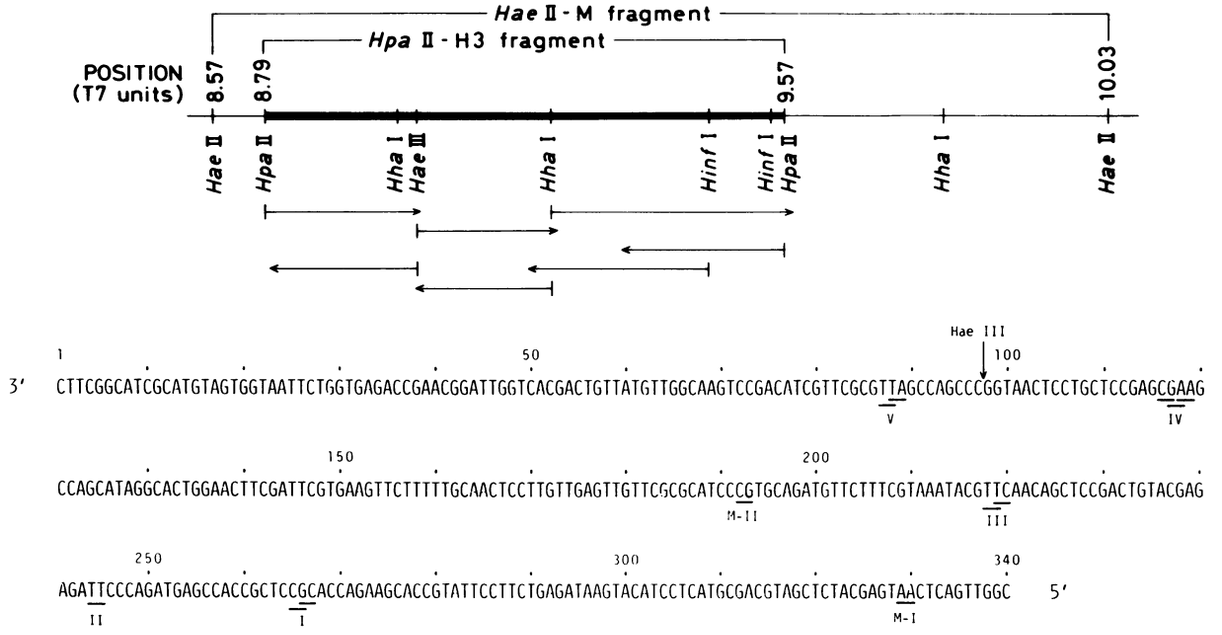

FIG. 1. (*Upper*) Strategy for determining the nucleotide sequence of fragment *Hpa* II-H3. Nucleotide sequence was determined by the method of Maxam and Gilbert (16) with *Hpa* II-H3 and *Hae* II-M fragments (14). Positions of the fragments on T7 phage DNA are given in T7 units according to Studier *et al.* (14). Endonuclease cleavage sites used for sequence determination are indicated on the fragments; horizontal arrows show the direction and extent of sequencing runs. (*Lower*) Nucleotide sequence of the H strand of fragment *Hpa* II-H3. Nucleotides are numbered from the 3′ end of the H strand. I–V, M-I, and M-II correspond to the locations of the 5′ termini of the DNA in bands I–V, M-I, and M-II, respectively, in Fig. 2. Dinucleotides found at the sites of transition from primer RNA to DNA synthesis of H strand are shown by bars. Cleavage site of *Hae* III is shown.

phosphodiesterase or by digestion with nuclease P1 (4). Mononucleotides were identified on polyethyleneimine-cellulose plates by successive development with 0.25 M formic acid and 0.5 M lithium formate (pH 3.1).

RESULTS

Nucleotide Sequence of Restriction Fragment *Hpa* II-H3. This study used the H3 restriction fragment of an *Hpa* II digest of T7 phage DNA (*Hpa* II-H3), which is about 0.8% of the total T7 genome (340 nucleotides) and is located 9% from the left end of the genetic map (14). Its nucleotide sequence was determined as described (16) (Fig. 1).

Purification of H-Strand Nascent DNA in the H3 Region of T7. RNA-linked nascent DNA pieces of bacteriophage T7 were prepared from T7 *ts136* phage-infected *E. coli* C-N3 cells briefly exposed to 43°C. The 5′-hydroxyl ends of DNA uncovered by alkaline treatment of the RNA-linked DNA pieces were labeled with T4 polynucleotide kinase and [γ-^{32}P]ATP at 0°C (7); 0.9% and 44.1% of the 5′-^{32}P-labeled DNAs thus obtained hybridized with *E. coli* DNA and T7 DNA respectively, immobilized on membrane filters. Because the hybridization efficiency of homologous DNA was 40–50%, the results indicate that 98% of the 5′-^{32}P-labeled DNA was derived from T7. This shows that the polynucleotide kinase reaction at 0°C was specific for 5′-hydroxyl ends of DNA and that exchange with 5′-phosphoryl-terminated DNA (18) was negligible; the RNA-linked T7 DNA fraction contained 3 times as many RNA-free DNA molecules, two-thirds of which were derived from the *E. coli* genome (3). The ^{32}P-labeled 5′-terminal nucleotides consisted of all four deoxyribonucleotides, but were clearly enriched for dTMP and dGMP, containing 30% dGMP, 46% dTMP, 14% dAMP, and 10% dCMP. The end-labeled T7 DNA was two-thirds H strand and one-third L strand.

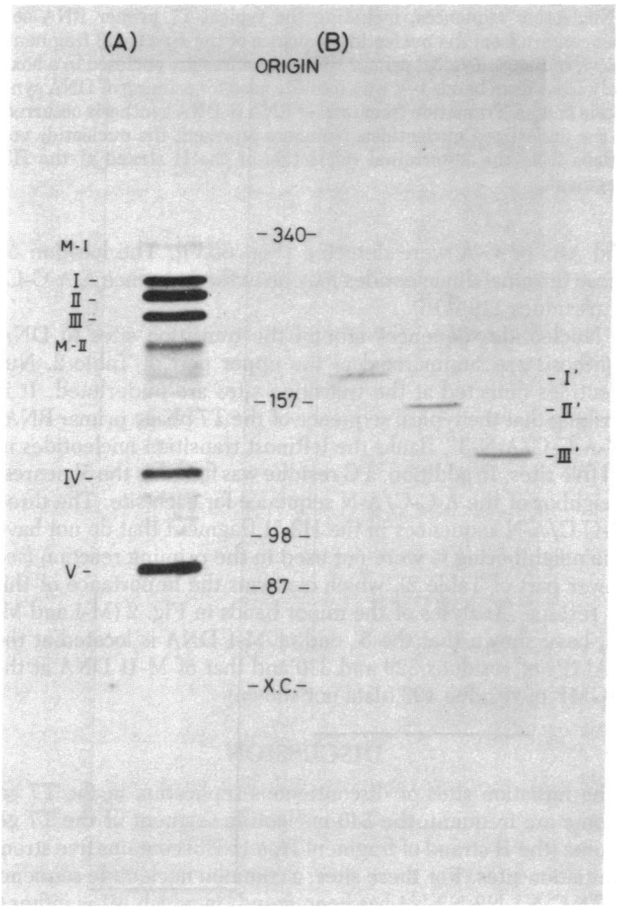

FIG. 2. Gel electrophoresis of 5′-^{32}P-labeled short chains of the H strand synthesized on the *Hpa* II-H3 region of T7 genome. (*A*) H-strand DNA chains that had been primed by RNA on the H3 segment of the genome were purified from the 5′-^{32}P-labeled DNA and trimmed at the 3′ ends. Resulting DNA chains were fractionated in a 10% polyacrylamide/7 M urea gel, and DNA bands were detected by autoradiography. Bands designated I–V are described in the text. Positions of chain-length markers are indicated as number of residues. (*B*) Bands I, II, and III in *A* were extracted from the gel and cleaved with *Hae* III. The digests were fractionated under the same conditions as for *A*. Bands I′, II′, and III′ were derived from bands I, II, and III, respectively. x.c., xylene cyanol FF.

The H strand of the end-labeled DNA was purified and hybridized to the L strands of fragment *Hpa* II-H3 (H3-L fragment); then the unhybridized portion of the DNA chains was digested with nuclease S1. The DNA was treated with 3′-to-5′ exonuclease of T4 DNA polymerase in the presence of dTTP in order to digest the single-stranded portion of DNA completely and to trim the 3′ ends of the H-strand short chains which are hybridized to the L strands at the first residue (dCMP) of the restriction fragment (Fig. 1). After these treatments, 1.2% of the ^{32}P radioactivity of the end-labeled H-strand DNA was excluded by a Sephadex G-100 column. In contrast, only 0.2% of the radioactivity was excluded by the Sephadex G-100 column in a control in which the end-labeled H-strand DNA had been treated by the nucleases without preceding hybridization with the H3-L fragments. Therefore, 1% of the end-labeled H-strand fragments are derived from the *Hpa* II-H3 region of the genome, the length of which (340 nucleotides) corresponds to 0.8% of the T7 DNA.

Location of Sites of Transition from Primer RNA to DNA Synthesis. The chain length of the ^{32}P-labeled DNA after T4 DNA polymerase digestion indicates the distance of transition sites from the first dTMP residue at the 3′ end of the H3-H restriction fragment because the exonuclease activity does not proceed beyond a dTMP residue on a duplex DNA in the presence of dTTP (19). The end-labeled DNA excluded by a Sephadex G-100 column was fractionated by polyacrylamide gel electrophoresis under denaturing conditions and autoradiographed (Fig. 2*A*). Five strong discrete bands were detected on the autoradiogram (bands I–V), each of which contained nearly an equal amount of ^{32}P. Several minor bands were also observed (M-I and M-II). The DNA chain length for each band (Table 1) was calculated from the mobility relative to marker DNAs (Fig. 2). The length of the DNA in bands I–III was more accurately determined by re-electrophoresis of each DNA after *Hae* III

Table 1. Characterization of the 5′-^{32}P-labeled H-strand DNA derived from the *Hpa* II-H3 segment of the genome

Fractionated DNA from Fig. 2	Chain length*	Mononucleotides, %†				Dinucleotides†
		G	T	A	C	
I	270 (169)	70	5	1	23	G-C, C-G
II	250 (148)	2	93	3	2	T-T
III	224 (125)	3	73	2	22	C-T, T-T
IV	119	30	10	58	2	A-A, G-C, A-G
V	90	7	46	47	0	A-T, T-T

The chain length (no. of nucleotides) of each DNA molecule was determined as described in the legend of Fig. 2. For the end-group analyses, DNAs in bands I′, II′, III′, IV, and V shown in Fig. 2 were extracted from the gel and analyzed as described in *Materials and Methods* and the legend of Fig. 3. ^{32}P radioactivity recovered was 200–1170 Čerenkov cpm.

* Numbers in parentheses are the chain lengths of the DNA molecules after *Hae* III digestion. Chain lengths are accurate to ±5 nucleotides.

† Nucleotides at the 5′ termini.

digestion. As shown in Fig. 1, an *Hae* III site lies between nucleotides 97 and 98. As expected, DNA from bands I–III was shortened by about 100 nucleotides, resulting in bands I′–III′ (Fig. 2B and Table 1).

To locate precisely the deoxynucleotides at the transition, we analyzed the 5′-terminal mono- and dinucleotides of the ^{32}P-labeled DNA in each band. The 5′-terminal mononucleotides of each DNA band are shown in Table 1. Except for DNA from band II, in which dTMP clearly predominates, two major nucleotides were detected from DNA in each band: dGMP and dCMP in band I; dTMP and dCMP in band III; dAMP and dGMP in band IV; and dTMP and dAMP in band V. The 5′-terminal dinucleotides revealed the exact location of the sites of transition (Fig. 3 and Table 1). The 5′-terminal dinucleotide sequence of the DNA from band II was T-T and it was 148 (±5) nucleotides from the *Hae* III cleavage site. This T-T sequence must be residues 244 and 245 in the H3-H fragment, because no other T cluster is present nearby (Fig. 1). The DNA in band III, whose 5′ termini were 125 (±5) nucleotides from the *Hae* III site, produced two dinucleotide spots, T-T and C-T. Approximately 125 nucleotides from the *Hae* III site is the sequence 5′-C-T-T-T-3′ (residues 220–218) in which DNA synthesis starts with T-T or C-T. Because the 5′-terminal mononucleotides of DNA from band III consist of 73% dTMP and 22% dCMP, the transition from primer RNA to DNA synthesis seems to occur mainly at the dTMP at residue 219 and partly at the dCMP at residue 220. Similarly, 5′ termini of the DNA from band V were located at the dAMP at residue 89 and the dTMP at residue 88. Because the chain length of band I DNA after *Hae* III digestion was 169 (±5) nucleotides (Fig. 2*B* and Table 1), the location of the 5′ termini is probably at the dGMP at residue 266 and the dCMP at residue 267. From the DNA of band IV, three dinucleotide spots corresponding to A-A, G-C, and A-G or G-A were detected [Fig. 3(IV)]. The location of these terminal dinucleotides may be in the sequence A-A-G-C-3′ (residues 119–116).

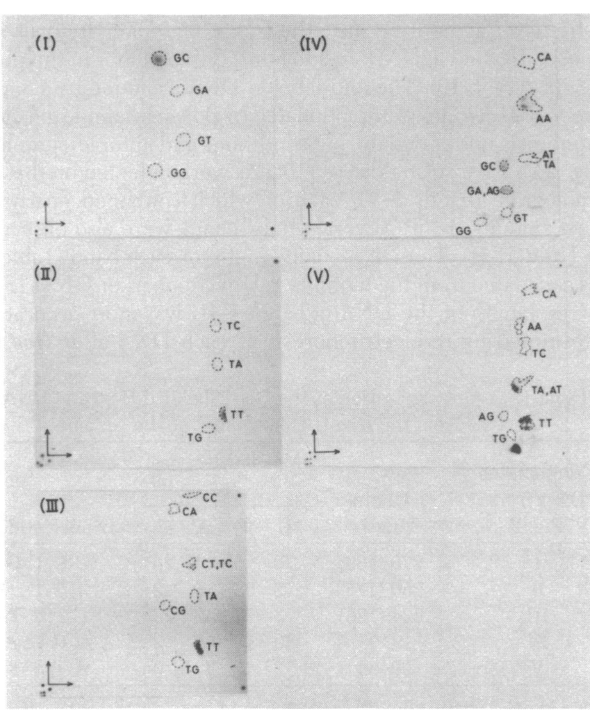

FIG. 3. Identification of the 5′-terminal dinucleotides of the 5′-^{32}P-labeled DNA chains shown in Fig. 2. DNAs in bands I′, II′, III′, IV, and V from Fig. 2 were extracted from the gel and completely digested with nuclease SW. Resulting ^{32}P-labeled dinucleotides were chromatographed on polyethyleneimine-cellulose plates with authentic dinucleotide markers and then autoradiographed. The positions of markers are indicated by broken lines. ↑, First dimension; →, second dimension.

Table 2. Primer RNA sequence for the H-strand H3 fragment

5′		3′	
(275) AGTCTTCCTTATGCCACGAAG	ACCA	CGCCTC (265)	I
(250) CCACGCCTCGCCACCGAGTAG	ACCC	TTAGAG (240)	II
(225) TTAGAGAGCATGTCAGCCTCG	ACAA	CTTGCA (215)	III
(125) AGCTTCAAGGTCACGGATACG	ACCG	AAGCGA (115)	IV
(95) AGCCTCGTCCTCAATGGCCCG	ACCG	ATTGCG (85)	V
(305) ATCTCGATGCAGCGTACTCCT	ACAT	GAATAG (295)	
(260) CCACGAAGACCACGCCTCGCC	ACCG	AGTAGA (250)	
(80) GCCCGACCGATTGCGCTTGCT	ACAG	CCTGAA (70)	

Nucleotide sequences, including the typical T7 primer RNA sequences, are from the nucleotide sequence of the *Hpa* II-H3 fragment shown in Fig. 1. Typical primer RNA sequences are enclosed in a box. Only DNA from bands I–V was actually used for priming of DNA synthesis *in vivo*. Transition from primer RNA to DNA synthesis occurred at the underlined nucleotides. Numbers represent the nucleotide positions from the 3′-terminal nucleotide of the H strand of the H3 fragment.

Nucleotide sequences around the transition sites to DNA synthesis are summarized in the upper part of Table 2. Nucleotides detected at the transition sites are underlined. It is striking that the typical sequence of the T7 phage primar RNA, 5′-A-C-C/A-N-3′, flanks the leftmost transition nucleotides in all five sites. In addition, a G residue was found as the 5′ nearest neighbor of the A-C-C/A-N sequence for each site. The three A-C-C/A-N sequences in the H3-H fragment that do not have the neightboring G were not used in the priming reaction (see lower part of Table 2), which confirms the importance of this G residue. Analyses of the minor bands in Fig. 2 (M-I and M-II) have shown that the 5′ end of M-I DNA is located at the dAMPs of residues 329 and 330 and that of M-II DNA at the dGMP of residue 193 (data not shown).

DISCUSSION

The initiation sites of discontinuous replication in the T7 genome are frequent; the 340-nucleotide segment of the T7 genome (the H strand of fragment *Hpa* II-H3) contains five strong initiation sites. For these sites, a common nucleotide sequence G-A-C-N1-N2-N3-N4 has been found, in which N1 is either C or A and N3 and N4 represent the nucleotides at the site of transition to DNA synthesis (Table 2). Conversely, all such sequences on the H strand of the restriction fragment have been strong priming sites. N1-N2 sequences found in the five sites were A-A, C-A, C-G, and C-C. Analyses of the minor bands in Fig. 2 have shown that the priming reaction occurs at low frequency at the sequence where N1 is either G or T (residues 193–198 and 329–335). No priming has been detected at the A-C-A-T, A-C-C-G, and A-C-A-G sequences, which lack the leftmost G residue (Table 2). In addition, priming has not been detected at the A-C-C-A sequence outside the H3 fragment

which also lacks the 5'-G residue (unpublished result). Therefore, we conclude that 3'-C-T-G-N'1-N'2-(N'3) on the template strand is the signal sequence for *in situ* synthesis of T7 primer RNA and that, at the sites where N'1 is G or T, priming reactions occur much more frequently than at the sites where N'1 is A or C. No evidence for a preferred primer synthesis at the sites with T or G at N'2 (A or C at N2) has been obtained; the five sites with A, C, and G at N2 in the H strand of the restriction fragment showed roughly equal initiation frequency. It remains to be clarified, however, whether or not the N1-N2 sequence is important for the priming reaction; only four of the eight possible dinucleotide sequences have been analyzed in the present work. Primer RNA synthesized on the whole T7 genome is rich in C and A at both the N1 and N2 positions; 70% and 20% of N1 and 50% and 30% of N2 is occupied by C and A, respectively (3). Such a bias in the nucleotide composition at N1 and N2 would result if some of the dinucleotide sequences that were not analyzed in this work (C-T, A-C, A-G, or A-T) are used less frequently for priming. Alternatively, the bias in the nucleotide composition of the primer RNA could reflect a bias in the nucleotide sequence of the T7 genome itself. The preferred recognition site for primer synthesis by gene *4* protein *in vitro* is 3'-C-T-G-G-G/T-5' (see ref. 20).

Although the initial nucleotide for primer RNA is precisely defined, the transition to DNA synthesis can vary within two, or less frequently, three nucleotides. Thus, tetra- and pentaribonucleotide primers may be synthesized at the same sites during different rounds of replication. The termination of primer synthesis seems to be influenced by the base composition at N3. When N3 is T, the switchover to DNA synthesis occurs exclusively at N3; when N3 is C, the switchover predominates at N4 (Tables 1 and 2). Consistent with this, the starting deoxyribonucleotides are biased toward dTMP and dGMP, in this order, although all four kinds of nucleotides are represented. This may be an intrinsic property of T7 primase, which preferentially uses rATP and rCTP and self-terminates synthesis mostly at tetranucleotides (5, 6).

The observed strong signal sequence would be present once every 128 nucleotides in a random base sequence if a possible effect of N'2 nucleotide were neglected. Because nascent short DNA is 1000–2000 nucleotide long (1), even the strong signal sequence is used only once every 10 rounds of replication. The frequency of signal usage could be determined by the number of signals exposed by unwinding of the parental duplex and by the number of primase molecules active at the replication fork. In addition, DNA synthesis competes with primer synthesis by converting the template strand into a duplex form because primase requires single-stranded DNA. Frequent short recognition signals may be advantageous because they provide frequent chances for chain initiation with minimal interference with coding functions.

RNA-linked DNA chains of T7 phage are composed of both complementary strands. The nascent H3-H-strand fragments would be the leading strand component if throughout DNA synthesis each T7 genome proves to be replicated from the same origin (15% from the genetic left end) that functions in the first replication cycle in both directions (21). However, the origin and direction of DNA synthesis late in T7 infection are unknown.

The primer for discontinuous DNA replication of bacteriophage T4 is a pentaribonucleotide also beginning predominantly with A-C (12). This pentaribonucleotide is made *in vitro* by T4 gene *41* and *61* proteins on the multiple initiation sites of single-stranded DNA (22, 23). Thus, a similar recognition mechanism for primer synthesis might operate for these two coliphages.

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