Volume 2 number 6 June 1975 Nucleic Acids Research

Determination of the sequences of 18 nucleotides from the 5'-end of the 1-strand and 15 nucleotides from the 5'-end of the r-strand of T7 DNA.†

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Received 26 March 1975

ABSTRACT

The sequences of 18 nucleotides from the 5'-end of the 1-strand and 15 nucleotides from the 5'-end of the r-strand of T7 bacteriophage DNA have been determined to be pT-C-T-C-A-C-A-G-T-G-T-A-C-G-T-C-C-C (1-strand) and pA-G-G-G-A-C-A-C-A-G-C-G-C-T-C (r-strand)*. The 5'-termini of whole DNA or separated strands were kinased using polynucleotide kinase and $(\gamma\text{-}^{32}P)$ rATP. The DNA was partially digested with pancreatic DNase and the fragments were separated by two dimensional electrophoresis and homochromatography. To complete the sequence, snake venom phosphodiesterase digestions of these fragments were carried out. The relationship of these sequences to the proposed cleavage of concatemeric DNA during DNA replication is discussed.

INTRODUCTION

DNA sequence analysis since 1970 has complemented certain biochemical and genetic studies of protein-nucleic acid interactions (1-6). However, very little sequence data has been made available for the coliphage T7 in spite of the extensive studies which have been carried out on the phage in other ways. Only a trinucleotide sequence at the 3'-end of the r-strand and a heptanucleotide sequence at the 3'-end of the 1-strand have been determined (7-9).

T7 DNA is known to contain unpermuted terminally redundant sequences of about 260 base pairs (10). A model has been proposed which links the origin of these repetitious ends to the cleavage of concatemeric DNA during DNA replication. One or two nucleases may be involved which recognize specific sequences in the concatemer (11). If this model is correct, the

* Abbreviations used: The symbols A, G, T and C are used throughout this paper to stand for deoxyribonucleosides. Ribonucleosides are prefixed with r, as in rATP, adenosine triphosphate. 2-D homochromatography denotes electrophoresis on a cellulose acetate strip at pH 3.5 (pyridine-acetate buffer) followed by homochromatography on a DEAE cellulose thin layer plate (20 x 40 cm) using 2% partially hydrolyzed yeast RNA in 7 M urea. Homo-mix was the hydrolyzed yeast RNA mixture prepared as described by Jay et al (18).

two sequences must be wholly contained within a unit length T7 genome. Furthermore their sequences can be determined by existing techniques.

This paper describes the determination of the sequences of 18 nucleotides at the 5'-end of the T7 l-strand and 15 nucleotides at the 5'-end of the r-strand. The complete sequence determined for T7 DNA is shown in Figure 1. The format which is to be utilized to deduce a further sequence within the locations of proposed nuclease cleavage is also described.

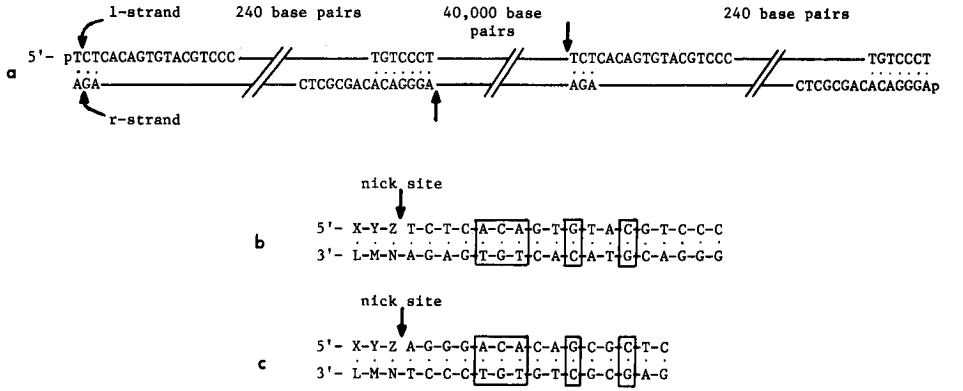

Figure 1. (a) The complete sequence of T7 DNA which has been determined in this and previous work (7-9). The sites of the proposed (11) nuclease cleavage are indicated by the arrows. The 3'-end sequences shown were determined by Price et al (7) and Englund (8) while the 5'-end dinucleotide sequences had been determined by Weiss et al (9).

(b) The sequence around one site of proposed nuclease cleavage involving the 5'-end of the l-strand. The nick occurs only on one strand as indicated by the arrow and the sequence is known only on one side of the nick

(c) The sequence around the second site of nuclease cleavage involving the 5'-end of the r-strand. The nick location on one strand is indicated by the arrow. The sequences shown in the boxes are common to both of the cleavage regions.

MATERIALS AND METHODS

DNA and nucleotides Nonradioactive T7 DNA was prepared by lysing CsCl purified T7 phage in 0.2 N KOH followed by centrifugation in 5-20% alkaline sucrose gradients using an SW 41 Beckman rotor. The peak of DNA was pooled and neutralized with KH_2PO_4. The DNA was then dialyzed against 10 mM Tris pH 7.6 and concentrated to 5 pmoles/ml by gentle evaporation under a stream of air. Dialysis against 10 mM Tris pH 7.6 was repeated and the DNA was stored at 5°C. The separated strands of T7 DNA were prepared by CsCl centrifugation in the presence of poly (U,G) (P.L. Biochemicals) as described by Szybalski et al (12). After dialysis to remove the CsCl, the separated strands were treated with 0.2 N KOH for 10 minutes at 37°C. The mixture was then centrifuged in a 5-20% alkaline sucrose gradient. The peak of DNA was pooled, neutralized and concentrated as described above.

$(\gamma\text{-}^{32}P)$ rATP was prepared by a modification of the method of Glynn and Chappell (13) to produce a specific activity of from 60 to 200 Ci/mmole. (^{32}P) phosphoric acid (New England Nuclear) was purified by passage through a 0.5 x 2.5 cm column of SP/C25 Sephadex (Sigma) and concentrated. The final reaction volume depended upon the specific activity required, with the concentration of rATP being maintained at 0.6 mM. Other constituents of the reaction mixture were: 67 mM Tris pH 7.6, 10 mM $MgCl_2$, 4 mM glutathione, 0.5 mM 3-phosphoglyceric acid, 200 µg/ml rabbit muscle 3-phosphoglycerate dehydrogenase (Sigma) and 10 µg/ml 3-phosphoglycerate kinase (Sigma). The reaction was usually complete (70-85% conversion of ^{32}Pi into $(\gamma\text{-}^{32}P)$ rATP) within 45 minutes as assayed by Norite adsorption.

Enzymes Pancreatic DNase, snake venom phosphodiesterase and bacterial alkaline phosphatase were purchased from Sigma. The bacterial alkaline phosphatase was further purified as described by Weiss et al (14). T4 induced polynucleotide kinase was purified as described by Panet et al (15) and Richardson (16). Before each series of kinase reactions, 100 units of polynucleotide kinase were fractionated on a 1 x 30 cm G100 Sephadex (Sigma) column equilibrated with 50 mM Tris pH 7.6, 0.1 M KCl, 10 µM rATP, 10 mM mercaptoethanol and 5% glycerol. The major peak of activity at the void volume was pooled and concentrated by dialysis against the same buffer with 50% glycerol. This kinase was used within one month since an endonuclease activity appeared after this time.

Labeling reactions The DNA or separated strands (5-10 pmoles/ml in 10 mM Tris pH 7.6) were treated with bacterial alkaline phosphatase (50 µg/ml) for 45 minutes at 37°C. For the subsequent kinase reactions, the solution contained the following reagents: 1 mM potassium phosphate pH 7.0, 70 mM Tris pH 7.6, 10 mM $MgCl_2$, 10 µM $(\gamma\text{-}^{32}P)$ rATP, 1 mM spermidine and 10 mM mercaptoethanol. This solution was incubated with 10 units/ml of polynucleotide kinase for 90 minutes at 37°C. KOH and EDTA were added to concentrations of 0.2 M and 20 mM respectively followed by fractionation on a 1 x 30 cm G50/SF Sephadex (Sigma) column equilibrated with 50 mM triethylammonium bicarbonate pH 7.6. The $5'\text{-}^{32}P$-labeled DNA eluted at the void volume and was located by Cherenkov counting in a scintillation counter. The DNA was then pooled and concentrated to dryness.

Enzyme digests A solution of $5'\text{-}^{32}P$-labeled DNA containing 40 mM Tris pH 7.6, 10 mM potassium phosphate pH 7.0, 1 mM pT and 10 mM $MgCl_2$ was incubated with various concentrations of pancreatic DNase (1, 5 and 50 µg/ml) at 37°C. for 15 minutes. EDTA pH 8.0 was added to a concen-

tration of 20 mM and the solution was evaporated to dryness. The resulting mixture of fragments was separated in two dimensions as described below.

Fragments recovered from the separation of pancreatic DNase digests containing 50 mM sodium glycinate pH 9.2, 10 mM $MgCl_2$ and 20 µg/ml snake venom phosphodiesterase were incubated for 20 minutes at 37°C. EDTA, pH 8.0, was added to a concentration of 10 mM and the solution was concentrated to dryness. This mixture was then separated in two dimensions.

Digestion to 5'-mononucleotides was carried out as described by Sgaramella et al (17).

Two dimensional electrophoresis and homochromatography The DNA digested with either pancreatic DNase or snake venom phosphodiesterase was dissolved in 5 µl of 1 M acetic acid. The fractionation of this solution was in two dimensions using cellulose acetate (Schleicher and Schuell) in the first dimension (I) and homochromatography in the second dimension (II). Thin layer plates were 1:7.5 DEAE cellulose to cellulose (MN 300 from Brinkmann). Homomixtures I, III and VI were prepared as described by Jay et al (18) and all other techniques were as described by either Barrell (19) or Jay et al (18). Fragments were located using Kodak RP-R14 X-ray film.

RESULTS

5'-Nucleotide Analyses Previous work had shown that the 5'-nucleotides of T7 DNA were pT on the l-strand and pA on the r-strand. The present work corroborated this as shown in Table I. Whole DNA contained ^{32}P-label in both pT and pA while the l-strand and r-strand contained predominantly pT and pA respectively. The proportion of pT to pA in the DNA fraction varied between preparations of the DNA and may reflect the amount or type of secondary structure in the various preparations which would affect the kinase reaction. Alternatively the kinase may have a higher affinity for a T end group. There was always a fairly high background of counts in the digests of the separated strands which may have arisen either from partial fragmentation of the strands or from the incomplete removal of poly (U,G) after the strand separation.

Also included in Table I are the 5'-nucleotide analyses of various fragments isolated from the 50 µg/ml pancreatic DNase digests shown in Figure 2. All of the fragments analyzed from the l-strand digest (L1-3, Figure 2b) contained mainly $(5'-^{32}P)$ pT while those from the r-strand digest (R1 and R2, Figure 2c) contained mainly $(5'-^{32}P)$ pA . If the corresponding fragments were isolated and analyzed from the DNA finger-

Table I

5'-Mononucleotide composition of various 5'-^{32}P-labeled DNAs

Experiment	DNA	pA	pG	pT	pC
			counts/minute		
1	T7 DNA	2058	30	3063	13
2	T7 r-strand	316	45	27	51
3	T7 l-strand	50	39	329	68
4	L1	50	69	359	68
5	L2	62	62	238	58
6	L3	47	49	202	57
7	R1	265	68	68	67
8	R2	539	70	74	76

print, Figure 2c, it was possible to identify which of the fragments came from which strand.

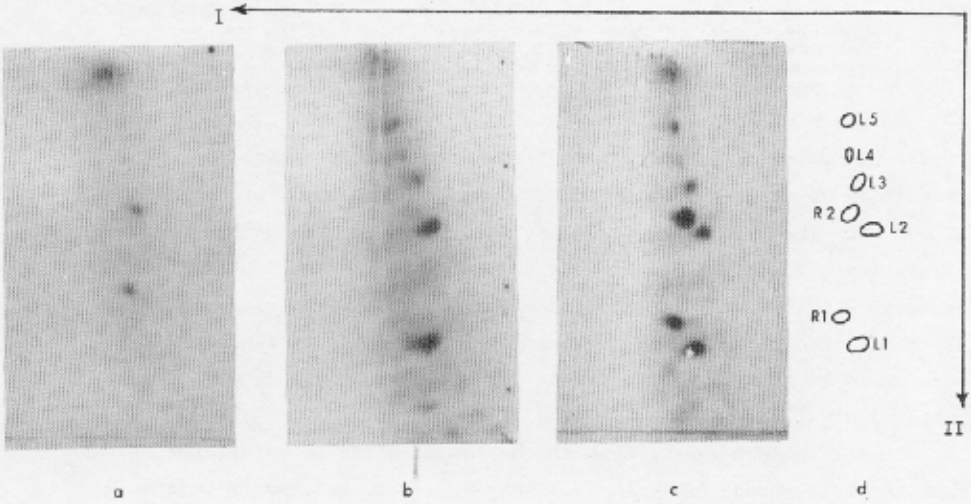

Figure 2. 2-D homochromatography (homo-mix VI, 20 x 40 cm) fingerprints of the partial pancreatic DNase digests (50 µg/ml of the nuclease) of: (a) [5'-^{32}P]T7 r-strand, (b) [5'-^{32}P] T7 l-strand and (c) [5'-^{32}P]T7 DNA. Part (d) is a schematic diagram of the spots in part (c) labeled as they are referred to in the text. The conditions for the digests are described in Materials and Methods.

Pancreatic DNase digests The two dimensional fingerprints of r- and l-strands and of whole DNA which were created by 50 and 1 µg/ml of pancreatic DNase are shown in Figures 2 and 3. For each nuclease concentration the fingerprint of whole DNA was a composite of the fingerprints of the separated strands. It should be stressed that the whole DNA was isolated from alkaline sucrose gradients. It was therefore not double stranded DNA but a mixture of the two separate strands.

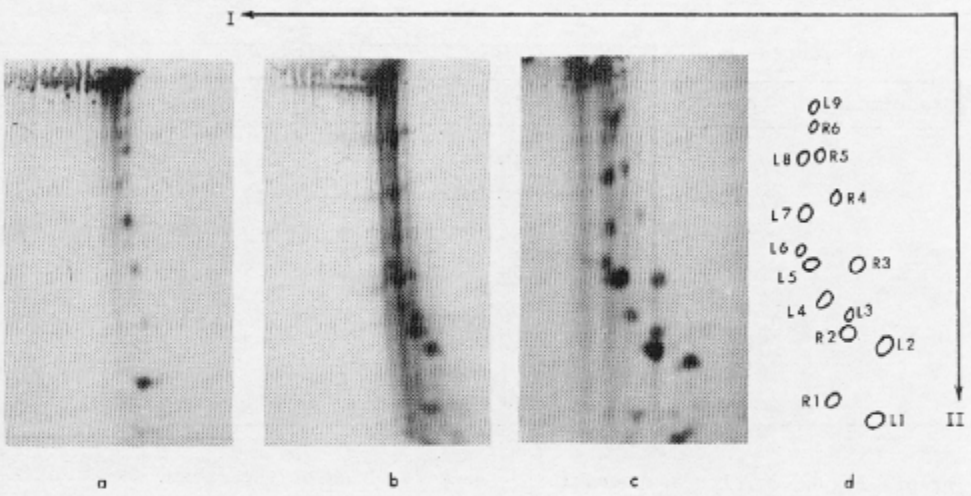

Figure 3. 2-D homochromatography (homo-mix III, 20 x 40 cm) fingerprints of the partial pancreatic DNase digests (1 μg/ml of the nuclease) of: (a) [5'-^{32}P]T7 r-strand, (b) [5'-^{32}P] T7 l-strand and (c) [5'-^{32}P]T7 DNA. Part (d) is a schematic diagram of the spots in part (c) labeled as they are referred to in the text. The conditions for the digests are described in Materials and Methods.

In the fingerprints created by 50 μg/ml pancreatic DNase shown in Figure 2, two major products were formed from each strand. They were identified as the tetranucleotides, L1 and R1, and the heptanucleotides, L2 and R2. The same sized fragments were isolated from both strands suggesting that the nuclease binds to tetra-and heptanucleotide lengths protecting them from further degradation. The slower migration of R1 and R2 relative to L1 and L2 in homochromatography in spite of having the same chain lengths was shown to be the result of R1 and R2 having a greater purine content (Table II).

Some fragments longer than the heptanucleotide L2 were formed in the case of the l-strand (L3 - L5 in Figure 2b). However, no fragments larger than the heptanucleotide R2 were produced from the r-strand. The additional fragments in the fingerprint of whole DNA must therefore have been derived from the l-strand. This was verified by both 5'-mononucleotide analysis (Table I) and partial venom phosphodiesterase digests (see below).

The preferential formation of the heptanucleotide, R2, from the r-strand was accentuated in the fingerprint created by 5 μg/ml of pancreatic DNase (not shown). The major product was R2 with only a small amount of R1 and no larger fragments being formed. Digestion of the l-strand or DNA with 5 μg/ml of pancreatic DNase (fingerprints not shown) yielded a number of fragments larger than the heptanucleotide L2, all of which were

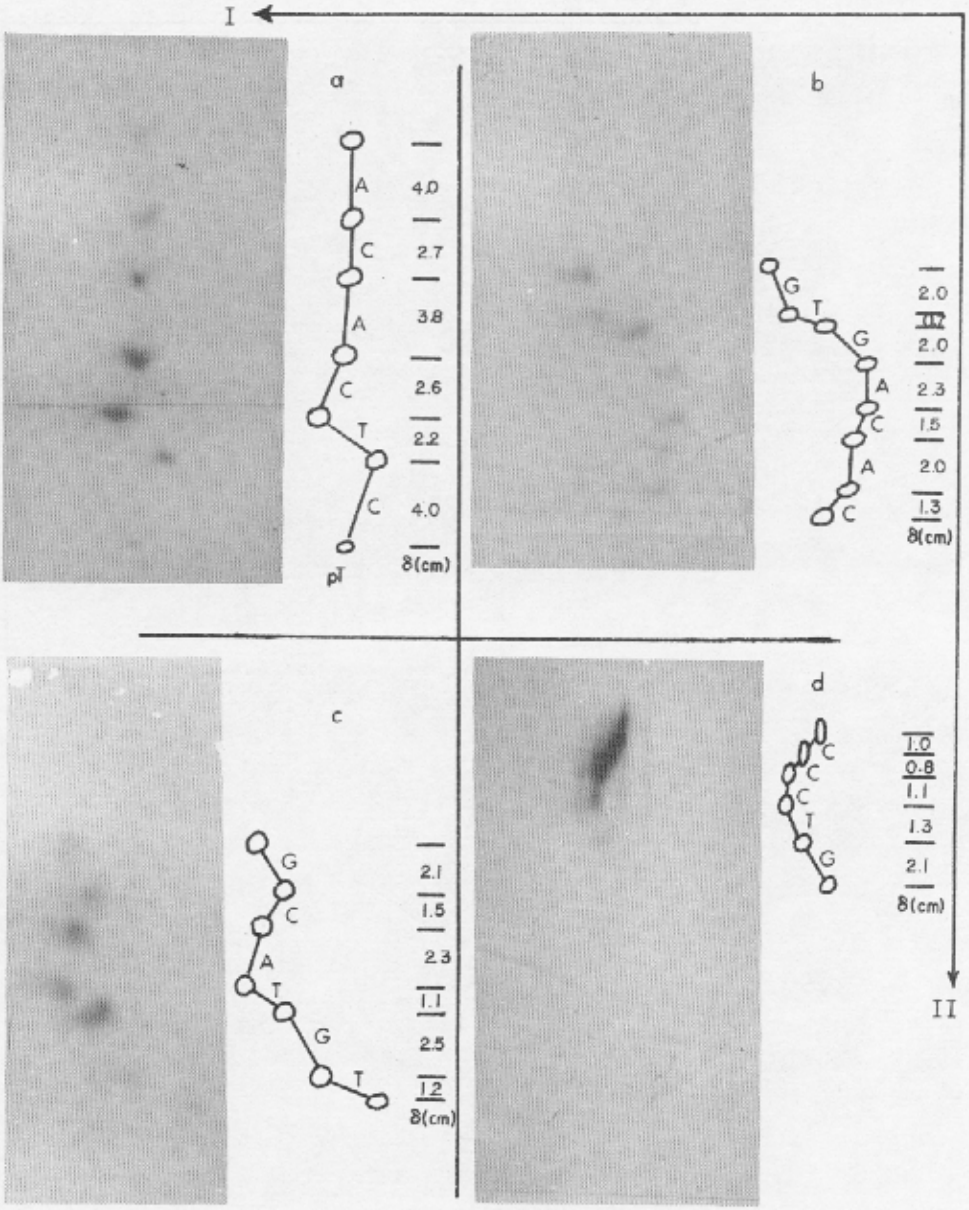

Figure 4. 2-D homochromatography (20 x 40 cm) fingerprints of partial venom phosphodiesterase digests of: (a) L1 (homo-mix VI), (b) L5 (homo-mix III), (c) L8 (homo-mix III) and (d) L9 (homo-mix III). The conditions for the digests are described in Materials and Methods.

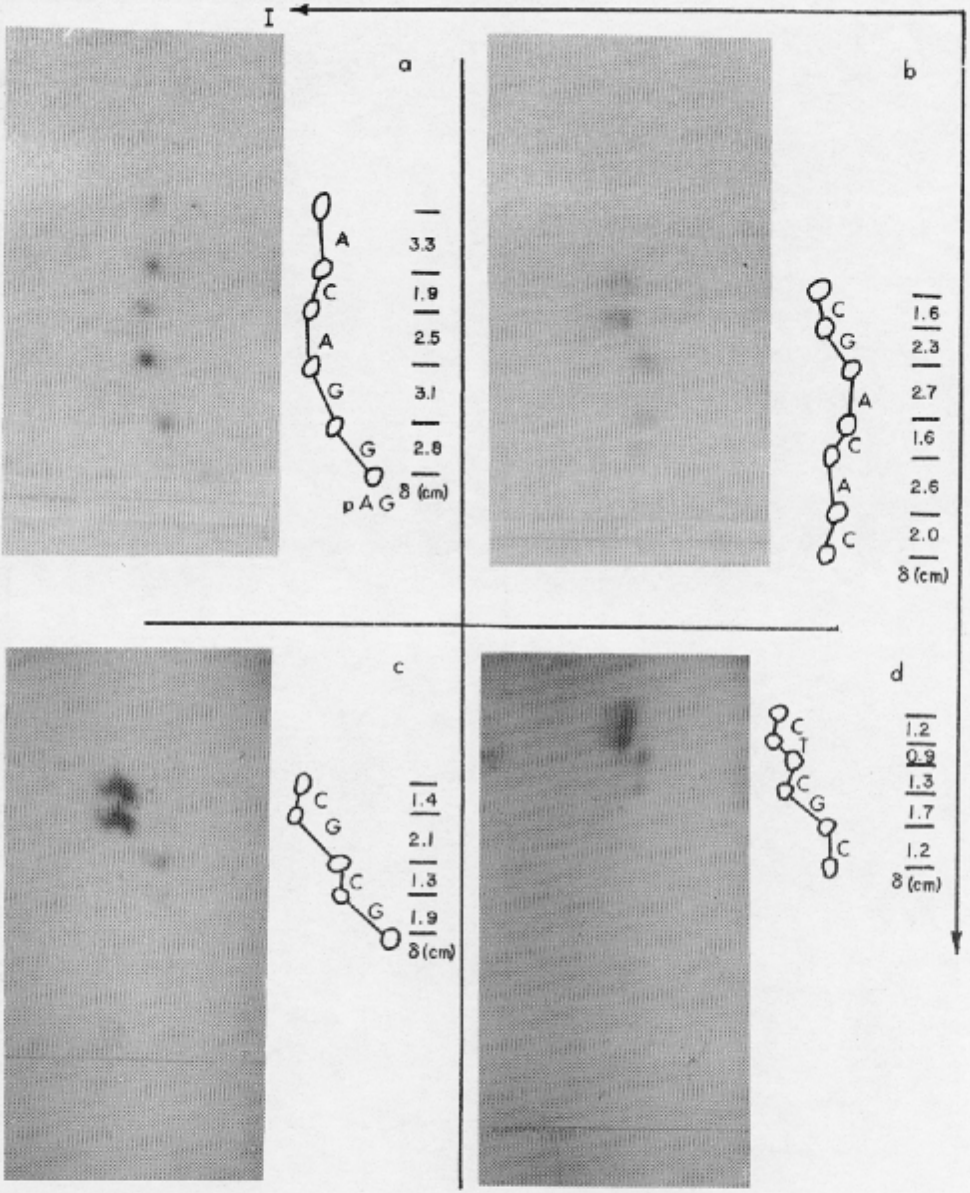

Figure 5. 2-D homochromatography (20 x 40 cm) fingerprints of the partial venom phosphodiesterase digests of: (a) R2 (homo-mix VI), (b) R4 (homo-mix III), (c) R5 (homo-mix III) and (d) R6 (homo-mix III). The conditions for the digests are described in Materials and Methods.

Table II

Comparison of the U_T (calculated) and the U_T (observed) for the various oligonucleotide fragments from the l-strand and the r-strand.

N	Oligonucleotide	Pancreatic Digest Designation	U_T(calc.)	U_T (obs.) venom digest	U_T (obs.) pancreatic digest
1	pT		1.00	1.00	
2	pTC		0.78	0.79	
3	pTCT		1.13	1.13	
4	pTCTC	L1	0.96	0.95	0.97
5	pTCTCA		0.94	0.89	
6	pTCTCAC		0.86	0.87	
7	pTCTCACA	L2	0.84	0.85	0.83
8	pTCTCACAG	L3	0.97	0.98	0.97
9	pTCTCACAGT	L4	1.10	1.11	1.10
10	pTCTCACAGTG	L5	1.17	1.17	1.16
11	pTCTCACAGTGT	L6	1.26	1.27	1.23
12	pTCTCACAGTGTA	L7	1.22	1.21	1.17
13	pTCTCACAGTGTAC		1.18	1.18	
14	pTCTCACAGTGTACG	L8	1.22	1.23	1.19
15	pTCTCACAGTGTACGT		1.29	1.27	
16	pTCTCACAGTGTACGTC		1.23	1.23	
17	pTCTCACAGTGTACGTCC		1.18	1.20	
18	pTCTCACAGTGTACGTCCC	L9	1.13	1.17	1.15
1	pA		0.30	0.37	
2	pAG		0.79	0.77	
3	pAGG		1.05	0.96	
4	pAGGG	R1	1.19	1.08	1.08
5	pAGGGA		1.14	1.03	
6	pAGGGAC		1.04	1.01	
7	pAGGGACA	R2	1.00	1.00	1.02
8	pAGGGACAC		0.93	0.96	
9	pAGGGACACA	R3	0.91	0.93	0.97
10	pAGGGACACAG		1.00	1.03	
11	pAGGGACACAGC	R4	0.95	1.03	1.06
12	pAGGGACACAGCG		1.02	1.11	
13	pAGGGACACAGCGC	R5	0.98	1.10	1.12
14	pAGGGACACAGCGCT		1.06	1.16	
15	pAGGGACACAGCGCTC	R6	1.02	1.14	1.16

derived from the l-strand.

When 1 µg/ml of pancreatic DNase was used, the larger fragments from both the r- and the l-strands became more prominant (Figure 3). The divergence of the patterns of sequentially larger fragments from the r- and l-strands is clearly evident in Figure 3. Up to the heptanucleotide stage, the r-strand fragments electrophoresed faster on cellulose acetate than the corresponding fragments from the l-strand. L-strand fragments larger than an octanucleotide migrated faster than those of corresponding size from the r-strand. This trend was maintained until the fragments reached 16 to 18 nucleotides in length when little difference in the relative electrophoretic mobilities could be seen.

A small amount of R2 could be seen in some l-strand digests (Figure 3b). This indicated that the l-strand preparations were slightly contaminated by some r-strand. The r-strand fingerprints did not show any contamination by l-strand fragments. While the T7 strands separated quite well (12), there must have been a small amount of tailing of the heavier r-strand peak into the lighter l-strand.

Two features of the pancreatic DNase action were interesting. The first was that the nuclease created tetra- and heptanucleotide fragments as the preferred smaller cleavage products. The second was that the enzyme created a different size range of products for each DNA strand. Since non-double stranded DNA was used for these experiments, these observations may have been a reflection of the different secondary structure at the ends of the two strands. The smaller tetra- and heptanucleotides may have been determined by the amount of DNA bound by the nuclease and protected from further degradation.

Snake venom phosphodiesterase digests All of the fragments isolated from the partial pancreatic DNase fingerprints were subjected to partial venom phosphodiesterase digestion. The two dimensional fingerprints of the digest mixtures necessary to complete the sequences are shown in Figures 4 and 5. Alongside each of the fingerprints is a schematic diagram of the fragment pattern and the distance between each subsequent fragment. The nucleotide which was concluded to have been removed at each step is also shown in the diagram.

The assignment of each removed nucleotide was based on two criteria: the M-values or distances between subsequent fragments in the homochromatography dimension (18) and the U_T or migration distances relative to pT during electrophoresis in cellulose acetate (20). The U_T (calculated)

and the U_T (observed) values for every fragment in the two sequences are contained in Table II. As well the U_T values observed in the fingerprints of the pancreatic digests are included for comparison. As expected there was a close correlation between the U_T values for fragments found in the pancreatic and the venom DNase digests. The U_T values observed from the fingerprints of the venom DNase digests agreed very well with the calculated values in the case of the l-strand fragments. However, the agreement was not as good for the r-strand fragments. When the fragments were small, as for Rl, the observed U_T values were smaller than the calculated U_T values. The converse was true for the larger fragments such as R5 and R6. Here the observed U_T values were larger than the calculated U_T values. This variation may be due to the greater proportion of purine bases in the r-strand sequence (9 of 15). Bambara et al (20) observed a similar variation for some small purine rich fragments which they analyzed. The variations which were observed in this work are also smaller than the largest variations observed by Bambara et al (20) for several of their fragments.

In Figure 4, the venom phosphodiesterase fingerprints for L2, L5, L8 and L9 are shown. L2 proved to be the heptanucleotide pT-C-T-C-A-C-A while L5 was the decanucleotide pT-C-T-C-A-C-A-G-T-G. Fragment L8 overlapped with L5 and L9 providing the final sequence pT-C-T-C-A-C-A-G-T-G-T-A-C-G-T-C-C-C, of eighteen nucleotides at the 5'-end of the T7 l-strand.

In Figure 5, the venom phosphodiesterase fingerprints of R2, R4, R5 and R6 are shown. R2 was the heptanucleotide pA-G-G-G-A-C-A while R4 was the undecanucleotide pA-G-G-G-A-C-A-C-A-G-C. R5 overlapped with R4 and R6 providing the final sequence pA-G-G-G-A-C-A-C-A-G-C-G-C-T-C of fifteen nucleotides at the 5'-end of the r-strand.

Polynucleotide kinase In order to label only the 5'-ends of such large DNA, it was very important that the enzymes used be free of all nuclease activities. Bacterial alkaline phosphatase was easily purified by the method of Weiss et al (14). The polynucleotide kinase, however, presented more difficulty (15). Three separate batches of the enzyme prepared by the methods of Panet et al (15) or of Richardson (16) were shown to be free of any nuclease activity immediately after their preparation. However, upon storage at -20°C for several months a nuclease activity appeared in each batch. Chromatography of a fraction of the kinase preparations on a G100 Sephadex column retarded the nuclease and separated it from the kinase which passed through the column at the void

volume. Within several weeks the nuclease activity had reappeared in the G100 kinase fraction. Whether there was a latent nuclease in the kinase preparations or the kinase tetramer (15) was breaking down into its subunits which possessed a nuclease activity remains to be determined. In view of this interesting but annoying aspect of the kinase structure, the kinase was purified before every set of experiments. The nature of the nuclease activity is presently under investigation.

DISCUSSION

The complete sequence of T7 DNA which has been determined is shown in Figure 1. The orientation of the sequences in the terminally redundant regions is also illustrated. Since both of the new sequences are repeated two times, the sequence of 66 base pairs at the ends of T7 DNA is known and 33 of these base pairs are situated about 260 pairs into the DNA molecule.

The sequences determined in this paper corroborate previous work (7) which showed that a heptanucleotide TGTCCCT existed at the 3'-end of the l-strand and a trinucleotide AGA existed at the 3'-end of the r-strand. In addition, it was shown (7) that there was a GPA transfer near the 3'-end of the l-strand and ApC and GpT transfers near the 3'-end of the r-strand. The complements to these transfers were found in the 5'-end sequences. Specifically, a T_pC dinucleotide was found 14 nucleotides from the 5'-end of the r-strand while GpT and ApC dinucleotides were found 8 and 5 nucleotides respectively, from the 5'-end of the l-strand.

Watson (11) has proposed a model in which the terminally redundant ends of T7 DNA allow DNA replication to proceed by a mechanism involving concatemeric DNA. Once formed the concatemeric DNA is cleaved by one or more specific nucleases to form unit length T7 DNA molecules. This mechanism produces staggered nicks with potential 5'-protruding segments of complementary sequence at the ends of the DNA. When these potential sticky ends are reconverted to duplex DNA by a DNA polymerase the terminally redundant sequences are created.

The sequences recognized by the putative specific concatemer nuclease (or nucleases) must be contained within the unit length DNA molecule. These locations are shown in Figure 1. In a concatemer molecule the two nicks on opposite strands would actually take place about 260 base pairs apart. It was this large gap between the two cut locations which suggested to Watson that two nucleases were involved, one for each nick. The DNA sequences determined in this paper are ambiguous in that they

do not clearly support arguments for either one or two nucleases.

An element of two fold symmetry has been found in all of the DNA sequences involved in protein-nucleic acid interactions. A similar feature should be exhibited by the sequences recognized by the putative concatemer nuclease or nucleases if they are to fit into the general trend. The known portions of the sequences around the proposed cleavage sites are also shown in Figure 1. The l-strand sequence, Figure 1b, begins with four pyrimidines, T-C-T-C, while the r-strand sequence in Figure 1c begins with four purines, A-G-G-G. Beyond these first four base pairs, a region of hyphenated homology exists which is shown in the boxed regions. This is not an extensive region of homology when compared to the λ (3) and tyrosine tRNA (5a, 5b) promoter regions. However, it would prove to be significant if a symmetric region containing this homology were to be found on the opposite or left side of the cleavage sites. Therefore the sequences on the left side of the nuclease cuts in Figures 1b and 1c must be determined in order to complete this study. The complementary sequences to those determined in this paper are being synthesized by chemical means. These synthetic oligonucleotides will then be annealed to the complementary separated strands of T7 DNA and elongated with DNA polymerase I into the unknown regions. This procedure of primer elongation by DNA polymerase I has been used successfully by several groups for DNA sequence determinations (1, 3, 4, 5a, 5b, 21).

† Research supported by the National Research Council of Canada and the University of Manitoba Faculty of Graduate Studies

REFERENCES

1. Wu, R. and Taylor, E. (1971) J. Mol. Biol. 57:491-511.
2. Gilbert, W. and Maxam, A. (1973) Proc. Nat. Acad. Sci. USA 70: 3581-3584.
3. Maniatis, T., Ptashne, M., Barrell, B. G., and Donelson, J. (1974) Nature 250:394-397.
4. Loewen, P. C., Sekiya, T. and Khorana, H. G. (1974) J. Biol. Chem. 249:217-226.
5a. Sekiya, T. and Khorana, H. G. (1974) Proc. Nat. Acad. Sci. USA 71:2978-2982.
5b. Sekiya, T., van Ormondt, H. and Khorana, H. G. (1975) J. Biol. Chem. 250:1087-1098.
6. In Handbook of Nucleic Acid Sequences Barrell, B. G., and Clark, B. F.C. p. 96-101 Joynson-Bruvvers, Oxford (1974).
7. Price, S. S., Schwing, J. M., and Englund, P. T. (1973) J. Biol. Chem. 248:7001-7006.
8. Englund, P. T. (1972) J. Mol. Biol. 66:209-224.
9. Weiss, B. and Richardson, C. C. (1967) J. Mol. Biol. 23:405-415.

10. Ritchie, D. A., Thomas, C. A. Jr., MacHattie, L. A. and Wensink, P.C. (1967) J. Mol. Biol. 23:365-376.
11. Watson, J. D. (1972) Nature New Biol. 239:197-201.
12. Szybalski, W., Kubinski, H., Hradecna, Z. and Summers, W. C. (1971) Methods in Enzymology 21:383-413.
13. Glynn, I. M. and Chappell, J. B. (1964) Biochem. J. 90:147-149.
14. Weiss, B., Live, T. R. and Richardson, C. C. (1968) J. Biol. Chem. 243:4530-4542.
15. Panet, A., vande Sande, J. H., Loewen, P. C., Khorana, H. G., Raae, A. J., Lillehaug, J. R. and Kleppe K. (1973) Biochemistry 12: 5045-5050.
16. Richardson, C. C. (1972) Proced. Nucleic Acid Res. 2:815-828.
17. Sgaramella, V., vande Sande, J. H. and Khorana, H. G. (1970) Proc. Nat. Acad. Sci. USA 67:1468-1475.
18. Jay, E., Bambara, R., Padmanabhan, R. and Wu, R. (1974) Nucleic Acids Research 1:331-353.
19. Barrell, B. G. (1972) Proced. Nucleic Acid Res. 2:751-779.
20. Bambara, R., Jay, E., and Wu, R. (1974) Nucleic Acids Research 1: 1503-1520.
21. Sanger, F., Donelson, J. E., Coulson, A. R., Kössel, H. and Fisher, D. (1973) Proc. Nat. Acad. Sci. USA 70:1209-1213.