SEQUENCE AT THE 5'-END OF BACTERIOPHAGE f2 RNA

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Summary. A sequence of 74 nucleotides has been determined at the 5'-end of bacteriophage f2 RNA. This sequence is identical to the 5'-end of the closely related phage R17 (4). This invariant region in the RNA may be important in the function of the 5'-end of these two phages.

R17 and f2 are closely related RNA bacteriophages whose RNAs serve as messengers in the host bacterium (1,2,3). Recently Adams and Cory (4) have determined the sequence of 74 nucleotides from the 5'-end of R17 RNA and have concluded that this portion of the phage RNA was not translated into any of the known R17 proteins. The exact function of this apparently untranslated region of the bacteriophage is not clear. Evidence is presented in this report to show that the sequence of the first 74 nucleotides from the 5'-end of f2 RNA is identical with that of R17.

MATERIALS AND METHODS

Bacteriophage f2 RNA uniformly labelled with ^{32}P-phosphate was prepared according to the method of Dahlberg (5). The labelled RNA was digested with ribonuclease T1 to yield a number of large fragments which were separated by electrophoresis on a 12.5 per cent polyacrylamide gel slab at pH 8.3 (6). The separated fragments were visualized by radioautography and a fragment containing the 5'-terminus was located by detecting the presence of pppGp in the alkaline hydrolysate of the isolated fragment (5). The sequence of this fragment was determined according to the methods developed by Sanger and co-workers (6-10).

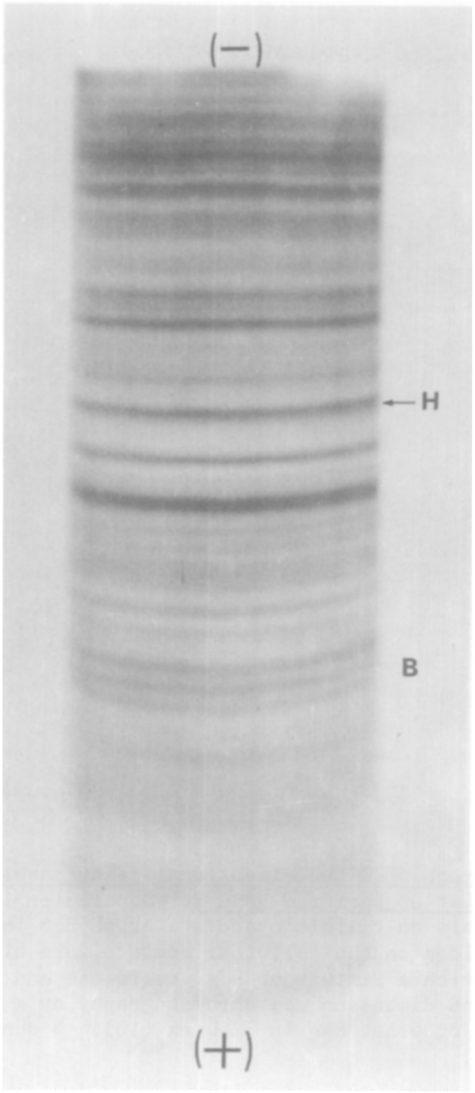

Fig. 1. Radioautograph of the polyacrylamide gel fractionation (6) of a partial ribonuclease T1 digest of 0.8 mg of ^{32}P-f2 RNA (specific activity 2.2 x 10^6 cpm per microgram). The enzyme to substrate ratio was 1:500 and the digestion was performed at 0° for 16 hours in 0.1 ml of a buffer consisting of 0.02M magnesium acetate and 0.05M Tris-HCl at pH 7.5. Band H is the major band containing the 5'-terminus. B is the position to which the bromphenol blue marker migrated in this gel.

RESULTS AND DISCUSSION

Fig. 1 shows the radioautograph of ^{32}P-labelled f2 RNA fragments separated on polyacrylamide gel. The major fragment containing the 5'-terminus is indicated by an arrow and labelled H. This fragment was eluted from the gel and when necessary re-purified by the two-dimensional thin-layer

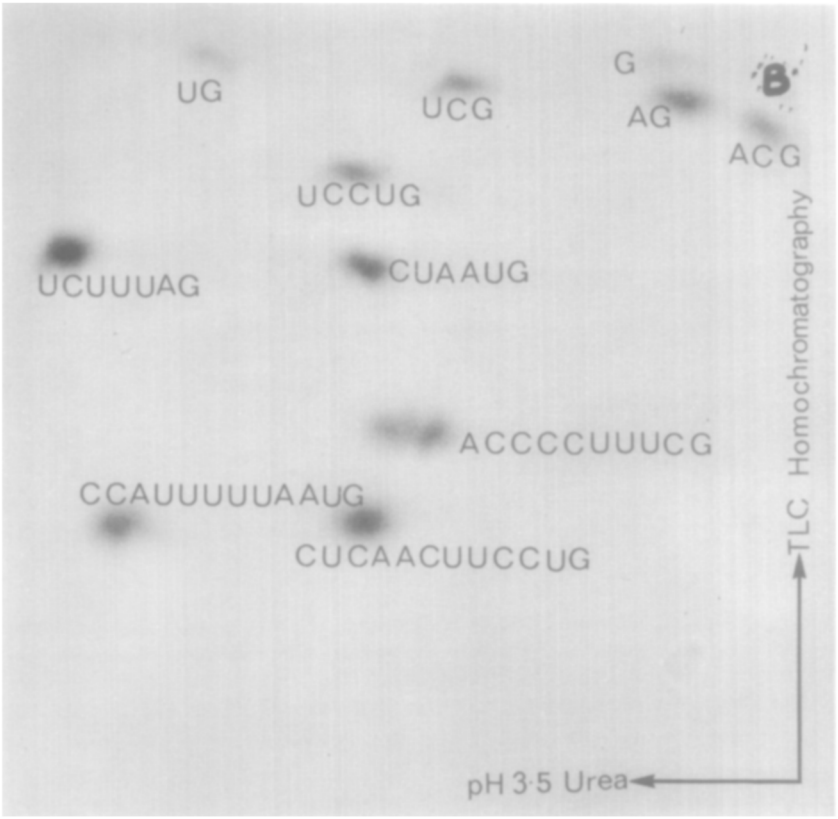

Fig. 2. Radioautograph of a two-dimensional fractionation (10) of a complete ribonuclease T1 digest of purified band H. Separation in the first dimension was by electrophoresis on cellulose acetate at pH 3.5 in 7M Urea containing 5 mM EDTA. Nucleotides on the cellulose acetate were blotted on to a thin-layer plate coated with a mixture of DEAE-cellulose and cellulose in a ratio of 1:7.5. The second dimension was chromatography by a 5% partially hydrolyzed solution of yeast RNA in 7M Urea (10). B denotes the position of the marker blue dye.

system described in Fig. 4. Fig. 2 shows the radioautograph of a two-dimensional fractionation by electrophoresis and thin-layer homochromatography (10) of a complete ribonuclease T1 digest of re-purified band H. The 5'-terminus pppGp migrates considerably faster than the other nucleotides in the first dimension (electrophoresis on cellulose acetate) and is therefore not included in this fingerprint; however, pppGp is recovered when the standard fingerprint on DEAE-paper (7, see also Fig. 3) was employed. The two large oligonucleotides ACCCCUUUCG and CUCAACUUCCUG observed here as being well separated on the thin-layer are not well resolved in the DEAE-paper system. Fig. 3 shows the

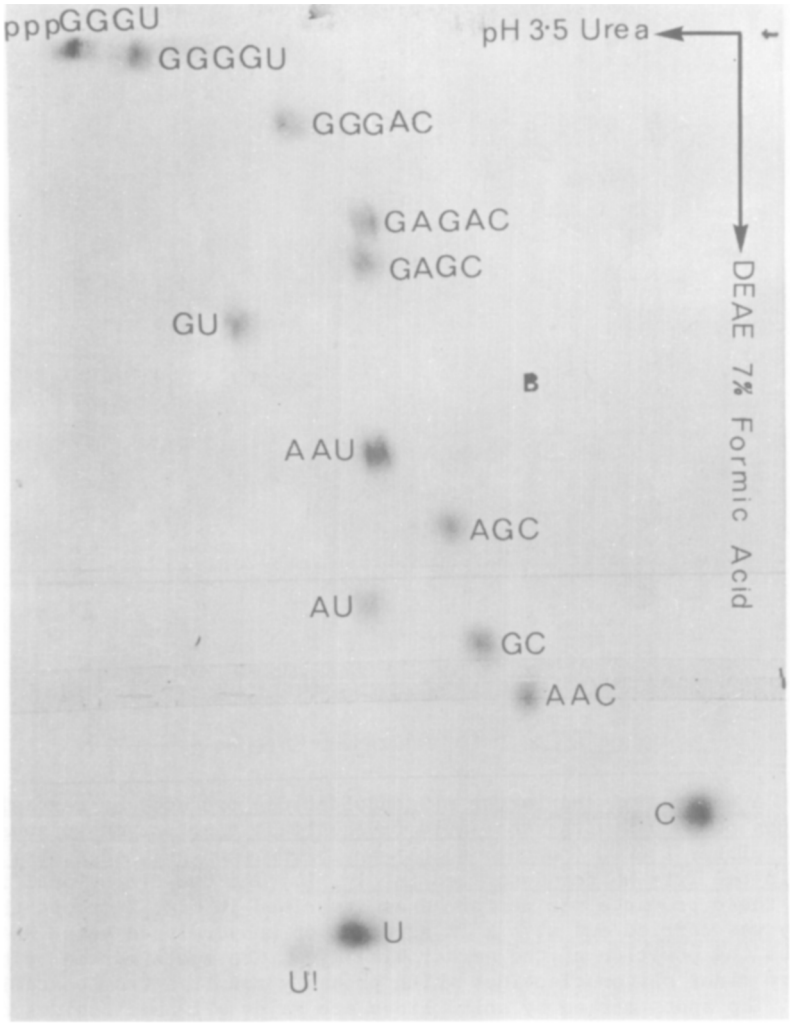

Fig. 3. Radioautograph of a two-dimensional fractionation of a complete pancreatic ribonuclease A digest of purified band H. The first dimension was by electrophoresis on cellulose acetate as in Fig. 2. The second dimension was by electrophoresis on DEAE-paper in 7% formic acid. Cyclic nucleotides are marked by "!". B denotes the position of the marker blue dye.

fingerprint on DEAE-paper of the products of band H resulting from a complete digestion with pancreatic ribonuclease A. The 5'-terminus is present as part of the oligonucleotide pppGGGU. The sequences of the oligonucleotides shown in Figs. 2 and 3 were determined by the techniques described by Sanger and co-workers (6-10). The ordering of these oligonucleotides was deduced from the analysis of fragments produced from partial digestions of band H with pancreatic ribonuclease (Fig. 4) and the sequence of band H is shown in Fig. 5.

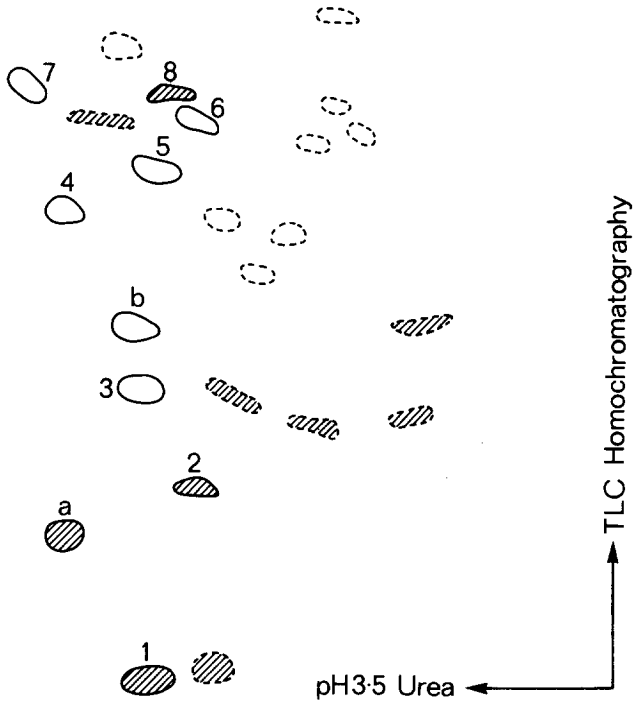

Fig. 4. A diagram of the larger oligonucleotides produced by partial digestions of band H with pancreatic ribonuclease A at enzyme to substrate ratios of either 1:4000 (hatched spots) or 1:1000 (non-hatched spots) at 0° for 15 min in the Tris buffer described in Fig. 1. The two-dimensional fractionation of these products was performed as described in Fig. 2 except that chromatography was carried out with a 5% solution of unhydrolyzed yeast RNA. B denotes the position of the marker blue dye. The spots marked by broken lines are minor oligonucleotides which probably resulted from contaminants of band H. The spots marked by solid lines are major oligonucleotides derived from band H. Spots a and b were still mixtures and were not used for sequence deduction. The sequence of band H was deduced by analysis of oligonucleotides 1 to 8 and their sequences are indicated in Fig. 5.

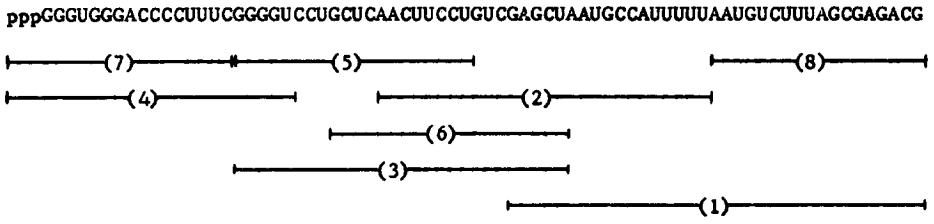

Fig. 5. The nucleotide sequence of band H. The sequence was deduced by analysis of fragments produced by partial digestions of band H with pancreatic ribonuclease A (see Fig. 4). The nucleotide sequence deduced for oligonucleotides 1 to 8 of Fig. 4 are indicated by solid lines.

This sequence is identical with the nucleotide sequence at the 5'-end of R17 (4).

Nichols and Robertson (11) have observed that the fingerprint pattern on thin-layer homochromatography of the large oligonucleotides produced by complete digestion of intact f2 RNA with ribonuclease T1 was quite different from that of R17 (12) indicating the presence of many non-identical sequences between these two phages. Further, on examination of two sequences located in the coat protein cistron of f2 RNA, they have found four differences between f2 and R17 in a total of 100 nucleotides analyzed (11). Gupta et al. (13) have also found two differences in a sequence of 39 nucleotides in the coat protein ribosome-binding site of f2 and R17. These results clearly establish the non-identity of f2 and R17. It is probably significant that a sequence of 74 nucleotides at the 5'-end of these two phages is completely identical and this invariant sequence may play an important role in the function of the untranslated 5'-end of the phage RNA.

While the actual function of the 5'-end of the RNA phages is not known, Adams and Cory (4) have pointed out that the 5'-terminal sequences of R17 and Qβ RNA are strikingly similar especially since these two phages are quite different and are not related serologically nor chemically. They have suggested that this similarity is unlikely to be accidental and that the 5'-terminal sequence must have some functional importance. It is perhaps not surprising therefore that the sequence at the 5'-end of R17 and f2 (two closely related phages) is identical.

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REFERENCES

1. Weissmann, C. and Ochoa, S., Prog. Nucleic Acid Res. 6, 353 (1967).

2. Lodish, H.F. and Robertson, H.D., Cold Spring Harbor Symp. Quant. Biol. 34, 655 (1969).

3. Valentine, R.C., Ward, R. and Strand, M., Adv. in Virus Res. 15, 1 (1969).

4. Adams, J.M. and Cory, S., Nature 227, 570 (1970).

5. Dahlberg, J.E., Nature 220, 548 (1968).

6. Adams, J.M., Jeppesen, P.G.N., Sanger, F. and Barrell, B.G., Nature 223, 1009 (1969).

7. Sanger, F., Brownlee, G.G. and Barrell, B.G., J. Mol. Biol. 13, 379 (1965).

8. Brownlee, G.G. and Sanger, F., J. Mol. Biol. 23, 337 (1967).

9. Brownlee, G.G., Sanger, F. and Barrell, B.G., J. Mol. Biol. 34, 379 (1969).

10. Brownlee, G.G. and Sanger, F., Europ. J. Biochem. 11, 395 (1969).

11. Nichols, J.L. and Robertson, H.D., Biochim. Biophys. Acta, in press 1970.

12. Jeppesen, P.G.N., Argetsinger-Steitz, J., Gesteland, R.F. and Spahr, P.F., Nature 226, 230 (1970).

13. Gupta, S.L., Chen, J., Schaefer, L., Lengyel, P. and Weissman, S.M., Biochem. Biophys. Res. Comm. 39, 883 (1970).