THE 3′-TERMINAL NUCLEOTIDE SEQUENCE (n = 16) OF BACTERIOPHAGE MS2 RNA

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1. Intoduction

The 3′-terminal nucleotide sequence of viral RNA is of particular interest. Indeed, as replication proceeds in an antiparallel direction, it is likely that the former region contains the recognition signal required for specific interaction with the viral RNA polymerase. Alternatively, or in addition, one may hope to find a termination signal for protein synthesis for the cistron nearest the 3′-end of the molecule, in casu RNA polymerase [1, 2].

De Wachter and Fiers [3] have previously reported that MS2 RNA ends in (G) U–U–A–C–C–A–C–C–C–A, and the same sequence was confirmed for the bacteriophage f2 RNA [4, 5] and R17 RNA [5], which are serologically closely related to MS2 RNA. Bacteriophage Qβ RNA has a very different 3′-terminal sequence [5, 6]. All four sequences, however, share in common the ...C–C–C–$A_{3'OH}$ end and the absence of a termination codon.

By applying partial instead of complete degradation with ribonuclease T1, followed by separation of the products by electrophoresis on polyacrylamide gel, longer oligonucleotides can be obtained for sequence analysis [7]. In this paper we described the analysis of such a fragment derived from the 3′-end. Its sequence is:

(G) G–C–U–A–G–U–U–A–C–C–A–C–C–C–A

The possible significance of the amber codon UAG is discussed.

2. Methods

^{32}P-Labelled MS2 RNA was prepared essentially as previously described [8]. The RNA was partially degraded with ribonuclease T1 and the hydrolysate separated on a 12% polyacrylamide gel slab at neutral pH [9]. The method used in similar to the one described for the isolation of R17 RNA fragments [10, 11]. After visualisation of the gel pattern by radioautography, bands were cut out and the RNA was extracted as described in reference [9]. The mixture of partial products from such bands was further separated into individual components by high-voltage electrophoresis at pH 3.5 in one dimension followed by "homo-chromatography" on thin-layer plates in the second dimension [12]. The methods for sequence analysis were essentially as described by Sanger and coworkers [10, 13, 14].

3. Results and discussion

When the mixture of partial products from band D6 of the polyacrylamide gel (D is the region of the 12% gel between the eosine and bromophenol blue dyes [9], D6 is the band near the bromophenol blue marker) is fractionated further, a pattern as shown in fig. 1 was obtained. A ribonuclease T1 map of component no 1 of this "homo-chromatogram" gave only three products (table 1), one of which was shown to be identical with the known 3′-terminal sequence of MS2 RNA [3]. The RNAase T1 and pancreatic ribonuclease products (table 1) from this fragment were sufficient to construct a unique sequence, located at the 3′-end of MS2 RNA:

(G) G–C–U–A–G–U–U–A–C–C–A–C–C–C–A.

It is of interest that this sequence contains an

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Table 1
The nucleotide sequence analysis of component no 1.

Material	Composition		RNAase U_2 products[c]	CMCT-blocked RNAase A products[d]	Structure deduced	Distribution of radioactivity[f]
	Alkaline hydrolysis	RNAase A or T1 products[b]				
RNAase T_1 products:[a]						
1	Gp				Gp	1.0
2		2Up, 3Cp, 2A–Cp	C–C–C–A U–U–Ap, C–C–Ap	A–Cp, Cp >, Cp U*–U*–A–Cp	U–U–A–C–C–A–C–C–C–A[e]	9.0
3		Up, Cp, A–Gp		C, C–U*–A–Gp* U*–A–Gp*	C–U–A–Gp	4.6
RNAase A products: [c]						
1	Up				Up	3.2
2	Cp				Cp	2.2
3	1Ap, 1Cp				A–Cp	4.0
4	1Gp, 1Cp				G–Cp	2.2
5		Up, A–Gp			A–G–Up	3.4

[a] The RNAase T1 products were separated by (two dimensional) electrophoresis on cellulose acetate at pH 3.5, followed by electrophoresis in 7% formic acid on DEAE-paper, or by (one dimensional) electrophoresis in the 7% formic acid system.

[b] The products from pancreatic (for T1 products) or T1 RNAase (for RNAase A products) were separated by electrophoresis on DEAE-paper at pH 3.5. They were identified from their positions on the electropherogram.

[c] Separated on DEAE-paper at pH 1.9. The products were characterized by their nucleotide composition, unless otherwise specified.

[d] CMCT (*N*-cyclohexyl-*N*-(β-morpholinyl-(4)-ethyl)carbodiimide-methyl-*p*-toluene sulphonate)-modified oligonucleotides were digested with pancreatic ribonuclease and the digestion products fractionated on Whatman no 3MM at pH 3.5. U* and G* indicate modified nucleoside residues. The products were characterized by their nucleotide composition. It follows from the results that hydrolysis by RNAase A was incomplete (presumably due to steric hindrance by the blocking group).

[e] The presence of the terminal adenosine was not proven in these experiments. However, the complete agreement of the sequence derived with the known 3′-end of MS2 RNA [3] allows an unambiguous identification.

[f] For the RNAase T1 products, the distribution was calculated on the basis of the known number of phosphate residues in the 3′-terminal sequence. For the RNAase A products, the two A–Cp sequences were taken as a reference.

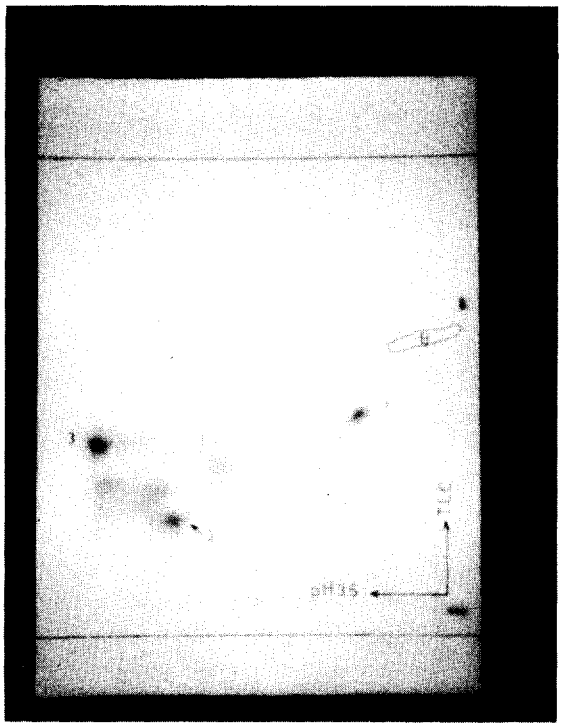

Fig. 1. A two dimensional separation of RNA fragments from gel band D6. Separation in the first dimension was by electrophoresis on cellulose acetate at pH 3.5 (in 7 M urea) until the distance between the blue and the pink marker was about 20 cm. The material in this region (containing most of the radioactivity) was transferred on to a thin-layer plate, which consisted of a mixture of DEAE-cellulose and cellulose in a ratio of 1:7.5. The plate was run in a 5 percent solution of RNA which had been dialysed against 7 M urea for 2 hr at 4° (homomixture b [12]).

amber (UAG) codon, although it is very doubtful that the latter is involved in polypeptide chain termination. Indeed, it is now generally believed that the chain termination signal is more complex than a single triplet. Direct evidence comes from the work of Nichols [11], who reported that the R17 coat protein cistron ends by a succession of two nonsense codons. Moreover, Weissmann and collaborators [15] found an untranslated stretch of over 60 nucleotides at the 5′-end of Qβ RNA, and by analogy, a similar situation may hold for the 3′-terminus of viral RNAs.

The same sequence as here reported was found by J.Adams and S.Corey in R17 RNA (personal communication).

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