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A nucleotide sequence from a ribonuclease III processing site in bacteriophage T7 RNA

(polycistronic mRNA/RNA hairpins/endoribonuclease III)

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ABSTRACT **Transcription of that portion of the bacteriophage T7 genome encoding early functions yields RNA molecules about 7500 nucleotides long representing this entire early region. These long transcripts can be cleaved *in vitro* by highly purified *Escherichia coli* ribonuclease III (endoribonuclease III; EC 3.1.4.24), yielding five messenger RNAs identical to those produced *in vivo*. During this reaction, a small RNA fragment called F5 RNA is released, which is specified by the region of the T7 genome between genes *1.1* and *1.3*. The following sequence of ^{32}P-labeled F5 RNA has been determined using standard RNA sequencing techniques: pU-A-A-G-G-U-C-G-C-U-C-U-C-U-A-G-G-A-G-U-G-G-C-C-U-U-A-G-U_{OH}. The relative contributions of sequence and structure to ribonuclease III processing signals are considered in light of these findings.**

Transcription of that portion of the bacteriophage T7 genome encoding early functions yields RNA molecules about 7500 nucleotides long representing this entire early region (1–4). These long transcripts can be cleaved *in vitro* by highly purified *Escherichia coli* ribonuclease III (RNase III; endoribonuclease III; EG 3.1.4.24), yielding five messenger RNAs identical to those produced *in vivo* (3–6). During this reaction, a small RNA fragment called F5 RNA is released, which is specified by the region of the T7 genome between genes *1.1* and *1.3* (7).

The highly reproducible cleavages of T7 RNA by RNase III constitute a good example of RNA processing—defined here as RNA cleavage occurring at a specific site *in vivo* or its exact equivalent *in vitro*. Such RNase III cleavage sites within RNA molecules have been defined by Dunn (8) as primary sites. Single-stranded RNA can also be cleaved by RNase III at secondary sites [those not cleaved *in vivo* (8)] under a variety of *in vitro* conditions‡ (8–10). RNase III can also cause extensive digestion of RNA·RNA duplexes at nonspecific sites to yield fragments about 15 bases long with random end groups (5, 11–12). Furthermore, double-stranded RNA is a competitive inhibitor of cleavage at both primary and secondary sites. In this communication we report the nucleotide sequence of the T7 RNA fragment F5, which, when considered together with the above modes of RNase III action, should help to assess the relative contributions of sequence and structure to RNase III processing signals.

MATERIALS AND METHODS

Materials. α-^{32}P-Labeled ribonucleoside triphosphates (specific activity about 100 Ci/mmol) were purchased from New England Nuclear. Materials and enzymes for RNA sequence analysis were those described by Pieczenik *et al.* (14) and Barrell (15). *E. coli* RNase III and RNA polymerase (RNA nucleotidyltransferase, EC 2.7.7.6) were prepared as described previously (5, 16). Double-stranded RNA from a virus of *Penicillium chrysogenum* (17) was a gift of D. Planterose, Beecham Research Laboratories, Betchworth, England.

Methods: Preparation of F5 RNA. ^{32}P-Labeled primary RNA transcripts from the early region of the T7 genome were isolated either after infection of an RNase-III-deficient strain of *E. coli* with T7 (4) or by *in vitro* transcription of T7 DNA with *E. coli* RNA polymerase (3). RNA for sequence analysis was prepared as before (3). *In vitro* cleavage by RNase III was carried out as before (3–5), and the F5 RNA cleavage product was isolated by electrophoresis on 20% polyacrylamide gels (18). The gel region containing F5 RNA was located by autoradiography and excised, and the RNA was eluted (19). Prior to sequence analysis, F5 RNA was further purified by two-dimensional fractionation including high voltage electrophoresis at pH 3.5 and thin-layer homochromatography using a 5% solution of unhydrolyzed yeast RNA in 7 M urea (14, 20). ^{32}P-Labeled mRNA transcribed from gene *1.1* was isolated from infected cells after electrophoresis on polyacrylamide gels (8).

RNA Sequence Analysis. Four separate preparations of F5 RNA, each containing a single different ribonucleoside [α-^{32}P]triphosphate, were synthesized as described above and subjected to RNase T_1 (guanyloribonuclease, EC 3.1.4.8) and pancreatic RNase (ribonuclease I, EC 3.1.4.22) fingerprinting analysis. RNase-T_1- and pancreatic-RNase-resistant oligonucleotides were subjected to further analysis and their sequences and neighboring bases were determined by standard techniques (ref. 15 and legend to Table 1).

RESULTS

Location of F5 RNA. RNase III cleavage of full-length RNA transcripts from the region of the T7 genome encoding early functions releases the five early mRNAs, three initiator RNAs, and the small RNA fragment derived from a double cleavage between the gene *1.1* and *1.3* RNAs (7) referred to as F5 RNA (Fig. 1*A*). *In vivo*, most of the F5 RNA is left attached to the 3′ end of *1.1* mRNA (8). The second RNase III cleavage required to release F5 RNA can be carried out *in vitro* (Fig. 1*B*, lanes a–c and e) and is inhibited by double-stranded RNA (dsRNA) (Fig. 1*B*, lane d; refs. 4 and 5).

Sequence of F5 RNA. F5 RNA preparations that had been labeled separately by one of the four ribonucleoside triphosphates were subjected to either RNase T_1 or pancreatic RNase digestion and two-dimensional fingerprinting analysis (Fig. 2). All oligonucleotides were eluted from the fingerprints and subjected to further analysis as outlined in Table 1. Table 1 also shows, in schematic form, the only nucleotide sequence consistent with these data.

Abbreviations: RNase, ribonuclease; *0.3* mRNA, *1.1* mRNA, and *1.3* mRNA, the messenger RNAs for the protein products of bacteriophage T7 genes *0.3*, *1.1*, and *1.3*; U_{OH}, a uridine residue lacking a 3′ monophosphate; dsRNA, double-stranded RNA.

‡ RNase III cleavage at primary sites (i.e., the authentic *in vivo* processing reaction) is favored by a low enzyme-to-substrate ratio and moderate ionic strength. Cleavage at secondary sites can be promoted either by conditions of low ionic strength (8, 9) or by a high enzyme-to-substrate ratio (10).

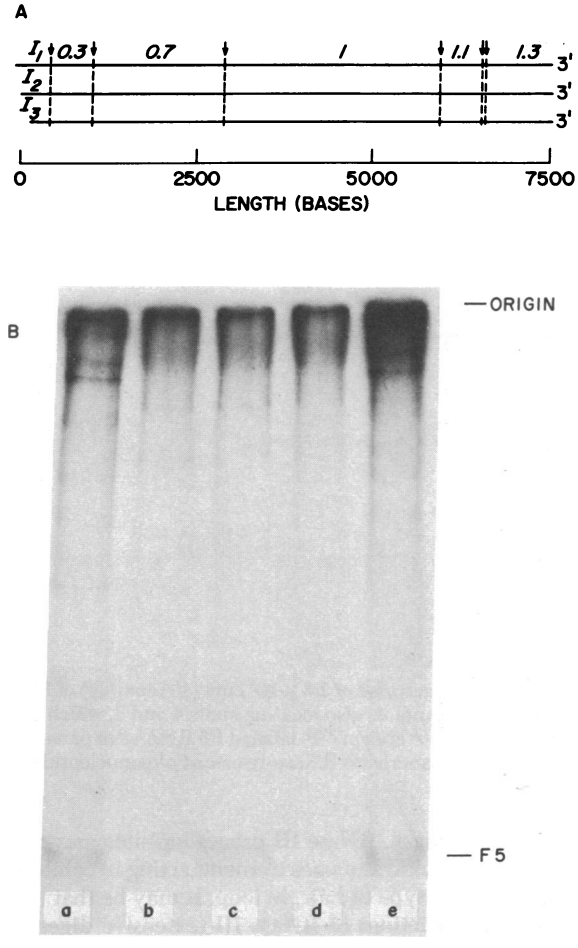


FIG. 1. RNA transcribed from the early region of phage T7 DNA is cleaved by RNase III at the positions indicated by the arrows in *Panel A*. When cleavage occurs at both sites between the *1.1* and *1.3* mRNAs, the RNA fragment F5 is released. F5 can be separated from the other cleavage products by electrophoresis on 20% polyacrylamide gels.

Panel B is an autoradiogram of a 20% polyacrylamide gel which shows that F5 RNA can also be released from *1.1* mRNA isolated from infected cells. Because most of the cleavage products do not enter the 20% polyacrylamide gel, the origin was cut off prior to autoradiography in order to avoid overexposure to the x-ray films. *1.1* mRNA and other high-molecular-weight species do not enter this gel and are thus not visible. The samples applied to the gel were: (a) Full-length early RNA synthesized *in vitro* and then incubated with RNase III. (b) *1.1* mRNA isolated from infected cells. (c) *1.1* mRNA isolated from infected cells and then incubated with RNase III. (d) *1.1* mRNA incubated with RNase III in the presence of an excess (20 μg/ml) of the RNase III competitive inhibitor, double-stranded RNA from the virus of *Penicillium chrysogenum*. (e) Mixture of the samples applied to lanes (a) and (c), showing that the F5 RNAs released from full-length early RNA transcripts and from the larger form of *1.1* mRNA have identical electrophoretic mobilities.

DISCUSSION

We have reported the sequence of a 29-base RNA fragment situated at the 3′ terminus of the larger form of T7 *1.1* mRNA. The cleavage event that generates the larger form of *1.1* mRNA from its precursor has all of the properties of a primary RNase III cleavage site (see above and ref. 8). In contrast, the cleavage event that generates the 5′ terminus of F5 RNA has properties in common with both primary and secondary cleavage sites. In particular, this cleavage occurs *in vivo*, albeit with low efficiency (F. W. Studier and J. J. Dunn, unpublished data); but it is optimized *in vitro* by conditions that favor cleavage at secondary sites‡ (8). Complete sequence analysis of the RNA in this region will be a prerequisite to determining which features convey the primary or secondary nature of these cleavage sites. As a first step in exploring this question, we have determined the sequence of F5 RNA.

Termini Produced by RNase III Cleavage. Table 2 shows the termini generated by RNase III cleavage of a variety of RNA molecules. All of the sites depicted are purely primary except that generating the 5′-terminus of F5 RNA (see above) and the two cleavage sites within bacteriophage T4 species whose cleavage is optimal under conditions favoring cleavage at secondary sites (8, 9). Although the terminal sequences in Table 2 are far from a random collection, they nevertheless establish that RNase III cleaves specifically within more than one unique sequence of bases. Thus, there is no single sequence element surrounding the site of cleavage uniquely capable of specifying the cleavage point. This does not rule out the idea that any one of these sequence elements could, by itself, specify cleavage by RNase III (21).

Comparison of F5 RNA with the 3′ Terminus of T7 Gene 0.3 mRNA. In an accompanying paper Rosenberg and Kramer (22) have studied the RNA region surrounding the RNase III cleavage site located between genes *0.3* and *0.7*. In contrast to the double cleavage by RNase III between genes *1.1* and *1.3* resulting in the release of F5 RNA, only a single cleavage occurs to separate *0.3* mRNA from *0.7* mRNA. However, when the 29 bases at the 3′ terminus of *0.3* mRNA are compared with the 29-base sequence of F5 RNA, a startling homology is revealed. Only 4 of the 29 bases are not homologous. The 5′ terminal pU of F5 is replaced by -C-; the nonbase-paired loop sequence -*U*-C-U-A-G- is replaced by -C-U-A-G-*C*- (see Fig. 3*A* for a potential hairpin structure for F5 RNA); and the 3′ terminal -*A*-*G*-U_{OH} is replaced by -*U*-*A*-U_{OH}. In both cases cleavage occurs 13 bases to the 3′ side of the top base pair of the stem (Fig. 3*A* and ref. 22). Despite these similarities, cleavage at a secondary site 29 bases from the 3′ terminus of *0.3* mRNA has not been observed (ref. 8 and J. J. Dunn, unpublished data) even under conditions favoring secondary cleavage.

Features of RNase III Processing Signals. The possibility that a short double-stranded region may also constitute part of RNase III processing signals must be explored. Although RNase III rapidly and efficiently digests a variety of synthetic and natural RNA·RNA duplex molecules (5, 11, 12), this reaction lacks the precision characteristic of *in vivo* processing of RNA precursors. In particular, termini generated by extensive digestion of dsRNA are nearly random (5), and, furthermore, no dsRNA fragment shorter than 20 base pairs can be cleaved (5, 13). For these reasons, the suggestion that the mere presence of a short region of dsRNA in an RNA molecule would fully specify an RNase III processing site is untenable. However, since dsRNA competes efficiently with the processing reaction (2, 5) it would appear that these two reactions share an active site.

Although a perfect double-stranded region cannot specify processing by RNase III, it might still be possible that loop-stem structures of the size and stability proposed for F5 RNA in Fig. 3*A* would suffice. However, the presence of such a region within the RNA genome of bacteriophage R17 (23–25) indicates that this possibility is also an oversimplification (Fig. 3*B*). Despite the numerous similarities between the RNA phage region and that from T7, no region of the RNA genomes of the f2 family of *E. coli* bacteriophages contains an RNase III processing site (8, 26). A second potential hairpin of comparable size and composition is present near the 3′-terminus of *E. coli* 16S RNA (27). At present there is no evidence that this sequence is cleaved by RNase III. Therefore, many hairpin loops lack features sufficient to specify cleavage by RNase III. At present

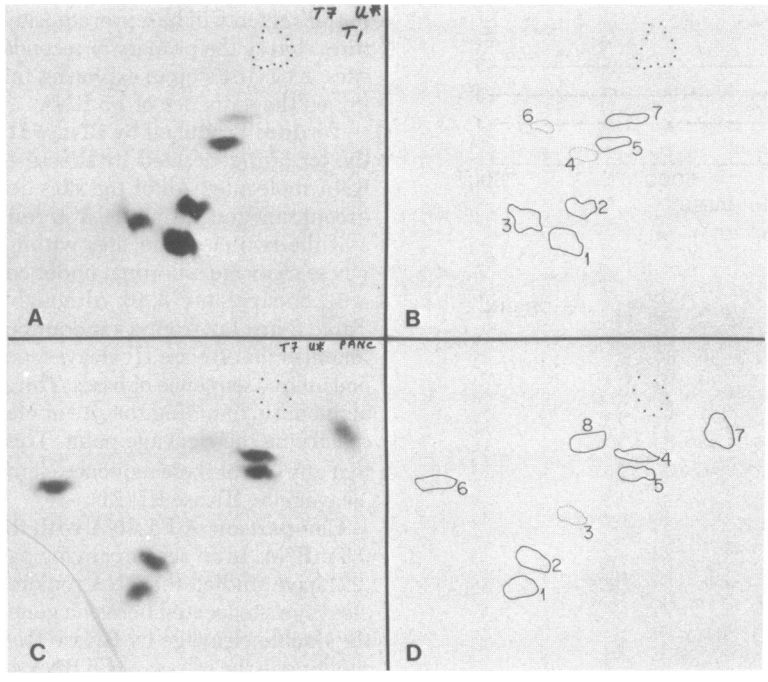


FIG. 2. RNA fingerprinting analysis of T7 F5 RNA. (*A*) Autoradiograph of an RNase T_1 fingerprint of 2.5×10^5 cpm (10^8 cpm/μg) of F5 RNA synthesized in the presence of [α-^{32}P]UTP. (*B*) Schematic drawing of the pattern shown in panel *A*, also locating spots 4 and 6, which are not labeled by UTP (see Table 1). (*C*) Autoradiograph of a pancreatic RNase fingerprint of 2.5×10^5 cpm of ^{32}P-labeled F5 RNA as in panel *A*. (*D*) Schematic drawing of the pattern shown in panel *C*, including the approximate location of pancreatic RNase-resistant oligonucleotide no. 3, not labeled by UTP (see Table 1).

it is not possible to determine whether the exact stability (28) of a potential hairpin plays a role in cleavage site selection by RNase III.

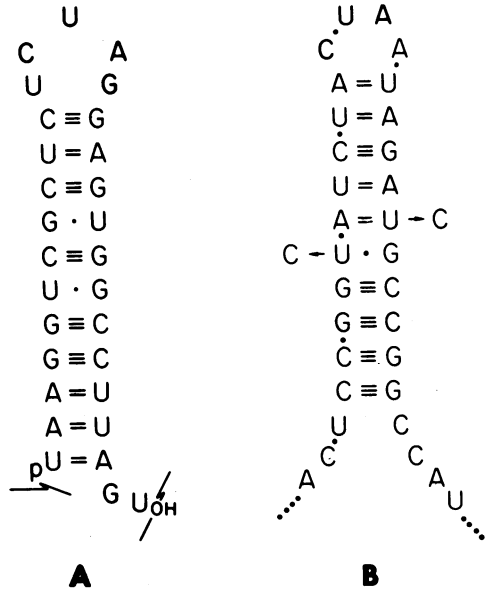


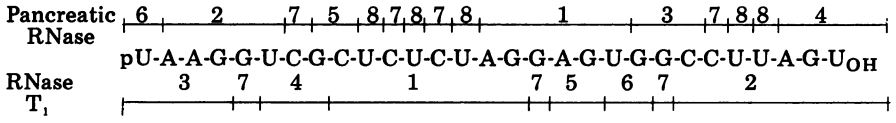
FIG. 3. Proposed structure of an RNase III cleavage site from bacteriophage T7 RNA and a similar uncleaved region from the RNA bacteriophage R17. (*A*) Schematic representation of a potential hairpin structure for F5 RNA. (*B*) Schematic representation of a potential hairpin structure at the 3′ end of the major coat protein cistron of bacteriophages R17, f2, and MS2. The sequence represented is that of R17 (23), while the two indicated C substitutions are found in the analogous regions of f2 (24) and MS2 (25). The U-A-A and U-A-G at the top are the actual terminators for this cistron *in vivo*, thus separating the coding region (left side; dots between triplets indicate reading frame) from the intercistronic space (right side). Horizontal lines (dots in the case of U·G) indicate potential base pairing.

Thus, in summary, RNase III processing sites may involve one of several specific sequence elements acting in combination with a particular type of hairpin loop. It may be that the two other *in vitro* reactions of RNase III (extensive digestion of RNA·RNA duplexes and cleavage of single-stranded RNA at secondary sites) could each represent a partial reaction involving recognition of only one of these signals. A more detailed consideration of these and other properties of the mechanism of RNase III action will appear elsewhere (29).

Biological Significance of T7 RNase III Processing Sites. While RNase III cleavage is not necessary for the translation of the T7 mRNAs encoding early functions (7, 30), processing stimulates translation of *0.3* mRNA (30). In addition, processing could have subtle effects on the stabilities of the various T7 mRNAs. While such minor effects on the level of translation may help T7 to grow, it also seems possible that these RNase III processing sites and similar regions in bacteriophage genomes (Fig. 3*B*) could be remnants of signals from portions of the bacterial genomes from which these phages evolved. However, in the case of the RNA phage, sufficient changes would have been necessary to inactivate any potential RNase III cleavage sites. Alternatively, these similarities (if not fortuitous) could be the result of a requirement for a high density of common biological signals (for example, those that specify initiation and termination of protein synthesis, ribosome binding, and codons, as well as RNA processing). Such interdependent constraints on the sequence would not only be expected to influence the mode of action of each of these biological processes, but also would narrow the choice of signals for RNase III processing. Evidence for such strong selection comes from comparative sequence analysis of phages R17, f2, and MS2 (23–25, 31), where we see that two Cs in f2 and MS2 coordinately replace two Us on opposite sides of the potential hairpin loop (Fig. 3*B*). This is the only known case of coordinate base changes within an RNA phage hairpin loop.

While a full understanding of processing by RNase III may require determination of the complete sequence and structure

Table 1. Analyses of RNase-T_1- and pancreatic-RNase-resistant oligonucleotides from T7 F5 RNA



RNase-T_1-resistant oligonucleotides						Pancreatic-RNase-resistant oligonucleotides				
No.	Proposed sequence	[^{32}P]-NTP	Composition	Pancreatic RNase products	RNase U2 products	No.	Proposed sequence	[^{32}P]-NTP	Composition	RNase T_1 products
1	CUCUCUAG[G][a]	U	$\overset{*}{C}$	$\overset{*}{C}$	$\overset{*}{C}U\overset{*}{C}U\overset{*}{C}UA$	1	AGGAGU[G]	U	$\overset{*}{G}$	$A\overset{*}{G}$[U]
		C	$\overset{*}{U}$	$\overset{*}{U}$	$C\overset{*}{U}C\overset{*}{U}CUA$			A	$\overset{*}{G}$	$\overset{*}{G}$[A]
		A	$\overset{*}{U}$	$\overset{*}{U}$	$CUCUC\overset{*}{U}A$			G	$\overset{*}{A}$, $\overset{*}{G}$	$\overset{*}{A}\overset{*}{G}$[G], $\overset{*}{A}G$
		G	$\overset{*}{A}$, $\overset{*}{G}$	$\overset{*}{A}\overset{*}{G}$[G]	$CUCUCU\overset{*}{A}$[G], $\overset{*}{G}$	2	AAGGU[C]	U	$\overset{*}{G}$	$\overset{*}{G}$[U]
2	CCUUAG[U][b]	U	$\overset{*}{C}$, $\overset{*}{U}$, $\overset{*}{G}$	$\overset{*}{C}$, $\overset{*}{U}$, $A\overset{*}{G}$[U]	$C\overset{*}{C}\overset{*}{U}UA$, $\overset{*}{G}$			C	$\overset{*}{U}$	$\overset{*}{U}$[C]
		C	$\overset{*}{C}$	$\overset{*}{C}$	$\overset{*}{C}CUUA$			A	$\overset{*}{A}$	$\overset{*}{A}AG$
		A	$\overset{*}{U}$	$\overset{*}{U}$	$CCU\overset{*}{U}A$			G	$\overset{*}{A}$, $\overset{*}{G}$	$A\overset{*}{A}\overset{*}{G}$[G]
		G	$\overset{*}{A}$	$\overset{*}{A}G$	$CCUU\overset{*}{A}$[G]	3	GGC[C]	C	$\overset{*}{G}$, $\overset{*}{C}$	$\overset{*}{G}$[C], $\overset{*}{C}$[C]
3	pUAAG[G][c]	U	$\overset{*}{p}Up$	$\overset{*}{p}Up$	$\overset{*}{p}UA$			G	$\overset{*}{G}$	$\overset{*}{G}$[G]
		A	$pU\overset{*}{p}$, $\overset{*}{A}$	$pU\overset{*}{p}$, $\overset{*}{A}AG$	$\overset{*}{p}UA$	4	AGU_{OH}[d]	U	$\overset{*}{G}$	$A\overset{*}{G}$[U]
		G	$\overset{*}{A}$, $\overset{*}{G}$	$A\overset{*}{A}\overset{*}{G}$[G]	$\overset{*}{A}$, $\overset{*}{G}$			G	$\overset{*}{A}$	$\overset{*}{A}G$
4	UCG[C]	C	$\overset{*}{U}$, $\overset{*}{G}$	$\overset{*}{U}$, $\overset{*}{G}$	$\overset{*}{U}C\overset{*}{G}$[C]	5	GC[U]	U	$\overset{*}{C}$	$\overset{*}{C}$[U]
		G	$\overset{*}{C}$	$\overset{*}{C}$	$UC\overset{*}{G}$			C	$\overset{*}{G}$	$\overset{*}{G}$[C]
5	AG[U]	U	$\overset{*}{G}$	$A\overset{*}{G}$[U]	$\overset{*}{G}$	6	pU[A]	U	$\overset{*}{p}Up$	$\overset{*}{p}Up$
		G	$\overset{*}{A}$	$\overset{*}{A}G$	$\overset{*}{A}$			A	$pU\overset{*}{p}$	$pU\overset{*}{p}$
6	UG[G]	G	$\overset{*}{U}$, $\overset{*}{G}$	$\overset{*}{U}$, $\overset{*}{G}$	$\overset{*}{U}\overset{*}{G}$[G]	7	C[U/G]	U	$\overset{*}{C}$	$\overset{*}{C}$
7	G[U/C/A]	U	$\overset{*}{G}$	$\overset{*}{G}$	$\overset{*}{G}$			G	$\overset{*}{C}$	$\overset{*}{C}$
		C	$\overset{*}{G}$	$\overset{*}{G}$	$\overset{*}{G}$	8	U[U/C/A]	U	$\overset{*}{U}$	$\overset{*}{U}$
		A	$\overset{*}{G}$	$\overset{*}{G}$	$\overset{*}{G}$			C	$\overset{*}{U}$	$\overset{*}{U}$
								A	$\overset{*}{U}$	$\overset{*}{U}$

The numbers refer to the fingerprints shown in Fig. 2. The 2′ or 3′ monophosphates and the hyphens for internal phosphodiester bonds have been omitted from the table for brevity. NTP refers to the ribonucleoside [α-^{32}P]triphosphate precursor used to make the oligonucleotides analyzed in a given line. When an oligonucleotide is not labeled by one or more of the four NTPs, this is indicated by omission from the table of data pertaining to that NTP. Base compositions were determined by alkaline hydrolysis [0.4 M NaOH, 37°, 18 hr (15)] and are listed under *Composition*. Pancreatic RNase, RNase U2, and RNase T_1 products were identified both by electrophoretic mobility on DEAE-paper (at pH 3.5, 1.9, and 3.5, respectively) and by base composition analysis. Asterisks denote those bases found to be labeled after base composition analysis. Square brackets indicate proposed nearest-neighbor bases.

[a] Sequence of T_1-resistant spot 1. Modification of the U residues with *N*-cyclohexyl-*N*′-[β-morpholinyl-(4)-ethyl]carbodiimide-methyl-*p*-toluene sulfonate (CMCT) followed by pancreatic RNase digestion yielded free C in UTP-labeled RNA digests and the absence of free U in the CTP-labeled RNA digests (data not shown), demonstrating that C is the 5′ terminal residue. This result together with analyses of spot 1 shown in Table 1 indicates that the sequence of spot 1 has the form $(C\text{-}U)_n$-A-G[G]. Because ATP and CTP both label U in this oligonucleotide, *n* must be 2 or greater. Size comparison of spots 1 and 2, as well as the major RNase U2 products thereof, shows that the sequence of spot 1 is longer than that of spot 2. Therefore, *n* must be greater than 2. Alkaline hydrolysis of total UTP-labeled F5 RNA reveals pUp, U-U, and C-U to be present in a ratio of 1:1.3:3.65. Since one C-U is present in spot 2, and no other CU sequences are present except in spot 1, we conclude that 2.35–2.65 mol of C-U are present in spot 1. These results are only compatible with the conclusion that $n = 3$ and that the sequence of spot 1 is C-U-C-U-C-U-A-G[G].

[b] Sequence of T_1-resistant spot 2. Because UTP was found to label C, U, and G in this sequence in a ratio of approximately 1:1:1 (data not shown), a single C-U, U-U, and G-U must be present. Taken together with the U2 products of oligonucleotide 2 and pancreatic-RNase-resistant oligonucleotide 4, this result indicates that the sequence of spot 2 must be C_n-U-U-A-G[U]. We know that *n* must be ≥ 2 here, because C is labeled by both UTP and CTP. Spot 2 contains the only adjacent Cs in the entire F5 sequence. These data also reveal the presence of only two G[C] sequences; furthermore, after alkaline hydrolysis of the entire CTP-labeled F5 RNA, the observed molar ratio of labeled G to labeled C is 2 to 0.8. This leads to the conclusion that $n = 2$, and that the sequence of spot 2 is CCUUAG[U].

[c] Sequence of T_1-RNase-resistant oligonucleotide 3. The identity of the unusual pancreatic RNase digestion product pUp arising from this spot was confirmed by two-dimensional electrophoretic analysis (5).

[d] While this oligonucleotide contains A-G[U], this U cannot be recovered from F5 RNA labeled by any of the four ribonucleoside [α-^{32}P]triphosphates. Thus, there must be a U_{OH} located at the 3′ end of spot 4 which, in turn, must be located at the 3′ terminus of F5 RNA.

Table 2. Termini produced by RNase III cleavage

RNA species cleaved	3'-Terminus	5'-Terminus	Refs.
	Cleavage point ↓		
Release of 17S RNA from *E. coli* 30S rRNA precursor			
Cleavage to produce 5' end	?	pU-G	21
Cleavage to produce 3' end	A-C-A-C-A_{OH}	?	21
T7 early RNA transcripts cleaved between:			
Initiator RNA and *0.3* mRNA	U-U-U-A-U_{OH}	pG-A-U	6
0.3 mRNA and *0.7* mRNA	U-U-U-A-U_{OH}	pG-A-U	6, 22
0.7 mRNA and *1.0* mRNA	U-U-U-A-U_{OH}	pG-A-U	6
1.0 mRNA and *1.1* mRNA	U-U-U-A-U_{OH}	pG-A-U	6, This paper*
1.1 mRNA and F5 RNA	?	pU-A-A	This paper
F5 RNA and *1.3* mRNA	U-U-A-G-U_{OH}	?	This paper
T4 species I RNA			
Cleavage 1	G-U-U-G-A_{OH}	pG-A-U	9
Cleavage 2	A-U-C-U-U_{OH}	pU-G-C	9

As discussed in the *text*, RNase III cleaves a number of RNA species in a highly specific fashion [yielding a 3'-hydroxyl and 5'-phosphate at each point of cleavage (5)]. All known sequences immediately adjacent to such cleavage sites have been collected and tabulated here to facilitate comparison in a search for sequence elements that may signal cleavage (see *text*).

* The 5' terminal sequence of *1.1* mRNA was determined using preparations of this mRNA (before and after F5 RNA removal) obtained from the same ^{32}P-labeled T7 RNA transcripts used for sequencing F5 RNA. Two-dimensional electrophoresis of alkaline digests (5) allowed us to assign the 5' terminal sequence pG-A, and subsequent isolation of a pancreatic-RNase-resistant oligonucleotide with the sequence pG-A-U confirmed and extended by this observation.

of both enzyme and substrates, it is hoped that future studies, including analysis of RNA molecules containing mutations affecting RNase III cleavage, will shed further light on (*i*) the relative contributions of sequence and structure to the RNase III recognition signal, (*ii*) mechanisms of protein–nucleic acid interaction, and (*iii*) possible evolutionary relationships between the genomes of viruses and their hosts.

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