Issell, B. F. (1980) *Cancer Treat. Rev. 7*, 73.
Joselow, M., & Dawson, C. R. (1951) *J. Biol. Chem. 191*, 11.
Legha, S. S., Keating, M. J., Zander, A. R., McCredie, K. B., Bodey, G. P., & Freireich, E. J. (1980) *Ann. Intern. Med. 93*, 17.
Lloyd, R. S., Haidle, C. W., & Robberson, D. L. (1978) *Biochemistry 17*, 1980.
Loach, P. A. (1976) *Handbook of Biochemistry and Molecular Biology* (Fasman, G. D., Ed.) Vol. 2, p 122, CRC Press, Cleveland, OH.
Marshall, L. E., Graham, D. R., Reich, K. A., & Sigman, D. S. (1981) *Biochemistry 20*, 244.
McCord, J. M., & Fridovich, I. (1969) *J. Biol. Chem. 244*, 6049.
Mirabelli, C. K., Mong, S., Huang, C. H., & Crooke, S. T. (1979) *Biochem. Biophys. Res. Commun. 91*, 871.
Mirabelli, C. K., Huang, C. H., & Crooke, S. T. (1980) *Cancer Res. 40*, 4173.
Nebesar, B. (1961) *Anal. Chem. 36*, 1961.
Ouannes, C., & Wilson, T. (1968) *J. Am. Chem. Soc. 90*, 6527.
Povirk, L. F., Hogan, M., & Dattagupta, N. (1979) *Biochemistry 18*, 98.
Que, B. G., Downey, K. M., & So, A. G. (1980) *Biochemistry 19*, 5987.
Rivera, G., Evans, W. E., Dahl, G. V., Yee, G. C., & Pratt, C. B. (1980) *Cancer Res. 40*, 4250.
Sausville, E. A., Peisach, J., & Horwitz, S. B. (1976) *Biochem. Biophys. Res. Commun. 73*, 814.
Sausville, E. A., Peisach, J., & Horwitz, S. B. (1978a) *Biochemistry 17*, 2740.
Sausville, E. A., Stein, R. W., Peisach, J., & Horwitz, S. B. (1978b) *Biochemistry 17*, 2746.
Shoemaker, D. D., Cysyk, R. L., Padmanabhan, S., Bhat, H. B., & Malspeis, L. (1982) *Drug Metab. Dispos. 10*, 35.
Sigman, D. S., Graham, D. R., Aurora, V. D., & Stern, A. M. (1979) *J. Biol. Chem. 254*, 12269.
Someya, A., & Tanaka, N. (1979) *J. Antibiot. 32*, 839.
Strong, J. E., & Crooke, S. T. (1978) *Cancer Res. 38*, 3322.
Suguira, Y. (1980) *J. Am. Chem. Soc. 102*, 5208.
Waring, M. J. (1976) *Eur. J. Cancer 12*, 995.
Wilson, W. R. (1973) *Chem. N. Z. 37*, 148.
Wilson, W. R., Giesbrecht, J. L., Hill, R. P., & Whitmore, G. F. (1981) *Cancer Res. 41*, 2809.
Wong, A., Huang, C.-H., & Crooke, S. T. (1984) *Biochemistry* (preceding paper in this issue).

# 7S RNA, Containing 5S Ribosomal RNA and the Termination Stem, Is a Specific Substrate for the Two RNA Processing Enzymes RNase III and RNase E†

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ABSTRACT: The 7S RNA, a precursor of 5S rRNA that contains 5S rRNA and the termination stem and loop, is a substrate for RNase E and is also a substrate for RNase III. The cleavage by RNase III is in the stem, 11 nucleotides downstream from the 3′ end of the mature 5S rRNA and 8 nucleotides downstream from the RNase E cleavage site. Near the cleaved nucleotides there are three base pairs that appear in the same relative positions in most known RNase III cleavage sites. The large product of the RNase III cleavage reaction, which is a 5S rRNA that contains 11 extra nucleotides at the 3′ end, is a substrate for RNase E. This suggests that the information for the 3′-end cleavage by RNase E resides mainly in the 5S rRNA itself. Using *rnc rne* strains, carrying the plasmid that leads to the accumulation of 7S RNA, we showed that the 7S RNA does not result from an RNase III cleavage but is apparently a proper transcription termination product.

The study of RNA processing enzymes is impaired to a large extent by the unavailability of large quantities of simple substrates. This is mainly due to the fact that the proper substrates are precursor RNAs that accumulate either in small quantities during the normal metabolism of RNA molecules or in larger quantities in appropriate mutants, blocked directly or indirectly in RNA processing reactions. This is also true for ribonuclease III (RNase III), the enzyme responsible for the primary processing of p16[1] and p23 rRNAs and for the cleavage of polycistronic mRNA precursors of some bacteriophages of *Escherichia coli* [for reviews, see Robertson (1982), Gegenheimer & Apirion (1981), and Pace (1984)]. These natural substrates of RNase III are several thousand nucleotides long, and obtaining them in significant quantities is a troublesome procedure.

We have been studying recently a precursor of 5S rRNA (designated 7S RNA) that accumulates in substantial quantities in the absence of functional RNase E in strains carrying a multicopy plasmid that contains an active 5S rRNA gene (Szeberényi & Apirion, 1983; Elford & Holmes, 1983; Szeberényi et al., 1983). The 7S RNA is only 165 nucleotides long and contains a perfect stem of 15 base pairs which could

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[1] Abbreviations: p16, p23, and p5 rRNAs, precursors to 16S, 23S, and 5S rRNAs, respectively; $Na_2EDTA$, ethylenediaminetetraacetic acid disodium salt; PEIC, poly(ethylenimine)–cellulose; Tris-HCl, tris(hydroxymethyl)aminomethane hydrochloride.

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be a potential substrate for RNase III. In this paper we show that indeed the 7S RNA is a substrate for RNase III and it is cleaved by it in the stem in a unique position. The position of the cleavage shares many similarities with other well-established RNase III cleavage sites.

## Materials and Methods

*Plasmid.* The recombinant plasmid pJR3Δ, which carries a 5S rRNA gene, has been described recently (Elford & Holmes, 1983; Szeberényi & Apirion, 1983).

*Bacterial Strains.* Strains N3438 (*recA*, *rne*) and N5706 (N3438, transformed with pJR3Δ) have been described by Szeberényi & Apirion (1983). Strain N5713 (*rne*, *rho*, carrying pJR3Δ) has been described by Szeberényi et al. (1983). Strains N7060 (*rna*, *rnb*, *pnp*), N2069 (*rna*, *rnb*, *rnc*, *pnp*), N3441 (*rna*, *rnb*, *rne*, *pnp*), and N3520 (*rnc*, *rne*) have been described previously (Apirion & Watson, 1975; Apirion & Gitelman, 1980). Strain N5722 has been derived from strain N3520 by transformation with pJR3Δ.

*Analytical Scale Labeling and Fractionation of RNA.* These procedures were described previously (Gegenheimer et al., 1977; Gegenheimer & Apirion, 1978). For some of the details, see also the legend to Figure 6.

*Isolation and Purification of 7S RNA.* $^{32}$P-Labeled 7S RNA was prepared from strain N5713 as described previously (Szeberényi & Apirion, 1983; Szeberényi et al., 1983) except that the cultures were treated with chloramphenicol (200 μg/mL) 60 min before being transferred to the nonpermissive temperature (44 °C). Chloramphenicol increases the relative yield of the 7S RNA species (H. C. Vaidya, J. Szeberényi, and D. Apirion, unpublished observations).

*In Vitro Processing of 7S RNA.* Processing with S30 preparations were carried out as described by Szeberényi et al. (1983). S30 extracts were prepared by using the method of Misra & Apirion (1979) except that sonication rather than alumina grinding was used to open the cells.

Processing of 7S RNA with partially purified RNase III (endoribonuclease III, EC 3.1.4.24) was performed in 20-μL reaction mixtures containing 0.02 M Tris-HCl (pH 8.0), 0.15 M $NH_4Cl$, 5 mM $MgCl_2$, 0.1 mM $Na_2EDTA$, 0.1 mM dithioerythritol, 1 μg of yeast RNA (carrier), $^{32}$P-labeled 7S RNA [(1–5) × $10^3$ cpm], and RNase III. Samples were incubated at 37 °C for 60 min, and reactions were terminated by the addition of stop solution and boiling (Gegenheimer et al., 1977). The products of the in vitro cleavage were fractionated in 5%/10% or 5%/15% tandem polyacrylamide gels containing 7 M urea. A good separation of p5L RNA from p5 RNA can be achieved in a 10% polyacrylamide gel, while the smaller products of the cleavage (products a and b; see Figure 1) can be displayed in a 15% gel. (p5L RNA is the large product of RNase III cleavage of 7S RNA.)

For large scale processing, $^{32}$P-labeled 7S RNA (5 × $10^6$ cpm) was incubated with RNase III in a 200-μL mixture in the presence of 10 μg of carrier RNA. All other components were used in the same concentrations as described for the analytical scale reactions. Reaction products were separated and purified in two successive polyacrylamide gels (5%/15% containing 7 M urea and 20%), excised from the second gel, and eluted as described by Gurevitz et al. (1982).

The RNase III preparation was a kind gift from Dr. Hugh Robertson. It was a fraction from a phosphocellulose column that was previously purified according to the procedure of Dunn (1976) up to and including the DEAE-cellulose column.

*Structural Analysis of 7S RNA and Its in Vitro Processing Products.* $^{32}$P-Labeled 7S RNA ($10^5$ cpm), p5L RNA (5 × $10^4$ cpm), and products a and b (2 × $10^4$ cpm) were digested with RNase T1 and fingerprinted by using the method of Volckaert et al. (1976). The oligonucleotides of the fingerprints were further analyzed after digestion with RNase A as described by Volckaert & Fiers (1977). If necessary, the oligonucleotides were also digested with RNase T2 or nuclease P1 and analyzed in cellulose plates by the two-dimensional chromatographic method of Saneyoshi et al. (1972) or in PEIC [poly(ethylenimine)–cellulose] plates that were developed with 1.25 M LiCl as described by Gegenheimer & Apirion (1980a). Quantitation of the oligonucleotides was carried out by liquid scintillation counting.

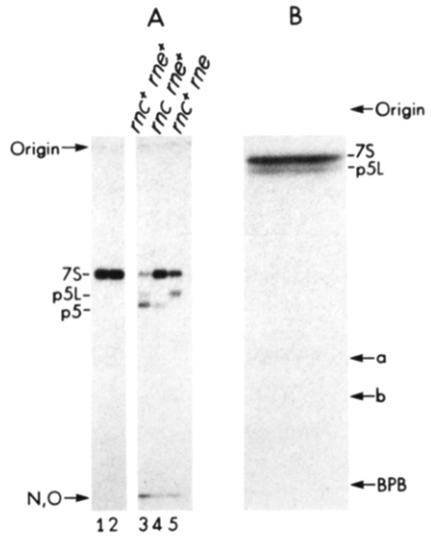


FIGURE 1: In vitro processing of 7S RNA. Panel A, S30 extracts; panel B, partially purified RNase III. (A) Substrates and products were analyzed in a 5%/10% tandem polyacrylamide gel containing 7 M urea. Lane 1, 7S RNA, no incubation; lane 2, 7S RNA after incubation under RNase III conditions (see Materials and Methods) without enzyme; lane 3, incubation of 7S RNA in the presence of 10 μg of S30 protein from strain N7060, N2069 (lane 4), or N3441 (lane 5). Panel B shows the separation of products from 7S RNA in a 5%/15% tandem polyacrylamide gel containing 7 M urea, after large-scale cleavage with partially purified RNase III. For details see Materials and Methods. The degradation products, nucleotides (N) and oligonucleotides (O), comigrated with bromphenol blue (BPB).

*In Vitro Processing with RNase E Preparations.* This was performed as described previously (Szeberényi et al., 1983). The RNase E preparations used for the in vitro cleavage reaction have been described by Roy & Apirion (1983); for further details, see the legend to Figure 5.

## Results

*In Vitro Cleavage of 7S RNA by RNase III.* It was known from earlier studies from this laboratory (Szeberényi & Apirion, 1983; Szeberényi et al., 1983) that 7S RNA is produced from a recombinant plasmid (pJR3Δ). It contains a 5S rRNA and an *rrn* terminator, and it accumulates when RNase E is inactivated. It is cleaved efficiently by RNase E, the enzyme responsible for the primary processing of 5S rRNA in *E. coli* (Gegenheimer & Apirion, 1981; Misra & Apirion, 1979). Extracts from strains in which RNase E is thermolabile fail to produce p5 from 7S RNA (lane 5 in Figure 1). Using S30s from different strains (with regard to the genotype of the known processing RNases) in addition to p5 rRNA, we observed another product (p5L RNA) which can be clearly distinguished from p5 RNA (Figure 1). This product was not related to the presence of RNase E in the extracts (see lane 4). On the other hand, all extracts containing RNase III (for example, lanes 3 and 5 in Figure 1) gave rise to p5L RNA. This product was never produced by extracts prepared from

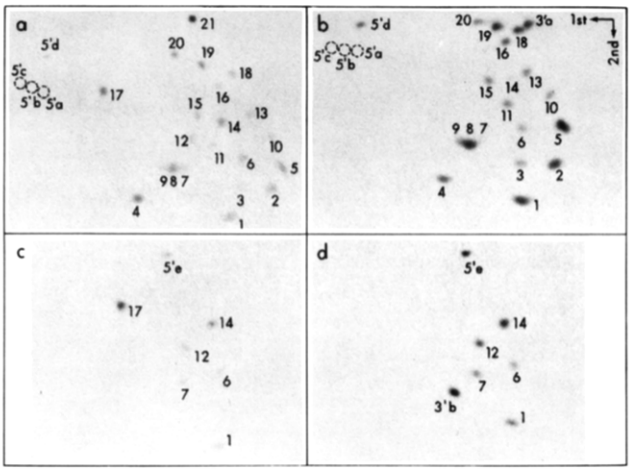


FIGURE 2: RNase T1 fingerprints of 7S RNA and its cleavage products generated by partially purified RNase III. 7S RNA (a); p5L RNA (b); product a (c); product b (d). For the composition of the various spots, see Table I.

RNase III$^-$ strains (for example, lane 4 in Figure 1).

To prove that RNase III is responsible for the production of p5L RNA, $^{32}$P-labeled 7S RNA was incubated with partially purified RNase III and the products were fractionated by polyacrylamide gel electrophoresis (panel B, Figure 1). One large and two small fragments were observed (a and b in Figure 1B); the large fragment (p5L) comigrated with p5L RNA produced by *rnc*$^+$ extracts from 7S RNA (not shown).

*Determination of the RNase III Cleavage Site.* In order to determine the RNase III cleavage site, large-scale processing of $^{32}$P-labeled 7S RNA was carried out with partially purified RNase III. The products were separated in a 5%/15% tandem polyacrylamide gel containing 7 M urea (Figure 1, panel B) and further purified in a 20% polyacrylamide gel. Products eluted from the 20% gel were subjected to fingerprinting after digestion with RNase T1. The fingerprints of 7S RNA, p5L RNA, and the two small products (a and b) are shown in Figure 2, and the composition of the T1 spots is summarized in Table I.

p5L RNA contains all the oligonucleotides present in 7S RNA up to position 119 (Figure 3), including the four different 5′-end oligonucleotides (5′a, 5′b, 5′c, and 5′d; see Figure 2 and Table I). The four different 5′-end oligonucleotides are also characteristic of p5 rRNA (Jordan et al., 1970, 1971). All the unique oligonucleotides from position 120 onward are missing, but there is one new oligonucleotide, 3′a (Figure 2b and Table I). Its analysis (Figure 4A) showed that it fits the sequence ACAUCAAAUAAAACp (Table I; positions 120–133 in Figure 3). We suggest that the last nucleotide in p5L RNA is $A_{OH}$ (position 134; for further details, see below).

Both small products, a and b, start with a 5′-phosphate (see oligonucleotide 5′e in Figure 2c,d and Table I) and contain all the nucleotides between positions 135 and 157. The 5′-end T1 oligonucleotides from both a and b were identical. After digestion with pancreatic RNase a single spot appeared (Figure 4B) that migrated in a typical position for an oligonucleotide that contains a 5′-phosphate. This oligonucleotide was eluted and digested with RNase T2, and three spots appeared corresponding to pAp, Ap, and Gp. The quantitative relationship among the spots suggested that the 5′e oligonucleotide contained one pAp, two Ap, and one Gp. These experiments indicated that oligonucleotide 5′e is pAAAGp (positions 135–138, Figure 3). The position of the RNase III cleavage as indicated in Figure 3 is further supported by the fact that the T1 3′-end oligonucleotide from p5L RNA (3′a; Figure 2b

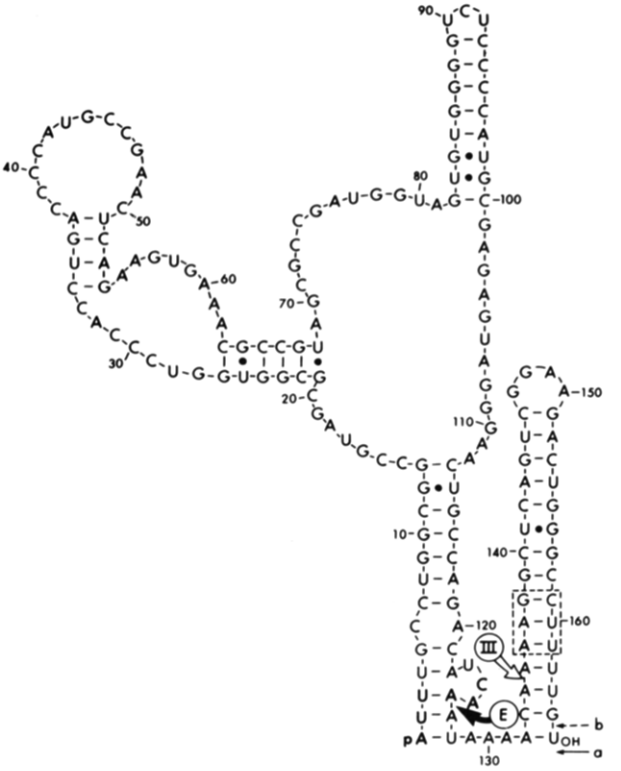


FIGURE 3: Sequence of 7S RNA. The cleavages by RNase E and RNase III are indicated by closed and open arrows, respectively. The 3′ ends of products a and b are also indicated by arrows after nucleotides 165 and 164, respectively. The consensus sequence characteristic of most of the known RNase III cleavage sites is boxed in. The secondary structure of 7S RNA was according to Pieler & Erdmann (1983), Singh & Apirion (1982), and Szeberēnyi & Apirion (1983). Mature 5S rRNA consists of the nucleotides from position 4 to position 123.

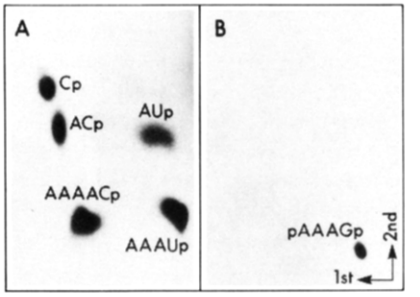


FIGURE 4: Redigestion of oligonucleotides flanking the cleavage site of RNase III in 7S RNA. Panel A, the 3′-end oligonucleotide of p5L RNA (3′a in Figure 2 and Table I); panel B, the 5′-end oligonucleotide of product a. These oligonucleotides were digested with RNase A and chromatographed in PEIC plates as described by Volckaert & Fiers (1977).

and Table I), when digested with pancreatic RNase (Figure 4A), did not show a spot that could correspond to $ApA_{OH}$, excluding a possible cleavage between nucleotides 135 and 136. The difference between product a and product b is at their 3′ ends. While product a contains oligonucleotide t17 (CCUUUUGp, positions 158–164 in Figure 3), in product b oligonucleotide t17 is replaced by a new spot, 3′b (see Figure 2c,d and Table I). Digestion of this oligonucleotide (3′b) with RNase T2 produced Up and Cp, while after treatment with nuclease P1, pC, pU, and pG have been identified. From this we concluded that 3′b is $CCUUUUG_{OH}$ (positions 158–163) and product b is one nucleotide shorter at its 3′ end than product a. Product a ends with a $U_{OH}$. This was indicated from studies with nuclease S1 (Szeberēnyi & Apirion, 1983).

Table I: Composition of the T1 Oligonucleotides of 7S RNA and Its Cleavage Products Generated by RNase III[a]

| oligo-nucleotide | composition | suggested sequence[b] | position[c] | presence in RNAs | | | |
|---|---|---|---|---|---|---|---|
| | | | | 7S | p5L | a | b |
| t1 | Gp | Gp | – | + | + | + | + |
| t2 | C, Gp | CGp | – | + | + | – | – |
| t3 | AGp | AGp | – | + | + | – | – |
| t4 | U, Gp | UGp | – | + | + | – | – |
| t5 | $C_2$, Gp | CCGp | – | + | + | – | – |
| t6 | AAGp | AAGp | – | + | + | + | + |
| t7 | (C, U)Gp | UCGp | 145 | + | +[d] | + | + |
| t8 | U, AGp | UAGp | – | + | + | – | – |
| t9 | AU, Gp | AUGp | 76 | + | + | – | – |
| t10 | $C_2$, AGp | CCAGp | 116 | + | + | – | – |
| t11 | ($C_2$, U)Gp | CCUGp | 6 | + | + | – | – |
| t12 | (AC, U)Gp | ACUGp | 152 | + | – | + | + |
| t13 | AAAC, Gp | AAACGp | 60 | + | + | – | – |
| t14 | ($C_2$, U)AGp | CUCAGp | 140 | + | +[d] | + | + |
| t15 | (AAC, U)Gp | AACUGp | 111 | + | + | – | – |
| t16 | (C, AAC, U)AGp | AACUCAGp | 48 | + | + | – | – |
| t17 | ($C_2$, $U_4$)Gp | CCUUUUGp | 158 | + | – | + | – |
| t18 | ($C_3$, AC, AU)Gp | ACCCCAUGp | 37 | + | + | – | – |
| t19 | ($C_4$, AC, $U_2$)Gp | UCCCACCUGp | 28 | + | + | – | – |
| t20 | ($C_5$, $U_2$, AU)Gp | UCUCCCCAUGp | 90 | + | + | – | – |
| t21 | (C, AC, AAAAC, AU, AAAU)AAAAGp | ACAUCAAAUAAAACAAAAGp | 120 | + | – | – | – |
| 5′a | pU, Gp | pUGp | 4 | + | + | – | – |
| 5′b | (U, pU)Gp | pUUGp | 3 | + | + | – | – |
| 5′c | ($U_2$, pU)Gp | pUUUGp | 2 | + | + | – | – |
| 5′d | ($U_2$, pAU)Gp | pAUUUGp | 1 | + | + | – | – |
| 5′e | pAAAGp | pAAAGp | 135 | – | – | + | + |
| 3′a | (C, AC, AAAAC, AU, AAAU)$X_{OH}$ | ACAUCAAAUAAAACA$_{OH}$ | 120 | – | + | – | – |
| 3′b | ($C_2$, $U_4$)$G_{OH}$ | CCUUUUG$_{OH}$ | 158 | – | – | – | + |

[a] The composition of the T1 oligonucleotides was determined after elution from the PEIC plates and digestion with pancreatic RNase. When necessary, redigestion with nuclease P1 and/or RNase T2 was also carried out (see Materials and Methods). The same oligonucleotide assignment is used as in Figure 2. [b] The sequence of 7S RNA is according to Duester & Holmes (1980) and Szeberényi & Apirion (1983). [c] The position of the 5′ end is indicated for the oligonucleotides that appear only once in the sequence. The complete sequence of 7S RNA is presented in Figure 3. [d] These oligonucleotides are not present in p5L RNA and appear in low molar yields. They represent a contaminating fragment derived from the termination stem [nucleotides 140–147 in Figure 3; see also Szeberényi et al. (1983)].

We assume that product b is not derived from product a but is produced independently during the reaction, since in various experiments we never have seen product a without product b regardless of the time of the assay. When the monocation concentration is relatively low in the RNase III reaction mixture (below 100 mM), we observed mainly product b, suggesting that product b is produced independently from product a.

From the structural analysis of the products of the RNase III reaction with 7S RNA, the following conclusions can be drawn: (i) the cleavage by RNase III occurs in the termination stem; (ii) the cleavage producing the 3′ end of p5L RNA is between nucleotides 134 and 135, eight nucleotides downstream from the RNase E site (Figure 3); (iii) RNase III, as with other substrates (Robertson, 1982; Gegenheimer & Apirion, 1981; Pace, 1984), generated products with 3′-OH and 5′-phosphate termini; (iv) one of the small products (product a) is the result of a single-stranded cleavage; (v) product b could have resulted from a staggered double-stranded cleavage and/or from exonucleolytic trimming of product a.

*In Vitro Processing of p5L RNA by RNase E.* In order to find out whether the double-stranded structure of the termination stem has a role in the RNase E cleavage that produces the 3′ end of p5 RNA, p5L RNA was incubated with RNase E preparations of different purity (Figure 5). Were the stem important in the recognition and/or cleavage reactions, p5L RNA, which is only eight nucleotides longer than p5 RNA and from which the termination stem and loop are completely missing, would not be a substrate for RNase E. This was not the case; p5L RNA was processed efficiently to p5 RNA by all the RNase E preparations tested. Fractionation of the products, in a sequencing-type gel (not shown), indicated that the majority of p5L RNA molecules were cut by RNase E at the same position (between nucleotides 126 and 127 in Figure 3) as in 7S (Szeberényi et al., 1983) or in 9S RNA (Roy et al., 1983). [The products of the reaction could be analyzed unambiguously in a sequencing gel since the vast majority of the original 7S RNA substrate was the longest form starting with pAUUUG (see Table I and Figure 2a,b).] The results of the experiment described here show that the termination stem and loop are not necessary for the RNase E cleavage near the 3′ end of 5S rRNA.

*In Vivo Labeling.* Since the rRNA termination stem is rather substantial (15 base pairs), and since RNase III can cleave double-stranded RNA (Robertson et al., 1968; Crouch, 1974; Robertson, 1982; Gegenheimer & Apirion, 1981; Pace, 1984) it was reasonable to raise the possibility that the termination stem itself is a product of an RNase III cleavage.

To clarify this point, we introduced the pJR3Δ plasmid into an *rnc rne* strain. If the 3′ ends of the plasmid-specific transcripts were produced by RNase III, one would expect the appearance of longer pJR3Δ-related RNA species in an *rnc rne* strain than in an *rnc*$^+$ *rne* strain, but this is not the case. Figure 6 shows the in vivo labeled extracts of *rnc*$^+$ *rne* (lanes 1 and 2), *rnc*$^+$ *rne* containing pJR3Δ (lanes 3 and 4), *rnc rne* (lanes 5 and 6), and *rnc rne* cells transformed by pJR3Δ (lanes 7 and 8) from permissive (lanes 1, 3, 5, and 7) and nonpermissive temperatures (lanes 2, 4, 6, and 8). The pattern of plasmid-specific RNAs accumulating when RNase E was inactivated was very similar in the *rnc*$^+$ *rne* (lane 4) and *rnc rne* (lane 8) cells. This experiment shows rather clearly that


FIGURE 5: In vitro processing of p5L RNA with various protein fractions containing RNase E. $^{32}$P-Labeled p5L RNA ($1 \times 10^3$ cpm) was incubated without enzyme (lane 1), with 10 $\mu$g of protein from the S200 fraction (lane 2), with 1 $\mu$g of protein from an RNase E fraction partially purified by isoelectric focusing (lane 3), or with 2 $\mu$g of RNase E further purified by a second isoelectrofocusing followed by chromatography on a Sephadex G-150 column (lane 4). [For details of RNase E purification, see Roy & Apirion (1983).] All three RNase E containing fractions produced p5 from p5L RNA. The products of the reactions were analyzed in a 5%/15% tandem polyacrylamide gel containing 7 M urea (see Materials and Methods).

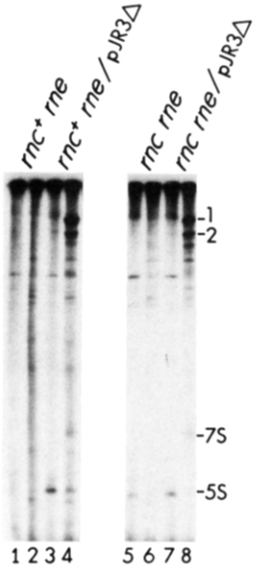


FIGURE 6: In vivo labeling of *rnc*$^+$ *rne* and *rnc rne* strains with or without the plasmid pJR3Δ. Strains N3438 (*rnc*$^+$ *rne*), N5706 (*rnc*$^+$ *rne*/pJR3Δ), N3520 (*rnc*, *rne*), and N5722 (*rnc*, *rne*/pJR3Δ) were grown at 37 °C, and portions of the cultures were shifted to 43 °C at an $A_{560}$ of 0.25. After 20 min, cultures were labeled with $^{32}P_i$ (500 $\mu$Ci/mL) for 1 h, and cell free extracts were prepared [for details, see Gegenheimer et al. (1977)]. Samples were fractionated in a 5%/8% tandem polyacrylamide gel containing 7 M urea (only the 8% part of the gel is displayed).

the termination stem is not a result of a processing event by RNase III.

## Discussion

The experiments presented here show that 7S RNA is an adequate substrate for the RNA processing enzyme RNase III. The ease of accumulation and purification of 7S RNA in large quantities and its relatively small size make it an ideal substrate to study various aspects of RNase III.

The RNase III cleavage site resembles other RNase III cleavage sites (Robertson, 1982; Gegenheimer & Apirion, 1981). For instance, most of the T7 mRNA RNase III sites (Dunn & Studier, 1981, 1983) and the 23S rRNA RNase III site (Bram et al., 1980; Gegenheimer & Apirion, 1980b), as well as the site studied here, contain the sequence $\frac{AAG}{UUC}$ and the cleavages are one nucleotide from the first A and three from the first U (see box in Figure 3). This also suggests that product b is not derived from product a but apparently results from a direct staggered cleavage by RNase III.

Using 7S RNA derived from *rrnB* as substrate for RNase III, we found that the cleavages were introduced at the same positions and were as efficient as with 7S RNA described in this paper (J. Szeberényi and D. Apirion, unpublished observations). The termination stem of the *rrnB* transcript contains a G-C base pair at positions 134 and 163 (Figure 3) rather than an A-U base pair in the substrate used in the studies reported here. [The 7S RNA used here has been derived from the *rrnD* gene [see Szeberényi & Apirion (1983)].]

The position of the RNase III cleavage in 7S RNA is very specific, and the fact that it occurs 12 base pairs from the termination loop is probably significant. In the case of p16 and p23 rRNAs, the T7 1.1–1.3 site and the λ *sib* site, the cleavage by RNase III occurs 10–13 base pairs from one end of the double-stranded stem (Robertson, 1982). It is also worth mentioning that when homopolymers of RNA are used as substrates, the final products of the RNase III reaction are double-stranded fragments of 12–15 nucleotides long (Robertson & Dunn, 1975). It should be of interest to find out if the same position in the 7S RNA will be recognized by RNase III if the stem will be separated from the 5S rRNA. Thus, the analysis of this simple substrate (7S RNA) can throw some light on the nature of the signal(s) that RNase III recognizes.

The RNase III cleavage reported here is most likely analogous to the primary RNase III cleavages observed in the precursor of rRNA (Robertson, 1982) and T7 early RNA (Dunn, 1976). Like in the case of T7 RNA the RNase III cleavage discussed here takes place in a wide range of monocation concentrations, but at the lower range additional cleavages occur. This suggests that 7S RNA is a very appropriate natural substrate to study the mechanism of action of RNase III.

These findings raise the question of the relevance of this reaction in vivo: Is it possible that the termination stem is removed in the cell by RNase III? We believe that this is not the case, since it seems that the cell contains a very efficient nuclease that can digest the stem. Only with purified preparations of RNase E (prepared from an RNase III$^-$ strain) can the stem be found after digestion of 7S RNA with RNase E (Szeberényi et al., 1983). Therefore, we suggest that in vivo RNase E acts first and then the fragment that contains the termination stem is digested by another enzyme(s) that has (have) not been yet identified and characterized.

Moreover, 7S RNA is apparently not attacked in vivo by RNase III, since it accumulates to the same extent in the absence or presence of RNase III (see Figure 6). We do not know why 7S RNA is a substrate for RNase III in vitro but not in vivo; several possibilities such as interference by the inactivated RNase E or other proteins could explain this difference.

The results presented here also show that the termination stem is not produced by RNase III since the same size 7S RNA and RNAs 1 and 2 (Szeberényi & Apirion, 1983) are produced in the presence or absence of RNase III (Figure 6).

The termination stem is *rho* independent since it appears in the same size in *rho*$^+$ and *rho* strains (J. Szeberényi, unpublished observations).

In summary, the results presented here show that 7S RNA is a very suitable substrate for the investigation of the RNA processing enzyme RNase III.

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**Registry No.** RNase III, 9073-62-5; RNase E, 76106-82-6.

References

Apirion, D., & Watson, N. (1975) *J. Bacteriol. 124*, 317–324.

Apirion, D., & Gitelman, D. R. (1980) *Mol. Gen. Genet. 117*, 339–343.

Bram, R. J., Young, R. A., & Steitz, J. A. (1980) *Cell (Cambridge, Mass.) 19*, 393–401.

Crouch, R. J. (1974) *J. Biol. Chem. 249*, 1314–1316.

Duester, G. L., & Holmes, W. M. (1980) *Nucleic Acids Res. 8*, 3793–3807.

Dunn, J. J. (1976) *J. Biol. Chem. 251*, 3807–3814.

Dunn, J. J., & Studier, F. W. (1981) *J. Mol. Biol. 148*, 303–330.

Dunn, J. J., & Studier, F. W. (1983) *J. Mol. Biol. 166*, 477–535.

Elford, R. M., & Holmes, W. M. (1983) *J. Mol. Biol. 168*, 557–561.

Gegenheimer, P., & Apirion, D. (1978) *Cell (Cambridge, Mass.) 15*, 527–539.

Gegenheimer, P., & Apirion, D. (1980a) *J. Mol. Biol. 143*, 227–257.

Gegenheimer, P., & Apirion, D. (1980b) *Nucleic Acids Res. 8*, 1873–1891.

Gegenheimer, P., & Apirion, D. (1981) *Microbiol. Rev. 45*, 502–541.

Gegenheimer, P., Watson, N., & Apirion, D. (1977) *J. Biol. Chem. 252*, 3064–3073.

Gurevitz, M., Watson, D., & Apirion, D. (1982) *Eur. J. Biochem. 124*, 553–559.

Jordan, B. R., Feunteun, J., & Monier, R. (1970) *J. Mol. Biol. 50*, 605–615.

Jordan, B. R., Forget, B. G., & Monier, R. (1971) *J. Mol. Biol. 55*, 407–421.

Misra, T. K., & Apirion, D. (1979) *J. Biol. Chem. 254*, 11154–11159.

Pace, N. R. (1984) in *RNA Processing* (Apirion, D., Ed.) pp 1–34, CRC Press, Boca-Raton, FL.

Pieler, T., & Erdmann, V. A. (1982) *Proc. Natl. Acad. Sci. U.S.A. 79*, 4599–4603.

Robertson, H. D. (1982) *Cell (Cambridge, Mass.) 30*, 669–672.

Robertson, H. D., & Dunn, J. J. (1975) *J. Biol. Chem. 250*, 3050–3056.

Robertson, H. D., Webster, R. E., & Zinder, N. D. (1968) *J. Biol. Chem. 243*, 82–91.

Roy, M. K., & Apirion, D. (1983) *Biochim. Biophys. Acta 747*, 200–208.

Roy, M. K., Singh, B., Ray, B. K., & Apirion, D. (1983) *Eur. J. Biochem. 131*, 119–127.

Saneyoshi, M., Ohashi, A., Harada, F., & Nishimura, S. (1972) *Biochim. Biophys. Acta 262*, 1–10.

Singh, B., & Apirion, D. (1982) *Biochim. Biophys. Acta 698*, 252–259.

Szeberényi, J., & Apirion, D. (1983) *J. Mol. Biol. 168*, 525–557.

Szeberényi, J., Roy, M. K., & Apirion, D. (1983) *Biochim. Biophys. Acta 740*, 282–290.

Volckaert, G., & Fiers, W. (1977) *Anal. Biochem. 83*, 228–239.

Volckaert, G., Min Jou, W., & Fiers, W. (1976) *Anal. Biochem. 72*, 433–446.