Ribonucleic Acid Processing Activity of Escherichia coli Ribonuclease III*

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SUMMARY

We have studied the nuclease activities present in preparations of Escherichia coli RNase III and the "sizing factor" responsible for specific processing of several RNA species. RNase III preparations contain three activities: one which solubilizes stable RNA:RNA duplexes; one which solubilizes the RNA of DNA:RNA hybrids; and one which processes the polycistronic mRNA of bacteriophage T7 in a manner identical with sizing factor. We show that the activity against the RNA of DNA:RNA hybrids can be removed, but that the activity which cleaves RNA:RNA duplexes and that responsible for specific processing of phase T7 polycistronic mRNA appear to be identical by several biochemical criteria. In addition, partially purified enzyme fractions from mutants lacking these two activities contain substantial amounts of activity against the RNA of DNA:RNA hybrids.

We have also defined several properties of the two activities which solubilize RNA:RNA duplexes and RNA of DNA:RNA hybrids. Average oligonucleotide chain length in an exhaustive digest of double-stranded RNA is about 15 bases, while that in a digest of the RNA in DNA:RNA hybrids is less than 10 bases. Direct analysis shows that both activities cleave RNA chains to yield 5' phosphate and 3'-hydroxyl termini. All four bases can reside at the 5' end of the resulting oligonucleotides, although both activities show a mild preference for certain bases. These results and previous findings allow us to specify the probable size and structure of potential cleavage sites for these enzymes in biological RNA molecules.

The role of specific RNase activities in cells has recently been the object of intensive study. Of particular interest have been those RNase activities which introduce cleavages at a limited number of specific sites during the maturation of RNA precursors. In Escherichia coli, several such RNases have recently been identified and the proper cleavage of their normal substrates have been reproduced in vitro by isolated enzyme (1-3).

Dunn and Studier (1) originally observed that the E. coli "sizing factor" which processes early T7 mRNA copurified with RNase III (4, 5). With the subsequent availability of an E. coli mutant lacking that activity in crude extracts which cleaves dsRNA (6), these authors were able to show that mutant cells failed to process early T7 mRNA and E. coli rRNA precursors which accumulated in vivo (2). These precursors could be processed in vitro with enzyme from wild type cells. Similar results have been obtained by Nikolaev et al. (7, 8).

RNase III was originally characterized as an enzyme preparation with endonucleolytic activities against both classes of stable double helical RNA—RNA:RNA duplexes and the RNA of DNA:RNA hybrids (5). Subsequent studies confirmed the presence of both activities after a number of purification steps (9-11). Activities specifically able to digest the RNA of DNA:RNA hybrids have since been found in eukaryotic nuclei (12), tumor virus reverse transcriptase (13), and E. coli (14-16), and have been given the generic name "RNase H." Such RNase H activities could play a role in removing the RNA primer which has recently been implicated in the initiation of DNA synthesis in a number of systems (17-20).

Crouch (21) has employed sedimentation on sucrose density gradients to fractionate further E. coli RNase III preparations. He obtained fractions of higher S value which were largely free of RNase H activity, but were still capable of digesting dsRNA; activity of lower S value contained largely RNase H. In addition, Nikolaev et al. (7) have stated that the mutant lacking activity against dsRNA still retains wild type levels of RNase H. It should be noted, however, that several groups (14-16, 21) have identified three different enzyme fractions in E. coli capable of digesting hybrid RNA.

In this communication, we will present evidence complementary to that of Crouch (21) that it is possible to obtain RNase III preparations which contain essentially no RNase H activity.

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The abbreviations used are: dsRNA, double-stranded RNA; pXp, a (2', 2'), 5'-nucleoside diphosphate; pAp, pGp, and pUp, the four common bases when involved in this configuration.
III preparations which lack RNase H activity. The key observation of these studies is that these fractions (lacking RNase H) are both able to solubilize stable dsRNA and to process phage T7 polycistronic mRNA in vitro. In order to describe these activities in more detail, we have also carried out experiments on the specificity, end group polarity, and size limit of the digestion products. The results of these studies place limitations upon the kinds of cleavage sites which we might expect to find in biological RNA molecules.

EXPERIMENTAL PROCEDURE

Materials

Nucleic Acids—Nonradioactive and [3H]dsRNA (50,000 cpm/µg at 15% counting efficiency) from a virus associated with the mold Penicillium chrysogenum were the kind gift of Dr. D. Planterosee, Beecham Research Laboratories, Surrey, England (22).

Doubly labeled DNA:RNA hybrids of bacteriophage f1 (10) were prepared in collaboration with Dr. P. Model, Rockefeller University. Phage f1 [3H]DNA, the gift of Dr. G. Vovis, Rockefeller University, was used as template for Escherichia coli RNA polymerase in a reaction containing 2.8 µg of f1 [3H]DNA (specific activity 6.3 X 107 cpm/µg) using reaction conditions described by Burgess (23). The four ribonucleotide triphosphates were adjusted to 60 µM each. In DNA:RNA hybrid Preparation A (that used for experiments of Figs. 1 and 3 and Tables I and II), the RNA was labeled using [α-32P]UTP adjusted to a final specific radioactivity of 0.7 µCi/µmol. Incubation was for 30 min at 37°C and the hybrid was purified using cellulose CF11 chromatography according to Robertson (30). The extent of synthesis was 95%, and the resulting hybrid regions had a DNA specific activity of 3.15 X 106 cpm/µg and an RNA specific activity of 2.25 X 107 cpm/µg. For the experiments in Figs. 3 and 4 and Table III, equal specific radioactivity for all four ribonucleotide triphosphates was sought so that the in vitro product would reflect accurately the base composition of the template. Therefore, in hybrid Preparation B, all four [α-32P]ribonucleotide triphosphates were added in the above reaction conditions to give a final specific radioactivity of 3 X 107 cpm of [32P] per µg.

Double-stranded RNA containing sequences from polyoma DNA was synthesized in vitro using the DNA (a gift of Dr. A. R. Hunter, Salk Institute, La Jolla, Calif.) from a defective polyoma virus strain which yields upon transcription by E. coli RNA polymerase large proportions of complementary RNA when assayed as described by Haas et al. (24). Synthesis was carried out as for DNA:RNA hybrids, substituting the polyoma DNA for the f1 DNA template. For the polyoma DNA used in Fig. 3 and Table III a preparation was synthesized of identical specific activity to DNA:RNA hybrid Preparation B described above (containing all four [α-32P]ribonucleotide triphosphates; final specific activity 3 X 107 cpm/µg). To maximize dsRNA content in the product of such reactions, they were heated at 60°C for 20 hours followed by mild RNase T1 digestion for 10 min at 37°C (0.01 µg/ml) in 0.1 M NaCl, 0.05 M Tris-HCl (pH 7.0), 0.001 M EDTA. Extraction with phenol and chromatography on cellulose CF11 were as described previously (25).

Polycistronic T7 [32P]mRNA was synthesized in vitro using E. coli RNA polymerase with [α-32P]GTP as label. Reaction conditions and subsequent purification by gel electrophoresis were as described previously (1, 2). Unlabeled RNA from the bacteriophage f2 was the gift of Dr. N. D. Zinder. [32P]RNA from bacteriophage f1 mRNA ribosome binding sites was synthesized and isolated by H. D. R. and Drs. G. Pieczekin and P. Model (26) using an adaptation of published procedures (27). Preparation I was prepared from E. coli MRE600 as previously described (5, 28) and from E. coli A19 with minor modifications: DEAE-cellulose and CM-cellulose were employed instead of DEAE-Sephadex and CM-Sephadex. Enzyme at this stage of purification is referred to as Fraction VI. An additional fractionation step was carried out on part of Fraction VI. Portions (2 ml) were applied to a column (0.9 x 130 cm) of Sephadex G-100 equilibrated and developed with 0.02 M Tris HCl (pH 7.0), 0.1 M KCl, 0.1 M EDTA, 0.1 M dithiothreitol, and 10% glycerol. Fractions (1.75 ml) were collected and assayed for their ability to solubilize [3H]poly(A-U) (1, 3). The peak fractions were pooled and stored on ice (Fraction VII). Sizing factor was purified using BioGel filtration and other procedures as before (1).

Pancreatic DNase (DPFF) was purchased from Worthington. RNase T1 was purchased from Calbiochem.

Other Materials—[α-32P]ribonucleotide triphosphates (ATP, GTP, CTP, and UTP), all with specific radioactivities in the range of 100 Ci/mmol, were purchased from New England Nuclear. Unlabeled RNA:dsRNA hybrid molecules were prepared as previously described (25). Standards of 3'-UMP, CMP, AMP, and GMP, as well as pUP, pCP, pAP, and pGP, were those used previously (25).

Methods

Solubilization of double helical RNA was assayed by precipitation with 5% trichloroacetic acid and filtration on Whatman GF/A filters as before (5). Sizing factor activity (processing of T7 polycistronic mRNA) was assayed as described previously (25).

Fingerprinting analysis of digested RNA was carried out according to the procedure of Brownlee and Sanger (29). End group analysis of dsRNA and hybrid RNA was performed using a modification first suggested by Dr. P. G. N. Jeppesen of the standard fingerprinting procedure for RNase T1 digests which has been reviewed by Barrell (30). RNA samples were exposed to alkaline digestion followed by resuspending the RNA in 1 M NaOH and incubating them 18 hours at 37°C in sealed capillaries. Digested RNA was then spotted near one end of a strip (2.5 X 85 cm) of Whatman No. 3 MM paper along with standard tracking dyes (30). The paper was moistened with pH 3.5 buffer containing 5% glacial acetic acid, 0.5% pyridine, and 0.005 M EDTA, and exposed to electrophoresis at 5000 volts until the blue dye (xylene cyanol FF) had migrated 13 cm towards the anode. The paper was then blotted onto a sheet (46 x 85 cm) of Whatman DE81 DEAE-paper, washed with 95% ethanol, and exposed to electrophoresis in a second dimension in 7% formic acid as described previously (30). These and other two-dimensional analyses were exposed to autoradiography with DuPont Cronex-X-ray film. Radioactive spots were located, cut out and their Cerenkov radiation was determined in a liquid scintillation counter set for tritium counting (efficiency of Cerenkov counting of 3H, 10%).

RESULTS

Section A: Further Purification of RNase III—As shown in Fig. 1a, RNase III preparations at the Fraction VI stage of purification contain activities which digest stable dsRNA and hybrid RNA. In the present discussion we refer to these activities as "RNase III" and "RNase H," respectively. As demonstrated earlier (5), at the Fraction VI stage of purification there is no DNase activity, and the DNA strands of the DNA:RNA hybrid are not digested. When the DNA:RNA hybrid is preincubated with pancreatic DNase sufficient to solubilize all of the hybrid DNA, the RNA which was originally contained in the hybrid is rendered resistant to digestion by the RNase H activity (Fig. 1b), presumably through its release in single-stranded form as suggested earlier (10). Fig. 1b also shows that pancreatic DNase has no effect on the ability of RNase III to digest stable dsRNA.

As shown in Fig. 2, RNase III preparations at this stage of purification are able to process T7 polycistronic early mRNA to yield the five monocistronic RNAs, indicated in the figure by the numbers 1, 0.7, 0.3, 1.3, and 1.1. A comparison of this activity with that of an Escherichia coli protein fraction "sizing factor," originally isolated solely on the basis of its ability to carry out this processing reaction (1), resulted in nearly identical RNA patterns following electrophoresis of the reaction mixtures on polyacrylamide gels. In particular, when reaction mixtures are adjusted to contain equal units of activity against dsRNA (5) the extent and...
FIG. 1. Enzymatic activities of Escherichia coli RNase III. 
RNase III was prepared from E. coli MRE600 according to the 
procedure of Robertson et al. (5, 28) through the Fraction VI 
stage. Reactions were carried out in a volume of 0.1 ml in a buffer 
containing 0.1 M Tris-HCl (pH 7.6), 0.01 M magnesium acetate, 
0.13 M NH₄Cl, and 5% sucrose and were incubated in silicenel 
treated glass tubes at 37° for the times indicated. a, digestion 
of dsRNA and DNA:RNA hybrids. Five nanograms of polyoma 
[32P]dsRNA (containing 1 × 10⁶ cpm as described under “Experi-
nmental Procedure”) were incubated with 1 unit of Fraction VI 
activity, and 15-μl aliquots were withdrawn at the times indi-
cated and assayed for radioactivity remaining acid-precipitable 
(●—●); 5 ng of φ1 DNA:RNA hybrid Preparation A containing 
9000 cpm of [3H]DNA (□—□) and 4000 cpm of [32P]RNA 
(△—△) was digested with 1 unit of Fraction VI. TCA, trichloro-
acetic acid. b, effect of preincubation with pancreatic DNase. 
Identical aliquots of the substrates described in a were pretreated 
with pancreatic DNase at a concentration of 20 μg/ml for 30 min 
at 37°, after which one unit of Fraction VI activity was added to 
the reactions and incubation continued for an additional 50 min. 
●—●, [32P]dsRNA; □—□, φ1 hybrid [3H]DNA; △—△, φ1 
hybrid [32P]RNA.

specificity of cleavage is the same for both (Fig. 2, Lanes b, b' 
and c, c'). Competition experiments suggest that processing activity 
and the ability to digest dsRNA are reactions catalyzed by the 
same enzyme. As shown in Fig. 2 the addition of a 60-fold weight 
excess of unlabeled dsRNA abolished cleavage (Lanes f, f') while 
neither a 60-fold nor a 600-fold weight excess of f2 ssRNA had 
any effect on the reactions (Lanes d, d'; e, e'). Similar weight 
excesses of tRNA or E. coli mature rRNAs also did not prevent 
cleavage of the T7 polycistronic mRNA (data not shown).

Table I shows digestion of dsRNA or hybrid RNA by three 
different enzyme preparations which were capable of specific 
cleavage of T7 polycistronic mRNA. All three (Fraction VI, 
Fraction VII, and sizing factor) were able to solubilize 60 ng of 
PC dsRNA at about the expected rate. RNase H activity is 
present in Fraction VI. However, Fraction VII, the peak of 
RNase III activity, fails to cleave dsRNA and T7 mRNA processing activity chromatographing on Sephadex G-100, is unable to digest hybrid RNA. Apparently gel filtration removes some component required 
for RNase H activity. Furthermore, the sizing factor preparation 
which is shown in Table I to be lacking RNase H, had been 
chromatographed on BioGel A.5M prior to ion exchange chromatography (1). Here again apparently a protein sizing step has re-
moved RNase H activity. Attempts to date to recover RNase H 
activity following Sephadex G-100 chromatography have failed.

Table II shows an independent experiment which suggests that 
the digestion of dsRNA or hybrid RNA must be due to dif-
ferent enzymes. In the presence of sufficient excess unlabeled 
dsRNA to abolish completely the ability of Fraction VI to 
solubilize dsRNA, its ability to digest hybrid RNA is retained 
with only an 11% drop in the amount solubilized.

If the activities against dsRNA and hybrid RNA are related, 
there might be an effect of the RNase III mutation (6) on both 
activities. Preliminary experiments similar to those in Fig. 1 
and Table I with extracts of the RNase III-deficient strain which had been fractionated through the ammonium sulfate stage as before 
(3, 5) reveal that while less than 5% of the parental strain's level 
of activity against dsRNA remains, there appears to be a full 
amount of RNase H activity still present (data not shown). The 
presence or absence of RNase H activity seems to have no effect 
on the ability of enzyme fractions to cleave T7 RNA. Both the 
RNase III preparation which had abundant RNase H activity 
and the sizing factor preparation which contained little or no 
RNase H activity cleaved the T7 polycistronic mRNA with a 
high degree of fidelity.

Section B: Size Distribution of Complete Digests of dsRNA and 
Hybrid RNA—Fig. 3 shows autoradiographs of two-dimensional 
analyses of several RNAs after nuclease treatment. The finger-
print technique employed is one in which oligonucleotides of 
a size 20 bases long or less will be denatured and incapable of
TABLE I

| Nucleic acid substrate | Per cent remaining acid-precipitable using |
|------------------------|------------------------------------------|
|                        | No enzyme | Fraction VI | Fraction VII | Digesting factor |
| PC [PH]dsRNA           | 100       | 15.8        | 24.5         | 16.1             |
| Hybrid [PH]DNA         | 100       | 91.0        | 100.0        | 100.0            |
| Hybrid [PP]RNA         | 100       | 3.9         | 100.0        | 96.4             |

TABLE II

| Nucleic acid substrate | Per cent remaining acid-precipitable when incubated with |
|------------------------|----------------------------------------------------------|
|                        | No additions Fraction VI [5X] + 125 X PC dsRNA Fraction VI [25 X] + 125 X PC dsRNA |
| [PP]dsRNA              | 100 | 14.9 | 87.0 | 100.0 |
| Hybrid [PH]DNA         | 100 | 100.0| 99.0 | 98.0  |
| Hybrid [PP]RNA         | 100 | 10.2 | 14.8 | 21.3  |

Fig. 3 shows the fingerprint of an exhaustive digest (incubated 9 times longer than necessary to obtain complete acid solubility) of polyoma dsRNA by RNase III (Fraction VI). The region of radioactivity in Fig. 3b can be compared directly with regard to mobility with the three 11- to 13-base long oligonucleotides shown in Fig. 3c. We conclude that the median size for the digested hybrid RNA is quite a bit smaller than that observed in Fig. 3b and that oligonucleotides of a wider size range (5 to 15 bases) are present.

Section C: End Group Analysis of Digests of dsRNA and Hybrid RNA—One way to distinguish among the various nuclease activities in Fraction VI might be endgroup analysis. If some activities release 5'- and others 3'-phosphate termini, it would be unlikely that they could be caused by the same enzyme. Fig. 4 shows such analyses for the two activities which release acid-soluble fragments, RNase III and RNase H. Fig. 4c shows a drawing of the separation obtained using the two-dimensional base composition analysis method described under “Experimental Procedure.” The four common (2',3')-nucleoside monophosphates produced by alkaline hydrolysis are readily distinguishable from each other and from the four (2',3'), 5'-nucleoside diphosphates (2Xp's) as indicated. Fig. 4a shows the autoradiograph of such an analysis of polyoma [PH]dsRNA which had been incubated in RNase III participating in any hydrogen-bonded complexes (29, 30). This is the case because both dimensions are run in 7 mM urea, while the second dimension is also carried out at 60°C. It is the second dimension, which separates oligonucleotide chains according to size (29), which allows us to estimate the average size of limit digestion products.

Since different homochromatography mixtures do not give exactly the same patterns, it was necessary to run the three analyses shown in Fig. 3 at the same time and in the same chromatography tank. Fig. 3a shows the pattern following RNase T1 digestion of the three principal ribosome binding sites of bacteriophage f1. Each of the major oligonucleotides shown here has been sequenced (26), and in particular, the three prominent spots lowest in the picture (about one-third from the origin to the top) are 11, 12, and 13 bases long, while the prominent topmost dark spot at the right side corresponds to GMP.
FIG. 4. Two-dimensional electrophoretic analysis of dsRNA and hybrid RNA following alkaline hydrolysis. Reactions carried out in 5 μl of 0.01 M Tris-HCl (pH 7.6), 0.01 M magnesium acetate, 0.13 M NH₄Cl, 5% sucrose and were terminated by mixing with 5 μl of 0.4 M NaOH and incubated as described under “Experimental Procedure.” After 18 hours, two-dimensional analysis was carried out also as described under “Experimental Procedure.” a, polyoma [32P]dsRNA (3 × 10⁶ cpm, specific activity 3 × 10⁷ cpm/μg) was incubated for 50 min in the above buffer at 37°. b, polyoma [32P]dsRNA was incubated as in a for 50 min with 0.3 unit of Fraction VI, rendering 85% of the radioactivity soluble in 5% reaction conditions in the absence of enzyme. Only the four (2', 3') nucleoside monophosphates are evident. After RNase III digestion, additional spots appear at positions characteristic of all four pXp's (Fig. 4b). This observation indicates that the activity in Fraction VI which cleaves dsRNA leaves a 5' phosphate terminus. Fig. 4, d and e shows analyses of similar incubations of φ1 DNA:RNA hybrids. Again, digestion releases all four pXp's in detectable quantities.

Table III shows the radioactivity recovered in the various nucleoside mono- and diphosphates for the two digestions analyzed in Fig. 4, d and e. Several further conclusions of interest may be drawn from this data. First, the 5' end groups released do not reflect the overall base composition of the dsRNA or hybrid RNA. In particular, digestion products of dsRNA are enriched for A and U at their 5' termini, while in hybrid RNA, pyrimidines are mildly favored.

Finally, the data of Fig. 4 and Table III allow us to calculate independently the size distribution of oligonucleotides in these digests. Although the digestion conditions were much less exhaustive than those in Fig. 3, we can see that the average size of trichloroacetic acid. c, drawing of Fig. 4b, indicating locations and identities of the spots visible in the autoradiographs. These locations were established both with unlabeled marker nucleotide compounds as before (25) and also with specific digests of [32P]-RNAs containing only one of the four pXp's (3, 32). d, φ1 DNA:RNA hybrid (1.5 × 10⁶ cpm [32P]RNA, specific activity 3 × 10⁷ cpm/μg of hybrid) was incubated for 50 min in the buffer described above; e, φ1 DNA:RNA hybrid was incubated as in d for 50 min with 0.3 unit of Fraction VI as in b. In these pictures, the first dimension is from right to left, while the second is from the bottom of the picture to the top.
The most important observation to emerge from the experiments in Figs. 1 and 2 and Table I is that the RNase III activity is probably responsible for processing polycistronic T7 mRNA. If this is the case, it puts limitations on both the sorts of cleavage sites we might expect within ssRNA and the endgroups we would obtain. The most straightforward model for cleavage sites would suggest that among many potential hairpin loops in an RNA precursor, only a few are large and stable enough to be recognized as dsRNA. Robertson and Hunter (28) have shown that RNase III preparations purified through the Fraction VI stage do not cleave ssRNA containing numerous potential hairpin loops, nor do such RNAs compete for digestion of stable dsRNA substrates. This point is also illustrated in Fig. 1, Lanes d, d' and e, e'. In addition, isolated bacteriophage f2 band 21, a 57-nucleotide RNA which can form a potential hairpin loop with 18 base pairs, is not cleaved by RNase III preparations (28). Since most hairpin loops found in Escherichia coli RNA are not cleaved by RNase III, and since little, if any, stable dsRNA is found in normal E. coli (31), there may be other features required for specific processing by RNase III. This is especially likely since preliminary studies on end groups on RNase III cleavage sites indicate that the same particular sequences are cleaved in each case. Thus a combination of structure and specific sequence may be required for the processing event to occur.

RNase III is the second E. coli enzyme to which specific processing functions have been ascribed. Factors which determine the specificity of the other such enzyme, RNase P which cleaves the precursor to tyrosine tRNA (3, 34), have also not been defined. However, it is likely that, as with RNase III, some combination of sequence and structure will be involved (3, 35).

Size Distribution of Oligonucleotide Digestion Products—The data in Table III (31) establish that an exhaustive digest of dsRNA by RNase III contains oligonucleotides averaging about 15 bases long, in agreement with previous observations (11). Crouch (21) has estimated 13 bases as the chain length of an exhaustive digest of poly(A-U) by RNase III. Using milder conditions of digestion, Crouch has estimated a chain length of 25 bases, in good agreement with our findings for such digests in Table III. The most striking aspect of the size distribution of RNase III digestion products of dsRNA is its narrow range, resulting in oligonucleotides between about 10 and 18 bases long (Fig. 3b).

End Group Analysis—Fig. 4 shows that both RNase III and RNase H cleave to form 5'-phosphate end groups. Robertson et al. (5) found that an exhaustive digest of poly(A-U) contained about 10% of its radioactivity in the form of 3'-UMP, while no 5' UMP was released. Fig. 3b shows that no significant radioactivity was released into any mononucleotides. Since the original purification of RNase III utilized E. coli K38, which contains normal E. coli (31), there may be other features required for the processing event to occur.

Our conclusion that RNase H activity present in E. coli RNase III preparations also forms 5' end groups (Fig. 4, d and e) is also in agreement with the suggestions of others using indirect assays (12, 13) or RNase H's from eukaryotic sources (15).

Finally, as observed by Rosenberg et al. (33) in vitro cleavage products of T7 polycistronic mRNA contain the same end groups (bearing 5'-phosphate) observed in monocistronic mRNAs isolated from infected cells. Furthermore, the two cleavages

**Table III**

| Oligonucleotide Production and End Group Analysis of digested dsRNA and hybrid RNA | Polyoma dsRNA | 4 Hybrid RNA |
|---|---|---|
| Total radioactivity (cpm)* | 28,642 | 12,583 |
| Per cent in nucleoside monophosphates | 93.5 | 81.4 |
| Per cent in pXp's | 6.5 | 18.6 |
| Chain length (bases)* | 30.7 | 10.7 |
| Proportion of nucleoside monophosphates in: | | |
| Cp | 0.346 | 0.240 |
| Ap | 0.185 | 0.223 |
| Gp | 0.251 | 0.248 |
| Up | 0.218 | 0.289 |
| Proportion in: | | |
| Ap + Up | 0.403 | 0.529 |
| Cp + Up | 0.597 | 0.471 |
| Gp + Cp | 0.100 | 0.366 |
| Ap + Gp | 0.266 | 0.217 |
| Proportion of pXp's in: | | |
| pCp | 0.154 | 0.111 |
| pAp | 0.390 | 0.317 |
| Proportion in: | | |
| pAp + pUp | 0.656 | 0.672 |
| pUp + pGp | 0.344 | 0.328 |

* Nucleotides shown in Fig. 4, b and e were cut out and their Cerenkov radiation was determined as described under "Experimental Procedure."

* Chain length was calculated by dividing the total RNA (100% by the per cent in pXp's and multiplying the quotient by 2.

In particular, Paddock and Abelson (32) have shown that bacteriophage T4 RNA Species I is cleaved specifically by E. coli extracts, and the activity responsible has been identified as RNase III (Robertson and Abelson, unpublished experiments). Furthermore, Rosenberg et al. (33) have found that similar specific end-guages are reproducibly generated in several of the RNase III mediated cleavages of T7 polycistronic mRNA.
made by highly purified RNase III preparations in T4 RNA
Species I both yield 5'-phosphate and 3'-hydroxyl termini. Our
finding that RNase III digests have the same end groups further
increases the probability that cleavage of dsRNA and processing
of T7 and rRNA precursors reflect different aspects of the same
enzyme.

In conclusion, we can expect the specific sites in cellular RNAs
which are processed by RNase III to have substantial double
helical structure; to be greater than 20 base pairs in length; to
contain 5'-phosphate and 3'-hydroxyl endgroups after cleavage;
and to contain, in all probability, at least one further charac-
teristic feature, either a common sequence or an additional struc-
tural element, to differentiate them from the many regions of
potential secondary structure now thought to reside at frequent
intervals in biological RNA sequences (36).

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