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Specific RNA-cleaving activities from HeLa cells

(RNA processing/heterogeneous nuclear RNA/RNA precursors)

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ABSTRACT **Subcellular fractionation of HeLa cells was carried out under gentle conditions to isolate enzymes that cleave RNA precursors in a specific manner. Four separate activities—cleavage of HeLa cell heterogeneous nuclear RNA, the HeLa cell 45S rRNA precursor, RNA·DNA hybrids (RNase H), and the *Escherichia coli* $tRNA^{Tyr}$ precursor (RNase P)—were revealed by these studies. The specificity and limited nature of these cleavages suggest that they are due to eukaryotic RNA-processing enzymes. The virtual absence of random nucleases from these enzymes was demonstrated by their inability to cleave the 8000-base early mRNA precursor of bacteriophage T7, *E. coli* 30S rRNA precursor, or HeLa cytoplasmic poly(A)-containing RNA.**

It is now evident that in primary transcripts of most prokaryotic and eukaryotic RNAs the regions destined to occur in the mature molecules are interspersed among regions that are removed during the maturation process (1–5). The role of RNA processing enzymes is the correct removal of these "extra" regions, leading to the formation of functional mature RNAs. Because of the availability of numerous well-defined prokaryotic RNA precursors, the enzymatic RNA processing reactions that occur in prokaryotes have been the most extensively studied (6–10). From these studies the conclusion has emerged that such enzymes retain the ability to cleave their natural substrates properly *in vitro*, even after extensive purification of both the enzyme and the RNA precursors. Considerably less information concerning the enzymes involved in specific cleavage of eukaryotic RNA precursors (11, 12) is available.

We have carried out gentle fractionation of HeLa cells to minimize release of random nucleases, and we have assayed nuclear and cytoplasmic fractions for their ability to cleave a wide spectrum of well-defined prokaryotic and eukaryotic RNA molecules. Four specific RNA-cleaving activities were detected: cleavage of heterogeneous nuclear RNA (hnRNA) in a characteristic fashion, specific cleavage of 45S rRNA precursor, cleavage of RNA·DNA hybrids (RNase H), and cleavage of a tRNA precursor (RNase P). Only the latter two activities have been described previously in mammalian cells.

MATERIALS AND METHODS

Cell Growth and Subcellular Fractionation. HeLa (human) cells (strain S3) from the collection of Igor Tamm, The Rockefeller University, were maintained in suspension culture [Joklik's modified Eagle's medium (GIBCO) containing 5% fetal calf serum (GIBCO)]. Cells from 1–3 liters of culture at 5–6 × 10^5 cells per ml were harvested and fractionated according to Bothwell and Altman's modifications (13) of methods published by Penman (14) and Amaldi and Attardi (15). Worthington DNase DPFF was used without further treatment in the preparation of nuclear lysates. Ammonium sulfate precipitation was performed by adding neutralized saturated ammonium sulfate (4°C) to obtain the desired final concentration, with constant stirring, incubating at 4°C for 20 min, and centrifuging at 20,000 × *g* for 20 min. Precipitates were resuspended in 10 mM Tris·HCl, pH 7.5/20 mM NaCl/2 mM $MgCl_2$/1 mM dithiothreitol, and dialyzed for 2 hr at 4°C against this buffer.

Labeled RNA Substrates. HeLa cells were grown in the presence of actinomycin D at 0.05 μg/ml and ^{32}P-labeled inorganic phosphate for 3 hr at a cell density of 5–10 × 10^6 cells per ml (16). hnRNA was extracted from isolated nuclei, fractionated on sucrose gradients to obtain RNA sedimenting faster than 32 S (16), and digested to yield double-stranded RNA (dsRNA) (16). Cytoplasmic poly(A)-containing RNA was removed from cytoplasmic fractions by oligo(dT)-cellulose chromatography on type 3 oligo(dT)-cellulose from Collaborative Research (Waltham, MA), according to the manufacturer's instructions. The 45S rRNA precursor was prepared from cells grown in the presence of [^{32}P]phosphate for 30 min and in the absence of actinomycin D, and was further fractionated on sucrose gradients (16). *Escherichia coli* 30S rRNA precursor was isolated from *E. coli* BL107 (the gift of F. W. Studier, Brookhaven National Laboratory, Upton, NY) grown in the presence of ^{32}P-labeled inorganic phosphate (17, 18).

Bacteriophage T7 early mRNA precursor was synthesized according to Dunn and Studier (19). RNA·DNA hybrids were synthesized by the action of *E. coli* RNA polymerase on single-stranded bacteriophage f1 DNA in the presence of [^3H]UTP (20). [^3H]Poly(A-U) (double-stranded alternating copolymer of A and U) was synthesized in a similar manner (6, 20), using poly[d(A-T)] as template. ^{32}P-Labeled *E. coli* tyrosine tRNA precursor (8) was the generous gift of S. Altman (Yale University).

Enzyme Assays. Protein concentrations were determined by measuring the spectral shift caused by Coomassie blue–protein interaction as described by Bradford (21). Comparable proportions of subcellular fractions were assayed for their ability to cleave RNA substrates by incubation in 10 μl of a standard buffer containing 30 mM Tris·HCl at pH 8.0, 100 mM NaCl, 5 mM $MgCl_2$, 0.1 mM EDTA, and 0.1 mM dithiothreitol for 30 min at 37°C. Reactions were terminated by adding 3 μl of 20% sucrose/1% sodium dodecyl sulfate/0.2% bromphenol blue/0.2 M EDTA. The extent of substrate cleavage was determined by acid precipitation, polyacrylamide gel electrophoresis, or sucrose gradient centrifugation, as detailed in the figure legends.

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Abbreviations: hnRNA, heterogeneous nuclear RNA; dsRNA, double-stranded RNA.

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RESULTS

HeLa cells were gently fractionated by using the procedures summarized in Fig. 1. Cytoplasmic fractions proved to be richer and more reliable sources of enzymatic activities than the nuclear fractions. Each subcellular fraction was tested for the presence of enzymes capable of cleaving four well-defined RNA substrates.

Specific hnRNA-Cleaving Activity. We have observed a highly specific RNase activity against hnRNA (Fig. 2*A*). The majority of this activity is reproducibly found in association with cytoplasmic membranes and is released by detergent treatment. The major shift in electrophoretic mobility of the HeLa hnRNA, after treatment with the membrane-rich fractions as shown in Fig. 2*A*, is in marked contrast to the low level of cleavage of the 8000-base phage T7 early mRNA precursor by the same fractions (Figs. 2*C* and 3). Although the nuclear lysate and the nuclear sap fractions also demonstrated some ability to cleave hnRNA (data not shown), the ratio of this activity to that against T7 mRNA precursor was much less favorable than for the cytoplasmic fractions. Limited cleavage of hnRNA has been observed previously as a result of treatment by *E. coli* RNase III (23), a dsRNA-specific RNA processing enzyme (6). To find out whether the hnRNA-specific enzyme in HeLa cytoplasmic fraction 9 is similar to RNase III, we have compared the abilities of these two activities to cleave hnRNA, T7 early mRNA precursor, and *E. coli* 30S rRNA precursor. As shown in Fig. 3, only hnRNA is cleaved by HeLa cell fraction 9, whereas all three substrates are digested by RNase III, demonstrating that the substrate specificities of these two activities differ.

The hnRNA-cleaving activity of fraction 9 can be quantitatively recovered by precipitation with ammonium sulfate at 20% saturation. hnRNA treated by the recovered activity is reduced in sedimentation rate from 32 S to 6–15 S, whereas poly(A)-containing cytoplasmic RNA is unaffected (Fig. 4).

HeLa cell fraction 9 was also compared with the random nuclease S1 as a further test of its degree of specificity. Unlike fraction 9, nuclease S1 readily digests both hnRNA and the T7 early mRNA precursor (data not shown). Under conditions that permitted nuclease S1 to digest hnRNA to the same extent as fraction 9, the relative abilities of unlabeled hnRNA and tRNA to compete with these reactions were determined. As shown in Table 1, hnRNA cleavage by nuclease S1 is equally inhibited by either unlabeled hnRNA or tRNA. In contrast, cleavage of hnRNA by the activity in HeLa cell fraction 9 is effectively inhibited by low quantities of hnRNA but is totally unaffected by the addition of tRNA.

Activity for HeLa 45S rRNA Precursor. Cytoplasmic subcellular fractions were screened for their ability to cleave HeLa 45S rRNA precursor, as shown in Fig. 2*B*. The 2% polyacrylamide/0.5% agarose gels used here provide an excellent assay

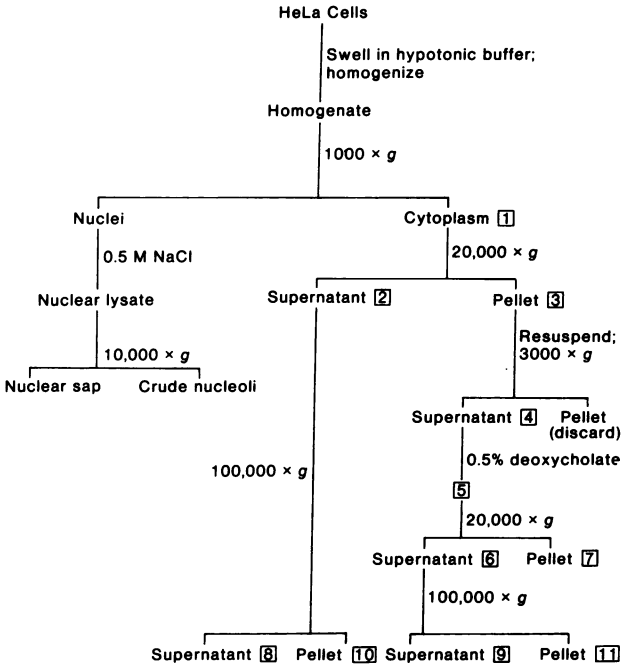

FIG. 1. Flow diagram of HeLa subcellular fractionation. Cells were swollen briefly in hypotonic solution and homogenized to release nuclei, which were resuspended in 10 mM Tris·HCl, pH 7.5/20 mM NaCl/5 mM dithiothreitol/50 mM $MgCl_2$, and lysed by addition of 0.5 M NaCl. DNase was added at 50 μg/ml, and the mixture was shaken and Vortex mixed until the viscosity was greatly diminished (1–2 min). The nuclear lysate was centrifuged at 4°C at 10,000 × *g* for 5 min, yielding a supernatant (nuclear sap) and a pellet, which was resuspended in the nuclear resuspension buffer (crude nucleoli). Low-speed centrifugation yielded the crude cytoplasm, fraction 1. The crude cytoplasm was subjected to higher-speed centrifugation (20,000 × *g*) to separate the soluble cytoplasm (fraction 2) from the membrane-containing pellet (fraction 3). The soluble cytoplasm was further fractionated by 100,000 × *g* centrifugation to yield the high-speed supernatant (fraction 8) and the ribosomal pellet (fraction 10). The membrane-containing pellet (fraction 3) was resuspended in 10 mM Tris·HCl, pH 7.5/20 mM NaCl/5 mM dithiothreitol and subjected to low-speed centrifugation to remove nuclei and large cellular debris. The resulting supernatant (fraction 4) was treated with detergent (0.5% sodium deoxycholate) to yield a solution of detergent-treated membranes (fraction 5). The material that was not solubilized by the detergent was removed by 20,000 × *g* centrifugation (fraction 7), and the soluble membrane fraction (fraction 6) was further fractionated by high-speed centrifugation (100,000 × *g*) into the ribosome pellet (fraction 11) and the detergent-soluble membrane fraction (fraction 9).

Table 1. Relative abilities of hnRNA and tRNA to inhibit cleavage of HeLa cell hnRNA by either HeLa cell activity or nuclease S1

Competitor added		Remaining activity	
RNA	μg	HeLa cell*	Nuclease S1†
hnRNA‡	0	+++	+++
	1	+++	−
	2	+	−
	5	−	−
	10	−	−
tRNA§	0	+++	+++
	1	+++	−
	2	+++	−
	5	+++	−
	10	+++	−

* HeLa cell hnRNA-cleaving activity was assayed by incubating 24,000 cpm of ^{32}P-labeled hnRNA (specific activity 330,000 cpm/μg) in 10-μl reaction mixtures along with 0.25 μl of the 20% ammonium sulfate precipitate of subcellular fraction 9 (see legend to Fig. 4) and the indicated amount of unlabeled RNA competitor. After polyacrylamide gel electrophoresis and autoradiography, remaining activity was estimated by visual inspection of the autoradiograms.

† Reactions were carried out as described in * footnote, except that each reaction mixture contained 0.01 unit of S1 nuclease (Bethesda Research Laboratories, Rockville, MD) instead of the HeLa cell activity.

‡ Unlabeled hnRNA was prepared by fractionating nuclei from normally growing HeLa cells and utilizing the fraction of RNA that sedimented faster than 32 S.

§ tRNA from *E. coli* (Sigma) was highly purified by three rounds of phenol extraction followed by cellulose CF11 chromatography and ethanol precipitation.

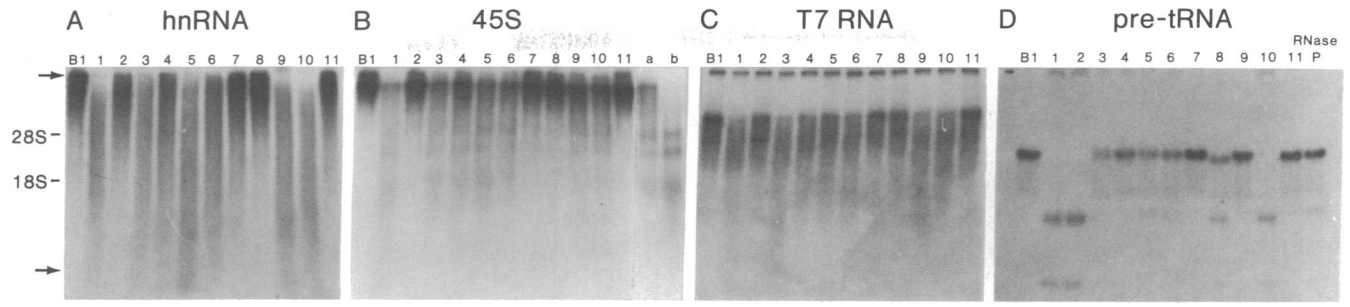

FIG. 2. Effect of HeLa subcellular fractions on four different ^{32}P-labeled RNA substrates. Each substrate was treated with comparable amounts (normalized to original number of cells extracted) of 11 different cytoplasmic subcellular fractions, numbered 1–11, as identified in Fig. 1 and Table 2.

In each of the four gel analyses shown here, the left-hand lane (marked B1) contained products of a reaction in which the substrate was incubated in buffer with no added enzyme. Except for the case of *E. coli* pre-tRNA^{Tyr}, 1 μg of bacteriophage f2 RNA was included in each reaction. Reactions were terminated and samples were prepared for electrophoresis and autoradiography as described in *Materials and Methods*. The upper left arrow in the autoradiograph indicates the origin of electrophoresis. The lower left arrow indicates the position of the bromphenol blue marker for gels *A*, *B*, and *C*. The bromphenol blue migrated to the bottom of gel *D*. The positions of HeLa cell 28S and 18S RNA are shown for *A*, *B*, and *C*. hnRNA (*A*, 30,000 cpm per reaction, specific activity 1.2×10^6 cpm/μg), 45S rRNA precursor (*B*, 6000 cpm per reaction, specific activity 11,000 cpm/μg), and bacteriophage T7 mRNA precursor (*C*, 7500 cpm per reaction, specific activity 2×10^6 cpm/μg) were analyzed on the 2% acrylamide/0.5% agarose gel system referred to in *Materials and Methods*, while the *E. coli* pre-tRNA^{Tyr} (*D*, 1000 cpm per reaction, specific activity about 1.5×10^6 cpm/μg) was assayed on 10% polyacrylamide gels as described above.

In *B*, lanes a and b represent the action of two concentrations of more highly purified HeLa cell material prepared by ammonium sulfate precipitation (as described in the text), followed by dialysis against the buffer indicated and DEAE-Sephadex chromatography in a gradient of the dialysis buffer ranging from 0.05 to 0.5 M NaCl. A major peak of activity eluted at about 0.1 M NaCl, and lanes a and b represent the action of 3 and 4 μl, respectively, of the peak fraction (whose total volume was 2 ml). The identical subcellular fractions were used in the experiments shown in *A*, *C*, and *D*, whereas a different set of fractions (made from about half as many HeLa cells) was used for *B*. In *D*, the lane entitled RNase P depicts analysis of the action of authentic *E. coli* RNase P upon the *E. coli* tRNA^{Tyr} precursor.

for this 20,000-base RNA, which migrates as a single sharp band. An activity that cleaves this substrate to yield two bands (approximately 28 S and 24 S) plus additional products (Fig. 2*B*) was observed with a subcellular distribution much the same as that observed for the hnRNA-cleaving activity. The two specific bands can be obtained in much higher yield after further fractionation of the HeLa cell activity on DEAE-Sephadex (Fig. 2*B*, lanes a and b). The observation that this 45S rRNA-cleaving activity disappears from HeLa cell fractions more rapidly during storage at 4°C than does the hnRNA-specific activity suggests that the two are distinct.

RNase H. As assayed by solubilization of [^{3}H]RNA·DNA hybrids, RNase H activity was detected in several cytoplasmic fractions (Table 2). In particular, most of the RNase H is found in those fractions that lack detergent (fractions 1, 2, 8, and 10). In other cell types (25, 26) RNase H is often found together with RNases that cleave single-stranded RNAs and dsRNAs. Therefore, we subjected single-stranded RNAs of comparable specific radioactivities (^{3}H-labeled phage T7 mRNA precursor and ^{32}P-labeled transcripts of HeLa cell repetitive DNA) and dsRNAs [^{3}H-labeled poly(A-U) and reovirus RNAs, and ^{32}P-labeled dsRNA prepared from HeLa cell hnRNA] to treatment with the cytoplasmic subcellular fractions. As assayed by solubilization in 5% trichloroacetic acid, none of these substrates was cleaved by any fraction (data not shown).

Table 2. RNase H activity in HeLa subcellular fractions

Cell fraction	Total protein, mg	Total RNase H,* units
1	64	4320
2	47	3868
3	17	1182
4	12	446
5	12	64
6	12	676
7	0.5	0
8	30	3804
9	10	1250
10	18	547
11	2	0

* Assays for RNase H were performed by using an amount of each subcellular fraction corresponding to about 1.5×10^4 cells. Thus, in the assays shown here, the quantity of protein present in the assay mixture varied from 0.75 (fraction 1) to 0.007 μg (fraction 7). RNase H assay mixtures contained 2600 cpm of ^{3}H-labeled RNA in the form of RNA·DNA hybrids (specific activity 10^5 cpm/μg). One unit of RNase H activity is defined as the amount of enzyme that solubilizes 1 nmol of ribonucleotides from RNA·DNA hybrids in 20 min at 37°C (24).

RNase P. Using the precursor to *E. coli* tRNA^{Tyr} as a probe, Koski *et al.* (12) have shown that subcellular fractions of human KB cells contain an enzyme similar to *E. coli* RNase P. We have used the same assay to seek tRNA-processing activities in HeLa cells. Nuclear fractions caused only limited nonspecific breakdown of *E. coli* pre-tRNA^{Tyr} (data not shown). On the other hand, cytoplasmic fractions prepared without detergent treatment introduce a single cleavage into the molecule (Fig. 2*D*). The resulting two specific fragments (corresponding to an 85-base and a 41-base segment) show electrophoretic mobilities comparable to those of the corresponding fragments produced by authentic *E. coli* RNase P (Fig. 2*D*).

DISCUSSION

The subcellular fractionation scheme for HeLa cells employed in this study of specific RNA cleavage revealed remarkably few interfering random RNase activities, making it an ideal system in which to seek RNA-processing enzymes (see also ref. 27). As shown in Figs. 2*C* and 3, under our standard assay conditions at pH 8.0, little nonspecific RNase activity is observed, even with a sensitive assay utilizing the electrophoretic mobility of the ^{32}P-labeled 8000-base T7 early mRNA precursor. Only at pH conditions below 6.5 were we able to detect significant nonspecific RNase activity (data not shown) of the kind described previously by Saha *et al.* (22).

These studies have uncovered four enzymes that could be important in the RNA metabolism of HeLa cells: (*i*) an

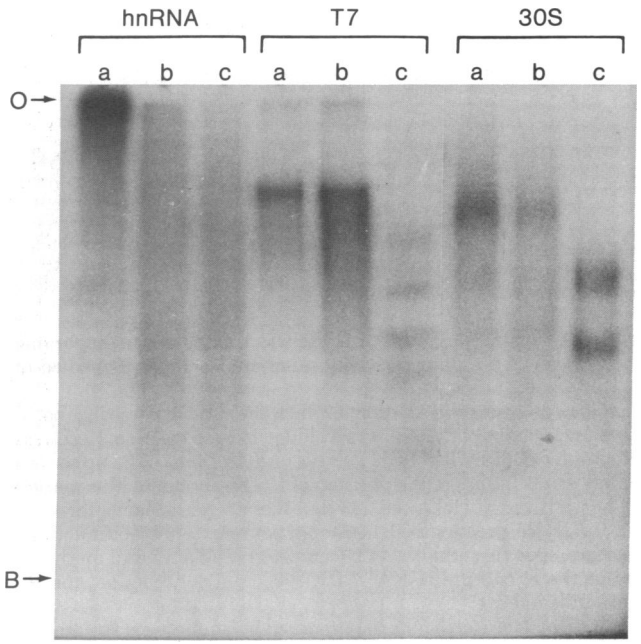

FIG. 3. Comparison of the HeLa hnRNA-cleaving activity and *E. coli* RNase III. Three ^{32}P-labeled substrates were assayed for cleavage as in Fig. 2. Origin (O) and position of bromphenol blue (B) are indicated on the left. Amounts and specific activities were as follows: hnRNA, 13,000 cpm per reaction, specific activity 1×10^5 cpm/μg; T7 mRNA precursor, 6000 cpm per reaction, specific activity 2×10^6 cpm/μg; *E. coli* 30S rRNA precursor, 12,000 cpm per reaction, specific activity estimated at 5×10^6 cpm/μg. In each of the three sets of assays depicted in the autoradiograms, lane a depicts the analysis of substrate incubated in buffer without enzyme; lane b depicts the effect of HeLa subcellular fraction 9; and lane c depicts the effect of 0.5 unit of *E. coli* RNase III purified to the fraction VI stage (22). In the analysis of T7 lane c, the bands produced by RNase III correspond to the T7 mRNA species previously observed (19). Furthermore, in the analysis of 30S rRNA precursor, the bands observed in lane c correspond to the previously identified intermediates in rRNA metabolism running at positions corresponding to 17 S and 25 S (18).

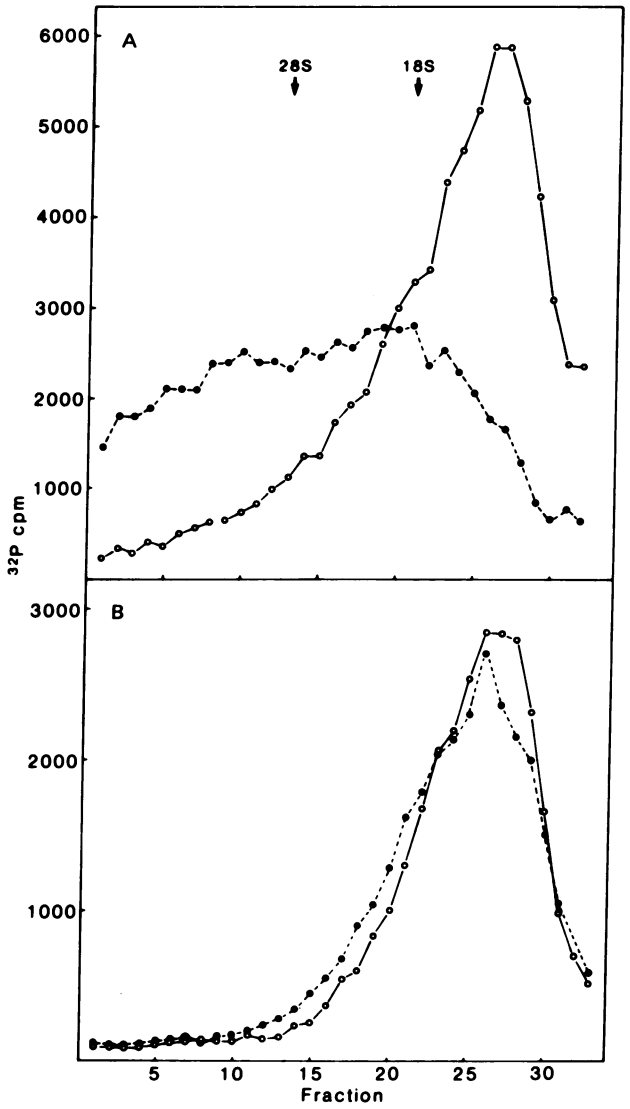

FIG. 4. Effect of HeLa hnRNA-cleaving activity upon hnRNA and poly(A)-containing cytoplasmic RNA. ^{32}P-Labeled RNAs were incubated for 30 min at 37°C in 20 μl of the reaction buffer described in *Materials and Methods*, except that it contained 0.1 M NH_4Cl instead of 0.1 M NaCl. Reactions were stopped by addition of 80 μl of 0.01 M Tris·HCl, pH 7.5/0.01 M EDTA/0.2% sodium dodecyl sulfate, heated at 65°C for 3 min, layered upon 5-ml 15–30% sucrose gradients in the above buffer containing 0.1 M NaCl, and centrifuged for 3 hr at 45,000 rpm in the SW 50.1 rotor of a Beckman L2-65B ultracentrifuge at 20°C. Collection was as before (15). (*A*) Analysis of hnRNA: 88,000 cpm (Cerenkov) of ^{32}P-labeled hnRNA (specific activity 110,000 cpm/μg), together with 2 μg of tRNA carrier, was incubated with buffer alone (●) or with 0.5 μl of HeLa subcellular fraction 9 after precipitation in 20% saturated ammonium sulfate and resuspension in half the original volume (○). The positions of unlabeled HeLa cell 28S and 18S rRNA species are indicated. (*B*) Analysis of poly(A)-containing cytoplasmic RNA: 32,000 cpm (Cerenkov) of RNA (specific activity 13,000 cpm/μg) was incubated in buffer alone (●), or with 0.5 μl of HeLa cell hnRNA-cleaving activity as described above (○).

hnRNA-cleaving enzyme, which may participate in HeLa cell mRNA processing; (*ii*) an enzyme that cleaves 45S rRNA precursor in a reproducible manner; (*iii*) an RNase H, which could be involved either in metabolizing RNA primers for DNA synthesis (28–30) or in removing RNA·DNA hybrid regions formed during transcription (20, 31); and (*iv*) an RNase P, which, like that reported in KB cells (12), is probably involved in the metabolism of HeLa cell tRNA precursors. Although all four of these activities would be expected to function mainly in the nucleus, our most active preparations were derived from cytoplasmic fractions. Numerous earlier studies have shared this property. For example, RNase P of KB cells (12) is found predominantly in the cytoplasm. Likewise, an enzyme involved in yeast tRNA processing was recovered not only from yeast nuclei (32) but also from the 0.2 M salt wash of yeast cytoplasmic ribosomes (33). Finally, the DNA polymerase α, initially recovered from the cytoplasm of various cell types (34), has more recently been found in association with the nucleus (35). Thus, although it appears that subcellular fractionation of eukaryotic cells may not preserve the *in vivo* distribution of enzymes involved in nucleic acid metabolism, this situation does not necessarily impede their study.

The 45S rRNA-cleaving activity detected in our study was recovered from cytoplasmic membrane-rich fractions (see Fig. 2*B*). Grummt *et al.* (36) detected such an activity in the nucleolar fraction of chicken embryos. Perhaps, in our studies, the activity was liberated from the nuclei and bound preferentially to membranes during subcellular fractionation. However, unlike the activity detected in chicken cells (36), and in contrast to reports concerning other cell types (24, 36–42), neither our 45S rRNA-cleaving preparations nor any other HeLa subcellular fraction in our study contained detectable activity against dsRNA. The possibility that such an activity could be present in an inactive form is raised by recent studies of Rech *et al.* (43), who demonstrated the presence of a

dsRNA-cleaving enzyme bound tightly to HeLa cell nuclear RNA that could be detected only when the RNA component was removed, either by RNase treatment or by DEAE-Sephadex chromatography.

As expected from the KB cell studies of Koski *et al.* (12), we found an RNase P activity in HeLa cells. However, HeLa cell RNase P was only slightly more dense than a marker protein in Cs_2SO_4 (data not shown). In contrast, KB cell RNase P has a buoyant density similar to that of *E. coli* RNase P (S. Altman, personal communication), which is composed of an RNA and a protein subunit and has a buoyant density much closer to that of RNA itself (9). It is possible that the HeLa cell RNase P detected in our studies contains a much smaller RNA species, or it may have been isolated as an RNA·protein·membrane complex with a net density close to that of protein.

Perhaps the most interesting of the specific enzymes reported here is that which cleaves hnRNA. The enzyme in our cytoplasmic membrane-rich fraction 9 and fractions prepared from it (Figs. 2–4, Table 1) is free of nonspecific nucleases and RNase P, and has a remarkable preference for the large heterogeneous RNAs isolated from HeLa cell nuclei (Fig. 3). The hnRNA-cleaving enzyme is inactive against all other substrates tested (Figs. 2–4, Table 1). Thus there must be a set of cleavage sites in most hnRNA molecules that is absent from all other RNA populations tested. It is therefore likely that the "smear" on polyacrylamide gels (See Figs. 2 and 3) that is characteristic of this enzyme's action on hnRNA results from the complexity of the mixed substrate population (44), not from lack of specificity. This activity has been obtained from a number of batches of cells, and has been taken through ammonium sulfate precipitation and DEAE-Sephadex and DEAE-cellulose chromatography.

Most eukaryotic mRNAs are now thought to be derived from larger precursors by a number of maturation steps. Specific cleavage is thought to initiate several important further steps, such as polyadenylylation and "splicing." Although the hnRNA-specific activity detected by us in HeLa cells may be involved in some of these reactions, its further study will have to be pursued by using specific mRNA precursor molecules such as those of adenovirus.

In conclusion, the purity of enzyme activities obtained by using the approach reported here was sufficient to permit determination of their substrate specificities. Furthermore, this study represents a starting point from which to examine the biological roles and mechanisms of action of these potential RNA-processing enzymes of HeLa cells.

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1. Altman, S. (1975) *Cell* **4**, 21–29.
2. Abelson, J. (1979) *Annu. Rev. Biochem.* **48**, 1035–1069.
3. Chow, L. T., Gelinas, R. E., Broker, T. R. & Roberts, R. J. (1977) *Cell* **12**, 1–8.
4. Tilghman, S. N., Tiemeier, D. C., Seidman, J. G., Peterlin, B. N., Sullivan, M., Maizel, J. V. & Leder, P. (1978) *Proc. Natl. Acad. Sci. USA* **75**, 725–729.
5. Robertson, H. D. (1977) in *Nucleic Acid-Protein Recognition*, ed. Vogel, H. (Academic, New York), pp. 549–568.
6. Robertson, H. D., Webster, R. E. & Zinder, N. D. (1968) *J. Biol. Chem.* **243**, 82–91.
7. Dunn, J. J. & Studier, F. W. (1973) *Proc. Natl. Acad. Sci. USA* **70**, 1559–1563.
8. Robertson, H. D., Altman, S. & Smith, J. D. (1972) *J. Biol. Chem.* **247**, 5243–5251.
9. Stark, B., Kole, R., Bowman, E. & Altman, S. (1978) *Proc. Natl. Acad. Sci. USA* **75**, 3713–3721.
10. Sogin, M. L., Pace, B. & Pace, N. R. (1977) *J. Biol. Chem.* **252**, 1350–1357.
11. Darnell, J. E. (1968) *Bacteriol. Rev.* **32**, 262–290.
12. Koski, R. A., Bothwell, A. L. & Altman, S. (1976) *Cell* **9**, 101–116.
13. Bothwell, A. L. & Altman, S. (1975) *J. Biol. Chem.* **250**, 1451–1459.
14. Penman, S. (1966) *J. Mol. Biol.* **17**, 117–130.
15. Amaldi, F. & Attardi, G. (1968) *J. Mol. Biol.* **33**, 737–749.
16. Robertson, H. D., Dickson, E. & Jelinek, W. (1977) *J. Mol. Biol.* **115**, 571–589.
17. Robertson, H. D., Pelle, E. G. & McClain, W. H. (1980) in *tRNA*, eds. Abelson, J., Schimmel, P. & Söll, D. (Cold Spring Harbor Laboratory, Cold Spring Harbor, NY), in press.
18. Robertson, H. D. & Barany, F. (1978) in *Proceedings of the 12th FEBS Congress*, (Pergamon, Oxford, England), pp. 285–295.
19. Dunn, J. J. & Studier, F. W. (1973) *Proc. Natl. Acad. Sci. USA* **70**, 3296–3300.
20. Robertson, H. D. (1971) *Nature (London)* **229**, 169.
21. Bradford, M. M. (1976) *Anal. Biochem.* **72**, 248–254.
22. Saha, B. K., Graham, M. Y. & Schlessinger, D. (1979) *J. Biol. Chem.* **254**, 5951–5957.
23. Robertson, H. D. & Dickson, E. (1974) *Brookhaven Symp. Biol.* **26**, 240–266.
24. Nikolaev, N., Birge, C. H., Gotoh, S., Glazier, K. & Schlessinger, D. (1975) *Brookhaven Symp. Biol.* **26**, 175–193.
25. Hall, S. H. & Crouch, R. J. (1977) *J. Biol. Chem.* **252**, 4092–4097.
26. Cathala, G., Rech, J., Huet, J. & Jeanteur, P. (1979) *J. Biol. Chem.* **254**, 7353–7361.
27. Udvardy, A. & Seifart, K. H. (1976) *Eur. J. Biochem.* **62**, 353–363.
28. Berkower, I., Leis, J. & Hurwitz, J. (1973) *J. Biol. Chem.* **248**, 2621–2624.
29. Busen, W., Peters, J. H. & Hausen, P. (1977) *Eur. J. Biochem.* **74**, 203–208.
30. Fujinaga, K., Parsons, J. T., Beard, J. W., Beard, D. & Green, M. (1970) *Proc. Natl. Acad. Sci. USA* **67**, 1432–1439.
31. Huet, J., Buhler, J. M., Sentenac, A. & Fromageot, P. (1977) *J. Biol. Chem.* **252**, 8848–8855.
32. O'Farrell, P. Z., Cordell, B., Valenzuela, P., Rutter, W. H. & Goodman, H. (1978) *Nature (London)* **274**, 438–445.
33. Knapp, G., Beckmann, J. S., Johnson, P. F., Fuhrman, S. A. & Abelson, J. (1978) *Cell* **14**, 221–236.
34. Chang, L. M. S. & Bollum, F. (1971) *J. Biol. Chem.* **246**, 5835–5837.
35. Herrick, G., Spear, B. B. & Veomett, G. (1976) *Proc. Natl. Acad. Sci. USA* **73**, 1136–1139.
36. Grummt, I., Hall, S. H. & Crouch, R. J. (1979) *Eur. J. Biochem.* **94**, 437–443.
37. Robertson, H. D. & Mathews, M. B. (1973) *Proc. Natl. Acad. Sci. USA* **70**, 225–229.
38. Busen, W. & Hausen, P. (1975) *Eur. J. Biochem.* **521**, 179–190.
39. Rech, J., Cathala, G. & Jeanteur, P. (1976) *Nucleic Acids Res.* **3**, 2055–2065.
40. Ohtsuki, K., Groner, Y. & Hurwitz, J. (1977) *J. Biol. Chem.* **252**, 483–491.
41. Hall, S. H. & Crouch, R. J. (1977) *J. Biol. Chem.* **252**, 4092–4097.
42. Berkower, I., Leis, J. & Hurwitz, J. (1973) *J. Biol. Chem.* **248**, 5914–5921.
43. Rech, J., Brunel, C. & Jeanteur, P. (1979) *Biochem. Biophys. Res. Commun.* **88**, 422–427.
44. Davidson, E. H. & Britten, R. J. (1973) *Q. Rev. Biol.* **48**, 565–613.