MOLECULAR AND CELLULAR BIOLOGY, Feb. 1982, p. 154–160
0270-7306/82/020154-07$02.00/0

Vol. 2, No. 2

Heterogeneous Nuclear RNA Promotes Synthesis of (2′,5′)Oligoadenylate and Is Cleaved by the (2′,5′)Oligoadenylate-Activated Endoribonuclease

TIMOTHY W. NILSEN,[1] PATRICIA A. MARONEY,[1] HUGH D. ROBERTSON,[2] AND CORRADO BAGLIONI[1]*

Department of Biological Sciences, State University of New York at Albany, Albany, New York 12222[1] and Rockefeller University, New York, New York 10021[2]

Received 2 July 1981/Accepted 30 September 1981

Heterogeneous nuclear RNA contains double-stranded regions that are not found in mRNA and that may serve as recognition elements for processing enzymes. The double-stranded regions of heterogeneous nuclear RNA prepared from HeLa cells promoted the synthesis of (2′,5′)oligoadenylate [(2′,5′)oligo(A) or (2′,5′)A_n] when incubated with (2′,5′)A_n polymerase. This enzyme is present in elevated levels in interferon-treated cells, and labeled heterogeneous nuclear RNA incubated with extracts of these cells is preferentially cleaved, since mRNA included in the same incubations is not appreciably degraded. The cleavage of heterogeneous nuclear RNA is caused by the synthesis of (2′,5′)A_n and by a "localized" activation of the (2′,5′)A_n-dependent endonuclease, since it was enhanced by ATP, the substrate of the (2′,5′)A_n polymerase, and inhibited by 2′-dATP and ethidium bromide. Both of these compounds suppress the synthesis of (2′,5′)A_n, the first by competitive inhibition and the latter by intercalating into double-stranded RNA. The possible role of double-stranded regions and of the (2′,5′)A_n polymerase-endonuclease system in the processing of heterogeneous nuclear RNA is discussed.

Nuclear heterogeneous RNA (hnRNA) obtained from a variety of eucaryotic cells contains duplex structures greater than 20 base pairs (12, 14, 15, 18, 25, 31), which represent approximately 4 to 8% of the hnRNA (31). This fraction of double-stranded (ds) RNA has been characterized in hnRNA extracted from cell nuclei (12, 14, 15, 18, 25, 31). However, Calvet and Pederson (7) have shown that some ds regions are present in the hnRNA of intact cells by cross-linking the RNA with the psoralen derivative 4′-aminomethyl-4,5′,8-trimethylpsoralen (AMT). Furthermore, ribonucleoprotein particles containing hnRNA complexed with protein bind the dye ethidium (29). Both AMT and ethidium intercalate preferentially in ds regions (13, 19). The dsRNA in hnRNA is also cleaved by the enzyme RNase III (5, 30).

The ds regions are interspersed in hnRNA and are not recovered in mRNA (14, 30). It has been suggested, therefore, that these ds regions could serve as recognition elements for enzymes which process hnRNA to mRNA (29, 31). The cleavage of these regions by RNase III, which is a processing enzyme of *Escherichia coli* (32), and the inhibition of processing of nuclear transcripts by agents which intercalate into dsRNA (4, 8, 39) are consistent with this suggestion. Moreover, when hnRNA is complexed with protein (hnRNP), the ds regions are apparently not associated with proteins (5, 6) and thus may serve as recognition sites for specific enzymes that may be activated by binding to dsRNA and could play some functional role in nuclear RNA metabolism.

Two enzymes known to be activated by dsRNA, a protein kinase and an oligoadenylate polymerase, are present in a basal level in animal cells (see reference 1 for a review). Interferon greatly enhances the synthesis of these two proteins, which may have an important role in the antiviral state (1). In particular, in cells infected by viruses which form replicative complexes containing dsRNA, the oligoadenylate polymerase forms oligonucleotides characterized by 2′,5′-phosphodiester bonds, collectively designated (2′,5′)oligo(A) or (2′,5′)A_n (1). These oligonucleotides are synthesized by the polymerase bound to dsRNA, and their chemical structure was determined by Kerr and Brown (16). The (2′,5′)A_n activates a latent endoribonuclease (RNase L) present in cells treated with interferon or untreated (3). This nuclease can be activated in a "localized" way, since under certain conditions single-stranded RNA linked to dsRNA is preferentially cleaved in incubations

with extracts from interferon-treated cells (26). The localized activity of the nuclease may result from limited activation near the site of the synthesis of $(2',5')A_n$ (26). The behavior of these two enzymatic activities caused us to ask whether there might be in normal cells a dsRNA moiety capable of activating this pathway. The ds regions of hnRNA are a logical candidate for such a role. Therefore, we carried out experiments designed to establish whether the $(2',5')A_n$ polymerase may be activated by ds regions of hnRNA and whether the RNase L could preferentially cleave hnRNA. If this were the case, a role for these enzymes in normal hnRNA metabolism may be suggested.

MATERIALS AND METHODS

Preparation and analysis of HeLa cell hnRNA. ^{32}P-labeled hnRNA was prepared by the method described by Robertson et al. (31); ^{3}H-labeled hnRNA was prepared by the same method, except that 5 mCi of [^{3}H]uridine (New England Nuclear Corp., Boston, Mass.) was added instead of 20 to 50 mCi of ^{32}P-labeled phosphate. The RNA labeled in the presence of 0.05 μg of actinomycin D per ml was fractionated by preparative sucrose gradient sedimentation, and only the fraction sedimenting at greater than 32S was utilized. Both ^{32}P- and ^{3}H-labeled hnRNA preparations were checked for the presence of ds regions by the conventional procedures described (31). Size analysis of hnRNA before and after various treatments was carried out on 2% polyacrylamide -0.5% agarose gels, as described by Ferrari et al. (10). The hnRNA had a specific activity of $\sim 1 \times 10^6$ cpm/μg.

$(2',5')A_n$ polymerase assay. A preparation of this enzyme free of 2'-phosphodiesterase activity (35) was obtained as previously described (21). The ribosomal pellet obtained from 10 ml of extract of HeLa cells treated for 18 h with 100 U of β-interferon per ml (23) was suspended in 0.5 ml of 0.5 M KCl; the supernatant obtained after spinning the salt-washed ribosomes at 150,000 × g was then chromatographed on DEAE-cellulose, as indicated (21). The assays for $(2',5')A_n$ synthesis contained 5 μl of enzyme preparation, 10 mM ATP, 30 mM magnesium acetate, 0.12 M potassium acetate, 3 mM fructose, 1,6-bisphosphate, and the indicated amount of RNA in a final volume of 25 μl. After 16 h at 30°C, the $(2',5')A_n$ synthesized was isolated by chromatography on DEAE-cellulose (23) and measured by competition with $(2',5')A_n$-[^{32}P]cytidine 3',5'-diphosphate for binding to a protein prepared from HeLa cells (27). This assay is a modification of that reported by Knight et al. (17) for the quantitation of $(2',5')A_n$.

Incubation and analysis of RNA. Samples of labeled hnRNA were diluted to have 20,000 to 50,000 cpm/μl. Two microliters of hnRNA solution was incubated at 30°C in a final volume of 10 μl with 0.5 or 1 μl of extract from HeLa cells treated with interferon as described above, 0.12 M potassium acetate, 4 mM magnesium acetate, 4 mM fructose, 1,6-bisphosphate, 0.1 μg of *E. coli* tRNA, and 10 mM HEPES (*N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid)-KOH buffer, pH 7.4.

The incubations were stopped by the addition of twice-concentrated electrophoresis sample buffer. Incubations containing vesicular stomatitis virus (VSV) mRNA were analyzed by chromatography on oligodeoxythymidylate-cellulose, as previously described (37). This RNA was prepared by in vitro transcription, as described elsewhere (26), and had a specific activity of 1×10^5 cpm/μg.

RESULTS

The hnRNA was prepared from HeLa cells labeled with [^{3}H]uridine or ^{32}P as described above. This hnRNA contained ds regions by the following criteria (33): (i) digestion with RNase III decreased the sedimentation of hnRNA by cleaving ds regions, since this effect could be inhibited by the addition of competitor dsRNA (Fig. 1); (ii) about 4% of the hnRNA was resistant to digestion with pancreatic RNase and RNase T_2 at high ionic strength (0.2 M NaCl); (iii) this RNA digested with RNase T_1 has the characteristic fingerprint pattern of HeLa hnRNA ds regions (31).

To assay the hnRNA for the activation of $(2',5')A_n$ polymerase, samples of the hnRNA were incubated with a partially purified preparation of this enzyme (see above). With this preparation of polymerase, the net synthesis of $(2',5')A_n$ can be detected by a competition binding assay (18, 29) with less than 0.25 ng of the synthetic dsRNA $I_n \cdot C_n$ as activator (Table 1). With hnRNA as an activator, the synthesis of a

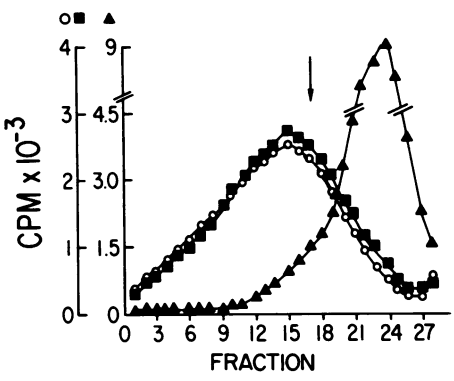

FIG. 1. Sucrose density gradient sedimentation of HeLa cell hnRNA after RNase III treatment. Isolated hnRNA was treated with RNase III for 30 min at 37°C as described (31, 32), diluted 10-fold with 0.2% sodium dodecyl sulfate, 0.01 M EDTA, and 0.01 M Tris-hydrochloride (pH 7.5), heated for 2 min at 65°C, layered onto 5-ml sucrose gradients (15 to 30% sucrose in the above buffer containing, in addition, 0.1 M NaCl), and centrifuged for 100 min at 45,000 rpm in a Beckman SW50.1 rotor. Symbols: ○, control reaction with no RNase III; ▲, reaction containing 0.6 U of RNase III; ■, reaction containing 0.6 U of RNase III and 0.5 μg of *Penicillium chrysogenum* competitor dsRNA. The arrow represents the position of 32S rRNA precursor sedimented under these conditions. The top of the gradient is at the right.

TABLE 1. Activation of the (2',5')A_n polymerase by hnRNA

Activator	Amt (ng) added	(2',5')A_n synthesized[a] (pmol)
$I_n \cdot C_n$	0.25	2,100
$I_n \cdot C_n$	0.75	10,700
hnRNA	100	75
hnRNA	20	10
hnRNA treated with RNase III	100	0
mRNA	250	0

[a] The assay for (2',5')A_n synthesis is described in the text.

compound which competed in the binding assay was detectable with 20 ng of RNA (Table 1). This binding assay is highly specific for (2',5')A_n (18, 29). In addition, we showed that the compound synthesized with hnRNA was cleaved by various nucleases like authentic (2',5')A_n (Table 2). No competition in the binding assay was obtained after digestion with phosphodiesterase or phosphatase, whereas RNase T_2 had no effect. This latter enzyme does not degrade 2',5'-oligonucleotides, whereas the other enzymes degrade (2',5')A_n (17, 32). The hnRNA digested with RNase III no longer promoted the synthesis of (2',5')A_n; mRNA in amounts up to 250 ng also failed to activate the synthesis of (2',5')A_n (Table 1).

An RNA containing ds regions is preferentially cleaved in extracts of interferon-treated cells with respect to other RNAs present in the same incubation (26). This preferential degradation is only observed when the ds regions can activate the (2',5')A_n polymerase, since it can be prevented by inhibiting the synthesis of (2',5')A_n with a variety of inhibitors (28). Since hnRNA contains ds regions, we examined whether it could be cleaved by a similar mechanism. In the following experiments, the degradation of [^{32}P]hnRNA was assayed by electrophoresis in

TABLE 2. Digestion with different nucleases of (2',5')A_n synthesized with $I_n \cdot C_n$ or hnRNA as activator

Treatment	Amt (pmol) of (2',5')A_n[a]	
	$I^n \cdot C_n$	hnRNA
Undigested	2.0	1.5
RNase T_2	1.9	1.4
Phosphodiesterase	<0.03	<0.03
Phosphatase	<0.03	<0.03

[a] A portion of the (2',5')A_n synthesized and purified by chromatography on DEAE-cellulose, as described in Table 1, was assayed by competition binding (27) before and after digestion with nucleases, under the conditions previously reported (23). The picomoles remaining after digestion are shown.

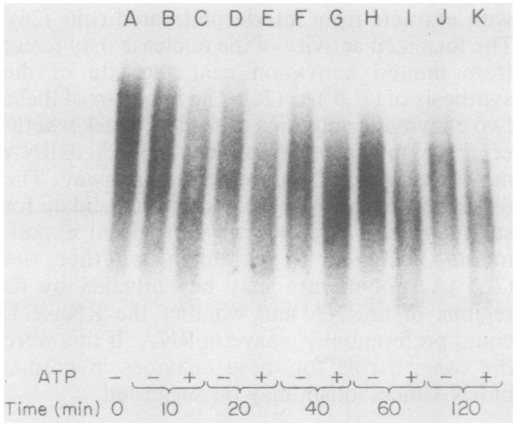

FIG. 2. Time course of degradation of hnRNA and effect of ATP addition. The [^{32}P]hnRNA was incubated under the conditions described in the text for the time indicated; 2 mM ATP was added where indicated.

polyacrylamide gels after incubation with cell extract. Undegraded hnRNA migrated as a rather diffuse band extending from the top of the gel; cleavage of the hnRNA resulted in a shift of this band toward the bottom of the gel (Fig. 2). The hnRNA was incubated with different amounts of extract prepared from interferon-treated cells, which contain a high level of (2',5')A_n polymerase. In this way we established that we could use relatively small amounts of cell extract and detect cleavage by (2',5')A_n-activated endonuclease over a background of other nuclease activities present in cell extracts (3). In assays containing 1/10 or less of the final volume of cell extract, the hnRNA was increasingly cleaved with time of incubation; addition of 2 mM ATP, the substrate for (2',5')A_n synthesis, caused a substantial increase in hnRNA degradation (Fig. 2). After 60 min of incubation, this degradation was maximal, since no further change in hnRNA migration was observed after another 60 min of incubation (Fig. 2, lanes I and K). In parallel experiments with extract obtained from cells not treated with interferon, we did not observe a comparable degradation of hnRNA (data not shown).

If the degradation of hnRNA were due to (2',5')A_n-activated endonuclease, inhibition of (2',5')A_n synthesis should prevent this degradation. The first inhibitor used, ethidium, prevents the activation of the (2',5')A_n polymerase by intercalating into ds RNA (2). When 0.2 mM ethidium was added to an incubation containing 2 mM ATP, there was little degradation of hnRNA (Fig. 3, lane C). The hnRNA was degraded even less than in incubations with no added ATP. Two other inhibitors of (2',5')A_n synthesis were used in subsequent incubations, carried out for 1 h with added ATP. The first,

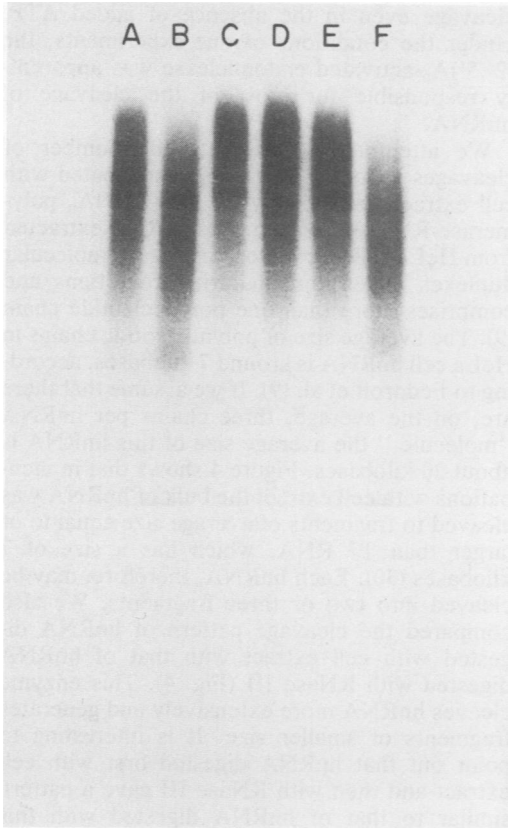

FIG. 3. Effect of inhibitors of $(2',5')A_n$ synthesis on the degradation of hnRNA. Incubations were for 1 h as described in the text, with 2 mM ATP added except in lanes A and F. Additions: lane C, 0.2 mM ethidium bromide; lane D, 2 mM EDTA; lane E, 2 mM 2'-dATP; and lane F, 0.5 μM $(2',5')A_n$.

EDTA, chelates magnesium, which is required by the $(2',5')A_n$ polymerase (22). The second, 2'-dATP, is a competitive inhibitor of ATP in the synthesis of $(2',5')A_n$ (21). Both of these compounds prevented the degradation of hnRNA (Fig. 3). The addition of 0.5 μM $(2',5')A_n$ promoted the degradation of hnRNA, as well as ATP (Fig. 3F).

Additional experiments to investigate the pattern of cleavage of hnRNA are shown in Fig. 4. A different preparation of hnRNA, of slightly greater size, was used in these experiments (Fig. 4, track A). The hnRNA was cleaved by RNase III to products migrating faster than those formed in incubations with extract of interferon-treated cells plus ATP (cf. Fig. 4, tracks B and D). In the absence of added ATP (Fig. 4, track C) or when GTP was substituted for ATP (not shown), no significant change in the electrophoretic mobility of the hnRNA was observed. The hnRNA digested with RNase III was no further degraded in a second incubation with cell extract, with or without added ATP, in agreement with the observation that RNase III digestion abolished the ability of hnRNA to promote the synthesis of $(2',5')A_n$ (Table 1). The hnRNA digested with cell extract plus or minus ATP and subsequently with RNase III gave the same pattern as hnRNA digested with RNase III alone. The hnRNA is apparently cleaved at fewer sites by RNase L than by RNase III. This latter enzyme cleaves duplexes as small as 15 base pairs (30, 32), whereas the synthesis of $(2',5')A_n$ and the activation of RNase L are promoted by longer duplexes (24). Moreover, the RNase III cleaves dsRNA itself, whereas RNase L does not (25, 28) but cleaves single-stranded RNA, yielding predominantly UpNp-terminated products (11, 38).

Under the incubation conditions of Fig. 4, little hnRNA was degraded to nucleotides. This was shown by precipitating samples of the reactions with 5% trichloroacetic acid. Less than 5%

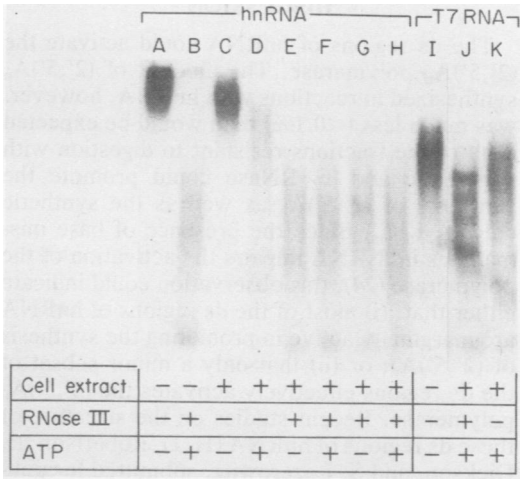

FIG. 4. Pattern of cleavage of hnRNA and phage T7 RNA by cell extract and RNase III. The RNAs were digested with cell extract or RNase III (indicated by a +) or both, with 2 mM ATP added where indicated. All of the samples were incubated for 90 min at 30°C. Lanes A and I were incubated with buffer alone. Lanes E and F were incubated 30 min with RNase III, and then cell extract was added for 60 min; lanes G, H, and J were incubated 60 min with cell extract, and then RNase III was added for 30 min. The incubations contained about 12,000 cpm of either ^{32}P-labeled hnRNA or T7 RNA, prepared as previously described (32). Lanes A, C, and D contained, in addition 6,000 cpm of [^3H]mRNA from VSV; one half of these incubations were analyzed by chromatography on oligodeoxythymidylate-cellulose; 96 and 92% of the input VSV mRNA was bound to oligodeoxythymidylate in lanes C and D, relative to 100% in lane A. The ratio of VSV mRNA to hnRNA in these incubations was 5:1.

of the counts were soluble (data not shown). As a control for the activity of RNase III in the presence of HeLa cell extract, a sample of ^{32}P-labeled T7 RNA was digested under the same conditions employed for hnRNA. The expected products of T7 RNA processing (32) were obtained (Fig. 4). When the T7 RNA was incubated with cell extract alone, little degradation was observed. The (2',5')A_n polymerase-RNase L system in the cell extract is apparently not activated by the short and imperfectly base-paired regions of T7 RNA (30, 32).

To establish whether the degradation of hnRNA was localized, we included in some incubations poly(A)-containing VSV mRNA labeled with [^{3}H]uridine. Half of each incubation was then analyzed by gel electrophoresis, whereas the other half was analyzed by oligodeoxythymidylate-cellulose chromatography (37). This chromatographic analysis showed that VSV mRNA was not degraded in incubations where hnRNA was cleaved (see the legend to Fig. 4).

DISCUSSION

The ds regions of hnRNA could activate the (2',5')A_n polymerase. The amount of (2',5')A_n synthesized in reactions with hnRNA, however, was much less (<0.1%) than would be expected if all of the fractions resistant to digestion with pancreatic and T_2 RNase could promote the synthesis of (2',5')A_n as well as the synthetic dsRNA $I_n \cdot C_n$. Since the presence of base mismatches in dsRNA impairs the activation of the polymerase (24), this observation could indicate either that: (i) most of the ds regions of hnRNA are marginally active in promoting the synthesis of (2',5')A_n; or (ii) that only a minor subset of the ds regions effectively activates the (2',5')A_n polymerase. Recent studies on the structure of these ds regions of hnRNA (H. D. Robertson, E. Dickson, and S. Lazarowitz, submitted for publication) and their DNA templates (34) suggest that most of them may well contain 10 to 15% mismatched bases, a finding that would favor option (i) above.

The hnRNA incubated with cell extract containing high levels of (2',5')A_n polymerase activity (extract of interferon-treated cells) was cleaved to a limited extent. This cleavage was enhanced by the addition of ATP and prevented by inhibitors of the synthesis of (2',5')A_n. With all of the inhibitors tested, less cleavage of hnRNA was observed than in incubations without added ATP. This suggests that at least some cleavage of hnRNA in the latter incubations may be due to (2',5')A_n synthesized from ATP present in the cell extracts and to endonuclease activation. This would explain why inhibitors of the synthesis of (2',5')A_n prevented hnRNA cleavage even in the absence of added ATP. Under the conditions of our experiments, the (2',5')A_n-activated endonuclease was apparently responsible for most of the cleavage of hnRNA.

We attempted to estimate the number of cleavages introduced in hnRNA incubated with cell extract, presumably by the (2',5')A_n polymerase-RNase L system. The hnRNA extracted from HeLa cells, however, forms intermolecular duplexes under nondenaturing conditions and comprises more than one polynucleotide chain (9). The average size of polynucleotide chains in HeLa cell hnRNA is around 7 kilobases, according to Fedoroff et al. (9). If we assume that there are, on the average, three chains per hnRNA "molecule," the average size of this hnRNA is about 20 kilobases. Figure 4 shows that in incubations with cell extract the bulk of hnRNA was cleaved to fragments of average size equal to or larger than T7 RNA, which has a size of 7 kilobases (30). Each hnRNA, therefore, may be cleaved into two or three fragments. We also compared the cleavage pattern of hnRNA digested with cell extract with that of hnRNA digested with RNase III (Fig. 4). This enzyme cleaves hnRNA more extensively and generates fragments of smaller size. It is interesting to point out that hnRNA digested first with cell extract and then with RNase III gave a pattern similar to that of hnRNA digested with this enzyme alone. This suggests that some corresponding regions of hnRNA were cleaved by both treatments, within duplexes by RNase III and near some duplexes by RNase L. This latter enzyme is presumably activated only near those duplexes that can promote synthesis of (2',5')A_n.

In incubations containing hnRNA and mRNA labeled with a different isotope, only the hnRNA was degraded. This result and the effect of inhibitors of (2',5')A_n synthesis indicate that the limited cleavage of hnRNA observed results from the activation of the (2',5')A_n polymerase by ds regions of hnRNA and localized activation of the (2',5')A_n-dependent endonuclease. Furthermore, these results show that a cellular RNA can promote synthesis of (2',5')A_n and suggest a possible role for the constitutive levels of both (2',5')A_n polymerase and endonuclease present in cells not treated with interferon.

Before we can fully interpret the significance of the findings reported here, it is necessary to consider what kind of experiments will yield useful information when using a substrate as complex and heterogeneous as hnRNA from HeLa cells. It has been estimated that the total complexity of hnRNA transcripts approaches 10^8 nucleotides (36). Therefore, it is clear that only studies which address a general property of

hnRNA, e.g., the presence of polyadenylate or a 5'-terminal cap structures (20), will yield information which can be meaningfully interpreted. The various studies on the ds regions of hnRNA reviewed in the introduction make it clear that dsRNA is present in most or all hnRNA species. Therefore, it made sense to consider their cleavage by RNase III in previous studies (30). Likewise, the finding that most or all hnRNA molecules undergo a significant size shift when incubated with cell extract under conditions which promote synthesis of $(2',5')A_n$ suggests that we are studying a property of hnRNA which could have general significance for nuclear RNA metabolism.

Several investigators have speculated that ds regions of hnRNA could serve as a signal for processing. Here we report that these ds regions can activate an enzyme which, in turn, forms an activator for a specific endonuclease. The significance of this observation remains to be determined. Preliminary results of experiments in progress indicate that most of the $(2',5')A_n$ polymerase is present in the nucleus of cells untreated with interferon; moreover, a significant amount of $(2',5')A_n$-activated endonuclease is present in the nuclei of HeLa cells (T. W. Nilsen, D. L. Wood, and C. Baglioni, J. Biol. Chem., in press).

The metabolism of any population of RNA molecules as complex as hnRNA must be carried out in a series of steps, including several which involve cleavage of the RNA chains. In light of the results reported here, it is possible that one function of the $(2',5')A_n$ polymerase and $(2',5')A_n$-activated endonuclease in normal cells is the cleavage of hnRNA.

ACKNOWLEDGMENTS

This work was supported by Public Health Service grants AI-16076 and CA-29895 to C.B., GM-28294 and GM-27168 to H.D.R.

We thank Kerry Willis for superb technical assistance.

LITERATURE CITED

1. **Baglioni, C.** 1979. Interferon induced enzymatic activities and their role in the antiviral state. Cell **17**:255–264.
2. **Baglioni, C., and P. A. Maroney.** 1981. Inhibition of double-stranded RNA activated protein kinase and 2'5'-oligo(A) polymerase by ethidium bromide. Biochemistry **20**:758–765.
3. **Baglioni, C., M. A. Minks, and P. A. Maroney.** 1978. Interferon action may be mediated by activation of a nuclease by pppA2'p5'A2'p5'A. Nature (London) **273**:684–687.
4. **Brinker, S. M., H. P. Madore, and L. S. Bello.** 1973. Stabilization of heterogeneous nuclear RNA by intercalating drugs. Biochem. Biophys. Res. Commun. **52**:928–934.
5. **Calvet, J., and T. Pederson.** 1977. Secondary structure of hnRNA: two classes of double-stranded RNA in native RNP. Proc. Natl. Acad. Sci. U.S.A. **74**:3705–3709.
6. **Calvet, J., and T. Pederson.** 1978. Nucleoprotein organization of inverted repeat DNA transcripts in hnRNA-ribonucleoprotein particles from HeLa cells. J. Mol. Biol. **122**:361–378.
7. **Calvet, J., and T. Pederson.** 1979. Heterogeneous nuclear RNA double-stranded regions probed in living HeLa cells by crosslinking with the psoralen derivative aminomethyltrioxalen. Proc. Nalt. Acad. Sci. U.S.A. **76**:755–759.
8. **Chiu, N. H., W. B. Bruszewski, and N. Salzman.** 1980. Evidence for the role of double-helical structures in the maturation of simian virus-40 messenger RNA. Nucleic Acid Res. **8**:153–168.
9. **Fedoroff, N., P. Wellauer, and K. Wall.** 1977. Intermolecular duplexes in heterogeneous nuclear RNA from HeLa cells. Cell **10**:597–610.
10. **Ferrari, S., C. Yehle, H. D. Robertson, and E. Dickson.** 1980. Specific RNA-cleaving activities from HeLa cells. Proc. Natl. Acad. Sci. U.S.A. **77**:2395–2399.
11. **Floyd-Smith, G., E. Slattery, and P. Lengyel.** 1981. Interferon action: RNA cleavage pattern of a (2'-5')oligoadenylate-dependent endonuclease. Science **212**:1030–1032.
12. **Harel, L., and L. Montagnier.** 1971. Homology of double-stranded RNA from rat liver cells with the cellular genome. Nature (London) New Biol. **229**:106–108.
13. **Isaacs, S. T., C. S. Shen, J. E. Hearst, and H. Rapoport.** 1977. Synthesis and characterization of new psoralen derivatives with superior photoreactivity with DNA and RNA. Biochemistry **16**:1058–1064.
14. **Jelinek, W., and J. E. Darnell.** 1972. Double-stranded regions on hnRNA from HeLa cells. Proc. Natl. Acad. Sci. U.S.A. **69**:2537–2541.
15. **Jelinek, W., G. Molloy, R. Fernandez-Munoz, M. Salditt, and J. E. Darnell.** 1974. Secondary structure in hnRNA: involvement of regions from repeated DNA sites. J. Mol. Biol. **82**:361–370.
16. **Kerr, I. M., and R. E. Brown.** 1978. pppA2'p5'A2'p5'A: an inhibitor of protein synthesis synthesized with an enzyme fraction from interferon-treated cells. Proc. Natl. Acad. Sci. U.S.A. **75**:256–260.
17. **Knight, M., P. J. Cayley, R. H. Silverman, D. H. Wreschner, C. S. Gilbert, R. E. Brown, and I. M. Kerr.** 1980. Radioimmune, radiobinding and HPLC analysis of 2-5A and related oligonucleotides from intact cells. Nature (London) **288**:189–192.
18. **Krohenberg, L. H., and T. Humphreys.** 1972. Double-stranded ribonucleic acid in sea urchin embryos. Biochemistry **11**:2020–2026.
19. **LePecq, J. B., and C. Paoletti.** 1976. A fluorescent complex between ethidium bromide and nucleic acids. J. Mol. Biol. **27**:87–106.
20. **Lewin, B.** 1980. Gene expression, vol. 2. John Wiley & Sons, Inc., New York.
21. **Minks, M. A., S. Benvin, and C. Baglioni.** 1980. Mechanism of $pppA(2'p5'A)_n2'p5'A_{OH}$ synthesis in extracts of interferon-treated HeLa cells. J. Biol. Chem. **255**:5031–5035.
22. **Minks, M. A., S. Benvin, P. A. Maroney, and C. Baglioni.** 1979. Metabolic stability of 2'5'oligo(A) and activity of 2'5'oligo(A)-dependent endonuclease in extracts of control and interferon-treated HeLa cells. Nucleic Acid Res. **6**:767–780.
23. **Minks, M. A., S. Benvin, P. A. Maroney, and C. Baglioni.** 1979. Synthesis of 2',5'-oligo(A) in extracts of interferon-treated HeLa cells. J. Biol. Chem. **254**:5058–5064.
24. **Minks, M. A., D. K. West, S. Benvin, and C. Baglioni.** 1979. Structural requirements of double-stranded RNA for the activation of 2'5'-oligo(A) polymerase and protein kinase of interferon-treated HeLa cells. J. Biol. Chem. **254**:10180–10183.
25. **Montagnier, L.** 1968. Présence d'un acide ribonucléique en double chaîne dans des cellules animales. C. R. Acad. Sci. Ser. D **267**:1417–1421.
26. **Nilsen, T. W., and C. Baglioni.** 1979. A mechanism for discrimination between viral and host mRNA in interferon-treated cells. Proc. Natl. Acad. Sci. U.S.A. **76**:2600–2604.
27. **Nilsen, T. W., P. Maroney, and C. Baglioni.** 1981. Double-

stranded RNA causes synthesis of 2'5'-oligo(A) and degradation of messenger RNA in interferon-treated cells. J. Biol. Chem. **256**:7806–7812.

28. **Nilsen, T. W., S. G. Weissman, and C. Baglioni.** 1980. Role of 2'5'-oligo(A) polymerase in the degradation of RNA linked to double-stranded RNA by extracts of interferon-treated cells. Biochemistry **19**:5574–5579.
29. **Paoletti, J., J. Rech, C. Brunel, and P. Jeanteur.** 1980. Constrained configuration of double-stranded ribonucleic acid in HeLa hnRNP and its relaxation by ribonuclease D. Biochemistry **19**:5223–5228.
30. **Robertson, H. D., and E. Dickson.** 1974. RNA processing and the control of gene expression. Brookhaven Symp. Biol. **26**:240–266.
31. **Robertson, H. D., E. Dickson, and W. Jelinek.** 1977. Determination of nucleotide sequences from double-stranded regions of HeLa cell nuclear RNA. J. Mol. Biol. **115**:571–589.
32. **Robertson, H. D., and J. J. Dunn.** 1975. Ribonucleic acid processing activity of *E. coli* ribonuclease III. J. Biol. Chem. **250**:3050–3056.
33. **Robertson, H. D., and T. Hunter.** 1975. Sensitive methods for the detection and characterization of double helical ribonucleic acid. J. Biol. Chem. **250**:418–427.
34. **Rubin, C. M., C. M. Houk, P. L. Deininger, T. Friedman, and C.W. Schmid.** 1980. Partial nucleotide sequence of the 300-nucleotide interspersed repeated human DNA sequences. Nature (London) **284**:372–374.
35. **Schmidt, A., A. Ziberstein, L. Shulman, P. Federman, H. Berissi, and M. Revel.** 1978. Interferon action: isolation of nuclease F, a translation inhibitor activated by interferon-induced (2'-5')oligo-isoadenylate. FEBS Lett. **95**:257–264.
36. **Spradling, A., S. Penman, M. S. Campo, and J. O. Bishop.** 1974. Repetitious and unique sequences in heterogeneous nuclear and cytoplasmic RNA of mammalian and insect cells. Cell **3**:23–30.
37. **Weber, L. A., M. Simili, and C. Baglioni.** 1979. Binding of viral and cellular messenger RNA to ribosomes in eukaryotic cell extracts. Methods Enzymol. **60**:351–360.
38. **Wreschner, D. H., J. W. McCauley, J. J. Skehel, and I. M. Kerr.** 1981. Interferon action-sequence specificity of the $ppp(A2'p)_nA$-dependent ribonuclease. Nature (London) **289**:414–417.
39. **Yannarell, A., M. Niemann, D. E. Schumm, and T. E. Webb.** 1977. Proflavin sensitivity of RNA processing in isolated nuclei. Nucleic Acid Res. **4**:503–511.