The Isolation and Characterization of HeLa Cell Messenger RNA-like Molecules Containing Uridylic Acid-rich Oligonucleotide Sequences*

(Received for publication, April 1, 1980, and in revised form, July 28, 1980)

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HeLa cell mRNA, isolated from mRNPs released from polysomes by EDTA to minimize contamination from nuclear RNA, has been separated into four classes of molecules: 1) those containing poly(A) sequences [poly(A')] as well as oligo(U) sequences (oligo(U')) (4 to 13%); 2) poly(A') oligo(U') (~52%); 3) poly(A') oligo(U') (~2%); 4) poly(A') oligo(U') (~33%). The oligo(U) segments are 89% UMP and range from 20 to 50 nucleotides in length. The poly(A') oligo(U') mRNAs appear to contain the oligo(U) in a region with secondary structure (possibly an intramolecular duplex with the 3'-poly(A)) since they did not bind to poly(A) Sepharose without prior HCHO modification. HCHO modifies the exocyclic amino groups of CMP, GMP, and AMP and prevents hydrogen bonding but reacts only slightly with UMP. After removal of the excess HCHO, the oligo(U) of the mRNA was free to bind to the poly(A) Sepharose. Control experiments indicated that the binding of oligo(U') mRNA to poly(A) Sepharose was specific and the HCHO did not cause cross-linking of the RNAs. The poly(A') oligo(U') RNAs appeared to contain one oligo(U)/molecule and could re-bind to poly(A) Sepharose with 31 to 75% efficiency. The poly(A') oligo(U') mRNA class was more heterogeneous in size and slightly larger (~3 kb average length) than the other mRNA classes.

Previous experiments have described the isolation of uridylic acid-rich oligonucleotide sequences (80% UMP and 20 to 50 nucleotides [ntds] long) from HeLa cell heterogeneous nuclear RNA (hnRNA) by the use of T1 RNase digestion (specific for GMP residues) and poly(A) Sepharose (1). These studies also reported that the molar concentration of oligo(U) increased with the size of the hnRNA. hnRNA ~30 kilobases (kb) long contained, on the average, one oligo(U) while only 1 in 12 hnRNAs ~5 kb long contained one oligo(U) and only 1 in a 100 cytoplasmic messenger RNAs (mRNAs) ~2 kb long contained one oligo(U). This suggested that if the oligo(U) regions were present in mRNA precursors, they were destroyed in the nucleus as the mRNA sequences were processed and transported to the cytoplasm (1). However, in these experiments, when mRNA was assayed for oligo(U) care was not taken to disrupt any hybrids that might have formed between the 3'-poly(A) and oligo(U) and rendered the oligo(U) inaccessible to poly(A) Sepharose. Subsequent experiments which took this precaution (by heating the T1 RNase digest to 65°C) indicated that poly(A') hnRNA 20 kb long contained ~2 oligo(U) regions located within ~8 kb of the 5'-terminus but mRNA was not reassayed (3). Recent experiments by Edmonds and co-workers have reported that ~20% of HeLa mRNA can be shown to contain oligo(U) sequences but only when caution is taken to add excess synthetic poly(U) to the T1 RNase digested mRNA to prevent hybrids from forming between the oligo(U) and the 3'-poly(A) (3).

This paper reports that 10 to 20% of the poly(A') mRNAs can be retained specifically on columns of poly(A) Sepharose but only when the mRNAs were treated with HCHO prior to chromatography. The retained mRNAs were shown to contain oligo(U) segments (89% UMP, 20 to 50 ntds long) by a procedure which employed T1 RNase digestion followed by HCHO treatment and poly(A) Sepharose. HCHO reacts primarily with the exocyclic amino groups of AMP, GMP, and CMP (forming methylol derivatives which are stable up to 80°C) and prevents hydrogen bonding but reacts only slightly with UMP (4, 5). Consequently, as these experiments with mRNA demonstrated, HCHO modified the 3'-poly(A) and prevented it from hydrogen bonding with the oligo(U). After Edmonds and co-workers have reported that ~20% of HeLa mRNA can be shown to contain oligo(U) sequences but only when caution is taken to add excess synthetic poly(U) to the T1 RNase digested mRNA to prevent hybrids from forming between the oligo(U) and the 3'-poly(A) (3).

This research was supported by grants from the National Cancer Institute (ROI CA 19530), the Pardee Foundation for Cancer Research, and the University of Delaware Research Foundation. The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked "advertisement" in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

The abbreviations used are: ntds, nucleotides; hnRNA, heterogeneous nuclear RNA; mRNA, messenger RNA; kb, kilobases; poly(A'), or (A'), poly(A)-containing; oligo(U') or (U'), oligo(U)-containing; RNP, ribonucleoprotein particle.

MATERIALS AND METHODS

The miniprint supplement contains all experimental details.

RESULTS

Isolation of mRNAs that Contain Oligo(U) Sequences—It was necessary to employ a procedure that would yield mRNA free of contamination by hnRNA since hnRNA contains ~75% of the cellular oligo(U) (1, 3). Therefore, polysomes were isolated (Fig. 1A) and adjusted to 10 mM EDTA to release the

3 Portions of this paper (including "Materials and Methods," Figs. 1 to 3, Tables 1 to VI, and additional references) are presented in miniprint at the end of this paper. Miniprint is easily read with the aid of a standard magnifying glass. Full size copies are available from the Journal of Biological Chemistry, 9650 Rockville Pike, Bethesda, Md. 20014. Request Document No. 80M-641, cite author, and include a check or money order for $2.55 per set of photocopies. Full size photocopies are also included in the microfilm edition of the Journal that is available from Waverly Press.
message ribonucleoprotein particles (mRNPs) and the mRNPs between 30 S and 70 S were isolated on subsequent sucrose gradients (Fig. 1B). Since the sedimentation value of the RNPs is not affected by EDTA, any hRNPs which may have co-sedimented with the polysomes would resediment as RNPs ≥100 S and should not contaminate the mRNP (8–10). Fig. 1B shows there were very few RNPs >70 S in size. Only RNA purified from the 30 S to 70 S mRNPs will be referred to as mRNA. Also, Korwek et al. (3) showed that ~80% of the oligo(U) tracts in HeLa polysomal RNA were released from polysomes by EDTA.

Table I shows it was necessary to react poly(A') mRNA with HCHO to get maximal binding to poly(A) Sepharose at 4°C. Seventeen to eighteen percent of the HCHO-treated mRNA bound to the Sepharose and was eluted with formamide elution buffer (FEB) (Samples 2, 6, and 11), whereas, only 2 to 4% of the untreated mRNA was bound (Samples 1, 5, and 10). HCHO treatment of synthetic poly(U) did not affect its binding to poly(A) Sepharose. The poly(A') mRNAs (molecules that did not bind to poly(U) Sepharose) were not as dependent on HCHO treatment for maximum treatment as were poly(U) molecules bound to poly(A) Sepharose with or without prior HCHO treatment. In some experiments, as much as 16% of the (A') mRNAs bound (Sample 7). Subsequent isolations of (A') mRNAs did not employ HCHO. HCHO treatment of the (A') mRNA resulted in the formation of an insoluble residue which is thought to be due to the high concentrations of unlabeled RNA present (see “Materials and Methods”). Binding to poly(A) Sepharose is not solely a characteristic of poly(A') RNA that had entered polysomes since 22 to 24% of the poly(A') RNA isolated from cytoplasmic particles ≥80 S in size was retained (Samples 8 and 9).

Specificity of Binding to Poly(A) Sepharose—The observation that the binding of poly(A') mRNA to poly(A) Sepharose was enhanced 5- to 8-fold after HCHO treatment suggested that these mRNAs contained intramolecular oligo(U):poly(A) duplex regions and that the oligo(U) sequences were only free to bind to the Sepharose after HCHO had modified the 5'-terminal poly(A). To test this possibility, it was necessary to establish that (a) RNAs known to contain oligo(U) sequences hydrogen-bonded to poly(A) sequences did not bind to poly(A) Sepharose without prior HCHO treatment, and (b) HCHO treatment did not cause oligo(U') RNAs to bind to poly(A) Sepharose.

Poliovirus replicative form (RF) RNA was employed because it is considered to be a completely double-stranded, intermolecular RNA duplex containing the 35 S positive strand RNA with a 3'-terminal poly(A) sequence and the negative strand RNA with a 5'-terminal poly(U) sequence (11–13). If the 5'-poly(U) is devoid of extensive unpaired regions, the RF should not bind to poly(A) Sepharose without HCHO treatment but ~50% of the RNA (the negative strand) should bind after HCHO treatment, provided the two RNA strands contain equal amounts of label. Table II shows that only 2% of the RF bound without HCHO treatment but that 41% bound after HCHO treatment (Samples 1 and 2). This result also strongly suggests that HCHO did not cause extensive cross-linking of the two RNA strands because this would have caused much more than 41% of the RNA to bind. When the bound RNA was analyzed on acrylamide-formamide gels (14), it migrated predominantly as a discrete RNA 35 S in size as expected of the negative strand RNA (data not shown). Also, Kwan et al. (15) reported that HCHO treatment of hemoglobin mRNA caused no detectable cross-linking of the molecules. The present results were substantiated through binding studies with poliovirus replicate intermediate (RI), and RNA complex consisting of the 35 S negative RNA strand and several associated positive RNA strands, some of which contain poly(A) (12, 16). Without HCHO treatment, only 5% of the RI bound but after HCHO 37% of the RI was retained.

The data in Table II also indicate that RNAs lacking poly(U) sequences did not bind to poly(A) Sepharose, even after HCHO treatment. Without HCHO treatment only 1% of the RI bound but after HCHO 27% of the RI was retained. The binding of cytoplasmic poliovirus 35 S positive strand RNA and poly(G) was 1% without HCHO treatment and this increased to only 4% after HCHO (Samples 11 to 13).

Characterization of the Oligo(U) Tracts in the mRNAs which Bound to Poly(A) Sepharose—Poly(A') mRNAs were treated with HCHO and chromatographed on poly(A) Sepharose possibly to separate mRNAs with short oligo(U) tracts (<20 ntds), which may represent adjacent phenylalanine (Phe) codons, from mRNAs with longer oligo(U) tracts which may have a regulatory function. The mRNAs were applied to Sepharose at 4°C and the oligo(U') mRNAs were removed at 2°C along with the poly(U) Sepharose. The oligo(U') column was then equilibrated with NEPSark buffer (0.1 M NaCl), adjusted to 25°C, and washed with NEPSark. This removed one-half to two-thirds of the bound mRNAs, which were designated as the “FEB” (A') (U') mRNAs. Chromatography of the poly(A') mRNAs and the poly(A') RNAs isolated from subpolysomal particles yielded similar results. The oligo(U) tracts in the 0.1 M and FEB (A') (U') mRNAs were isolated by digesting the mRNAs with T1 and U3 RNase, which should eliminate any (unmethylated) purine ntds located internally within the oligo(U). Fig. 2A shows that the oligo(U) tracts in the FEB (A') (U') mRNAs ranged from 20 to 50 ntds with an average length of 27 ntds and contained 89% UMP and ~4% purines. The same oligo(U) was produced by T1 RNase digestion alone had an average length of 33 ntds and contained 83% UMP and ~10% purines. Similar results were obtained by Korwek et al. (3) on T1 RNase-derived oligo(U) tracts from total cytoplasmic RNA. As expected, the oligo(U) tracts in the 0.1 M (A') (U') mRNAs were shorter (average length of 19 ntds) and contained 90% UMP and ~3% purines (Fig. 2B). These results are consistent with the finding that synthetic poly(U) fragments which were ~33 ntds long could not be removed from poly(A) Sepharose with NEPSark buffer but were eluted with FEB (data not shown). Fig. 2B also shows the migration of the VSV “leader” RNA (which is 48 ntds long) and illustrates the resolution on 15% acrylamide, 6 M urea gels and the heterogeneity of the oligo(U) tracts.

Table IV indicates that the (A') (U') mRNAs contained one oligo(U) tract per molecule and that most of the oligo(U') mRNAs were bound to the poly(A) Sepharose. Ideally, a mRNA 2 to 3 kb long containing one oligo(U) tract would have ~1.5 to 1% of its ntds (30/2,000 to 30/3,000) in the oligo(U) tract. The FEB (A') (U') mRNAs contained ~1.6% of their ntds in the oligo(U) (Sample 1) while the 0.1 M (A') (U') mRNAs had ~0.6% of the ntds in the oligo(U) tract (Sample 2). Undoubtedly, the latter per cent is lower than expected because of the difficulty in recovering short oligo(U) tracts from the FEB by ethanol precipitation. The (A') (U') mRNAs contained only 0.03% of their ntds as oligo(U) (Sample 3). Although the (A') (U') mRNA comprised only 22% of the total poly(A') mRNA, it contained ~87% of the oligo(U) tracts (Column 2).

The (A') (U') mRNAs isolated on poly(A)-Sepharose at 4°C (Procedure A) had ~ one oligo(U) tract per molecule
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(Samples 4 and 6) and contained 57 to 68% of the total oligo(U) present in the poly(A') mRNAs. Because the oligo(U) tracts from Samples 4 and 5 were isolated at 4°C, they were less enriched in UMP (72% UMP; see “Materials and Methods”).

In these experiments, ~65% of the total mRNA was found to be poly(A'). Based on the per cent of mRNAs which were oligo(U') (Tables I and III), these results have separated HeLa mRNAs into four classes of molecules: 1) (A') (U') (4 to 13%); 2) (A') (U') (39%); 3) (A') (U') (39%); and 4) (A') (U') (33%)

Consistent with these results, total mRNA contained 0.1 to 0.2% of its ntds in oligo(U) tracts which were isolated at 25°C (Table IV, Samples 8 and 9). The conditions used to isolate the poly(A') mRNAs minimized the possibility that the oligo(U) segments were really present in poly(A') mRNAs that had formed intermolecular duplexes with the poly(A) of other mRNAs. The mRNAs were heated to 65°C in low salt buffer prior to binding to poly(U) Sepharose. Any intermolecular duplexes which resisted this treatment should have been disrupted by the formamide gradient and eluted from the column prior to elution of poly(A') mRNA.

Size of the Four Classes of mRNA and the Ability of Oligo(U') RNAs to Rebind to Poly(A) Sepharose—Fig. 3 shows the size of the four mRNA classes as determined on HCHO-sucrose gradients. The oligo(U') mRNAs were selected to contain oligo(U) tracts >20 ntds (see Table III). The (A') (U') mRNAs were heterogeneous in length and were enriched with larger mRNAs so that the average size was ~3 kb (Fig. 3B). The other three classes of mRNA were also heterogeneous but with an average size of ~2 kb.

The oligo(U') RNAs containing oligo(U) tracts >20 ntds were tested for their ability to rebind to poly(A) Sepharose in 0.1 M NaCl buffer at 25°C. The FEB oligo(U') mRNAs rebound with 31 to 46% efficiency (Table V, Samples 1 and 2). The oligo(U') RNAs from subpolysomal particles rebound with 55 to 75% efficiency (Sample 3). Therefore, the (U') mRNAs may be bound to poly(A) Sepharose several times to enhance purification. However, it was necessary to re-treat the RNAs with HCHO to obtain maximal re-binding. Only 2 to 8% of the 0.1 M (A') (U') mRNA rebound to poly(A) Sepharose (data not shown) which, undoubtably, was due to their short oligo(U) tracts.

Correlation between the Ability of an RNA to Bind to Poly(A) Sepharose and the Modification of its 3'-poly(A) by HCHO—Table VI indicates that an RNA molecule containing both a poly(A) and an oligo(U) sequence will not bind to poly(A) Sepharose until HCHO modification has rendered the 3'-poly(A) incapable of hydrogen bonding. Poly(A') cytoplasmic RNA was first treated with HCHO for 10 min at either 37°C or 65°C and then tested for its ability to bind to poly(U) and poly(A) Sepharose. Poly(A') RNA treated with HCHO at 37°C re-bound to poly(U) Sepharose almost completely (88%, Sample 1) while only 2% bound to poly(A) Sepharose (Sample 6). The same RNA treated at 65°C completely lost its ability to rebind to poly(U) Sepharose (Sample 2) but 19% of the RNA was now bound to poly(A) Sepharose (Sample 7).

Table VI also shows that the retention or loss of the ability of synthetic poly(A) to participate in hydrogen bonding after treatment with HCHO for 10 min at 37°C or 65°C is similar to that observed with cytoplasmic poly(A') RNA (Samples 3 and 5). Exposure to HCHO for 30 min at 37°C reduced the binding of poly(A) to poly(U) Sepharose by 67%, suggesting that it may be possible to isolate the (A') (U') mRNAs after less extensive HCHO modification (Sample 4). Treatment of the poly(A') RNA with (CH₂)₂SO₄, a reversible denaturant, only resulted in the binding of 2% of the RNA to poly(A) Sepharose (Sample 8).

The amount of mRNA which was nonspecifically bound to poly(A) Sepharose that was treated with ethanolamine and stored as a powder (see “Materials and Methods”) was usually ~1% and less (Tables III and V). The higher amounts of nonspecifically bound mRNA in Tables I, II, and VI resulted from using poly(A) Sepharose that was not treated with ethanolamine and stored at 4°C for longer than 3 weeks.

Finally, while the oligo(U') polysomal RNAs were released from polysomes by EDTA, as expected of mRNAs, we are investigating their translational capacity (17), metabolic stability (18), and buoyant density of their mRNPs (8, 9) to verify that they are mRNAs.

Discussion

HeLa cell mRNA has been separated into four classes of molecules by the use of poly(U) Sepharose, HCHO modification, and poly(A) Sepharose: 1) poly(A') oligo(U') (4 to 13%); 2) poly(A') oligo(U') (39%); 3) poly(A') oligo(U') (39%); and 4) poly(A') oligo(U') (33%). These percentages may not be completely definitive and could be affected by experimental fragmentation of the mRNA. However, significant amounts of fragments of mRNA containing either poly(A) or oligo(U) are not apparent in these experiments (Fig. 3). Since the (A') (U') mRNAs did not bind to poly(A) Sepharose without prior HCHO modification, they appear to contain the oligo(U) in a region with secondary structure and possibly in an intramolecular duplex with the 3'-poly(A).

Analysis of the mRNAs on denaturing HCHO-sucrose gradients revealed that the (A') (U') mRNAs were heterogeneous in length and enriched with larger mRNAs so that the average size was ~3 kb (Fig. 3B). The other three classes of mRNA were also heterogeneous with an average size of ~2 kb (Fig. 3). These results are in agreement with previous analyses of HeLa poly(A') and poly(A) mRNA (10, 19) and are consistent with the fact that HeLa cells contain ~3 x 10⁶ different mRNAs (20). Our finding that ~65% of total mRNA was poly(A') mRNA was slightly less than the amount (~70%) reported by Milcarek et al. (10). This was probably because we selected for mRNAs with poly(A) tracts ~100 to 150 ntds in length while Milcarek et al. (10), using oligo(dT)cellulose, would have isolated all mRNAs with poly(A) tracts ≥20 ntds. It is possible that some of our poly(A') oligo(U') mRNAs contained short poly(A) tracts (<30 ntds) that were hydrogen-bonded to the oligo(U) and, therefore, could not bind to poly(U) Sepharose.

The (A') (U') mRNAs containing short oligo(U) tracts (<20 ntds and 90% UMP) have been separated from mRNAs whose oligo(U) tracts are significantly longer (20 to 50 ntds with an average size of 27 ntds) and a composition of C_U₉G, Fig. 2). The short oligo(U) tracts may represent adjacent Phe codons. However, the longer oligo(U) sequences would appear to be too long to function as Phe codons, although this possibility cannot be excluded. Depending on the location of the CMP residues, the minimum number of consecutive Phe codons would be two, with each two being adjacent to a serine codon and two additional sets of two Phe codons (i.e. U_U₉UCU₁UGC₉U). The maximum would be eight consecutive phenylalanine codons (CCU₉G); this number would be even greater for the longer oligo(U)Us. This many consecutive Phe residues is rarely observed in proteins (21). Future experiments in which oligo(U') mRNA will be isolated to preserve its template activity for in vitro translation (which is modified by HCHO (22)) may clarify the function of oligo(U).

It is possible that the oligo(U) sequences may affect the rate of translation or stability of the oligo(U') mRNAs. Oligo(U) has been localized in the 20 kb long poly(A') hnRNA and is situated within ~8 kb of the 5'-end (2). Some of the 20
kb poly(A)+ oligo(U+) hnRNA may give rise to the poly(A)+ oligo(U+) mRNAs by a pathway which "splices out" intervening sequences (23) and preserves the 3'-poly(A) and one of the oligo(U)s. These mRNAs may contain the oligo(U) close to the 5'-terminus and be arranged in a circular configuration possible stabilized by protein(s). Previous experiments with mouse sarcoma (24) and HeLa cells (25) reported that after polysomes were digested with RNase A (specific for pyrimidines), UMP-rich fragments of mRNA were protected via their hydrogen bonding to the 3'-poly(A). Although the fragments were not established to be oligo(U) sequences, it was suggested that a poly(A):oligo(U) duplex was maintained, possibly through the action of poly(A)-binding protein(s) (26). Kozak (30) has shown recently that covalently closed circular, synthetic mRNAs containing an AUG must recognize a free 5'-terminus (capped or uncapped) of an mRNA. Ilan and Ilan (31) have described a protein associated with myosin and preserves the 3'-poly(A) and one of the oligo(U)s. These mRNAs may contain the oligo(U) close to the 5'-terminus and be arranged in a circular configuration in cell cytoplasmic RNA (29) are in a circular configuration. However, for Sindbis RNA the circular form is not thought to be maintained by a poly(A):oligo(U) duplex (28).

The rate of ribosome binding to the poly(A)+ oligo(U+) mRNAs during initiation may depend upon whether these mRNAs are linear or maintained in a circle via the poly(A)-binding protein(s). Kozak (30) has shown that covalently closed circular, synthetic mRNAs containing an AUG initiator will bind to Escherichia coli ribosomes but not to wheat germ or reticulocyte ribosomes. Linearization of these mRNAs restored their ability to bind to the eukaryotic ribosomes, suggesting that during initiation eukaryotic ribosomes must recognize a free 5'-terminus (capped or uncapped) of an mRNA. Ilan and Ilan (31) have described a protein associated with reticulocyte initiator factor 3 (eIF-3) that denatures synthetic poly(rA:rU) and globin mRNA. When AUG(U)n was used as an mRNA, polyphenylalanine synthesis could be inhibited completely when the polymer (A)n(U)n was hybridized to the AUG(U)n. The addition of eIF-3 restored 80% of translation (31). Such a protein could also disrupt the poly(A):oligo(U) duplexes in HeLa cell mRNA and facilitate ribosome binding.

It should be noted that the present experiments were not designed to detect any small UMP-rich RNAs which may have been intermolecularly hydrogen-bonded to mRNA. Such a RNA has been reported to be associated with myosin mRNA in a manner to possibly affect translation (32).
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SUGGESTED MATERIALS AND METHODS

Materials

Antibodies to oligo(U) sequences from HeLa cells were used as the probes for the hybridization reactions. The antibodies were produced by incubating the cells with different concentrations of oligo(U) sequences and were detected using an indirect immunofluorescence technique. The antibodies were affinity-purified using a protein A-Sepharose column.

Preparation of RNA

Total RNA was isolated from HeLa cells using the guanidinium thiocyanate-cesium chloride method. The RNA was then fractionated into poly(A)+ and poly(A)- fractions using oligo(dT) cellulose chromatography. The poly(A)+ RNA was further purified by size exclusion chromatography on a 1.5 M NaCl column. The poly(A)+ RNA was then used as the template for the transcription reactions.

Transcription

The poly(A)+ RNA was transcribed using SP6 RNA polymerase and [35S]UTP to generate cRNA probes. The cRNA probes were then hybridized to the filters containing the oligo(U) sequences to detect the presence of mRNA-like molecules.

Hybridization

The filters were baked at 80°C for 2 hours and then hybridized with the cRNA probes at 65°C for 20 hours. The filters were then washed at high stringency (0.1× SSC, 0.1% SDS) and autoradiographed for 48 hours.

Results

The hybridization experiments revealed the presence of mRNA-like molecules containing oligo(U) sequences. These molecules were detected in the nuclear fraction of HeLa cells, indicating that they are likely to be nuclear transcripts. The hybridization signals were specific to the oligo(U) sequences, and no cross-hybridization was observed with other RNA sequences.

Discussion

The results of the hybridization experiments suggest that HeLa cells synthesize mRNA-like molecules containing oligo(U) sequences. These molecules may play a role in the regulation of gene expression in HeLa cells. Further studies are needed to characterize these molecules and their function in the cell.
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![Graphs and Data](image)

**Figure 1.** Isolation of poly-(U) and mRNA by sucrose gradient centrifugation. (A) The poly-(U) oligo(U) was centrifuged as described in Methods. (B) The poly-(U) oligo(U) was centrifuged as described in Methods. The poly-(U) oligo(U) was centrifuged as described in Methods.

**Figure 2.** Isolation and characterization of poly-(U) oligo(U) treated from the HeLa cell line. The poly-(U) oligo(U) was centrifuged as described in Methods. The poly-(U) oligo(U) was centrifuged as described in Methods.

**Figure 3.** The four classes of mRNA were hybridized and analyzed on 1.5% agarose gel as described in Methods.

**Table 1.** The effect of HEC treatment on the size of HeLa cell mRNA to Poly(U) sequence at 4°C using procedure A described in Methods.

| Sample | Unbound | Hybridized | Nonspecifically Bound |
|--------|---------|------------|----------------------|
| Experiment 1: | | | |
| 1. Poly(A)+RNA | 95 | 3 | 1.0 |
| 2. Poly(A)+RNA + poly(U) (2) | 73 | 6 | 1.0 |
| 3. Poly(A)+RNA + poly(U) (2) | 85 | 5 | 3.9 |
| 4. Poly(A)+RNA + poly(U) (2) | 87 | 5 | 4.3 |
| Experiment 2: | | | |
| 5. Poly(A)+RNA + poly(U) (2) | 95 | 1 | 0.7 |
| 6. Poly(A)+RNA + poly(U) (2) | 75 | 3 | 3.4 |
| 7. Poly(A)+RNA + poly(U) (2) | 80 | 2 | 1.7 |

Poly(A)+RNA from supercoiled DNA particles

1. DNA from 105 particles (2) | 67 | 5 | 2.2 |
2. DNA from x50 particles (2) | 71 | 5 | 7.1 |
3. DNA from 105 particles (2) | 95 | 1 | 1.1 |
4. DNA from x50 particles (2) | 77 | 3 | 2.1 |
5. Synthetic Poly(A)+RNA (2) | 1 | 0 | 0.8 |
6. Synthetic Poly(A)+RNA (2) | 1 | 0 | 1.0 |

*In all samples, the number in brackets is the number of samples performed on a separate preparation of poly-(U). The average of the results is presented for each sample. The input of the other samples ranged from 2 x 10^5 to 1 x 10^6 particles. Non-specifically bound DNA is the DNA which remained on the membrane after washing with 90% formamide. All values have been adjusted to the nearest whole number except the nonspecifically bound DNA.
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Table I
The effect of HCHO treatment on the binding of natural and synthetic RNA molecules to Poly(A) particles at 4°C according to procedure B.

| Sample                                      | Amount (%) | Retained (%) | Non-specifically Bound (%) |
|---------------------------------------------|------------|--------------|-----------------------------|
| 1. Polynucleos H (+HCHO)                    | 97         | 2            | 0.5                         |
| 2. Polynucleos H (+HCHO) (^)                | 57         | 43           | 7.2                         |
| 3. Polynucleos H (-HCHO)                    | 97         | 2            | 0.5                         |
| 4. Polynucleos H (+HCHO)                    | 99         | 77           | 3.5                         |
| 5. 25S pre-RNA (-HCHO)                      | 98         | 1            | 1.0                         |
| 6. 25S pre-RNA (+HCHO)                      | 98         | 1            | 0.5                         |
| 7. Poly(A) (+HCHO)                          | 44         | 7            | 0.9                         |
| 8. Poly(U) (+HCHO)                          | 91         | 1            | 1.5                         |
| 9. Mitochondrial Poly(A) + RNA (-HCHO)       | 94         | 3            | 0.7                         |
| 10. Mitochondrial Poly(A) + RNA (+HCHO)      | 95         | 3            | 1.5                         |
| 11. Polynucleos S5S RNA (-HCHO)              | 90         | 1            | 0.9                         |
| 12. Polynucleos S5S RNA (+HCHO)              | 94         | 6            | 2.4                         |

Act of Poly(A), Poly(U), and Poly(C) was retained when HCHO was not employed. The total RNA for samples 1-4 was 1 x 10^6 cpm, input of other samples ranged from 1 to 2 x 10^6 cpm.

Table II
Retention of HeLa cell RNA on Poly(A) particles at 25°C using procedure B.

| Sample                                      | Unbound (%) | Retained (%) | Non-specifically Bound (%) |
|---------------------------------------------|-------------|--------------|-----------------------------|
| 1. [3P]Poly(A) (+HCHO)                      | 97          | 33           | 0.5                         |
| 2. [3P]Poly(A) (+HCHO)                      | 63          | 14           | 1.4                         |
| 3. [3P]Poly(A) (+HCHO)                      | 82          | 9            | 0.3                         |
| 4. [3P]Poly(A) (+HCHO)                      | 94          | -            | 0.9                         |

RNA isolated from subnuclear RNP particles

5. [3P]Poly(A) (+HCHO) in HCHO solution: 95
6. [3P]Poly(A) (+HCHO) in HCHO solution: 95

*These RNAs were directly applied to the sepharose at 25°C in MTris buffer, the column was washed with MTris, and the bound RNAs were eluted with TES. The input RNA for each was 1 to 2 x 10^6 cpm. Input of the other samples ranged from 1 to 2 x 10^6 cpm.

Table III
Nucleotide composition and yield of oligo(U) from [3P]poly(U) oligo(oligo(oligo(U)), and poly(U) oligo(oligo(oligo(U)) isolated from Poly(A) particles at 4°C or 25°C.

| Sample                                      | Total yield (cpm x 10^6) | [3P]oligo(U) (cpm x 10^6) | Composition of oligo(U) |
|---------------------------------------------|--------------------------|----------------------------|-------------------------|
| Experiment 1: Messenger RNA chromatographed at 25°C (procedure B) | 1. Poly(U) oligo(U) RNA (+HCHO) | 18.0 | 75.1 (1.5) | 85.0: 45.1 85.0 |
| 2. Poly(U) oligo(U) RNA (-HCHO)              | 14.4                     | 20.3 (0.58)               | 85.0: 71.0            |
| 3. Poly(U) oligo(U) RNA (-HCHO)              | 17.6                     | 5.9 (0.33)                | 85.0: 52.0            |
| Experiment 2: Messenger RNA chromatographed at 4°C (procedure A) | 4. Poly(U) oligo(U) RNA (+HCHO) | 6.0 | 9.8 (1.17) | 31.1: 31.1 31.1 |
| 5. Poly(U) oligo(U) RNA (-HCHO)              | 3.1                     | 7.4 (0.24)               | 41.5: 65.5            |
| Experiment 3: RNA isolated from subnuclear RNP particles chromatographed at 4°C (procedure A) | 6. Poly(U) oligo(U) RNA (+HCHO) | 3.6 | 3.3 (0.68) | 31.1: 31.1 31.1 |
| 7. Poly(U) oligo(U) RNA (-HCHO)              | 9.0                     | 1.6 (0.24)               | 31.1: 65.5            |
| Experiment 8: | 8. Total RNA | 7.4 | 5.7 (0.33) | 31.1: 31.1 31.1 |
| 9. Total RNA | 7.4 | 4.8 (0.24) | 31.1: 65.5 |

*Note: isolation of the isolated RNAs appears in Table I, sample 2. Nuclear RNA appears in Table I, sample 3. Poly(U) RNA was quantified in ['P]acrylamide gels by densitometry and corrected for the lower counting efficiency (50%) with creatine (radiation sample 0 cpm.)

Table IV
The ability of [3P]RNA to react to Poly(A) particles at 25°C. The [3P]RNA containing Oligo(U) tract (20 kbp) were isolated and reacted to Poly(A) particles.

| Sample                                      | Unbound (%) | Retained (%) | Non-specifically Bound (%) |
|---------------------------------------------|-------------|--------------|-----------------------------|
| 1. [3P]Poly(A) (+HCHO)                      | 9            | 85           | 1.3                         |
| 2. [3P]Poly(A) (+HCHO)                      | 64           | 67           | 1.7                         |

RNA isolated from subnuclear RNA particles

3. [3P]Poly(A) (+HCHO)  
Experiment 1: 95 65 0.7  
Experiment 2: 75 3.6  

The input RNA ranged from 1 to 2 x 10^6 cpm.

Table V
Chromatography on Poly(A) particles at 25°C

| Sample                                      | Unbound (%) | Retained (%) | Non-specifically Bound (%) |
|---------------------------------------------|-------------|--------------|-----------------------------|
| 1. Poly(A) RNA (+HCHO) 37°C-10 min | 9           | 83           | 3.5                         |
| 2. Poly(A) RNA (+HCHO) 65°C-10 min | 98           | 1            | 0.9                         |
| 3. Synthetic Poly(A) (+HCHO) 37°C-20 min | 1           | 93           | 0.5                         |
| 4. Synthetic Poly(A) (+HCHO) 37°C-30 min | 60           | 10           | 0.4                         |
| 5. Synthetic Poly(A) (+HCHO) 65°C-10 min | 90           | 0            | 0.6                         |

Chromatography on Poly(A) particles at 65°C

| Sample                                      | Unbound (%) | Retained (%) | Non-specifically Bound (%) |
|---------------------------------------------|-------------|--------------|-----------------------------|
| 1. Poly(A) RNA (+HCHO) 37°C-10 min | 11           | 2            | 0.7                         |
| 2. Poly(A) RNA (+HCHO) 65°C-10 min | 78           | 19           | 3.0                         |
| 3. Poly(A) RNA (+HCHO) 65°C-30 min | 91           | 2            | 7.3                         |

The input RNA ranged from 1 to 2 x 10^6 cpm.