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A Method for Isolation of Oligo(uridylic acid)-Containing Messenger Ribonucleic Acid from HeLa Cells†

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ABSTRACT: When cytoplasmic polyadenylated ribonucleic acid [poly(A+) RNA] from HeLa cells was treated with ribonuclease H (RNase H) and oligodeoxythymidylate [oligo(dT)] to remove its 3′-poly(A) tail, an increased binding to poly(A)–agarose was observed. The bound material, which comprised 4–6% of the initial RNA, contained 65–80% of the oligo(uridylic acid) [oligo(U)] sequences generated by RNase T_1 digestion. Oligo(U) isolated from the bound fraction was shown to be 83% U and to have a U/G ratio of 33. In contrast, oligo(U) from the unbound material was 77% U and had a U/G ratio of 13, suggesting that it is shorter and less U rich than the oligo(U) in the bound fraction. On sucrose gradients, oligo(U+) RNA consistently sedimented with a larger *s* value than oligo(U−) RNA. The oligo(U) content of oligo(U+) RNA suggests one oligo(U) tract of 33 nucleotides per RNA molecule of 2000–3000 residues.

It has been previously reported that RNA[1] fractions from HeLa cells with the properties of mRNA contained oligo(U) sequences ~30 nucleotides in length (Korwek et al., 1976). These sequences were present in both poly(A+) and poly(A−) mRNA fractions (Korwek et al., 1976) and have been demonstrated in hnRNA from a variety of sources (Molloy et al., 1972; Korwek et al., 1976; Bajszar et al., 1976; Peunova et al., 1979). In hnRNA the oligo(U) tracts were preferentially located near the 5′ ends of the molecules (Molloy et al., 1974; Bajszar et al., 1976; Peunova et al., 1979). Molloy et al. (1972) reported that oligo(U) was essentially absent from mRNA fractions from HeLa cells. In contrast, when caution was taken to compete out interfering poly(A) with excess synthetic poly(U), Korwek et al. (1976) demonstrated that 20–25% of the oligo(U), from RNA labeled for 2.5 h with $^{32}PO_4$ in HeLa cells, was localized in the cytoplasmic fraction. Furthermore, the size distribution of molecules in which oligo(U) was located differed between nucleus and cytoplasm, suggesting that the cytoplasmic oligo(U) sequences were not simply a result of nuclear contamination, unless a size-selective leakage is proposed.

The previous observations prompted us to attempt to isolate cytoplasmic oligo(U)-containing RNA, which calculations had predicted could represent as much as 15–20% of the poly(A+) mRNA population, if there is only one oligo(U) sequence per poly(A)-containing molecule. Preliminary attempts to isolate RNA molecules containing oligo(U) by affinity chromatography resulted in poor enrichment of oligo(U) in selected fractions (D. W. Kopp, W. M. Wood, and M. Edmonds, unpublished observations). However, a consistent factor in all of these experiments was the coexistence of short oligo(U) tracts with an excess of longer poly(A) segments, and we reasoned that poly(A), especially of intramolecular origin, may be inhibiting selection of oligo(U) by competitive hybridization. The following report describes a method whereby oligo(U)-containing RNA was quantitatively isolated from an mRNA fraction after removal of the 3′-poly(A) tail. Some properties of this novel RNA species are discussed.

Experimental Procedures

Materials. Sources for most of the materials used have been described previously (Korwek et al., 1976). Formamide (stabilized) was originally supplied by Fisher Chemical Co. and later obtained, redistilled, from Bethesda Research Laboratories. Before use, both were deionized by stirring for several hours with a mixed-bed ion-exchange resin AG501-X8 (Bio-Rad Laboratories), filtered, and stored at −20 °C. Ribonuclease H (RNase H) from calf thymus was initially a gift from Dr. J. Stavrianopoulos and later purified in this laboratory by the method of Stavrianopoulos (personal communication). Sephadex G-75 was obtained from Pharmacia Fine Chemicals. Oligo(dT)–cellulose was either synthesized in the

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[1] Abbreviations used: RNA, ribonucleic acid; oligo(U), oligo(uridylic acid); poly(A), poly(adenylic acid); poly(A+) RNA, poly(A)-containing RNA; poly(A−) RNA, poly(A)-lacking RNA; oligo(U+) RNA, oligo(U)-containing RNA; oligo(U−) RNA, oligo(U)-lacking RNA; C, cytidylic acid; A, adenylic acid; U, uridylic acid; G, guanylic acid; dT, deoxythymidylic acid; mRNA, messenger ribonucleic acid; hnRNA, heterogeneous nuclear RNA; tRNA, transfer RNA; DNA, deoxyribonucleic acid; cDNA, complementary DNA; RNase H, ribonuclease H; oligo(dT), oligo(deoxythymidylic acid); $NaDodSO_4$, sodium dodecyl sulfate; EDTA, ethylenediaminetetraacetic acid; Tris, tris(hydroxymethyl)aminomethane; ETS, 0.01 M EDTA, 0.01 M Tris-HCl (pH 7.5), and 0.2% $NaDodSO_4$; 0.4 M NETS, same as ETS plus NaCl at 0.4 M; F/ETS, same as ETS with formamide as indicated (percent, v/v).

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laboratory by the method of Gilham (1964) or supplied by P-L Biochemicals (Type 7) as was poly(A)–agarose (Type 6). Oligodeoxythymidylate [oligo(dT)$_{12-18}$] was supplied by Collaborative Research Inc. or by P-L Biochemicals.

Cell Culture, Labeling, Fractionation, RNA Extraction, and Oligo(dT)–Cellulose Chromatography. HeLa cells were maintained and labeled with carrier-free $^{32}PO_4$, and the RNA was extracted with hot phenol and $NaDodSO_4$ as previously described (Korwek et al., 1976). In all experiments cells (8×10^7) were labeled for 4 h in the presence of 1.25 mCi of $^{32}PO_4$/mL. Separation of poly(A+) and poly(A–) RNA was as reported (Nakazato & Edmonds, 1974), except that bound fractions were subjected to further selection as indicated.

Removal of 3′-Poly(A) Tails from Poly(A+) RNA. Poly(A+) RNA was ethanol precipitated at −20 °C with 100 μg of yeast RNA as carrier. The resulting precipitate was dissolved in 100 μL of sterile water, and 20 μL of oligo(dT) (~1 mg/mL) was added. After incubation at 63 °C for 3 min, to allow maximum exposure of poly(A) for hybridization with oligo(dT), the sample was cooled to room temperature in the presence of 0.1 M NaCl, 0.05 M Tris-HCl, pH 8, and 0.01 M $MgCl_2$. RNase H (200–1000 units; see Stavrianopoulos & Chargaff (1978) for the definition of a unit) was then added, and the sample (total volume of 200 μL) was incubated at 23 °C for 30 min. The resulting sample was then applied to a column of Sephadex G-75 (0.7 × 29 cm), which had been preequilibrated with ETS (0.01 M Tris-HCl, pH 7.4, 0.01 M EDTA, and 0.2% $NaDodSO_4$), the column was developed, at room temperature, with the same buffer, and 0.4-mL fractions were collected. The material eluting in the void volume was pooled (pool A, Figure 1) and precipitated with 2.5 volumes of 95% ethanol at −20 °C. As can be seen from Figure 1, Sephadex G-75 effectively separates RNA from oligo(dT), identified as the middle peak in Figure 1 by its A_{260}/A_{280} ratio (1.47). The absorbance peak eluting after oligo(dT) represents phenol (A_{260}/A_{280} ratio = 2.6) added as a low molecular weight marker.

Poly(A)–Agarose Affinity Chromatography. Ethanol-precipitated samples for poly(A)–agarose chromatography were dissolved in 0.25 mL of 0.4 M NETS (as for ETS but with NaCl at a concentration of 0.4 M) and applied to a small column (0.7 × 3 cm) of poly(A)–agarose (~1 mL of packed gel). The poly(A)–agarose had been previously washed with 10 volumes of 90% F/ETS (ETS containing 90% formamide) and equilibrated with 10 volumes of 0.4 M NETS. After binding was allowed to occur for 15–30 min at room temperature, the column was washed with 20 mL of 0.4 M NETS. In later experiments to avoid temperature fluctuations, a jacketed column was used and the temperature maintained throughout at 25 °C. The initial 6 mL was shown to contain >95% of the unbound material, so this volume was pooled and designated oligo(U–) RNA. Subsequently, the bound material [oligo(U+) RNA] was eluted with 5 mL of 90% F/ETS. Because of technical problems with subsequent analysis of formamide-containing eluates, it was decided to elute first with ETS alone which comprised 80–90% of the bound material and then with 30% or 90% F/ETS for equating with previous elution conditions. Both oligo(U+) and oligo(U–) fractions were precipitated with 2.5 volumes of 95% ethanol at −20 °C (after adjustment to 0.1 M NaCl) with addition of 100 μg of yeast RNA carrier if necessary. In some experiments, the bound fractions were reselected on a fresh poly(A)–agarose column under identical conditions.

Isolation and Quantitation of Poly(A) and Oligo(U). Poly(A) fragments were generated by using RNases A and

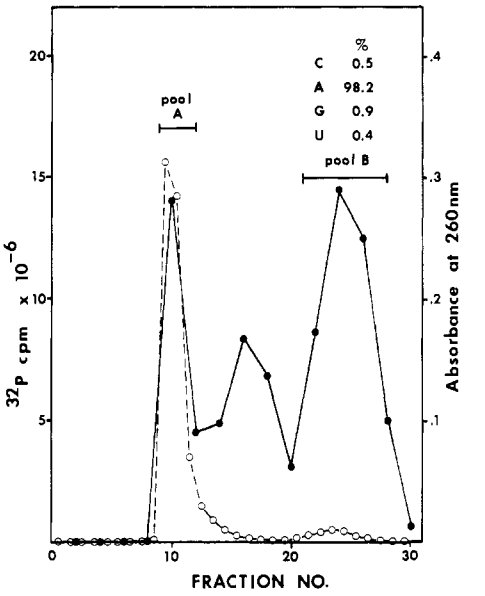

FIGURE 1: Sephadex G-75 profile of cytoplasmic poly(A+) RNA after treatment with RNase H and oligo(dT). Gel filtration was carried out as described under Experimental Procedures. The absorbance profile at 260 mm (●) shows the relative elution positions of RNA, oligo(dT), and phenol in order of elution. The radioactivity profile (○) represents the products of RNase H treatment in the presence of oligo(dT). The excluded volume (pool A) was collected and ethanol precipitated for further analysis. The low molecular weight product fractions (pool B) were combined, and an aliquot was analyzed for base composition as outlined under Experimental Procedures (shown in inset as percent C, A, G, and U).

T_1 and isolated by a procedure that permits quantitative binding of small poly(A) sequences to oligo(dT)–cellulose (Nakazato et al., 1974). Electrophoresis of poly(A) in 98% formamide polyacrylamide gels has been described (Nakazato et al., 1975). Oligo(U) sequences from RNase T_1 digests were isolated by affinity chromatography on a poly(A) resin in the presence of carrier poly(U) and quantitated by 10% aqueous polyacrylamide gel electrophoresis by methods described elsewhere (Venkatesan et al., 1976; Korwek et al., 1976). Base composition determinations were carried out by using high-voltage paper electrophoresis as described earlier (Korwek et al., 1976).

Results

Removal of 3′-Poly(A) Tails from Poly(A+) RNA. Since removal of substantial poly(A) from the 3′ end of mRNA appears to be crucial for quantitative binding of RNA containing oligo(U)-rich regions to poly(A)–agarose, several criteria have to be established to see that this has been achieved. Figure 1 shows a Sephadex G-75 profile obtained after RNase H treatment of $^{32}PO_4$-labeled poly(A+) RNA in the presence of oligo(dT). The majority of the applied radioactivity eluted in the excluded volume suggesting that no excessive degradation of the RNA had occurred. However, a small but reproducible amount (i.e., 6–10%) eluted later than oligo(dT) (average of 15 residues) in the same position as phenol. Base composition analysis of this material (pool B, Figure 1), after alkali digestion, revealed it to be >98% adenylate (Figure 1, inset), indicative of the fact that an amount of radioactivity equivalent to that expected to represent the poly(A) region of the molecule was digested to low molecular weight products. Further evidence is presented in Figure 2 that little, if any, nonspecific degradation of the RNA portion of the molecule is occurring after treatment to remove poly(A). Comparison of the sucrose density gradient profiles of untreated and RNase H–oligo(dT)-treated aliquots is shown in

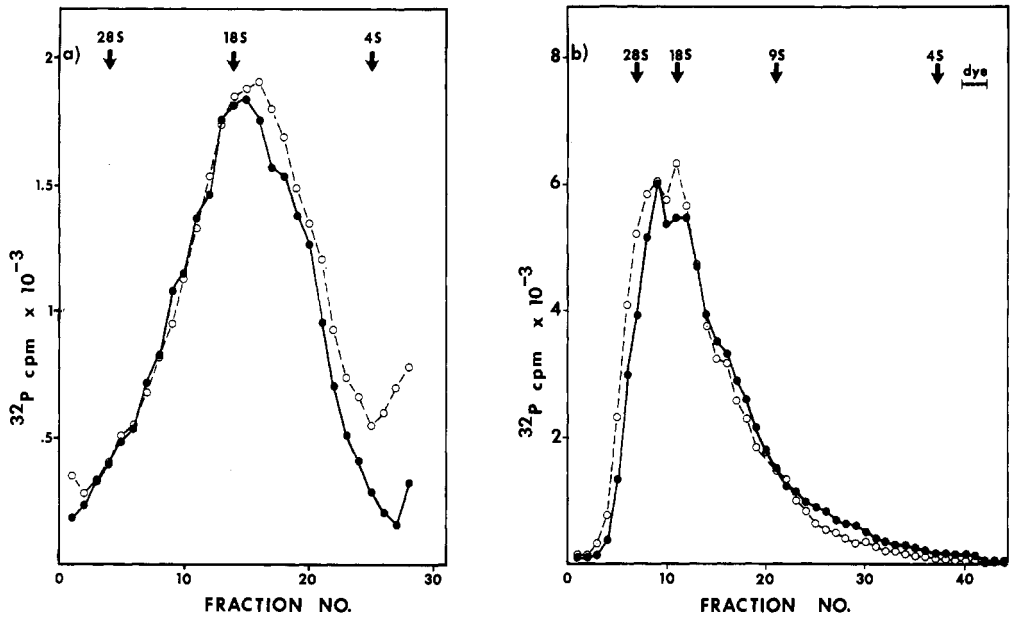

FIGURE 2: (a) Sucrose density gradient analysis of cytoplasmic poly(A+) RNA before (●) and after (○) treatment with RNase H and oligo(dT). Samples for analysis were preincubated at 63 °C for 3 min in the presence of 70% Me_2SO. Gradients (15–30% sucrose in 0.1 M NETS) were centrifuged for 18 h at 26000 rpm on a Beckman SW-40 rotor. Arrows represent the positions of HeLa cytoplasmic poly(A–) markers sedimented in a parallel gradient. (b) 98% formamide polyacrylamide gel electrophoresis of cytoplasmic poly(A+) RNA treated with RNase H in the absence (●) or presence (○) of oligo(dT). Samples were ethanol precipitated and resuspended in 98% formamide in 10 mM sodium phosphate buffer, pH 7.0 (formamide buffer). After being heated at 63 °C for 3 min and rapidly cooled to 0 °C, aliquots were electrophoresed in 4.2% polyacrylamide gels as previously described (Nakazato et al., 1975). Arrows denote the approximate positions in parallel gradients of HeLa cytoplasmic poly(A–) markers and 9S rabbit globin mRNA (Bethesda Research Laboratories) labeled at its 3′ end with [^{32}P]pCp (New England Nuclear) by using RNA ligase (P-L Biochemicals).

Figure 2a. As can be seen, the removal of poly(A) had essentially no effect on the sedimentation profile of the RNA. Because of poor resolution on sucrose gradients at *s* values lower than 18 S, aliquots were also electrophoresed on 4.2% polyacrylamide gels in 98% formamide (Figure 2b). In this case the depolyadenylated sample was compared to an aliquot subjected to RNase H in the absence of oligo(dT). Again, virtually identical profiles were observed, and in both cases the bulk of the material was larger than 9 S globin mRNA (~670 residues), providing additional evidence that the enzymic treatment was causing negligible damage to the treated RNA. Admittedly, slight degradation or cleavage adjacent to the ends of the molecules would not be detectable by the methods employed. This is discussed further in the following paper in this issue, describing the 5′ termini of this preparation (Wallace et al., 1981). Removal of poly(A) should result in decreased binding to oligo(dT)–cellulose. This is indeed the case where the ability of poly(A+) RNA to rebind consistently dropped from 87–97% to <1% after RNase H–oligo(dT) treatment (data not shown). Poly(A) sequences were isolated by using RNases T_1 and A and very stringent conditions of oligo(dT)–cellulose selection (see Experimental Procedures). The RNase H–oligo(dT)-treated product was shown to be devoid of poly(A) segments, selectable under these conditions, by polyacrylamide gel electrophoresis. Even though not detectable as a distinct peak on polyacrylamide gels, a residual level of acid-precipitable radioactivity did bind to oligo(dT)–cellulose and comprised 0.016% of the initial RNA (i.e., ~0.3 adenylate residues per RNA molecule). If this residual material remained attached exclusively to oligo(U)-containing molecules and was long enough to interfere with oligo(U) hybridization to poly(A)–agarose (i.e., 10–20 residues), it could conceivably prevent 10–20% of the potential oligo(U)-containing species from being selected.

Selection of Oligo(U+) RNA on Poly(A)–Agarose. The results in Table I (specifically, experiments 1 and 5) clearly demonstrate that prior removal of poly(A) from poly(A+) mRNA using RNase H and oligo(dT) enhanced its binding to poly(A)–agarose. It thus appears that, as suspected, poly(A) was indeed inhibiting the selection of oligo(U+) RNA, probably by competing with poly(A) residues on the agarose. Although, before poly(A) removal, material selected once on oligo(dT)–cellulose had a higher degree of binding to poly(A)–agarose than that 3 times bound, the net increase in binding achieved after depolyadenylation represented 4–6%

Table I: Effect of Poly(A) Removal on Binding of Cytoplasmic Poly(A+) RNA to Poly(A)–Agarose[a]

expt no.	% unbound	% bound	av ± SD
1[b]	94.1	4.0	
1	89.0	9.9	9.7 ± 0.7
2	88.4	10.4	
3	88.0	9.0	
4	89.5	6.5	
4[c]	18.7	81.0	
5[b]	99.2	0.6	
5	96.0	4.0	
6	95.6	4.0	
7	94.8	4.7	4.5 ± 0.5
8	94.2	5.1	
9	94.8	4.7	
9[c]	27.9	71.0	

[a] Samples were bound to poly(A)-agarose as described under Experimental Procedures. Bound material was eluted with ETS + 30% F/ETS except in experiment 1 where 90% F/ETS was the elution buffer. [b] Denotes that RNA samples were bound to poly(A)-agarose prior to treatment with RNase H and oligo(dT) to remove poly(A) as described under Experimental Procedures. [c] Denotes that the ETS fraction was adjusted to 0.4 M NETS, reapplied to a fresh poly(A)-agarose column, and eluted by using conditions similar to those used in the initial binding. In experiments 1-3, poly(A+) RNA was isolated by binding once to oligo(dT)–cellulose, in experiment 4 by binding twice, and in experiments 5-9 by binding 3 times.

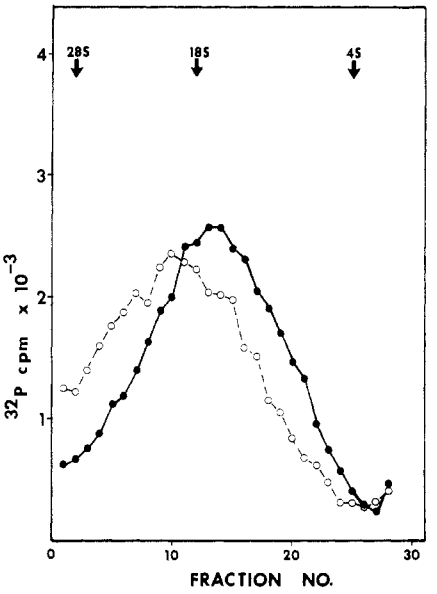

FIGURE 3: Sucrose density gradient centrifugation of oligo(U−) RNA (●) and oligo(U+) RNA (○) was carried out as described in the legend to Figure 2.

of the initial poly(A+) RNA population. The nature of the fraction selected on poly(A)–agarose before poly(A) removal is not clear, but it could represent poly(A)-lacking species containing oligo(U) which are lost by further purification on oligo(dT)–cellulose. Generally, 15–20% of the initial poly(A+) fraction was lost on successive bindings to oligo(dT)–cellulose. We are at present investigating this fraction for its content of poly(A) and oligo(U).

The oligo(U+) and oligo(U−) RNAs were analyzed by sucrose density gradient centrifugation (Figure 3). No further degradation was detectable as a result of poly(A)–agarose chromatography; in fact, the oligo(U+) RNA consistently sedimented with an *s* value slightly greater than that of the oligo(U−) fraction. When the oligo(U+) fraction was reapplied to poly(A)–agarose under similar selection conditions, 70–80% was found to be rebindable, demonstrating the specific nature of the binding (Table I, experiments 4[c] and 9[c]).

Distribution of Oligo(U) Sequences on Poly(A)–Agarose. It was necessary to show that the binding to poly(A)–agarose was due to selection of molecules containing oligo(U)-rich sequences. The poly(A)–agarose-bound and unbound fractions were analyzed for oligo(U) sequences as described under Experimental Procedures. It was shown that 65–80% (three experiments) of the oligo(U) that is bindable to the poly(A) resin was localized in the RNA fraction selectable on poly(A)–agarose. Polyacrylamide gel electrophoresis in 10% aqueous gels demonstrated that the oligo(U) stretches generated by RNase T_1 digestion migrated between oligo$(A)_{25}$ and tRNA (75 residues) (Figure 4). Analysis of the respective base compositions revealed that the oligo(U) in the oligo(U−) fraction was less U rich (77%) and shorter (13 uridylate residues per 3′-terminal guanylate) than that in the oligo(U+) fraction (83% U; 33 uridylate residues per 3′-terminal guanylate) (Figure 4, inset). Therefore, it appears that the poly(A)–agarose binding conditions employed favor selection of molecules containing oligo(U) sequences of a certain length and U content. RNAs with shorter and less U-rich regions are excluded. Calculations based on the oligo(U) content of oligo(U+) RNA (approximately 1–1.5%, Table II) are compatible with the presence of one oligo(U) tract of 30 nucleotides per RNA molecule of 2000–3000 residues, which was the approximate average size of the oligo(U+) population as

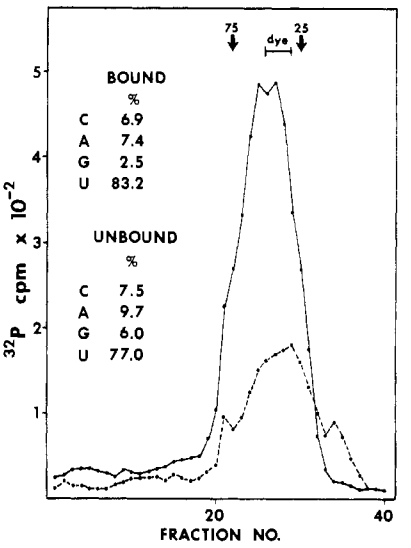

FIGURE 4: Polyacrylamide gel electrophoresis of oligo(U). RNase T_1 generated oligo(U) fragments from oligo(U+) (solid line) and oligo(U−) (broken line) RNAs were purified by binding to a poly(A) resin and quantitated by electrophoresis on 10% aqueous polyacrylamide gels as described under Experimental Procedures. The inset shows the base compositions of oligo(U) from oligo(U+) RNA (bound) and oligo(U−) RNA (unbound) carried out as described under Experimental Procedures.

Table II: Oligo(U) Content of Poly(A)–Agarose-Bound RNA[a]

expt no[b]	fraction[c]	RNA (cpm × 10^{-5})	oligo(U) (cpm × 10^{-3})	% oligo(U)/ RNA
1	90% F/ETS	3.66	4.23	1.15
2	90% F/ETS	9.10	12.25	1.35
4	ETS + 30% F/ETS	3.96	3.36	0.85
9	ETS	3.84	4.70	1.22

[a] RNA fractions were digested with RNase T_1, and the oligo(U) sequences were quantitated by selection on a poly(A) resin and electrophoresis in 10% aqueous polyacrylamide gels as described under Experimental Procedures (see Figure 4). [b] Refers to the experiment number from Table I. [c] Denotes the particular poly(A)-agarose fraction analyzed.

displayed on sucrose gradients (Figure 3).

Discussion

This report describes a method for the quantitative isolation of RNA molecules containing oligo(U) sequences from HeLa cells. These sequences are present in hnRNA and in both poly(A+) and poly(A−) molecules with the properties of mRNA (Korwek et al., 1976) and absent from the 45S ribosomal precursor (Korwek, 1974). We reasoned that as long as poly(A) sequences were present in excess, quantitative isolation of oligo(U+) RNA by affinity chromatography would result in limited recovery of such molecules. This problem might be circumvented either by excising the poly(A) tails from the poly(A+) RNA or by confining our study to those molecules lacking poly(A). Initially, we adopted the former approach, since poly(A−), RNA, although inherently interesting because it contains more oligo(U) sequences than poly(A+) RNA, represents a highly heterogeneous mixture of cytoplasmic RNAs of which the material of interest comprises considerably <1%.

Obviously, the method used to selectively remove the poly(A) must be one that results in negligible damage to the RNA portion of the molecule. An ideal enzyme for this task is ribonuclease H, a nuclease which specifically hydrolyzes the ribo strand of a deoxyribo–ribo hybrid (Stavrianopoulos &

Chargaff, 1973). The use of this enzyme, in conjuction with oligodeoxythymidylate, to remove the poly(A) tail from globin mRNA has been described, resulting in little detectable damage to the RNA per se, as measured by fidelity of translation in a cell-free system (Sippel et al., 1975). We have obtained preliminary evidence that removal of poly(A) from HeLa cytoplasmic poly(A+) RNA, using RNase H and oligo(dT), had little effect on its translational capacity in a cell-free system from wheat germ (D. W. Kopp, W. M. Wood, and M. Edmonds, unpublished results).

Figures 1 and 2 describe the results of several analyses designed to test the efficacy of a highly purified preparation of RNase H for poly(A) removal from HeLa cytoplasmic poly(A+) RNA. Digestion of poly(A+) RNA in the presence of RNase H and oligo(dT) yielded a product displaying, on sucrose density gradient centrifugation and gel electrophoresis, a size similar to that of the untreated control or that product treated with RNase H in the absence of oligo(dT). It was unable to rebind to oligo(dT)–cellulose and contained negligible residual poly(A). Furthermore, the enzymic treatment also resulted in the generation of a low molecular weight product separable on Sephadex G-75, with a content equivalent to the poly(A) region of the initial RNA fraction. Base composition determination confirmed this material to be derived from poly(A) (>98% adenylate).

Use of RNase H to remove poly(A) from poly(A+) RNA resulted in the ability to select a fraction, on poly(A)–agarose, which contained 65–80% of the oligo(U) sequences and had a content equivalent to one oligo(U) tract of 30 nucleotides per RNA molecule of 2000–3000 residues (Table II). This fraction, which comprised 4–6% of the poly(A+) RNA labeled in a 4-h pulse with $^{32}PO_4$, represents a unique population of messenger RNA molecules which contain oligo(U) in addition to poly(A). The oligo(U+) RNA had a slightly greater sedimentation coefficient than the remainder [oligo(U–) fraction] of the poly(A+) mRNA (Figure 3). The significance of this finding is not clear, but kinetic experiments are underway to determine if this population represents a cytoplasmic precursor from which oligo(U) is excised during its conversion to mature mRNA. The possibility of cytoplasmic splicing or processing has been invoked as one explanation for the decrease in absolute frequency of N^6-methyladenosine in HeLa cell mRNA with increasing labeling time (Sommer et al., 1978).

Although the size distribution of molecules containing oligo(U) differed between nucleus and cytoplasm (Korwek et al., 1976), this in itself is not confirming evidence that the cytoplasmic oligo(U) sequences are not a result of nuclear contamination. In order to further verify that the oligo(U+) RNA isolated from cytoplasmic fractions is messenger in nature, we have examined its 5′-terminal cap structure (Wallace et al., 1981). Further studies on the relative metabolic stability and translational capacity of oligo(U+) RNA are planned. Experiments are also underway to determine the location of the oligo(U) tract within the oligo(U+) RNA molecule.

Oligo(U) stretches of the size described in this report do not exist in presently sequenced mRNAs, perhaps because these species represent a limited number of very specialized gene products. However, the presence of oligo(U)-rich sequences in cytoplasmic RNA molecules with the properties of a messenger have been described. Burdon et al. (1977) reported that a fraction of pulse-labeled polysomal-derived RNA from BHK-21 cells possessed affinity for poly(A) but that this type of poly(A)-binding species was undetectable in the corresponding poly(A+) fractions. This could be explained by the inability to select molecules containing oligo(U) in the presence of intramolecular poly(A), as discussed earlier in this report. They further demonstrated little sequence homology between the poly(A)-binding RNA and poly(A+) RNA from Friend murine leukemia cells (Katinakis & Burdon, 1978). In a recent report, Molloy (1980), using formaldehyde to disrupt secondary structure in a poly(A+) RNA fraction derived from HeLa polysomes, showed that such a messengerlike fraction contained molecules with oligo(U)-rich sequences and that this oligo(U+) poly(A+) population comprised 4–13% of the total mRNA. The length and base composition of the (U)-rich sequences bore a striking resemblance to those described in this report. Finally, Baranov et al. (1977) reported that 15–17% of globin mRNA from pigeon reticulocytes was selectable on poly(A)–Sepharose.

Oligo(dT) tracts of length and dT content comparable to those of the oligo(U) segments herein described have been reported from several genomic sources. The intervening sequences of β-globin minor and ovalbumin genes contain such dT-rich regions, although they are sometimes interrupted by other bases (Breathnach et al., 1978; Konkel et al., 1979). A family of actin genes sequenced from *Dictyostelium discoideum* contains extremely dT-rich regions very close to the presumptive AUG initiation condons, which, on transcription, would give rise to very U-rich sequences (Firtel et al., 1979). Flavell et al. (1977) reported that oligo(dA-dT) clusters, as large as 23 residues, are interspersed throughout the rabbit genome and that some of these are adjacent to unique-sequence DNA. More specifically, Tokarskaya et al. (1980) showed that unique mouse DNA restriction fragments, of a size equivalent to that of mRNA, were hybridizable both to cDNA copies of mRNA and to oligo(dT) indicating close proximity of coding regions and oligo(dA-dT) sequences.

The function of oligo(U) in some mRNA molecules remains unknown, although intuitively some form of interaction with poly(A) seems likely. The in vivo association of oligo(U) and poly(A) promoted by a binding protein in HeLa cells was proposed by Kish & Pederson (1976, 1977). Another possibility, that U-rich regions in mRNA code for phenylalanine-rich protein domains, cannot be discounted, but it appears unlikely since several consecutive phenylalanine residues are an extremely rare feature of proteins. For a further discussion of this point see Molloy (1980).

What properties oligo(U) confers on mRNA remain to be elucidated. The types of experiments we have proposed to further characterize this unique fraction of RNA may help to answer these questions.

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5′-Terminal Cap Structures of Oligo(uridylic acid)-Containing Messenger Ribonucleic Acid from HeLa Cells: Comparison with Other Ribonucleic Acid Subpopulations†

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ABSTRACT: The 5′-terminal cap structures of ^{32}P-labeled oligo(uridylic acid)-containing messenger ribonucleic acid [oligo(U+) mRNA] isolated from HeLa cell polyadenylated [poly(A+)] mRNA were analyzed and compared to those of the poly(A+) mRNA. A method employing P1 nuclease, alkaline phosphatase, and adsorption to activated charcoal showed that the types of cap core (m^7 GpppXm) in oligo(U+) mRNA were essentially identical with those in poly(A+) mRNA. Analysis of RNase T_2 digestion products of oligo(U+) mRNA demonstrated the presence of both cap 1 (m^7GpppXmpYp) and cap 2 (m^7GpppXmpYmpZp) in this subpopulation, confirming its cytoplasmic location. The base compositions of these two types of caps were different from each other and nonrandom but did not differ significantly between oligo(U+) and poly(A+) mRNA. The only observed difference between the mRNA populations was a higher ratio of cap 1 to cap 2 in the former. Possible implications of these findings for the relationship between oligo(U+) mRNA and poly(A+) mRNA are discussed.

Some of the poly(A)-containing messenger ribonucleic acid [poly(A+) mRNA][1] molecules in the cytoplasm of HeLa cells also contain an oligo(U) sequence (Korwek et al., 1976; Molloy, 1980). A procedure developed in this laboratory for the isolation of this subset of mRNA molecules (Wood & Edmonds, 1981) has allowed us to examine other structural features of oligo(U)-containing molecules that may differentiate them from mRNA molecules that do not contain oligo(U). One structure, the modified 5′ terminus of mRNA molecules designated as the cap [m^7GpppXmpY(m)p], appears to be present in most of the poly(A)-containing mRNA molecules of HeLa cells (Salditt-Georgieff et al., 1976). It could not be assumed, however, that oligo(U)-containing mRNAs were capped. An uncapped subpopulation of this size

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[1] Abbreviations used: RNA, ribonucleic acid; oligo(U), oligo(uridylic acid); poly(A), poly(adenylic acid); poly(A+) mRNA, poly(A)-containing messenger RNA; oligo(U+) mRNA, oligo(U)-containing mRNA; oligo(U−) mRNA, oligo(U)-lacking mRNA; hnRNA, heterogeneous nuclear RNA; rRNA, ribosomal RNA; DNA, deoxyribonucleic acid; pC, cytidine 5′-monophosphate; pA, adenosine 5′-monophosphate; pG, guanosine 5′-monophosphate; pU, uridine 5′-monophosphate; m^7pG, 7-methylguanosine 5′-monophosphate; pNp, 3′,5′-nucleoside diphosphate; RNase H, ribonuclease H; DBAE, (dihydroxyboryl)aminoethyl; DEAE, diethylaminoethyl; EDTA, ethylenediaminetetraacetic acid; Tris, tris(hydroxymethyl)aminomethane; RSA, relative specific activity; cpm, counts per minute.

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