OLIGO(A) AND OLIGO(A)-ADJACENT SEQUENCES PRESENT IN NUCLEAR RIBONUCLEOPROTEIN COMPLEXES AND mRNA

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SUMMARY: Twenty to thirty percent of the nuclear oligo(A) sequences of mouse ascites cells were found associated with the 30S ribonucleoprotein (RNP) subunit of the larger hnRNA:protein fibril (hnRNP). The oligo(A) present in 30S RNP subcomplexes was found covalently linked to non-oligo(A) RNA. The sequences adjacent to the 5' end of this oligo(A) have been transcribed into complementary DNA (cDNA) with reverse transcriptase. From analysis of RNA·cDNA hybridization kinetics utilizing 30S RNP-RNA and cytoplasmic messenger RNA (mRNA), the following observations were made: 1. Approximately twenty-five percent of the oligo(A)-adjacent sequences constituted a single abundant class with a combined nucleotide complexity of ca. 500 nucleotides. 2. These abundant oligo(A)-adjacent sequences were also found in the abundant class of cytoplasmic mRNA.

INTRODUCTION

The large, heterogeneous nuclear RNA (hnRNA) of eukaryotes is complexed with proteins (1-5). The native hnRNP complex has been shown to be composed of 30S RNP subunits (1,4,6), and in many cell types the 30S RNP complex is the major extractable form of hnRNP due to endogenous nucleases. The 30S subparticles contain most of the pulse-labeled nuclear RNA including sequences homologous to cytoplasmic poly(A) plus mRNA (7).

Nuclear RNA contains a number of definable sequence elements including oligo(A) sequences. Nuclear oligo(A) of

approximately 30 nucleotides occurs internally in hnRNA and is transcribed from DNA, in contrast to poly(A) (8). To further understand the organization of nuclear RNA in ribonucleoprotein complexes we have examined our nuclear RNP extracts for oligo(A) sequences and have found a substantial amount associated with 30S RNP. We have also studied the sequences which are adjacent to the 5' end of the oligo(A). These sequences include a highly abundant class of nuclear RNA sequences which are also present in cytoplasmic poly(A) plus mRNA. These sequences may therefore be utilized in mRNA processing as possible sites for the cleavage of hnRNA.

MATERIALS AND METHODS

Taper ascites cells were grown in mice as described (3). Preparation of 30S RNP and the various RNA fractions have been described (3, 7). Complementary DNA was prepared with avian myeloblastosis virus reverse transcriptase (7). The cDNA was purified by oligo(dA)-cellulose affinity chromatography using binding and elution conditions described by Aviv and Leder (9). Hybridization reactions, S_1-nuclease digestions, and polyacrylamide gel electrophoresis were performed as described.

RESULTS

Oligo(A) sequences in 30S RNP-RNA were prepared by T_1 and pancreatic RNase digestion of [^{3}H]-adenosine labeled 30S particle RNA. When this material was analyzed on 12% acrylamide gels we found a large proportion of the radioactivity that migrated as a single peak of 20-40 nucleotides, as expected for oligo(A) (Fig. 1) (8). Some RNA migrates heterogeneously and consists of double-stranded RNA (our unpublished results). When undigested 30S RNP-RNA was analyzed after chromatography on oligo(dT)-cellulose both the bound [oligo(A) plus] and unbound [oligo(A) minus] fractions contained RNA identical in size to total 30S RNP-

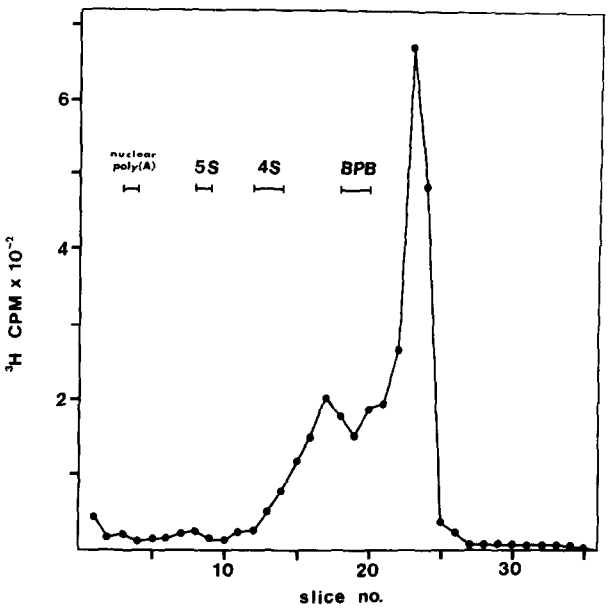

Figure 1. Acrylamide gel electrophoresis of pancreatic and T_1 RNase resistant [^{3}H]-adenosine labeled 30S RNP-RNA. Cells were labeled for 20 min with 10 μC/ml of [^{3}H]-adenosine and the nuclear extract prepared and centrifuged on a 15-30% sucrose gradient (2). RNA was extracted (7) from the 30S region on this gradient. The RNA was digested for 40 min with 2 μg/ml of pancreatic and 10 U/ml of T_1 RNase, purified, and electrophoresed on a 12% acrylamide gel for 2.5 hr at 5 ma/gel (7). 4S and 5S RNA were included in this gel as molecular weight markers, and nuclear poly(A) [from nuclear poly(A) containing RNP] was run in a parallel gel. BPB, bromphenol blue tracking dye.

RNA (results not shown). Therefore the oligo(A) sequences present in 30S RNP-RNA were linked to RNase sensitive sequences. We determined that 20-30% of the nuclear oligo(A) sequences were in 30S RNP subcomplexes. The remaining oligo(A) sedimented at less than 30S in our RNP gradients (results not shown). This would indicate that these oligo(A) sequences are present in the nuclease-sensitive regions of the larger hnRNP fibril.

TABLE I

REVERSE TRANSCRIPTASE TEMPLATE ACTIVITY OF NUCLEAR 30S RNP-RNA AND CYTOPLASMIC POLY(A) PLUS RNA

RNA[a]	Primer[b]	[^{3}H]dCTP Incorporated[c] (CPM X 10^{-3})	Stimulation By Primer (X)
Cytoplasmic poly(A) plus RNA	none	26.5	---
	oligo(dA)$_{12-18}$	24.2	1.0
	oligo(dT)$_{12-18}$	217.2	8.2
Total 30S RNP-RNA	none	6.0	---
	oligo(dA)$_{12-18}$	5.8	1.0
	oligo(dT)$_{12-18}$	38.6	6.4

a. 0.5 µg poly(A) plus RNA and 1.5 µg 30S RNP-RNA per 100 µl reaction were assayed as described (7).

b. Primer was present at a final concentration of 7.5 µg/ml.

c. [^{3}H]dCTP specific activity was 2,500 cpm/pmole.

The presence of oligo(A) sequences in 30S RNP-RNA allowed the transcription of the RNA sequences adjacent to the 5' end of the oligo(A) into complementary DNA (cDNA) with reverse transcriptase (Table I). Since poly(A) is found in a distinct 15-17S RNP subcomplex (10) and not in 30S RNP (see Fig. 1), only sequences adjacent to oligo(A) will be transcribed. The use of an oligo(dT) primer, but not an oligo(dA) primer, promoted efficient transcription of both 30S RNP-RNA and cytoplasmic mRNA into cDNA (Table I). In subsequent experiments the cDNA complementary to the sequences adjacent to the 5' end of oligo(A) [oligo(A) adjacent] was purified by affinity chromatography on an oligo(dA)-cellulose column to ensure the purity of the probe.

When 30S RNP-RNA was hybridized (in large excess) with our cDNA probe a very rapidly hybridizing component was observed (Fig. 2). This component comprised approximately 25% of the cDNA to oligo(A)-adjacent sequences. Since the nucleotide complexity of an RNA population is proportional to the $R_0t_{1/2}$ of its homologous cDNA hybridization, the complexity of the oligo(A)-adjacent sequences could be calculated (11). Using a globin mRNA standard (7) the complexity of the rapidly hybridizing oligo(A)-adjacent sequences is 500-1000 nucleotides. This estimate most likely represents an upper limit since the actual proportion of 30S RNP-RNA driving the reaction is not known and its smaller size (40-90 nucleotides, 7) will reduce its relative hybridization rate. The slower hybridizing component is at least 10^4 times more complex. Due to the problems in securing the relatively high concentrations

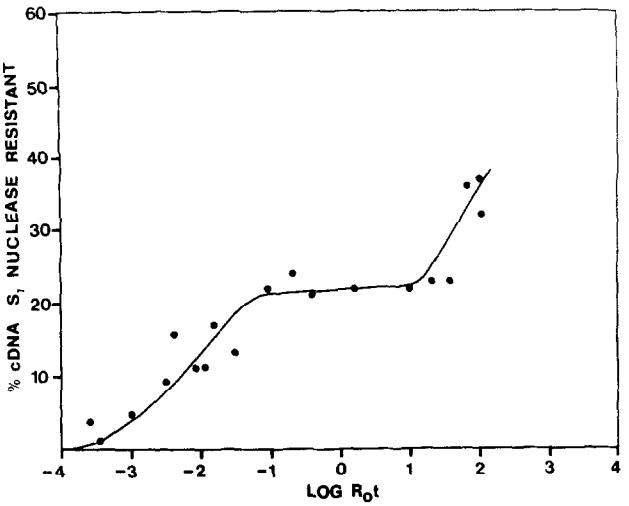

Figure 2. Hybridization kinetics of 30S RNP-RNA and homologous cDNA. 30S RNP-RNA was prepared from pelleted complexes and cDNA prepared with avian myeloblastosis virus reverse transcriptase and oligo(dT) primer. Hybridization was performed as described (7). R_0t is the product of RNA concentration (in moles of nucleotides/ℓ) and time (in seconds).

of 30S RNP-RNA needed to obtain more detailed data for this component, more precise estimates cannot be made at this time.

Since oligo(A) is present internally in non-adenylated hnRNA and not detectable in cytoplasmic mRNA (8, 12), it was important to establish whether any of the oligo(A)-adjacent sequences were homologous to mRNA or were entirely nucleus-restricted. When cytoplasmic poly(A) plus mRNA was reacted with cDNA to oligo(A) adjacent sequences it hybridized to the low complexity cDNA (Fig. 3). Purified polysomal mRNA also hybridized with this cDNA, suggesting that some functional mRNA does indeed contain these simple

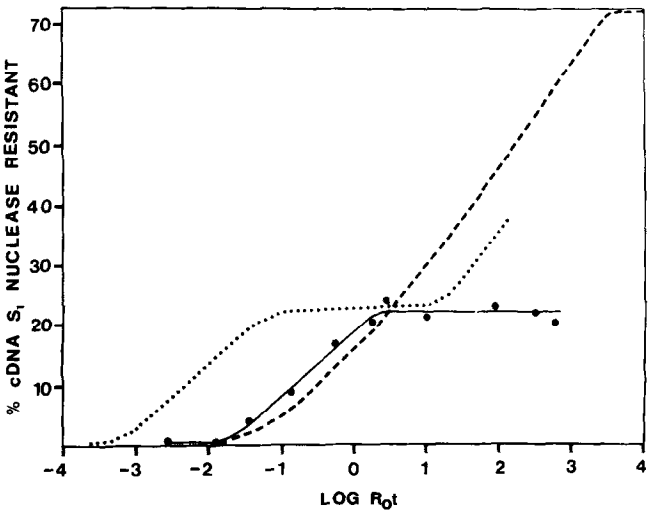

Figure 3. Hybridization kinetics of cytoplasmic poly(A) plus RNA with cDNA to oligo(A) adjacent sequences. Cytoplasmic poly(A) plus RNA was hybridized to 30S RNP-RNA specific cDNA as described (7). Poly(A) plus RNA hybridization with 30S RNP-RNA specific cDNA, (●--●); 30S RNP-RNA hybridization with homologous cDNA from Figure 2, (····); poly(A) plus RNA hybridization with homologous cDNA from ref. 7, (---).

sequences. The kinetics were 30-50 times slower than the homologous reaction, but as fast as the most abundant class of poly(A) plus mRNA hybridized with homologous cDNA (Fig. 3). At the R_0t values achieved no hybridization with the more complex oligo(A) adjacent sequences occurred. Much larger quantities of purified RNA are needed to test homology between poly(A) plus mRNA and these more diverse sequences adjacent to oligo(A).

DISCUSSION

The hybridization data presented have established that the sequences adjacent to the 5' end of oligo(A) se-

quences found in 30S RNP contain a simple class of sequences amounting to ca. 25% of the total sequences. Kinetic analysis yields a complexity of 500-1000 nucleotides. The complexity may actually be less since the true amount of 30S RNP-RNA driving the reaction cannot be firmly established. Cytoplasmic poly(A) plus mRNA has sequences homologous to these simple complexity oligo(A)-adjacent sequences. The kinetics of hybridization suggest that abundant mRNA's contain these common sequences. The abundant nuclear RNA sequences may therefore represent sequences which are important in the selection of mRNA sequences for transport to the cytoplasm such as recognition sites for processing enzymes, or sequences involved in specific folding of the nuclear molecules. It is possible that some mRNA molecules of mouse ascites cells are derived from internal regions of poly(A) minus hnRNA. If the initial cleavage occurred at the 3' end of the oligo(A) sequence terminal riboadenylate transferase [which *in vitro* prefers adenylate rich primers (13)] could then extend this oligo(A) into the 200 nucleotide poly(A) sequence found on newly synthesized mRNA. With other processing events, such as "capping" and methylation (14, 15), the finished mRNA would then be transported to the cytoplasm. Recently, Edmonds and co-workers (12) have proposed a similar model for mRNA processing in HeLa cells, though direct homologies between oligo(A) adjacent sequences and mRNA were not shown. We recognize that the relative abundancies of the oligo(A) adjacent sequences in nuclear and cytoplasmic RNA are not fully explained by such a simple model and more data are required to assess the role

of the sequences in intranulcear processes and mRNA maturation.

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