Translation of nonpolyadenylated messenger RNA of sea urchin embryos

[translation/oligo(dT)-cellulose/nonhistone mRNA]

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Communicated by Wilder Penfield, October 9, 1975

ABSTRACT A large proportion of newly synthesized polyribosomal RNA of sea urchin blastulae is not polyadenylated. The size distributions of the polyadenylated and nonpolyadenylated RNA are indistinguishable (mean size of 26 S). Upon translation of sea urchin polyribosomal RNA containing poly(A) and that without poly(A) in a wheat embryo cell-free protein-synthesizing system, where polypeptide synthesis is dependent on added messenger, both classes of RNA support peptide synthesis to the same extent. A preliminary analysis of the proteins synthesized in response to added mRNA (polyadenylated and nonpolyadenylated) indicates that these classes of mRNA molecules may code for different populations of proteins.

The existence of polyadenylated mRNA and heterogeneous nuclear RNA is now well established (1–6). Recent studies indicate that in addition to polyadenylated mRNA, there are also nonpolyadenylated mRNA molecules in a number of eukaryotic cell types, including HeLa cells (7), sea urchin embryos (8, 9), and slime molds (10). The functional significance of poly(A) tracts in mRNA is not yet established (see ref. 11). The lack of poly(A) in the histone mRNA prevents it neither from being transported from the nucleus to the cytoplasm nor from being translated (12). When poly(A) is enzymatically removed from L cell mRNA, these mRNA molecules supported protein synthesis in vitro effectively (13). Nevertheless, poly(A) may influence the half-life of mRNA (see refs. 11 and 14). In sea urchin embryos a large proportion of the mRNA in polyribosomes is not polyadenylated, and we describe here some of the properties of these naturally occurring mRNA molecules without poly(A) [poly(A)] mRNA. These two classes of mRNA exhibited similar size distributions and supported protein synthesis in vitro to almost the same extent. A preliminary characterization of the peptides synthesized in vitro suggests that poly(A) and poly(A) mRNA code for distinct populations of protein.

MATERIALS AND METHODS

Animals. Sea urchins (Lytechinus pictus) were purchased from Pacific Biomanine Supply Company, Venice, Calif. Gametes were obtained by intracoelomic injections of 0.5 M KCl; they were fertilized and raised on synthetic sea water at 18°C (containing 50 μg/ml of streptomycin) to mesenchyme blastulae (17 hr after fertilization). Only those embryos with >95% normal development were used.

Isolation of Polyribosomes and Polyribosomal RNA. When labeled RNA was desired, mesenchyme blastulae were incubated with 50 μCi/ml of [³H]uridine (25–28 Ci/mm) or with 0.1 μCi/ml of [¹⁴C]uridine (472 mCi/mm)

Abbreviations: poly(A) mRNA and poly(A) mRNA, mRNA containing and not containing polyadenylate, respectively; TEAKM buffer, 0.05 M triethanolamine, 0.4 M KCl, 0.01 M MgCl₂ (pH 7.8).
RESULTS

Approximately 65% of the small polyribosomal RNA (2 to 6 ribosomes per mRNA) and 55% of the large polyribosomal RNA (>6 ribosomes per mRNA) do not bind to oligo(dT)-cellulose, suggesting that they lack poly(A). That oligo(dT)-cellulose chromatography reliably separated poly(A)− and poly(A)+ RNA is indicated in the following experiment. Mesenchyme blastulae were incubated with [3H]adenosine for 60 min. Polyribosomal RNA was fractionated on oligo(dT)-cellulose columns. Both poly(A)− and poly(A)+ fractions were digested with pancreatic and T1 RNase under conditions shown to leave intact poly(A) tracts (8). After RNase digestion, 14.2% of the adenine-labeled poly(A)+ RNA remained precipitable by trichloroacetic acid while only 0.6% of the labeled poly(A)− RNA was acid-precipitable. This suggests that there is very little, if any, polyadenylated polyribosomal RNA in the poly(A)− RNA fraction (see also ref. 9). Since these embryos at this stage of development do not synthesize appreciable amounts of rRNA (25), the nonpolyadenylated, newly synthesized RNA in polyribosomes is most likely functional mRNA (see ref. 8).

To determine whether there were discernible differences in the size distribution of poly(A)− and poly(A)+ RNA, [3H]uridine-labeled RNA was analyzed by polyacrylamide gel electrophoresis. Fig. 1 shows that these two populations of molecules are similar with respect to size. They are relatively large (mean size 26 S) and heterodisperse. It is unlikely that the nonpolyadenylated molecules are derived from larger polyadenylated mRNA molecules by some nonspecific endonuclease activity (see also ref. 8).

The functional integrity of these RNA molecules was examined by translating nonpolyadenylated and polyadenylated RNA in a wheat embryo cell-free protein-synthesizing system (21, 22, 26). Both RNA fractions stimulated the incorporation of labeled leucine and tryptophan into hot trichloroacetic acid-precipitable material (Table 1). The incorporation was linearly dependent on the amount of added sea urchin poly(A)− and poly(A)+ mRNA. Although the poly(A)− RNA fraction contained substantial amounts of rRNA, its presence neither inhibited nor stimulated the incorporation supported by either class of mRNA (data not shown).

It is known that histone mRNA is the only identified nonpolyadenylated mRNA (27, 28), and that the histone proteins lack the amino-acid tryptophan (29, 30). Therefore, the incorporation of tryptophan in response to the added poly(A)− RNA suggests that this class of RNA does not exclusively code for histone proteins. Translation of these two classes of mRNA in the wheat embryo cell-free system is independent of any tissue specific factor(s). Since this system is capable of initiation and reinitiation (31) as well as release of completed polypeptide chains (21, 22), the amino-acid incorporation supported by poly(A)− and poly(A)+ sea urchin RNA suggests that both fractions are true messengers. Aurintricarboxylic acid, a potent inhibitor of peptide chain initiation, completely inhibits the amino-acid incorporation supported by both poly(A)− and poly(A)+ mRNA (Table 1).

The efficiency of translation of poly(A)− and poly(A)+ mRNA in vitro was estimated by measuring the amount of labeled amino-acid incorporation into protein per unit of lab.
beled RNA. The labeled RNA was separated into poly(A)− and poly(A)+ fractions. It was assumed that the uridine precursor pools for these two RNA fractions are the same, and that both species have the same specific activity. Since the half-life of poly(A)− and poly(A)+ mRNA is the same (36), it is unlikely that the specific activities of the two classes of RNA are different. Table 2 shows that both classes of mRNA stimulated the incorporation of [3H]leucine in vitro into hot trichloroacetic acid-precipitable material to almost the same extent.

The question of whether poly(A)− and poly(A)+ mRNA code for different populations of proteins was examined as follows. Nonpolyadenylated or polyadenylated RNA was translated in the wheat embryo cell-free system using either [3H]leucine or [3H]tryptophan. After translation, the ribosomes were removed by ultracentrifugation. H2SO4 was added to the supernatant (which contains the released polypeptides) to a final concentration of 0.2 M. The reaction mixtures were kept on ice for 3 hr, and then centrifuged at 15,000 × g for 15 min to obtain the H2SO4-soluble and -insoluble fractions. The H2SO4-insoluble fraction was resuspended. Both fractions were then treated with hot 20% trichloroacetic acid. The precipitates were collected and radioactivity was determined. This procedure distinguishes histone-like proteins from other cellular proteins (32). [3H]Leucine incorporation in vitro into H2SO4-soluble and -insoluble material was determined. The data (Table 3) show that these ratios are different for poly(A)− and poly(A)+ mRNA, and indicate that they may code for different populations of proteins. This conclusion is reinforced by the [3H]tryptophan incorporation ratios, which are very different for the two mRNA fractions. Thus, a substantial part of the poly(A)− mRNA may code for nonhistone nuclear proteins.

**DISCUSSION**

Approximately half of the polyribosomal RNA of sea urchin blastulae lacks poly(A). Nemer et al. (9) also found that 50% of the [3H]uridine-labeled polyribosomal RNA is nonpolyadenylated in sea urchin blastulae, and showed that this RNA was not histone mRNA, as judged by size criteria. As much as 30% of HeLa cell mRNA is poly(A)+ (7), and this mRNA has also been detected in slime molds (10). Thus, there appears to be growing evidence suggesting that the histone mRNA is not the only nonpolyadenylated mRNA in eukaryotes (see refs. 27 and 28).

To substantiate the idea that the nonpolyadenylated polyribosomal RNA is a functional message, this RNA was translated in vitro in a messenger-dependent, cell-free protein-synthesizing system. Our data show that poly(A)− polyribosomal RNA of sea urchin blastulae supports the incorporation of [3H]leucine and [3H]tryptophan into polypeptides. This incorporation was directly proportional to the amount of RNA added, and 60-70% of the newly synthesized polypeptides were released from the ribosomes (data not shown). Since histones lack tryptophan, the incorporation of [3H]tryptophan in response to poly(A)+ mRNA suggests that these RNA sequences code for some proteins in addition to histones. Aurintricarboxylic acid completely inhibited peptide synthesis supported by poly(A)+ and poly(A)+ mRNA of sea urchin embryos. This suggests that the nonpolyadenylated polyribosomal RNA is a natural mRNA, and therefore it is unlikely that poly(A)+ mRNA is derived from larger poly(A)+ mRNA molecules by limited endonuclease activity.

Translation of mRNA is not dependent on the presence of poly(A), but does require specific recognition sequences before it can act as messenger (13, 33, 34). We show that naturally occurring poly(A)+ mRNA is translatable in vitro. The efficiency of translation (the amount of amino-acid incorpor-

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**Table 2. Efficiency of translation of poly(A)− and poly(A)+ mRNA in vitro**

<table>
<thead>
<tr>
<th>Source of mRNA</th>
<th>Incorporation of [3H]leucine per 1000 cpm RNA</th>
<th>Incorporation of [14C]uridine per 0.05 ml of RNA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sea urchin poly(A)− RNA</td>
<td>2569</td>
<td>15,958</td>
</tr>
<tr>
<td>Sea urchin poly(A)+ RNA</td>
<td>4306</td>
<td>22,439</td>
</tr>
</tbody>
</table>

Mesenchyme blastulae were labeled with [14C]uridine (0.1 μCi/ml) for 60 min and the poly(A)− and poly(A)+ RNA was prepared from polyribosomes. An aliquot of each RNA was translated in the wheat embryo system as described in Table 1. The radioactivity of an aliquot of the [14C]uridine-labeled RNA was determined.

**Table 3. Differential synthesis of H2SO4-soluble and -insoluble polypeptides by poly(A)− and poly(A)+ mRNA**

<table>
<thead>
<tr>
<th>Source of mRNA</th>
<th>Labeled amino acid</th>
<th>Incorporation into H2SO4-soluble polypeptides (cpm)</th>
<th>Incorporation into H2SO4-insoluble polypeptides (cpm)</th>
<th>Soluble (cpm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sea urchin poly(A)+ RNA</td>
<td>[3H]Leucine</td>
<td>5,548</td>
<td>5,206</td>
<td>1.07</td>
</tr>
<tr>
<td>Sea urchin poly(A)− RNA</td>
<td>[3H]Leucine</td>
<td>3,665</td>
<td>2,247</td>
<td>1.63</td>
</tr>
<tr>
<td>Sea urchin poly(A)+ RNA</td>
<td>[3H]Tryptophan</td>
<td>21,258</td>
<td>21,500</td>
<td>0.99</td>
</tr>
<tr>
<td>Sea urchin poly(A)− RNA</td>
<td>[3H]Tryptophan</td>
<td>15,491</td>
<td>5,532</td>
<td>2.80</td>
</tr>
</tbody>
</table>

Translation of poly(A)− and poly(A)+ mRNA in vitro was carried out as in Table 1 with [3H]leucine or [3H]tryptophan. After incubation the ribosomes were removed and H2SO4 was added to the postribosomal supernatant to a final concentration of 0.2 M. Radioactivity in the H2SO4-soluble and -insoluble material was measured after precipitation with hot trichloroacetic acid. The histone-like proteins are soluble in 0.2 M H2SO4. The amounts of poly(A)+ and poly(A)− mRNA added to each reaction mixture were not identical, and therefore the amounts of amino-acid incorporation reflect the difference in the concentration of mRNA (see Table 1).
rated into polypeptides per unit of mRNA) was essentially the same for poly(A)$^-$ and poly(A)$^+$ mRNA. The implications of this observation are limited, since in the wheat embryo system the mechanisms that regulate the stability of mRNA may not have been operative. Also, since the reaction mixtures were incubated for only 60 min, it is likely that the stability of the mRNA molecules did not enter into these efficiency measurements. Huez et al. (35) have shown that polyadenylated globin mRNA decays more rapidly than the unaltered globin mRNA when these mRNA molecules were injected into Xenopus oocytes and translated in vitro.

That poly(A)$^-$ and poly(A)$^+$ mRNA are two different classes of molecules is indicated by several lines of evidence. The products of translation appear to be distinct based upon acid-soluble/acid-insoluble incorporation ratios with both $[^3]H$leucine and $[^3]H$tryptophan. Based on the solubility data, a major portion of the poly(A)$^-$ mRNA may code for nonhistone nuclear proteins. An analysis of the products synthesized in vitro is in progress. The differences in these two mRNA populations are also suggested by the hybridization data of Nemer et al. (9), who used DNA polymerase I to prepare DNA complementary to poly(A)$^+$ mRNA, and reported a negligible amount of hybridization between the complementary DNA copy and poly(A)$^-$ mRNA. Similar results were obtained with HeLa cells (7). Thus, these experiments suggest that there is little sequence homology between polyadenylated and nonpolyadenylated mRNA molecules.

We thank Drs. G. A. MacLachlan and B. Brandhorst for helpful suggestions in the preparation of this manuscript and Ms. Heidi Lloyd-Price for the gel electrophoresis. We also thank Mr. André Duchastel for technical assistance. This research was supported by grants from the National Research Council of Canada and the Quebec Ministry of Education.