POLYADENYLIC ACID SEGMENT IN POLYRIBOSOMAL RIBONUCLEIC ACID OF CULTURED THYROID CELLS

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1. Introduction

Segments of polyribosomal RNA rich in adenylate residues [poly(A)] have been shown to be covalently bound to messenger RNA (mRNA) of several animal cell types [1–3]. Poly(A) sequences have also been found in the RNA of both DNA [4–5] and RNA viruses [6–8]. These segments appear to be 40 to 250 nucleotides in length and to consist of 90% adenylate [9–11]. Poly(A) segments are attached to mRNA in the nucleus and thus might play a role in transport of mRNA from nucleus to cytoplasm [12]. However, the presence of poly(A) in mRNA of viruses replicating in the cytoplasm [13] has suggested that it can exert a special cytoplasmic function in some translational event(s).

We report here that newly synthetized mRNA isolated from polyribosomes of adult thyroid cells in culture contain poly(A) segments similar to that found in other cell and virus mRNA.

2. Materials and methods

Thyroid cells were isolated by discontinuous flow trypsinization [14] from porcine thyroid glands. Cells (6×10^7) were cultured in 75 cm^2 Falcon plastic flasks for 4 days in the presence of porcine TSH (40 mU/ml). Before labeling, cells were preincubated for 1 hr at 35° in fresh Eagle minimum essential medium containing 20% calf serum and then for 30 min with 40 ng/ml of actinomycin D. After this period, 0.5 mCi/flask of either [^{3}H]adenosine (12 Ci/mmole) or [^{3}H]uridine (20 Ci/mmole) was added and incubation was continued for 4.5 hr. The cells were then rinsed with cold Eagle medium without serum, removed by gentle scraping in the presence of 20 ml cold Eagle medium without serum and centrifuged for 5 min at 500 *g*. Cells were dispersed in 20 ml of 50 mM Tris-Cl pH 8.5, 0.3 M KCl, 10 mM Mg acetate and 1 mM dithiothreitol, pelleted at 500 *g* and resuspended in 4 ml of this medium containing 0.5% Nonidet P_{40} in a Dounce glass homogenizer. After 5 min at 0°, the cells were disrupted by 5 strokes of a tightly fitting pestle. In these conditions, almost all cells were broken but, as shown by light microscopy, nuclei remained intact. Nuclei and mitochondria were eliminated by consecutive centrifugations at 700 *g* (5 min) and 15 000 *g* (10 min). Polyribosomes were obtained by centrifugation of the 15 000 *g* supernatant through layers of 0.5 M and 2.0 M sucrose for 4 hr at 100 000 *g* and 0°. The polyribosomal pellet was rinsed and suspended in a small volume of water. A_{260}/A_{280} ratio was 1.85. Polyribosomal RNA was extracted according to Perry et al. [15]. One volume of 2 × solution containing 10 mM Na acetate pH 6.0, 100 mM NaCl and 1 mM EDTA was added to one volume of polyribosomal suspension and SDS was added immediately to 0.5% final conc. Two volumes of a mixture of water saturated phenol and chloroform (1:1, v:v) were added and shaken at room temp. for 5 min. The aqueous phase was deproteinized two times more in the absence of SDS and RNA was precipitated overnight by ethanol at −20°, centrifuged and dissolved in water. Analysis of labeled RNA was performed in sucrose gradients made in 10 mM Tris-Cl

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pH 7.6, 100 mM NaCl, 1 mM EDTA and 0.05% SDS at 39 000 rpm (Spinco rotor SW 41) for 6 hr at 20°.

For selective absorption of poly(A)-containing RNA, polyribosomal RNA was diluted 5-fold with 500 mM KCl, 10 mM Tris-Cl pH 7.6 and 1 mM $MgCl_2$, filtered (0.5 ml/min) through HAWP Millipore filter presoaked and postwashed with 5 vol of the same solution. RNA retained on filter was extracted with 1 ml 10 mM Tris-Cl pH 9 containing 0.5% SDS and then with 1 ml 100 mM Tris-Cl pH 9.0. The combined extracts were cooled, adjusted to 0.2 M NaCl, carrier tRNA added and RNA was precipitated overnight with 2.5 vol ethanol at −20°. RNA not retained on filter was precipitated in the same conditions but tRNA was omitted. In all cases, one part of labeled polyribosomal RNA was treated with pancreatic RNAase (1.6 μg/ml) and T_1 RNAase (10 U/ml) in 15 mM sodium acetate, 1.5 mM sodium citrate at 37° for 45 min [13]. The mixture was cooled and analyzed on 5–20% sucrose gradient either directly or after deproteinization with phenol–chloroform. Gradient fractions were precipitated with cold 5% TCA, filtered on GF/C glass fiber filter discs, dried and their radioactivity estimated after addition of scintillation mixture in a Packard Tricarb spectrometer.

Electrophoretic analyses were performed in 2.5% acrylamide gels (0.6 × 10 cm) supplemented with 0.5% agarose in Tris-acetate pH 7.2–EDTA–0.2% SDS buffer [16] at 25° for 105 min at 4 mA per tube. Gels were frozen in liquid nitrogen, sliced, dissolved with H_2O_2 and radioactivity estimated as above.

3. Results

In the course of our current investigations on the isolation of thyroglobulin mRNA, we have been interested to know whether polyribosomal RNA of cultured thyroid cells contained poly(A) sequences and, whether these segments were lost during extraction and fractionation of the various classes of polyribosomal RNA as has been noted in some instances [15,2].

Selective absorption on Millipore filter and digestion with RNAases were used to demonstrate the presence of poly(A) segments in polyribosomal RNA.

TSH-stimulated cultured thyroid cells incubated in the presence of actinomycin D (40 ng/ml) were used as a source of tritiated-adenosine or -uridine labeled

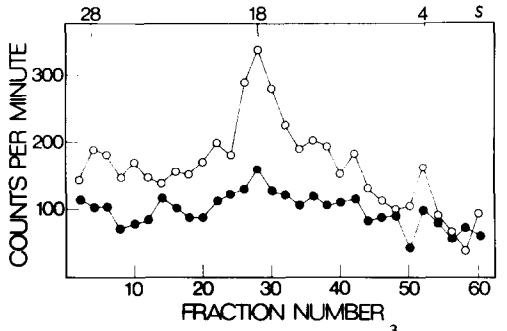

Fig. 1. Sucrose density gradient profiles of [^{3}H]adenosine (○–○–○) or [^{3}H]uridine (●–●–●) labeled polyribosomal RNA of TSH-stimulated porcine thyroid cells in culture.

polyribosomal RNA. This concentration of the antibiotic was shown to inhibit at least 95% of rRNA synthesis in this system [17].

Analysis of total labeled polyribosomal RNA on sucrose gradient showed an heterogeneous population of RNA molecules (fig. 1). After treatment with both pancreatic and T_1 RNAases, all the radioactive peaks of [^{3}H]uridine labeled polyribosomal RNA disappeared, whereas a new about 2–4 S peak appeared in the case of [^{3}H] adenosine-labeled RNA (fig. 2).

About 30% of the radioactivity of polyribosomal RNA was bound to Millipore filter. In some experiments in which a limited action of actinomycin D was observed, 18 S and 28 S ribosomal RNA form the largest part of RNA non retained to Millipore filter. After the action of both RNAases, sucrose gradient centrifugation of bound polyribosomal RNA showed the presence of segments resistant to the RNAases action whereas unbound RNA did not (fig. 3) suggesting that mRNA molecules having poly(A) sequences are specifically bound to membrane filter.

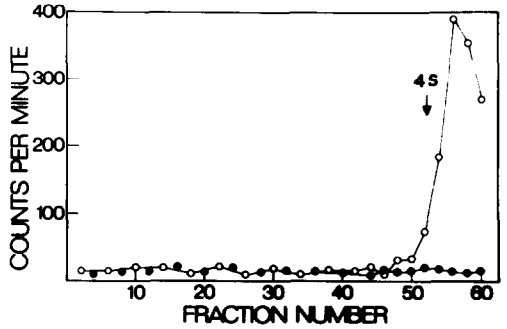

Fig. 2. Sucrose density gradient profiles of [^{3}H]adenosine (○–○–○) or [^{3}H]uridine (●–●–●) labeled polyribosomal RNA after treatment with pancreatic and T_1 RNAases.

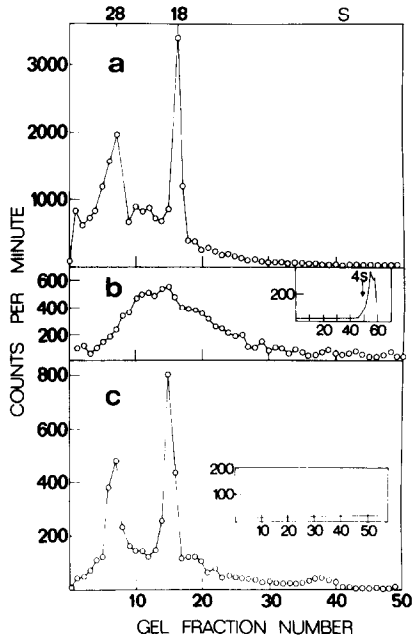

Fig. 3. Electrophoretic pattern of [^{3}H]adenosine labeled polyribosomal RNA: (a) before filtration through Millipore filter; (b) RNA retained on filter; insert, sucrose density gradient profile after treatment with pancreatic and T_1 RNAases; (c) RNA non retained on filter; insert, sucrose density gradient profile after treatment with pancreatic and T_1 RNAases.

It is therefore clear that TSH-stimulated cultured thyroid cells which show all the organizational [18] and the specific metabolic properties of gland follicular cells [19] contain cytoplasmic RNA molecules having poly(A) sequences. It is interesting to note that the average size of the main fractions of these RNA (about 22 S, 18 S and 12–14 S, fig. 1) agrees with the size of mRNA previously found in polyribosomal RNA of whole thyroid glands [20].

Eventually, experiments in progress indicate that both membrane-bound and free polyribosomes contain mRNA molecules having poly(A) sequences.

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