Vol. 87, No. 2, 1979 BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS
March 30, 1979 Pages 461-468

MESSENGER RNA PROPERTIES OF PRIMARY CULTURES OF THYROID CELLS ISOLATED FROM NORMAL THYROID TISSUE AND FROM PATIENTS WITH VARIOUS THYROID DISEASES

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Received January 20,1979

SUMMARY : When compared to cells isolated from normal thyroid tissue, cells isolated from colloid adenoma have the same total polyadenylic acid content and total template activity. However, in both the cells isolated from diffuse non toxic goiter and from toxic adenoma, these two values are increased, the most striking effect being observed in the latter case. Moreover, as compared to normal thyroid tissue, in the three thyroid diseases and particularly in toxic adenoma, we observed an increase in the polyadenylic acid and messenger activity associated to RNA sedimenting at greater than 30 S, which correspond probably to thyroglobulin messenger RNA.

In recent years several studies have been performed on thyroid RNA from mammalian glands and particularly on thyroglobulin messenger RNA and its relation to the subunit of the thyroglobulin molecule (1-7).

Up to date, no data have been published concerning messenger RNA isolated from human thyroid cells in the normal man and in its thyroid diseases.

In the present paper, we have compared some properties of messenger RNA of human thyroid cells cultured from normal thyroid tissue and from diffuse non toxic goiters, "cold nodules" (colloid adenoma) and toxic "hot" nodules. Messenger RNA was characterized by its poly(A)$^+$ tract and by its template activity in the unfractionated RNA preparation and after separation by sucrose density centrifugation. Despite the fact that in tissue culture the capacity of binding for ^{125}I-thyrotropin and the cyclic AMP level were identical in the various diseases investigated (8-9), our results suggest differences in messenger RNA in the various thyroid diseases when compared to normal thyroid cells.

$^+$Abbreviations : Poly(A), polyadenylic acid.
Poly(A) RNA, polyadenylic acid containing RNA.
SDS, sodium dodecylsulfate.

0006-291X/79/060461-08$01.00/0

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MATERIALS AND METHODS

Primary cultures of human thyroid cells were prepared from surgically removed thyroid gland as previously described (10). Diffuse non toxic goiter appeared as an enlargement of the thyroid gland with in some cases presence of nodules. These glands fix normally 131 iodine. Patients with toxic adenoma had hot nodule appearing at the scintigraphy. Patients with colloid- or macrofollicular adenoma- had cold nodule. Histological aspects of the various diseases were those described by Meissner (11). The normal thyroid tissue was the tissue adjacent to a hot or a cold isolated nodule. It was considered as normal on the basis of absence of enlargment of the gland, normal RIA scan and histological examination.

After 4 to 8 days of culture, RNA was extracted from a post-nuclear fraction according to the procedure of Buckingham et al. (12), using 3 ml breaking buffer per 4 10 cm Petri dishes.

Poly(A) content of RNA preparations was estimated by hybridisation reactions with (^{3}H) labelled synthetic polyuridilic acid according to the procedure described by Bishop et al. (13). A linear relationship was obtained between the TCA precipitable radioactivity and the amount of commercial poly(A) (Boehringer, Mannheim, Germany) added in the assay.

The messenger activity of extensively washed RNA preparation was evaluated in a wheat germ derived protein synthetizing system. The preparation of the wheat germ S 30 fraction and conditions of the translation have been described by Marcy and Dudock (14) with the following modifications : each 50 µl reaction contained 2 mM MgAC, 78 mM KCl and 0.5 mM Spermidine (15). Assays and control (no mRNA added) were incubated for 90 min. at 25° C with (^{3}H) leucine (50 Ci/mM, 2.5 µCi/ assay Amersham, England) and (^{3}H) glutamic acid (30 Ci/mM, 2.5 µCi/assay, Amersham, England). The template activity of thyroid RNA was calculated as the difference between TCA insoluble radioactivity in 5 µl assay and control. Unfractionated RNA were tested using 3 µg of RNA. It was controled that this amount was, in each case, in the linear portion of the curve between the template activity and the amount of RNA added in the assay. When the synthetized peptides were submitted to analysis, (35 S) methionine (1100 Ci/mM, 10 µCi/assay, Amersham, England) was used instead of leucine and glutamic acid.

5 % polyacrylamide gel electrophoresis in SDS was performed on glass plates by using the discontinous buffer system of Neville (16). Dog thyroglobulin subunit (Mr 330,000) and reduced myosin (Mr 220,000) were used for markers.

Sucrose density gradient centrifugation of RNA was performed according to Bucingham et al. (12) except that a Beckman SW 39 L rotor was used (4 hours at 34,000 rpm). After centrifugation, 25 to 30 fractions were collected through the bottom of the tube. 20-50 µl aliquots were analyzed for poly(A) content and for optical density. RNA was precipitated from the remainder by the addition of 5 µg carrier tRNA (Sigma Chemical Company, St. Louis, MO) 0.1 vol. 3.0 M Na acetate, pH 5.9 and 2 vol. ethanol, and after extensive washing was analyzed for messenger activity.

The RNA concentration was measured by using an $E^{1\%}_{1cm}$ value of 250 at 260 nm. DNA measurement in the nuclear pellet was performed by the procedure of Mc. Intyre and Sproull (17). Calf thymus DNA (Sigma Chemical Co, St. Louis, MO) was used as reference. DNA was used as reference in our different experiments since in all the cases a linear and identical relationship was found between the DNA content and the number of cells.

TABLE 1. Poly(A) content and template activity of unfractionated RNA extracted from human thyroid cells.

Thyroid cells	Poly(A) [a] ng μg DNA^{-1}	Cpm into proteins [b] μg DNA^{-1}
Normal thyroid tissue (6)	0.64 ± 0.15	2,500 ± 500
Diffuse non toxic goiter (5)	1.38 ± 0.24	5,700 ± 800
Colloid adenoma (4)	0.67 ± 0.17	3,100 ± 550
Toxic adenoma (5)	2.40 ± 0.50	18,900 ± 1,500

RNA was extracted from different types of cells. The number of experiments are in brackets. Poly(A) content (a) and template activity (b) were determinated as described in "Materials and Methods" and calculated as reference to the amount of DNA. Mean average values are given ± 1 SD.

RESULTS AND DISCUSSION

Characterization of the unfractionated RNA

Since it has been well established that in mammalian cells a high proportion of the messenger RNA contains poly(A) sequence (18), we used this property to analyze the unfractionated RNA of cells isolated from normal thyroid tissue and from various thyroid diseases (Table 1a). The poly(A) content of cells expressed as reference to the amount of DNA was similar in both the cells isolated from normal thyroid tissue and in cells isolated from colloid adenoma. It was markedly increased in the diffuse non toxic goiter (2 times) and in the toxic adenoma (4 times).

These increases in the poly(A) content must reflect an increase in the mean lenght of the poly(A) segments attached to the different RNA molecules, differences in the size distribution of the poly(A) RNA (19-20) or an increase in the number of molecules of poly(A) RNA.

Unfractionated RNA has been further investigated for its messenger functional property in a wheat germ derived protein synthetizing system (Table 1b). When compared to normal thyroid cells, the template activity was markedly increased in the toxic adenoma; less striking but consistent increase was observed in the diffuse non toxic goiter.

Therefore, variations in the poly(A) content were in good correlation with variations in the template activity of the unfractionated RNA.

RNA characterization after sucrose gradient centrifugation

When RNA of cells isolated from human thyroid cells were separated in a sucrose gradient centrifugation, poly(A) associated to RNA with sedimentation velocity higher than 30 S was detected.

The relative proportion of poly(A) attached to a particular class of RNA is not only a function of its own lenght and number of molecules but also of the properties of all the other poly(A) RNA species. Therefore, it is indicative of the total poly(A) RNA population metabolism. We compared the relative distribution of poly(A) in a sucrose gradient obtained with RNA of cells isolated from normal and diseased thyroid glands and calculated the percentage of poly(A) associated to RNA sedimenting at greater than 30 S (Table 2a). In RNA of normal thyroid tissue and of diffuse non toxic goiter, very similar results were obtained, whereas in the colloid adenoma and in the toxic adenoma,the values are higher.

When the poly(A) associated to RNA sedimenting at greater than 30 S was calculated as reference to the DNA content (Table 2b), the values are two times higher in cells isolated from diffuse non toxic goiter and in cells isolated from colloid adenoma and 8 times higher in cells isolated from toxic adenoma, when compared to normal human thyroid cells.

These results suggest that cells isolated from colloid adenoma are comparable to cells isolated from toxic adenoma, and cells from diffuse non toxic goiter to cells from normal thyroid tissue in so far as the relative distribution of poly(A) is concerned. Howver, in the diffuse non toxic goiter and in the toxic adenoma, we observe in addition an increase in the total poly(A) content (Table 1a), resulting in a higher amount of poly(A) associated to RNA sedimenting at greater than 30 S.

We have further compared, in cells isolated from the various thyroid glands, the template activity associated to RNA with high sedimentation velocity (> 30 S) using for reference the amount of DNA. A consistent increase is observed both in the colloid adenoma and in the diffuse non toxic goiter and a striking

TABLE 2. Poly(A) and template activity of RNA isolated from human thyroid cells and sedimenting at greater than 30 S.

Thyroid cells	Poly(A) % [a]		Poly(A) ng μg DNA^{-1} x 100 [b]		Template activity [c] cpm μg DNA^{-1}	
	I	II	I	II	I	II
Normal thyroid tissue	11.2	13.4	7.2	6.7	441	1,223
Diffuse non toxic goiter	11.5	15.2	15.9	18.0	1,089	2,144
Colloid adenoma	20.4	22.5	13.7	15.0	1,042	1,609
Toxic adenoma	25.2	28.3	60.5	70.2	3,705	9,034

RNA isolated from normal thyroid tissue and from diseased thyroid glands were fractionated on 5 - 20 % sucrose gradient; fractions were analyzed for poly(A) content and for template activity (see "Materials and Methods"). The poly(A) associated to RNA sedimenting at greater than 30 S was calculated as percent of the total poly(A) content in the gradient (a) or calculated as reference to the amount of DNA (b). The template activity associated to these heavy fractions was expressed as reference to the amount of DNA (c). Table shows the results of two sets of experiments (I and II) using different preparations of RNA and different badges of wheat germ.

increase is obtained in the toxic adenoma as compared to cells isolated from normal thyroid tissue (Table 2c).

The importance of the poly(A) and template activity associated to thyroid RNA sedimenting at greater than 30 S is assessed by the fact that several authors found thyroglobulin messenger activity in this heavy fraction of RNA isolated from animal thyroid glands (1-7) and promoting the synthesis of thyroglobulin subunit with 300,000 Mr (1,2,4,6,7). It is therefore likely that RNA sedimenting at greater than 30 S represent thyroglobulin messenger RNA and an increase in the template activity associated to this fraction could be correlated with an increase in the 300,000 Mr peptide synthesis. The products of translation were analyzed by polyacrylamide gel electrophoresis in denaturating conditions and we investigated the amount of radioactivity migrating in the 300,000 mol. wt region of the gel. Table 3 shows two typical experiments with unfractionated RNA of cells isolated from toxic adenoma and from the surrounding diffuse non toxic goiter. Starting with identical amounts of TCA preci-

TABLE 3. 300,000 Mr peptide synthesis by RNA isolated from human thyroid cells.

Thyroid cells	cpm incorporated into 300,000 Mr peptide	
	Experiment 1	Experiment 2
Diffuse non toxic goiter	1,067	605
Toxic adenoma	6,211	1,806

Unfractionated RNA of cells isolated from toxic adenoma and from the surrounding diffuse non toxic goiter were assayed in a wheat germ derived protein synthetizing system. Aliquots of the (35 S) methionine labeled synthetized peptides (100,000 cpm TCA precipitable in each case) were reduced by 2-mercaptoethanol in the presence of SDS and urea and analyzed on SDS polyacrylamide gel electrophoresis (see "Materials and Methods"). Gels were cut and the radioactivity associated to peptides migrating in the 300,000 mol wt region was counted. The results of two separate experiments using different preparations of RNA are given.

pitable radioactivity, a net increase in the messenger activity for thyroglobulin like peptide was observed in the toxic adenoma when compared to the diffuse non toxic goiter, which was correlated positively with the increase in the poly(A) and template activity associated to the heavy fraction (> 30 S). However, using DNA complementary to thyroglobulin mRNA as a probe will allow to elucidate whether this increase is really correlated with difference in the number of thyroglobulin mRNA sequences.

It is a striking fact that despite the impairment in the thyroid hormones production observed in vitro in diffuse non toxic goiter (21-22), in the benign solid "cold" nodule (23-25) and in the "hot" nodule (24-25), we observed in the three thyroid diseases an increase in the poly(A) and template activity associated to RNA sedimenting at greater than 30 S.

ACKNOWLEDGEMENTS

We are indebted to Prof. F. Gros and Dr. R. Whalen, Institut Pasteur, Paris, who initiated us to the technics of RNA chemistry; we are grateful to Prof. J. Gielen and Drs. A. Belajew and R. Kaufman, Laboratoire de Chimie Médicale, Liège, for providing us the translation cell free system. We thank Prof. A. Nizet for his continous interest during the course of this work.

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