PURIFICATION AND SOME PROPERTIES OF SHEEP THYROID AND RAT LIVER MESSENGER RNAs

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Several attempts to purify m-RNAs have already been made mostly using bacterial cells (1, 2). Experiments using Hela cells (3), red blood cells (4-7), mammalian liver tissue (8-11) and thymus (12) have lead to the isolation of RNA preparations, the messenger character of which was detected by DNA-like base composition, competence to stimulate amino acid incorporation into proteins or hybridization with homologous DNA (13, 14).

This note summarizes results obtained in the purification of sheep thyroid and rat liver RNAs. The stimulation of incorporation of L-leucine-^{14}C into protein material by a cell-free extract from E.coli was used as a test for m-RNA activity. E.coli RNA was used a reference material.

Methods and material : Sheep thyroid glands were collected at the local slaughter-house, freed of fat and connective tissue, immediately frozen (-60°), ground in a mortar in the presence of dry ice, rapidly thawed, prehomogenized in the convenient medium (1 : 5), in the Servall omnimixer (4,000 RPM) for 1min and then homogenized in a loosely fitting glass-teflon homogenizer (one stroke at 1,000 RPM). Rat livers were treated in the same way. Cytological control revealed 95 % broken cells and 90 % intact nuclei. The methods proposed by Barondes et al (9), DiGirolamo et al (15) and Braverman et al (10) to purify m-RNA from rat liver were also applied to thyroid tissue starting with 500 g sample of an homogenate or with purified nuclei. The method of Scherrer and Darnell (3) described for Hela cells was used for thyroid or liver nuclei and for E. coli. Differential phe-

nol extraction of RNA with respect to temperature was done according to Georgiev et al (11) starting with 600 g sample of thyroid or rat homogenates. The method of Nirenberg and Matthaei (1) was followed for the preparation of E.coli (ML 308) extracts (preincubated, DNAase treated, S 30 fraction) and for reaction mixtures (with the addition of ammonium acetate) used for determining L-leucine-^{14}C incorporation into proteins. The test volume was 0,25 ml. Specific stimulatory activity (S.A.) is expressed as µmole leucine incorporated in 45min per mg RNA per mg S 30-proteins.

Results and Discussion. E.coli RNA prepared according to Scherrer and Darnell exhibited a better stimulating activity in the E.coli system than RNA extracted by phenol at low temperature. Total RNA from E.coli was twice as active as ribosomal RNA, exhibiting a S.A. of 300 to 500 and the quantity of leucine-^{14}C incorporated into proteins increased linearly with the amount of RNA between 0.1 and 1 mg concentration per ml of reaction mixture. RNAs were extracted by Scherrer and Darnell's method from subcellular fractions of liver or thyroid homogenates : maximal yield of stimulatory RNA was found for both tissues in the nuclear fraction (table I).

Fig.1 shows results obtained with rat liver and sheep thyroid nuclear RNA prepared by the methods of Barondes et al, Hiatt and Scherrer et al, all using SDS at various final concentrations (respectively 0.1, 1 and 0.5 %). Results obtained with RNAs extracted by the method of Braverman et al are also shown but it should be noted that they concern the fraction extractible at pH 7.6 while RNA obtained by extraction at pH 8.8 was less active.

Table II summarizes the yields, specific activities and some physical constants of these RNA preparations. Rat liver RNA prepared according to Hiatt or Barondes et al exhibits a higher stimulating activity than sheep thyroid RNA but the extraction yield is low. RNA extracted from both tissues by Scherrer's method stimulated incorporation to the same extent but the extraction yield is better.

The use of thermal fractionation in the last method incited us to check a more elaborate process of phenol extraction at different temperature. This was done according to Georgiev et al (11) starting with nuclei (600 g fraction of a 1:5 homogenate

Nature of fractions	mg RNA extracted from 100g wet tissue (1)	specific activity (2)	total stimulating activity (1)x(2)
Nuclear sheep thyroid	100	186	18,600
Nuclear rat liver	183	171	31,300
Sheep thyroid mitochondria	35	46	1,611
Rat liver mitochondria	4	42	168
Sheep thyroid microsomes	6	123	738
Rat liver microsomes	23	69	1,590
Sheep thyroid 75,000g supernatant	3	8	24
Rat liver 75,000g supernatant	36	4	144

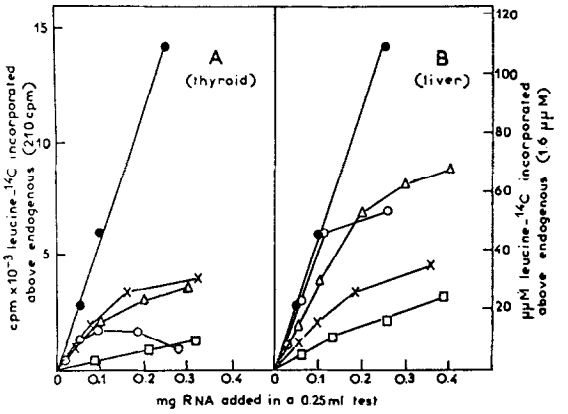
Table I. Localization of stimulating RNAs in the subcellular fractions of rat liver and sheep thyroid : 100 g wet tissue are extracted by the hot phenol method. Specific activity is calculated from stimulation obtained by 0.1mg RNA in the test.

Extraction method	Tissue or bacteria	mg RNA extracted from 100g wet tissue or bacteria	260/280	specific activity	Sedimentation*
Barondes et al	Rat liver	2	1.93	406	30S, 12S
	Sheep thyroid	4	1.90	136	30S, 12S
Hiatt	Rat liver	4	1.85	290	10S
	Sheep thyroid	3	1.85	163	10S
Braverman et al	Rat liver	100	1.75	85	28S, 16S
	Sheep thyroid	115	1.76	35	28S, 16S
Scherrer et al	Rat liver	183	1.80	171	18S, polydisperse
	Sheep thyroid	100	1.80	186	18S, polydisperse
	E. Coli	3,000	2.05	460	23S, 16S, 4S

Table II. Specific activity, extraction yield, optical and sedimentation properties of rat liver and sheep thyroid nuclear RNAs prepared by various methods. The same properties of E.coli total RNA are also given.
* Value of S for the main (s) fraction (s). The major component is expressed by underlining the S value.

in 0.14 M NaCl) extracted repeatdly with phenol at pH 6.0 and 4°, the interphase beeing successively extracted with phenol at 45°, 55° and 65°.

Fig.2 shows typical stimulating activity of rat liver and sheep thyroid nuclear RNA obtained in this way. Further purification with phenol-SDS and further precipitation with 2.5 M



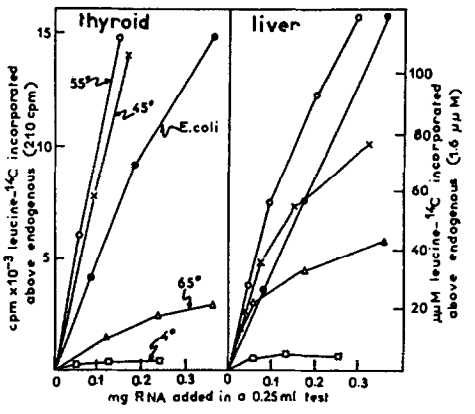


Fig.1 (left)- Stimulation of leucine-^{14}C (S.A. : 100 uC/uM) incorporation into protein by sheep thyroid (A) and rat liver (B) nuclear RNAs prepared according to Barondes et al (O), Scherrer et al (X), Hiatt (Δ) and Braverman et al (□). Results obtained with total E.coli RNA (●) prepared according to Scherrer et al are also indicated.

Fig.2 (right)- Stimulation of leucine-^{14}C incorporation into protein by sheep thyroid and rat liver RNAs prepared by thermal fractionation at 4° (□), 45° (X), 55° (O) and 65° (Δ). Stimulation obtained with total E.coli RNA is also indicated. The 3 numbers in the brackets correspond respectively to : mg RNA extracted from 100 g tissue, A 260/A 280 ratio and S.A. of each fraction.

A : sheep thyroid RNA
4° (56 ; 2.05 ; 23), 45° (3.2 ; 1.92 ; 615)
55° (2.6 ; 1.90 ; 730) and 65° (4 ; 1.85 ; 77)

B : rat liver RNA
4° (125 ; 2.00 ; 42), 45° (10.3 ; 1.95 ; 410)
55° (8 ; 1.89 ; 570) and 65° (4.5 ; 1.87 ; 260)

NaCl has been omitted. The 55°-RNA fraction is always the most active and has a S.A. higher than the S.A.of total E.coli RNA. The stimulation is linear between 0.1 and 0.8 mg/ml of incubation mixture. The 4° fraction is always practically inactive. The other fractions (45° and 65°) are also stimulatory but to a lesser extent than the 55° fraction. No protein (or DNA) is present except in the 65° fraction (5 % DNA).

Fig.3 shows the centrifugation pattern of the thyroid RNA fractions ; r-RNA and t-RNA are evidently only present in the 4° fraction, the other ones disclosing a pattern of polydis-

persity. Similar results are obtained with liver RNA fractions. When spun for a shorter time, the combined 45° and 55° fractions of thyroid RNA show the presence of a large zone the sedimentation coefficient of which is higher than 30. After pooling the tubes into 3 fractions as indicated in the figure, RNA was isolated and tested for stimulation of protein synthesis. High-molecular weight fraction 1 had the highest activity, 2- and 3- times higher than fractions 2 and 3 respectively and of the same order of magnitude as the unfractionated material.

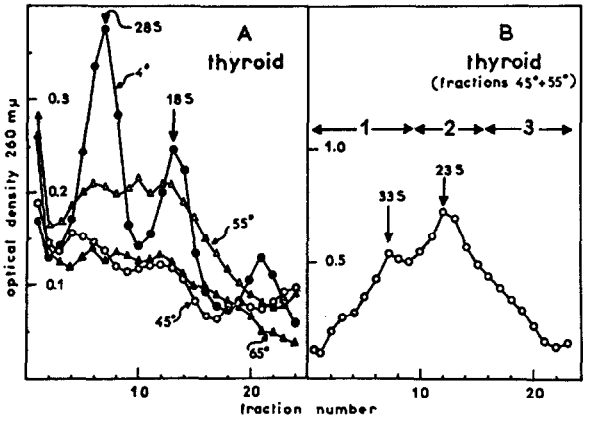


Fig.3- Sucrose density gradient centrifugation of sheep thyroid nuclear RNA prepared by phenol fractionation.
A- thyroid RNA obtained at 4° (●), 45° (○), 55° (△), and 65° (▲) ; 0.3 mg RNA centrifuged on a 20-5 % sucrose linear gradient containing 10^{-1} M NaCl, 10^{-2} M acetate buffer pH 5.0 for 14 hr at 23.000 RPM in the SW 25 rotor of Spinco L ; 0.5 ml fractions collected.
B- thyroid RNA obtained at 45° + 55° ; 1 mg RNA spun at the same speed but for 10 hr.

Thermal extraction in the presence of phenol of liver or thyroid nuclei enabled us to carry out a differential purification of m-RNA fractionsin which a very high stimulating activity was associated with high-molecular weight components (30 S) and which were essentially free of r-RNA, t-RNA and DNA. A detailed report and discussion will be published elsewhere.

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