HIGH MOLECULAR WEIGHT DNA-LIKE RNA IN RAT THYROID

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Thyroid glands provide an adequate material to study the relationship between messenger RNA and the biosynthesis of specific proteins in animal cells. It is shown here that very "heavy" DNA-like RNA (dRNA) can be isolated from fetal rat thyroid glands maintained in organ culture.

MATERIAL AND METHODS : Thyroid glands from 21 day CF Wistar rat fetuses, were cultured by a modified watch glass method (Elias and Rivera 1965). Each culture dish contained 1 ml of medium NCTC 109 on which a piece of dacron organdy was floated to support 2-4 isolated thyroid lobes (Nataf, Rivera and Chaikoff 1965). The dishes were incubated as described elsewhere (Nataf, Malaise and Tubiana 1966) for 48 hours, 24 hours before the end of each experiment ^{32}P (100 µc/ml) was added to 10 dishes, in 3 experiments ^{131}I (50 µc/ml) was added to one other dish.

^{131}I measurements : Explants were washed and homogenized in 0.3 ml of NaCl 0.14 M, Tris 0.05 M. Total ^{131}I incorporation was measured on an aliquot of the homogenate. The rest of the homogenate was digested with pronase and chromatographed, to separate the various labelled components (Tong, Raghupathy and Chaikoff 1963). Radioautograms were prepared from the dried chromatograms. Sections of the chromatograms corresponding to the radioautographic bands were cut out and counted in a scintillation counter.

RNA preparation : Explants were frozen at - 76°C and homogenized at 2°C in 2 ml of phosphate buffer (PO^4) 0.002 M, $MgCl^2$ 0.002 M with bentonite. RNA which will be refered to as "Phenol-RNA" was extracted by 4 successive phenol treatments. The initial interphase was washed with PO^4 0.002 M, suspended overnight in 80 % ethanol, 20 % NaCl 0.2 M, collected and incubated for 2 hours at 37°C in 2 ml of PO^4 0.01 M, NaCl 0.2 M with 4 mg of pronase. RNA which will be refered to as "Interphase-RNA" was extracted with 1 % SDS and phenol and precipitated with ethanol and Sodium citrate (up to 0.1 M) or carrier RNA from mouse cells. Precipitates were dissolved and dialyzed with bentonite (20 µg/ml) at 2°C, against PO^4 0.002 M, $MgCl^2$ 0.002 M for 48 hours. RNA was freed from DNA either by coprecipitation with carrier rRNA in $MgCl^2$ 0.2 M (Harel et al. 1964), or as indicated in the comment to Fig. 3. RNA amounts were estimated by measuring U.V. absorbancy. Radioactivity was counted on aliquots in a low back ground flow counter. Centrifugations were performed in glycerol gradients (Huppert et al. 1966). Labelled fractions, the S values of which were estimated (Nomura, Hall and Spiegelman 1960), were precipitated with carrier RNA and their base composition determined as previously described (Harel et al. 1963). In a "chase" experiment, after labelling 24 hours, half of the explants were frozen, the others were reincubated for 8 hours in non radioactive medium plus PO^4 (up to 0.01 M).

Experiment N^r		1		2		3	
Per cent ^{131}I uptake		0.51	0.47	1.33	1.34	0.57	0.45
Per cent ^{131}I distribution on chromatograms	origin	2.2	2.0	2.9	2.5	2.6	2.0
	MIT	35.1	35.0	43.6	49.0	41.1	38.6
	DIT	17.2	17.4	28.0	26.4	15.5	23.5
	T4	1.6	0.8	1.8	1.6	1.2	1.4
	T3	0.1	0.2	0.2	0.2	0.3	0.2
	I	42.2	47.0	22.9	19.7	38.5	33.9
	Front	1.0	0.6	0.6	0.6	0.8	0.4

Table 1 : ^{131} measurements (2 déterminations in each experiment).

RESULTS : Table I shows that fetal glands cultured in a purely synthetic medium are able to metabolize ^{131}I. However the amounts of T 4 synthezised in the present conditions were quite low as compared to the results obtained with another medium containing insulin and, or TSH (Nataf and Chaikoff 1965).

RNA yields : In 2 experiments both the amount and the specific radioactivity of carrier free thyroid RNA could be estimated (a : 60 µg-10, 500 cpm/µg for phenol-RNA, 10 µg-11, 400 cpm/µg for Interphase-RNA. b : 49 µg-11, 700 cpm/µg for Phenol-RNA, 11 µg-12, 000 cpm/µg for Interphase-RNA). In 4 other experiments radioactive Interphase-RNA represented 14 % - 23 % of total extracted RNA. The 8 hours chase resulted in a decrease in radioactivity of 20 % for Phenol-RNA and 63 % for Interphase-RNA.

Phenol-RNA represented uniformly labelled rRNA and sRNA as shown by gradient centrifugation (Fig. 1) and base composition (Table 2). A very small proportion sedimented above 60 S.

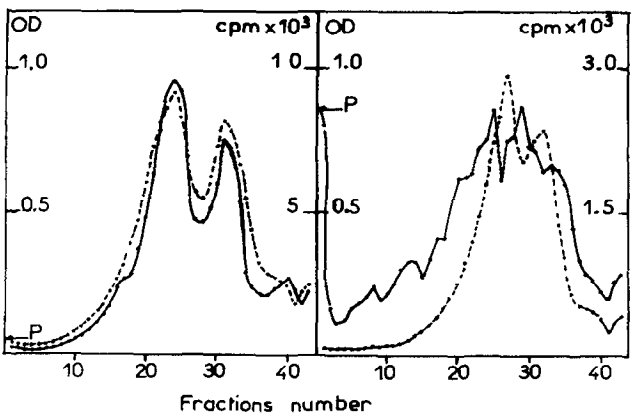

Fig. 1 (left) : Labelled Phenol-RNA centrifuged in 5-20 % glycerol gradient, PO_4 0.01 M at 39, 000 rpm for 210 minutes in the SW 39 of Spinco ultracentrifuge.

Fig. 2 (right) : Labelled Interphase-RNA centrifuged in 5-30 % glycerol gradient, PO_4 0.01 M for 150 minutes. Solid lines : radioactivity, dotted lines : A 260 of carrier RNA, P : cpm in pellets.

	RNA Fraction		Moles per cent C	A	G	U	$\frac{A+U}{C+G}$
PHENOL-RNA	25 - 30 S		28.2	19.0	34.6	18.2	0.59
	sd		0.3	0.3	0.4	0.3	0.010
	15 - 20 S		26.1	22.9	29.1	21.9	0.81
	sd		0.4	0.3	0.25	0.2	0.016
	s RNA		28.0	19.3	31.3	21.3	0.68
	sd		0.6	0.5	0.3	0.2	0.012
INTERPHASE-RNA	90 S	1	23.6	24.0	24.7	27.8	1.07
		2	23.4	24.0	22.6	30.0	1.17
		3	22.0	26.1	25.0	26.9	1.13
		4	22.3	22.3	25.0	30.5	1.12
	60-80 S	1	23.7	24.6	24.6	27.1	1.07
		2	21.2	26.0	25.3	27.5	1.15
		3	22.1	27.2	23.6	27.1	1.19
	45-60 S	2	22.4	21.7	29.7	26.2	0.92
		3	25.0	24.1	26.4	24.1	0.95
	30-45 S	1	25.7	20.3	27.9	26.1	0.87
		2	22.5	20.5	29.8	27.2	0.91
		3	23.9	22.9	29.1	24.1	0.89
		4	23.5	21.1	27.9	27.5	0.95
	20-30 S	1	21.8	25.3	29.1	23.8	0.96
		3	25.4	22.5	28.1	24.0	0.87
		4	23.5	22.8	26.8	27.0	0.99

Table 2 : Base composition of ^{32}P Phenol-RNA (means of 4 experiments, sd : standard deviation) and ^{32}P Interphase-RNA (1, 2, 3, 4 : experiment number).

Interphase-RNA : A major proportion sedimented between 20-45 S (Fig. 2, 3) and had a G-C content lower than that of rRNA (table 2). Heavier fractions $>$60 S, represented 15-25 % of total Interphase-RNA and were distinctly more DNA like. From experiments such as that reported in Fig. 3, high S values did not seem to correspond to RNA associated with DNA or proteins, or to RNA aggregates.

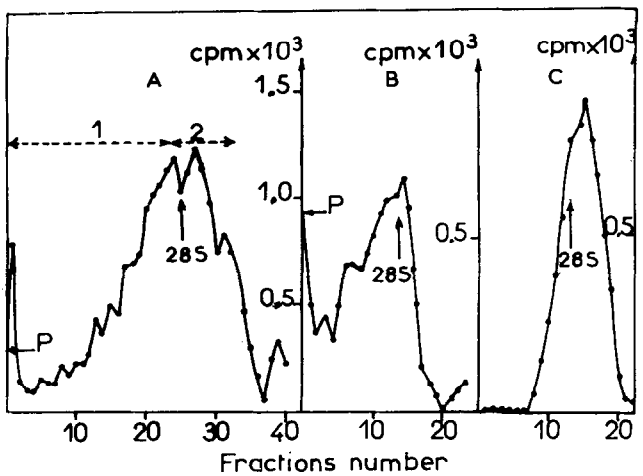

Fig. 3 : A Interphase-RNA treated with DNAse (2 µg/ml, 30 minutes) and pronase (100 µg/ml, 60 minutes), reextracted with phenol, centrifuged as in Fig. 2. Pooled fractions were precipitated with citrate and ethanol, dialysed ovenight against PO_4 0.01 M, EDTA 0.01 M. B : fraction 1, C : fraction 2, centrifuged in 5 - 30 % glycerol, EDTA 0.01 M for 225 minutes.

DISCUSSION : From the present study it appears that Phenol-RNA consists mostly of cytoplasmic RNA, and Interphase-RNA represents nuclear RNA (in agreement with Georgiev and Mantieva, 1962). Base composition indicate that in the later, fractions 30-45 S may correspond to a mixture of dRNA and rRNA precursors (Scherrer, Latham and Darnell 1963) and that heavier ones mostly represent dRNA. The relative proportion and G-C content of the various fractions permit the estimation that 50 - 60 % of nuclear RNA and 8 - 12 % of total RNA consisted of dRNA. It is noteworthy that in conditions which allow the synthesis of only a small amount of thyroxin, heavy nuclear dRNA, presumably polycistronic, was found. Pulse labelled heavy dRNA has been isolated from growing culture cells (Yoshikawa, Fukada and Kawade 1965. Warner et al. 1966), and from duck erythroblasts (Scherrer et al. 1965). Nuclear RNA (30 S) which stimulated incorporation of amino acids "in vitro" has been extracted from sheep thyroid (Cartouzou, Manté and Lissitsky 1965). Further comparative study of RNA and ^{131}I metabolism, might contribute to a better knowledge of polycistronic dRNA.

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