RNA REPLICATION BY NUCLEAR SATELLITE DNA IN DIFFERENT MOUSE CELLS

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A "light" nuclear satellite DNA (LS-DNA), amounting to 10 p.cent of the total DNA in all mouse cells, and consisting of many repetitive sites, has been isolated by isopycnic centrifugation (Kit 1961, Flamm et al, 1966, Waring and Britten 1966, Bond et al 1967). Flamm et al (1967) have separated its complementary strands which contain 45 p.cent thymidylic acid (T) or deoxyadenylic acid (dA) respectively. It is shown here that rapidly labeled RNA of high molecular weight, from liver, kidney and L cells, is hybridizable with purified LS-DNA. Experiments with ^{32}P nuclear RNAs from L cells which had a high U content, and both separated strands of LS-DNA, indicated that only the "dA rich" strand seems to be replicative.

MATERIALS and METHODS. "Interphase" (i) RNA labeled with ^{32}P was extracted from liver, kidney and cultivated L cells as described elsewhere (Hanania and Harel, 1968, a,b). Nuclear (n) RNA was prepared by the method of Penman (1966). All RNAs were fractionnated by gradient centrifugation and their base composition was determined as indicated before (Harel et al 1963).

DNA was extracted from mouse liver or from nuclei of FLS ascites tumor cells (Lacour et al 1960) by the procedure of Marmur (1961), treated with ribonuclease (RNase) and pronase (Gillepsie and Spiegelman 1965) and reextracted with phenol. Purified DNA was dissolved (0.6-1 mg/ml) in 0.1 x SSC (1xSSC=0.15 M NaCl, 0.015 M Sodium citrate), denatured by heating for 10 min. at 100°C, followed by quick cooling at 0°C, thereafter "renatured" by adjusting the solution to 2xSSC and incubating for 2 hr at 66°C. DNA was dialyzed 24 hr at 2°C against 0.1 M Tris, pH 8.2, and centrifuged twice in CsCl by the method of Flamm et al. (1966), modified as indicated in the comment of Fig. 1. Purified LS-DNA and the main DNA band (M-DNA) were dialyzed 48 hr against 0.1xSSC. Both strands of LS-DNA were separated by centrifuging 120 µg at pH 12.8 (Flamm et al, 1967). The "heavy" fractions (T strand) and "light" fractions (dA strand) were pooled separately and dialyzed 48 hr against 2xSSC. Analytical ultracentrifugations of DNA were carried out (in

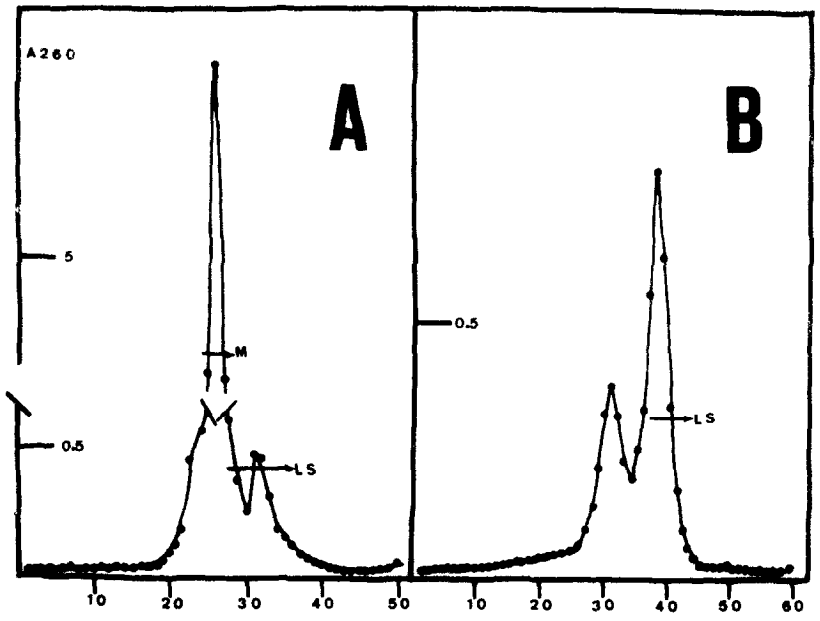

Fig.1. Fractionnation of DNA from mouse tumor cells. A : First centrifugation CsCl (Merck) was added to 35-50 ml of the DNA solution (12.785g per 10 ml). Aliquots of 10.5 ml were distributed in 4-6 tubes of the Spinco L 40 fixed angle rotor. The tubes were completed with paraffin oil and centrifuged at 33,500 rpm, for 25 hr at 20°C. Fractions were collected from the bottom of each tube (bottom at left in the graph) and measured at 230,260 and 280 mμ. Those fractions delimited by horizontal arrows were pooled, dialyzed 24 hr against 0.1 M Tris and recentrifuged in CsCl. B: Second centrifugation of LS-DNA.

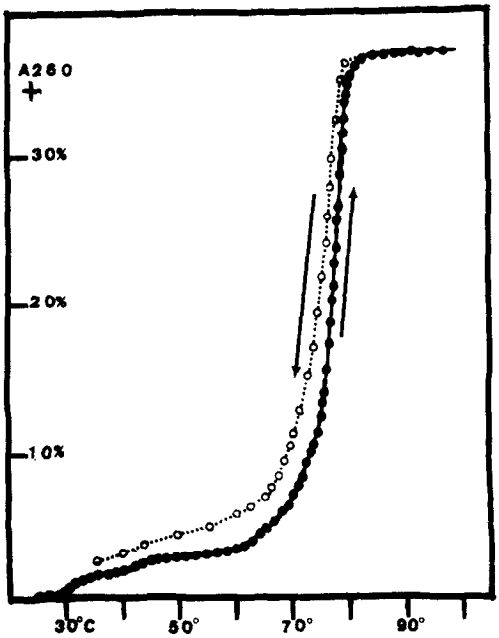

Fig. 2. Thermal denaturation of LS-DNA (full circles), followed by renaturation (empty circles), was studied in a Zeiss spectrophotometer, using a 1 cm path stoppered cell and 20 μg/ml (0.4 A 260 unit) of reannealed LS-DNA in 0.15 M NaCl. The heating device insured a temperature increase of about 3°C per min. After switching it off, it took 50 min to cool the cell from 98°C to 35°. Hyperchromicity expressed in p.cent of the initial OD (at 23°C). Tm : 77.5°C.

CsCl solution of density : 1.700 g cm3) under the conditions specified by May et al (1966). Thermal denaturation was studied as shown in Fig. 2.

RNA DNA hybridization assays were performed with DNA trapped on nitrocellulose membranes (see table 1) or in liquid (see table 3).

RESULTS. DNA fractionnation by the first centrifugation in CsCl is shown in Fig. 1A. Similar results were obtained with 5 preparations of liver DNA and 2 preparations of FLS tumor DNA. Modification of the method of Flamm et al (1966) offered several advantages : DNA bands appeared sharper, greater quantities were prepared in a same run, and the centrifugation time could be decreased to 25 hr, since no better resolution was observed when centrifuging 48 hr or more. LS-DNA amounted to 10-11 p.cent of the total DNA in both liver and tumor cells. The second centrifugation (Fig.1B) seemed to provide highly purified LS-DNA. When similarly recentrifuged, M-DNA appeared as a single peak with an occasionnal tiny shoulder.

The purity of LS-DNA was verified by analytical ultracentrifugation in CsCl, which confirmed that its apparent buoyant density is 0.014g cm3 less than that of M-DNA (see Bond et al, 1967) and by spectrophotometric studies (Fig.2). Reannealing of LS-DNA during the incubation at 66°C, which preceded DNA fractionnation, was evidenced by a 36 p.cent increase in A 260 and a sharp thermal transition in the course of a second thermal denaturation. Its subsequent thermal renaturation was attested by the proximity of the hypochromicity and hyperchromicity curves. In contrast, M-DNA showed the usual characteristics of denatured bulk animal DNA : small hyperchromicity (12 p.cent) and broad thermal transition.

RNA DNA hybridization. Using relatively great amounts of both DNA and RNA (table 1), rapidly labeled iRNA from liver, kidney and L cells, which sedimented

^{32}P for :	Input iRNA From	µg	cpm	^{32}P iRNA fixed by 10 µg: E Coli DNA cpm	Mouse liver DNA M-DNA cpm	LS-DNA cpm	(+)
120 min	liver	88	55,000	53	270	321	(124%)
120 min	kidney	65	11,200	18	144	182	(130%)
30 min	L cells	20	65,300	31	396	647	(169%)
24 hr	L cells	10	211,000	52	1495	778	(50%)

Table 1. Hybridization of mouse 32P iRNAs (> 20S) with M-DNA or LS-DNA from mouse liver. DNA denatured at pH 12.8 and trapped on nitrocellulose filters (Gillepsie and Spiegelman 1965) was incubated for 30 hr at 66°C with RNA in 1.5 ml of 4xSSC. Each filter was washed twice at 60°C (by keeping it for 30 min in 20 ml of 2xSSC), thereafter incubated for 30 min at 37°C with 40µg of pancreatic RNase (Worthington) in 2 ml of 2xSSC, washed again, dried up on a planchette and counted for 10-30 min in a low back ground (0.3 cpm) Tracerlab counter. (+): Annealing rates expressed in p.cent of cpm fixed by M-DNA after deduction of the control (E Coli) value.

above 20 S and contained "messenger" like (m) RNAs (Hanania and Harel, a), hybridized more efficiently with LS-DNA than with M-DNA. In contrast uniformly labeled iRNA hybridized more efficiently with M-DNA.

Other experiments were carried out with smaller quantities of each separated strand of LS-DNA and fractionnated ^{32}P L-cell nRNA which had a sufficient specific radioactivity, and a base composition intermediate between those of mRNAs and ribosomal RNAs, with a hig U content (table 2).

^{32}P for:	nRNA fraction	Moles per cent C	A	G	U	$\frac{U}{A}$
90 min	> 40 S	25.2	16.9	31.5	26.4	1.57
	30-40 S	26.0	18.1	28.4	27.5	1.53
	20-30 S	24.2	21.6	27.0	27.2	1.26
	5-10 S	22.0	19.1	30.1	28.8	1.50
24 hr	30-40 S	25.4	18.7	32.1	23.8	1.27
	5-10 S	22.4	23.9	26.2	27.5	1.15

Table 2. Base composition of different fractions of ^{32}P L-cell nRNA separated by sucrose gradient centrifugation. C: cytidylic, A: adenylic, G: guanylic, U: uridylic acids.

All rapidly labeled RNA fractions, hybridized at different rates with both M-DNA, and the "dA strand" of LS-DNA, but not with the "T strand" A high molecular weight 24 hr labeled fraction hybridized with M-DNA, and less efficiently with the "dA strand", whereas a low molecular weight fraction hybridized only with M-DNA (table 3).

^{32}P for :	Input mRNA Fraction	µg	cpm	^{32}P nRNA fixed by : control cpm	4µg M-DNA cpm	2µg LS-DNA dA strand cpm	(+)	T strand cpm	(+)
90 min	> 40 S	3	16,200	16	125	60	(40%)	14	(0%)
	30-40 S	10	32,000	5	109	66	(59%)	6	(1%)
	20-30 S	3	9,300	2	46	19	(39%)	1.5	(0%)
	id	10	31,000	10	123	49	(34%)	12	(2%)
	5-10 S	10	34,500	14	112	45	(32%)	14	(0%)
24 hr	30-40 S	5	135,000	103	740	271	(26%)	79	(0%)
	5-10 S	5	127,000	62	995	67	(0.5%)	81	(2%)

Table 3. Hybridization of fractionnated ^{32}P L-cell nRNA with M-DNA or each separated strand of LS-DNA, from tumor cell nuclei. DNA was added to RNA in a final volume of 1 ml of 4xSSC, incubated for 1 hr at 66°C and thereafter loaded on nitrocellulose filters. Filters were treated with RNase, washed and counted for 10-120 min, as indicated in the comment of table 1. Controls : no DNA or E coli DNA. (+) : P.cent rates expressed as shown in table 1. Means of 2 samples not differing by more than 2-6 p.cent.

DISCUSSION of the present data must envisage several misleading possibilities. Fixation of ^{32}P RNA by LS-DNA might be due to residual M-DNA or to other contaminants, radioactive impurities being excluded, because in each RNA preparation all of the ^{32}P was acid precipitable and located in the ribonucleotides. As shown in table 1 purified LS-DNA fixed greater quantities of "pulse" labeled iRNAs, as compared with M-DNA, obviously such results could not be attributed to tiny amounts of residual M-DNA. Random fixation of RNA by contaminants such as basic proteins was unprobable, since the chemical purity of LS-DNA has been previously investigated (Flamm et al, Waring and Britten) and we used similar methods of purification. Anyhow random fixation could not explain why, rapidly labeled RNAs hybridized with LS-DNA more efficiently than 24 hr labeled RNAs and why a low molecular weight fraction of the latter RNAs was not linked to LS-DNA, although it hybridized with M-DNA. Furthermore, annealing of LS-DNA with ribosomal RNA was unsignificant as compared with that of heavier DNA fractions (Tapiero et al, in preparation).

A recent finding suggested another possibility : in L cells a very small amount of cytoplasmic DNA which had a buoyant density close to that of LS-DNA, represented DNA from mycoplasma (Pratt et al, 1968). If assuming that all our animals and cell cultures were similarly infected, microbial nucleic acids corresponding to a negligeable fraction of the total, could presumably not provide hybridization figures greater still than those obtained with mRNAs from the host cells. Although the specific radioactivity of mycoplasmic RNA could much exceed that of mouse RNAs, "pulse" labeling of animal cells being equivalent to long term labeling of microorganisms, radioactive microbial RNAs would mainly consist of stable RNA which anneals with DNA much less efficiently than mRNAs.

Finally, the results of experiments using L-cell nuclear RNA and both separated strands of LS-DNA seemed to exclude any interference of cytoplasmic agents. It is tempting to assume that the high U content of rapidly labeled nRNAs from L-cells, was due to many "U rich" RNA sequences complementary to "dA rich" sites of the replicative strand of LS-DNA.

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