MYELOMA TUMOR SATELLITE DNA: A Role in Ribosomal RNA Synthesis*

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Satellite DNA components have been described in association with chloroplast-containing cells of Euglena gracilis (Brawerman & Eisenstadt, 1964; Ray & Hanawalt, 1964; Edelman et al., 1964) while mutants of Euglena which lack the capacity for chloroplast formation also lacked detectable satellite (Leff et al., 1963) or contained only a low molecular weight satellite DNA (Ray & Hanawalt, 1965). A satellite DNA of buoyant density 1.692 has been shown to be associated with purified chloroplasts (Brawerman & Eisenstadt, 1964; Edelman et al., 1964) and to have a base composition similar to that of the RNA of chloroplast-associated ribosomes (Brawerman & Eisenstadt, 1964) which the authors thought suggestive of satellite DNA control of RNA synthesis. Satellite DNA has also been described in mammalian cells (Borst & Ruttenberg, 1966; Flamm et al., 1966). It has been shown to be of nuclear origin, easily renatureable and distinct from mitochondrial DNA (Flamm et al., 1966). These observations, however, have not established the biological role of satellite DNA.

This communication describes experiments designed to analyze the role of satellite DNA in RNA synthesis by the technique of DNA-RNA hybridization. DNA's from three distinct myeloma tumors and normal mouse thymus, all of which have satellite components, were isolated and characterized. High specific activity RNA from one tumor (MPC_{11}) was also purified and fractionated for

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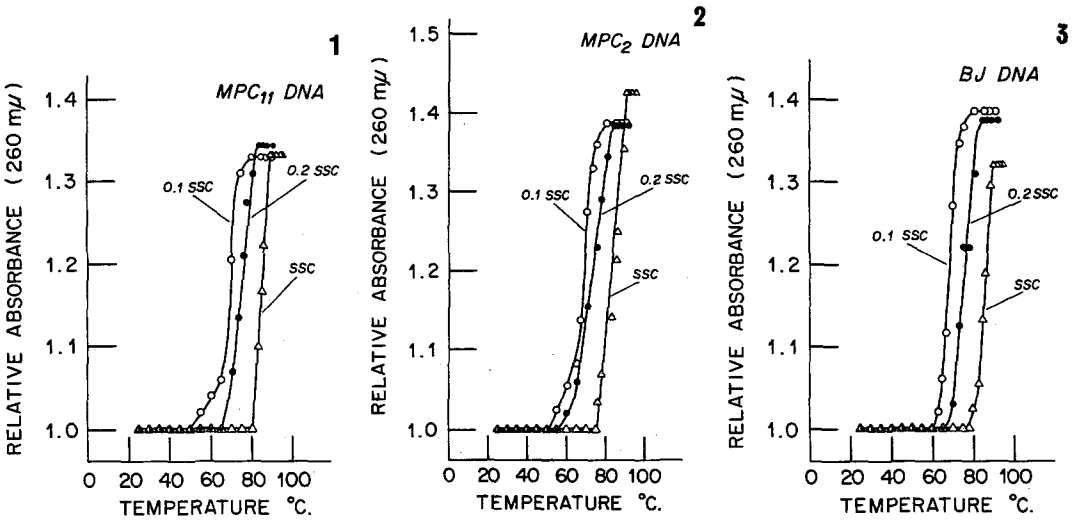
use in hybridization experiments.

EXPERIMENTAL The myeloma tumors MPC_{11}, MPC_2 and BJ used in this study are solid tumors carried by serial transplantation into Balb/c mice. The MPC_{11} synthesizes γG globulin; MPC_2, γA, and BJ, primarily L-chains. Nuclei were isolated from the three tumors and normal Balb/c thymus (Widnell and Tata,1964) and their DNA's extracted by modification (Wallace & Birnstiel, 1966) of the method of Marmur (1961). DNA's were fractionated by centrifugation in the Spinco 40 rotor at 33,000 rev/min for 60 hr in a CsCl density gradient at 25° (Flamm et al., 1966a). RNA was purified by modification of the hot phenol-SDS procedure (Scherrer & Darnell,1962). Sedimentation of RNA through linear sucrose gradients resulted in peak fractions corresponding to 28, 16, 10 and 4 S molecules. The 28, 16, and 10 S fractions were separately pooled, precipitated with ethanol, resedimented through sucrose and the resultant homogeneous peak fractions employed. High specific activity RNA was obtained by injecting mice intraperitoneally with 500 uc ^{32}P per mouse two hours prior to sacrifice. Hybrids were formed by heating RNA and denatured DNA together in 2 X SSC at 70° for 4 hr. This time and temperature were optimum for hybrid formation. Hybrids were assayed for by the CsCl method of Wallace & Birnstiel (1966) and by modification of the filtration technique of Gillespie & Spiegelman (1965) for liquid hybrids. Both methods showed reasonable agreement and therefore the latter procedure was employed throughout these studies.

RESULTS The three tumor DNA's display characteristic thermal denaturation profiles in varying SSC concentrations with increases in hyperchromicity between 30-40% and Tm's of 85° in 1 X SSC (Figs. 1-3). It can be seen in Fig. 4 that the tumor DNA's and normal thymus DNA each have a satellite component of buoyant density in CsCl of 1.69 g/cm^3 and a major band of buoyant density of 1.70 g/cm^3. A comparison of the G-C content as calculated from Tm and buoyant density shows reasonable agreement for the major band DNA's but consistently higher G-C values calculated from Tm's for the satellite DNA's (Table I). Chemical analysis is now under way in an effort to explain this



Figs.1-3. Thermal denaturation profiles of tumor DNA's in varying SSC concentrations (SSC=0.15M sodium chloride and 0.015M sodium citrate, pH 7.0). DNA's were dialyzed against the respective SSC concentrations for 36 hr at 0°. Melting curves were determined in a Gilford Spectrophotometer equipped with a thermosensing probe. Absorbances were corrected for thermal expansion.

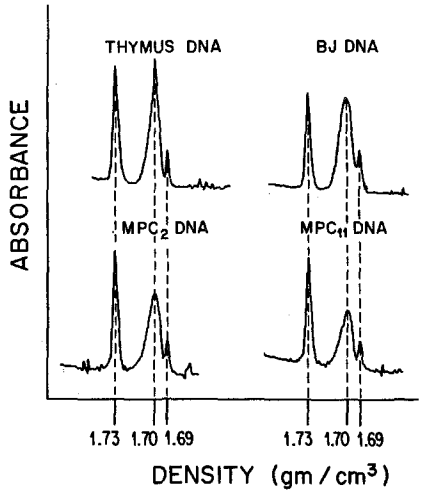


Fig.4. Densitometer tracings of DNA's isolated from tumor and thymus nuclei centrifuged to equilibrium in a CsCl density gradient. All samples were centrifuged at 44,000 RPM for 24 hr at 25° in a Spinco Model E centrifuge equipped with ultraviolet optics. M.lysodeikticus was used as a density marker.

variation.

Percent composition analysis of tumor DNA's and normal mouse thymus DNA (Table II) reveals a higher satellite content in the tumor DNA's than in the normal mouse tissue. The significance of this observation is not understood

TABLE I

DNA	Tm (1.0xSSC)	Buoyant density	G-C (Mole Percent) Tm	Buoyant density
MPC11				
Total	85		38.3	
Satellite	86.5	1.692	42	32.7
Major	86	1.702	40.7	42.9
MPC2				
Total	85		38.3	
Satellite	86	1.692	40.7	32.7
Major	86.5	1.702	42	42.9
BJ				
Total	85.5		39.5	
Satellite	88	1.693	45.5	33.7
Major	87	1.702	43.2	42.9

TABLE II

DNA	Percent Composition: Major band	Satellite
MPC_{11}	88.35	11.65
MPC_2	89.65	10.35
BJ	88.10	11.90
Thymus	92.60	7.40

at present. In order to investigate the involvement of satellite DNA in RNA synthesis, saturation experiments were conducted for both major band and satellite DNA using 28, 16, and 10 S RNA's. The data is presented in Figs. 5-7. It is apparent (Fig.5) that 10 S RNA hybridizes with the major band DNA only. The amount of hybrid formed with 16 S RNA and satellite DNA is approximately four times that formed with the major band DNA (Fig.6). 28 S RNA (Fig.7) hybridizes with both major band and satellite DNA, although slightly more hybrid is formed with the major band.

CONCLUSIONS Experiments with bacteria (Yankofsky & Spiegelman, 1962; Yankofsky & Spiegelman, 1963) and mammalian cells (McConkey & Hopkins, 1964) indicate that there are duplicated cistrons which code for the synthesis of ribosomal RNA. Davison (1966) using CsCl fractionated DNA from the same bacterial source found that the DNA which hybridized with ribosomal RNA was distributed eccentrically in the band formed by the total DNA. This eccentric distribution was interpreted to mean that the duplicated sequences coding for ribosomal RNA are adjacent. The existence of duplicated cistrons could

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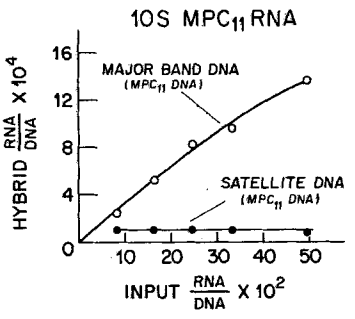

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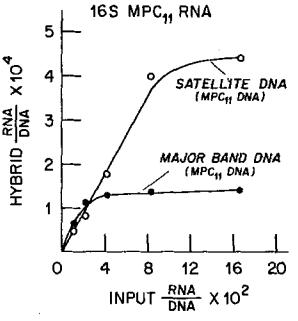

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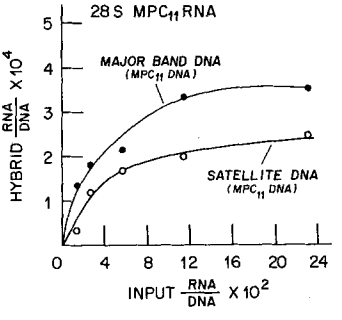

Figs.5-7. Saturation curves for annealing major band and satellite MPC_{11} DNA with 10, 16 and 28 S MPC_{11} RNA. 20ug aliquots of denatured DNA were mixed with various amounts of labeled RNA in 2 X SSC and incubated at 70° for 4 hr. Each point is the average of quadruplicate determinations which were corrected for control values differing only by the absence of an annealing period.

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account for the ease with which liver satellite DNA renatures (Flamm et al., 1966). This satellite DNA (Flamm et al.,1966) is thought to be the same as the "stable" DNA fraction of Walker and McLaren (1965). Walker and McLaren (1965) have pointed out that a cleaner fractionation of "stable" DNA on hydroxyapatite occurs when the DNA molecular weight is reduced, because a larger

proportion of the "stable" molecules are in the duplex state. In terms of DNA-RNA hybridization, however, this reduction in DNA molecular weight would minimize the chance for DNA-RNA duplex formation. It is for this reason that the satellite DNA's in the present study were fractionated on CsCl with resultant molecular weights in the 10-40 million range as compared to 200,000-500,000 molecular weight fragments employed in the hydroxyapatite procedure.

SUMMARY The data in the present study is consistent with the concept of duplicated adjacent stretches of DNA coding for 16 and 28 S ribosomal RNA. The four-fold increase in hybrid formation between 16 S RNA and satellite DNA strongly suggests that satellite DNA codes for this RNA. The capacity for both DNA's to form hybrids with respect to 28 S RNA could mean that the DNA stretches coding for 28 S RNA are adjacent to those coding for 16 S RNA but are of slightly higher buoyant density. 10 S RNA appears to be coded for by the major band DNA only.

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