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Nucleotide Clusters in Deoxyribonucleic Acids. Pyrimidine Oligonucleotides of Mouse L-Cell Satellite Deoxyribonucleic Acid and Main-Band Deoxyribonucleic Acid†

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ABSTRACT: Satellite DNA of mouse L cells was isolated by neutral CsCl density gradient centrifugation. Electron micrographs of the DNA preparation indicated a molecular weight range of 4×10^6 to 20×10^6 daltons with no one size predominating. ^{32}P-Labeled double-stranded satellite DNA and satellite DNA H and L separated strand preparations were hydrolyzed with formic acid–diphenylamine and the pyrimidine oligonucleotides released separated by DEAE-cellulose column chromatography. For a comparative study the distribution of pyrimidine oligonucleotides in main band DNA of mouse L cells was also investigated. The distribution in satellite DNA was nonrandom and characterized by a high concentration of pyrimidine tetra- and hexanucleotides and a low occurrence of tri- and pentanucleotides compared to main band DNA. Octanucleotides were the longest oligonucleotides clearly identified in satellite DNA. The most common pyrimidine oligonucleotides in the H strand of the satellite DNA were C_2T_4, C_2T_2, CT, C_2, CT_4, CT_3, CT_5, and C_3T_5 and in the L strand CT, T_2, CT_2, and C_2T. The results are consistent with the theory that the satellite DNA of mouse L cells is highly repetitious but indicate that the basic repeating unit is longer than suggested from other preliminary chemical studies.

The renaturation properties of mouse satellite DNA have led to the suggestion that this DNA fraction is composed of a short highly repetitive base sequence (Waring and Britten, 1966) (for a review, see Walker, 1971, and Flamm, 1972). No function has been defined for mouse satellite DNA and conflicting reports concerning transcription from it have appeared (Harel *et al.*, 1968; Flamm *et al.*, 1969; Cohen *et al.*, 1973). In order to speculate on the possible function of mouse satellite DNA it would be very useful to have information on the primary structure of this DNA fraction.

At the present time, there are considerable difficulties in sequencing DNA, mainly due to methodological problems.

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The analysis of the pyrimidine oligonucleotides released following specific depurination and hydrolysis of DNA by formic acid–diphenylamine (Burton and Petersen, 1960) is often used as the initial investigation of a DNA. The majority of analyses of pyrimidine oligonucleotide distribution have been performed on bacteriophage DNAs in attempts to ascertain whether there is any correlation between structure, transcriptional function, and the occurrence of poly(G) binding sites in the DNA (Černý *et al.*, 1969; Mushynski and Spencer, 1970a,b; Darby *et al.*, 1970; Kizer and Saunders, 1972).

The pyrimidine oligonucleotide distribution method was used partly in elucidation of the basic repeating sequence of guinea pig α satellite DNA (Southern, 1970). The study demonstrated that the repeating unit length computed from reassociation kinetic data (Waring and Britten, 1966) did not correlate with the repeating unit determined by sequence analysis. In the report of the sequence of guinea pig α satellite DNA Southern (1970) also quoted unpublished results for mouse satellite DNA. Some of these results have recently appeared in a review by Walker (1971) who suggested that the repeating unit in mouse satellite DNA is not as simple a sequence as that in guinea pig α satellite DNA.

The present study is the first in a series to determine the sequence of the repeating unit of mouse L-cell satellite DNA and provides the complete nonisomeric pyrimidine oligonucleotide catalog of mouse satellite DNA. The results substantiate the claim that the repeating unit in mouse satellite DNA is not a simple short sequence and, because of this, additional degradation methods must be used to determine the primary structure. Comparative studies on mouse L-cell main-band DNA provide further evidence that satellite DNA is a unique DNA fraction in the nucleus of mouse cells.

Materials and Methods

Isolation of Mouse L-Cell Main-Band and Satellite DNA. Mouse L cells labeled with ^{32}P were generously provided by Drs. A. F. Graham and S. Millward. L-929 mouse fibroblasts were grown in spinner modified Eagles minimum essential medium (Grand Island Biological Co.) supplemented with 5% fetal calf serum. When the cell concentration reached 0.5×10^6 cells/ml, the cells were harvested, washed three times in fresh medium, and then transferred to phosphate-free medium containing 2% fetal calf serum which had previously been dialyzed against phosphate-free medium. $H_3{}^{32}PO_4$ (New England Nuclear) (10 μCi/ml) was added, the cells were grown for a further 24 hr, and then harvested by centrifugation. The cells were washed in phosphate-buffered saline (0.13 M NaCl–2.7 mM KCl–0.82 mM Na_2HPO_4–1.5 mM KH_2PO_4–0.91 mM CaCl–0.5 mM $MgCl_2$, pH 7.3) and the nuclei were isolated and washed according to the procedure described by Penman (1966). The nuclei were gently disrupted with sodium dodecyl sulfate and the DNA was deproteinized with isoamyl alcohol according to Marmur (1961). Purification of the DNA was completed by cesium chloride density gradient centrifugation at a starting density of 1.708 g/cm^3 in a Beckman type 50 rotor for 60 hr at 35,000 rpm at 20°, as described by Flamm *et al.* (1967). After the first cesium chloride density gradient centrifugation, the distinct shoulder on the light side of the main-band DNA was collected from the top of the centrifuge tube by displacement with a heavy cushion of cesium chloride. The peak fractions of main-band DNA were pooled at this stage in certain experiments. The light shoulder fractions were pooled and recentrifuged in a cesium chloride density gradient under identical conditions. The second gradient resulted in a distinct light satellite peak separated from the main-band DNA. The material was again collected from the top of the tube and pooled, and the preparation was monitored in the Model E analytical ultracentrifuge. When necessary the material was recentrifuged in an identical cesium chloride gradient for a third time.

Strand Separation of Mouse L-Cell Satellite DNA. This was performed according to Flamm *et al.* (1967) except that 0.1% sarcosyl NL97 (Geigy Industrial Chemicals) was used in place of sodium dodecyl sulfate.

Pyrimidine cluster analyses were performed as described by Černý *et al.* (1968, 1969). In the present series of experiments 50 mg of calf thymus DNA was added to the satellite DNA preparations as carrier prior to hydrolysis.

Identification and Quantitation of Oligonucleotides. In the shorter isostich fractions of the main-band DNA where all possible oligonucleotides were present the order of elution from the chromatograms gave the oligonucleotide composition and no further identification was performed. For the longer isostichs unlabeled S13 DNA oligonucleotides of known composition (Černý *et al.*, 1969) were cochromatographed with the corresponding ^{32}P-labeled isostich fractions from the mouse main-band DNA. This allowed construction of a grid described in Results which provided a correlation of the concentration of the eluting salt with the base composition of the oligonucleotide eluted in the pH 3 chromatograms. The base compositions of the oligonucleotides from the satellite DNA were assigned using this grid.

To quantitate the individual radioactive oligonucleotides separated in the pH 3 chromatograms and the isostichs in the pH 5 chromatograms the fractions corresponding to each oligonucleotide or isostich were pooled, the volume was measured, and 10-ml samples were transferred to scintillation vials and Čerenkov radiation (Clausen, 1968) measured directly in a Beckman LS 250 liquid scintillation counter. The use of Čerenkov radiation for direct measurement in this way has been found to be entirely quantitative and eliminates the use of scintillation fluids.

Sedimentation Analyses. Buoyant densities of the DNA preparations were determined by isopycnic centrifugation in cesium chloride at 44,000 rpm for 20 hr at 25° in a Beckman Model E analytical ultracentrifuge with the use of Kel-F centerpieces and an An-D rotor (Mandel *et al.*, 1968). Densitometer tracings of the photographs were obtained with a Beckman Acta III spectrophotometer fitted with a scanning device using the 0.05-mm slit assembly. All buoyant densities were determined using *Micrococcus lysodeikticus* DNA as a density reference $\rho = 1.731$ g/cm^3 in neutral CsCl (Vinograd *et al.*, 1963).

Electron Microscopy. The DNA (in 50 mM Tris-HCl buffer, pH 8) was diluted tenfold into 1 M ammonium acetate solution (pH 7.2), containing 0.1% cytochrome *c* and spread on a 0.15 M ammonium acetate hypophase (pH 7.0) (Kleinschmidt and Zahn, 1959). The concentrations of the DNA in the cytochrome *c* solution ranged from 1.5 to 2.5 μg per ml. Collodion-coated grids with an ultraviolet-irradiated carbon coating were used to pick up the film, were then dehydrated, fixed with uranyl acetate, and dried. The grids were rotary shadowed with uranium oxide at an angle of 5° and examined in a Philips EM 300 electron microscope. Photographs of DNA molecules were projected and traced, and the lengths were measured with a map measurer.

Base Ratio Analyses. These were performed as described previously (Spencer *et al.*, 1972), except that Čerenkov radiation was used for determination of radioactivity as described above.

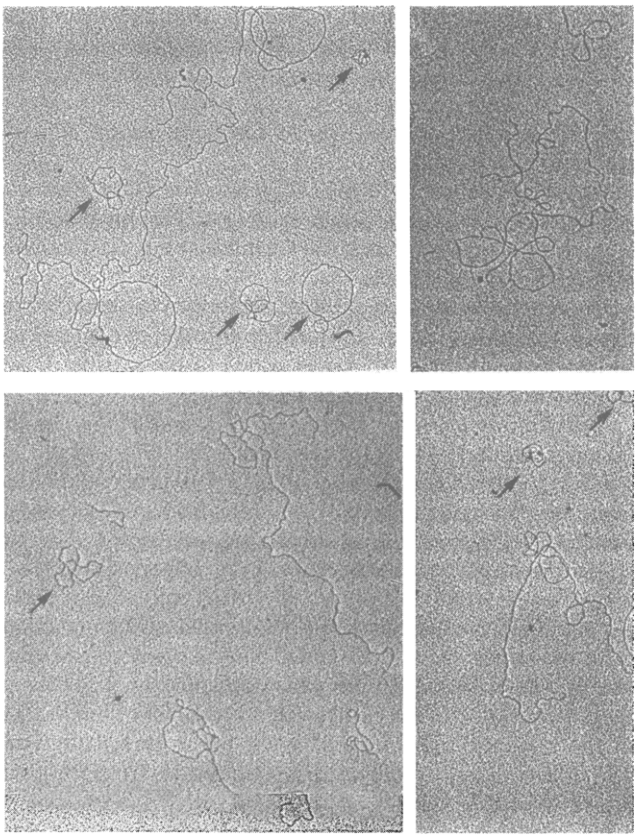

FIGURE 1: Electron micrographs of mouse satellite DNA. $\phi X174$ replicative-form DNA (arrows) was added to the preparation as marker. Magnification 20,000.

Results

Figure 1 is an electron micrograph of a mouse satellite DNA preparation used in the present study. Bacteriophage $\phi X174$ RF DNA (a gift from Dr. D. Denhardt) was included in the preparation as a reference marker for length measurements and molecular weight calculations. Measurement of the molecules in the electron micrographs showed 85% of them had a length of 2–10 μm corresponding to a molecular weight of 4×10^6 to 20×10^6 daltons, assuming a mass per unit length of 1.92×10^6 daltons/μm (MacHattie and Thomas, 1964).

Preparations of purified satellite DNA were monitored in the Model E analytical ultracentrifuge and the buoyant density in neutral CsCl was determined to be 1.690 g/cm^3.

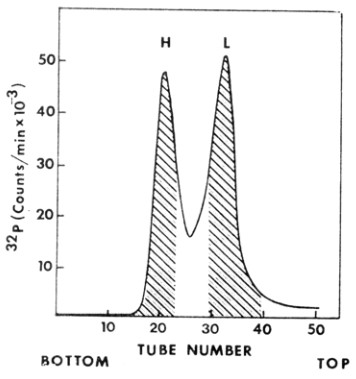

FIGURE 2: Separation of the complementary strands of ^{32}P-labeled mouse L-cell satellite DNA by preparative alkaline CsCl density gradient centrifugation. The shaded areas represent the portions of the density gradient which were pooled for subsequent analyses, fractions 14 to 22 for strand H and fractions 29 to 40 for strand L.

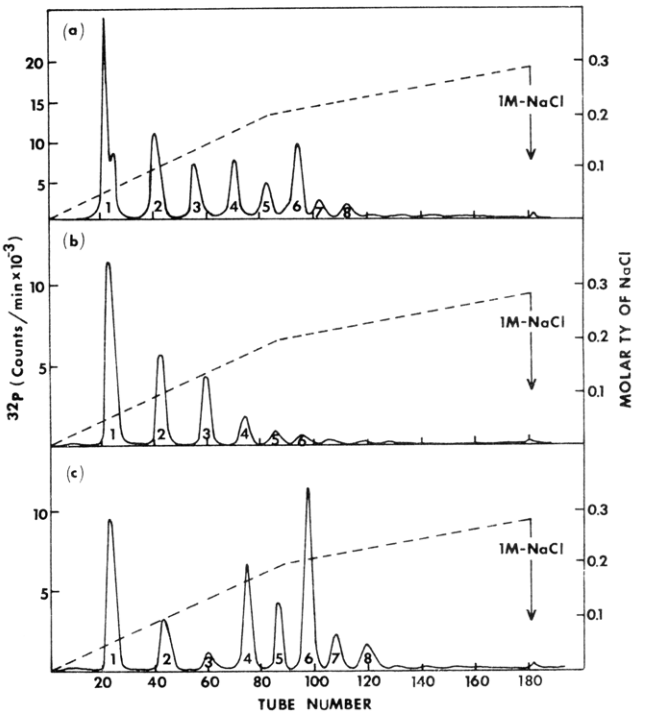

FIGURE 3: Chromatography of formic acid–diphenylamine hydrolysates of ^{32}P-labeled mouse satellite DNA and the separated strands H and L on DEAE-cellulose (5.0 g, 325 mesh, 28 × 1.5 cm, Cl^- form). The hydrolysates (^{32}P-labeled DNA, 50 mg of calf thymus DNA carrier, 67% formic acid–2% diphenylamine, 30°, 18 hr) freed of formic acid and diphenylamine were applied to the columns. The exchanger was washed with 0.05 M sodium acetate buffer in 7 M urea (pH 5.5) to remove unabsorbed purines and then eluted with a linear gradient of NaCl from 0 to 0.2 M in 0.1 M sodium acetate buffer in 7 M urea (pH 5.5), followed by a second gradient of NaCl, 0.2–0.3 M in the same buffer. Total volume of eluent 2 l., 10-ml fractions were collected. After the gradient elution the column was washed with 1.0 M NaCl. Fractions were collected in scintillation vials and Čerenkov radiation measured directly. (——) ^{32}P radioactivity; (----) NaCl molarity; (a) total satellite DNA 3.5×10^5 cpm; (b) L strand (2.7×10^5 cpm normalized to 1.75×10^5 cpm); (c) H strand (2.0×10^5 cpm normalized to 1.75×10^5 cpm).

No main-band DNA was observed contaminating the satellite preparation. The complementary strands of satellite DNA were separated by alkaline CsCl density gradient centrifugation shown in Figure 2. The symmetry of the peaks and the clear separation of the two strands was used as a criterion for the absence of main-band DNA contamination in the satellite DNA preparations (Tobia *et al.*, 1971). Base analyses of the separated strands confirmed the values reported from other laboratories (Flamm *et al.*, 1967; Corneo *et al.*, 1968).

The ^{32}P-labeled satellite DNA and separated strand H and L DNAs were hydrolyzed with formic acid–diphenylamine and the pyrimidine oligonucleotides released were separated according to chain length on DEAE-cellulose–urea columns as shown in Figure 3. The purines and P_i were eluted during the procedures of loading and washing the columns. Figure 3a and Table I show the longest oligonucleotides in total satellite DNA of any significant occurrence were the octanucleotides. Very minor quantities of nona- and decanucleotides were observed but the amounts were too small to determine quantitatively. The occurrence of hexanucleotides was very common, while tri- and pentanucleotides were relatively rare. This unusual oligonucleotide cluster distribution was further emphasized when the individual strands of the satellite DNA were analyzed separatedly as shown in Figure 3b,c. The values for counts per minute in Figure 3b,c have been normal-

TABLE I: Distribution of Pyrimidine Isostichs in Mouse L-Cell Main-Band, Satellite, and Strands H and L of Satellite DNA.[a]

Pyrimidine Isostich	Mol of Pyrimidine/100 g-atoms of DNA-P			
	Main Band	Total	Satellite H Strand	L Strand
1	10.82	11.32	9.54	11.33
2	9.75	7.70	6.08	7.81
3	8.73	5.91	1.91	6.70
4	6.92	6.51	10.82	2.97
5	4.78	4.11	7.08	1.46
6	3.03	8.79	20.39	1.31
7	2.05	2.63	5.23	0.74
8	1.35	2.42	5.66	0.52
9	0.92	0.36	1.31	0.20
10	0.60	0.25	0.17	0.16
11	0.43			
12	0.26			
13	0.15			
14	0.08			
15 and longer	0.13			
Total	50.00	50.00	67.20	33.20
Uncorrected total	51.26	55.38	64.72	35.10

[a] The values for main-band DNA and total satellite DNA have been adjusted on the assumption that moles of pyrimidine account for 50% DNA phosphorus (DNA-P). The values for the separated strands have been adjusted to total 67.2% DNA phosphorus for the H strand and 33.2% for the L strand, determined from the base compositions of the separated strands (Corneo *et al.*, 1968).

ized to provide a direct comparison of the separated strands with the main band (Mushynski and Spencer, 1970a). The quantitative results of these analyses are presented in Table I. The H strand is the pyrimidine-rich strand. In the L-strand hepta- and octanucleotides occur in very small amounts only, whereas the mono-, di-, and tripyrimidine oligonucleotides are more common than in the H strand. Also presented in Table I are data from an experiment on main-band DNA. The elution profile from a DEAE-cellulose column for this experiment is shown in Figure 4. Isostichs of up to 14 residues were clearly resolved and longer isostichs also present were eluted by the 1 M NaCl wash.

All isostichs from total satellite DNA and the separated strand H and L DNAs were subfractionated according to base composition by chromatography on DEAE-cellulose columns at pH 3. The elution profiles of isostichs 2, 4, 5, 6, 7, and 8 are shown in Figures 5, 6, and 7, respectively, and the quantitative results are summarized in Table II. In total satellite DNA all possible pyrimidine oligonucleotides were present in isostichs 1–5 inclusive except for C_4 and C_5. In isostichs 6, 7, and 8 the oligonucleotides not present were those with high proportions of C. The most commonly occurring hepta- and octanucleotides were CT_6, C_2T_5, and C_3T_5. The distribution of pyrimidine oligonucleotides in the separated strands was completely asymmetric. The reproducibility of the results is exemplified by comparing the experiment on total satellite DNA with those on the separated strand H and L DNAs which were from a different satellite DNA preparation. Addition of the quantitative results of the separated strands and division of the

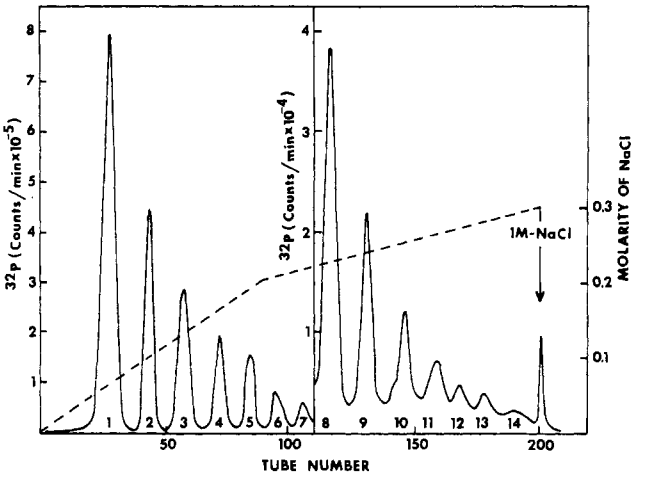

FIGURE 4: Chromatography of a formic acid–diphenylamine hydrolysate of ^{32}P-labeled mouse main-band DNA (16×10^6 cpm) on a column of DEAE-cellulose. Conditions as in Figure 3.

summation by two provides a direct comparison with the quantitative results from total satellite DNA.

For comparative purposes the quantitative results of the subfractionation of isostichs 1–10 of main-band DNA are shown in Table II also. Except that C-rich components were absent from the longer isostichs, the amount of each compo-

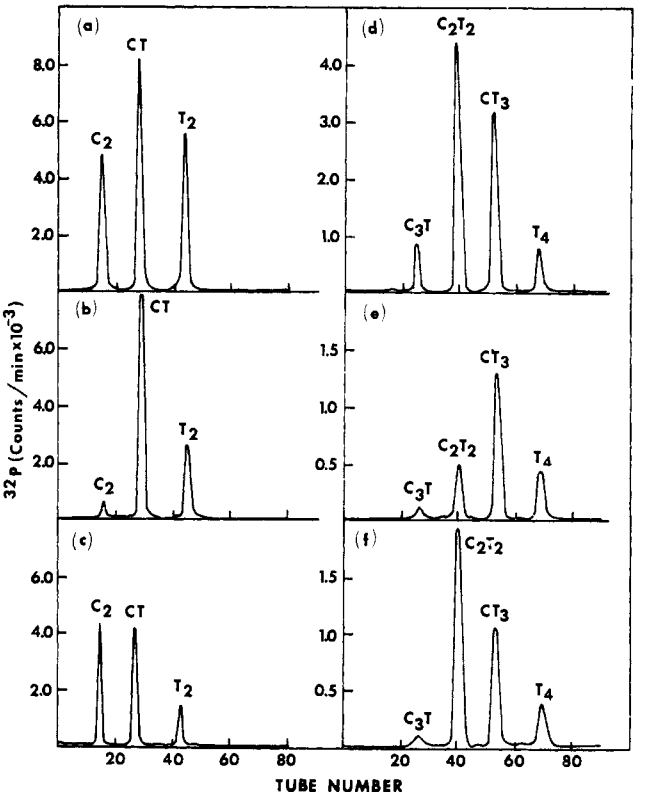

FIGURE 5: Chromatography of pyrimidine isostichs 2 and 4 from total satellite DNA and strands H and L on DEAE-cellulose (5.0 g, 325 mesh, 25 × 1.5 cm, formate form). The pooled isostich fractions, diluted with distilled water, were applied to the columns. The exchanger was washed with 0.1 M formic acid and then eluted with a linear gradient of ammonium formate from 0 to 0.5 M, pH 3.0. Total volume of eluent, 1 l.; 5-ml fractions collected. Fractions were collected in scintillation vials and Čerenkov radiation measured directly; (a) total satellite DNA dinucleotides; (b) L-strand dinucleotides; (c) H-strand dinucleotides; (d) total satellite DNA tetranucleotides; (e) L-strand tetranucleotides; (f) H-strand tetranucleotides.

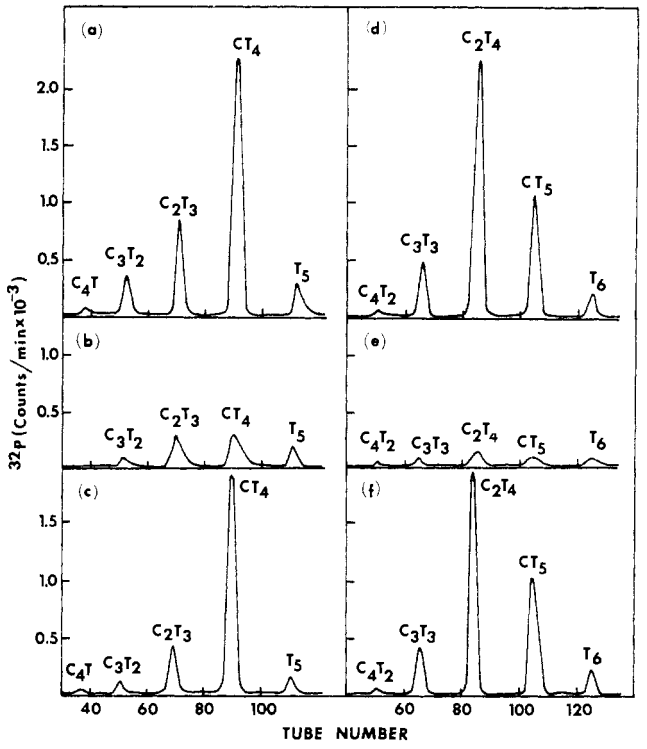

FIGURE 6: Chromatography of pyrimidine isostichs 5 and 6 from total satellite DNA and separated strands H and L on DEAE-cellulose. Conditions as in Figure 5, except that the columns were eluted with a linear gradient of ammonium formate from 0 to 1.0 M pH 3.0 and the total volume of eluent was 2 l.; (a) total satellite DNA pentanucleotides; (b) L-strand pentanucleotides; (c) H-strand pentanucleotides; (d) total satellite DNA hexanucleotides; (e) L-strand hexanucleotides; (f) H-strand hexanucleotides.

nent in main band DNA followed a normal distribution in all isostichs in agreement with studies on other nonrepetitive

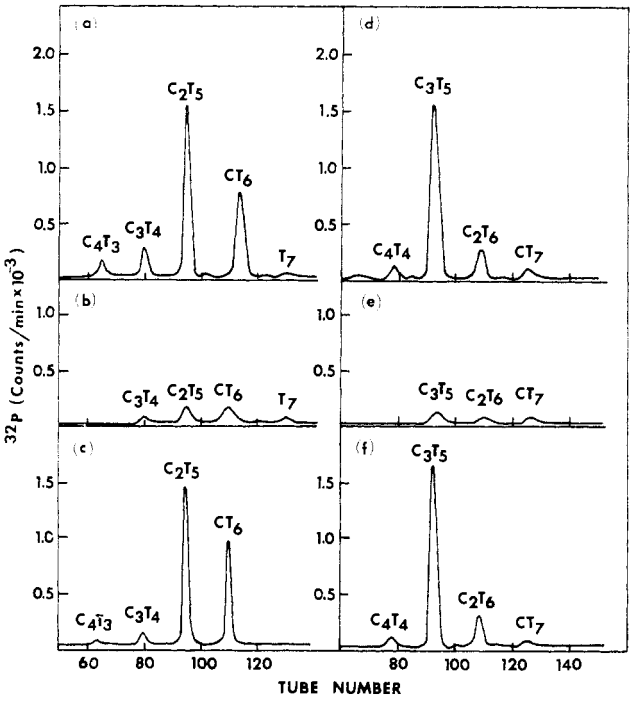

FIGURE 7: Chromatography of pyrimidine isostichs 7 and 8 from total satellite DNA and separated strands H and L on columns of DEAE-cellulose. Conditions as in Figure 6; (a) total satellite DNA heptanucleotides; (b) L-strand heptanucleotides; (c) H-strand heptanucleotides; (d) total satellite DNA octanucleotides; (e) L-strand octanucleotides; (f) H-strand octanucleotides.

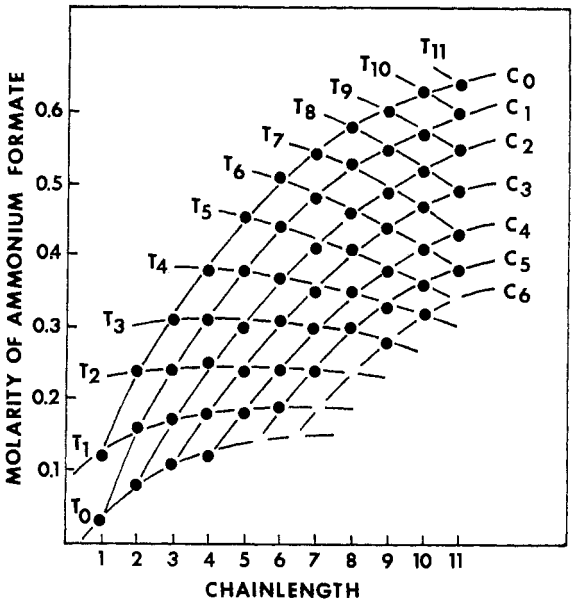

FIGURE 8: Elution grid of pyrimidine oligonucleotides constructed from data on mouse main-band DNA. For details, see Results.

DNAs (*e.g.*, Černý *et al.*, 1968; Sneider, 1971; Rudner *et al.*, 1972; Bellett *et al.*, 1972). Component C_5 was the longest cytidylic acid run found in the main band DNA and component T_{10} was the longest thymidylic acid run that could be clearly identified. However, indications that T_{11} was also present were observed on subfractionation of isostich 11 (not shown).

The data presented in columns 7 and 8 of Table II were calculated by assuming that the most infrequently occurring oligonucleotides in columns 5 and 6 would be unique in occurrence in any theoretical repetitive sequence. This assumption then allows the calculation of the frequency of occurrence of the other oligonucleotides present and the size of the theoretical repetitive sequence which in this case is 15,000 bases corresponding to a molecular weight of 5×10^6 daltons. Presentation of data in this manner provides ready comparison with frequency distributions in other DNAs and within the molecule itself.

When the pyrimidine oligonucleotides which occur most frequently (>~100 copies) in the separated strand DNAs are tabulated separately, those in the H strand can be grouped into two major categories: (1) containing one cytosine per oligonucleotide and (2) containing two cytosines per oligonucleotide. The exception is the component C_3T_5 which could be considered a third category. The data organized in this manner are presented in Table III.

Assignment of the base composition for many of the oligonucleotides in the present study was by means of a grid which was constructed from data of the components which occurred in the main band DNA after subfractionation. Accurate assignment of base compositions of main band DNA oligonucleotides was accomplished by cochromatography with selected oligonucleotides of known base composition from S13 DNA (Černý *et al.*, 1969). A plot of the chain length and base composition of each component of the main-band DNA *vs.* the molarity of ammonium formate which eluted the component from the DEAE-cellulose column at pH 3.0 was made and is presented in Figure 8. The use of this elution grid was aided considerably by the fact that up to 6 DEAE-cellulose columns were eluted simultaneously from the same gradient bottle allowing accurate comparison of one column elution profile with another for isostichs of consecutive size.

TABLE II: Distribution of Pyrimidine Isostich Components in Mouse L-Cell Main-Band, Satellite, and Strands H and L of Satellite DNA.

Isostich	Component	Mol of Pyrimidine/100 g-atoms of DNA-P[a]				No. of Tracts/DNA Molecule[b] (DNA Mol Wt 5×10^6 daltons: 15,000 bases) Satellite	
		Main Band	Total	Satellite H Strand	L Strand	H Strand	L Strand
1	C	3.69	3.75	2.67	3.68	400	550
	T	7.13	7.57	6.86	7.65	1030	1150
2	C_2	1.63	1.11	2.39	0.39	180	30
	CT	4.86	4.79	2.84	5.43	215	410
	T_2	3.26	1.79	0.85	2.00	65	150
3	C_3	0.59	0.22	0.08	0.13	4	7
	C_2T	2.63	2.08	0.61	2.36	30	120
	CT_2	3.45	2.24	0.63	2.45	30	120
	T_3	2.06	1.36	0.58	1.77	30	90
4	C_4	0.14					
	C_3T	1.15	0.58	0.21	0.10	8	4
	C_2T_2	2.35	2.89	6.16	0.59	230	20
	CT_3	2.18	2.43	3.90	1.63	145	60
	T_4	1.09	0.60	0.53	0.66	20	25
5	C_4T	0.37	0.06	0.04		1	
	C_3T_2	1.18	0.41	0.18	0.14	5	4
	C_2T_3	1.65	0.85	0.81	0.51	25	15
	CT_4	1.19	2.48	5.66	0.46	170	15
	T_5	0.39	0.31	0.38	0.36	10	10
6	C_5T	0.05					
	C_4T_2	0.37	0.13	0.04	0.04	1	1
	C_3T_3	0.83	0.88	1.76	0.14	45	4
	C_2T_4	1.02	4.80	11.63	0.46	290	10
	CT_5	0.61	2.40	5.58	0.45	140	10
	T_6	0.15	0.57	1.36	0.23	35	6
7	C_5T_2	0.11					
	C_4T_3	0.41	0.14	0.12		3	
	C_3T_4	0.63	0.37	0.31	0.09	7	2
	C_2T_5	0.54	1.10	2.43	0.26	50	6
	CT_6	0.28	0.97	2.37	0.28	50	6
	T_7	0.07	0.06		0.11		2
8	C_5T_3	0.15					
	C_4T_4	0.32	0.16	0.23		4	
	C_3T_5	0.42	1.79	4.60	0.22	85	4
	C_2T_6	0.27	0.38	0.77	0.14	15	3
	CT_7	0.15	0.09	0.06	0.17	1	3
	T_8	0.04					
9	C_6T_3	0.05					
	C_5T_4	0.17					
	C_4T_5	0.24					
	C_3T_6	0.22					
	C_2T_7	0.13					
	CT_8	0.07					
	T_9	0.04					
10	C_6T_4	0.06					
	C_5T_5	0.12					
	C_4T_6	0.15					
	C_3T_7	0.13					
	C_2T_8	0.08					
	CT_9	0.04					
	T_{10}	0.02					

[a] All values are corrected to the values in Table I. [b] For details of the calculation of number of tracts, see Results.

TABLE III: Frequency of Occurrence of the Major Pyrimidine Oligonucleotides in Mouse L-Cell Satellite DNA.[a]

Component	No. of Tracts/DNA Molecule (DNA Mol Wt 5×10^6 daltons: 15,000 bases)	
	H Strand	L Strand
CT	215	410
CT_2		120
CT_3	145	
CT_4	170	
CT_5	140	
T_2		150
C_2	180	
C_2T		120
C_2T_2	230	
C_2T_4	290	
C_3T_5	85	

[a] For details of the calculation of number of tracts, see Results.

Discussion

The satellite DNA used in the present study was not subjected to either deliberate mechanical shearing or denaturation and renaturation during its preparation (Flamm *et al.*, 1967). The molecular weight of the DNA was 4×10^6 to 20×10^6 daltons corresponding to 2 to 10 μm in length with no particular size predominating. A previous electronmicrograph study of L-cell satellite DNA reported four size classes of DNA molecules, from 1 to 4 μm long (Salomon *et al.*, 1969). The difference in the two studies is probably due to the different procedures used to isolate the DNA.

The distribution of pyrimidine oligonucleotides in mouse total satellite DNA and the separated strand H and L DNAs appears similar to the distribution reported in a review article by Walker (1971). However, a direct comparison of the results is impossible as no quantitative data were given in the review article, some of the more common pyrimidine sequences were listed in a table only. Total satellite DNA has a most unusual distribution of pyrimidine oligonucleotides unlike any other distribution previously reported for any DNA, even from small phage DNAs of molecular weight less than 2×10^6 (Černý *et al.*, 1969; Darby *et al.*, 1970; Spencer and Boshkov, 1973). Also the distribution between the strands is asymmetric. An interesting observation is the distribution within isostich 8 where C_3T_5 accounts for more than 80% of the total isostich in the H strand and thus belongs to the group of more common pyrimidine tracts in satellite DNA (Table III). In the chain length distribution chromatograms oligonucleotides of chain length 9 and 10 were observed in total satellite DNA, but were found to be less than 1% of the total amount of radioactivity, which was insufficient for further analysis. No attempt was made to investigate the distribution of 5-methylcytosine in any of the isostichs.

The pyrimidine cluster data support the theory that satellite DNA of mouse L cells is highly repetitious. The unusual and nonrandom distribution and the restricted number of oligonucleotides observed, together with the fact that the satellite DNA had a molecular weight of $4–20 \times 10^6$ daltons also supports the concept of satellite DNA being a tandem series of repetitive sequences. Southern (1970) determined the basic repeating sequence of guinea pig α satellite DNA to be 6 nucleotides long and suggested that the basic repeating unit in mouse satellite DNA was between 8 and 13 nucleotides. Our pyrimidine cluster data do not support such a small repeating unit for mouse satellite DNA. Examination of the most frequently occurring oligonucleotides in Table III and assuming that one component from each group occurs at least once, then the minumum size repeat unit for mouse L-cell satellite DNA is much larger than Southern's outside estimate of 13 nucleotides.

Although the results are consistent with the theory of satellite DNA being made up of a short tandem repeating sequence with some sequence heterogeneity introduced by point mutations (Southern, 1970), they are also consistent with the alternative theory that satellite DNA is a tandem repetition of a basic short sequence spaced by a variable sequence (Hutton and Wetmur, 1973). Further speculation on the size of the satellite DNA repeating sequence should wait until sequence studies in progress on the longer frequently occurring pyrimidine oligonucleotides are completed.

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Inhibition of *Azotobacter vinelandii* Ribonucleic Acid Polymerase by Glutamyl,Tyrosyl Copolymers†

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ABSTRACT: Of a series of glutamyl containing copolypeptides tested, poly(Glu^1,Tyr^1) is the most effective inhibitor of RNA polymerase. Inhibition is a consequence of binding of the poly(Glu^1,Tyr^1) to the polymerase which blocks the subsequent formation of the enzyme–template complex. The preformed holoenzyme–d(A-T) complex is much more resistant to dissociation by poly(Glu^1,Tyr^1) than is the core polymerase–d(A-T) complex.

A variety of polyanionic inhibitors of RNA polymerase have been studied, including polyribonucleotides (Dubert and Hirschbein, 1969; Tissières *et al.*, 1963; Krakow and Ochoa, 1963), heparin (Walter *et al.*, 1967), and the azosulfonic acid dye, Congo Red (Krakow, 1965; Smuckler, 1972). These inhibitors bind to RNA polymerase and block the formation of the enzyme–template complex. In this paper a novel class of RNA polymerase inhibitors will be described; these are synthetic copolypeptides containing glutamyl and tyrosyl or other aminoacyl residues which act by interfering with template binding.

Methods and Materials

Tris, ATP, UTP, EDTA, and mercaptoethylamine were products of Sigma Chemical Co. Labeled and unlabeled d(A-T) and d(I-C) were prepared using *Escherichia coli* DNA polymerase I (Jovin *et al.*, 1969). Glu-Tyr-Glu was obtained from Fox Chemical Co. and the polypeptides were obtained as follows (the molecular weights listed were determined by the manufacturer): poly(aspartic acid) (mol wt 20,000–50,000) and poly(glutamic acid) (mol wt 40,000–100,000) from Pierce Chemical Co.; poly(Glu^9,Tyr^1) (mol wt 85,000) and poly(Glu^1,Tyr^1) (mol wt 22,000) from Miles Laboratories. The following were generously donated by Dr. G. Fasman, Brandeis University: poly(Glu^{95},Tyr^5), poly(Glu^{95},Trp^5), poly(Glu^9,Leu^1), and poly(Glu^3,Phe^1). [^{32}P]PP$_i$ was purchased from New England Nuclear and *p*-[^{14}C]chloromercuribenzoate from Schwarz/Mann.

Nitrocellulose membrane filters (0.45-μ pore size, 25-mm diameter) were obtained from Matheson-Higgins, Woburn, Mass. Prior to use the filters were soaked in 0.1 M KOH at room temperature for 30 min (Smolarsky and Tal, 1970) and then placed in 0.02 M Tris-HCl (pH 7.8)–0.05 M NaCl. This procedure was used to lower the blank adsorption of the labeled deoxypolynucleotides.

Azotobacter vinelandii RNA polymerase was purified by a modification of the published procedure (Krakow and Horsley, 1968). In the final step RNA polymerase holoenzyme ($\beta'\beta\alpha_2\sigma$) and core ($\beta'\beta\alpha_2$) were resolved by gradient elution from phosphocellulose (Whatman P-11) and each form was essentially homogeneous as determined by sodium dodecyl sulfate–acrylamide gel electrophoresis.

Results

Although poly(glutamic acid) and poly(aspartic acid) do not apparently affect RNA polymerase activity, the random copolymer, poly(Glu^1,Tyr^1) completely inhibits [^{32}P]PP$_i$ exchange (Table I). The extent of inhibition is related to the relative amounts of glutamyl and tyrosyl residues in the synthetic polypeptides tested; no inhibition is seen with poly(glutamic acid) or poly(Glu^{95},Tyr^5), poly(Glu^9,Tyr^1) elicits a 19% inhibition, and the poly(Glu^1,Tyr^1) elicits a 95% inhibition of the d(A-T) directed [^{32}P]PP$_i$ exchange reaction (Krakow and Fronk, 1969). The ability to inhibit RNA polymerase is not limited to those polypeptides containing glutamyl and tyrosyl residues since poly(Glu^3,Phe^1) and poly(Glu^{95},Trp^5) are also effective.

At low ionic strength RNA polymerase sediments as a dimer or higher aggregate (Berg and Chamberlin, 1970) (Figure 1A); under similar ionic conditions the tRNA–enzyme complex sediments as a 12S protomer form (Figure 1B). Incubation of RNA polymerase with poly(Glu^1,Tyr^1) also results in loss of the polymerase dimer and the appearance of a protomer form sedimenting at a position identical with that of the tRNA protomer complex (Figure 1C). An ad-

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