paper and treated with 7 M ammonia for 2 hr at room temperature. The deblocked products were then analyzed by degradation with alkaline phosphatase and snake venom phosphodiesterase followed by ion-exchange chromatography (Ho and Gilham, 1973). With this procedure the dA-dTp produced equal quantities of dA and pdT while the pdTp gave thymidine as the only product.

The unmodified tetranucleotide, pdT-dA-dT-dA (6 ODU_{260nm}), was treated with micrococcal nuclease under identical conditions and the products were separated by paper chromatography as described above. The products, dA (R_F 0.68), dT-dA (R_F 0.55), dTp (R_F 0.43), dAp (R_F 0.35), and pdTp (R_F 0.12), were identified by the comparison of their ultraviolet spectra and R_F values with those of authentic compounds.

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Studies on the Relationship between Deoxyribonucleic Acid Polymerase Activity and Intracisternal A-Type Particles in Mouse Myeloma†

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ABSTRACT: Previous studies have demonstrated a DNA polymerase activity in preparations of mouse intracisternal A particles. In the current study the relationship between this DNA polymerase activity and A particles in mouse myeloma was investigated. Reaction conditions were adjusted for specific measurement of the type of DNA polymerase activity that was found in isolated A particles. This type of DNA polymerase activity was detected in several A-particle containing tissues but not in tissues devoid of or containing very low numbers of A particles. Similarly, four enzymes isolated from myeloma that appear to correspond to mouse cellular DNA polymerases were not active under the reaction conditions used for measurement of the A particle associated DNA polymerase. During subcellular fractionation, the enzyme activity behaved as a particulate cytoplasmic component and was concentrated 30-fold in purified A particles relative to the crude homogenate. A very similar subcellular distribution was observed for an antigen associated with the main A-particle structural protein. The DNA polymerase activity cosedimented with A particles in isopycnic sucrose gradients and was not solubilized by treatment with 1 M KCl or several surfactants. Implications of these findings are discussed.

Mammalian cells contain a number of DNA polymerase activities distinguished on the basis of physical properties, intracellular localization, template specificity, and requirements for maximal activity. Biochemical characterization of these DNA polymerases and elucidation of their physiological roles in DNA replication and repair are matters of current investigation.

In a previous study (Wilson and Kuff, 1972), DNA polymerase activity was detected in preparations of intracisternal A-type particles (Bernhard, 1960; Provisional Committee for Nomenclature of Viruses, 1966) from several mouse tumors. The enzyme possessed an unusual biochemical property in that it was active with poly(riboadenylate) as template, but virtually inactive with either natural or synthetic DNA templates. The suggestion was therefore made that the DNA polymerase activity was not due to contamination by cellular DNA polymerase and was A-particle specific. However, discoveries of normal cellular DNA polymerase activities that copy

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poly(rA) containing templates with higher efficiency than DNA templates (Maia *et al.*, 1971; Stavrianopoulos *et al.*, 1971; Ward *et al.*, 1972; Chang and Bollum, 1972; Fridlender *et al.*, 1972) raised the possibility that a contaminating general cellular enzyme was responsible for the activity observed in isolated A-particle fractions. This question of the relationship between A particles and cellular DNA polymerase activities has been examined in greater detail in the current study using extracts primarily from mouse myeloma.

The results are quite clear in indicating that (1) a type of DNA polymerase activity was physically associated with A particles and was not removed by treatment with high salt or surfactant; (2) virtually all of this enzyme activity in myeloma MOPC-104E was A-particle associated, and like the main A-particle structural protein, was concentrated approximately 30-fold in A-particle preparations relative to the whole homogenate; and (3) this enzyme activity was unique among murine DNA polymerase activities tested in that it was detected only in A-particle containing tissues, was active with poly(rA)·oligo(dT) as template primer in the presence of 250 mM KCl and 12.5 mM Mg^{2+}, and was concentrated in A-particle preparations. The enzyme activity was thus A-particle specific.

Materials

Reagents. Unradioactive nucleoside 5′-triphosphates were from Calbiochem. [*methyl*-^{3}H]Thymidine 5′-triphosphate (dTTP), lithium salt (13.4 Ci/mmol), was from Schwarz/Mann. Bovine liver nucleoside diphosphokinase (EC 2.7.4.6) was from Boehringer Mannheim (Catalog No. 15448, lot No. 05). *Escherichia coli* B DNA polymerase I, fraction VII, was from General Biochemicals (Catalog No. D-101, lot No. M-7). Poly(riboadenylate) [poly(rA)] and poly(deoxyadenylate) [poly(dA)], potassium salts, were from Miles Laboratories (Catalog No. 11-301, control No. 18430, and Catalog No. 11-331, lot No. 5, respectively). Molecular weights of these polymers were assessed by polyacrylamide gel electrophoresis (Dingman and Peacock, 1971) in collaboration with Dr. C. W. Dingman, National Institutes of Health. Their electrophoretic mobilities suggested that molecules in each of the preparations were heterodisperse with respect to molecular weight. The ranges of molecular weights and the molecular weights of the forms present in highest concentration were as follows: poly(rA), $1.3–21 \times 10^5$, 9.4×10^5; poly(dA), $0.6–4.2 \times 10^5$, 3.7×10^5. The oligodeoxythymidylate primer, $(pdTpdT)_{6-9}$, average of $(pdTpdT)_7$, is termed $(dT)_{14}$ and was from Collaborative Research (Catalog No. M-5, lot No. 336-60A). Native calf-thymus DNA was from Worthington. It was activated maximally by digestion for 15 min with beef pancreas DNase I from Worthington (DPFF; lot No. OCH) according to Aposhian and Kornberg (1962). Activation was tested using *E. coli* DNA polymerase.

Tissues. All cell lines and tissues were of mouse origin except the rat hepatoma tissue culture (HTC) cells (Thompson *et al.*, 1966) provided by Dr. E. B. Thompson, National Institutes of Health. Myeloma tumors MOPC-104E and MOPC-321 were obtained from Dr. M. Potter, National Institutes of Health, and propogated as solid tumors by subcutaneous transplantation in female BALB/c mice that were free of mammary tumor agent (Kuff *et al.*, 1972). Spleen, liver, and liver mitochondria were from 2-month-old BALB/c females, and embryo membranes were from whole BALB/c embryos at 16 days of gestation. Newborn thymus was from a cross between C57BL and A/H mice.

The neuroblastoma C-1300 (clone N4) (Schrier *et al.*, 1974) and the hybrid clone NLI (Minna *et al.*, 1971) from the cross between neuroblastoma C-1300 (clone N4TG1) and L cell (clone B82) were provided by Drs. M. Nirenberg and J. Minna, National Institutes of Health. Swiss-3T3 cells (Matsuya and Green, 1969) and L cells (clone A9) (Littlefield, 1966) were provided by Dr. E. B. Thompson, National Institutes of Health. The JLSV-9 cells (Wright *et al.*, 1967) were purchased from Electro-Nucleonics, Inc. Homogenates of confluent cultures of these cell lines were prepared by the scraping and sonication method of Wilson *et al.* (1972b).

Preparation of Mouse Myeloma DNA Polymerases. Fractionation of mouse myeloma DNA polymerases has been described (Matsukage *et al.*, 1974). Myeloma MOPC-104E was homogenized in the presence of 500 mM KCl and the extract was fractionated by ammonium sulfate precipitation. The cellular DNA polymerases were then separated and purified using DEAE-cellulose and phosphocellulose column chromatography essentially as described by Baril *et al.* (1971). The enzymes eluted from phosphocellulose columns were further purified by hydroxylapatite column chromatography according to Yang *et al.* (1972). Sedimentation coefficients were determined in linear 10–30% glycerol gradients using bovine plasma albumin (4.3 S) as marker. The enzymes are designated DNA polymerases I–IV as follows. DNA polymerase I is an *N*-ethylmaleimide[1]-sensitive 6.1S enzyme localized in the cytoplasmic 100,000*g* supernatant fraction that was more active with activated DNA than poly(rA) as template; DNA polymerase II is a 2.5S enzyme that was resistant to MalNEt and was much more active with poly(rA) than activated DNA as template; DNA polymerase III is a MalNEt-sensitive 7.9S enzyme localized primarily in the cytoplasmic membrane fraction that was active with poly(rA) as template but not with activated DNA; and DNA polymerase IV is a MalNEt-sensitive 5.9S enzyme localized exclusively in the nuclear fraction that was more active with activated DNA as template than with poly(rA). Four enzymes have been detected in normal mouse liver that possess very similar characteristics to those of the four myeloma enzymes (A. Matsukage *et al.*, manuscript in preparation).

Methods

Miscellaneous Methods. The protein concentration was determined by a modification of the method of Lowry *et al.* (1951) using 3–20 μg of protein per reaction. The concentration of antigen associated with the main A-particle structural protein was determined by a microtiter complement fixation assay (Sever, 1962), using rabbit antiserum as described (Kuff *et al.*, 1972). Ultracentrifugation procedures were performed in a Beckman L2-65B centrifuge operated at 4°. Sedimentation forces are average values and concentrations of sucrose (RNase free from Schwarz/Mann) solutions refer to weight per volume values. Sucrose gradient fractions of 200 μl were collected from above. Their density at 5° was determined by measurement of the refractive index at 24° and reference to the relationship between the density and refractive index. Gradient fractions were negatively stained with phosphotungstic acid and examined electron microscopically by Dr. William Hall, Electro-Nucleonics, Inc.

Tissue Fractionation. MOPC-104E tumors, passage 131-2, weighing 2–2.5 g each, were excised 17 days after transplanta-

[1] Abbreviations used are: MalNEt, *N*-ethylmaleimide; HTC, hepatoma tissue culture.

tion, placed in solution A (250 mM sucrose, 50 mM Tris-HCl buffer (pH 7.6) at 25°, 25 mM KCl_3 and 5 mM $MgCl_2$) at 0–1° and weighed. All subsequent operations were performed at 0–5°. The tumors were minced and homogenized in 4 vol of solution A using 15 strokes with a hand-operated Potter-Elvehjen homogenizer and the homogenate was filtered through two layers of gauze. Subcellular fractions and intracisternal A-type particles were isolated from this homogenate on the day of its preparation as described in detail elsewhere (Kuff *et al.*, 1968) and as outlined in Figure 1. In the present study, sucrose-washed nuclei were isolated from the initial 700*g* pellet (nuclear fraction) as follows: the pellet was resuspended by hand homogenization in solution A plus 60% sucrose, layered over 5 ml of additional solution A plus 60% sucrose, and centrifuged at 53,500*g* for 1 hr in a SW 25.1 rotor to yield a pellet containing nuclei.

Unless otherwise indicated all pellet fractions were resuspended in ice-cold 10 mM Tris-HCl buffer (pH 7.4 at 25°) at a protein concentration of ~10 mg/ml. Measurements of the volume of each fraction were made; then fractions that were not used in a subsequent step were divided into aliquots, frozen in solid CO_2, and stored in the vapor phase of liquid N_2.

Assays of DNA Polymerase Activity. DNA polymerase activity was measured by determining the amount of incorporation of tritium-labeled deoxynucleoside triphosphate into cold acid-insoluble material. Preparations for enzyme assay were thawed immediately before use and mixed with 1 vol of 20 mg/ml of crystalline bovine plasma albumin (Armour Pharmaceuticals); then 1 vol of this extract–bovine plasma albumin solution was mixed with 9 vol of 60% glycerol. Nine volumes of the extract–bovine plasma albumin–glycerol solution was then mixed with 1 vol of solution B (1.25 M Tris-HCl buffer (pH 8.3) at 50 mM and 37°, 1.0 M KCl, 313 mM magnesium acetate, and 25 mM dithiothreitol) and added to reaction mixtures.

Reactions performed under conditions for measurement of activity in A-particle preparations (Wilson and Kuff, 1972), termed A conditions, contained in a final volume of 25 μl: 50 mM Tris-HCl buffer (pH 8.3) at 37°, 19% (v/v) glycerol, 360 μg/ml of bovine plasma albumin, 250 mM KCl, 12.5 mM magnesium acetate, 1 mM dithiothreitol, 1% Tween 80 (Wilson *et al.*, 1972a), 0.5 mM ATP, 10 μg/ml of nucleoside diphosphokinase, 230 μg/ml each of poly(rA) and $(dT)_{14}$ as indicated, 0.3–3.3 μg of enzyme extract protein, and 0.5 mM [^{3}H]deoxythymidine 5′-triphosphate (200–300 cpm/pmol). Reactions containing all components except nucleotides and nucleoside diphosphokinase were incubated at 37° for 20 min before incorporations were begun by addition of the remaining components. Incorporations were at 37° for 75 min in silicon-treated soft glass tubes, 10 × 75 mm.

Reactions performed under M conditions were identical with A-condition reactions except that they did not contain Tween 80 and they contained 75 mM KCl, 0.5 mM $MnCl_2$ instead of magnesium acetate, 40 μg/ml of $(dT)_{14}$, and 200 μg/ml of poly(rA) or poly(dA) as indicated, and they were incubated for 60 min at 37° without a preincubation. Reactions performed under D conditions were identical with A-condition reactions except that they did not contain Tween 80 and they contained 40 mM KCl, 10 mM magnesium acetate, 0.5 mM dATP, dCTP, and dGTP, and 230 μg/ml of maximally activated calf-thymus DNA instead of poly(rA)·$(dT)_{14}$. Incubation was for 60 min at 37° without a preincubation.

Acid-precipitable radioactivity was measured as previously described (Wilson and Kuff, 1972). All DNA polymerase activities reported were obtained from reactions performed in

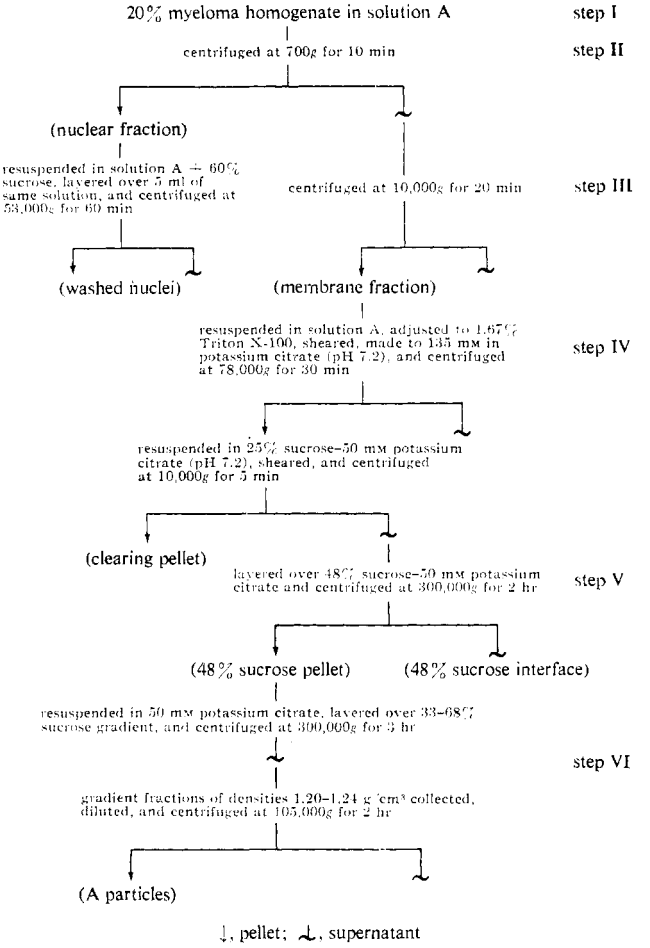


FIGURE 1: Schematic representation of the tissue fractionation and A-particle isolation procedures.

the range of linearity between both time of incubation and concentration of enzyme protein. Values reported as Δpicomoles of [^{3}H]dTMP incorporated had "blank" values subtracted from them. "Blank" values were determined in reactions containing no enzyme protein and were equal to 0.4–0.6 pmol of [^{3}H]dTMP incorporated per reaction. Activities reported as "less than" a certain level of incorporation refer to values that were less than 33% more than the reaction "blank."

Results

Assay for the Type of DNA Polymerase Activity in A-Particle Preparations (A Conditions). The poly(rA)·$(dT)_{14}$-directed DNA polymerase activity of A-particle preparations was characterized (Wilson and Kuff, 1972) in order to find reaction conditions which would permit its specific measurement in crude tissue extracts (Wilson *et al.*, 1972a). These previously reported results demonstrated that the enzyme activity could be measured in reactions containing 250 mM KCl, 12.5 mM Mg^{2+}, and poly(rA)·$(dT)_{14}$ as a template primer. The specificity of these assay conditions (termed A conditions) for the polymerase in A-particle preparation and the relationship between this enzyme and other mouse DNA polymerases were investigated in two types of experiments: first, by comparing the activity of the A-particle preparation DNA polymerase with other mouse DNA polymerases, and second, by comparing the amount of A-condition DNA polymerase activity with the number of A particles in a variety of murine tissues.

TABLE I: Comparison of Activities of DNA Polymerases under Different Types of Reaction Conditions.

Enzyme Prepn from Myeloma MOPC 104E	[^{3}H]dTMP Incorpd (Δpmol)/min per mg of Protein under Reaction Conditions[a]			
			M	
	A	D	Poly(rA)	Poly(dA)
A particle	675	0	0	0
DNA polymerase I	0	7	6	
DNA polymerase II	0	7	75	153
DNA polymerase III	2	0	242	40
DNA polymerase IV	0	10	5	

[a] Reactions were performed as described under Methods and contained 1–1.5 μg of protein except with DNA polymerase IV where 5 μg of protein was used.

In the first experiment, shown in Table I, the activity of enzyme preparations was compared under four different reaction conditions including those for assay of natural DNA-directed DNA polymerase activities (termed D conditions) and synthetic template-directed manganese-dependent activities (termed M conditions). It is important to note that M conditions were suitable for detection of the poly(rA)-directed DNA polymerase activities reported by Maia *et al.* (1971), Fridlender *et al.* (1972), and Ward *et al.* (1972). Although the precise relationship is unclear, either DNA polymerase II or III from myeloma could correspond to the enzymes described by Weissbach and coworkers (Fridlender *et al.*, 1972; Fry and Weissbach, 1973), while the myeloma DNA polymerase III could correspond to the enzyme reported in rat liver by Ward *et al.* (1972). The A-particle preparation was not active under M conditions or D conditions while the four mouse myeloma DNA polymerases were much less active under A conditions than at least one of the other three reaction conditions.

In the second experiment, shown in Table II, the amount of A-condition DNA polymerase activity was compared with the abundance of A particles in several mouse cell types and in rat HTC cells. Unfractionated homogenates and other extracts prepared from tissues containing numerous A particles (myelomas MOPC-104E, neuroblastoma clone N4, and a neuroblastoma–L cell hybrid clone) all possessed activity under A conditions that was completely dependent upon poly(rA)·$(dT)_{14}$ and sensitive to MalNEt in the absence of dithiothreitol. The whole homogenate from myeloma MOPC-321, a tumor containing only a few A particles as determined by electron microscopy, possessed no significant activity; however, activity was detected in the cytoplasmic particulate fraction from this tumor. Similarly prepared whole homogenates and particulate fractions from tissues that did not contain A particles possessed no detectable activity under A conditions, and no activity was detected in fractions from embryos and newborn thymus (Aoki *et al.*, 1970) in which occasional A-type particles have been observed by electron microscopy. Mixing of a previously characterized sample of A particles with the unfractionated homogenates resulted in little or no inhibition of the A-particle sample DNA polymerase activity, except with the homogenate from MuLV-infected JLSV9 cells where 56% inhibition was observed.

Subcellular Distribution of A-Condition DNA Polymerase Activity in Myeloma MOPC-104E. The subcellular distribution of the A-condition DNA polymerase activity was determined by assay of the fractions produced during the isolation of A particles. The isolation procedure, outlined in Figure 1, was based upon purification of particulate material that was resistant to treatment with 1.67% Triton X-100 and 135 mM potassium citrate, and is known, from previous electron microscopy (Kuff *et al.*, 1968; Wivel *et al.*, 1973), to yield preparations consisting of A particles contaminated by small amounts of membrane vesicles and amorphous material. The various fractions were assayed for total protein, DNA polymerase activity, and antigen associated with the main A-particle structural protein. The results are shown in Table

TABLE II: Comparison of the Content of A Particles and the Amount of A-Condition DNA Polymerase Activity in Various Tissues.[a]

Type of Murine[b] Tissue and Extract	Content of A Particles in Tissues as Judged by Electron Microscopy	Amt of [^{3}H]dTMP Incorpd per min	
		Δpmol/mg of Extract Protein[i]	Δpmol/mg of Added A-Particle Protein[j]
A particles from			
Myeloma MOPC-104E		200	
None		0	250
(A) Unfractionated homogenates			
Myeloma MOPC-104E	High[c]	9	320
Neuroblastoma clone N4	High[d]	7	
Neuroblastoma–L cell hybrid clone NL1	High[d]	16	
Myeloma MOPC-321	Low[c]	<2	230
JLSV9 cells	Absent[d]	0	220
MuLV-infected JLSV9 cells	Absent[d]	<2	110
3T3 cells	Absent[d]	0	280
L(A9) cells	Low[d]	<2	200
Adult spleen	Extremely low[e]	0	280
Adult liver	Extremely low[e]	0	280
HTC cells	Absent[d]	0	270
(B) Subcellular fractions			
Myeloma MOPC-104E[f]		110	320
Myeloma MOPC-321[f]		3	
Embryo[g]		0	
Newborn thymus[h]		0	

[a] The sources and procedures for preparation of enzyme extracts are described under Methods and in the table. Reactions were performed under A conditions as described under Methods. The myeloma MOPC-104E A particles possessed a specific activity under A conditions of 213 pmol of [^{3}H]-dTMP incorporated/min per mg of protein. [b] HTC cells were of rat origin; all other tissues were of mouse origin. [c] Kuff *et al.* (1972). [d] Electron micrographs provided with cells. [e] Wivel and Smith (1971). [f] Pellet after treatment with Triton X-100–potassium citrate as in step IV, Figure 1. [g] Membrane pellet as in step III, Figure 1. [h] Nuclear supernatant as in step II, Figure 1. [i] No modification. [j] Plus 1 μg of A-particle protein.

TABLE III: Cumulative Recoveries of Protein, DNA Polymerase Activity, and A-Particle Antigen in Subcellular Fractions of Myeloma MOPC-104E.[a]

Subcellular Fraction[b]	Amt of Protein Recovd		DNA Polymerase Act				A-Particle Antigen			
			Amt Recovd				Amt Recovd			
	mg	%	Units[c]	%	Units/mg of Protein	Fold Purification	CF Units[d] $\times 10^{-3}$	%	CF Units/mg of Protein $\times 10^{-3}$	Fold Purification
Whole 20% homogenate	396	100	6819	100	17	1	1988	100	5.0	1
Nuclear fraction	110	27.8	1353	19.8	12.3		164	8.3	1.5	
Nuclear supernatant	262	66.2	5756	84.4	22	1.3	2540	128	9.7	1.9
Membrane supernatant	162	40.9	0	0	0		121	6.1	0.7	
Membrane fraction	106	26.8	6463	94.8	61	3.6	2805	141	26.4	5.3
Supernatant after Triton X-100–citrate treatment	43.3	11.0	0	0	0		11	0.5	0.2	
Pellet after Triton X-100–citrate treatment	27.6	7.0	5396	79.1	196	11.5	1937	98	70.4	14.1
Clearing pellet	2.4	0.6	334	4.9	139		242	12.2	101	
48% sucrose interface	11.2	2.8	1042	15.3	93		484	24.3	43.2	
48% sucrose pellet	4.5	1.1	1332	19.6	296	17.4	1160	58.4	257	51.2
A particles	2.3	0.6	1131	16.6	492	29	291	14.6	133	26.6
Total recovered in fractions	331.4	83.7	3860	56.6			1313	66		

[a] Four grams of fresh murine myeloma MOPG-104E, passage 131-2, was used. The homogenate was prepared and fractionated as shown in Figure 1. Each value for DNA polymerase activity represents the average of measurements at two levels of extract protein (between 0.6 and 1.9 μg per reaction) performed under A conditions as described under Methods. [b] Fractions prepared as shown in Figure 1; fractions in italics were used in the next step of the isolation procedure. [c] One unit of DNA polymerase activity is equivalent to 1 pmol of [^{3}H]dTMP incorporated per min at 37°. [d] One unit of complement fixation is equivalent to the reciprocal of the end-point dilution.

III. In each fraction that possessed DNA polymerase, all of the activity was dependent upon poly(rA)·$(dT)_{14}$, negligible in reactions containing MalNEt in the absence of dithiothreitol, and proportional to the amount of tissue extract in reaction mixtures. No effectors of activity were detected in mixing experiments using a previously characterized sample of A particles.

As may be seen in Table III, the isolation procedure gave a recovery in A particles of 0.6% of the crude homogenate protein and 17% of the DNA polymerase activity resulting in a 29-fold purification of activity. Very little activity was recovered in the two supernatant fractions derived from the postnuclear supernatant. Some of the activity in the nuclear fraction may have been due to contamination by A particles since only 30% of this activity remained associated with nuclei after they were washed by sedimentation through 68% sucrose. Note that the fold purification and subcellular distribution of the A-particle antigen were similar to those of the DNA polymerase activity.

In Figure 2 are shown typical isopycnic sucrose gradient profiles of absorbance at 260 nm (Kuff *et al.*, 1968) and DNA polymerase activity obtained during the final step of the isolation procedure. Purified A particles were recovered from gradient fractions 18–22, which contained the congruent peaks of absorbance and DNA polymerase that sedimented at an average density of 1.22 g/ml.

As seen in Table III, much of the total A-condition DNA polymerase activity was not recovered in the final A-particle preparation. This loss of activity occurred primarily at step V, in which A particles were separated from other Triton X-100–potassium citrate resistant particulate material. The nature of this loss was investigated by comparing the isopycnic sedimentation properties of A particles and the DNA polymerase activity in the Triton X-100–potassium citrate resistant particulate fraction. The A particles were located in the gradient both by electron microscopic examination and by measurement of the antigen associated with the A-particle structural protein. The results are shown in Figure 3. It may be seen that the distribution of DNA polymerase activity was different from the distribution of activity in the 48% sucrose pellet shown in Figure 2. However, the distribution of activity was very similar to that of the A-particle antigen, and typical A particles were observed in fractions 10 and 16 where the peaks of DNA polymerase activity were found. This apparent cosedimentation of activity and A-particle structural components was not the result of enzyme effectors since the activity of a characterized sample of A particle was not changed after mixing with gradient fractions.

As shown in Figure 3, approximately 40% of the enzyme and antigen-containing material (fractions 6–12) was recovered at densities lighter than 48% sucrose (1.18 g/cm^3). This indicated that some of the loss of DNA polymerase activity and A-particle structural protein during step V of the isolation procedure was the result of exclusion of this light polymerase and A-particle containing material. No evidence was obtained for the association of activity with particulate material other than A particles.

Separation of A-Particle DNA Polymerase from Other DNA Polymerases. In order to assess the possibility of contamination of the gradient purified A particles by other cellular DNA polymerase, the subcellular distribution was determined of activities measured under D conditions, M conditions, and

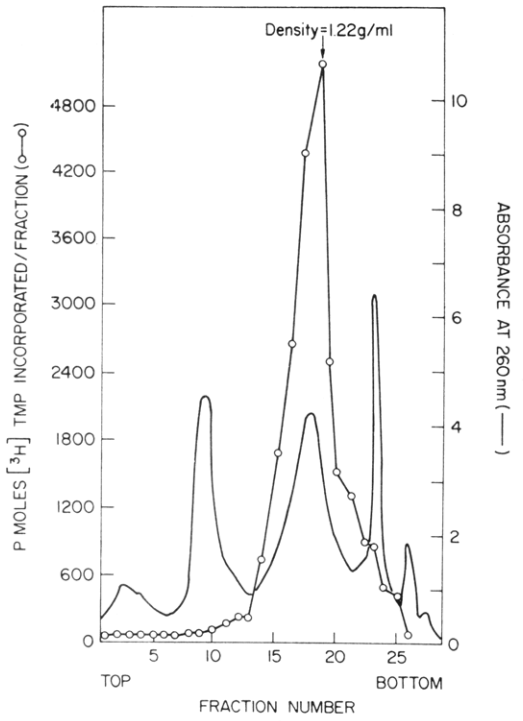


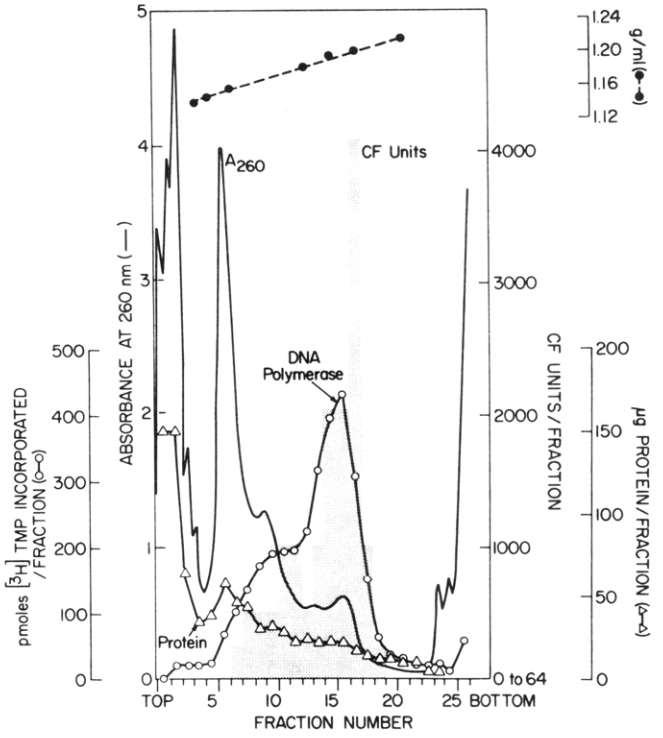
FIGURE 2: Density equilibrium centrifugation of the "48% sucrose pellet" from step V of the A-particle isolation procedure. A "48% sucrose pellet" from myeloma MOPC-104E (passage 127-2) was resuspended in 50 mM potassium citrate (pH 7.2). A portion of the suspension containing 1.99 mg of protein and 295 units of DNA polymerase activity was layered over a 5.0-ml 33–68% linear sucrose gradient at 3–5° containing 50 mM potassium citrate (pH 7.2). Centrifugation was for 4 hr at 234,000*g* in an SW 50.1 rotor. DNA polymerase assays were performed under A conditions as described under Methods using 1–5 μl of each 200-μl gradient fraction. A total of 313 units of DNA polymerase was recovered in gradient fractions. One unit of DNA polymerase activity (○) was equal to 1 pmol of [^{3}H]dTMP incorporated per min at 37°. Absorbance at 260 nm (–) was measured in a continuous-flow Gilford spectrophotometer.

M conditions using poly(dA) as a template instead of poly(rA). As shown in Table IV, the activity under A conditions was the only activity concentrated in the gradient-purified A particles. The highest activity in the whole homogenate was observed using M conditions with poly(dA) as template. This activity was present in the ratio of 100:18:14 in the nuclear pellet, membrane fraction, and 105,000*g* supernatant fraction, respectively. After the treatment of the membrane fraction with Triton X-100–potassium citrate during step IV of the fractionation procedure, most of this poly(dA)-directed activity was separated from the A particles and recovered in the supernatant, in contrast to the A-condition activity.

TABLE IV: Comparison of DNA Polymerase Activities in Myeloma Homogenate and A Particles under Different Types of Reaction Conditions.

Subcellular Fraction	Δpmol of [^{3}H]dTMP Incorpd per min per mg of Protein for Reaction Condns			
			M	
	A	D	Poly(rA)	Poly(dA)
20% whole homogenate	23	3	9	72
A particles	707	2	7	17

[a] Myeloma MOPC-104E (passage 140-2) was fractionated as shown in Figure 1. Each reaction contained 0.7 and 0.5 μg of protein for the homogenate and A-particle fraction, respectively, and was performed as described under Methods.



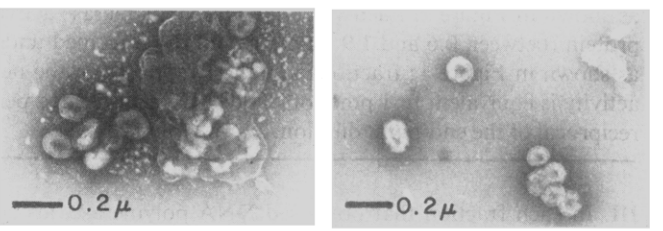


FIGURE 3: Density equilibrium centrifugation of the Triton X-100–potassium citrate resistant pellet fraction. A portion of the pellet fraction that was produced during step IV of the A-particle isolation procedure using myeloma MOPC-104E, passage 131-2, was layered over a 5-ml 33–68% linear sucrose gradient containing 100 mM potassium chloride and 50 mM Tris-HCl (pH 7.4 at 25°). The material layered over the gradient contained 1.04 mg of protein, 67 units of DNA polymerase activity, and 16,384 complement fixation units of antigen associated with the main A-particle structural protein. Centrifugation was for 3 hr at 300,000*g* in a SW 50.1 rotor. Absorbance at 260 nm (–) was measured in a Gilford continuous-flow spectrophotometer. DNA polymerase activity (○) was measured under A conditions using 5 μl of each 200-μl gradient fraction. Complement fixation assays (bar graph) and negative stain electron microscopic examination of fractions 10 and 16 both were performed using 25 μl of gradient fractions. One unit of DNA polymerase activity was equal to 1 pmol of [^{3}H]dTMP incorporated per min at 37°; one unit of antigen complement fixation was equal to the reciprocal of the end-point dilution of the gradient fraction. A total of 43 units of DNA polymerase and 15,104 complement fixation units of antigen were recovered in gradient fractions. Results are expressed as the amounts of protein, complement fixation, and DNA polymerase activity per total gradient fraction.

Attachment of the DNA Polymerase to A Particles. The precise co-fractionation and co-sedimentation of the DNA polymerase activity and A particles suggest that the enzyme was physically attached to the particle. This apparent association between the DNA polymerase and A particles was

investigated by exposing particles to various surfactants and a high concentration of KCl. Mixtures were then layered over a solution containing 30% glycerol and centrifuged for 1 hr at 49,000 rpm in a SW 50.1 rotor. Particulate components (A particles and subparticle material) were recovered in the bottom of the centrifuge tube in pellet form. Similar procedures have been shown to result in the removal of a portion of the outer membrane shell of the A particle (Wivel *et al.*, 1973). The results of DNA polymerase assays using the supernatant, the pellet, and material that was exposed to solubilization agent but not centrifuged are shown in Table V. DNA polymerase activity was not recovered in the supernatant after any of the treatments and activity equal to that of the uncentrifuged sample was recovered in the pellet. When Rauscher MuLV was handled similarly using the same surfactant solubilization agents, its DNA polymerase activity was recovered in the supernatant indicating that the procedure was suitable for release and detection of at least one type of particle-associated DNA polymerase activity. Thus, it is unlikely that the association between the polymerase and particle was solely the result of electrostatic interaction involving the outer membrane shell of the particle.

TABLE V: Effect of Potassium Chloride and Surfactants on the Sedimentation of the A-Particle Preparation DNA Polymerase Activity.

Treatment of A Particles in Solution C[a]	[^{3}H]dTMP Incorpd per Reaction (pmol)			
	Not Centrifuged	Supernatant	Pellet	Minus A Particles
None (solution C alone)	6.4	0.3	6.8	0.4
(+) 900 mM KCl	8.5	0.3	6.8	0.3
(+) 1% sodium deoxycholate	3.2	0.3	4.0	0.3
(+) 1% Tween 80	5.6	0.3	6.7	0.4
(+) 1% Triton N-101	3.6	0.4	2.9	0.4

[a] Aliquots (30 μl) of a preparation of gradient-purified A particles (from myeloma MOPC-104E passage 140-2) containing 120 μg of protein and 58.9 pmol of [^{3}H]dTMP incorporated per min of A-condition DNA polymerase activity were mixed at 0–2° with 30 μl of an appropriate solution so that the resulting mixture contained solution C [20% (v/v) glycerol, 10 mM KCl, 0.1 mM EDTA, 2 mM dithiothreitol, 5 mg/ml of bovine plasma albumin, 50 mM Tris-HCl (pH 8.5) at 3°) and the surfactant or 900 mM KCl as indicated in the table. The mixture (57 μl) was layered over 200 μl of solution D (solution C + 10% glycerol) and centrifuged at 2° for 60 min at 49,000 rpm (SW 50.1 rotor). The remaining 3 μl of each mixture was mixed with 10 μl of solution D and held at 0–3° during the centrifugation. The supernatants in the six tubes were withdrawn and the pellets were resuspended in 30 μl of solution C containing the appropriate surfactant or KCl and 200 μl of solution C. A 1-μl portion of the three solutions (supernatant, pellet, and uncentrifuged portion) from each of the treatments was then tested for DNA polymerase activity under A conditions.

Discussion

Homogenates and particulate fractions prepared from mouse tissues rich in intracisternal A-type particles exhibited poly(dT) synthetic capacity in reactions containing poly(rA)·$(dT)_{14}$ as template primer, 250 mM KCl, and 12.5 mM Mg^{2+} (A conditions). The activity required a sulfhydryl reducing agent and was inhibited by *N*-ethylmaleimide. There are several reasons why this activity does not appear to be due to a normal cellular DNA polymerase. Most of this type of DNA polymerase activity in the total myeloma homogenate fractionated as a particulate cytoplasmic component that was not solubilized when the membrane fraction was treated with Triton X-100 and 135 mM potassium citrate. The activity appeared to be firmly associated with A particles. No similar subcellular fractionation was observed for the other types of DNA polymerase activity tested (Table IV). In addition, a variety of cells that do not contain abundant A particles showed no detectable activity under A conditions and none of the four myeloma enzymes that appear to correspond to mouse cellular DNA polymerases exhibited high activity in the A-condition assay. Conversely, the A-particle associated enzyme was essentially inactive with calf-thymus DNA as template primer and under other conditions favoring activity of myeloma cellular DNA polymerases.

It is pertinent to consider the relationship between the A-particle associated activity and the various other DNA polymerase activities capable of using poly(rA) as template that have been reported in a number of normal and malignant cells, including mouse myeloma (Maia *et al.*, 1971; Scolnick *et al.*, 1971; Stavrianopoulos *et al.*, 1971; Penner *et al.*, 1971; Chang and Bollum, 1972; Fridlender *et al.*, 1972; Bolden *et al.*, 1972; Ward *et al.*, 1972; Fry and Weissbach, 1973; Persico *et al.*, 1973; Matsukage *et al.*, 1974). It is an important feature that all of these enzyme activities were found in the soluble phase when cells were disrupted in mild buffers or were readily solubilized by salt extraction or treatment with nonionic surfactant. In the cases tested, these ribopolymer-transcribing activities preferred Mn^{2+} to Mg^{2+} when poly(rA)·oligo(dT) was the template primer. This behavior contrasts clearly with that of the A-particle associated activity. It appears then that the A-particle associated enzyme activity, by virtue of its restriction to particle-containing cells, its resistance to solubilization, and its activity requirements, can be distinguished from other known DNA polymerase activities.

Finally, it is important to note the possibility that a cellular DNA polymerase not uniquely in association with the particle could have been responsible for the A-particle activity. A cellular enzyme in firm association with the A particle might assume reaction properties different from those of identical enzyme molecules not associated with the particle.

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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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