a determination. The discovery that DNA polymerase δ is associated with an active 3′ to 5′ exonuclease suggests that this mammalian DNA polymerase is able to correct mistakes made during DNA polymerization and that one of the mechanisms whereby replication fidelity is maintained in eukaryotic cells may be similar to that of the prokaryotes.

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Complexity and Specificity of Polysomal Poly(A+) RNA in Mouse Tissues†

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ABSTRACT: Base sequence complexities of polysomal poly(A+) RNA from mouse embryo, brain, and liver have been estimated by hybridization to homologous cDNA to be approximately 7×10^9, 1.5×10^{10}, and 7×10^9 daltons, respectively. By annealing each cDNA with a large excess of total mouse embryo DNA, the genes coding for the polysomal poly(A+) sequences were shown to be unique. Heterologous hybridization experiments showed that the high abundance class of poly(A+) sequences in one tissue is not identical with the high abundance class in other tissues. However, at least 55%, and possibly more, of the poly(A+) RNA in one tissue is present in the poly(A+) RNA of another tissue.

There is now a variety of techniques which can be used to examine heterogeneous populations of messenger RNA (mRNA) prepared from eukaryotic cells. A question of immediate interest is the number of different sequences present in such a population. Galau et al. (1974) hybridized excess polysomal RNA prepared from sea urchin embryos to purified unique DNA and demonstrated that approximately 14 000 different mRNA sequences were present on the polysomes. An alternative approach is to use a viral reverse transcriptase to prepare a complementary DNA (cDNA) to the polyadenylated [poly(A+)] RNA sequences present in the cytoplasm or on the polysomes. By following the hybridization kinetics of the cDNA with its homologous RNA, it has been possible to estimate the number of different RNA sequences present in a number of cell types, e.g., 40 000 cytoplasmic sequences in HeLa cells (Bishop et al., 1974), 8000 polysomal sequences in mouse Friend cells (Birnie et al., 1974) and 8000 cytoplasmic sequences in mouse L cells (Ryffel and McCarthy, 1975). All these studies indicate that there is a wide range in abundance in such RNA populations.

As reviewed by Lewin (1975), DNA reannealing experiments indicate that most mRNA sequences are transcribed from unique DNA. Hence, the total proportion of unique eukaryotic DNA which codes for structural genes will be related to the degree of specificity of mRNA populations in different cell types. Ryffel and McCarthy (1975) demonstrated that cDNA from L cell cytoplasmic poly(A+) RNA can be hybridized efficiently to cytoplasmic poly(A+) RNA from mouse

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liver and kidney. By contrast, 35% of cDNA prepared from brain cytoplasmic poly(A^+) RNA failed to hybridize with L cell cytoplasmic poly(A^+) RNA. This was interpreted as being due to 11 000 mRNA sequences which are present in brain cells but not in L cells.

We have compared poly(A^+) RNA sequences present on polysomes purified from mouse embryo, brain, and liver tissues. There were two reasons for using only carefully purified high molecular weight polysomes, rather than the total cytoplasmic fraction. Firstly, in view of parallel studies with nuclear poly(A^+) RNA, we wished to avoid the possibility of contamination with nuclear RNP particles, as discussed by Galau et al. (1974). Secondly, it was desirable to consider only those sequences which were actively being translated since, by definition, only the mRNA on polysomes can be assumed to represent structural genes. This is important since it has recently been shown by MacLeod (1975) using pulse-chase labeling experiments in mouse myeloma cells that not all cytoplasmic poly(A^+) RNA sequences ultimately reach polysomes. Using a cDNA transcribed from each poly(A^+) RNA population, we have investigated, firstly, the base sequence complexity of each population and, secondly, the degree of specificity between the populations.

Experimental Procedures

Preparation of Tissues. Uteri were removed from 14-day pregnant mice and chilled in ice-cold isotonic phosphate buffered saline (PBS[1]). The embryos, after removal from the uteri, were minced through a 10-ml syringe without a needle, and washed several times in PBS to remove red blood cells.

Prior to removal the livers were perfused with warm (25–35 °C) 0.25 M sucrose in buffer A (0.025 M KCl, 0.05 M Tris-HCl (pH 7.6), 0.003 M $MgCl_2$) in order to reduce the amount of blood cells in the preparation. Perfusion was carried out by puncturing the hepatic portal vein with a fine needle and passing up to 10 ml of buffer into the liver from a syringe. The livers were separated from the gall bladders, removed, and minced with scissors. The minced tissue was washed several times in 0.25 M sucrose in buffer A.

Adult mice (3 months old) were killed and decapitated, and the brains were removed and placed in ice-cold 0.25 M sucrose in buffer A. The tissue was minced with scissors and washed several times in 0.25 M sucrose in buffer A.

Preparation of Polysomes. The minced tissue was resuspended in approximately 10 volumes of ice-cold 0.25 M sucrose in buffer A and ribonuclease inhibitor (Searle, England) was added to a concentration of 3 units/ml. The tissue was gently homogenized (about 5–6 strokes) with a hand-held Teflon ball homogenizer. It was found in practice that the use of a motor-driven homogenizer caused breakage of the high molecular weight polysomes. The homogenate was centrifuged at 800*g* (2000 rev/min) for 15 min at 4 °C in order to pellet nuclei and other debris. The post-nuclear supernatant was centrifuged at 10 000*g* (8000 rev/min) for 10 min at 4 °C to remove mitochondria. Triton X-100 was added to the post-mitochondrial supernatant to a final concentration of 1% w/v and 14 ml was layered over a 5-ml cushion of 2 M sucrose in buffer A in 21-ml polycarbonate tubes. The tubes were centrifuged at 230 000*g* (55 000 rev/min) for 3.5 h at 4 °C. The supernatant was removed carefully by aspiration and the inside walls of the tube were wiped with sterile gauze. The polysomes, which formed a translucent pellet, were stored at −20 °C.

The polysome pellets were resuspended in buffer B (0.14 M NaCl, 0.01 M Tris-HCl (pH 7.4), 0.0015 M $MgCl_2$) and 1-ml portions were centrifuged through 20 ml of 15–40% (w/w) sucrose gradients in buffer B at 29 000 rev/min for 1 h at 4 °C. $s_{20,w}$ values were computed by a modification of the method of Funding and Steensgaard (1973) and polysomes sedimenting >100 S were collected by centrifugation at 55 000 rev/min for 3 h. Typical polysome profiles are shown in Figure 1.

Deproteinization of Polysomes. The polysome pellets were dissolved in buffer B containing 1% w/v sodium lauryl sulfate to a final concentration of 1–2 mg of polysomes/ml. Solid CsCl, which had previously been passed through Chelex to remove heavy metal ions, was added (1.4 g per ml of original solution) and dissolved by vortex mixing. In order to reduce protein contamination of the RNA, diethyl pyrocarbonate was added (5 μl per mg of protein) and the mixture vortexed again. The mixture which contained a thick precipitate of cesium lauryl sulfate and protein was centrifuged at 10 000*g* (8000 rev/min) for 30 min at room temperature. The CsCl solution which contained the deproteinized RNA was removed by carefully inserting a long hypodermic needle through the densely packed pellicle which was formed by centrifugation. Any small particles from the pellicle were removed by passage through a Swinnex adaptor containing a disc of Whatman GF/C glass-fiber paper. Three volumes of distilled water was added to the CsCl solution and the RNA was precipitated by addition of 8 volumes (relative to the original volume) of cold ethanol. After at least 4 h at −20 °C, the RNA precipitate was recovered by centrifugation at 10 000*g* (8000 rev/min) for 10 min, washed with ethanol, and dried in a stream of nitrogen.

Preparation of Poly(A^+) RNA. Poly(A^+) RNA was isolated by passage through a 1-ml column of oligo(dT)cellulose (Collaborative Research Inc.) as described by Aviv and Leder (1972). RNA bound on the first passage was passed through again and only RNA which could rebind to the column was precipitated with ethanol, redissolved in sterile water, and desalted by gel filtration through Sephadex G-25 with a pad of Chelex equilibrated with water. In order to avoid cross-contamination, separate columns were used for each RNA preparation.

Synthesis of cDNA. Reverse transcriptase was prepared from avian myeloblastosis virus as described by Kacian et al. (1971). The template poly(A^+) RNA (8 μg) was incubated for 2 h at 38 °C in a 0.5-ml mixture containing 8 μg of oligo$(dT)_{17}$ (P-L Biochemicals), 0.2 μmol of dATP, 0.2 μmol of dGTP, 0.2 μmol of dTTP, 20 nmol of [^{3}H]CTP (4.85 Ci/mmol; Radiochemical Centre, Amersham, England), 50 μg of actinomycin D, 60 μg of bovine serum albumin, 50 mM Tris-HCl, pH 8.2, 50 mM KCl, 10 mM dithiothreitol, 5 mM magnesium acetate, and 100 μl of reverse transcriptase in 0.15 M potassium phosphate (pH 8) containing 50% glycerol and 1 mM dithiothreitol. The cDNA was isolated by chromatography on Sephadex G-50, after addition of 50 μg of *Escherichia coli* DNA as carrier and was centrifuged on a 4–11% sucrose gradient in 0.9 M NaCl, 0.1 M NaOH, at 29 000 rev/min for 24 h at 20 °C. cDNA which sedimented faster than 2.5 S was recovered by neutralization and precipitation with ethanol. Sedimentation coefficients were calculated by the methods of Funding and Steensgaard (1973) and molecular weights were calculated according to the equation $s_{20,w} = 0.0528M^{0.4}$ (Studier, 1965).

[1] Abbreviations used: PBS, phosphate buffered saline; Tris, tris(hydroxymethyl)aminomethane; Hepes, *N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid; EDTA, ethylenediaminetetraacetic acid; RNP, ribonucleoprotein.

Annealing of Mouse Embryo DNA and cDNA. DNA was isolated from whole mouse embryos by the procedure of Hell et al. (1972), sonicated, and fractionated as described by Harrison et al. (1974). The size of the DNA was determined on an alkaline sucrose gradient as described above for cDNA.

Mixtures of mouse embryo DNA (100 μg) and cDNA (0.2 ng) in 20 μl of 0.12 M sodium phosphate buffer (pH 6.8) were heat denatured (100 °C for 5 min), annealed at 60 °C for appropriate times, and fractionated on water-jacketed columns of hydroxylapatite at 60 °C (Harrison et al., 1974). Single-stranded and double-stranded DNAs were eluted with 0.16 and 0.4 M sodium phosphate buffer (pH 6.8), respectively, and the A_{260} and radioactivity of each fraction were measured.

RNA-cDNA Hybridization. Appropriate volumes of RNA and cDNA solutions in sterile, distilled water were mixed, lyophilized, and redissolved in hybridization buffer (0.5 M NaCl, 25 mM Hepes, 0.5 mM EDTA, pH 6.8, 50% (v/v) formamide; the salt solutions, before addition of formamide, were passed through Chelex-100 resin, treated with diethyl pyrocarbonate, and autoclaved). Portions of the solution (0.5–10 μl) were sealed in glass capillaries which had been siliconized (Repelcote; Hopkins and Williams, Ltd., Chadwell Heath, Essex, England) and washed with 0.1% aqueous diethyl pyrocarbonate. The capillaries were heated at 60 °C for 5 min and then incubated at 43 °C for the appropriate times. The contents of each capillary was flushed out with 0.25 ml of buffer comprised of 0.07 M sodium acetate, 2.8 mM $ZnSO_4$, 0.14 M NaCl, pH 4.5, and 14 μg/ml of heat-denatured mouse-embryo DNA. The proportion of cDNA in hybrid was determined by adding 0.1 ml of S1 nuclease (prepared from takadiastase as described by Sutton, 1971) and measuring the proportion of radioactivity rendered acid soluble by incubation at 37 °C for 2 h. The amount of nuclease added was sufficient to ensure complete degradation of unhybridized cDNA within 1 h.

Polyacrylamide Gel Electrophoresis. Aqueous 2.6% polyacrylamide gels (11 cm) were set up and run, according to Loening (1969), at 75 V for up to 2.5 h. Denaturing formamide gels (3.2% polyacrylamide) were prepared according to Staynov et al. (1972). Electrophoresis was carried out at 150 V for 2.5 h. An 18S RNA prepared from human reticulocyte ribosomal RNA was used as a marker.

Results

Polyadenylated Polysomal RNA. Polysomes, including both free and membrane bound, were prepared from the cytoplasmic fractions by pelleting through heavy sucrose. As a further purification step, however, the polysomes thus obtained were sedimented on a sucrose gradient and only those sedimenting >100 S were pooled for further analysis. This effectively removes any possible contamination with nuclear RNP particles (Galau et al., 1974). The use of this gradient had the further advantage that the integrity of the polysomes could be examined prior to extraction of RNA. This is particularly important when preparing polysomes from tissues which may contain high concentrations of nucleases since it is desirable to obtain full-length poly(A+) RNA from such polysomes. Figures 1a–c illustrate typical polysome profiles obtained from the three tissues.

Although phenol-based techniques are most frequently used for removing protein from RNA, there are two objections to their use in this study. Firstly, Macnaughton et al. (1974) have reported that phenol causes aggregation of RNA which would prevent complete purification of the poly(A+) sequences. Secondly, Perry et al. (1972) have shown that phenol preferentially removes poly(A+) sequences from the RNA population. For these reasons, we have developed a new deproteinization technique, based on that reported by Glisin et al. (1974) for preparing nuclear RNA by centrifugation in CsCl. Protein, DNA, and RNA have different buoyant densities in CsCl and, therefore, if the correct density of CsCl is chosen, centrifugation will cause protein to float, DNA to band in the solution, and RNA to pellet. However, since there is no DNA in our polysome preparation, it is only necessary to float out the proteins leaving the RNA in solution. Hence our technique requires only a short, low-speed centrifugation to cause the proteins to form a thick pellicle over the CsCl solution from which the RNA can be ethanol precipitated directly. The level of protein contamination of the RNA was estimated by the method of Schaffner and Weissmann (1973) to be approximately 1.5%. However, if diethyl pyrocarbonate is added, after the addition of CsCl, the level of protein contamination is reduced to less than 0.26%.

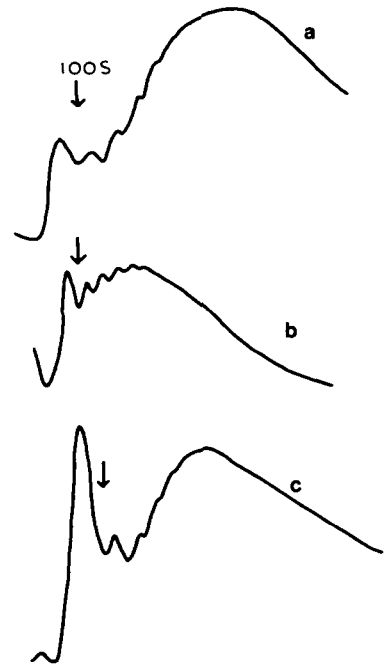

FIGURE 1: Centrifugation in neutral sucrose gradients of polysomes from (a) mouse embryo, (b) mouse liver, and (c) mouse brain. See text for experimental details.

Measured optically, the yield of polysomal RNA by this technique is 90–95% of the theoretical maximum. Furthermore, the A_{260}/A_{280} ratio of the purified RNA is always greater than 2. The integrity of total polysomal RNA prepared by this method was verified by polyacrylamide gel electrophoresis (Figure 2) since the major ribosomal RNA species, 28 S and 18 S, and the transfer RNA species, 4 S, are all intact.

Polyadenylated RNA was prepared by oligo(dT)cellulose chromatography and, in order to reduce contamination with poly(A−) species, only RNA which could bind twice was used for further analysis [denoted poly(A+)]. Poly(A+) RNA and poly(A−) RNA, which are mostly 18S and 28S ribosomal RNA, were electrophoresed on 3.2% polyacrylamide–formamide gels. The gel patterns of poly(A+) RNA from mouse embryo, brain, and liver polysomes are shown in Figure 3 and the positions of the 28S and 18S ribosomal components of the poly(A−) RNA are also indicated. It is clear that the 28S component is absent from the poly(A+) RNA and that the poly(A+) RNA populations are heterogeneous in size.

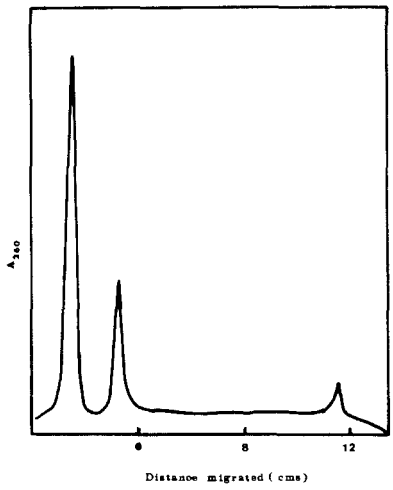

FIGURE 2: Electrophoresis in aqueous 2.6% polyacrylamide gels of ribosomal RNA prepared by CsCl deprotenization technique.

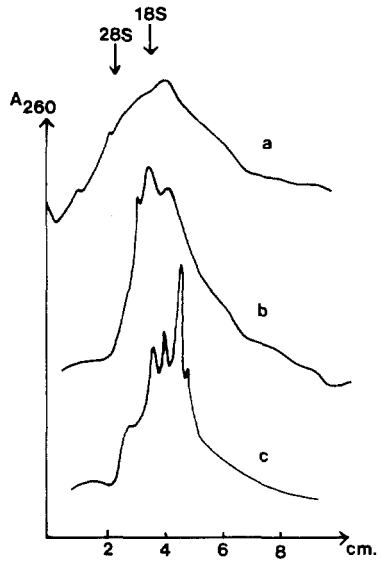

FIGURE 3: Electrophoresis in formamide gels (3.2% polyacrylamide) of poly(A^+) RNA prepared from (a) mouse embryo, (b) mouse brain, and (c) mouse liver.

Complementary DNA. Alkaline sucrose centrifugation showed that each cDNA had a peak size at 50 000 daltons with half-peak values at 10 000 and 110 000 daltons. Only cDNA sedimenting faster than 2.5 S was pooled for further use.

Each cDNA was annealed with a large excess of mouse embryo DNA under standard conditions (0.12 M PB 60 °C). The $C_0t_{1/2}$s ($C_0t_{1/2}$ = C_0t for 50% annealing) of the cDNA reactions are very similar and average approximately 1700 mol s $l.^{-1}$, whereas the $C_0t_{1/2}$ of the final transition of the mouse-embryo DNA reannealing is approximately 1300 mols $l.^{-1}$. However, if the difference in mean size between the cDNA (50 000 daltons) and the total DNA (100 000 daltons) is taken into account (Wetmur and Davidson, 1968), total DNA of the same size as the cDNA would have a $C_0t_{1/2}$ of 1300 × 1.4 = 1800. This value is very close to the cDNA $C_0t_{1/2}$s of 1700 and, hence, the large bulk of the three cDNA populations represent unique DNA sequences.

Annealing of Polysomal cDNA with Homologous RNA. Each of the three polysomal cDNAs was incubated with a large excess of its own template RNA and the hybridization of the cDNA was measured, using single-strand-specific nuclease (S1). The results are shown in Figure 4, where it can be seen that at least 70% of each cDNA can form nuclease-resistant hybrids with its template RNA. It has previously been shown by Harrison et al. (1974) that such RNA-DNA hybrids, formed under the conditions used (50% formamide, 0.5 M NaCl, 43 °C), are of high quality and, therefore, each cDNA probably represents a reasonably faithful copy of its own template RNA.

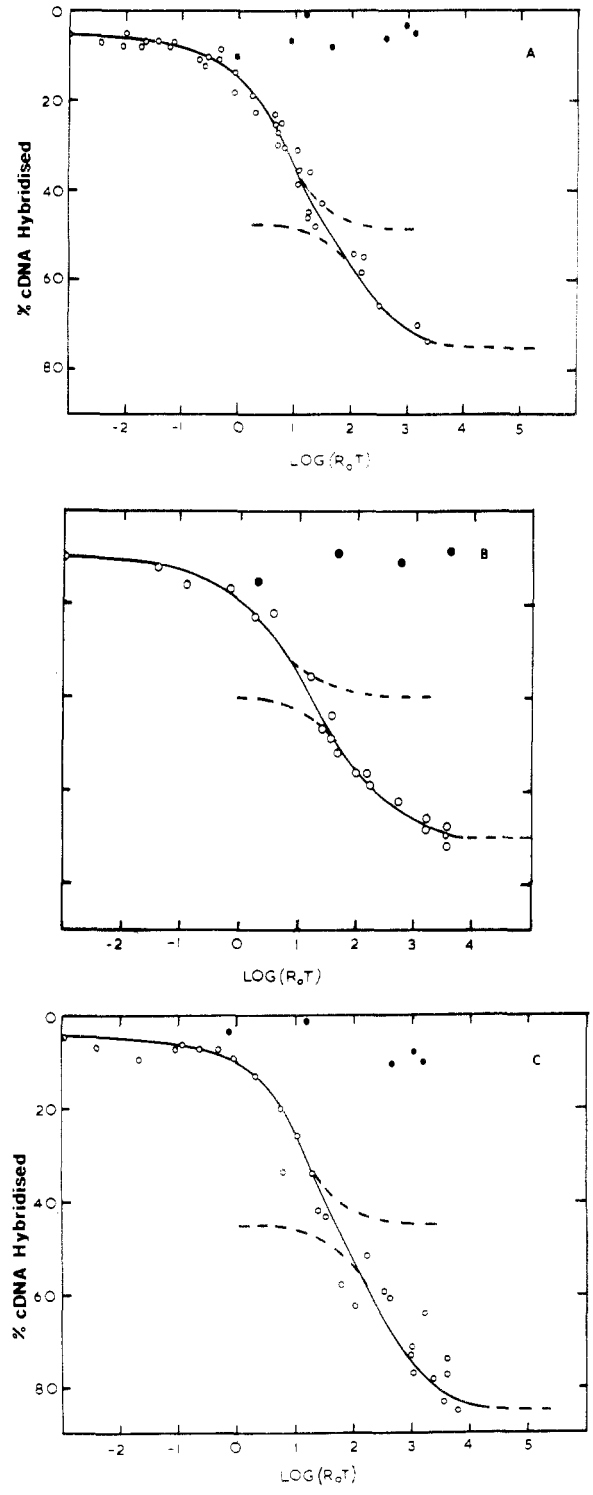

FIGURE 4: Hybridization of cDNA with homologous RNA. (A) mouse embryo poly(A^+) RNA and cDNA, (B) mouse liver poly(A^+) RNA and cDNA, (C) mouse brain poly(A^+) RNA and cDNA. (●) Represents hybridization of each cDNA with *E. coli* ribosomal RNA.

TABLE I: Base Sequence Complexity of Poly(A+) RNA Estimated from Hybridization Kinetics Presented in Figure 4.[a]

		I (Fast)	II (Slow)	% of Genome
Embryo	Complexity (daltons)	1.8×10^8	6.6×10^9	0.7
	Obsd $R_0t_{1/2}$	5	150	
	% hybridizable cDNA	56	44	
Liver	Complexity (daltons)	1.5×10^8	7×10^9	0.8
	Obsd $R_0t_{1/2}$	3	140	
	% hybridizable cDNA	50	50	
Brain	Complexity (daltons)	5×10^8	1.5×10^{10}	1.7
	Obsd $R_0t_{1/2}$	10	300	
	% hybridizable cDNA	50	50	

[a] The haploid mouse genome is assumed to be 1.8×10^{12} daltons.

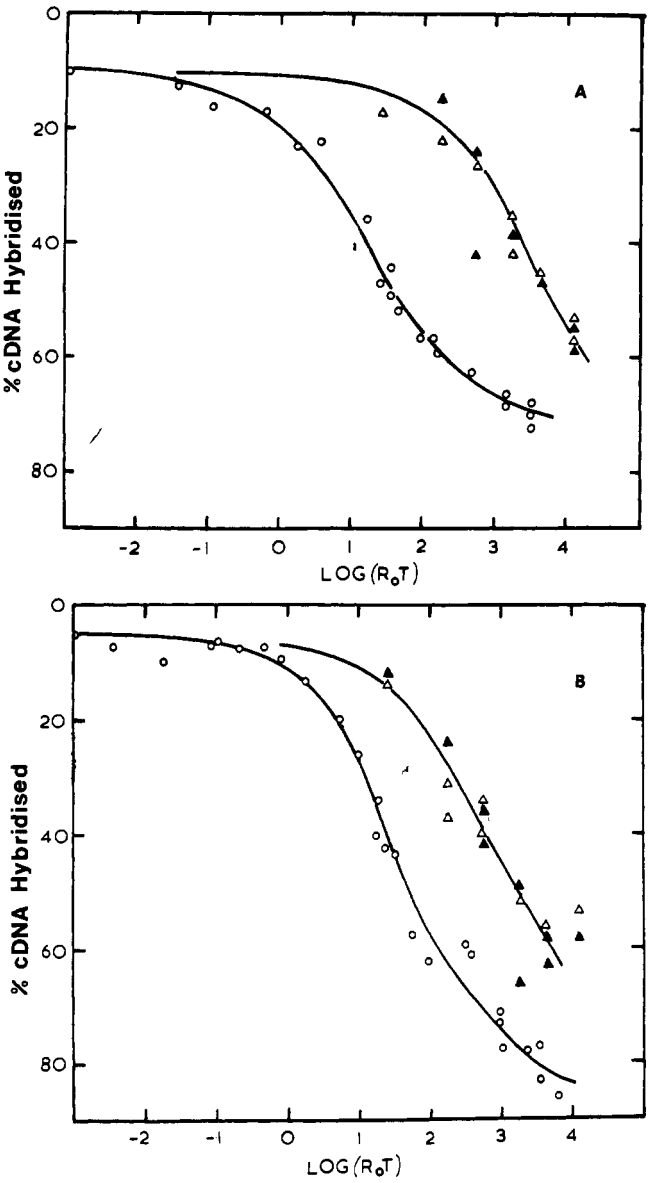

FIGURE 5: Hybridization of liver and brain cDNA. (A) Liver cDNA hybridized to liver poly(A+) RNA (○), to brain poly(A+) RNA (△), and to embryo poly(A+) RNA (▲). (B) Brain cDNA hybridized to brain poly(A+) RNA, (○) to liver poly(A+) RNA (△), and to embryo poly(A+) RNA (▲).

These low temperature conditions were chosen because RNA is much more stable at 43 °C than at the higher temperatures (about 70 °C) which are more commonly used for this type of reaction. Although this results in lower reaction rates, this can be compensated for by longer incubation times (up to 21 days). The specificity of the reaction was confirmed when each cDNA was annealed with *E. coli* ribosomal RNA and no hybridization above the background level of 5–10% was obtained (Figure 4). This small background level of nuclease resistance has been found with all cDNAs and may be due to secondary structure.

As in previous studies (Birnie et al., 1974; Getz et al., 1975), we have used the mouse globin mRNA–cDNA reaction as a kinetic standard with which to compare more complex hybridizing systems. Mouse globin mRNA which consists of α and β globin sequences with a total complexity of 4×10^5 daltons (Williamson et al., 1971) hybridizes with a $R_0t_{1/2}$ of 4×10^{-3} mol s l.$^{-1}$ (Young et al., 1974). As discussed by Getz et al. (1975), the fact that the range of this reaction on the R_0t scale is approximately 2.5 log units rather than the 1.5 log units expected for a pseudo-first-order reaction may be due to the range in size of the cDNA population. Larger cDNA transcripts tend to hybridize faster than small transcripts (Young et al., 1974) and, hence, the reaction may cover a wide range. Since the cDNA transcripts used in this study exhibit a similar size range, we have taken the experimental globin mRNA–cDNA reaction as being characteristic of the hybridization of a single abundance class with its cDNA.

It is clear from Figure 4 that the homologous reactions cover a range wider than 2.5 log units and, therefore, cannot be represented by a single abundance class. We have chosen to resolve the data into the minimum number of components necessary to describe adequately the complete reaction. These components are shown in Figure 4 by broken lines and it is clear that each reaction requires a minimum of two such abundance components. By comparing the $R_0t_{1/2}$ of each component with that of the globin mRNA–cDNA reaction, it is possible to estimate the base sequence complexity of each component (Table I). It should be stressed that the data could be resolved into a much larger number of components but this would not significantly improve the degree of fit. Furthermore, the division into abundance classes is not quantitatively exact in the sense, that, within a given class, not all sequences will have exactly the same abundance. Some sequences will be slightly higher than average and some slightly lower.

The greatest contribution to the total base sequence complexity of a heterogeneous population of RNA sequences is from the least abundant classes forming a significant fraction of the RNA population, that is, from the most slowly hybridizing component. Hence it is possible that a very low abundance component of high complexity may have been missed and therefore the data in Table I should be regarded as minimum estimates.

Annealing of Polysomal cDNA with Heterologous RNA. The tissue specificity of the polysomal poly(A+) RNA was investigated by annealing brain and liver cDNA with the heterologous RNAs. The results of these four hybridization experiments are shown in Figure 5 where it can be seen that there is a considerable degree of sequence homology between

the RNA populations. In all cases, more than 55% of the brain and liver cDNA can be hybridized by a heterologous RNA at a R_0t of 12 000 mol s $l.^{-1}$. Furthermore, it appears that these reactions are not complete by this R_0t value, although it is not possible to determine from these data what the final level will be. Because of the technical problems of lengthy incubation times and the quantity of RNA available, it was not possible to extend the experiments. It is clear from the data in Figure 5 that not only are the overall heterologous reactions much slower than the homologous reactions but there is complete absence of a fast annealing component. This implies that the RNA sequences driving a heterologous reaction are present in lower abundance than are the same sequences in the homologous RNA.

Discussion

Our preparation of polysomes included treatment with Triton X-100 to release any membrane-bound polysomes, which are thought to have a different function from free polysomes (Tata, 1972). Hence the poly(A^+) RNA used in this study was derived from a preparation which included all polysomes but excluded free messenger RNP particles not in the act of translation. It must be emphasized that this study concerns only polyadenylated polysomal RNA. Milcarek et al. (1974) have shown that the 30% of mRNA in HeLa cells, which is not polyadenylated, is not derived by cleavage of polyadenylated RNA and, in fact, represents a different set of sequences. Also in early sea urchin embryos there appears to be a distinct population of poly(A^-) messenger RNA (Nemer et al., 1974). Hence our studies say nothing about any possible poly(A^-) fraction of mRNA in mouse tissues.

The annealing of cDNA with total mouse embryo DNA indicates that most poly(A^+) mRNA sequences are transcribed from unique sequence DNA. This is consistent with a similar finding using cDNA transcribed from poly(A^+) polysomal RNA from mouse Friend cells (Birnie et al., 1974) and with the findings of Galau et al. (1974) using a different technique. It appears to be a general feature of mRNA populations (Lewin, 1975) that most sequences are unique DNA transcripts, but a small fraction, less than 10%, of the cDNA may correspond to intermediate-repetitive transcripts as reported for HeLa cells (Klein et al., 1974; Bishop et al., 1974).

The complexity of the embryo poly(A^+) RNA is of the same order of magnitude as that of the two adult tissues. This is somewhat surprising since in a 14-day-old mouse embryo, a high degree of differentiation into a heterogeneous population of cells has taken place. However, the relative contribution of the different embryonic tissues to the total polysome preparation is unknown; hence a large fraction of polysomes may be derived from one of the major tissues. It is apparent from Table I that brain poly(A^+) RNA is approximately twice as complex (1.5×10^{10} daltons) as that of the other two tissues. Using similar techniques, Ryffel and McCarthy (1975) have estimated that the complexity of cytoplasmic poly(A^+) RNA in mouse brain is approximately 1.14×10^{10} daltons. The difference between brain poly(A^+) RNA and that of other tissues may be related to the finding that total RNA from brain is three to four times more complex than total RNA from liver, kidney, or spleen (Hahn and Laird, 1971; Brown and Church, 1972; Grouse et al., 1972).

The heterologous hybridization experiments represent the first direct examination of the specificity of polysomal poly(A^+) RNA from whole tissues. It is not possible to analyze these curves as done for the homologous reactions since the RNA/DNA ratio for each sequence is completely unknown in a heterologous reaction and cannot be determined. However, it is possible to draw a number of important general conclusions. Firstly, the fact that at least 55% of brain and liver cDNA can be hybridized by the heterologous RNAs indicates that at least 55% of the poly(A^+) RNA present in one tissue is also present in the other. Since it is not entirely clear where these curves will finish, the final degree of homology may be much higher.

Using similar techniques, Ryffel and McCarthy (1975) have compared cytoplasmic poly(A^+) RNA from mouse tissues with that from mouse L cells. It was concluded that, whereas 35% of brain poly(A^+) RNA is not present in L cell poly(A^+) RNA, there is almost complete homology between liver and L cell poly(A^+) RNA and also between kidney and L cell poly(A^+) RNA. There is, however, one important difference between these results and those presented in Figure 5. Whereas Ryffel and McCarthy (1975) observed only a slight difference in rate between the heterologous and homologous reactions, our results show that, when compared with the homologous reactions, the heterologous reactions are reduced in rate by a factor of about 25 for brain cDNA and about 100 for liver cDNA. The sequences driving the hybridization of the cDNA in our heterologous reaction are present at a much lower abundance than the same sequences are in the homologous RNA. In other words, the sequences present at relatively high abundance in one tissue are also present in another tissue but at much lower abundance. The data in Table I predicts that, due to its higher complexity, a fraction of brain cDNA should not be hybridized by the other two heterologous RNAs. Unfortunately, the data in Figure 4 are insufficient to prove or disprove this point. It may be that Ryffel and McCarthy (1975) observed almost no difference in rate between homologous and heterologous reactions because total cytoplasmic preparations contain nuclear RNP particles (Galau et al., 1974) which might be expected to have less specificity with respect to cell type. Alternatively, in a particular cell type, there may be a degree of control over the incorporation of cytoplasmic mRNP particles into polysomes, although such a form of control could not be absolute since sequences present on polysomes at high abundance in one tissue are present at low abundance in other tissues. It has recently been shown by Humphries et al. (1975) that mouse globin RNA sequences can be detected in nuclear and cytoplasmic RNA not only from erythroid cells, but also in low amounts in nonerythroid cells. This result for a single sequence is in agreement with our conclusions concerning populations of mRNA sequences.

Finally, there is one general conclusion that can be drawn from the experiments reported here. The homologous reactions imply that the fraction of the genome expressed as poly(A^+) RNA in the polysomes of all the cell types of a particular tissue is relatively small, probably less than 2.5%. The heterologous reactions imply that there is a high level of homology between the polysomal poly(A^+) RNA of different tissues. Taken together these results indicate that a relatively small fraction of the large coding potential of unique eukaryotic DNA actually codes for structural genes; moreover, they suggest that the phenotype of a eukaryotic cell may be determined more by the relative abundance of mRNA species on the polysomes than by the presence or absence of specific mRNA sequences.

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Circular Dichroic Studies of the DNA and RNA of Nucleoli†

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ABSTRACT: Circular dichroism (CD) in the 240–300-nm region was used to study the conformation of DNA and RNA complexed with proteins in isolated nucleoli from HeLa cells. Deoxyribonuclease or ribonuclease digestion was employed to obtain (1) the individual CD spectra of nucleolar DNA or RNA in complex form with proteins, or in free form; and (2) the experimental CD baseline correction to exclude contributions from nonnucleic acid sources such as light scattering artifacts and proteins. The CD spectrum of nucleolar DNA in DNA–protein complexes was highly reduced in ellipticity in comparison with protein-free DNA. It showed a positive peak at 283 nm with a molar ellipticity $[\theta]_{283} = 1200$ deg cm^2 dmol^{-1} and a crossover at 262 nm. Addition of sodium dodecyl sulfate shifted the peak to 276 nm with $[\theta]_{276} = 8000$ deg cm^2 dmol^{-1} and a crossover at 254 nm. The CD spectrum of nucleolar RNA in RNA–protein complexes was also reduced in comparison with protein-free RNA, showing a peak at 269 nm ($[\theta]_{269} = 6900$ deg cm^2 dmol^{-1}), and a crossover at 250 nm. Addition of sodium dodecyl sulfate shifted the peak to 265 nm with $[\theta]_{265} = 18\,000$ deg cm^2 dmol^{-1} and a crossover at 246 nm. The low ellipticity of both nucleolar DNA and RNA when complexed with proteins was increased by treatment with sodium chloride, urea, or heparin. This suggests that some ionic, hydrophobic, and hydrogen bondings are involved in the nucleic acid–protein interaction in nucleolar chromatin similar to that observed in nuclear chromatin.

A number of investigators have pointed out that the functional and structural study of eukaryotic chromatin is made difficult by its complexity. In an attempt to simplify the problem, Polisky and McCarthy (1975) have used SV-40 chromatin. We have directed our attention to the nucleolus, which has the following advantages: (1) it is a component of the genome of normal cells; (2) it is isolated easily and in a highly reproducible form, preserving at the same time some of its functional activities (Busch and Smetana, 1970; Muramatsu et al., 1974); (3) it constitutes 3–5% of the total genome (McConkey and Hopkins, 1964; Steele, 1968; Busch and Smetana, 1970; Wilhelm et al., 1972; Schmid and Sekeris, 1975); (4) it has a functional specialization (Amalric and Zalta, 1975), that is, the synthesis of ribosomal RNA, which constitutes about 40% of total nuclear RNA synthesis (Reeder and Roeder, 1972); (5) the main product of the nucleolus,

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