COMPLEXITY AND COMPLEXITY OVERLAP IN MOUSE LIVER POLYADENYLATED AND NONADENYLATED MESSENGER RNA FRACTIONS

By

CHERYL LEE COURCHESNE

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Northern Michigan University

Marquette, Michigan

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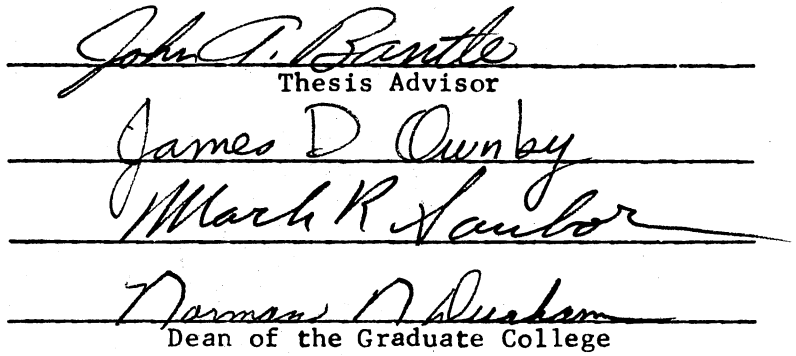

Thesis Advisor

Dean of the Graduate College

PREFACE

This study is concerned with the complexity of the polyadenylated and nonadenylated messenger RNA fractions in mouse liver. The primary objective of this study was to determine whether polyadenylated and nonadenylated messenger RNA molecules code for the same or different proteins in mouse liver. This research was supported by a National Science Foundation Grant to Dr. John A. Bantle of the Oklahoma State University.

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CHAPTER 1

INTRODUCTION

It is well known that all somatic cells of a particular organism possess the same kind and number of genes, although only a fraction of these genes are actually expressed in a given cell type (Galau et al., 1976). The mechanism by which genes are selectively activated or repressed during differentiation and development remains poorly understood. Furthermore, those genes expressed in certain tissues and organs such as liver, have been incompletely characterized.

This study is specifically concerned with structural gene expression in mouse liver. The ultimate transcriptional products of structural genes are messenger RNA (mRNA) molecules that may be translated directly into proteins. One method of measuring gene expression in liver is to measure by estimate the number of different kinds of sequences in a mRNA population. Since eukaryotic mRNAs are monocistronic (Lewin, 1975), the number of different proteins potentially expressed after the translation of each unique mRNA molecule can also be derived from sequence complexity estimates. The complexity and complexity overlap of both polyadenylated (poly(A^+)mRNA) and nonadenylated mRNA (poly(A^-)mRNA) were examined in this study. These two types of mRNAs have been demonstrated to be equally capable of synthesizing proteins (Fromson and Duchastel, 1977), and recent research has been

conderned with whether there are any functional differences between poly(A^+)mRNA and poly(A^-)mRNA in various eukaryotic tissues and organs.

With these considerations in mind, there were two objectives in this study. The first goal was to measure the sequence complexity of poly(A^+)mRNA, poly(A^-)mRNA and total polysomal mRNA (mRNA which contains both poly(A^+) and poly(A^-) sequences). The second objective was to establish the extent to which poly(A^+)mRNA and poly(A^-)mRNA share the same sequences in mouse liver. The first goal of this study will determine the number of different sequences contributed separately by the poly(A^+)mRNA and poly(A^-)mRNA populations while the latter goal will ascertain whether poly(A^+)mRNA and poly(A^-)mRNA populations code for the same or different proteins in liver.

A substantial amount of terminology exists that is exclusive to this subject. For this reason, a glossary of terms is provided in the appendix.

CHAPTER II

LITERATURE REVIEW

A great deal of experimental evidence suggests that most mRNAs are processed from heterogeneous nuclear RNAs (hnRNAs) (Lewin, 1975; Perry, 1976) that have been transcribed from the unique portion of the genome (Goldberg, 1973; Davidson and Britten, 1974).

Some mRNAs possess a poly(A) tract on the 3' terminus (Kates, 1970; Lim and Canallakis, 1970; Karpetsky et al., 1979). Initially it was believed that all biologically active eukaryotic mRNAs, except histone mRNAs were polyadenylated (Adesnik et al., 1972; Greenberg and Perry, 1972). However, it is now known that a significant fraction of mRNAs in eukaryotes are nonadenylated (Karpetsky et al., 1979). In sea urchin (Nemer et al., 1974; Fromson and Verma, 1976), Hela cells (Milcarek et al., 1974), mouse L cells (Greenberg, 1976), and mouse kidney and liver (VanNess and Hahn, 1980a), 30-50% of mRNAs by weight are nonadenylated (poly(A^-)mRNA). This lack of poly(A) does not seem to be an extraction artifact (Nemer et al., 1975; Greenberg, 1976) and the natural diminution of the poly(A) tract in poly(A^+)mRNA (Gorski et al., 1975; Brandhorst and Bannet, 1978) cannot account for all of the poly(A^-)mRNA in the cytoplasm. Poly(A^-)mRNA molecules possess a 5' cap which suggests that these mRNAs are functional (Faust et al., 1976; Surrey and Nemer, 1976). Much evidence suggests

that poly(A^-)mRNAs are synthesized, processed, transported, and translated without the addition of poly(A) (Mendecki et al., 1972; Milcarek et al., 1974; Fromson and Verma, 1976; Greenberg, 1976). Several proteins are known to be coded by either poly(A^+)mRNA or poly(A^-)mRNA. These include: globin, casein, actin, protamine, and histone (Cann et al., 1974; Houdebine, 1976; Hunter and Garrels, 1977; Iatrou and Dixon, 1977; Ruderman and Pardue, 1977). Fromson and Duchastel (1977) found that both poly(A^+)mRNA and poly(A^-)mRNAs from sea urchin are translated with equal effeciency in vitro. However, Sonenshein et al. (1976) reported that the effeciency of translation for poly(A^-)mRNAs from sarcoma 180 ascites cells were lower that that of poly(A^+)mRNAs in vitro. In addition, Nemer (1974) suggested that poly(A^-)mRNA may not bind as effeciently to ribosomes as poly(A^+)mRNA.

The function of poly(A) remains unknown (Adams, 1977), although polyadenylation has been theorized to play a role in translation, transport, stability, and splicing (eg., Greenberg, 1975; Brawerman, 1976; Shafritz, 1977; Revel and Groner, 1978; Karpetsky et al., 1979; Bina et al., 1980; Marbaix and Huez, 1980; Rogers and Wall, 1980). Of these four possibilities, it seems unlikely that poly(A) has some function in mRNA transport for numerous reasons, the most significant being that both nonadenylated and polyadenylated mRNAs enter the cytoplasm rapidly and at similar rates(Nemer, 1975) or at rates which vary according to the functional state of the cell (Chernovskaya, et al. 1976). In addition, poly(A^-)mRNA does not appear to arise in the cytoplasm mainly from the deadenylation of poly(A^+)mRNA molecules

(Milcarek et al., 1974).

The amount of poly(A^+)mRNA versus poly(A^-)mRNA changes during development (Nemer, 1975; Fromson and Duchastel, 1975; Chernovskaya et al., 1976; Iatrou and Dixon, 1977) and in response to differing environmental states (Shaposhnikov and Ratovitski, 1978; Bantle et al., 1980a). Therefore, it appears that regulatory mechanisms may be involved in controlling the levels of poly(A^+)mRNA and poly(A^-)mRNA in the cell. This suggest that poly(A^-)mRNA molecules may be composed of different sequences than poly(A^+)mRNA molecules. Much of the present literature existing on poly(A^+)mRNA and poly(A^-)mRNA sequence homology are conflicting. Clearly, poly(A^+)mRNAs and poly(A^-)mRNAs have been demonstrated to be nonhomologous in mouse brain (VanNess et al., 1979) and rat brain (Chikaraishi, 1979). Little or no homology between poly(A^+)mRNA and poly(A^-)mRNA has also been suggested for Hela cells (Milcarek et al., 1974), mouse liver (Grady et al., 1978), and sea urchin embryos (Nemer et al., 1974). On the other hand, poly(A^+)mRNAs and poly(A^-)mRNAs have been reported by other investigators to be completely (or almost completely) homologous in sea urchin embryos (Brandhorst et al., 1979), Hela cells (Kaufmann et al., 1977) mouse kidney (Ouellette and Ordahl, 1979), and AKR-2B cells (Siegal et al., 1980).

The number of different sequences or genes potentially expressed in a tissue or organ is frequently established by determining the sequence complexity of messenger RNA fractions using molecular hybridization techniques. Sequence complexity is measured by one of two methods. This has lead to serious discrepancies in the literature

concerning estimates of total gene expression in various tissues and organs in different animals.

The first technique makes use of a viral reverse transcriptase to produce a labeled DNA probe complementary to poly(A)mRNA (cDNA). The rate of reaction is directly proportional to the sequence complexity. cDNA probes can also be synthesized from nonadenylated mRNA molecules by first using terminal transferase to add a poly(A) tract to nonadenylated mRNAs before the addition of reverse transcriptase to the reaction mixture (Bender and Davidson, 1976). Reverse transcriptase requires a double-stranded primer region in order for the enzyme to synthesize the cDNA probe. This primer region is provided by hybridizing an oligo(dT) tract to the 3' poly(A) terminus of the mRNA. The advantages of using a cDNA probe are the high amount of radioactivity obtained for hybridization and the absence of DNA-DNA renaturation since cDNA probes are sense strand only. A major disadvantage of this technique is that a cDNA probe represents those RNA sequences occurring most frequently in the cell. Such a probe is biased for identical mRNAs, presumably coding for a particular protein, of which the cell has many copies. This can be a serious problem since mRNA sequences present in high abundance are of low complexity (Galau et al., 1974). Typically, the use of a cDNA probe leads to an underestimation of complexity because of the difficulty in estimating the kinetic transition of the low-abundance high-complexity or complex mRNA class. This is because the accuracy of mRNA complexity estimates using a cDNA probe relies on the precision that the rate constant (Rot 1/2) for the final transition of the slowly

reacting low-abundance high-complexity mRNA class is determined. Since the final transition typically represents only a small percentage of total reactable RNA mass, the determination of such small increments in hybridization is difficult to access (Ryffel and McCarthy, 1975). Furthermore, if the cDNA probe does not completely represent the complex mRNA class (Ordahl and Caplan, 1978) the Rot 1/2 value for the final transition of the complex class cannot be accurately determined (VanNess et al., 1980b).

The second technique of determining the complexity of mRNA populations is the saturation hybridization of mRNA to trace quantities of labeled unique sequence DNA (usDNA). The major advantage of this method is that every mRNA sequence is equally represented irrespective of its abundance in the cell. However, there are limitations with using a usDNA probe for hybridization studies. DNA-DNA reassociation (noise) will take place concurrently with RNA-DNA hybridization (signal). Thus control experiments are necessary in order to determine the percent of double-stranded molecules due to DNA renaturation. Furthermore, the technique is not sensitive to the high-abundance low-complexity class of mRNA sequences although reliable estimates for total base complexities are obtained. Since the majority of mRNA complexity is represented by mRNA transcripts of low-abundance, the use of a usDNA probe seems better suited than a cDNA probe for sequence complexity estimates. However, Kiper (1979) has argued that hybridization of usDNA leads to routine overestimates of mRNA complexity. This seems unlikely for several reasons (Goldberg and Timberlake, 1980; Hahn et al., 1980) the most significant being

that several investigators have obtained equivalent results using either a cDNA or usDNA probe (Axel et al., 1976; Hereford and Roshbash, 1977; Ordahl and Caplan, 1978; Savage et al., 1978; Aziz et al., 1979; Capetanaki and Alonso, 1980; VanNess and Hahn, 1980b). At the time this research was initiated; it had not yet been proven that the cDNA approach could accurately measure the complex class of mRNA because of the difficulties previously described. Although the usDNA had several disadvantages, it was nevertheless possible to design an experiment that allowed complete titration of all expressed genes. Therefore, the usDNA technique was selected for this study and the validty of this decision has been borne out by the close agreement of cur data with other researchers in this field (Savage et al., 1978; Chikaraishi, 1979; VanNess and Hahn, 1980b; Wilkes et al., 1979).

In designing a valid usDNA experiment, usDNA probes must be enhanced for sequences present only in the RNA population being studied. When it is considered that less than 2% of the poly(A^+)mRNA hybridizes with usDNA, small variations in methodology account for significant differences in complexity. The resolution of the technique must be increased by removing noncomplementary sequences from the usDNA probe. Galau et al. (1976) was first to use the term mDNA to refer to a usDNA probe of which most sequences noncomplementary to the mRNA population being studied had been removed. Unfortunately, few investigators have attempted to determine liver mRNA complexity with such a probe.

There have been a number of measurements on the complexity of liver poly(A^+)mRNA from rodent liver. Comparatively few investigators have attempted to study poly(A^-)mRNA complexity. Table 1 summarizes

current complexity estimates of poly(A^+)mRNA and poly(A^-)mRNA from rodent liver.

As shown in Table 1, the sequence complexity of liver poly(A^+)mRNA varies from 1.3×10^7 nucleotides (Sipple et al., 1977) to 1.0×10^8 nucleotides (Wilkes et al., 1979). Depending upon the estimated length of the average mRNA molecule, sequence diversity measurements (sequence complexity ÷ number average nucleotide length of a mRNA molecule) vary from 8600 (Hastie and Bishop, 1976; Grady et al., 1978) to 50,000 (Wilkes et al., 1979) different sequences. Diverse sequences are assumed to code for different proteins. Both Grady et al. (1978) and Hastie and Bishop (1976) estimated 8600 diverse sequences for poly(A^+)mRNA alone, although their sequence complexity estimates vary considerably from one another (1.5×10^7 nucleotides and 2.4×10^7 nucleotides, respectively). Differences in mRNA size may be somewhat attributed to degradation or variations in sizing techniques. However, current diversity estimates based on the average mRNA size may be overestimated since Meyuhas and Perry (1979) have argued that mRNA molecules of the complex class (accounting for the majority of the complexity but only a small percentage of total mRNA mass) are larger than mRNA molecules of the prevalent class (which account for the bulk of mRNA mass).

Most investigators estimating mRNA complexity by the hybridization of a cDNA probe with poly(A^+)RNA of cytoplasmic or polysomal origin in rodent liver (Ryffel and McCarthy, 1975; Hastie and Bishop, 1976; Young et al., 1976; Colbert et al., 1977; Sippel et al., 1977; Towle et al., 1978) resulted in lower measurements of complexity as

compared to more recent studies utilizing the hybridization of a usDNA or cDNA probe to rodent poly(A^+)mRNA (Savage et al., 1978; Wilkes et al., 1979; Capetanaki and Alonso, 1980; Jacobs and Birnie, 1980; VanNess and Hahn, 1980b) (see Table 1). Many of the studies listed in Table 1 exploiting the kinetic method of determining complexity used cDNA probes less than 500 nucleotides in length in comparison to the 1350-2000 mean nucleotide length of a liver mRNA molecule (Ryffel and McCarthy, 1975; Hastie and Bishop, 1976; Young et al., 1976; Towle et al., 1978; Jacobs and Birnie, 1980). This apparantly leads to underestimates of mRNA complexity because some mRNA sequences of the complex class are either only partially represented or completely absent in the cDNA population (Ordahl and Caplan, 1978). The net result is the early termination of hybridization which leads to erroneous Rot 1/2 estimates. Studies utilizing cDNA probes of equivalent size to the mean length of the mRNA yield higher complexity estimates. For example, Capetanski and Alonso (1980) using cDNA from 1800-2400 nucleotides in length determined liver polysomal polu(A^+)mRNA complexity as 4.8×10^7 nucleotides. Jacobs and Birnie (1980) also reported the same value for liver polysomal poly(A^+)mRNA complexity when using a usDNA probe but determined a slightly lower complexity for liver poly(A^+)mRNA (3.1×10^7 nucleotides) when using a cDNA probe of a mean length of 350 nucleotides. Similarly, Savage et al. (1978) using a cDNA of 1100 nucleotides in mean length found a slightly lower complexity for liver poly(A^+)mRNA than when using usDNA (4.1×10^7 nucleotides and 5.6×10^7 nucleotides, respectively). However, the complexity values for liver poly(A^+)mRNA reported by Jacobs and Birnie

TABLE 1. COMPLEXITY ESTIMATES OF RODENT LIVER mRNA

Animal	Organ	Subcellular fraction	mRNA class	Number Ave. mRNA size	cDNA or usDNA	Sequence complexity (nucleotides)	Diversity	References
Mouse	liver	cytoplasmic	poly(A)mRNA	1900	cDNA	1.4×10^7	8000	Ryffel and McCarthy, 1975
Mouse	liver	cytoplasmic	poly(A)mRNA	1900	cDNA	2.4×10^7	8600	Hastie and Bishop, 1976
Mouse	liver	polysomal	poly(A)mRNA	1350	cDNA	2.1×10^7	15,600	Young et al., 1976
Rat	liver	polysomal	poly(A)mRNA	2100	cDNA	3.3×10^7	15.609	Colbert et al., 1977
Rat	liver	polysomal	poly(A)mRNA	1300	cDNA	1.3×10^7	10,000	Sippel et al., 1977
Mouse	liver	polysomal	poly(A)mRNA	1750	usDNA	1.5×10^7	8600	Grady et al., 1978
Mouse	liver	polysomal	poly(A$^-$)mRNA	1750	usDNA	3.1×10^6	5700	Grady et al., 1978
Rat	liver	polysomal	poly(A)mRNA	1800	usDNA	5.6×10^7	31,000	Savage et al., 1978
Rat	liver	polysomal	poly(A)mRNA	1800	cDNA	4.1×10^7	23,000	Savage et al., 1978
Rat	liver	total cellular	poly(A)mRNA	1400	cDNA	1.6×10^7	11,756	Towle et al., 1978
Rat	liver	cytoplasmic	cytoplasmic	1500	usDNA	8.6×10^7	57,000	Chikaraishi, 1979
Rat	liver	polysomal	poly(A)mRNA	2000	usDNA	1.0×10^8	50,000	Wilkes et al., 1979
Rat	liver	polysomal	poly(A)mRNA	1800	cDNA	4.8×10^7	26,928	Capetanaki and Alonso, 1980
Rat	liver	polysomal	poly(A)mRNA	2000	cDNA	3.1×10^7	15,379	Jacobs and Birnie 1980
Rat	liver	polysomal	poly(A)mRNA	2000	usDNA	4.8×10^7	24,000	Jacobs and Birnie 1980

(1980) and Savage et al. (1978) are in close agreement with each other and support the hypothesis that when the complex mRNA class is completely represented by the cDNA population, cDNA-mRNA hybridization is essentially equivalent to those results obtained using usDNA-mRNA hybridization. Similar estimates of the complexity of mouse liver poly(A^+)mRNA (VanNess and Hahn, 1980b) and poly(A^+)mRNA and poly(A^-)mRNA from mouse brain (VanNess et al., 1979) using either a usDNA probe or cDNA probe have been reported. Hence it appears that reverse transcriptase does not preferentially copy some mRNA sequences.

The highest estimates of liver poly(A^+)mRNA complexity have been determined by saturation hybridization of this RNA class to usDNA. According to Table 1, these upper estimates include: Jacobs and Birnie (1980), 4.8×10^7 nucleotides; Wilkes et al. (1979), 1.0×10^8 nucleotides; Savage et al. (1978), 5.6×10^7 nucleotides. These values represent enough diverse mRNA sequence to encode 24,000, 50,000 and 31,000 proteins, respectively based on an average mRNA length of 1800-2000 nucleotides. In addition, Chikaraishi (1979) determined the complexity of rat liver cytoplasmic RNA as 8.6×10^7 nucleotides (57,000 proteins) through saturation hybridization of this RNA fraction to usDNA. The difference in the complexity estimates by these investigators appears to be primarily due to the insensitivity of the technique.

The lowest estimate of liver mRNA complexity listed on Table 1 using a usDNA probe was determined by Grady et al. (1978). They presented data suggesting that the complexity of poly(A^+)mRNA was only 1.5×10^7 nucleotide and poly(A^-)mRNA only 3.1×10^6 nucleotides, enough to encode 8600 and 5700 sequences, respectively. In addition,

Grady and coworkers found that poly(A^+)mRNA and poly(A^-)mRNA share few of the same dequences and so suggested that poly(A^+)mRNA and poly(A^-)mRNA code for different proteins. However, the validity of their data is questionable for several reasons. First of all, Grady et al. (1978) utilized a usDNA probe which had been enriched for sequences complementary to total nuclear RNA. Accurate complexity estimates can only be achieved with such a probe when all species of the mRNA population are fully represented. When preparing the probe, they drove only 3% of usDNA into hybrids with total liver RNA. Recent research has shown that nuclear RNA saturates 6.1% (Tedeschi et al., 1978), 6.8% (Wilkes et al., 1978), and 10.9% (Chikaraishi, et al., 1978) of a usDNA probe in rodent liver at saturation. Based on the very low saturation of unique sequence DNA with total liver RNA, it is very doubtful that the probe used by Grady et al. (1978) fully represented the poly(A^+)mRNA and poly(A^-)mRNA population in liver. Furthermore, because Grady et al., (1978) used such a low concentration of poly(A^+)mRNA for hybridization, they never achieved a Rot much higher than 2000. This value is well below the Rot required to titrate all complex mRNA sequences (Chikaraishi, 1979). Also, the hybridized DNA was not established to be of single copy origin and evidence that nuclear ribonucleoprotein was effectively removed was not provided as observed by VanNess et al. (1979).

No additional estimates of poly(A^-)mRNA complexity have been made for liver. Thus, it is still unclear whether poly(A^+)mRNA and poly(A^-)mRNA are composed of essentially overlapping on nonoverlapping sequences in this organ. If the sequence composition of

poly(A^+)mRNA and poly(A^-)mRNA molecules is essentially identical, then poly(A^+)mRNAs and poly(A^-)mRNAs are derived from the same genes and code for identical proteins. On the other hand, if poly(A^+)mRNAs are of different base composition than poly(A^-)mRNAs, then current determinations of total liver complexity based only on polyadenylated transcripts are gross underestimates.

CHAPTER III

METHODS AND MATERIALS

Preparation of Nuclei

ICR strain mice, 22-30 g (Timco) were killed by cervical dislocation. The gall bladders were removed and the livers dissected free. Approximately 25-50 mice were used for each nuclei preparation. All glassware used in this study was rinsed in triple distilled water and heat-treated for 3 hours at 350°C to destroy RNase activity. Plasticware was purchased sterile or treated with a saturated aqueous solution of diethylpyrocarbonate (Sigma) to eliminate RNAse contamination.

Whole livers or crude nuclear pellets (obtained by methods described in the Preparation of Polysomes section) were homogenized in 8 volumes per liver of 0.32 M Sucrose (RNase free), 0.001 M KCl, 0.001 M $MgCl_2$, 0.01 M Na acetate, pH 6.0, 0.25% v/v Triton X-100 for DNA isolation or 8 volumes per liver of 0.32 M Sucrose (RNase free), 0.1 M KCl, 0.001 M $MgCl_2$, 0.01 M Na acetate, pH 6.0, 0.25% v/v Triton X-100, 0.5 mg/ml heparin for nuclear RNA isolation. A motorized Teflon-glass (Potter-Elvejhem) tissue grinder was used to homogenize the crude nuclear pellet or whole livers by 5 complete up-down strokes with the pestle. Following homogenization, the preparation was passed directly over two layers of sterile cheesecloth and centrifuged at 2500 x g average, 10 minutes, 0-4°C. The nuclear pellet was resuspended in the

same buffer, lacking Triton X-100, with 5 strokes of a Teflon-glass homogenizer and centrifuged at 2500 x g average, 10 minutes, 0-4°C. For DNA isolation the pellet was then resuspended in 190 ml of 2.2 M Sucrose (RNase-free), 0.001 M KCl, 0.001 M $MgCl_2$, 0.01 M Na acetate, pH 6.0. For nuclear RNA isolation the pellet was resuspended in 190 ml of 2.2 M Sucrose (RNase-free), 0.1 M KCl, 0.001 M $MgCl_2$, 0.01 M Na acetate, pH 6.0, 0.5 mg/ml heparin. A Teflon-glass tissue grinder was used to resuspended the nuclear pellets. The homogenate was layered over 5 ml pads of the same buffered sucrose, lacking heparin, in Beckman 1" x 31/2" cellulose-nitrate centrifuge tubes. The interface was then gently stirred, and the tube centrifuged in a Beckman SW 27 or SW 28 rotor at 110,000 x g average, 90 minutes, 0-4°C. The nuclei which pelleted through the sucrose pad were then rinsed with 0.05 M Na acetate, 0.001 M EDTA, pH 5.2, and resuspended in the same buffer using a Dounce homogenizer (Type A pestle) and the RNA or DNA extracted.

Extraction and Purification of Nuclear RNA

Nuclei were isolated from whole livers as previously described. After pelleting through the 2.2 M Sucrose pads nuclei were resuspended in 0.1 M NaCl, 0.001 M EDTA, 0.01 M Tris, pH 7.5 (NETs buffer) with a Teflon-glass tissue grinder. Unlabeled total DNA was then prepared exactly as described for the preparation of ^3H-labeled nonrepeated DNA from mouse L cells except that repetitive sequences were not removed following shearing.

Nuclei that had pelleted through the 2.2 M sucrose pads were

resuspended in 0.05 M Na acetate, 0.001 M EDTA, pH 5.2 and disrupted by the addition of 0.25% w/v SDS. An equal volume of phenol (Bethesda Research Laboratories or Mallinckrodt) (containing 0.1% w/v 8-hydroxyquinoline) which had been liquified in 0.05 M Na acetate, 0.001 M EDTA, pH 5.2 was then added to the preparation. The RNA was extracted once for 6 minutes at 67°C by constant agitation as described by Edmonds and Carmella (1969). This method serves to preserve poly(A) RNA but eliminates most of the DNA by selective retention in the phenol phase. After centrifugation at 8000 x g average, 10 minutes, 0-4°C, the aqueous phase was re-extracted with an equal volume of pH 5.2 phenol for 5 minutes at 25°C and precipitated in 2.5 volumes of 100% ethanol at -20°C.

Liver nuclear RNA was extensively purified of DNA and heparin by salt precipitation followed by DNase treatment. After precipitating in ethanol, the nuclear RNA was pelleted by centrifugation at 8000 x g average, 10 minutes, 0-4°C and dissolved in 5 ml of 0.1 M NaCl, 0.001 M EDTA, 0.05 M Tris-HCl, pH 7.5. Three µg of diethylpyrocarbonate (Sigma) were then added to inhibit ribonuclease activity and the salt concentration was increased to 3 M NaCl. The preparation was kept at 0°C for 12-24 hours. The precipitated RNA was pelleted by centrifugation at 8000 x g average, 10 minutes, 0-4°C. This was followed by resuspension in 75% ethanol, 0.2 M Na acetate, pH 6.0. After centrifugation, 2.5 volumes of 100% ethanol were added to the RNA pellet followed by 1 volume of 0.1 M NaCl, 0.001 M EDTA, 0.01 M Tris-HCl, pH 7.5 (NETs buffer), and precipitated in ethanol at -20°C. The RNA was then resuspended in 5 ml 0.1 M NaCl, 0.01 M $MgCl_2$, 0.01 M Tris-HCl,

pH 7.5 and approximately 130 µg/ml DNase (Miles) were added. The preparation was incubated for 4 hours, 37°C, after which time 83 µg/ml Proteinase K (Sigma), predigested for 20 minutes at 37°C, were added. The preparation was allowed to incubate for an additional hour at 37°C. The volume of buffer was then increased two-fold with NETs buffer, 0.25% w/v SDS was added and the RNA extracted at 25°C by the addition of an equal volume of 50% phenol/50% chloroform, pH 7.5 (Perry et al., 1972). The aqueous phase was precipitated in 2.5 volumes of 100% ethanol and stored at -20°C.

After precipitating in ethanol, the RNA was pelleted by centrifugation at 8000 x g average, 10 minutes, 0-4°C, resuspended in NETs buffer, and passed through G100 Sephadex (Pharmacia). A second DNase treatment was performed using 440 µg/ml DNase (Miles) for 2 hours, 37°C, followed by treatment with Proteinase K, phenol-chloroform, and gel filtration chromatography as previously described. After the second DNase treatment, the RNA eluting as the excluded fraction from the G100 Sephadex column was quantified by the absorbance at 260 nm.

As a control for DNA contamination, 50 µg of RNA were removed and treated with 30 µg/ml RNase A and 3 units/ml Rnase T_1 (Sigma) for 1 hour at 37°C. After this time the mixture was passed through G100 Sephadex. Any DNA contaminating the RNA preparation would be observed as excludable material as determined by the continual monitoring of absorbance at 254 nm.

Preparation of Polysomes

Polysomal RNA was isolated following the method of Sala-Trepat

et al. (1978) in 4 volumes per liver of 0.1 M KCl, 0.04 M NaCl, 0.0075 M $MgCl_2$, 0.050 M NH_4Cl, 0.025 M Tris-HCl, pH 7.5, containing 0.5 mg/ml heparin (Sigma), 0.75 mg/ml yeast RNA Type III (Sigma) as ribonuclease inhibitors. The homogenate was centrifuged at 15,000 x g average, 10 minutes, 0-4°C, and the postmitochondrial supernatant containing the polysomes decanted from the crude nuclear pellet. The concentration of heparin in the postmitochondrial supernatant was then increased from 0.5 mg/ml to 3 mg/ml. This was followed by the addition of 1/9 volume, 10% v/v Triton X-100/10% w/v sodium deoxycholate solution. The detergent solution was added slowly with constant agitation. After approximately 10 minutes at 0-4°C, the detergent-treated postmitochondrial supernatant was layered over 5 ml of 1 M sucrose, 0.05 M Tris-HCl, pH 7.5, 0.005 M $MgCl_2$ in cellulose-nitrate centrifuge tubes. The tubes were centrifuged for 2 hours in a Beckman SW 27 rotor at 25,000 rpm. Polysomes which pelleted through the sucrose pad were rinsed with 0.1 M NaCl, 0.001 M EDTA, 0.01 M Tris, pH 7.5 (NETs buffer) and resuspended by homogenization in a Dounce tissue grinder (Type A pestle) in the same buffer.

Extraction and Purification of Polysomal RNA

Polysomes which were resuspended by homogenization in NETs buffer were disrupted by the addition of 0.25 w/v SDS. An equal volume of 50% phenol/50% chloroform, pH 7.5 was then added as described by Perry et al. (1972). This method serves to extract poly(A) containing mRNA. Two, 5-10 minute extractions at 25°C were performed. The phenol (Bethesda Research Laboratories or Mallinckrodt) was liquified in NETs

buffer and contained 8-hydroxyquinoline. The aqueous phase after the second extraction was precipitated in 2.5 voulmes of 100% ethanol and stored at -20°C.

Some insoluble material was extracted with the polysomal RNA. This was removed by dissolving the polysomal RNA in 0.1 M NaCl, 0.01 M Tris-HCl, pH 7.5 and centrifuged in a Beckman SW 27 rotor at 100,000 x g average, 4 hours, 0°C, over a 5 ml pad of 20% w/v sucrose. In this procedure, insoluble material pellets while RNA remains in the supernatant. Following centrifugation, supernatant RNA was ethanol precipitated.

Affinity Chromatography of Poly(A^+)mRNA

Poly(A^+)mRNA was purified by oligo(dT) cellulose (Collaborative Research) chromatography after treatment with 80% dimethysulfoxide (Baker) as described by Bantle et al. (1976) except that 0.01% w/v diethylpyrocarbonate and 0.1% w/v SDS were added as ribonuclease inhibitors. Bantle et al. (1976) have demonstrated that this technique removes 98% of rRNA.

Affinity Chromatography of Poly(A^-)mRNA and Total mRNA

Poly(A^-)mRNA and total mRNA were purified by benzoylated cellulose chromatography as described by VanNess et al. (1979). Poly(U) (Sigma) was bound to cyanogen bromide activated Sepharose 4B (Sigma) by the method of Poonian et al. (1971). All poly(A^-)RNA was passed twice over poly(U) Sepharose in order to eliminate poly(A^+)mRNA with short poly(A) tracts which failed to bind to oligo(dT) cellulose.

Sucrose Gradient Centrifugation

Nucleic acids stored as ethanol precipitants were pelleted by centrifugation, dried, and resuspended in 25 µl 0.01 M Tris-HCl, pH 7.5. This was followed by the immediate sequential addition of 250 µl DMSO, 25 µl LiCl buffer (1 M LiCl, 0.05 M EDTA, 0.2% w/v SDS, 0.1 M Tris-HCl, pH 7.5), and 200 µl 0.01 M Tris-HCl, pH 7.5. The preparation was layered over 5-20% w/v sucrose gradients containing 50% v/v DMSO, 0.1 M LiCl, 0.005 M EDTA, 0.02% SDS, 0.01 M Tris-HCl, pH 7.5. Gradients were centrifuged in a Beckman SW 41 rotor at 32,500 rpm for 16.5 hours at 20°C and fractionated using ISCO equipment with optical density recorded continuously at 254 nm.

For mRNA sizing, mice were injected intraperitoneally with 520 µCi of ^{3}H-uridine. A 50 minute labeled period was allowed prior to RNA extraction. Polysomal RNA was measured by continuous scanning of the gradient at 254 nm during fractionation. Each fraction was then transferred to scintillation vials and 4 ml of Biocount (Research Products International) were added to determine the size of labeled putative total mRNA. The size of poly(A^{+})mRNA was determined by both absorbance and labeling following isolation by oligo(dT) cellulose chromatography. For accurate nuclear RNA sizing, unlabeled nuclear RNA with contaminating DNA (prior to DNase treatment) was sedimented into the gradient. Following fractionation, each fraction was treated with 0.2 N NaOH, 10 hours, 37°C to degrade only RNA. Calf thymus DNA (40 µg) was added to each sample followed by the addition of 1 volume 10% w/v trichloroacetic acid. The precipitated DNA was then removed by centrifugation and the $A_{(260nm)}$ of the supernatant read. For all RNA fractions the

number average nucleotide lengths were calculated using the expression:

$$Ln = \frac{NiLi}{Ni}$$

where Ln is the number average length in nucleotides, Ni is the number of individual molecules in a given size class and Li is the length in nucleotides of individual molecules in a given size class (Bantle and Hahn, 1976). The sedimentation coefficients for each different RNA fraction relative to the migration of the mouse rRNA markers (4-5S, 18S, 28S) were converted to nucleotide length according to the method of Granboulan and Scherrer (1969).

Preparation of ^{3}H-labeled Nonrepeated DNA

Labeled DNA was prepared from mouse L cells that were plated to one-half confluency in L-15 medium (GIBCO) containing 5-10 μCi/ml ^{3}H-thymidine (6.7 Ci/m mole , New England Nuclear), 10% fetal calf serum (GIBCO), and 30 units/ml penicillin -30 mg/ml Streptomycin (GIBCO). The mouse L cells were kindly donated by Dr. Franklin Leach of the Oklahoma State University. After 3-5 days of growth at 37°C, cells were dispersed by treatment in Hanks balanced salt solution, Ca^{++} and Mg^{++} free, 0.5 g/l trypsin, 0.2 g/l EDTA. The cells were then pelleted by centrifugation at 1000 x g average, 10 minutes, 0-4°C. Following centrifugation, the cells were resuspended in 5 ml of 0.1 M NaCl, 0.001 M EDTA. 0.01 M Tris, pH 7.5 (NETs buffer) and homogenized in a Teflon-glass tissue grinder. DNA was then released from protein by treatment with 0.2 mg/ml Proteinase K (Sigma) in the homogenization buffer for 30 minutes at 37°C. The volume of buffer was then increased

5-fold with NETs and the preparation was extracted twice with 0.50% w/v SDS-50% chloroform, pH 7.5 at 25°C (Perry et al., 1972). The phenol (Bethesda Research Laboratories or Mallinckrodt) contained 0.1% w/v 8-hydroxyquinoline. This was followed by two chloroform/octanol (12:1) extractions, pH 7.5 at 25°C. After precipitation of the aqueous phase in 2.5 voulmes of 100% ethanol at -20°C, the crude DNA was dissolved in NETs buffer, spooled, and reprecipitated in ethanol at -20°C. The DNA was further purified by treatment with 100 µg/ml RNase A and 10 units/ml RNase T_1 (Sigma) for 45 minutes at 37°C followed by the addition of 0.2 mg/ml Proteinase K for 1 hour at 37°C. An additional 0.5% w/v SDS-50% phenol/50% chloroform, pH 7.5, extraction was performed at 25°C. The purified DNA was precipitated in 2.5 volumes of 100% ethanol, -20°C, then resuspended in NETs buffer and sheared at 20-25,000 psi in a pressure cell (American Instruments) to a number average size of 380 nucleotides. After precipitating the sheared DNA in ethanol, the DNA was resuspended in 0.5 ml NETs buffer, passed through G100 Sephadex (Pharmacia) to remove small fragments and reprecipitated in ethanol. Repetitive sequences were then removed from the labeled sheared DNA by dissolving the DNA in 0.4 M Na phosphate buffer, pH 6.8, 0.2% w/v SDS, 0.001 M EDTA. The DNA was heat-denatured at 102°C for 6 minutes then allowed to renature at 2-4 mg/ml to a Cot value of 300-500. The partially renatured DNA was diluted to 0.06 M Na phosphate buffer, pH 6.8, and applied to a 1.2 x 7.0 cm hydroxylapatite (BioRad DNA grade) column at 60°C as described in the Analysis of Hybrid Content section. About 66% of the DNA remained single stranded. The unrenatured DNA was concentrated to a volume of

0.5 ml in NETs buffer by hollow fiber filtration (Spectrum) and precipitated in 2.5 volumes of 100% ethanol at -20°C. After precipitation, the nonrepeated, single-stranded, ^{3}H-labeled DNA was resuspended in NETs buffer and passed through G100 Sephadex in NETs buffer to remove oligonucleotides and reprecipitated in ethanol. The DNA was then again renatured to Cot 300-500, processed as described above, and stored as an ethanol precipitant at -20°C. An aliquot of ^{3}H-labeled nonrepeated DNA was coprecipitated with unlabeled total sheared DNA in ethanol at -20°C for analysis of the renaturation kinetics. The ^{3}H-labeled nonrepeated DNA used in these experiments was prepared by Mary d'Arcy Doherty.

Preparation of ^{3}H-labeled Nonrepeated DNA Complementary to Nuclear RNA

Excess liver nuclear RNA (15 mg/ml) was coprecipitated with ^{3}H-labeled nonrepeated DNA (10 µg/ml) in 2.5 volumes of 100% ethanol at -20°C. After centrifugation at 8000 x g average, 10 minutes, 0-4°C, the nucleic acids were dried and resuspended in 0.02% w/v SDS, 0.001 M EDTA, 0.01 M Tris-HCl, pH 7.5, after which 1 M Na phosphate buffer, pH 6.8, was added to a final concentration of 0.4 M. The mixture was sealed in a capillary tube, heat-denatured at 102°C, 4 minutes, and incubated to an equivalent Rot of 32,000 or more. The reaction was stopped by freezing at -20°C in 100% ethanol. The preparation was then passed over G100 Sephadex in 0.3 M NaCl, 10^{-5} M $ZnSO_4$, 0.01 M Na acetate, pH 4.5, and treated with 4000 U/ml of S^1 nuclease (Sigma) for

80 minutes at 37°C to degrade single-stranded molecules. After this time, the solution was made 0.2% w/v SDS and extracted with an equal volume of pH 7.5 50% phenol/50% chloroform at 25°C. The aqueous phase was passed over G100 Sephadex in 0.12 M Na phosphate buffer, pH 6.8, and the excluded material applied to a 1 ml hydroxylapatite (Bio-Rad, HTP grade) column. Nucleotides were eluted by 14, 0.5 ml applications of 0.12 M Na phosphate buffer, pH 6.8. Double stranded molecules were eluted by 6, 0.5 ml washes of 0.4 M Na phosphate buffer pH 6.8. Double stranded nucleic acids in 0.4 M Na phosphate buffer were divided into two, 1.5 ml aliquots and applied separately to a G100 Sephadex column in NETs buffer. The excluded material was made 0.1 N NaOH and incubated for 3 hours at 25°C to separate hybrids and destroy all RNA After incubation, the solution was made 0.05 M Tris-HCl, pH 7.5, then neutralized to a pH 6.5-7.5 with HCl. This fraction enriched for DNA complementary to RNA was termed nuclear DNA or nDNA. The RNA fractions to be hybridized with nDNA were mixed with aliquots of nDNA and coprecipitated in ethanol.

Preparation of ^{3}H-labeled Nonrepeated DNA Complementary to Poly(A^{+})mRNA

The ^{3}H-labeled nonrepeated DNA complementary to poly(A^{+})mRNA was prepared as described by Bantle and Hahn (1976). Unlabeled nonrepeated DNA was coprecipitated with an aliquot of ^{3}H-labeled DNA complementary to poly(A^{+})mRNA in ethanol at -20°C.

Hybridization of Nucleic Acids

For high Rot reactions, 6-15 ug/ul RNA in 0.4 M Na phosphate buffer, pH 6.8, 0.01% w/v SDS, 0.001 M EDTA was mixed with either heat-denatured (104°C, 6 minutes) ^{3}H-labeled nonrepeated DNA or nDNA, and 3-5 µl aliquots (3000-5000cpm) were sealed in glass capillary tubes. Reaction mixtures were incubated for various periods (hours) at 67°C. RNA concentrations were determined by absorbance at 260 nm. At 260 nm, 1 mg/ml solution of RNA equals 22 absorbance units.

For DNA reassociation, 15 µg/ml unlabeled total sheared DNA in 0.4 M Na phosphate buffer, pH 6.8, 0.01% w/v SDS, 0.001 M EDTA was mixed with heat-denatured (104°C, 6 minutes) ^{3}H-labeled nonrepeated DNA and 3-5 µl (3000-5000cpm) were sealed in glass capillary tubes. The reaction mixtures were incubated for various periods (hours) at 67°C.

Analysis of Hybrid Content

Following hybridization, each sample point was diluted in 0.5 ml of 0.01 M Na phosphate buffer, pH 6.8, and passed over G100 Sephadex to remove small fragments. The excluded fraction was then immediately applied to a 1.2 x 7.0 cm column containing 1-1.5 ml of hydroxylapatite (HAP) (Bio-Rad) at 60°C. Single stranded molecules were eluted with 9, 1 ml washes of 0.01 M Na phosphate buffer, pH 6.8, while double stranded nucleic acids were eluted with 4, 1 ml washes of 0.4 M Na phosphate buffer, pH 6.8. The eluted fractions were counted under similar salt conditions. S^{1} nuclease (Miles), prepared by the method of VanNess et al. (1979) was used to digest single strand tails from

the hybrids of terminal Rot points. Hybridization samples were diluted with 500 µl of 0.1 M NaCl, 0.03 M Na acetate, 10^{-5} M $ZnSO_4$, pH 4.5. Next 6 µl (5.4 units) of S^1 nuclease, free of double-stranded activity was added and the sample incubated for 60 minutes at 37°C. The sample was then passed through G100 Sephadex in 0.01 M Na phosphate buffer, pH 6.8, then applied to HAP as described in the above.

DNA-DNA annealing was measured by releasing RNA-DNA hybrids with 30 µg/ml RNase A and 3 units/ml RNase T_1 (Sigma) for 15 hours, 37°C, in 0.05 M Na phosphate buffer, pH 6.8, followed by HAP chromatography as previously described. In all experiments, Biocount (Research Products International) was used as a scintillant. Thermal melt curves were performed on liver nuclear RNA:usDNA hybrids and native DNA by the method of Martinson (1973).

CHAPTER IV

RESULTS

Purity of RNA Preparations

All RNA populations were treated extensively with DNase after sizing. The extent of DNA contamination was determined to be less than 0.001%. The level of contamination was determined by treating 100 ug of the RNA sample with 20 µg/ml RNase A in NETs buffer. Following digestion of the RNA, the sample was passed through G-100 Sephadex. Contaminating DNA was excluded in this column as it was estimated that 0.001 µg of DNA would be detected at a monitor setting of 0.1 $A_{(254nm)}$. In all cases there was no observable peak attributable to DNA. Poly(A^+)mRNA was essentially free of contaminating rRNA due to DMSO and heat treatment which disrupts aggregates of RNA and reduces secondary structure (Bantle et al., 1976). However, poly(A^-)mRNA and total mRNA were contaminated with approximately 50% rRNA (VanNess et al., 1979).

The efficiency of the separation of poly(A^+)mRNA from poly(A^-) mRNA was measured by the hybridization of ^{3}H-labeled poly(U) with the poly(A) tracts of both poly(A^+)mRNA and polysomal RNA that had been chromatographed to remove poly(A^+)mRNA. This hybridization was performed by adding 2 µg of ^{3}H-labeled poly(A) (Miles, $1x10^6$ dpm/µg) to separate mixtures containing 25 µg purified poly(A^+)mRNA and 1 mg

of chromatographed polysomal RNA in NETs buffer. The reaction was carried out at 37°C for 1 hour. Then, 10 µg/ml RNase A was added to digest unreacted ^{3}H-poly(U). The ^{3}H-poly(U) hybrids were precipitated in 5% w/v TCA, collected on filters and counted at 18% efficiency. The poly(A^{+})mRNA fraction bound 252,000 cpm while the background (^{3}H-poly(U) alone) reaction bound 2,000 cpm. This was equivalent to 1.4 µg of poly(A) in the poly(A^{+})mRNA fraction or 5.6% of the total RNA. Only 2500 cpm bound to the chromatographed polysomal RNA and, after deducting the 2000 cpm background, the 500 cpm bound represented a poly(A) content of only 0.002 µg or 0.02% of the estimated 11.4 µg of poly(A^{-})mRNA (Table 2).

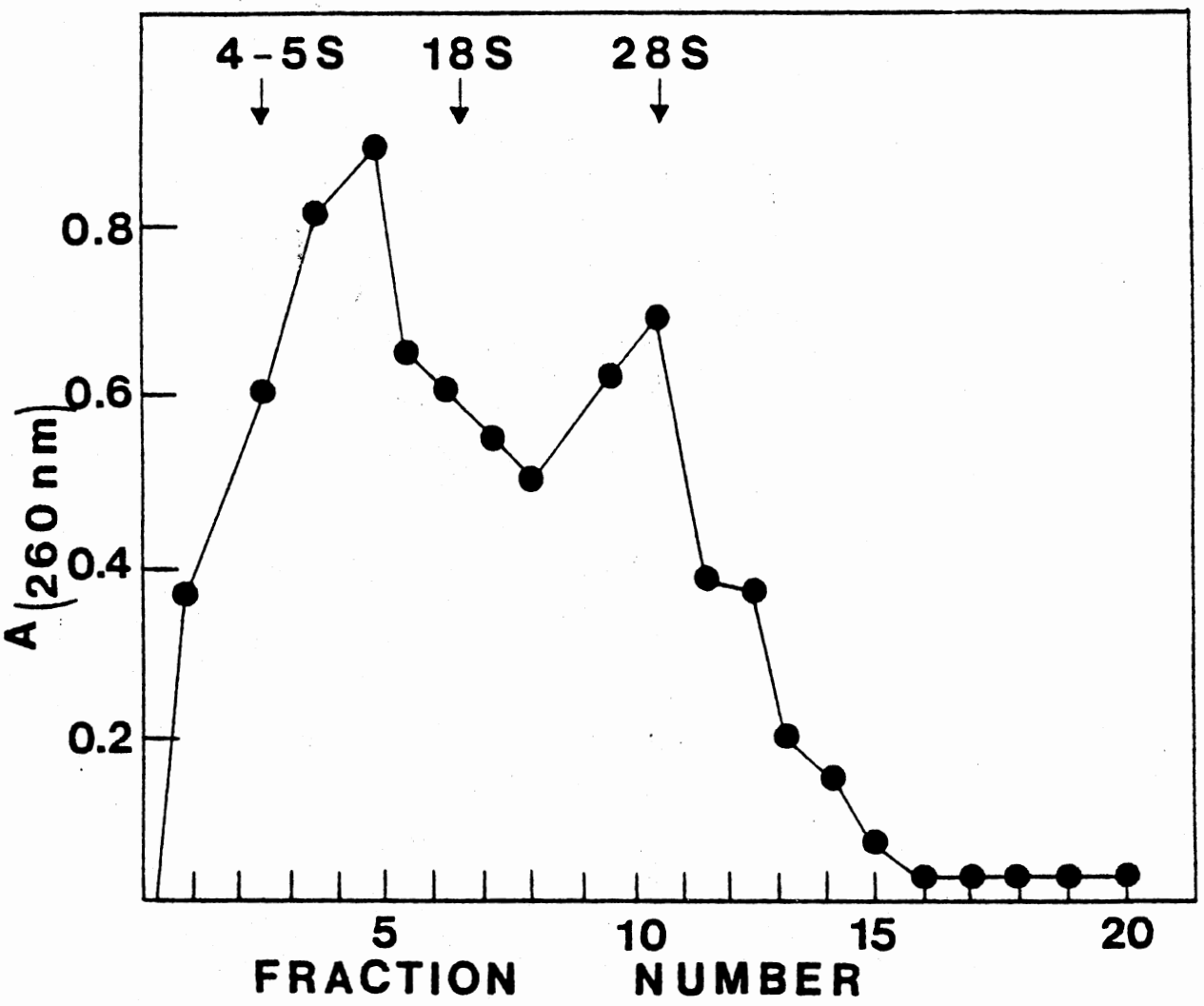
Yield and Size Estimates of Liver RNA

The yield of each RNA fraction was determined spectrophotometrically by the absorbance at 260 nm. The yield of nuclear RNA was 94 µg/g liver and the number average nucleotide length as analyzed by absorbance after sedimentation in DMSO-sucrose gradients was 1430 (Table 2, Figure 1). While most of the RNA sedimented less than 2200 nucleotides, an appreciable amount of nuclear RNA sedimented up to 26,000 nucleotides. This RNA profile was similar to that reported by Bastian (1980) for rat liver. The analysis of nuclear RNA size is made very difficult by RNA aggregation and enzymatic degradation (Naora, 1977). The use of dimethylsulfoxide (DMSO) in the sucrose gradient prevented aggregation of nuclear RNA (Bantle et al., 1976) as indicated by the migration of 4-5S, 18S, and 28S rRNA markers (Figure 1).

Table 2. Yield, size and complexity of nuclear and polysomal RNA from mouse liver[a].

Preparation	Average yield (pgRNA/g liver, % of RNA in Sub-cellular Fraction)	Number Average Size (Nucleotides)	Number of Preparations Hybridized	Net Saturation of Nonrepeated DNA[c] (%)	Corrected Saturation Value for Single Stranded Tails[d] (%)	Net Saturation of DNA Complementary to Nuclear RNA (%)	Total Nonrepeated DNA Complexity[g] (%)	Complexity[i] (Bases x 10^{-8})
Nuclear RNA	94	1430[a]	3	13.93	11.93	-	23.9	4.41
Poly(A^+)mRNA	-	1480[a]	2	-	-	9.9[e]	4.4[h]	0.81
Poly(A^+)mRNA	12.5(1.16%)	1500[a]	1	-	-	8.1[f]	4.1[h]	0.76
Poly(A^-)mRNA	11.0(1.0%)	1300[b]	1	-	-	7.65[f]	3.9[h]	0.72
Total mRNA	23.5(2.16%)	1460[b]	1	-	-	7.5[f]	3.8[h]	0.71

[a] Value based on absorbance scan of the H_2O-sucrose density gradient.
[b] Value based on radioactivity of 50 min [^{3}H]uridine labeled RNA sedimented in H_2O-sucrose density gradients.
[c] Saturation by each RNA population was determined from computer generated plateau values based on a least squares analysis of hybridization data (45). An RNA equivalent R_oT of greater than 35,000 was achieved for each RNA population and analysis was by hydroxylapatite chromatography.
[d] Terminal reaction points were treated with S_1 nuclease (Sigma) for 30 min at 37°C. Following SDS-phenol/chloroform extraction, samples were analyzed using hydroxylapatite chromatography. The S^1 nuclease assay of Maxwell et al. (46) was also used on terminal reaction points.
[e] Nuclear RNA saturated 53% of this preparation of [^{3}H]DNA complementary to nuclear RNA(nDNA).
[f] Nuclear RNA saturated 47% of this preparation of [^{3}H]DNA complementary to nuclear RNA(mDNA).
[g] Asymmetric transcription of DNA is assumed. Therefore, the percentage of total complexity transcribed is twice the corrected saturation value of nonrepeated DNA.
[h] % total nonrepeated DNA complexity = saturation value of nuclear RNA with nonrepeated DNA + saturation value of nuclear RNA with mDNA x saturation value of mRNA with mDNA x 2(correction for asymmetric transcription).
[i] Percentage of nonrepeated DNA hybridized is expressed as base complexity where nonrepeated DNA comprises two-thirds of a haploid mouse genome composed of 2.8×10^9 nucleotide pairs.



Figurel. Sedimentation analysis of liver nuclear RNA. Extracted liver nuclear RNA with contaminating DNA was sedimented into the gradient. Following fractionation, DNA was removed as described in the Materials and Methods section.

The yield of mRNA fractions was based on material bound to oligo(dT)-cellulose and benzoylated cellulose assuming that 50% of the bound material from benzoylated cellulose was ribosomal RNA as previously demonstrated by VanNess et al., 1979. The yield of polysomal RNA was 1.1 mg/g liver (Table 2). Of this, 2.14% was total mRNA, 1.14% poly(A^+)mRNA, and 1.0% poly(A^-)mRNA (Table 2). Therefore, approximately 50% of the mass of the mRNA in liver is non-adenylated. The DMSO-sucrose gradient sedimentation profiles of mouse liver polysomal RNA and poly(A^+)mRNA (Figure 2) were similar to that reported for rat liver (Sala-Trepat et al., 1978). Labeled poly(A^+)mRNA had the same profile as poly(A^+)mRNA analyzed by asorbance (Figure 2) and the average poly(A^+)mRNA size was 1500 nucleotides (Table 2). Assuming that the profiles of total mRNA (Figure 2) and poly(A^-)mRNA (Figure 2) as analyzed by radioactivity accurately measured the true size of these mRNA fractions, the size of total mRNA was 1300 nucleotides and poly(A^-)mRNA 1460 nucleotides (Figure 2, Table 2). The poly(A^-) profile was determined by subtracting the poly(A^+)mRNA profile from the total mRNA profile.

Characterization of ^{3}H-labeled Nonrepeated DNA from Mouse L Cells

The RNA-driven hybridization reactions on which all complexity estimates were based require a single copy DNA tracer of high specific activity. This can be accomplished by labeling DNA <u>in vitro</u> by either nick translation or the addition of a radioisotope to a cell

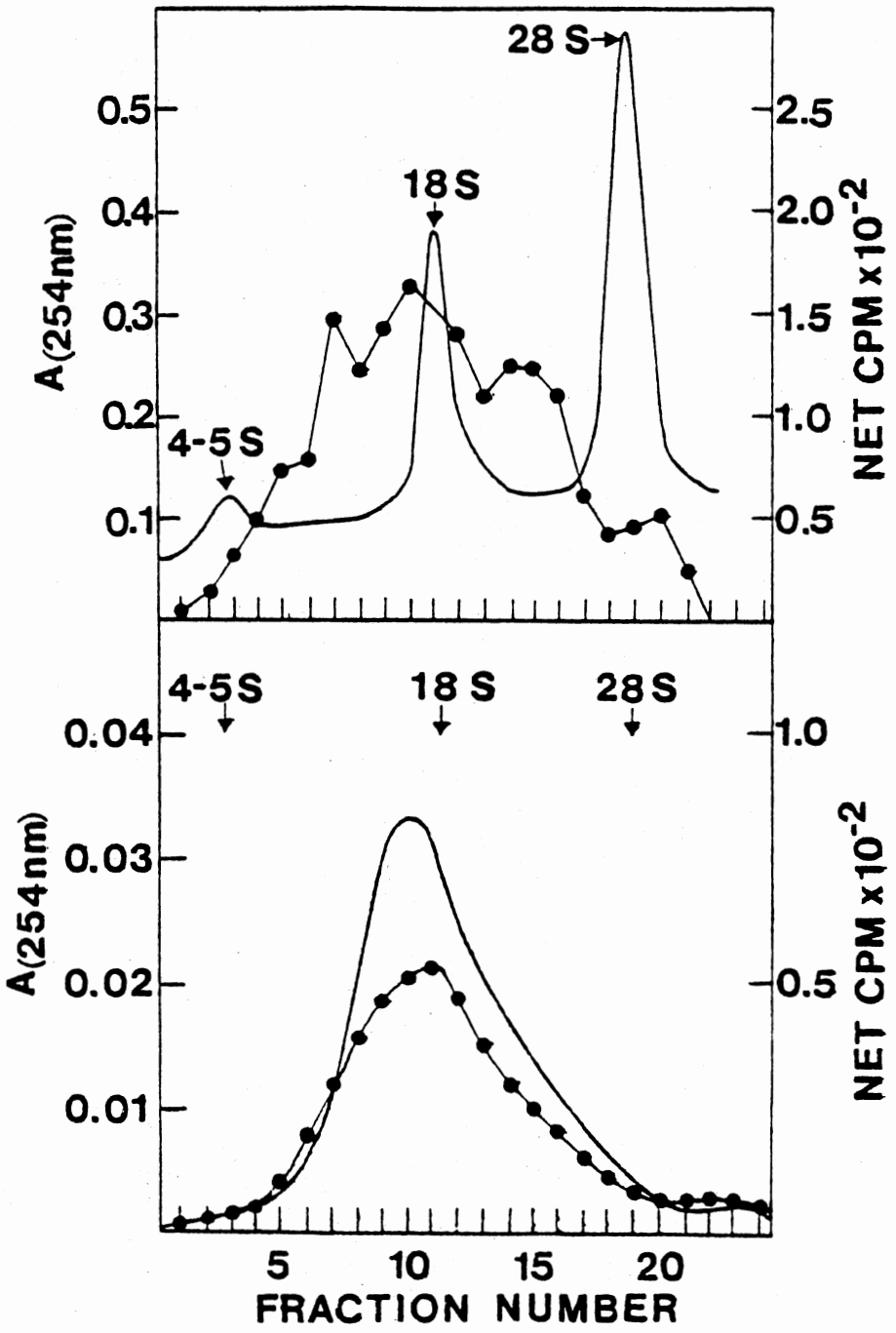


Figure 2. Sedimentation analysis of liver polysomal RNA and poly(A^+)mRNA. Mice were labeled for 50 minutes with 250 µCi of ^{3}H-uridine prior to extraction of RNA. Top panel: polysomal RNA absorbance (—), labeled putative total mRNA (●). Bottom panel: poly(A^+)mRNA absorbance (—), labeled poly(A^+)mRNA (●).

culture medium. Using this latter technique, a specific activity of 925,000 cpm/ug at 39% counting effeciency was obtained for ^{3}H-thymidine labeled DNA.

After isolation by conventional techniques, the ^{3}H-labeled nonrepeated DNA was sheared to a number average size of 380 nucleotides (Figure 3). The repetitive sequences were reduced to unique sequence concentration by reassociating the DNA twice to Cot 300-500. ^{3}H-labeled nonrepeated DNA renatured 86.8% with freshly denatured unlabeled total sheared DNA at an equivalent Cot of 60,000 as shown in Figure 4. To verify that the saturation experiments for various RNA classes gave true measurements of complexity, it was demonstrated that repetitive sequences had been removed from the probe by observing the reassociation kinetics of ^{3}H-labeled nonrepeated DNA which had been previously hybridized to poly(A^{+})mRNA with unlabeled total sheared mouse DNA (Figure 4). Since total mouse DNA renatures 30-35% at Cot 100 due to repetitive sequences (Laird, 1970) the lack of such renaturation in Figure 4 demonstrates that the ^{3}H-labeled nonrepeated probe used in these experiments was essentially free of contaminating repetitive sequences. In addition, the Cot 1/2 for the renaturation of excess unlabeled DNA with ^{3}H-nonrepeated DNA can also be derived from Figure 4. The Cot 1/2 represents that Cot value at which the renatuation reaction is proportional to the complexity of the unique sequence content of the given genome since it describes the rate at which nonrepeated DNA fragments renature. The fragment length of nonrepeated DNA plays an important role in the rate of renaturation. Genomes of high complexity (eg. mouse) take longer to

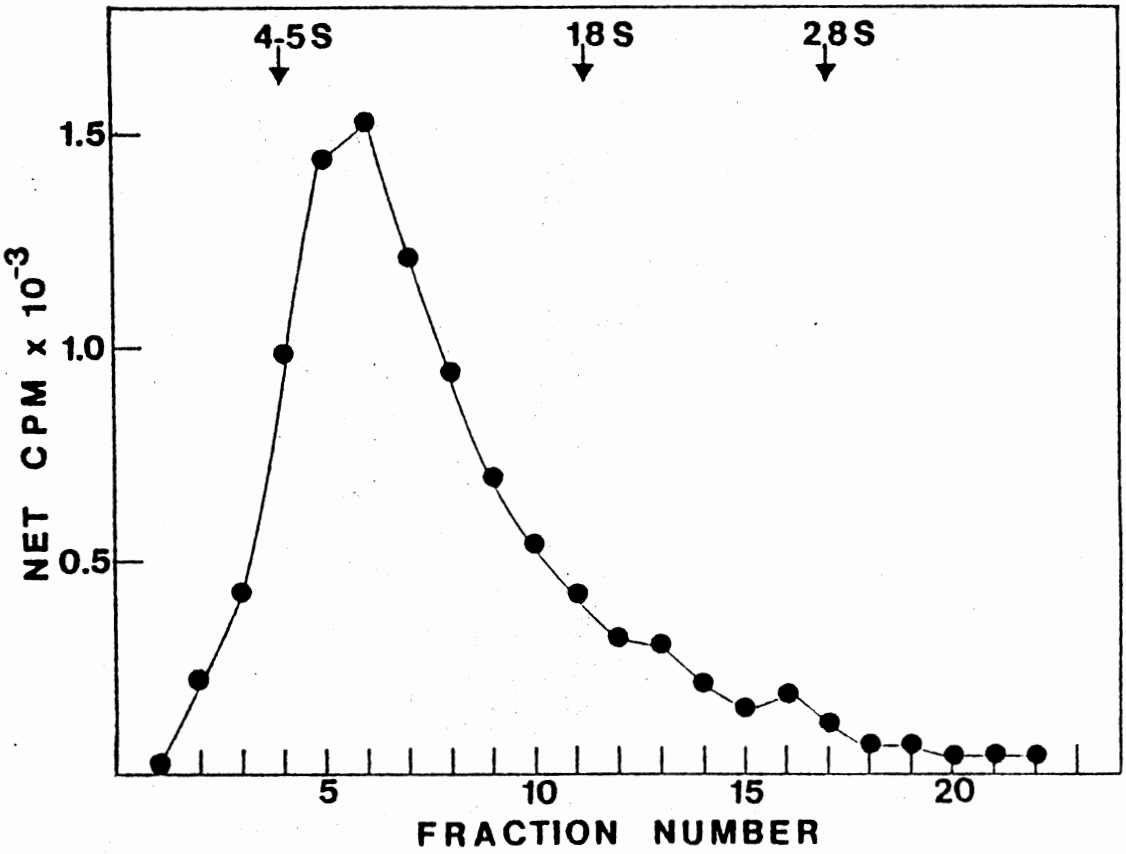


Figure 3: Sedimentation analysis of sheared ^{3}H-nonrepeated DNA.

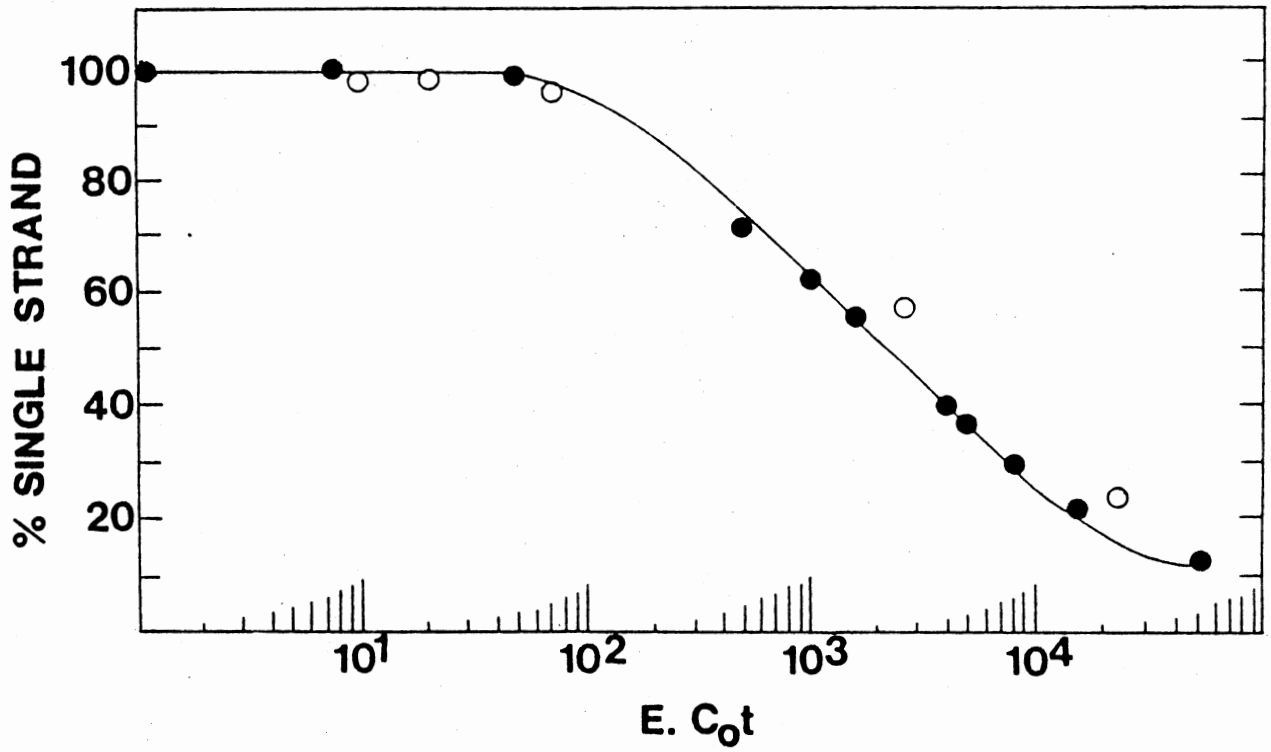


Figure 4: Renaturation of excess sheared unlabeled DNA with nonrepeated DNA (●) and with nonrepeated DNA complementary with poly(A^+)mRNA (○).

renature than genomes of low-complexity (eg. E. coli). This is because a given fragment comprises less of the percentage of the total genome in a large genome than in a small genome. Hence, by mass law, the rate of reaction will be slower for the large genome. For the mouse genome, a Cot 1/2 value of 2000 (Figure 4) at a fragment size of 380 nucleotides for nonrepeated DNA (Figure 3) agrees well with that obtained by Hahn and Laird (1971) for the renaturation kinetics of nonrepeated mouse DNA.

DNA-DNA Renaturation During DNA-RNA Hybridization and Zero Time Binding

DNA-DNA renaturation was monitored by treating DNA-RNA hybrids with RNase under low salt conditions. The amount of ^{3}H-DNA as DNA-DNA was determined by hydroxylapatite (HAP) chromatography. The appropriate RNAse control was used to correct each Rot point for DNA-DNA renaturation. Each reaction point was also corrected for zero time T_o duplex material as determined by HAP chromatography. For the hybridization of ^{3}H-labeled nonrepeated DNA with nuclear RNA, T_o samples were 1% and DNA-DNA renaturation at saturation was less than 3% of the duplex material.

The capacity of a particular lot of hydroxylapatite to bind single and double stranded molecules in 0.10 M or 0.12 M and 0.4 M Na phosphate buffer, pH 6.8, at 60^{o}C was determined using ^{3}H-labeled native sheared DNA. All HAP columns used in these experiments bound greater than 95% double stranded molecules and less than 1% single

stranded molecules in 0.10 M or 0.12 M phosphate buffer.

Complexity of Nuclear RNA

The saturation curve for the hybridization of ^{3}H-labeled nonrepeated DNA with nuclear RNA is shown in Figure 5. Nuclear RNA saturated 13.9% of nonrepeated DNA at Rot 36,000, when the hybridization reaction was assayed by hydroxylapatite chromatography (Figure 5, Table 2). This is equivalent to 23.9% of the total nonrepeated DNA complexity (Table 2). The use of a S^1 nuclease, an endonuclease which digests single stranded molecules, reduced the value of ^{3}H-labeled nonrepeated DNA hybridized with nuclear RNA at saturation to 11.9% (Table 2). This is equivalent to a complexity of 4.4×10^8 nucleotides (Table 2) as determined by the following equation (see Table 2 for data):

Complexity of Nuclear RNA	=	Net Saturation of Nonrepeated DNA Hybridizing with Nuclear RNA	X	Fraction of Haploid Mouse Genome that is Nonrepeated	X	Complexity of Haploid Mouse Genome

The percent of nonrepeated DNA hybridizing with nuclear RNA could not be due to repetitive sequence contamination since the renaturation of nonrepeated DNA with total DNA followed usDNA kinetics. No correction was made for ^{3}H-DNA "unavailable" for hybridization. During DNA renaturation, 12% of the ^{3}H-labeled usDNA fails to react (Figure 4). When the unreacted DNA is isolated by hydroxylapatite chromatography and renatured a second time with a fresh addition of denatured DNA to a Cot of 20,000, only 20% of the DNA reacts. However, excess nuclear RNA still reacts with this DNA to a level of 14% which is the same level of reaction as with total usDNA. The reason for this

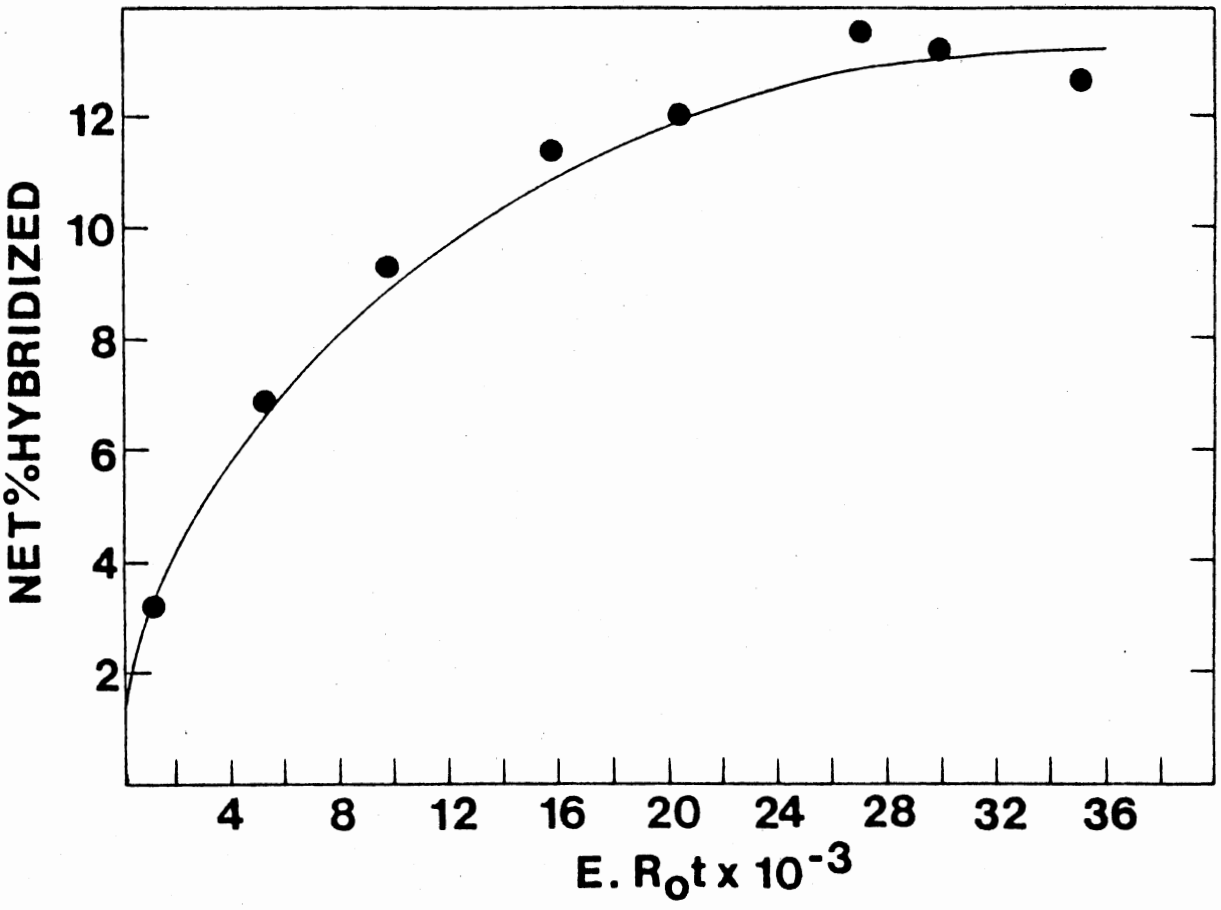


Figure 5. Hybridization of liver nuclear RNA with ^{3}H-labeled nonrepeated DNA.

result is unclear at present, but it must be concluded that virtually all of the probe DNA is available for hybridization with DNA.

Thermal melt analysis of hydroxylapatite bound hybrids (Figure 6) demonstrated that the Tm of sheared native DNA was 89° C while the Tm for ^{3}H-nonrepeated DNA-nuclear RNA was 82.5°C. This revealed 8-10% mismatch (Martinson, 1973).

Complexity of mRNA Fractions

Since other studies showed that liver poly(A^+)mRNA saturates only 1.9% of unique sequence DNA, it was necessary to increase the percentage of probe DNA that was complementary to mRNA in order to accurately measure the complexity of the mRNA fractions. Because nuclear RNA saturates a considerably higher quantity of unique sequence DNA than mRNA and serves as the probable precursor of mRNA, ^{3}H-nonrepeated DNA complementary to nuclear RNA (nuclear DNA or nDNA) was used to estimate mRNA complexity.

With the first preparation of nDNA, nuclear RNA hybridized with nDNA to a level of 53% (Figure 7). Since the parental single copy tracer reacted 13.9% (Figure 5, Table 2) with nuclear RNA, nDNA represents a 3.8 fold enrichment in DNA sequences expressed in liver. Poly(A^+)mRNA saturated 9.9% of the first nDNA probe (Figure 7, Table 2). Poly(A^+)mRNA that had been isolated by a means known to eliminate nuclear RNA contamination (Goldberg et al., 1973) reacted the same extent as poly(A^+)mRNA that had not been specially processed (Figure 7). Hence nuclear RNA contamination was too low to influence results. This is important to demonstrate since extensive nuclear

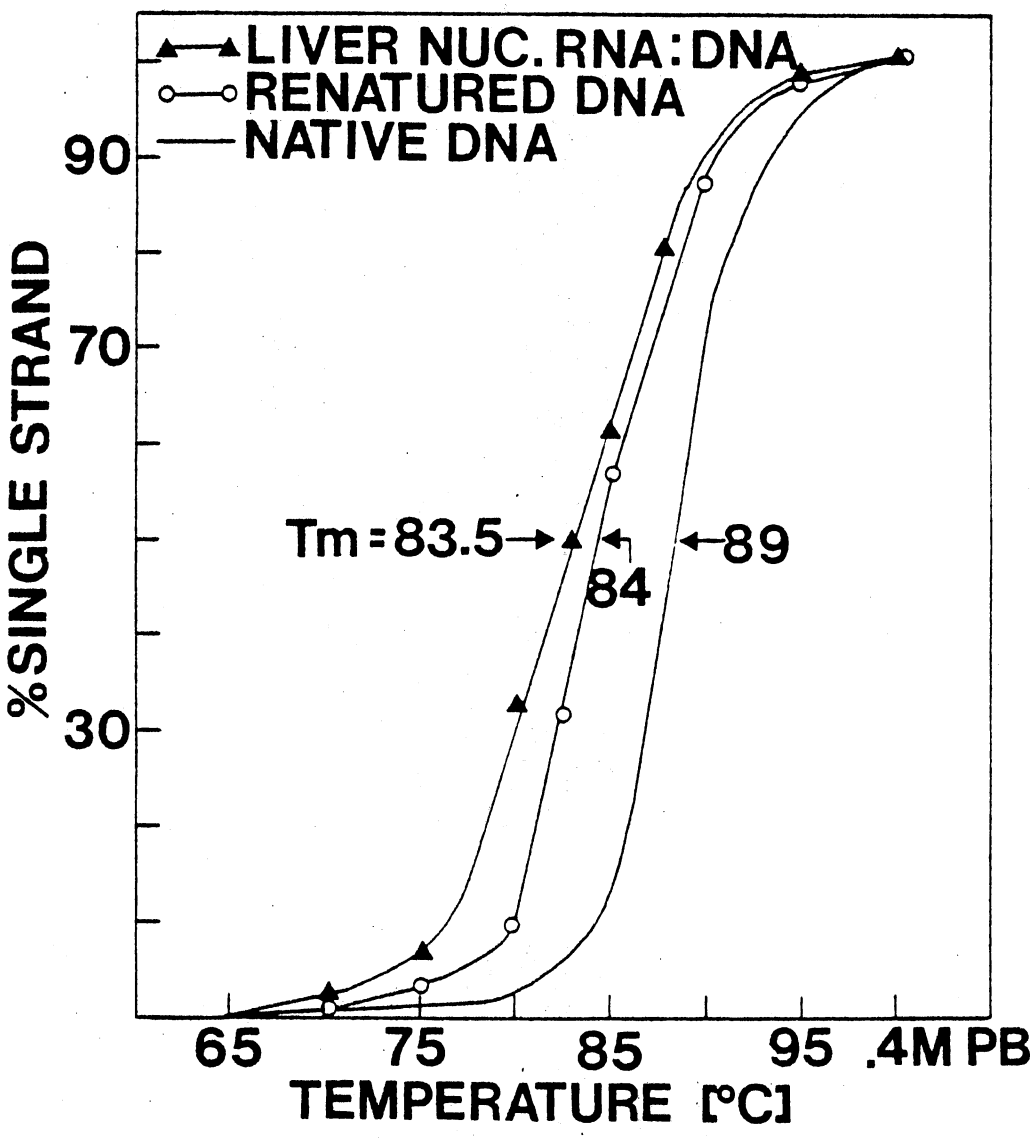


Figure 6. Thermal melt curves on liver nuclear RNA-non-repeated DNA hybrids and native DNA. Technique used was that of Martinson (1973).

RNA contamination would result in erroneous mRNA complexty estimates due to the presence of nuclear RNA sequences complementary to the nuclear DNA probe.

Nuclear RNA saturated 4.7% of a second nDNA probe (Table 2) while poly(A^+)mRNA saturated 8.1% (Figure 8, Table 2). Based on the two different nDNA probes, the complexity of poly(A^+)mRNA in liver is 7.6-8.1x10^7 nucleotides (Table 2). This was determined using the following equation (see Table 2 for data):

$$\text{Complexity of mRNA} = \text{Complexity of Nuclear RNA} \times \frac{\text{Saturation of mRNA with nuclear DNA}}{\text{Saturation of nuclear RNA with nuclear DNA}}$$

Poly (A^-)mRNA and total mRNA (poly(A^+)mRNA and poly(A^-)mRNA) saturated 7.5% and 7.6% of the second nDNA probe respectively (Figure 7, Table 2). These saturation values resulted in complexity estimates of 7.2x10^7 nucleotides for poly(A^-)mRNA and 7.2x10^7 nucleotides for total mRNA (Table 2) using the above equation. The percent of nuclear DNA hybridizing with the mRNA fractions could not be due to the preferential hybridization of mRNA with repetitive sequence DNA since poly(A^+) mRNA hybridized with nonrepeated DNA (Figure 4) following usDNA kinetics.

In these experiments, total mRNA and poly(A^-)mRNA Rot values were not corrected for the presence of 50% ribosomal RNA. This should have little effect on complexity estimates since rRNA hybridizes only with repetitive sequence DNA and such DNA has been demonstrated (Figure 4) to be reduced to a level so low it can be ignored. No differences in saturation values of terminal reaction points was observed after treatment with S^1 nuclease, thus indicating excellent fidelity of the

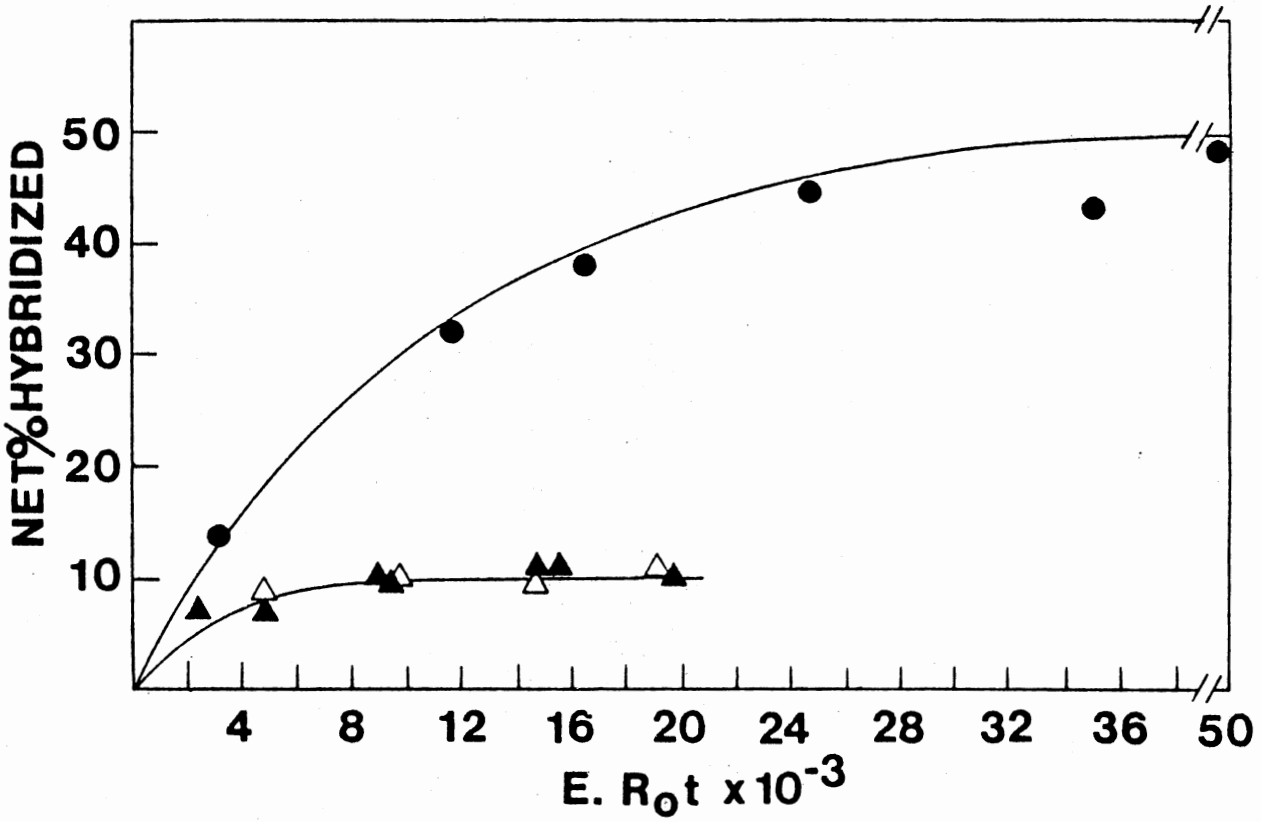


Figure 7. Hybridization of nuclear RNA and poly(A^+)mRNA with nDNA. Nuclear RNA (●), poly(A^+)mRNA (▲), poly(A^+)mRNA without nuclear contamination (△). Curves based on a least squares analysis of data by computer. (Pearson et al., 1977).

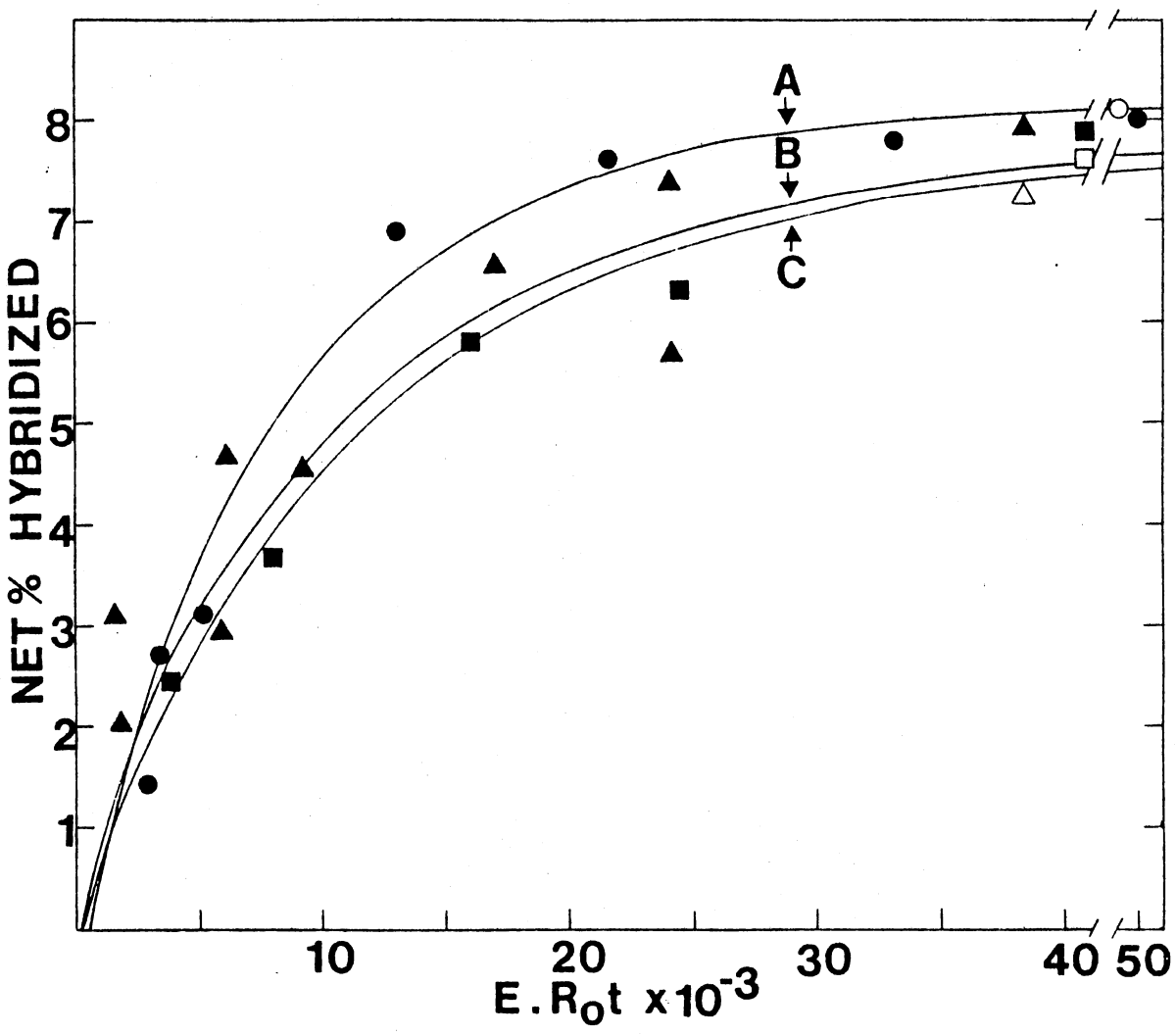


Figure 8. Hybridization of poly(A^+)mRNA, poly(A^-)mRNA and total mRNA with nDNA. A: poly(A^+)mRNA (● , S^1 nuclease ○). B: poly(A^-)mRNA (■ , S^1 nuclease □), C: total mRNA (▲ , S^1 nuclease, △). Curves based on a least squares analysis of data by computer (Pearson et al., 1977).

nDNA probe.

The values obtained for the saturation of nuclear DNA with poly(A^+)mRNA, poly(A^-)mRNA, and total mRNA are essentially identical. The slight differences observed for the saturation of nuclear DNA with the three mRNA fractions are within the range of satistical error ($\pm$1%) as determined by least squares analysis for the computer drawn curves (Figure 8). The almost identical complexity estimates obtained for poly(A^+)mRNA, poly(A^-)mRNA and total mRNA from mouse liver indicate that poly(A^+)mRNA and poly(A^-)mRNA possess essentially all of the same sequences. Had the poly(A^+) and poly(A^-)mRNA population in liver been of similar complexity but composed primarily of different sequence content, the complexity for total mRNA would have been the complexity of the poly(A^+) and the poly(A^-)mRNA population added together. As seen in Figure 8, this was not the case. Based on the complexity estimates for the three mRNA populations and the average mRNA size for each population, liver could potentially express 50-54,000 different proteins with the infrequent poly(A^+)mRNA and poly(A^-)mRNA classes coding for the same proteins.

A difference of 0.6% was obtained in the saturation of mDNA by three mRNA populations. Since a variation of 1% is common in RNA-DNA hybridization experiments (Bantle and Hahn, 1976), the 0.6% difference in results could solely be due to experimental error. There are an estimated 50,000 sequences expressed in liver free polyribosomes and this is equivalent to a saturation of nDNA of 8%. Therefore, a 0.6% variation represents an error of 37,000 distince sequences in this experiment or 7.5% of all expressed genes.

CHAPTER V

DISCUSSION

The purpose of this study was to estimate the complexity of both poly(A^+)mRNA and poly(A^-)mRNA and to determine the extent that these two RNA populations are shared in mouse liver. Numerous measurements of liver mRNA complexity exist in the literature (see Table 1). However, becasue of the difficulity in obtaining accurate Rot 1/2 estimates for the complex class (Ryffel and McCarthy, 1975) and the incomplete representation the mRNA population (Ordahl and Caplan, 1978), many early studies using cDNA probes resulted in underestimates of liver mRNA complexity (eg. Ryffel and McCarthy, 1975; Hastie and Bishop, 1976; Young et al., 1976; Colbert et al., 1977; Sippel et al., 1977; Towle et al., 1978). In addition, underestimates of liver mRNA complexity using usDNA probes have been reported as well (eg. Grady et al., 1978), apparantly the result of not reaching saturation during hybridization.

The complexity estimates of the mRNA fractions measured in this study were dependent upon the satisfactory preparation of a probe which completely represented all sequences in each mRNA class. The probe used in this study was prepared from nuclear RNA and termed nuclear DNA or nDNA. Nuclear DNA isolated by hybridizing nuclear RNA with ^{3}H-labeled nonrepeated DNA to saturation. In this investiga-

tion, nuclear RNA saturated 13.9% of nonrepeated DNA as determined by HAP chromatography (Table 2). When the nuclear RNA-nonrepeated DNA hybrids were treated with S^1 nuclease, 11.9% of nonrepeated DNA remained bound to nuclear RNA (Table 2). This is equivalent to a complexity of 4.41×10^8 nucleotides (Table 2). This decline in the percent of nonrepeated DNA hybridized with nuclear RNA after S^1 treatment was probably due to the removal of single stranded tails from the ends of hybrid molecules although some degradation of double-stranded DNA cannot be ruled out. In addition, the decline may have been due to the digestion of intervening sequences (Kiper, 1979) present in the DNA but absent in the nuclear RNA.

During the preparation of the nuclear DNA probe, double-stranded molecules were isolated by HAP chromatography after S^1 nuclease treatment. The double-stranded molecules were then subjected to 0.1 M NaOH for 3 hours at 37°C. This technique effectively separates hybrids whether they be of DNA-RNA or RNA-DNA origin. To reduce the extent of DNA-DNA renaturation when preparing the probe, only trace quantities of ^3H-labeled nonrepeated DNA were used in comparison to the vast excess of unlabeled RNA in the reaction. The final product of this procedure is a ^3H-labeled, single-stranded, nonrepeated DNA probe which has been enriched for sequences present in the nuclear RNA population.

The fidelity of nuclear DNA is demonstrated in Figure 7. The hybridization of nuclear RNA to two different nuclear DNA preparations revealed that 53% of the first probe (Figure 7, Table 2) and 47% of the second probe (Table 2) was reactable. This represents a 3.8 and 3.3 fold enrichment for DNA sequences expressed in liver, respectively.

This technique serves to improve the resolution normally achieved with the hybridization of total unique sequence DNA with an RNA preparation since noncomplementary sequences are removed.

Nuclear DNA was used to measure polysomal mRNA complexity in this study for two basic reasons. First, relatively accurate estimates of complexity can be obtained for nuclear RNA through saturation hybridization with usDNA, whereas mRNA saturates considerably less of usDNA hence inherent difficulties in the technique are more likely to result in erroneous mRNA complexity estimates. Second, it was desirable to demonstrate that mRNA sequences are represented in the nuclear RNA class.

One criticism of this work is that any error in the determination of nuclear RNA complexity will lead to erroneous estimates of mRNA complexity. While this is true, it is believed that the complexity of nuclear RNA (4.4×10^8 nucleotides, Figure 5, Table 2) was determined accurately in this study. This is based on several observations. First, all RNA preparations in this study were isolated in the presence of heparin and yeast RNA which serve to inhibit ribonuclease activity. Thus, nuclear RNA degradation was minimized. Second, the sedimentation profile obtained for nuclear RNA in this study (Figure 1) is similar to that reported by Bastian (1980) for rat liver. Third, and most importantly, the extent of saturation of usDNA with nuclear RNA obtained after S^1 nuclease treatment (11.93%, Table 2) is in close agreement to the 10.9% obtained by Chikaraishi et al. (1979) for rat liver.

The preparation of nDNA and the estimation of nuclear RNA comp-

lexity allowed the complexity of various mRNA fractions to be determined. Through saturation hybridization of poly(A^+)mRNA, poly(A^-)mRNA, and total mRNA with nDNA, the complexity of these mRNA fractions was estimated to be 7.6-8.1x10^7, 7.2x10^7, and 7.1x10^7 nucleotides respectively (Figure 8, Table 2). This represents enough sequences to encode 50,000-54,000 different proteins. This demonstrates that the poly(A^+)mRNA and poly(A^-)mRNA populations in mouse liver are composed essentially of the same sequences. Had poly(A^+)mRNA and poly(A^-)mRNA been of similar complexity but composed essentially of nonhomolgous sequences, then one would expect total mRNA (containing both poly(A^+) mRNA and poly(A^-)mRNA sequences) to saturate twice the quantity of the nDNA probe as did poly(A^+)mRNA and poly(A^-)mRNA alone. Figure 8 shows that this did not occur. These results are in direct conflict with those obtained by Grady et al. (1978) for mouse liver. However, it is believed that Grady et al. (1978) failed to prepare a DNA probe which adequately represented each mRNA population. As a result, Grady obtained false saturation values for the hybridization of ^{3}H-DNA with poly(A^+)mRNA and poly(A^-)mRNA (for additional criticisms of Grady et al., 1978, see Chapter II).

The complexity obtained in this study for poly(A^+)mRNA is closer to the 1.0x10^8 nucleotide estimate obtained by Wilkes et al. (1979). When the data of Wilkes et al. (1979) is not corrected for the percent of DNA "unavailable" to the reaction (as was the case in this study) a complexity of approximately 7.5x10^7 nucleotides for poly(A^+)mRNA is obtained. Chikaraishi (1979) also determined a complexity estimate very close to those obtained in this study for mRNA using total

cytoplasmic RNA in rat liver (0.86×10^8 nucleotides).

The determination that poly(A^+)mRNA and poly(A^-)mRNA code for identical proteins in liver has also been reported for mouse kidney (Ouellette and Ordahl, 1979). On the other hand VanNess et al. (1979) using mouse brain and Chikaraishi (1979) using rat brain have clearly demonstrated that poly(A^+)mRNA and poly(A^-)mRNA are of different sequence composition in this organ and so give rise to different proteins.

The determination that poly(A^+)mRNA and poly(A^-)mRNA code for identical proteins in some organs such as kidney (Ouellette and Ordahl, 1979) and liver (as established in this study) suggests that poly(A) may serve to regulate the quantity of proteins synthesized in a cell. This supposition is supported by several observations reported in the literature. First, the ratio of poly(A^+)mRNA to poly(A^-)mRNA fluctuates during development (Nemer, 1974; Fromson and Duchastel, 1975; Chernovskya et al., 1976; Iatrou and Dixon, 1977) and in response to differing environmental states (Shaposhnikov and Ratovitski, 1978; Bantle et al., 1980b). Second, preliminary evidence exists which suggests tha poly(A^-)mRNA is derived from poly(A^-)hnRNA and poly(A^+) mRNA is derived from poly(A^+)hnRNA (Bantle, et al., 1980a; VanNess and Hahn, 1980a). Third, poly(A^+)mRNA and poly(A^-)mRNA enter the cytoplasm at similar rates (Nemer, 1975) or at rates which fluctuate depending upon the functional state of the cell (Chernovskaya et al., 1976). Fourth, poly(A^+)mRNA and poly(A^-)mRNA are translated with equal efficiency (Fromson and Duchastel, 1977) and although the poly(A) segment appears to be unnecessary for translation, it is

required to sustain prolonged translation (Huez et al., 1974). Fifth, the amount of poly(A) polymerase, the enzyme responsible for the addition of the poly(A) tract to the 3' end of mRNA molecules declines during fasting (Jacobs et al., 1976) with a concomitant decline in protein synthesis.

The suggestion that poly(A) functions in stability may help explain why poly(A^+)mRNA and poly(A^-)mRNA in brain are composed of different sequences while poly(A^+)mRNA and poly(A^-)mRNA in kidney and liver are composed of identical sequences. Recently, VanNess and Hahn (1980a) demonstrated that 60-70% by mass of polyadenylated mRNA in brain is homologous with kidney or liver polysomal RNA (poly(A^+)mRNA and poly(A^-)mRNA) while nonadenylated mRNA transcripts in brain appear to be, for the most part, absent in liver and kidney. One interpretation of this result is that the bulk of genes expressed in kidney and liver perform some type of basic metabolic function common to all cells while brain (which has been demonstrated to be the most complex of all tissues and organs studied thus far) possesses a unique set of mRNA sequences transcribed from a different region of the genome that those sequences expressed in all cells. The quantity of protein synthesized by those mRNA sequences shared in all cell types may be regulated by the number of polyadenylated transcripts since the increase in stability acquired with the addition of a poly(A) segment may permit a polyadenylated molecule to be translated more times before degrading. For those mRNA sequences of which a particular cell type need only translate a few times in order to synthesize enough protein required for highly specialized functions (perhaps poly(A^-)mRNAs in brain), the addition of a poly(A) tract may be unnecessary.

CHAPTER VI

SUMMARY AND CONCLUSIONS

The number average nucleotide size of liver nuclear RNA, poly(A^+) mRNA, poly(A^-)mRNA, and total mRNA was 1430, 1500, 1300, and 1460 nucleotides, respectively. Approximately 50% of the mass of polysomal mRNA was found to be nonadenylated. The complexity of nuclear RNA, poly(A^+)mRNA, poly(A^-)mRNA, and total mRNA was 4.4×10^8, 7.9×10^7, 7.2×10^7 and 7.1×10^7 nucleotides, respectively. This complexity is sufficient to encode 50,000-54,000 proteins in liver if each mRNA is 1500 nucleotides in length. The overlap in complexity between the complex, infrequently copied poly(A^+)mRNA and poly(A^-)mRNA was essentially 100%, thus indicating that these two mRNA populations code for the same proteins. One possible explanation offered for the finding that polysomal poly(A^+)mRNA and poly(A^-)mRNA code for identical proteins in liver was that poly(A) serves as a means of regulating the quantity of protein synthesized in a post-transcriptional level since the presence of a poly(A) tract may augment the stability of a mRNA molecule. This increase in stability may allow a mRNA molecule more time in the cytoplasm to be utilized for translational processes before being degraded.

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APPENDIX

GLOSSARY OF TERMS

Abundance class.....A group of mRNA sequences with approximately the same copy frequency.

cDNA.....Labeled DNA complementary to mRNA which has been produced by reverse transcription. The DNA sequences are present in the same copy frequency as the mRNA population.

Complexity.....The total number of nucleotides present in diverse sequences of a RNA population.

Complex class.....The class of mRNA molecules whose sequences are present in low-abundance in the cell but contain most of the complexity.

Cot.....The product of DNA concentration (moles nucleotide per liter) and time (seconds).

Diversity.....The number of different sequences in a RNA population. Typically diversity is calculated by dividing the value for complexity by the number average nucleotide length for the RNA species.

E. Cot.....Equivalent Cot. The same as Cot but a correction factor has been applied for the salt concentrations since it is other than 0.18 M.

E. Rot.....Equivalent Rot. The same as Rot but a correction factor has been applied for the salt concentration since it is other than 0.18 M. The corrected reaction rate will be comparable to the standard rate of reaction in 0.18 M NaCl.

EDTA.....Ethylene dinitrilo tetraacetic acid. A chelating agent for divalent cations.

Genome.....The total haploid DNA content of a cell consisting of both repeated and nonrepeated sequences.

Heterogeneous nuclear RNA (hnRNA).....RNA contained in the nucleus believed to transcribed directly from the unique portion of the genome. The sequences are heterogeneous in size.

Hybrid.....As used in this study, a DNA molecule bound to a RNA molecule by complementary base pairing.

Housekeeping gene.....A gene expressed as a mRNA molecule which is present in all cells of an organism.

Labeled DNA.....In this study, DNA which has incorporated ^{3}H-thymidine.

Messenger DNA (mDNA).....^{3}H-labeled DNA which has been enriched for sequences present in mRNA.

Messenger RNA (mRNA).....That RNA which is transcribed from the unique portion of the genome processed from hnRNA.

Nonrepeated DNA.....See unique sequence DNA.

Nuclear RNA (nRNA).....^{3}H-labeled DNA which has been enriched for sequences present in nRNA.

Polysomal RNA.....RNA extracted from polysomes containing transfer RNA, ribosomal RNA and messenger RNA.

Poly(A)mRNA or poly(A^{+})mRNA.mRNA which possesses a poly(A) tract on the 3' end.

Poly(A^{-})mRNA.....mRNA which lacks a poly(A) tract on the 3' end.

Poly(A)RNA.....RNA which possesses a poly(A) tract on the 3' end. Not exclusively mRNA.

Poly(A) tract or tail.....A homopolymer of adenine containing residues attached post-transcriptionally to the 3' end of a RNA molecule. Typically 30-300 nucleotides in length.

Poly(U).....Polyuridylic acid. A homopolymer of uracil containing nucleotides.

Prevalent class.....The class of mRNA molecules whose sequences are present in high abundance in the cell but contain only a small fraction of the complexity.

Probe.....In this study, the ^{3}H-labeled DNA always present in minimal quantities during hybridization.

Repetitive DNA.....Sequences of DNA present more than one copy per haploid genome. Typically repetitive sequences are present many times on the DNA. Both rRNA, tRNA and some histone mRNAs are transcribed from this region of the genome.

Rot.....The product of RNA concentration (moles nucleotide per liter) and time (seconds).

Saturation Hybridization....In this study, the hybridization of unlabeled RNA or DNA in vast excess to ^{3}H-labeled usDNA to the point where no additional reaction can be observed.

SDS.....Sodium dodecylsulphate. A detergent.

Sequence complexity.....See complexity.

Sequence diversity.....See diversity.

Shared sequences.....mRNA sequences expressed in more than one tissue or organ.

Single copy DNA.....See unique sequence DNA.

Specific sequences.....mRNA sequences unique to a particular tissue or organ.

Structural genes.....Genes which code for mRNA molecules that are translated into proteins.

T_o.....A zero time binding point of a hybridization reaction.

Tm.....In a thermal melt curve of DNA-RNA or DNA-DNA hybrids it is the temperature at which one half of the hybrids become single strands and elute from the hydroxylapatite column.

Total mRNA.....mRNA containing both polyadenylated and nonadenylated molecules. In this study total mRNA was polysome bound.

Total sheared DNA.....Unlabeled DNA containing both repetitive and unique sequences which has been sheared to produce fragments of smaller size.

Unique sequence DNA (usDNA).Sequences of DNA which on the average occur only once per haploid genome. All mRNAs except most histone mRNAs are transcribed from usDNA.

VITA 2

Cheryl L. Courchesne

Candidate for the Degree of

Master of Science

Thesis: COMPLEXITY AND COMPLEXITY OVERLAP IN MOUSE LIVER POLYADENYLATED AND NONADENYLATED mRNA FRACTIONS.

Major Field: Zoology

Biographical:

Personal Data: Born in Springfield, Massachusetts, November 6, 1956.

Education: Graduated from Trenton High School, Trenton, Michigan; in June, 1974; received Bachelor of Science degree from Northern Michigan University, Marquette, Michigan, in May, 1978; completed requirements for the Master of Science Degree at Oklahoma State University in December, 1980.

Professional Experience: Laboratory analyst for the Monsanto Corporation, May to August, 1978; Teaching Assistant for the School of Biological Sciences or Research Assistant for Dr. John A. Bantle at Oklahoma State University from August 1978 to present.