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Mouse Kidney Nonpolysomal Messenger Ribonucleic Acid: Metabolism, Coding Function, and Translational Activity†

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ABSTRACT: To elucidate the distribution and function of mRNA in mouse kidney cytoplasm, we compared mRNA isolated from polysomal (>80S) and native postpolysomal (20–80S) ribonucleoproteins with respect to synthesis and lifetime, sequence content, and translational activity. The 20–25% of cytoplasmic mRNA recovered from postpolysomal ribonucleoprotein is similar to polysomal mRNA in size (20–22 S), in apparent half-life (11–13 h), in major products of cell-free translation, and in nucleotide complexity ($\sim 4 \times 10^7$ nucleotides). The labeling kinetics of polysomal and postpolysomal mRNA suggest these mRNA populations are in equilibrium. [^{3}H]cDNAs transcribed from polysomal and from postpolysomal poly(A)-containing mRNAs react with template mRNA and with the heterologous mRNA at the same rate ($C_0t_{1/2}$ ~6.3 mol·s/L) and to the same extent (95%). Therefore, these mRNAs are equally diverse and homologous and occur at similar relative frequencies. Postpolysomal mRNA directs cell-free protein synthesis at only ~30% of the rate of polysomal mRNA and to only 30% of the extent of mRNA from polysomes. Postpolysomal mRNA is ~3-fold less sensitive than polysomal mRNA to inhibition of translation by m^{7}GMP, suggesting postpolysomal mRNA contains a greater fraction of molecules deficient in 5′-terminal caps. Postpolysomal mRNA may derive from renal mRNAs that initiate translation inefficiently and thus accumulate as postpolysomal ribonucleoproteins.

In mammalian cells, newly synthesized messenger RNA exists both in polysomes and in native 20–80S postpolysomal ribonucleoprotein (RNP)[1] particles (Henshaw et al., 1965; Henshaw, 1968; Perry & Kelley, 1968; Spirin, 1969; Spohr et al., 1970). For example, 20–60% of newly synthesized cytoplasmic mRNA in cultured Vero cells (Lee & Engelhardt, 1978), HeLa cells (Spohr et al., 1970), Taper hepatoma cells (McMullen et al., 1979; Kinneburgh et al., 1979), and mouse sarcoma 180 cells (Geoghegan et al., 1979) is in the postpolysome region. The distribution of mRNA between the polysomal and postpolysomal cytoplasmic fractions can be modulated by the growth state of cells (Lee & Engelhardt, 1978), by starvation (Sonenshein & Brawerman, 1977), and

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[1] Abbreviations: poly(A+) mRNA, mRNA that contains poly(adenylate); RNP, ribonucleoprotein; mRNP, messenger ribonucleoprotein; C_0t, in hybridization studies initial concentration of RNA in moles of nucleotide per liter × time of reaction in seconds; EDTA, ethylenediaminetetraacetic acid; Tris, tris(hydroxymethyl)aminomethane.

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by heat shock (Schochetman & Perry, 1972).

The role and the origins of mRNA in postpolysomal mRNPs are unclear, perhaps in part because postpolysomal mRNA has not been characterized in a sufficient number of systems. Nevertheless, comparisons of cell-free translation products of polysomal and postpolysomal mRNAs from neuroblastoma cells (Croall & Morrison, 1980), Taper hepatoma cells (McMullen et al., 1979), Vero cells (Lee & Engelhardt, 1979), and developing chick muscle (Bag & Sarkar, 1976) suggest that some postpolysomal mRNAs are unique to that fraction. Furthermore, postpolysomal mRNA in Taper hepatoma cells has only 2–5% of the sequence content of polysomal mRNA (400–500 different mRNAs), providing additional evidence for unique properties in postpolysomal mRNA (Kinneburgh et al., 1979). In the sea urchin, however, polysomal and postpolysomal mRNAs direct the same translation products, but the translational activity of postpolysomal mRNA is inferior to that of polysomal mRNA (Rudensey & Infante, 1979).

To elucidate the common and unique properties of polysomal and postpolysomal mRNAs in a mammalian organ, we compared polysomal and postpolysomal poly(A+) mRNA from mouse kidney. In mouse kidney, 20–25% of cytoplasmic poly(A)-containing mRNA and poly(A)-deficient mRNA is postpolysomal, values that remain unchanged during compensatory renal hypertrophy (Ouellette & Malt, 1979; Ouellette et al., 1981). Although some authors consider mRNA in nonpolysomal RNP as mRNA sequestered from translation by the action of regulatory proteins (MacLeod, 1975; Rudland et al., 1975), the function of postpolysomal mRNA and its relation to mRNA being translated actually are not well established. Thus, we investigated renal postpolysomal mRNA by comparing the metabolism, template activity, and coding function of mRNA from polysomal and postpolysomal RNP. These populations of mRNA are similar in most respects, but postpolysomal mRNA has relatively low translational activity.

Materials and Methods

Animals. Young adult male mice (40–50 days, 30–35 g, Charles River Laboratories, North Wilmington, MA) were fed freely and were kept in alternating 12-h cycles of light. Radiochemicals were administered by dorsal subcutaneous injection.

Preparation of Polysomes and Postpolysomal Ribonucleoproteins by Sucrose Density Gradient Centrifugation. Decapsulated kidneys homogenized in ice-cold buffer consisting of 10 mM Tris-HCl (pH 7.4), 0.15 M NaCl, 3 mM $MgCl_2$, and 0.25 M sucrose were centrifuged 10 min at 10000 rpm in the Sorvall SS-34 rotor to prepare postmitochondrial supernatants. Polysomes (>80 S) were separated from postpolysomal (20–28S) ribonucleoproteins by centrifugation in 36 mL of linear 7–47% (w/w) sucrose density gradients in the Beckman SW27 rotor for 3 h at 26500 rpm at 4 °C. Alternatively, optimal resolution of postpolysomal RNPs was obtained by sedimentation of postmitochondrial supernatants in 32 mL of 15–30% (w/w) sucrose density gradients formed on 4 mL of 2 M sucrose (Lee & Brawerman, 1971; Ouellette et al., 1981) in the SW27 rotor for 17 h at 22000 rpm at 4 °C. Sucrose solutions contained 10 mM Tris-HCl (pH 7.4), 0.5 M NaCl, and 50 mM $MgCl_2$.

Total cytoplasmic messenger ribonucleoproteins (mRNPs) were prepared from dissociated polysomes by sedimentation of postmitochondrial supernatants in gradients containing 10 mM EDTA in place of $MgCl_2$. Polysomes and postpolysomal RNPs identified by continuous monitoring of A_{260nm} were pooled and were stored at −20 °C as precipitates in 2 volumes of 95% ethanol.

Preparation of Poly(A+) mRNA. Precipitated RNP dissolved in buffer containing 10 mM Tris-HCl (pH 9.0), 0.1 M NaCl, 10 mM EDTA, and 0.2% sodium dodecyl sulfate was deproteinized by several extractions with phenol/chloroform/isoamyl alcohol (Perry et al., 1972). Polysomal and postpolysomal poly(A+) RNA was purified by two cycles of affinity chromatography on oligo(dT)–cellulose (type T-2, Collaborative Research, Inc., Waltham, MA) (Aviv & Leder, 1972). RNA purified for translation was extracted with guanidine hydrochloride 3 times before deproteinization with phenol/chloroform/isoamyl alcohol (Cox, 1968; Strohman et al., 1977). RNA was stored in 2 volumes of 95% ethanol at −20 °C.

Buoyant Density of Polysomal and Postpolysomal Ribonucleoproteins. Buoyant densities of polysomal and postpolysomal RNPs were compared by isopycnic centrifugation of glutaraldehyde-fixed RNP in CsCl gradients (Spirin et al., 1965; Irwin et al., 1975). Equal volumes of polysomal RNP >80 S prepared from sucrose density gradients were mixed with 1 mL of 24% (v/v) glutaraldehyde (pH 8.0), incubated at 4 °C for 24 h, and stored at −20 °C. Aliquots (1 mL) of these fixed preparations were layered on 4.5 mL of CsCl solution (ρ = 1.445 g/mL) in 10 mM Tris-HCl (pH 7.4), 10 mM NaCl, 3 mM $MgCl_2$, and 0.8% Brij 35 and overlain with light mineral oil. Gradients centrifuged at 35000 rpm in the Beckman SW41 rotor at 4 °C for 20 h were fractionated, and the radioactivity in acid precipitates of each gradient fraction collected on glass fiber filters was counted in 5 mL of scintillation fluid containing 4 g of Omnifluor per L of toluene. Densities of selected gradient fractions were measured by weighing 10 μL of each fraction in tared micropipets.

mRNA-Directed Cell-Free Translation. Rabbit reticulocyte lysate was obtained from New England Nuclear Corp. as the [^{35}S]methionine and [^{3}H]leucine translation kits. Reaction mixtures (12.5 μL) containing 5 μL of micrococcal nuclease digested lysate (Pelham & Jackson, 1976), 0.5 μg of poly(A+) mRNA, and 1 μL of cocktail containing ATP, GTP, polyamines, creatine phosphate, and creatine phosphokinase as supplied with the kit, plus 22 μCi of L-[^{35}S]methionine (1118 Ci/mmol) or 17 μCi of L-[^{3}H]leucine (145 Ci/mmol), were incubated 45 min at 37 °C. Amino acid incorporation was assayed as alkali-stable, acid-insoluble radioactivity in 1-μL samples.

Translation products were analyzed in linear 6–15% gradient polyacrylamide slab gels (1.5 mm) (Laemmli, 1970). Samples containing approximately 500000 cpm of ^{35}S-labeled proteins in 62.5 mM Tris-HCl (pH 6.8), 2% sodium dodecyl sulfate, 10% (v/v) glycerol, 5% (v/v) β-mercaptoethanol, and 0.001% (w/v) bromphenol blue were boiled 3 min and applied to the gel in 15–20 μL. Gels run 2 h at 120 V were stained with 0.2% Coomassie blue to locate the molecular weight markers and were prepared for fluorography (Laskey & Mills, 1975).

Hybridization with [^{3}H]Poly(uridylic acid). RNA samples (1–100 ng) were incubated with excess [^{3}H]poly(U) in 200-μL reactions containing 100 nCi of [^{3}H]poly(U) (New England Nuclear Corp., 5.8 Ci/mmol UMP), 0.3 M NaCl, and 30 mM sodium citrate (2 × SSC) at 37 °C for 15 min (Wilt, 1973). Samples diluted 10-fold with 2 × SSC were incubated an additional 15 min at 37 °C with 10 μg of pancreatic RNase A. RNase-resistant polynucleotides in hybrid form were precipitated with 1 volume of ice-cold 20% (w/v) trichloroacetic acid immediately after addition of 50 μg of yeast tRNA. Precipitates collected on nitrocellulose filters were counted in

5 mL of toluene-based scintillation fluid.

Preparation and Analysis of cDNAs. cDNAs to poly(A+) mRNA isolated from polysomal and from postpolysomal RNP were synthesized in reaction mixtures containing 50 mM Tris-HCl (pH 8.3), 8 mM $MgCl_2$, 0.2 mM dithiothreitol, 50 mM KCl, 4 mM sodium pyrophosphate, 200 μM each of dTTP, dGTP, and dATP, 1 mCi of dCTP (5 Ci/mmol), 10 μg/mL oligo(dT), 5 μg of template RNA, and 45–60 units of reverse transcriptase from avian myeloblastosis virus (obtained from Dr. Joseph Beard, Life Sciences, Inc., St. Petersburg, FL) (Kacian & Myers, 1976; Ordahl & Caplan, 1978). After 1 h at 41 °C, reactions were adjusted to 0.3 M NaOH, heated at 65 °C for 5 min, neutralized, extracted with chloroform, and chromatographed on 10-mL columns (packed volume) of Sephadex G-100 (Pharmacia Fine Chemicals, Piscataway, NJ) in H_2O. cDNA was precipitated from the column void volume with 2 volumes of ethanol or was dried in vacuo. The average cDNA length was 800–1000 nucleotides determined by agarose gel electrophoresis.

Hybridizations were conducted either in 0.4 M sodium phosphate buffer (pH 6.8) or in 0.12 M sodium phosphate buffer at 65 °C (Ordahl & Caplan, 1978). The equivalence of C_0t values was computed as described (Britten et al., 1974). The fraction of [^{3}H]cDNA hybridized was quantitated by acid precipitation of cDNA/mRNA duplexes resistant to digestion by S1 nuclease. Less than 5% of labeled polysomal cDNA was resistant to S1 digestion after reaction with *Escherichia coli* RNA to a C_0t of 10^3.

Saturation Hybridization Analysis. Hybridization reactions contained 0.12 or 0.4 M sodium phosphate (pH 6.8), 0.2% sodium dodecyl sulfate, 5 mM EDTA, (1–5) × 10^3 cpm of gap-translated, single-copy DNA (Van Ness et al., 1979), and 0.05–9 μg of RNA depending on the C_0t value desired. Reaction mixtures sealed in glass capillary tubes were incubated at 64 °C for intervals up to 150 h, frozen rapidly, and stored at −20 °C. ^{3}H-Labeled single-copy DNA in duplex form was quantitated by the S1 nuclease–DEAE-cellulose filter procedure (Maxwell et al., 1978; Van Ness et al., 1979).

Radiochemicals. [^{35}S]Methionine (1118.3 Ci/mmol), L-[3,4,5-^{3}H(N)$_3$]leucine (135 Ci/mmol), [5-^{3}H]orotic acid (20 Ci/mmol), and [*uridylate*-5,6-$^3H_2$]poly(uridylic acid), sodium salt, were purchased from New England Nuclear Corp. [5,5′-$^3H_2$]dCTP was purchased from New England Nuclear Corp. (24.9 Ci/mmol) or from ICN Pharmaceuticals, Inc., Irvine, CA (16 Ci/mmol).

Results

Cytoplasmic Distribution of Renal mRNA. Approximately 25% of mouse kidney mRNA is in native 20–80S mRNP particles separate from polysomes (Table I, Ouellette et al., 1976). This 25% value holds for newly synthesized poly(A+) mRNA and for poly(A+) mRNA in the steady state measured by [^{3}H]poly(U) hybridization (Table I). Newly synthesized poly(A+) mRNA in RNP separated in sucrose density gradients (Figure 1A,B) was quantitated as the radioactivity in poly(A+) RNA after oligo(dT)–cellulose chromatography (Table I). Regardless of the conditions of sedimentation, 20% of renal poly(A+) mRNA was found in the postpolysomal region. For example, 20–80S RNP from the gradient centrifuged 3 h contained 19.3% of labeled poly(A+) mRNA (fraction 1, experiment C). Similarly, 20.7% of newly synthesized poly(A+) mRNA (fractions 1–5, experiment A) was recovered from 20–80S RNP prepared by overnight centrifugation. In six different experiments, these values ranged only from 18% to 22%, suggesting that variable recovery of RNA from individual gradient regions probably was not an important source of error. In the same six experiments, the fraction of rRNA in native 20–80S RNP ranged from 20% to 22.2% (data not shown), a range agreeing closely with the distribution of poly(A+) mRNA. Since unfractionated polysomal and postpolysomal RNAs contain 8.6 and 7.9 pg of poly(A) per μg of RNA, respectively (Table I), we infer the mRNA:ribosome ratio is the same for polysomal and for postpolysomal RNP. No poly(A) or poly(A+) mRNA was observed in RNP sedimenting <20 S.

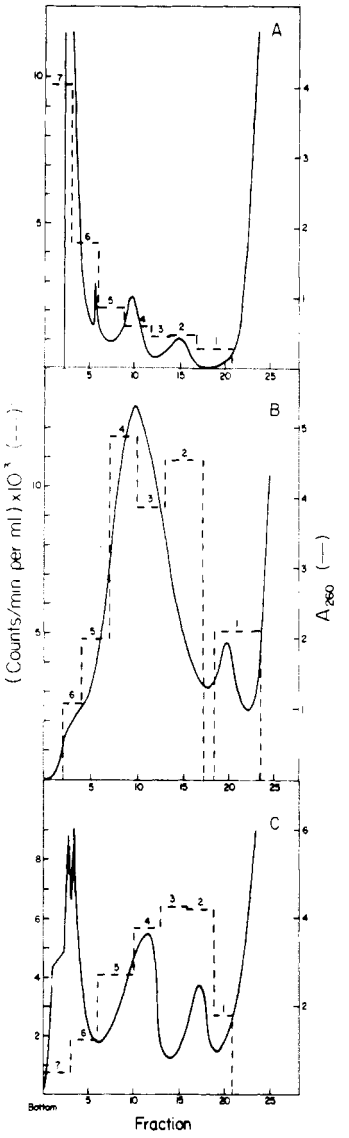

FIGURE 1: Cytoplasmic distribution of renal poly(A+) mRNA. Four mice were labeled 4 h with 250 μCi each of [^{3}H]orotic acid. Equal volumes of postmitochondrial supernatant were sedimented as follows: (A) 22 000 rpm at 4 °C for 15.5 h in the SW27 rotor on a 32-mL linear 15–30% sucrose density gradient in 10 mM Tris-HCl (pH 7.4), 0.50 M NaCl, and 50 mM $MgCl_2$ on a 4-mL cushion of 2.0 M sucrose in the same buffer; (B) 26 500 rpm at 4 °C for 3 h in the SW27 rotor on a 36-mL linear 7–47% sucrose gradient in the same buffer; (C) as described in panel A, except sucrose solutions contained 10 mM Tris-HCl (pH 7.4), 0.25 M NaCl, and 10 mM EDTA to dissociate polysomes. Gradient fractions were pooled as indicated by the broken lines, and poly(A+) mRNA in each gradient region was quantitated by chromatography on oligo(dT)–cellulose (see Materials and Methods). The solid line denotes A_{260nm} per milliliter.

Size of Polysomal and Native Postpolysomal Poly(A+) Messenger Ribonucleoproteins. Sedimentation of renal postmitochondrial supernatants in sucrose density gradients containing EDTA (Figure 1C) provided estimates of poly(A+) mRNP size. In 10 mM EDTA, approximately 95% of poly(A+) mRNA originally in polysomal RNP ≥80 S dissociated

Table I: Distribution of Poly(A+) mRNA in Renal Cytoplasm[a]

expt	fraction	radioactivity cpm	radioactivity % of total	pg of poly(A)/ng of RNA	total RNA µg	total RNA % total	poly(A) ng	poly(A) % total
A	2–6			8.57 ± 0.23	222	75	1909	76
	1			7.90 ± 0.20	74.4	25	588	24
B	1	4047	2.9					
	2	5166	3.7					
	3	3301	2.4					
	4	6588	4.8					
	5	9498	6.9					
	6	19718	14.3					
	7	44460	32.2					
	pellet	43354	32.8					
C	1	43858	19.3					
	2	66330	27.8					
	3	42561	17.9					
	4	53487	22.5					
	5	21991	9.3					
	6	7974	3.3					

[a] Postmitochondrial supernatant from four mice labeled 3 h with 100 µCi each of [^{3}H]orotate was sedimented in $MgCl_2$-containing sucrose density gradients (Figure 1). Ribonucleoproteins in the gradient region designated in Figure 1A,B were deproteinized, and RNA recovered from each region was chromatographed on oligo(dT)-cellulose (see Materials and Methods). Poly(A) was quantitated by hybridization of unfractionated RNA prepared from polysomal and postpolysomal RNP with excess [^{3}H]poly(U). Concentrations of unfractionated RNA were measured by the absorbance at 260 nm. Radioactivity in poly(A+) mRNA was measured by counting aliquot samples in a gel of sample, H_2O, and 10 mL of xylene-based scintillation fluid. Values shown represent total poly(A+) mRNA radioactivity recovered from each gradient region. Experiments A and B refer to gradient regions from Figure 1A; experiment C refers to gradient regions from Figure 1B.

Table II: Sedimentation Distribution of Renal Poly(A+) Messenger Ribonucleoproteins[a]

expt	fraction	radioactivity cpm	radioactivity % of total
A	>80 S	2003	6.4
	20–80 S	29117	93.6
B	1	8176	6.3
	2	28802	22.3
	3	29237	22.6
	4	25979	20.1
	5	24749	19.1
	6	8462	6.5
	7	3407	2.6
	pellet	475	0.4

[a] Postmitochondrial supernatants from four mice labeled 3 h with 100 µCi each of [^{3}H]orotate were sedimented in EDTA-containing sucrose density gradients (see Materials and Methods). Ribonucleoproteins from gradient regions were analyzed for [^{3}H]poly(A+) mRNA as in Table I. Experiment A: cytoplasm was sedimented 3 h in 7–47% (w/w) sucrose density gradients (14, not shown), and the indicated fractions were pooled and analyzed. Experiment B: gradient fractions are those designated in Figure 1C.

and sedimented as derived 20–80S mRNP (Table II). The procedure provided separation of renal mRNPs by size (Figure 1C), in addition to demonstrating that the poly(A+) RNA in RNP >80 S was polysomal. Comparison of labeled poly(A+) mRNA recovery from RNP ≥80 S from gradients containing $MgCl_2$ (Figure 1A, pellet plus fractions 6 and 7) (Table II) confirmed the effectiveness of polysome dissociation by EDTA. In the presence of $MgCl_2$, 79.3% of labeled poly(A+) mRNA was found in RNP ≥80 S; however, in 10 mM EDTA, only 9.5% of poly(A+) mRNP sedimented ≥80 S [Table II and Ouellette et al. (1981)].

Mouse kidney poly(A+) mRNP derived from polysomes by EDTA sedimented 20–120 S with a modal sedimentation coefficient of 55 S, as determined by the recovery of labeled poly(A+) mRNA from mRNP fractionated by overnight centrifugation (Figure 1C and Table II). Approximately 84% of poly(A+) mRNA was recovered from mRNPs 30–90 S; 30–40S, 40–55S, 55–65S, and 65–90S RNP contained equivalent amounts of labeled poly(A+) mRNA. Poly(A+) mRNA from rapidly sedimenting RNP was more heterogeneous and of greater molecular weight than mRNA from more slowly sedimenting RNP (data not shown). For example, poly(A+) mRNA from 70–80S mRNP (Figure 1C, region 5) sedimented with a broad peak with a modal value of 23–26 S, but the modal value of poly(A+) mRNA from 10–30S mRNP (Figure 1C, region 1) was 16 S. In addition, only 19% of poly(A+) mRNA from 75–80S mRNP was <18 S, but 60% of poly(A+) mRNA from 10–30S mRNP sedimented <18 S.

Native postpolysomal poly(A+) mRNP was similar in size to EDTA-derived poly(A+) mRNP (Figure 1A and Table I). Native postpolysomal mRNP sedimented 20–80 S and was not clustered in the 40S region, suggesting that postpolysomal mRNA is not restricted to 40S translation initiation complexes but exists free of ribosome associations. Poly(A+) mRNAs isolated from polysomal and postpolysomal RNP were indistinguishable in size as judged by sedimentation velocity analysis (not shown).

Buoyant Density of Polysomal and Postpolysomal Ribonucleoproteins. The buoyant densities of fixed native postpolysomal RNP confirmed that most native postpolysomal mRNP is free of association with ribosomes. Renal mRNP released from polysomes by EDTA has a density of 1.40–1.44 g/mL by equilibrium centrifugation in CsCl (Irwin et al., 1975), readily distinguishable from that associated with ribosomes and polysomes. Polysomes showed a single peak with a density of 1.54 g/mL (Figure 2A), but postpolysomal RNP contained a second labeled peak of 1.41–1.46 g/mL in addition to the 1.55 g/mL fraction, indicative of free mRNP (Figure 2B). Consistent with the fraction of labeled postpolysomal RNA retained on oligo(dT)–cellulose, the low-density peak represented ~30% of the radioactivity in postpolysomal mRNA.

Kinetics of Accumulation of Polysomal and Postpolysomal Poly(A+) mRNA. Newly synthesized poly(A+) mRNA accumulated in native 20–80S postpolysomal RNP faster than in polysomes (Figure 3A). Newly synthesized poly(A+) mRNA accumulated linearly for only 45 min in postpolysomal RNP (Figure 3A) but continued to accumulate for over 3 h in polysomes (Figure 3B). The relative amount of labeled mRNA in each fraction became consistent after the first hour of labeling (Figure 3A). Polysomal mRNA contained 4 times more *total* radioactivity than postpolysomal mRNP. However, because the data in Figure 3 are expressed as counts per minute per A_{260} unit of poly(A−) RNA and the mass ratios of polysomal and postpolysomal mRNA:rRNA are similar, the difference in total radioactivity is not apparent.

Kinetics of Polysomal and Postpolysomal Poly(A+) mRNA Decay. Polysomal and postpolysomal poly(A+) mRNA exhibited similar apparent half-lives of 11–13 h (Figure 4). If postpolysomal poly(A+) mRNAs include stable sequences segregated from translation, postpolysomal poly(A+) mRNA should be more long-lived than polysomal mRNA. Pulse–chase measurements of [^{3}H]orotate label re-

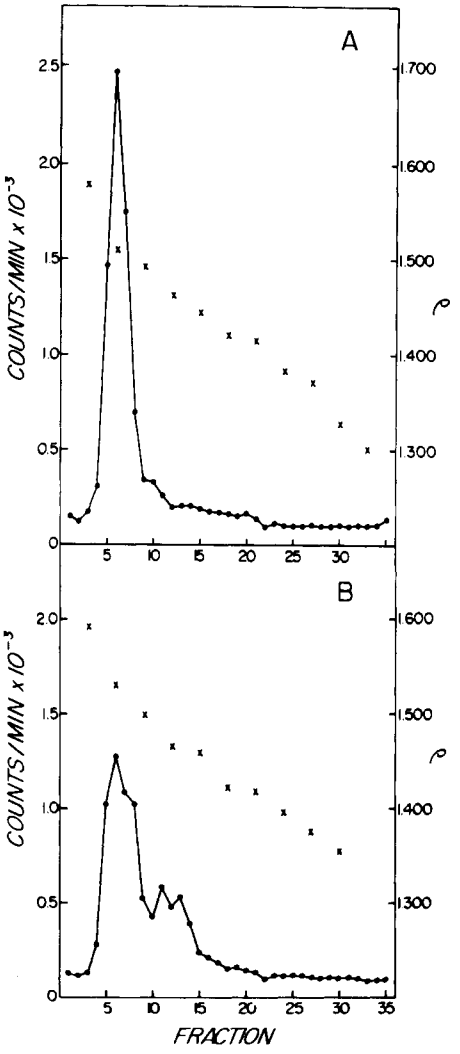

FIGURE 2: Buoyant density of polysomal and native postpolysomal ribonucleoproteins. Postmitochondrial supernatants from four mice each labeled with 250 μCi of [^{3}H]orotic acid were sedimented in $MgCl_2$-containing 7–47% (w/w) sucrose density gradients (see Figure 1B). RNPs from the 80S polysomal and the 20–80S postpolysomal regions were fixed in glutaraldehyde and analyzed in CsCl gradients (see Materials and Methods). (●) Acid-insoluble radioactivity in each gradient fraction; (×) density of selected fractions.

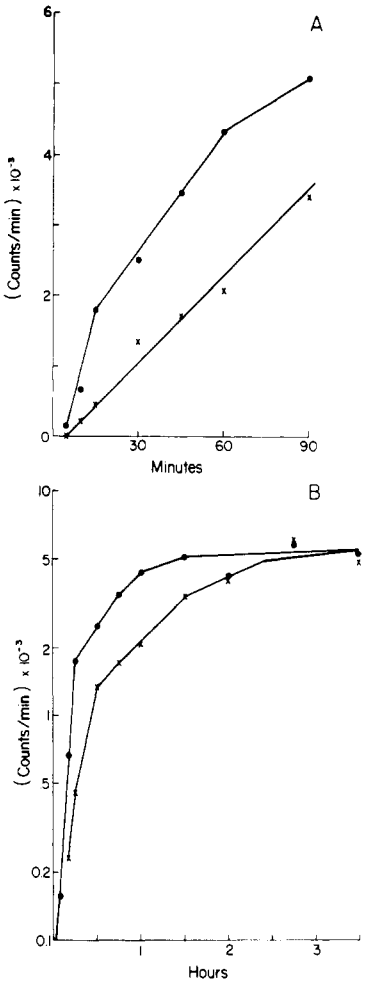

FIGURE 3: Accumulation of newly synthesized poly(A+) mRNA into polysomal and native postpolysomal regions. Mice (two per time point) were labeled with 100 μCi each of [^{3}H]orotate for the intervals shown. Radioactivity in poly(A+) mRNA from >80S polysomal and native 20–80S postpolysomal RNPs (Figure 1B) was quantitated by oligo(dT)–cellulose chromatography (see Materials and Methods). (●) Label in postpolysomal mRNA; (×) label in polysomal mRNA.

maining in polysomal and postpolysomal poly(A+) mRNA showed that the half-lives of these mRNAs were actually similar (Figure 4). Because these values represent averages of highly diverse populations, differences in the lifetimes of *individual* polysomal and postpolysomal mRNAs are possible, and thus minor differences in the mRNA lifetime cannot be excluded. Furthermore, these comparisons of mRNA half-life predominantly measure mRNA in tubular cells of the kidney, since orotic acid labels these cells selectively (Ross et al., 1975).

Coding Function of Postpolysomal mRNA. Except for a polypeptide of molecular weight ~15 000 abundantly coded only by polysomal mRNA, polysomal and postpolysomal mRNAs directed the same translation products when analyzed in one-dimensional polyacrylamide gels (Figure 5). In each case, [^{35}S]methionine-labeled cell-free translation products with molecular weights ranging from 8000 to 90 000 were present at the same apparent relative abundance when evaluated densitometrically (data not shown). These results suggest that the *abundant*, biologically active mRNAs in these cytoplasmic subfractions potentially encode the same proteins, but the possibility of different polypeptides migrating in the gels with the same apparent molecular weight cannot be discounted, since differences in translation products of polysomal and postpolysomal mRNA may only be apparent after analysis in two-dimensional gels (Lee & Engelhardt, 1979; Croall & Morrison, 1980; McMullen et al., 1979).

Nucleotide Complexity of Postpolysomal mRNA. Because the 30–40 mRNA-directed polypeptides in Figure 5 represent only a small fraction of renal mRNAs, we examined polysomal and postpolysomal mRNA further by nucleic acid hybridization to compare their total nucleotide complexity and relative sequence homology. The nucleotide complexity of polysomal and postpolysomal poly(A+) mRNAs was equivalent (Table III). As measured by saturation hybridization, both mRNA populations derive from 2% of single-copy DNA (scDNA) or ~4% of the genome, assuming asymmetric transcription. Thus, in mouse kidney, postpolysomal mRNA has the same diversity of coding potential as mRNA recovered from the translation apparatus. Because complexity estimates by saturation hybridization include low-abundance mRNAs, these findings complement those of cell-free translation (Figure 5) which provided comparisons of abundant, biologically active mRNAs. Furthermore, the complexities of poly(A+) RNA and unfractionated RNA from polysomes and postpolysomal RNPs are equivalent (not shown), suggesting that neither RNA population contains a class of diverse mRNAs that exclusively lacks or is deficient in poly(A) (Ouellette & Ordahl, 1981).

The number of different mRNAs contained in polysomal

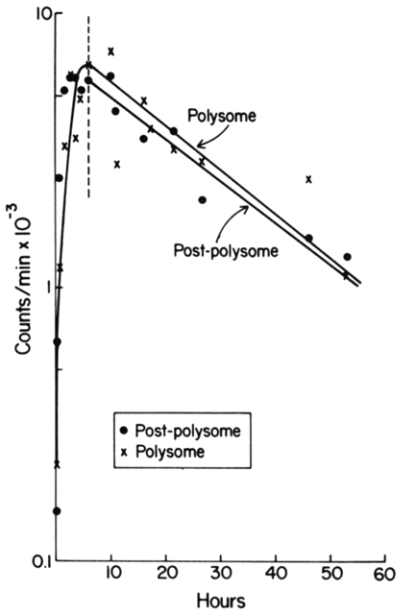

FIGURE 4: Decay of radioactivity from polysomal and native postpolysomal poly(A+) mRNA. Radioactivity in polysomal and native postpolysomal poly(A+) mRNA and poly(A−) RNA was measured as described in Figure 3.

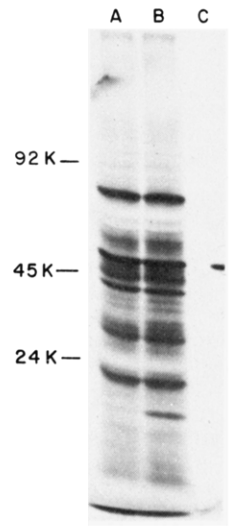

FIGURE 5: Electrophoretic analysis of translation products directed by polysomal and postpolysomal renal mRNA. [^{35}S]Methionine-labeled translation products of polysomal and postpolysomal mRNA were electrophoresed in linear 6–15% polyacrylamide gradient gels, and cell-free synthesized polypeptides were detected radiographically (see Materials and Methods). Approximately 500 000 cpm was applied to lanes A and B; the gel was exposed at −85 °C for 18 h. Products directed by postpolysomal mRNA (A) and polysomal mRNA (B); lane C shows only the left half of a reaction to which no mRNA was added.

mRNA, postpolysomal mRNA, unfractionated cytoplasmic RNA, and poly(A+) mRNA isolated from EDTA-derived 20–80S RNP (see Figure 1C) is equivalent, as the complexity of each preparation corresponded to ~2% scDNA (Table III). mRNAs in unfractionated renal cytoplasm and from EDTA-derived mRNP are represented in both polysomal and postpolysomal mRNA; thus, the combined nucleotide complexities of polysomal and postpolysomal mRNA are not additive but overlap in sequence content. Within the accuracy of the hybridization analysis, all renal mRNAs are, therefore, represented in the postpolysomal fraction.

Polysomal and postpolysomal renal poly(A+) mRNAs occur at similar relative frequencies as judged by cDNA hybridization. The relative abundance of mRNAs in these populations was evaluated by reacting [^{3}H]cDNAs transcribed both from polysomal and from postpolysomal poly(A+) mRNAs with template RNA and heterologous RNA (Figure 6). The results confirmed nucleotide complexity measurements (Table III), because each cDNA hybridized to 90% completion regardless of the reactant mRNA (Figure 6). The kinetics of postpolysomal cDNA hybridization with its template mRNA and with polysomal mRNA also were similar, demonstrating the relative abundance of mRNAs was equivalent in each population. These hybridization experiments provided additional and more detailed information relevant to abundant mRNAs, because cDNAs transcribed from complex mixtures of mRNA reflect the relative abundance of mRNA templates.

Table III: Total Nucleotide Complexity of Renal Postpolysomal Poly(A+) mRNA[a]

polysomal poly(A+) mRNA		postpolysomal poly(A+) mRNA		total cytoplasmic poly(A+) mRNA	
C_0t	scDNA in duplex (%)[b]	C_0t	scDNA in duplex (%)	C_0t	scDNA in duplex (%)
22 000	1.93 (1.96)	24 000	2.10 (1.98)	23 000	2.16 (2.01)

[a] Poly(A+) mRNA purified from >80S polysomes and from 20–80S postpolysomal RNP was hybridized to gap-translated single-copy mouse DNA (scDNA) (see Materials and Methods). [^{3}H]scDNA in the duplex was determined as the fraction of input scDNA radioactivity [(1–5) × 10^3 cpm] resistant to digestion with S1 nuclease. Total cytoplasmic poly(A+) mRNA was isolated from mRNP <100 S prepared in EDTA-containing sucrose density gradients (Figure 1C). [b] Values in parentheses represent the means of three determinations made after hybridization saturation (C_0t > 12 000).

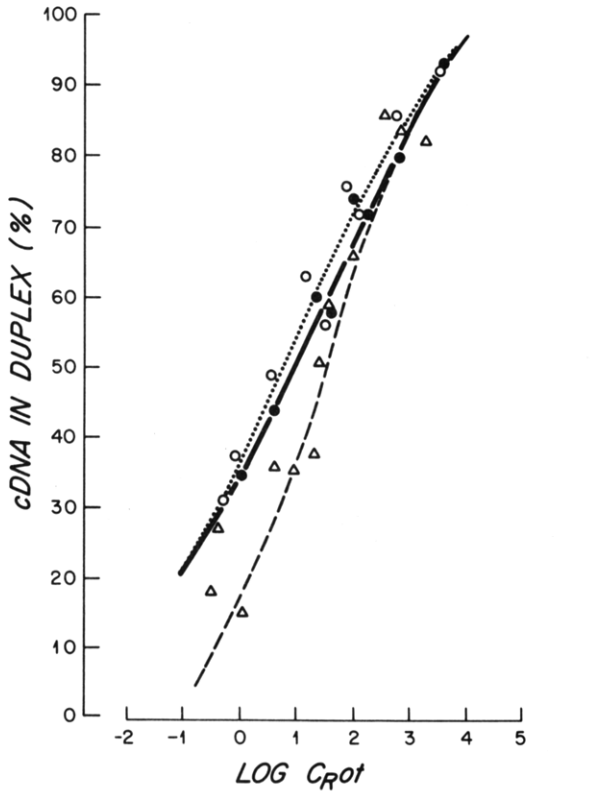

FIGURE 6: Hybridization of polysomal and postpolysomal mRNA to homologous and heterologous cDNA. Hybridizations were performed in 0.12 or 0.40 M phosphate buffer as described under Materials and Methods. Open circles, postpolysomal mRNA vs. postpolysomal cDNA; closed circles, polysomal mRNA vs. postpolysomal cDNA; triangles, postpolysomal mRNA vs. polysomal cDNA.

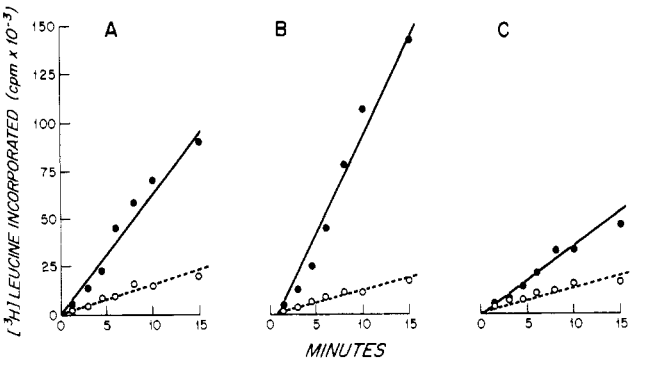

FIGURE 7: Cell-free protein synthesis directed by polysomal and postpolysomal poly(A+) mRNA. Reaction mixtures (50 μL final volume) prepared as described under Materials and Methods and containing 20 (A), 40 (B), and 160 ng/μL (C) of the respective poly(A+) mRNA were incubated in stoppered tubes at 37 °C. Incorporation of [^{3}H]leucine into mRNA-directed polypeptides was measured as alkali-stable, acid-insoluble radioactivity in 5-μL samples removed at the times shown. Closed circles denote incorporation directed by polysomal mRNA; open circles denote postpolysomal mRNA-directed synthesis.

These findings are consistent with results of cell-free translation, complement the saturation hybridization experiments, and show that the potential coding functions of polysomal and postpolysomal mRNAs are nearly indistinguishable.

Translational Efficiency of Postpolysomal Poly(A+) mRNA. Postpolysomal mRNA has deficient template activity in the reticulocyte lysate translation system. Preliminary experiments had shown that the extent of postpolysomal mRNA-directed protein synthesis was only 25% of that of polysomal mRNA (Ouellette et al., 1980). Therefore, we compared rates of cell-free protein synthesis directed by these two mRNA populations. As shown in Figure 7, the rate of [^{3}H]leucine incorporation directed by postpolysomal poly(A+) mRNA [0.46 pmol of leucine min^{-1} (μg of RNA)$^{-1}$] was only 30% of that directed by polysomal mRNA [1.57 pmol of leucine min^{-1} (μg of RNA)$^{-1}$]. The presence of inhibitory substances in postpolysomal RNA is unlikely, because translation of polysomal mRNA was not inhibited by the addition of postpolysomal mRNA to reaction mixtures. Discrepancies in the amount of mRNA added to reaction mixtures also were unlikely, because mRNA concentrations were verified both by the absorbance at 260 nm and by [^{3}H]poly(U) hybridization. The size of polysomal and postpolysomal mRNA was indistinguishable in sucrose density gradients (not shown).

Selective Inhibition of Postpolysomal mRNA by m^{7}GMP. The inhibitory effects of the cap analogue m^{7}GMP on the translation of polysomal and postpolysomal mRNA were compared to investigate the possible relation between translational deficiency and 5′-terminal caps in postpolysomal mRNA. If more postpolysomal poly(A+) mRNA molecules were cap deficient, m^{7}GMP should inhibit translation of postpolysomal mRNA less than polysomal mRNA (Both et al., 1975; Hickey et al., 1976; Muthukrishnan et al., 1976). In the presence of 500 μM m^{7}GMP, translation of postpolysomal mRNA continued at 40% of the control rate (Figure 8B), but translation of polysomal mRNA was inhibited to less than 10% of the control rate under the same conditions (Figure 8A). We noted that polysomal and postpolysomal mRNAs exhibit the same translational activity in 500 μM m^{7}GMP, but whether this results from common cap structures of the active mRNAs or the inability of 500 μM m^{7}GMP to inhibit translation completely is uncertain. Basal activity of the translation system, i.e., without added mRNA, was unaffected

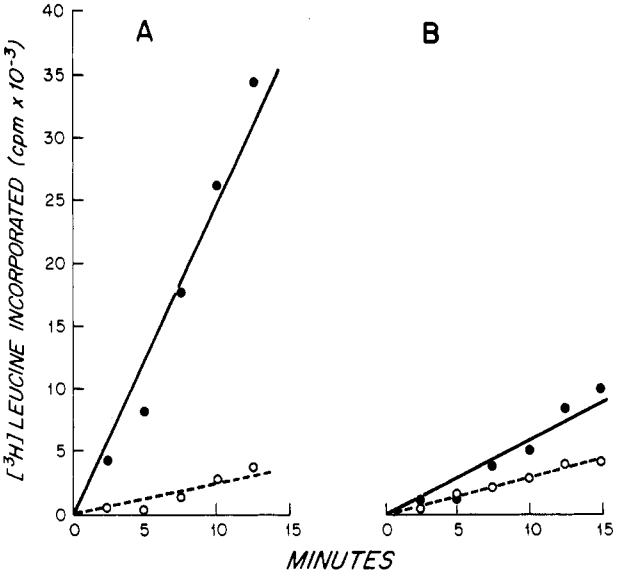

FIGURE 8: Selective inhibition of mRNA-directed translation by m^{7}GMP. Reaction mixtures (50 μL) containing 20 ng of poly(A+) mRNA/μL were prepared and assayed as described in Figure 7. (A) [^{3}H]Leucine incorporation directed by polysomal poly(A+) mRNA in control reactions (closed circles) and in reactions containing 500 μM m^{7}GMP (open circles); (B) as in panel A, but containing postpolysomal mRNA. In reactions lacking mRNA, background [^{3}H]leucine incorporation increased from 925 cpm at 0 min to 1280 cpm at 15 min; values shown denote net radioactivity with this basal incorporation subtracted.

by 500 μM m^{7}GMP; values shown in Figures 7 and 8 represent incorporation of [^{3}H]leucine with the basal activity of the translation system subtracted (see legends). The relative insensitivity of postpolysomal mRNA to m^{7}GMP inhibition suggests a greater content of cap-defective molecules contributes to its low translational activity.

Discussion

We summarize the understanding of nonpolysomal mRNA in mouse kidney as follows. Newly synthesized polysomal and postpolysomal mRNAs accumulate in the cytoplasm with similar kinetics, have similar apparent lifetimes, are similar in size and in poly(A) content, and contain similar mRNA sequences that occur at equivalent relative abundance. However, postpolysomal mRNA is translationally inefficient as compared with polysomal mRNA and, as measured indirectly by selective m^{7}GMP inhibition, seems to contain a higher proportion of cap-deficient molecules. Perhaps as a result of cap deficiency and other structural defects, the entry of these mRNAs into polysomes may be impaired, causing them to accumulate in 20–80S RNPs. Thus, we propose that cytoplasmic mRNAs in mouse kidney are structurally heterogeneous, and molecules that are translationally deficient constitute a major fraction of mRNAs in native 20–80S mRNP.

The lower sensitivity of renal postpolysomal mRNA to m^{7}GMP suggests a greater fraction of cap-deficient molecules, but defective caps may not be the sole determinants of its impaired biologic activity. For example, some inactive postpolysomal mRNAs may constitute inaccurately or incompletely spliced mRNA molecules. Second, proteins that regulate translation may be recalcitrant to deproteinization from their associated mRNA and may inhibit translation. Third, intermediates in mRNA degradation would be isolated from postpolysomal mRNAs and further contribute to the overall lower translational activity of this mRNA population. Finally, not all structurally intact mRNAs initiate at uniform rates. In the case of globin mRNA, for example, the relative content

of α- and β-globin mRNA is inversely proportional to the rate at which these mRNAs initiate translation (Lodish, 1971).

The major distinguishing characteristic of postpolysomal mRNA in mouse kidney is its low translational activity. Because postpolysomal and polysomal mRNAs are the same size, this property is probably not caused by selective postpolysomal mRNA degradation. Copurification of an inhibitor of translation from 20–80S postpolysomal RNP also is discounted, inasmuch as postpolysomal mRNA does not inhibit translation of polysomal mRNA when added to reaction mixtures (not shown). Deficient postpolysomal mRNA translation seems unrelated to cryptic RNA strand scissions or intermolecular RNA/RNA hybrids in postpolysomal mRNA. For example, translation of postpolysomal RNA after heating at 95 °C for 5 min improved its activity by only $\sim$10%, demonstrating few RNA/RNA duplexes or cryptic strand scissions. Furthermore, mRNA from EDTA-derived 10–80S RNP has translational activity comparable to that of mRNA from polysomal RNP. While these findings agree closely with those previously described from the sea urchin (Rudensey & Infante, 1979), they are, to our knowledge, unique in mammalian studies. Most other investigators of polysomal and postpolysomal mRNA translation have utilized the wheat germ lysate cell-free system (Lee & Engelhardt, 1979; McMullen et al., 1979). Our studies and those of the sea urchin (Rudensey & Infante, 1979) were done by using the rabbit reticulocyte lysate, which may be more sensitive to altered mRNA structures or less capable of repairing deficient caps or other anomalies in mammalian mRNA. Given the diversity of systems examined so far, conclusions about the role and properties of postpolysomal mRNA should await additional data from other systems.

A consistent pattern of mRNA utilization has not emerged yet from investigations of the coding functions of postpolysomal mRNA in other mammalian systems, perhaps because cell types and their growth conditions have varied greatly. For example, the mRNA-directed translation products of neuroblastoma cells (Croall & Morrison, 1980), Taper hepatoma cells (McMullen et al., 1979), and Vero cells (Lee & Engelhardt, 1979) contain numerous abundant polypeptides coded only by postpolysomal mRNA. Furthermore, in Taper hepatoma cells, cDNA hybridization experiments demonstrated that postpolysomal mRNA had low complexity relative to polysomal mRNA, containing only $\simeq$500 different mRNAs (Kinneburgh et al., 1979). In contrast, the diversity, coding properties, and relative abundance of mouse kidney polysomal and postpolysomal mRNAs are equivalent. Differences between kidney and Taper hepatoma cells may reflect dissimilar patterns of mRNA regulation, or the heterogeneity of renal cell populations may mask mRNAs unique to postpolysomal mRNA of one particular cell type.

Newly synthesized poly(A+) mRNA and translatable poly(A+) mRNA from native postpolysomal RNP may constitute separate pools, diluting translatable mRNA with stable, nontranslatable sequences. Conceivably, long-lived postpolysomal mRNAs may label slowly and initiate protein synthesis inefficiently compared with postpolysomal mRNAs that enter polysomes. If most postpolysomal poly(A+) mRNA is stable and translates poorly, our analysis of labeled postpolysomal mRNAs would not describe the kinetic behavior of all postpolysomal poly(A+) mRNAs. However, polysomal and postpolysomal mRNAs have similar specific activities [radioactivity per microgram of poly(A+) mRNA; data not shown], suggesting that mRNA unique to postpolysomal mRNA is unlikely.

The kinetics of postpolysomal mRNA labeling are inconsistent with the existence of a pool of postpolysomal mRNA segregated from translated mRNA molecules. The labeling time required to reach a constant ratio of labeled mRNA in polysomal and postpolysomal RNA is short and illustrates that most mRNAs of these fractions are in apparent equilibrium. Some postpolysomal poly(A+) mRNAs, however, may decay without entering polysomes (Mauron & Spohr, 1978). If postpolysomal poly(A+) mRNA mainly consisted of stable mRNAs, radioactivity should have accumulated in postpolysomal poly(A+) mRNA throughout the labeling period. The opposite was observed, however: radioactivity in postpolysomal mRNA (per microgram of RNA) reached the maximum value 2–3 h *before* labeled mRNA ceased accumulating in polysomes. Thus, if mRNAs encoding individual proteins are heterogeneous in translational efficiency, less efficient molecules could accumulate in the postpolysomal region but still exhibit the same kinetic behavior as corresponding polysomal poly(A+) mRNAs. This hypothesis is supported by the coding function, translational activity, and the kinetics of synthesis and decay of postpolysomal mRNA.

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Optical Model Studies of Salt-Induced Conformational Transitions in the Nucleosome†

Rodney E. Harrington

ABSTRACT: Optical modeling of the DNA conformation has been used to interpret the results of highly sensitive flow birefringence and extinction angle studies on nucleosome core particles over a range of counterion strength (using KCl as the supporting electrolyte) from <0.15 mM to >0.6 M. Results are consistent with an oblate disk or wedge of axial ratio $p^{-1} \simeq 2$ over an intermediate salt concentration range from about 1.5 to 450 mM. Below ~1.5 mM, the particle appears to unfold into an extended prolate or oblate structure which can be modeled as a uniform superhelix of DNA. Above ~0.45 M, the particle unfolds into a conformation which is hydrodynamically similar to but optically quite different from the low-salt structure. This form can be modeled as a partially unfolded disk in which only the nucleosomal DNA ends become dissociated and the central region remains bound to the histone core. A description of the optical modeling methods is presented, and the results are correlated with hydrodynamic property changes.

A fascinating question related to the molecular architecture of eukaryotic chromatin is the seemingly paradoxical fact that transcription can occur readily even though the DNA is packaged with extraordinary efficiency. A typical nucleus may contain 2 m of DNA with a packing ratio of 10^4 to 1. In *Escherichia coli*, RNA polymerase may bind to promoter regions as large as 30–40 base pairs [for a review, cf. Siebenlist et al. (1980)], and insofar as these concepts apply also to eukaryotic systems [cf. Ziff (1980)], it is clear that significant structural changes must occur in active chromatin [for a review, cf. Mathis et al. (1980)]. At the nucleosomal level, altered conformational states have been reported in replicating (Seale, 1978) and in transcriptionally active (Weintraub & Grondine, 1976; Garel & Axel, 1976) chromatin. Furthermore, the role of RNA polymerase activity seems to be modulated by salt concentration (Williamson & Felsenfeld, 1978; Wasylik & Chambon, 1979; Wasylik et al., 1979). Hence, the question of salt-dependent nucleosomal structures seems to be relevant to an understanding of transcriptional processes at the molecular level.

Conformational transitions in nucleosomes have been observed in solution under extremes in ionic strength conditions. Single transitions at ionic strengths of around 1 mM or less have been reported by Libertini & Small (1980), Dieterich et al. (1979, 1980), and Wu et al. (1979) and around 5 mM by Dieterich et al. (1980) and Martinson et al. (1979). Two defined transitions have been reported by Gordon et al. (1978, 1979) and in an earlier communication from this laboratory (Harrington, 1981). These transitions occur also around 1 and 5–7 mM salt. A conformationally metastable region appears to exist from about 10 to 350 mM ionic strength, and an additional broad transition is observed between approximately 0.4 and 0.6 M salt (Dieterich et al., 1979; Wilhelm & Wilhelm, 1980; McGhee et al., 1980). Below ~0.6 M, the nucleosome evidently remains intact, but at higher salt concen-

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