Accumulation and Decay of Messenger Ribonucleic Acid in Mouse Kidney†

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ABSTRACT: The stability of polyadenylated messenger ribonucleic acid (mRNA) from cytoplasmic structures sedimenting faster than 40 S was analyzed in normal mouse kidney. Incorporation of radioactivity into poly(A)-containing and poly(A)-lacking cytoplasmic RNAs separated by oligo(dT)-cellulose chromatography was determined after sedimentation of RNA in sodium dodecyl sulfate containing sucrose density gradients. Radioactivity accumulated in poly(A)-containing RNA during the first 6 h and then decayed exponentially. Beginning 8–12 h after administering label, two components were evident in the decay curve of poly(A)-containing RNA; the short-lived component (approximately 57% of newly synthesized molecules) had an apparent half-life of 6 h, and the second class (approximately 43% of new mRNA) was more stable, decaying with a 24-h half-life. These studies provide the basis for examining the regulation of mRNA stability during compensatory renal hypertrophy.

Compared with bacterial mRNA, poly(A)-containing (poly(A)$^+$) mRNA[1] in cultured mammalian cells is stable (Greenberg, 1975). Early estimates of mRNA stability in mammalian organs were made in liver, using inhibition with actinomycin D (Trakatellis et al., 1964; Endo et al., 1971) or selective degradation of polyribosomal mRNA with RNase (Tanaka et al., 1970; Tominaga et al., 1971). These experiments were indirect and were complicated by the nonspecificity inherent in inhibition with actinomycin D (Singer and Penman, 1972). Recently, rat liver mRNA labeled with $^{32}P_i$ was shown to have a single decay component with a half-life of 11.6 h (Tweedie and Pitot, 1974). The presence of 5S and 10S RNase-resistant fragments in liver poly(A)$^-$ RNA, however, suggested incomplete separation of poly(A)$^-$ and poly(A)$^+$ RNA, making accurate estimations of radioactivity in mRNA difficult.

To reexamine the stability of poly(A)$^+$ mRNA in mammalian organs and as a preliminary to studying the regulation of mRNA metabolism during compensatory renal growth, we now describe the kinetics of cytoplasmic accumulation and decay of poly(A)$^+$ mRNA from mouse kidney. Our experiments showed two apparent decay components: one with an apparent half-life of 11 h, corrected to 6 h, and a second with a 24-h half-life.

Experimental Procedure

Animals. Young adult male Charles River mice (40–50 days, 30–35 g, Charles River Laboratories, North Wilmington, Mass.) were fed ad lib. and kept on alternating 12-h cycles of light and dark.

Preparation of Polysomes and RNA Extraction. At times from 0.5 to 72 h after a single injection of [5-^{3}H]orotic acid, mice were killed by cervical dislocation. Kidneys were disrupted by Dounce homogenization in ice-cold buffer containing 10 mM Tris-HCl (pH 7.4), 2 mM $MgCl_2$, and 10 mM NaCl (Ouellette et al., 1975). Homogenates were centrifuged at 10 000 rpm for 10 min in the Sorvall SS-34 rotor at 4 °C. Supernatants were centrifuged in linear 7–47% (w/w) sucrose density gradients in 10 mM Tris-HCl (pH 7.4), 0.50 M NaCl, and 0.05 M $MgCl_2$ in the Beckman SW27 rotor at 26 500 rpm for 3 h at 4 °C (Ouellette et al., 1976a). The polysome (>80 S) and postpolysome (40–80 S) regions were pooled separately; ribonucleoproteins and RNA were precipitated at −20 °C with 2 volumes of 95% ethanol.

Polysomal and postpolysomal RNA samples were deproteinized separately by a modification of the procedure of Perry et al. (1972).

Oligo(dT)-Cellulose Chromatography. RNA was separated into poly(A)$^+$ and poly(A)$^-$ fractions with oligo(dT)-cellulose (type T-2, Collaborative Research, Waltham, Mass.) chromatography by a modification of the procedure of Aviv and Leder (1972). Less than 1 mg of RNA from structures sedimenting faster than 40 S was dissolved in 10 mM Tris-HCl (pH 7.4), 0.45 M NaCl, and applied to 15 × 0.5 cm columns of oligo(dT)-cellulose equilibrated with the same buffer. Poly(A)$^+$RNA bound to the column was eluted with 10 mM Tris-HCl (pH 7.4). Fractions were assayed for radioactivity by counting 50-μl aliquots in 5.0 ml of scintillation fluid containing 2 volumes of xylene, 1 volume of Triton X-100 (Rohm & Haas Co., Philadelphia, Pa.), and 8 g of Omnifluor (New England Nuclear Corp., Boston, Mass.) per l.

Sedimentation Analysis of RNA. RNA samples dissolved in NETS buffer (10 mM Tris-HCl (pH 7.4), 0.10 M NaCl, 10 mM EDTA, and 0.5% sodium dodecyl sulfate) were layered onto linear 15–30% (w/w) sucrose density gradients in NETS buffer and centrifuged in the SW27 rotor as indicated in legends to the figures. Aliquots of 1.4-ml fractions were counted in a gel of 3.0 ml of H_2O and 10 ml of xylene-based scintillation fluid.

Radiochemicals. [5-^{3}H]Orotic acid (11.1 Ci/mmol) was purchased from New England Nuclear Corp.

Results

Fractionation of Cytoplasmic RNA by Oligo(dT)-Cellu-

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[1] Abbreviations used: RNA, ribonucleic acid; poly(A)$^+$ RNA, RNA containing poly(A); poly(A)$^-$ RNA, RNA lacking poly(A); NETS buffer, 10 mM Tris-HCl (pH 7.4), 0.1 M NaCl, 10 mM EDTA, 0.5% sodium dodecyl sulfate; mRNA, messenger RNA; HnRNA, heterogeneous nuclear RNA; rRNA, ribosomal RNA; tRNA, transfer RNA; Tris,tris-(hydroxymethyl)aminomethane; EDTA, ethylenediaminetetraacetic acid; UDP, uridine diphosphate.

TABLE I: Oligo(dT)-Cellulose Chromatography of Cytoplasmic RNA.[a]

Hours	Radioactivity (cpm)		
	Poly(A)$^-$ RNA	Poly(A)$^+$ RNA	Poly(A)$^+$/Total (%)
1	13 415	3 901	22.5
2	19 055	4 492	18.7
3	27 226	5 652	17.2
4	29 134	6 491	18.2
6	48 615	10 702	18.0
8	43 204	6 662	13.3
12	28 046	1 972	6.6
18	50 178	2 756	5.2
24	50 268	2 568	4.9
48	55 102	1 670	3.0
72	21 467	429	1.9

[a] Mice (four per group) were injected intraperitoneally with 100 μCi of [^{3}H]orotate. At the indicated intervals after administration of label, RNA was extracted from structures sedimenting faster than 40 S (Experimental Procedure) and chromatographed on oligo(dT)-cellulose. Radioactivity in 50-μl aliquots from each 1.0-ml column fraction was assayed by counting in 5.0 ml of xylene-based scintillation fluid. Indicated values represent the sum of the radioactivity in 50-μl samples under the respective peaks. Data are not normalized with respect to RNA content or recovery.

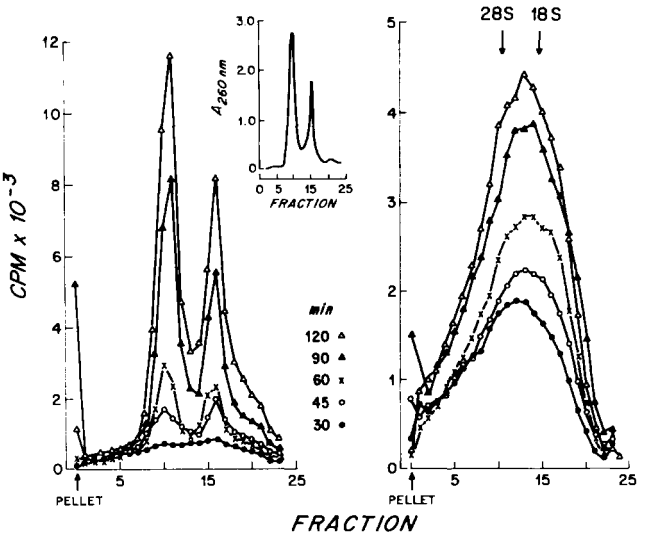

FIGURE 1: Kinetics of rRNA and poly(A)$^+$ mRNA appearance in kidney cytoplasm. Four mice were labeled with 100 μCi of [^{3}H]orotate. RNA from structures ≥40 S was fractionated on oligo(dT)-cellulose. Poly(A)$^+$ mRNA and poly(A)$^-$ RNA containing ^{14}C-labeled 28S RNA marker were dissolved in 1.0 ml of NETS buffer for sedimentation in 34 ml 15–30% sucrose density gradients in NETS at 20 000 rpm for 16 h at 23 °C in the Beckman SW27 rotor. (Left panel) Poly(A)$^-$ RNA; (right panel) poly(A)$^+$ RNA; (inset) poly(A)$^-$ RNA.

lose Chromatography. Poly(A)$^+$ mRNA was purified from polysomes and structures sedimenting faster than 40 S by selection of RNA on oligo(dT)-cellulose. Earlier studies have shown that poly(A)$^+$ mRNA is released from polysomes with EDTA, and that newly synthesized polysomal and postpolysomal poly(A)$^+$ mRNA are in rapid equilibrium (Ouellette et al., 1976a). The percent of total radioactivity in the poly(A)$^+$ RNA fraction was unchanged from 18.7% at 2 h to 18.0% by 6 h (Table I), demonstrating the relative cytoplasmic accumulation of newly synthesized poly(A)$^-$ RNA and poly(A)$^+$ mRNA molecules was constant by the second hour of labeling. Beginning 6–8 h after injection of label, however, the ratio of poly(A)$^+$ RNA to poly(A)$^-$ RNA decreased continuously, suggesting either reduced accumulation of mRNA relative to rRNA or decay mRNA without rRNA turnover.

Early Kinetics of Poly(A)$^+$ mRNA and Ribosomal RNA Accumulation in the Cytoplasm. The data in Table I suggested that poly(A)$^+$ mRNA appeared in the cytoplasm more rapidly than rRNA during the first 2 h of labeling or that there was a lag in the accumulation of rRNA. The early accumulation kinetics of mRNA and rRNA were examined by labeling mice for intervals from 30 min to 2 h. Within 30 min, there was a substantial amount of poly(A)$^+$ mRNA in the cytoplasm, but 28S RNA and 18S RNA were barely detectable in the corresponding poly(A)$^-$ RNA (Figure 1). After 45 min of labeling, 28S and 18S rRNA were both present in the cytoplasm, and by 90 min the labeling pattern of newly synthesized molecules was already in equilibrium with the mass ratios of the rRNA species (Figure 1, inset). This rate of cytoplasmic appearance seems faster than in rat liver (Chaudhuri and Lieberman, 1968a,b), but this apparent difference could result from differential utilization of precursor in the two tissues.

Sedimentation Properties of Labeled Poly(A)$^+$ and Poly(A)$^-$ RNA. Radioactivity in poly(A)$^-$ and poly(A)$^+$ RNA was quantitated after sedimentation in sucrose density gradients. This method had three advantages: it provided a test of RNA integrity as determined by the sedimentation rate, it allowed correction for possible rRNA contamination in poly(A)$^+$ RNA, and it permitted recovery of purified 18S and 28S rRNA for measurements of specific activities.

The sedimentation of fractionated RNAs for representative time points is shown in Figure 2. The poly(A)$^+$ RNA sedimented heterogeneously as expected for renal mRNA (Ouellette et al., 1975, 1976a), but by 12 h after labeling slight contamination of poly(A)$^+$ mRNA with 28S rRNA was apparent (Figure 2, stippled regions). This rRNA contamination (0.15–0.20% of total 28S rRNA radioactivity) was subtracted from radioactivity in poly(A)$^+$ mRNA. In contrast to heterogeneously sedimenting mRNA, poly(A)$^-$ RNA fractions displayed characteristic 28S and 18S rRNA species and low molecular weight RNA containing 5S RNA and polysome-associated tRNA (Ouellette et al., 1975).

A comparison of sedimentation patterns of poly(A)$^+$ mRNA labeled for up to 6 h (Figures 1 and 2a) with patterns of mRNAs labeled 18 h or longer (Figure 2a) showed perceptible differences in the size of these mRNA populations. Relative to ^{14}C-labeled 18S rRNA markers (arrows), mRNA from early time points sedimented faster than that from long labeling periods. Similar observations have been made for both poly(A)$^+$ mRNA (Singer and Penman, 1973) and poly(A)$^-$ mRNA from HeLa cells (Milcarek et al., 1974). This small difference suggested that the newly synthesized mRNAs present at short labeling intervals may consist of larger molecules possibly different from the more stable molecules assayed from long-term labeled kidneys.

Half-life of Kidney Poly(A)$^+$ mRNA. The half-life of renal poly(A)$^+$ mRNA was quantitated by measurement of radioactivity after centrifugation in sodium dodecyl sulfate containing sucrose gradients (Figure 2). Radioactivity in rRNA was determined by summing the counts under the 28S and 18S peaks (Figure 2). [^{3}H]Orotate was incorporated linearly into both ribosomal RNA and poly(A)$^+$ mRNA for the first 4 h (Figure 3). Total radioactivity continued to accumulate in rRNA for 8–12 h, remained unchanged for the next day, and declined slightly by 72 h. This result agrees closely with an earlier report (Malt and Stoddard, 1966) and with measure-

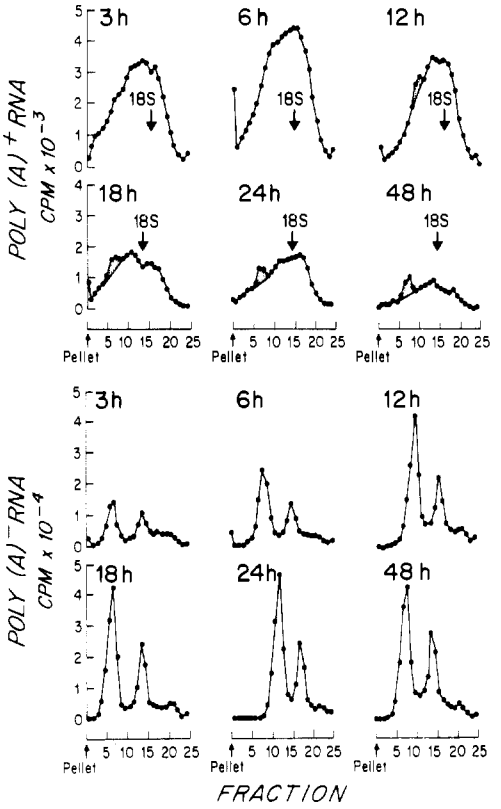

FIGURE 2: Sedimentation analysis of labeled poly(A)$^+$ and poly(A)$^-$ cytoplasmic RNAs. Four mice were labeled as in Figure 1. Combined polysomal and postpolysomal RNA fractionated on oligo(dT)-cellulose was dissolved in 1.4 ml of NETS buffer and was sedimented at 23 000 rpm in 34 ml 15–30% sucrose density gradients at 23 °C. Time of centrifugation was 16.5 h, except for the following: 12-h-labeled poly(A)$^+$ RNA and 24-h-labeled poly(A)$^-$ RNA centrifuged 15 h; 18-h-labeled poly(A)$^+$ RNA and 3-h- and 18-h-labeled poly(A)$^-$ RNA centrifuged 18.5 h. Total 1.4-ml fractions from poly(A)$^+$ mRNA gradients were counted, but only 0.50-ml aliquots of poly(A)$^-$ RNA gradients were assayed for radioactivity.

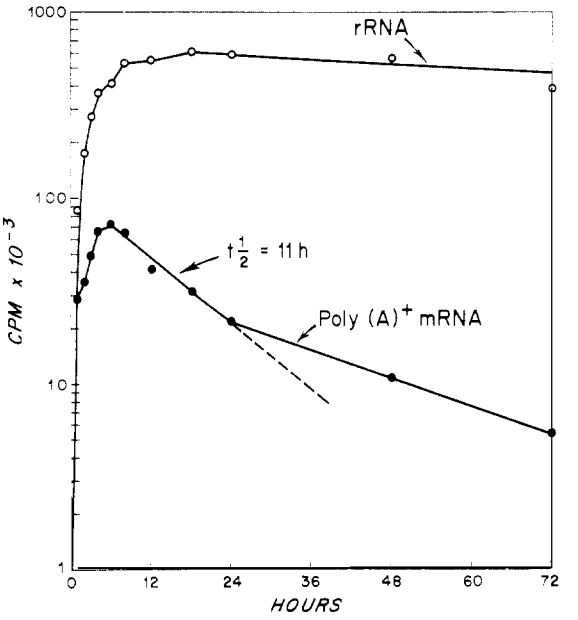

FIGURE 3: Kinetics of rRNA and poly(A)$^+$ mRNA accumulation and decay in mouse kidney cytoplasm. Poly(A)$^+$ and poly(A)$^-$ RNAs were analyzed in sodium dodecyl sulfate containing sucrose density gradients (Figure 2). Total poly(A)$^+$ radioactivity was taken as the total cpm in each gradient; radioactivity in rRNA was quantitated planimetrically as the sum of cpm under the 28S and 18S peaks. Since only 0.5 ml of 1.45 ml fractions was counted in poly(A)$^-$ RNA gradients (Figure 2), measured radioactivity was corrected to total radioactivity when normalized to the amount of rRNA in the 48-h time point. Data were normalized on this basis since the cellular RNA content of normal kidneys from mice over 30 days old is constant (Priestley and Malt, 1968) and since 85% of renal RNA is rRNA (Melvin et al., 1976).

ments of the specific activities of 18S and 28S RNA (data not shown). Since rRNA in kidney has a 5-day half-life (Melvin et al., 1976), incorporation of labeled precursors into RNA should be negligible after rRNA reaches constant specific activity, permitting decay analysis of unstable molecules (Singer and Penman, 1973).

Incorporation of [^{3}H]orotate into poly(A)$^+$ RNA abruptly terminated after 4 h; by 6 h, labeled molecules began to decay exponentially. Two classes of poly(A)$^+$ mRNA with different half-lives were apparent from the decay curve (Figure 3). The first component had a measured half-life of 11 h; the second class of mRNA was more stable, decaying with a 24-h half-life. When label in the 24-h component was subtracted from radioactivity in the 8–24-h time points, a corrected half-life of 6 h was estimated for the more rapidly metabolized mRNA.

The initial relative amounts of newly synthesized mRNA molecules were estimated by extrapolation of both decay curves in Figure 3 to zero time (Singer and Penman, 1973). By this approximation, 57% of the newly synthesized molecules were of the rapidly metabolized component, and the remaining 43% of the mRNA consisted of more stable molecules. These data closely agree with a similar analysis of HeLa cell mRNA in which 60% of newly synthesized messages are of the rapidly turning over class and 40% turn over slowly (Singer and Penman, 1973).

Discussion

The principal growth response of the mouse kidney to unilateral nephrectomy is tubular cell hypertrophy (Halliburton and Thomson, 1965; Threlfall et al., 1967; Kurnick and Lindsay, 1968; Malt and Lemaitre, 1968; Dicker and Shirley, 1971), primarily the cells of the proximal tubules. Autoradiographic evidence shows that 1 h after injection of [^{3}H]orotic acid, 89% of the total radioactivity in RNA is in the proximal tubular cells and 8% in the distal tubules (Ross et al., 1975). This distribution of label was similar in the remaining kidney following unilateral nephrectomy. Since in the present study we have determined the stability of mRNA labeled with orotic acid, it is likely that this mRNA is derived from the proximal tubular cells, although it is not clear that both components are derived from the same cell type. These findings, therefore, provide the basis for examining possible changes in the stability of mRNA during compensatory renal growth, induced neoplasia (Hard and Shaw, 1974), burn trauma (Asko-Seljevaara, 1975), and other growth induction models (Baserga et al., 1968; Malamud and Malt, 1971).

Renal rRNA labeled with orotic acid turns over slowly, with a 5-day half-life (Melvin et al., 1976), but some nucleotides released in rRNA degradation could have been salvaged and reincorporated. Assay of acid-soluble radioactivity does not adequately measure disappearance of label from the precursor pool because the nuclear and cytoplasmic nucleotide pools are compartmented (Plagemann, 1971a,b) and because labeled orotic acid enters stable acid-soluble fractions such as UDP-sugars (Bucher and Swaffield, 1966). However, since the pools for 45S pre-rRNA and HnRNA synthesis seem to equilibrate rapidly (Wu and Soeiro, 1971), and rRNA in mouse kidney is relatively stable (Melvin et al., 1976), radioactivity incor-

porated into rRNA was followed as an index of the pool specific activity since rRNA specific activity would be constant only after the precursor pool was depleted. The extent of [^{3}H]pyrimidine reutilization, although possible, does not seem extensive. After 30–40 min of orotate labeling, ^{3}H-labeled rRNA began accumulating in the cytoplasm at a faster rate than mRNA. If substantial reutilization of label had occurred, reincorporation into rRNA should have been detected but, between 24 and 72 h after injection of label, the specific activities of both 28S RNA and 18S RNA decreased in accordance with previously measured half-lives (Malt and Lemaitre, 1968; Melvin et al., 1976).

Unstable mRNAs with half-lives much less than 6–8 h were not detectable in these experiments, because of the 8-h delay necessary to effect a chase. Recently, labeling with L-[*methyl*-^{3}H]methionine, a rapidly chased RNA precursor (AB and Malt, 1970), we have found a class of mRNA with a 1–2-h half-life (Ouellette et al., 1976b). Poly(A)$^+$ mRNA purified from mouse kidney labeled with orotic acid contains two classes of mRNA: one decaying with a half-life of 6 h, the other with a half-life of 24 h. HeLa cells growing exponentially have two stable components with half-lives of 7 and 24 h (Singer and Penman, 1973), plus an additional labile component with a 1–2-h half-life (Puckett et al., 1975). In liver (Tweedie and Pitot, 1974) and most systems examined, however, mRNA decays as a single class (Greenberg, 1972, 1975; Abelson et al., 1974; Perry and Kelley, 1973).

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