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Messenger RNA in Compensatory Renal Hypertrophy

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Experiments that deal with the stability of messenger RNA (mRNA) in normal mouse kidney, and, to some extent, the stability of mRNA during renal growth will be described. We have found a population of mRNA in the cytoplasm of mouse kidney that is short-lived. Such a class of rapidly metabolized mRNA could play an adaptive role at the translational or cytoplasmic level in determining gene expression and may be important during the early phases of compensatory hypertrophy.

INTRODUCTION

The 3'-OH termini of most mRNA molecules terminate in a homogeneous tract of 80–180 adenylate (poly-A) residues [1,2]. Although the function of poly(A) is not known, since approximately 70 percent of non-histone mammalian cytoplasmic mRNA contains poly(A) [3,4], it provides a useful marker for the isolation of mRNA. The 5' end of the molecule contains a complex structure termed a "cap" [5,6,7,8,9,10]. The general chemical features of the 5' terminus are that it (1) terminates in 7-methyl guanosine, and (2) contains 2–4 methylated residues. In addition to the cap methyl sequences, there may be 3–5 methyl bases uniformly distributed in the molecule [5,6,7,11].

The main features of translation include an initiation step at a site near the 5' terminus and elongation where the ribosomes then travel down the mRNA strand in the 5' to 3' direction translating the nucleotide sequence into the amino acid sequence of the growing polypeptide chains. In the bacteria, mRNA has a mean lifetime of minutes and the regulation of protein synthesis is transcriptional, but mRNA in mammalian cells is more stable, thus increasing the likelihood of translational or cytoplasmic control mechanisms. Examples of such control could include increased rate of initiation or elongation, an increase or decrease in the amount of mRNA, or recruitment of existing mRNAs by some chemical activation process.

Since there is a precedent for marked accumulation of mRNA following induction of cell proliferation [12,13,14], since Melvin, Kumar and Malt [15] had demonstrated increased stability of ribosomal RNA (rRNA) during compensatory renal growth, and since intact methylated 5' termini seem to be necessary for efficient initiation of mammalian messenger RNAs [16,17,18,19], we asked the following questions regarding the metabolism of messenger RNA in mouse kidney. First, what is the stability of messenger RNA in normal mouse kidney and 'parenthetically' does the kidney contain more than one decay class of messenger RNA? Second, does the stability of renal mRNA increase during renal growth? And third, how are the methyl sequences of renal mRNA metabolized? That is, do methyl residues in cap structures

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selectively turn over to provide for rapid turning on and turning off of existing cytoplasmic messenger RNAs?

RESULTS AND DISCUSSION

The stability of mRNA was measured using pulse-chase analysis. Mice were labelled by single subcutaneous injection and at intervals after injection of label, kidneys were removed and polyribosomal RNA was chromatographed on oligo(dT)-cellulose to isolate poly(A)-containing mRNA (poly(A)$^+$ mRNA). Since the specific activity of rRNA increases as long as the precursor pool is radioactive, by monitoring the specific activity of rRNA one can determine when "chase" conditions are in effect, or the time after which labelled mRNA molecules cease to appear in the cytoplasm. Once rRNA specific activity becomes constant, disappearance of radioactivity from labile molecules such as mRNA can be observed.

The accumulation and decay of [^{3}H]-orotic acid, a general pyrimidine precursor, in both rRNA and poly(A)$^+$ mRNA is shown in Fig. 1. Since 8–12 hours were required to effect "chase" conditions, there was little possibility of detecting rapidly turning-over mRNA with orotate labelling. However, after the 8–12 hour pulse period, orotate-labelled mRNA exhibited biphasic decay kinetics (Fig. 1). The stable

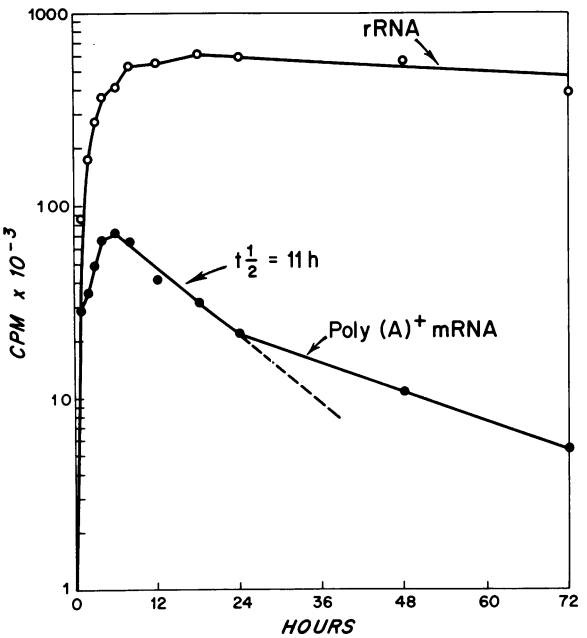

FIG. 1. Accumulation and decay of mRNA and poly(A)$^+$ mRNA labelled with [^{3}H]-orotate. Mice (four per time-point) were labelled with 100 μCi [^{3}H]-orotic acid (New England Nuclear Corp., Boston, Mass.). At the times shown, kidneys were removed, pooled, and disrupted by Dounce homogenization in 10 mM Tris-HCl (pH 7.4), 100 mM NaCl, and 2 mM $MgCl_2$. Postmitochondrial supernatants, prepared by centrifuging homogenates at 10,000 rpm for 10 minutes, were layered onto 36 ml 7–47 percent linear sucrose density gradients in 10 mM Tris-HCl, 0.5 mM NaCl, and 50 mM $MgCl_2$. Gradients were centrifuged in the Beckman SW27 rotor at 26,500 rpm for 3 hours at 4° C. Material sedimenting faster than 40S was precipitated with 2 vol 95 percent ethanol, and subsequently deproteinized by the phenol-chloroform procedure of Perry et al. [30] as described [31]. Polyribosomal RNA was fractionated into poly(A)$^+$ and poly(A)$^-$ fractions by single passage on oligo(dT)-cellulose [20,32]. Radioactivity in poly(A)$^+$ RNA and rRNA was quantitated after sedimentation in 15–30 percent sucrose density gradients containing 10 mM Tris-HCl (pH 7.4), 0.10 M NaCl, 10 mM EDTA and 0.5 percent sodium dodecyl sulfate [20].

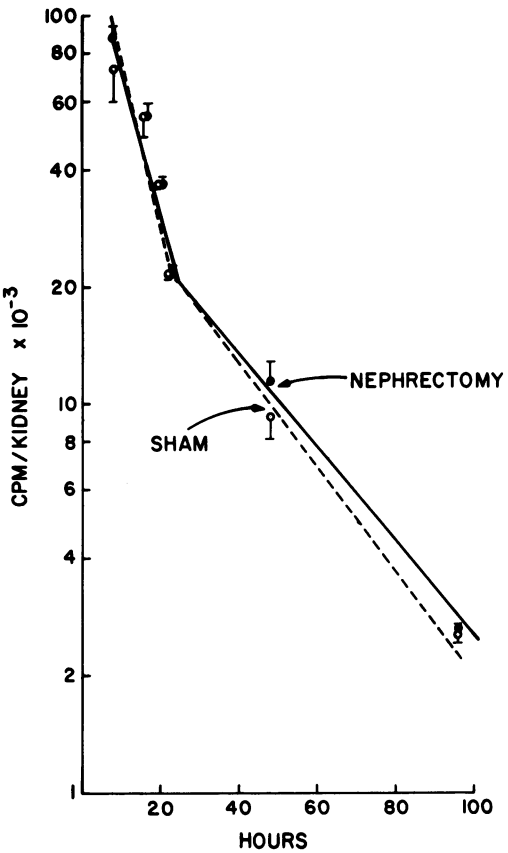

FIG. 2. Decay of [^{3}H]-orotic acid-labeled poly(A)$^+$ mRNA during compensatory renal hypertrophy. Left unilateral nephrectomy and sham operation were performed under light ether anesthesia. One hour post operation, mice were given a single injection of 50μ Ci [^{3}H]-orotic acid. At the times shown, right kidneys were disrupted in 10 mM Tris-HCl (pH 7.4), 0.25M sucrose, 0.15 M NaCl and 3 mM $MgCl_2$ by Dounce homogenization. Polyribosomes were precipitated from postmitochondrial supernatants with 70 mM $MgCl_2$ [33,34,35,26]. Polyribosomal RNA was fractionated by double passage on oligo(dT)-cellulose chromatography, and aliquots of poly(A)$^+$ and poly(A)$^-$ RNA were assayed for radioactivity by counting in a gel of 3 ml H_2O plus 10 ml xylene-based scintillation fluid [26]. Points are mean values of three replicate samples ± S.E.M.; each replicate consists of right kidneys from two animals.

component decayed with a half-life of 24 hours, and the less stable class had an adjusted half-life of approximately 6 hours [20]. These values are essentially identical to those of Singer and Penman [21] in HeLa cells, but in most other systems mRNA has been observed to decay as a single decay component [2,22,23,24,25].

Poly(A)$^+$ mRNA in growing kidneys and sham-nephrectomized controls was analyzed in a similar way to see if the stability of total renal mRNA increased during growth. Mice were labelled one-hour post operation, then kidneys were analyzed as before. Only the decay of radioactivity from poly(A)$^+$ RNA is shown in Fig. 2, and within the accuracy of this type of analysis, the two decay curves are not distinguishable. In addition, the decay components of orotic acid-labelled poly (A)$^+$ mRNA from growing and control kidney exhibit the same half-lives as mRNA from normal resting kidney. Therefore, for these populations of stable mRNA, there is no alteration in turnover rate as a function of induced growth, at least not for molecules made shortly after unilateral nephrectomy. However, since orotic acid does not label

all classes of mRNA, these data do not show the complete picture of mRNA metabolism in renal growth.

Since there is evidence for methylated 5′ terminal methyl residues being required for efficient translation of mRNA in cell-free systems [16,17,18,19], we wanted to test the possibility that the methyl residues in caps were metabolized selectively, and therefore studied the decay kinetics of mRNA dual-labelled with [^{14}C]-orotic acid and [^{3}H]-methyl methionine in normal mice (Fig. 3). There was a marked difference in the accumulation of these two precursors into rRNA (Fig. 3, left panel). As in Fig. 1, the specific activity of orotic acid in rRNA increased for 8–12 hours after injection of label, but the uptake of [^{3}H]-methyl label into rRNA increased only 1–2 hours before rRNA methyl specific activity remained constant. The right panel (Fig. 3) shows the decay of [^{3}H]-methyl label in mRNA. Because all points were taken within 18 hours of injection of label, little decay of orotic acid-labelled mRNA was observed, but methyl label in mRNA decayed very rapidly, with a half-life of 1–2 hours [26], a value similar to that reported in HeLa cells [27].

There are two possible interpretations of these decay kinetics. The first is that the kinetics describe a class of rapidly turning-over mRNA preferentially labelled during the brief pulse obtained with [^{3}H]-methyl labelling. It is also possible that the 5′ terminal methyl label turned over selectively, while the remainder of the molecule remained intact. However, since there was little change in the intramolecular 3′ to 5′ distribution of methyl radioactivity over the period when approximately 90 percent of methyl radioactivity decayed from the mRNA population [26], I conclude that the mouse kidney contains a population of mRNA that has a half-life of only 1–2 hours.

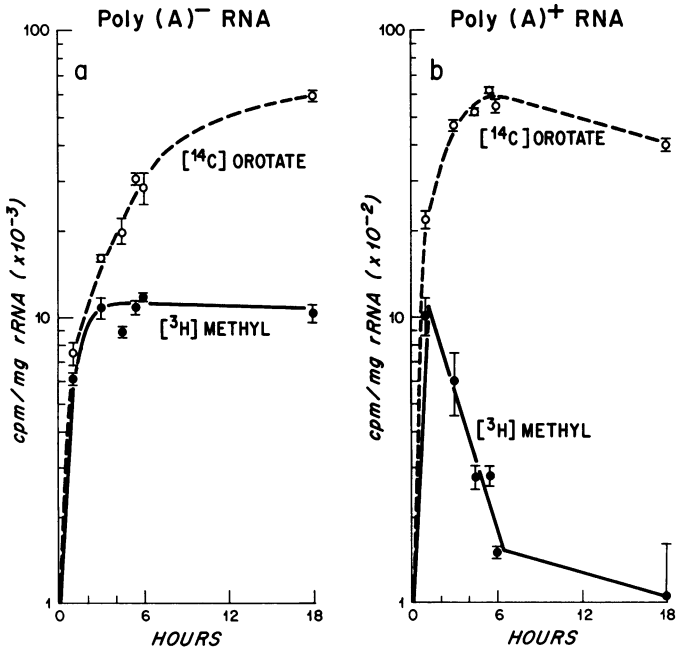

FIG. 3. Accumulation and decay of [^{3}H] methyl label in mRNA. Mice were labelled with 2.5μCi of [6-^{14}C]-orotic acid and 500 μCi of L-[methyl-^{3}H] methionine for the times shown. Radioactivity in poly(A)$^{+}$ and poly(A)$^{-}$ RNA was quantitated as in Fig. 2. Points are mean values of three replicate samples ± S.E.M.; each replicate consists of both kidneys from two labelled animals. The slope of the methyl-labelled mRNA decay curve was determined by single linear regression. (a) Poly(A)$^{-}$RNA; (b) Poly(A)$^{+}$RNA.

Although the pattern of metabolism of short-lived mRNA during compensatory renal growth is not known, it is possible to construct a working model for examining a possible role of this mRNA population in renal growth.

Ryffel and McCarthy [28] have shown that there are three classes of mRNA in mouse kidney which differ in regard to the number of copies of each mRNA sequence present in the cytoplasm. Class 1 is the class of lowest complexity since there are only 5–10 different genes represented, but there are several thousand copies of each mRNA molecule per cell. In contrast, there is a highly complex class of mRNA comprised of several thousand different RNA sequences, each present in only a few copies per cell. There is a third complexity class containing 400 different mRNAs, and each mRNA is present in approximately 500 copies per cell. Since the mouse kidney contains three distinct mRNA stability classes, it is interesting to speculate on a possible relationship between the clustering of mRNA in classes of varying complexity, and the rate at which mRNAs decay. Messenger RNAs of greatest complexity, i.e., messengers present in only a few copies per cell, might also be the mRNAs that turn over most rapidly; there is some suggestion that this may be the case in cultured insect cells [29]. If mouse kidney is similar, then one might expect quantitative or qualitative changes in rapidly turning over mRNA as a function of environmental changes. Such a mechanism could provide flexibility in gene expression in response to changes associated with induced growth.

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