*GIANT NUCLEOPLASMIC RNA IN THE SWITCH-ON OF COMPENSATORY RENAL GROWTH**

By Margherita Willems, Hana A. Musilova, and Ronald A. Malt

SURGICAL SERVICES, MASSACHUSETTS GENERAL HOSPITAL, BOSTON; AND DEPARTMENT OF SURGERY, HARVARD MEDICAL SCHOOL

Communicated by Paul C. Zamecnik, January 31, 1969

Abstract.—Heterogeneous RNA (HnRNA) (*ca.* 10*S*–70*S*) labeled for ten minutes with ^{3}H-uridine was identified by polyacrylamide-gel electrophoresis in the nucleoplasm of kidney cells. In the region >28*S* several discretely migrating species were present. Ten minutes after the removal of one kidney, labeled HnRNA >28*S* ("giant" HnRNA) in the remaining kidney began to decrease, and by 60 minutes it had fallen drastically. The presence of labeled giant HnRNA during a pulse label after uninephrectomy and its disappearance after longer labeling suggest that the decrease is a consequence of faster processing. Accelerated processing of giant HnRNA is antecedent to the increased synthesis of renal cytoplasmic RNA that follows uninephrectomy.

No function has heretofore been definitively associated with the heterogeneous nuclear RNA. At first it was thought to be a precursor of messenger RNA because of its rapid turnover, heterodisperse sedimentation properties, DNA-like base composition, hybridization with DNA, and presence in several varieties of plant and animal cells.[1–8] If so, only a small proportion of it can function in this manner, and identification of this moiety would be difficult in the presence of the vast majority of heterogeneous RNA employed in ways as yet unknown.[9, 10] Here we report the identification of this RNA in mouse kidney and present evidence that almost immediately after unilateral nephrectomy there is an acceleration in processing of nucleoplasmic HnRNA >28*S* as a preliminary step in compensatory renal hypertrophy.

Materials and Methods.—Preparation of nuclei and nucleoplasm: Left nephrectomy or sham operation was performed in young adult male Charles River mice. Beginning immediately thereafter and at intervals for the next 2 days the mice were labeled with an intraperitoneal injection of 250 μCi 5-^{3}H-uridine (20 Ci/mmole, Schwarz BioResearch Co.).

Four kidneys from decapitated control and uninephrectomized mice were disrupted in 10 ml homogenization medium (0.25 *M* sucrose in 0.003 *M* $MgCl_2$) with ten strokes of the loose pestle and ten of the tight pestle in a glass Dounce homogenizer. All procedures were carried out at 4°C. The volume was doubled with homogenization medium and the mixture was centrifuged at 1000 × *g* for 3 min.

Resuspended pellets were washed three times by centrifugation, and nuclei were separated by a modification of the method of Chauveau *et al.*[11] The pellets were resuspended in 7 ml of 2.2 *M* sucrose (0.00005 *M* $CaCl_2$, 0.001 *M* $MgCl_2$) with three strokes of a Ten-Broeck homogenizer for transfer to a cellulose nitrate tube that fit the Spinco SW-41 rotor. After being filled with homogenization medium above the 2.2 *M* sucrose suspension, the tubes were centrifuged at 40,000 rpm for 25 min to deposit the nuclei in a pellet directly at the bottom. The supernatant was decanted, and the tube was cut over the pellet, which was resuspended in 3 ml homogenization medium and 0.45 ml of a mixture of 10% Tween 40 and 10% deoxycholate (2:1), and briefly agitated.[12] Ten ml homogenization medium was added, and the pellet was deposited by low-speed centrifugation as before.

The purified pellet of nuclei was then digested with electrophoretically purified DNase (100 μg, Worthington Biochemical Co.) in 1 ml high-salt buffer (0.01 *M* Tris, pH 7.4, 0.5 *M* NaCl, 0.05 *M* $MgCl_2$) for 30 min.[12] Separation of nucleoplasm from nucleoli was effected by layering the digest on an 11-ml, linear 15–30% sucrose gradient in high-salt buffer and centrifuging for 15 min at 22,000 rpm (Spinco SW-41 rotor). The nucleoli at the bottom were reserved, and the nucleoplasmic RNA was precipitated from the supernatant with 2 volumes of ethanol at −20°C and collected by centrifugation.

Extraction of RNA: Iced precipitate was suspended with 2 ml of a buffer containing 0.01 *M* Tris, pH 7.4, 0.1 *M* NaCl, 0.001 *M* EDTA. Sodium dodecyl sulfate (SDS) was added to 0.5% and stirred continuously at 30°C until the mixture cleared (about 2 min). An equal volume of phenol (Mallinckrodt 88%) was immediately added, and extraction was performed as described by Penman,[12] except that the temperature was 22°C. Before precipitation with ethanol, $^1/_2$ vol of 5 × high-salt buffer was added to chelate the EDTA. (Kidney RNA could not be recovered undegraded from extractions at higher temperatures.) Phenol-extracted RNA was dissolved in 1 ml 10 × RSB buffer (RSB is 0.01 *M* Tris, pH 7.4, 0.01 *M* NaCl, 0.0015 *M* $MgCl_2$) and again digested with DNase for 10 min at 4°C. After the addition of SDS to 0.5% and EDTA to 0.01 *M*, an equal volume of phenol was added and another extraction carried out, after which the purified RNA was precipitated by ethanol.

Display of RNA: All phenol-extracted RNA recovered was layered on a 2.8% acrylamide gel[13] run at 5 ma/gel for the periods specified in the figures. Gels automatically scanned at 260 mμ were sliced, and the slices were hydrolyzed for 2 hr with 0.5 ml conc. NH_4OH.[13] The hydrolysate was mixed with 10 ml Aquafluor (Pilot Chemical Co.), and the radioactivity was counted in a Nuclear-Chicago Mark I spectrometer.

Results.—Labeling with ^{3}H-uridine for one hour normally results in the incorporation of radioactivity into all species of kidney RNA from precursors to finished products.[14] The nucleoplasm of mouse kidney cells in the present experiments contained 18*S* and 28*S* RNA, identified by parallel electrophoresis with cytoplasmic RNA, in amounts detectable by absorbance measurements. In normal kidneys labeled for ten minutes with ^{3}H-uridine, heterogeneous RNA of high specific activity was the only radioactive nucleoplasmic RNA (Fig. 1, *left*). After 60 minutes of labeling, 28*S* RNA—but not 18*S*—was also labeled (Fig. 2, *left*). Experiments in which longer periods of electrophoresis were used showed that the HnRNA was composed of several discrete species and that molecules with nominal sedimentation values >50*S* entered the gel (Fig. 3).

In animals uninephrectomized two days earlier, labeling for ten minutes showed a decreased amount of label in giant HnRNA (Fig. 1). When labeling was for 60 minutes (Fig. 2), practically no HnRNA >28*S* could be detected in the uninephrectomized animals. The absence of labeled giant HnRNA was also demonstrated in a 60-minute label one hour after uninephrectomy, although the pattern of labeling was normal 10 minutes after uninephrectomy (Fig. 4).

Discussion.—The nucleoplasm of mouse kidney cells therefore contains a heterodisperse (10*S*–70*S*), rapidly labeled RNA similar in these characteristics to the HnRNA recognized in duck erythroblasts, cultured cells, amphibian embryos, and insects.[1–8] In retrospect, HnRNA has probably been identified in rat liver as well.[15–18]

In a ten-minute exposure to ^{3}H-uridine, heterogeneous RNA is the major species of mouse kidney RNA labeled; much less label appears in the nucleoli. Gel electrophoresis for six hours to display the slowest-migrating HnRNA reveals distinct classes of molecules. No functional significance can now be

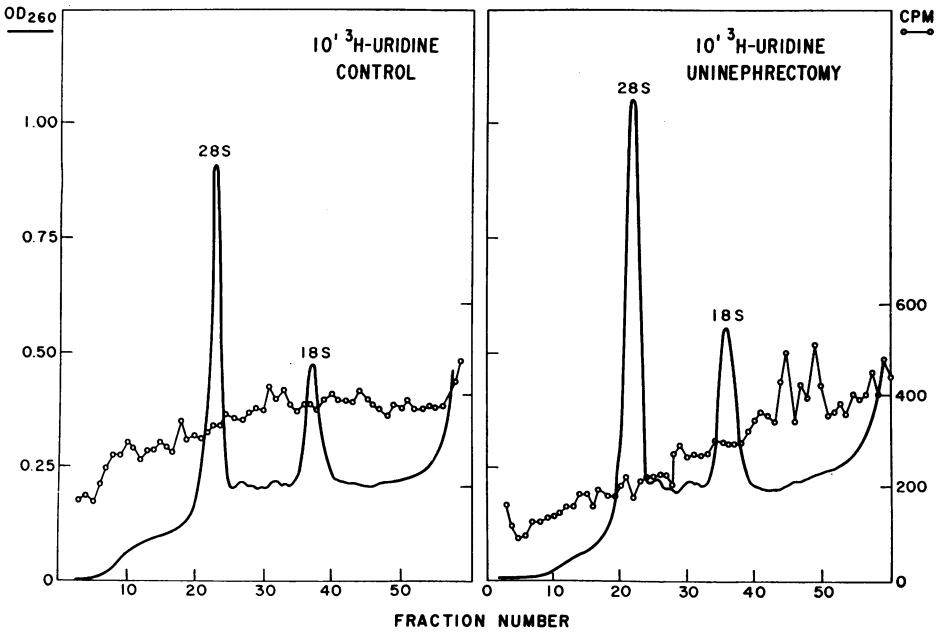

FIG. 1.—Electrophoretic analysis of renal nucleoplasmic RNA. Kidneys from control mice and from mice uninephrectomized 2 days earlier were labeled for 10 min with ^{3}H-uridine and processed as described in the text. Electrophoresis was for 3 hr. Migration proceeded from left to right.

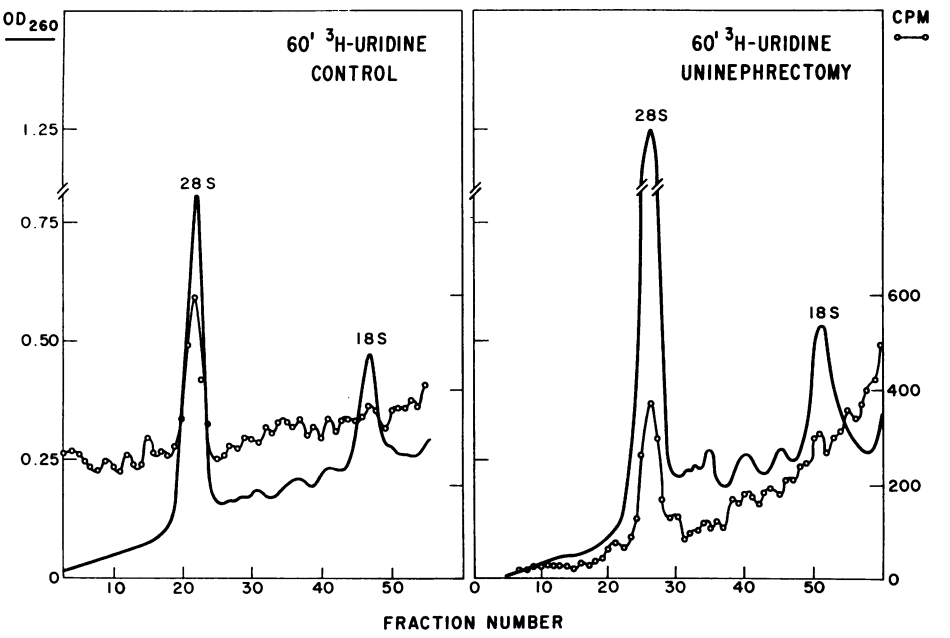

FIG. 2.—Electrophoretic analysis of renal nucleoplasmic RNA. Conditions were as shown in Fig. 1, except that labeling was for 60 min and electrophoresis for 5 hr.

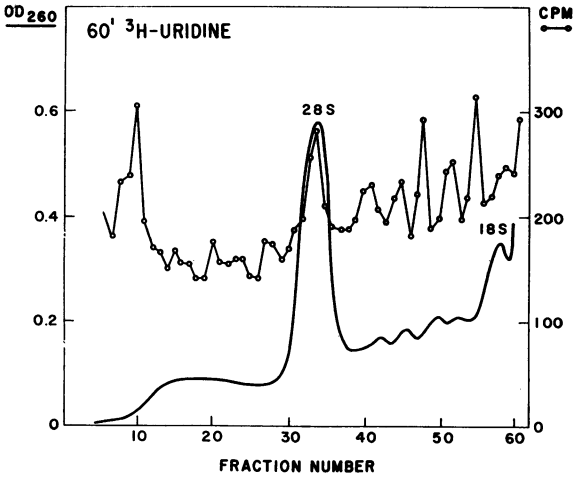

FIG. 3.—Prolonged electrophoresis of nucleoplasmic RNA. Normal kidneys were labeled for 60 min and nucleoplasmic RNA displayed by electrophoresis for 6 hr. The peaks at intervals >28S are constant findings.

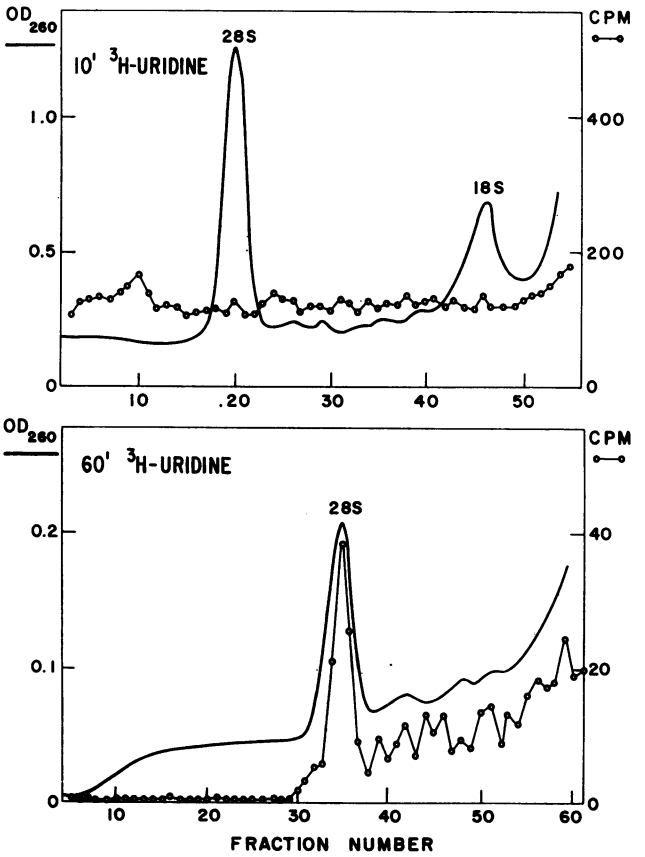

FIG. 4.—Nucleoplasmic RNA soon after nephrectomy. Mice were injected with ^{3}H-uridine immediately after uninephrectomy. Kidneys were harvested and processed either 10 or 60 min later. Electrophoresis was for 4.5 hr (*above*) and 5 hr (*below*).

attributed to these separate species; their roles might also be discrete, and kinetic experiments and analyses of base ratios might show how they differ.

For at least two days after uninephrectomy, practically no labeling of HnRNA migrating slower than 28*S* is demonstrable when animals are labeled for 60 minutes with ^{3}H-uridine. In a pulse of only ten minutes, the heavy high-specific-activity molecules are present, though in an amount decreased relative to the molecules migrating faster than 28*S*. The decrease is present as early as one hour after uninephrectomy. From the fact that renoprival mice labeled for ten minutes with high-specific-activity uridine ($\backsim 10^{-8}$ mole/animal) have radioactive HnRNA $>28S$ and that the label is virtually gone in 60 minutes, we infer that uninephrectomy speeds processing of giant HnRNA rather than inhibiting its synthesis; giant HnRNA is probably broken down[9] or transported elsewhere more rapidly in uninephrectomized than in control animals. Changes in pool sizes themselves cannot be responsible for the diminished label in giant HnRNA after nephrectomy, unless the pools are compartmented, because HnRNA with electrophoretic mobility $<18S$ actually seems to be labeled faster after uninephrectomy.

In addition to the use of a precursor of high specific activity to obtain these results, modifications of conventional methods of preparing RNA were necessary in order to avoid degradation by the active nucleases in kidney.[19] Evidence against appreciable degradation was the presence of the easily degraded HnRNA itself; moreover, added labeled cytoplasmic 28*S* RNA was completely recovered. The existence of labeled giant HnRNA in the solitary kidney after a ten-minute period of labeling argued against the presence of higher concentrations of nuclease in the kidney remaining after uninephrectomy as compared with the normal kidney. Although a detailed comparison of several methods of extraction will be presented elsewhere, a few essentials of the technique are noted here. The method of harvesting the nuclei reduced by one half the length of time previously required here for fractionation, and the digestion with DNase at 4° was essential. Purified undegraded RNA could be obtained only if the cellular fractions were resuspended at 4°, then treated with SDS for only a few minutes before phenol was added. Extractions had to be conducted at room temperature to avoid nucleolytic digestion.

From the presence of substantial amounts of 18*S* RNA in these nucleoplasmic preparations compared with HeLa cells, it might be argued that the preparations were contaminated with adherent cytoplasmic ribosomal RNA.[12] Evidence against this contention is that the nuclear 18*S* RNA is not labeled in one hour although renal cytoplasmic 18*S* RNA is,[14] that the nuclei are thoroughly washed with a combination of detergents known to clean at least HeLa nuclei effectively,[12] and that kidney cells have fewer perinuclear ribosomes than many other cells.[20]

Thus, the experiments indicate faster processing of giant HnRNA within an hour of uninephrectomy. This phenomenon is probably related to the "switch-on" of renal RNA synthesis that is also responsible for the hypertrophy of the proximal tubule cells, the 20–33 per cent increase in RNA/DNA concentration within two days, the faster rate of labeling of ribosomal RNA, and the increase

in polyribosomes.[21-24] This appears to be the first detection of a physiologic alteration in HnRNA. For example, heterogeneous RNA in HeLa cells is not specifically affected either by progressive inhibition of RNA synthesis with actidione or by poliovirus infection.[25]

Many of the peculiarities of gene control in animal cells may be related to the poorly understood pathways of HnRNA metabolism.[9] That the population of messenger and nuclear RNA's changes during differentiation, regeneration, and carcinogenesis seems clear;[26-29] but inferences derived from competitive hybridization experiments about the species of RNA involved are not exact. The experiments reported in this paper show that the metabolism of giant nucleoplasmic RNA is specifically affected at the onset of renal compensation. This may be a more general feature of the regulation of organ growth. Its occurrence within an hour of uninephrectomy is additional evidence in support of the hypothesis that loss of renal tissue almost immediately initiates the events leading to compensatory hypertrophy.

* This work was supported by the Shriners Burns Institute, American Heart Association, and National Institutes of Health (HD-01988 and AM-12769).

[1] Scherrer, K., L. Marcaud, F. Zajdela, I. M. London, and F. Gros, these PROCEEDINGS, **56**, 1571 (1966).

[2] Warner, J. R., R. Soeiro, H. C. Birnboim, M. Girard, and J. E. Darnell, *J. Mol. Biol.*, **19**, 349 (1966).

[3] Soeiro, R., H. C. Birnboim, and J. E. Darnell, *J. Mol. Biol.*, **19**, 362 (1966).

[4] Attardi, G., H. Parnas, M.-I. H. Hwang, and B. Attardi, *J. Mol. Biol.*, **20**, 145 (1966).

[5] Penman, S., I. Smith, and E. Holtzman, *Science*, **154**, 786 (1966).

[6] (*a*) Birnboim, H. C., J. J. Pène, and J. E. Darnell, these PROCEEDINGS, **58**, 320 (1967); (*b*) Brown, D. D., and E. Littna, *J. Mol. Biol.*, **20**, 81 (1966).

[7] Edström, J. E., and B. Daneholt, *J. Mol. Biol.*, **28**, 331 (1967).

[8] Retèl, J., and R. J. Planta, *European J. Biochem.*, **3**, 248 (1967).

[9] Soeiro, R., M. H. Vaughan, J. R. Warner, and J. E. Darnell, *J. Cell Biol.*, **39**, 112 (1968).

[10] Penman, S., C. Vesco, and M. Penman, *J. Mol. Biol.*, **34**, 49 (1968).

[11] Chauveau, J., Y. Moulé, and C. Rouiller, *Exptl. Cell Res.*, **11**, 317 (1956).

[12] Penman, S., *J. Mol. Biol.*, **17**, 117 (1966).

[13] Weinberg, R. A., U. Loening, M. Willems, and S. Penman, these PROCEEDINGS, **58**, 1088 (1967).

[14] Malt, R. A., *J. Exptl. Med.*, **124**, 679 (1966).

[15] Muramatsu, M., J. L. Hodnett, W. J. Steele, and H. Busch, *Biochim. Biophys. Acta*, **123**, 116 (1966).

[16] Muramatsu, M., and T. Fujisawa, *Biochim. Biophys. Acta*, **157**, 476 (1968).

[17] Dingman, C. W., and A. C. Peacock, *Biochemistry*, **7**, 659 (1968).

[18] Shütz, G., D. Gallwitz, and C. E. Sekeris, *European J. Biochem.*, **4**, 149 (1968).

[19] Malt, R. A., in *Renal Compensatory Hypertrophy*, ed. W. W. Nowinski and R. J. Goss (Academic Press, in press).

[20] Latta, H., A. B. Maundsbach, and L. Osvaldo, in *Ultrastructure of the Kidney*, ed. A. J. Dalton and F. Haguenau (New York: Academic Press, 1967).

[21] Halliburton, I. W., and R. Y. Thomson, *Cancer Res.*, **25**, 1882 (1965).

[22] Malt, R. A., and D. A. Lemaitre, *Am. J. Physiol.*, **214**, 1041 (1968).

[23] Malt, R. A., and D. A. Lemaitre, *Biochim. Biophys. Acta*, **145**, 190 (1967).

[24] Malt, R. A., and W. L. Miller, *J. Exptl. Med.*, **126**, 1 (1967).

[25] Willems, M., unpublished.

[26] Brown, D. D., and J. B. Gurdon, *J. Mol. Biol.*, **19**, 399 (1966).

[27] Denis, H., *J. Mol. Biol.*, **22**, 285 (1966).

[28] Church, R. B., and B. J. McCarthy, *J. Mol. Biol.*, **23**, 459 (1967).

[29] Drews, J., G. Brawerman, and H. P. Morris, *European J. Biochem.*, **3**, 284 (1968).