Newberry, V., Yazuchi, M., & Garrett, R. A. (1977) *Eur. J. Biochem. 76*, 51.
Peretz, H., Towbin, H., & Elson, D. (1976) *Eur. J. Biochem. 63*, 83.
Rummel, D. P., & Noller, H. F. (1973) *Nature (London) 245*, 72.
Shih, C. Y., & Craven, G. R. (1973) *J. Mol. Biol. 78*, 651.
Sommer, A., & Traut, R. R. (1976) *J. Mol. Biol. 106*, 995.
Spitnik-Elson, P., & Breiman, A. (1971) *Biochim. Biophys. Acta 254*, 457.
Stöffler, G., & Wittmann, H. G. (1977) *Molecular Mechanisms of Protein Biosynthesis*, pp 117–202, Academic Press, New York.
Tischendorf, G. W., Zeichardt, H., & Stöffler, G. (1975) *Proc. Natl. Acad. Sci. U.S.A. 72*, 4820.
Voynow, P., & Kurland, C. G. (1971) *Biochemistry 10*, 517.
Wittmann, H. G., Stöffler, G., Hindennach, I., Kurland, C. G., Randall-Haxelbauer, L., Birge, E. A., Nomura, M., Kaltschmidt, E., Mizushima, S., Traut, R. R., & Bickle, T. A. (1971) *Mol. Gen. Genet. 11*, 327.

Accumulation of Polyadenylated mRNA during Liver Regeneration†

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ABSTRACT: Cytoplasmic and polysomal polyadenylated mRNA [poly(A)$^+$-mRNA] increased by 120% prior to the onset of DNA synthesis during the regeneration of rat liver following partial hepatectomy. Despite this large change in cytoplasmic mRNA and an approximately 50% increase in total nuclear RNA, the amount of polyadenylated nuclear RNA increased by only 15–20% during this time. Neither the average size of nuclear or of cytoplasmic polyadenylated mRNA nor the length of their poly(adenylic acid) [poly(A)] tracts changed during liver regeneration. Polysomal poly(A)$^+$-mRNA increased proportionately more and at a faster rate than rRNA during the first day following partial hepatectomy. Normal livers contained a substantial proportion of cytoplasmic poly(A)$^+$-mRNA not associated with polysomes but this proportion was not altered in 3-h regenerating liver. Thus, in regenerating liver, most preexisting cytoplasmic mRNA does not appear to be recruited into polysomes prior to the substantial increase in the amount of cytoplasmic poly(A)$^+$-mRNA.

The regenerative response of the liver following removal of two-thirds of its mass provides a suitable in vivo system for studying the stimulation of cell replication. The early stages of this response in rats consist of a hypertrophic phase, lasting approximately 12–16 h, during which the rate of protein synthesis rises, and a subsequent hyperplastic phase which is characterized by a peak in DNA synthesis at about 24 h followed 6–8 h later by mitosis (reviewed by Bucher & Malt, 1971).

Qualitative and quantitative alterations in mRNA populations may be expected to be important determinants of the type and amounts of protein synthesized during liver regeneration. The complexity of nuclear and cytoplasmic mRNA at the early stages of liver regeneration as well as the homology between RNA populations from normal and regenerating liver have been determined by molecular hybridization methods (Colbert et al., 1977; Tedeschi et al., 1978). However, these methods do not provide a direct measurement of the total amounts of liver mRNA. Although the rate of synthesis of mRNA is probably increased very shortly after partial hepatectomy (Glazer, 1976, 1977), little is known about the actual amounts of mRNA present in regenerating livers, though previous studies suggest that they may be increased (Greene & Fausto, 1974). Therefore, we determined the absolute amounts of poly(A)$^+$-mRNA[1] present in various subcellular fractions of the liver at different stages of hepatic regeneration following partial hepatectomy. For these estimations it was necessary to determine the poly(A) content, the size of the poly(adenylic acid) tracts present in RNA preparations, as well as the average length of poly(A)$^+$-mRNA molecules in normal and regenerating liver.

Materials and Methods

Materials. Ribonuclease-free deoxyribonuclease was treated with iodoacetate (Zimmerman & Sandeen, 1966) prior to use. Bentonite was prepared according to the method of Brownhill et al. (1959). Solutions and plastic ware were sterilized by autoclaving. All glassware was siliconized (1% dichlorodimethylsilane in toluene) and kept at 300 °C for at least 6 h.

Animals. Three to eight male rats (Holtzmann; Charles River Breeding Labs.), weighing 120–160 g, were used for each RNA preparation. The animals were kept in a temperature-controlled room under alternating 12-h light–dark cycles.

Surgical procedures were performed under continuous ether–oxygen anesthesia, which is known not to affect hepatic levels of nucleoside triphosphates or of cyclic nucleotides (Bucher & Swaffield, 1966; Fausto & Butcher, 1976). Control animals were sham-operated; i.e., they were laparotomized and their livers manipulated but not removed. Partial hepatectomy, resulting in the removal of 70% of the liver mass, was done according to the method of Higgins & Anderson (1931). All

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[1] Abbreviations used: poly(A), poly(adenylic acid); poly(A)$^+$-mRNA, polyadenylated mRNA; poly(U), poly(uridylic acid); rRNA, ribosomal RNA; HnRNA, heterodisperse, high molecular weight nuclear RNA; SET, 10% sodium dodecyl sulfate, 50 mM EDTA, 250 mM Tris-HCl, pH 7.4; $NaDodSO_4$, sodium dodecyl sulfate; TNMDH, 50 mM Tris-HCl, pH 7.4, 25 mM NaCl, 5 mM $MgCl_2$, 1 mM dithiothreitol, 100 μg/mL sodium heparin.

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animals were killed at 9–10 a.m. after being fasted for 17 h.

Isolation of Cytoplasmic RNA. Livers were quickly excised and minced in 250 mM sucrose, 25 mM Tris-HCl, pH 7.5, 25 mM NaCl, 5 mM $MgCl_2$, 20 μg/mL poly(vinyl sulfate). The minced tissue was rinsed several times to remove blood cells and homogenized in 5 volumes (mL/g of liver) of buffer in a Potter-Elvehjem homogenizer with motor-driven Teflon pestle at 1500 rpm. The homogenate was centrifuged at $13000g_{av}$ for 10 min at 2 °C to pellet lysosomes, mitochondria, nuclei, and cellular debris. Bentonite (20% w/v) was added to the supernatant to a final concentration of 2%, followed by the addition of 1/9th volume of 10× SET (10% sodium dodecyl sulfate, 50 mM EDTA, 250 mM Tris-HCl, pH 7.4). The suspension was extracted at room temperature with an equal volume of 1× SET-saturated phenol:chloroform (1:1, phenol:chloroform). The interface and organic phase were extracted with 0.5 volume of 1× SET and the aqueous phase pooled with that of the first extraction. The pooled aqueous phases were extracted four more times with the phenol: chloroform mixture and twice with an equal volume of a mixture of isoamyl alcohol and chloroform (1:24). Two volumes of 95% ethanol containing 0.2 M sodium acetate were added and the RNA precipitated overnight at −20 °C. The precipitate was washed with 95% ethanol prior to use.

Isolation of Nuclear RNA. Nuclei were isolated by a method modified from that described by Busch et al. (1972). Livers were homogenized as described above but in 2% citric acid. The homogenate was filtered through two layers of gauze and centrifuged at 600*g* for 10 min at 2 °C. The pellets were resuspended with 6 volumes (mL/g of liver) of 250 mM sucrose, 0.1% Tween-80, 2% citric acid, and homogenized with eight strokes of the "B" pestle in a Dounce homogenizer (Kontes Glass Co.). Twenty-five mL of this suspension was layered onto 15 mL of 880 mM sucrose, 0.1% Tween-80, 2% citric acid and centrifuged at 800*g* for 10 min at 2 °C. The pellets were resuspended in 3 volumes (mL/g of liver) of 25 mM sucrose, 3 mM $CaCl_2$, 10 mM Tris-HCl, pH 7.4, and centrifuged at 600*g* for 10 min at 2 °C. The nuclei were resuspended in 3 volumes (mL/g of liver) of 50 mM Tris-HCl, pH 7.1, 25 mM NaCl, 5 mM $MgCl_2$, and incubated with 20 μg/mL of iodoacetate-treated deoxyribonuclease at 4 °C for 10 min with vigorous shaking. The suspension was adjusted to 2% bentonite and shaken an additional 15 min. Following the addition of 1/9th volume of 10× SET, the nucleic acids were extracted with phenol:chloroform and with isoamyl alcohol:chloroform, as described above.

The ethanol precipitate was dissolved in approximately 1 volume (mL/g of liver) of 2 mM $MgCl_2$, 10 mM Tris-HCl, pH 7.4, and the residual DNA digested with 80–160 μg/mL deoxyribonuclease for 30 min at 37 °C. Digestion was terminated by addition of 1/9th volume of 10× SET, and the nucleic acids were extracted as described above. Contaminating oligodeoxyribonucleotides were removed by chromatography on Sephadex G-75. The precipitated RNA was washed with 95% ethanol prior to use.

Determination of Poly(A) Content. Poly(A) content of purified RNA was determined by a method similar to that of Rosbash & Ford (1974). Six to 12 μg of RNA was annealed with 1.5 μg of [^{3}H]poly(U) (20–40 μCi of p/μmol) in 0.5 mL of 2× SSC (300 mM NaCl, 30 mM sodium citrate) for 30 min at 45 °C. Two milliliters of 25 μg/mL RNase A in 2× SSC were added and the samples incubated for 90 min at 37 °C. Bovine serum albumin was added to a final concentration of 100 μg/mL, and the hybrids were precipitated with an equal volume of cold 10% trichloroacetic acid. The precipitates were collected on Whatman GF/C filters. The filters were rinsed with cold 5% Cl_3CCOOH and with cold ethanol, dried, treated with Hyamine hydroxide at 70 °C for 90 min, and counted in 10 mL of Omniflour–toluene in a Nuclear Chicago scintillation counter. Samples containing known amounts of poly(A) standard and reagent blanks containing [^{3}H]poly(U) but no RNA were included in all asssays. The radioactivity of the reagent blanks was subtracted from that of the experimental and standard samples. The values obtained from the poly(A) standards were used to construct a standard curve from which the poly(A) contents of the experimental samples were determined. RNA concentrations were determined spectrophotometrically using an $E_{260}^{0.1\%}$ of 24 in 2× SSC.

After determining the percentage of total RNA which is comprised by poly(A)$^+$RNA, the absolute amount of cytoplasmic or nuclear poly(A)$^+$RNA/mg of DNA was calculated using the total RNA/DNA ratios obtained from whole homogenates or from purified nuclei, respectively, thus obviating problems associated with variability in RNA recovery.

Determination of the Length of Polyadenylated mRNA. Sucrose density gradient centrifugation under denaturing conditions was performed according to the method of Bantle et al. (1976). Approximately 0.4 mg of RNA was layered onto 35-mL gradients and centrifuged in an International SB 110 rotor at $105000g_{max}$ for 24 h at 20 °C. The fractions from the gradients were adjusted to 130 μg/mL *E. coli* tRNA and precipitated with 3 volumes of 95% ethanol containing 0.2 M sodium acetate. The distribution of poly(A)$^+$-RNA along the gradient was determined by dissolving the ethanol precipitates with 0.2 mL of 2× SSC and hybridizing with 0.6 μg of [^{3}H]poly(U) as described above.

The mean $s_{20,w}$ of each fraction was determined using 4, 18, and 28S RNA as standards. The mean nucleotide length was determined from each fraction according to the method of Spirin (1963). The weight average nucleotide length was calculated using the formula: $L_w = (cpm_i)(L_i)/(cpm_i)$, where L_i is the mean nucleotide length of the poly(A)$^+$-RNA in each fraction.

Centrifugation under nondenaturing conditions was performed using a 35-mL gradient of 15–30% sucrose in 100 mM NaCl, 1 mM EDTA, 0.5% $NaDodSO_4$, and 10 mM Tris-HCl, pH 7.4 at $105000g_{max}$ for 15 h at 16 °C. The fractions were precipitated with ethanol and hybridized with [^{3}H]poly(U) as described above.

Determination of Poly(A) Tract Length. Poly(A) tracts were obtained by digesting 0.5 mg of RNA in 1 mL of 500 mM NaCl, 1 mM EDTA, 10 mM Tris-HCl, pH 7.5, with 2 μg of RNase A and 5 units of RNase T_1 for 30 min at 37 °C. The solution was brought to 0.2% in sodium dodecyl sulfate and extracted with an equal volume of isoamyl alcohol: chloroform (1:24) at room temperature. The interface and the organic phase were extracted with 0.5 volume of the above buffer containing 0.2% $NaDodSO_4$. The pooled aqueous phases were reextracted twice more and 1.5 A_{260} units of *E. coli* tRNA was added as carrier. An equal volume of cold 2 M NaCl, pH 5.0, was added followed by 2 volumes of cold 95% ethanol. The samples were precipitated overnight at −20 °C. The precipitates were washed with 95% ethanol and dissolved in 0.21 mL of H_2O.

Forty-eight microliters of each sample was electrophoresed in parallel with poly(A) standards of known length in 6-mm thick 2.5% acrylamide, 0.5% agarose slab gels (Peacock & Dingman, 1968) for 4h at 100 V. Lanes containing poly(A) standards were excised, stained with "stains-all" (Dahlberg et al., 1969), and scanned at 570 nm. Lanes containing

experimental samples were cut into 0.33-cm fragments. The material was eluted from diced fragments by shaking with 1 mL of 2× SSC for 16 h at room temperature. The amount of poly(A) in each fraction was determined by hybridizing 0.8 mL of the eluted material with 0.125 μg of [^{3}H]poly(U) using the procedure described above.

The mean nucleotide length of the poly(A) in each gel fragment was determined from a standard curve constructed by plotting the log of the nucleotide lengths of the standards vs. distance migrated. Weight average nucleotide lengths were calculated as described above.

Cytoplasmic Distribution of Poly(A)$^+$-RNA. Cytoplasmic extracts were prepared according to the method of Sippel et al. (1977) with minor modifications. Livers from five or more rats were homogenized in 5 volumes (mL/g of liver) of 0.25 M sucrose–TNMDH (50 mM Tris-HCl, pH 7.4, 25 mM NaCl, 5 mM $MgCl_2$, 1 mM dithiothreitol, 100 μg/mL sodium heparin) in a Potter-Elvehjem homogenizer at 200 rpm. The homogenate was adjusted to 1% Triton X-100 and 1% deoxycholate, stirred for 2 min in the cold, and centrifuged at $13000g_{av}$ for 10 min at 2 °C. The resultant supernatant was recentrifuged as above and diluted threefold with 0.25 M sucrose–TNMDH. Aliquots of 0.3 mL were layered onto 34-mL gradients of 10–50% (w/v) sucrose in TNMDH and centrifuged, along with ribonuclease A treated samples, at $105000g_{max}$ for 3 h at 4 °C in an International SB110 rotor. The monoribosome peak was identified by comparing the polysome profiles with those of ribonuclease treated samples. Gradients were fractionated, as shown in Figure 5, into subpolysomal and polysomal fractions and the fractions precipitated with 2 volumes of 95% ethanol after addition of 0.25 volume of 4.0 M sodium acetate, pH 5.0. The precipitates were dissolved in 1× SET and extracted with SET-saturated phenol:chloroform and with isoamyl alcohol:chloroform, and the RNA was precipitated as above. The precipitates were dissolved in 2× SSC and assayed for poly(A) content.

To ensure that differences in poly(A) distribution were not due to variability in RNA yields following extraction, the poly(A) values were corrected for losses in the aqueous volume incurred during extraction. Volume loss was shown to be directly proportional to RNA loss by extracting the two gradient fractions together with a known amount of added ^{14}C-labeled rat liver nuclear RNA. The proportion of volume lost was found to be equivalent to that of radioactivity lost. Such corrections had an insignificant effect on the calculated distribution of cytoplasmic poly(A).

The proportion of polysomes with nine or more ribosomes was determind by cutting out tracings of the appropriate fractions and weighing the paper.

RNA and DNA Determinations. RNA was assayed according to the method of Munro & Fleck (1966). DNA was determined according to the method of Burton (1956), using calf thymus DNA as standard.

Results

Size Distribution of Poly(A)$^+$-RNA. The size distribution of either cytoplasmic or nuclear poly(A)$^+$-RNA was not altered during liver regeneration. This agrees with our previous report of a lack of difference in the sizes of polysomal poly(A)$^+$-RNA from normal (sham-operated) and 12-h regenerating livers (Colbert et al., 1977). Representative sucrose density gradient profiles are shown in Figure 1. Cytoplasmic poly(A)$^+$-RNA had an average sedimentation coefficient of approximately 15 S with a range of about 4–30 S (Figure 1a). These values are similar to those reported by Sippel et al. (1977) and Moore et al. (1977) for polysomal poly(A)$^+$-RNA

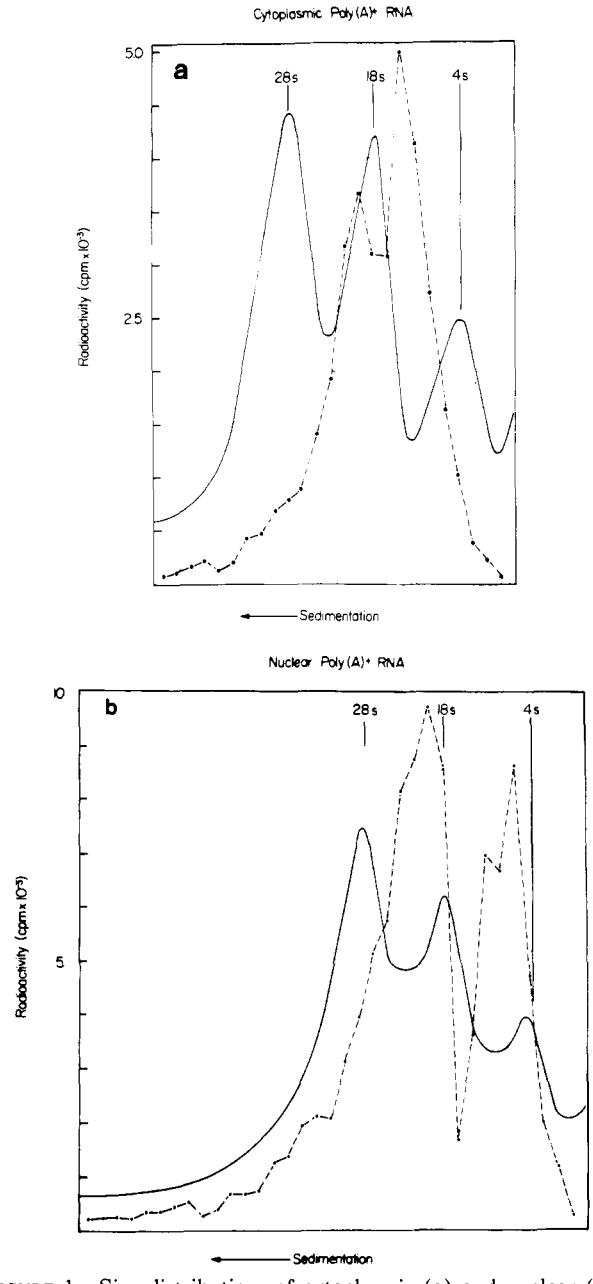

FIGURE 1: Size distributions of cytoplasmic (a) and nuclear (b) poly(A)$^+$-RNA. RNA was extracted from postmitochrondrial supernatant or isolated nuclei, and centrifuged through denaturing sucrose density gradients, and the fractions were hybridized with [^{3}H]poly(U) to localize poly(A)$^+$-RNA. A_{260} (—); cpm (---).

from normal rat liver. The weight average nucleotide length was calculated to be approximately 1500 nucleotides (about 16 S). Cytoplasmic poly(A)$^+$-RNA was often resolved into two peaks (Figure 1a) regardless of the regenerative state of the liver.

Nuclear poly(A)$^+$-RNA (Figure 1b) was invariably resolved into two peaks with average sedimentation coefficients of approximately 8 and 19 S. The overall size distribution ranged from less than 4 S to greater than 45 S. In contrast, Sippel et al. (1977) found only one size class with an average sedimentation coefficient somewhat greater than 18 S. The weight average nucleotide length of our nuclear poly(A)$^+$-RNA preparations was calculated to be approximately 4100 nucleotides (about 26 S). Thus liver nuclear poly(A)^1-RNA molecules are on the average larger than their cytoplasmic counterparts, in agreement with the report of Sippel et al. (1977).

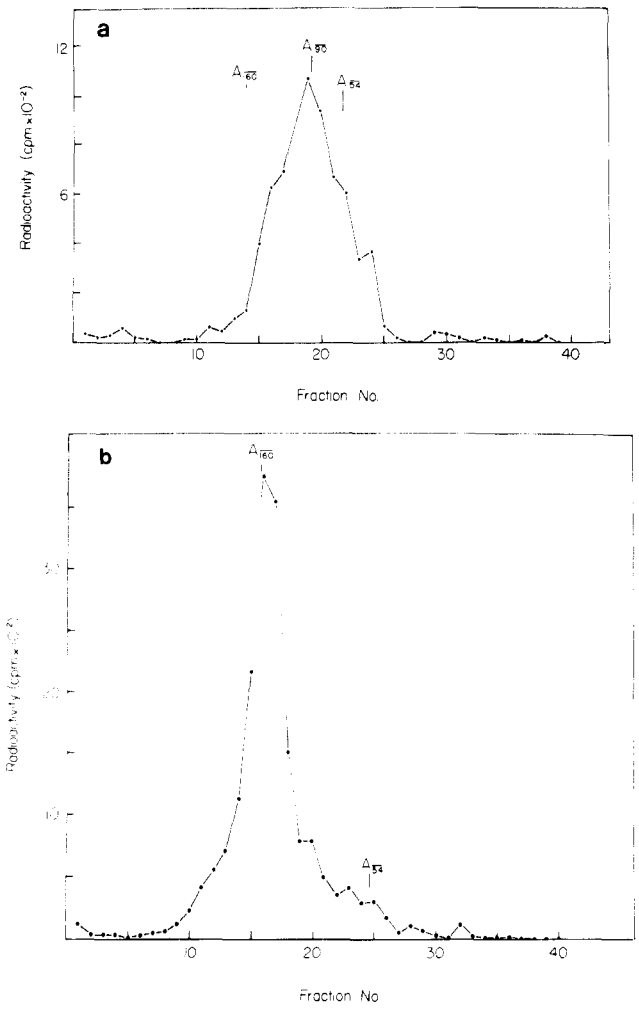

FIGURE 2: Size distributions of cytoplasmic (a) and nuclear (b) poly(A) tracts. RNA digested with RNase A and T_1 was electrophoresed and poly(A) localized by hybridization with [^{3}H]poly(U). Positions of poly(A) markers of known nucleotide length are indicated.

Nucleotide Length of Poly(A) Tracts. Nucleotide lengths were determined for nuclear and cytoplasmic poly(A) obtained from normal livers and from livers of sham-operated and partially hepatectomized rats 12 and 24 h after the operation. Representative size distribution profiles of nuclear and cytoplasmic poly(A) tracts are shown in Figure 2. No discrete size subclasses were unambiguously evident in any of the preparations. The weight average nucleotide lengths of cytoplasmic and of nuclear poly(A) tracts ranged from 113 to 141 and 165 to 195 nucleotides, respectively. The nucleotide lengths of poly(A) were independent of the regenerative state of the liver. Therefore, the average values of 124 and 175 nucleotides for cytoplasmic and nuclear poly(A), respectively, were used in all subsequent calculations.

Hepatic Content of Poly(A)$^+$-RNA Molecules. The proportions of poly(A) in nuclear RNA and in RNA extracted from postmitochondrial supernatants were used together with the ratios of nuclear or total RNA to DNA to calculate the absolute amount of nuclear or cytoplasmic poly(A)/mg of DNA. This value, together with the weight average nucleotide length of the poly(A) and Avogadro's number, was used to calculate the number of poly(A) tracts/mg of total DNA. This number is equivalent to the number of poly(A)$^+$-RNA molecules/mg of DNA, since each molecule contains one poly(A) tract (Brawerman, 1974).

As shown in Figure 3, the number of liver cytoplasmic poly(A)$^+$-RNA molecules/mg of DNA increased approximately 2.2-fold by 12 h after partial hepatectomy, remained elevated until 24 h and decreased by 48 h to a value still greater than that of sham-operated controls. The DNA content per nucleus remains constant during at least the first 12 h after partial hepatectomy (Van Lancker & Sempoux, 1958). Thus the doubling in number of poly(A)$^+$-RNA molecules/mg of DNA reflects a doubling in the content of poly(A)$^+$-RNA molecules per cell. Livers of sham-operated animals had a much smaller increase in the number of cytoplasmic poly(A)$^+$-RNA molecules/mg of DNA, with a maximal value only about 35% greater than that of unoperated controls (Figure 3).

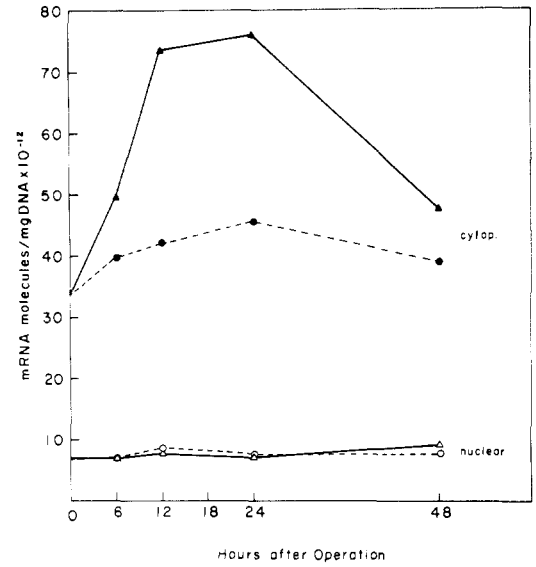

FIGURE 3: Content of nuclear (○, △) and cytoplasmic (●, ▲) poly(A)$^+$-RNA in livers of sham-operated (---) and partially hepatectomized (—) rats at various times after operation. Nuclear and cytoplasmic RNA was extracted and the number of poly(A)$^+$-RNA molecules/mg of DNA was determined as described in Materials and Methods. Each point in the graph corresponds to three different experiments. In each experiment an average of four animals was used.

In contrast to cytoplasmic poly(A)$^+$-RNA, the number of nuclear poly(A)$^+$-RNA molecules/mg of DNA in sham-operated and partially hepatectomized animals increased by only 30 and 40%, respectively, during the 48-h period studied (Figures 3 and 4). The ratio of the number of cytoplasmic to the number of nuclear poly(A)$^+$-RNA molecules increased twofold at 12 and 24 h after partial hepatectomy, while in sham-operated animals this ratio remained essentially constant.

The changes in poly(A)$^+$-RNA/mg of DNA were not due to alterations in the proportion of polysomes included in the postmitrochondrial supernatants because for these calculations RNA/DNA ratios of total homogenates were taken into account and the values obtained were thus independent of polysome yields. In addition, the RNA content of postmitochondrial supernatants relative to that of total homogenates did not change during liver regeneration, suggesting that the proportion of polysomes in the postmitochondrial extracts remains constant.

As shown in Figure 4, the accumulation of cytoplasmic poly(A)$^+$-RNA in regenerating livers occurred at a faster rate and was proportionately greater than that of total cellular RNA, most of which is ribosomal RNA (Adelman et al., 1973). At the same time, the amount of nuclear poly(A)$^+$-RNA rose only 15–20% despite an approximately 50% increase in total nuclear RNA after partial hepatectomy. In sham-operated rats, total cellular and nuclear RNA did not change while nuclear and cytoplasmic poly(A)$^+$-RNA rose by 30 and

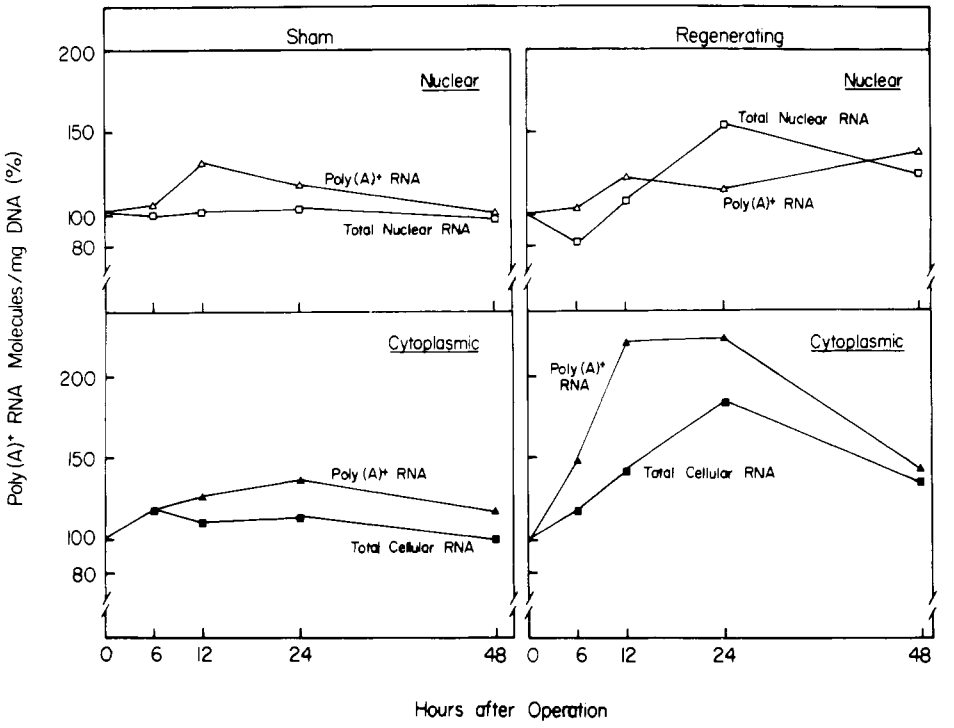

FIGURE 4: Changes in the number of poly(A)$^+$-RNA molecules (△, ▲) and in total RNA content (□, ■) per mg of DNA in nuclei (△, □) and cytoplasm (▲, ■) of sham-operated and partially hepatectomized rats relative to that of unoperated controls. The values for unoperated controls per mg of DNA are: 2.29 mg of total cytoplasmic RNA, 0.178 mg of total nuclear RNA, 33.5×10^{12} cytoplasmic poly(A)$^+$-RNA molecules. The number of animals used is the same as that indicated for Figure 3.

35%, respectively, during this time.

The amounts of mitochondrial RNA and DNA were not taken into account in the results presented in Figures 3 and 4. When the data were corrected using our own unpublished data and those of Price & Laird (1950), the absolute estimates of the number of poly(A)$^+$-RNA molecules/mg of DNA were lower but the relative magnitude and the pattern of increase of polyadenylated mRNA in regenerating livers were the same as those presented in Figures 3 and 4.

Cytoplasmic Distribution of Poly(A)$^+$-mRNA. The reported increase in protein synthesis during the first 3 h following partial hepatectomy (Tsukada et al., 1968) might be caused in part by a shift of preexisting mRNA from subpolysomal particles into polyribosomes. Postmitochondrial supernatants were fractionated by sucrose density gradient centrifugation and the amount of poly(A)$^+$-RNA in polysomes and subpolysomal fractions was determined.

Increases in the size of polysomes during liver regeneration have been described by Webb et al. (1966), Cammarano et al. (1965), and Zweig & Grisham (1971). Using methods which do not require prior polysomal separation, we detected an increase in polysomal size as early as 3 h after partial hepatectomy (Figure 5). The proportion of polysomes containing nine or more ribosomes changed from 51% in livers of unoperated rats to 59, 64, and 67% in 3-, 12-, and 24-h regenerating livers.

Treatment of postmitochondrial supernatants from normal or regenerating livers with ribonuclease or 25 mM EDTA caused a shift of practically all of the A_{260}-absorbing material to the subpolysomal region of the gradients. This is further confirmation that the material contained in the denser region of the gradient is almost entirely polysomal material.

Polysomes of normal, 3-, 12-, and 24-h regenerating liver contained, respectively, 61, 57, 70, and 60% of the total cytoplasmic polyadenylated mRNA. Thus, although a substantial fraction of poly(A)$^+$-RNA is not associated with

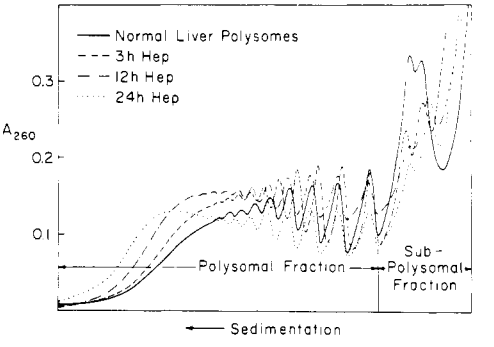

FIGURE 5: Distribution of polysomes from normal and regenerating livers. In all cases, 0.3 mL (equivalent to 0.02 g of liver) of postmitochondrial supernatant was layered onto sucrose density gradients and centrifuged as described in Materials and Methods. Portions of the gradient corresponding to subpolysomal and polysomal fractions are indicated.

polysomes in normal and regenerating liver, recruitment of a major portion of this preexisting mRNA does not occur prior to the time of maximal increase of cytoplasmic mRNA. Figure 6 shows that the absolute number of polyadenylated mRNA molecules/mg of DNA increases after partial hepatectomy both in polysomes and in subpolysomal fractions. The pattern of change in the amount of polysomal mRNA during liver regeneration roughly parallels that of whole cytoplasmic mRNA shown in Figures 3 and 4. However, the poly(A)$^+$-RNA in the subpolysomal fraction may contain a substantial proportion of partially degraded RNA since it sedimented at approximately 8 S (Figure 7) compared with about 15 S for total cytoplasmic poly(A)$^+$-RNA (Figure 1a).

Discussion

Although there are several studies comparing the synthesis, size distribution, and complexity of polyadenylated mRNA in resting and proliferating cells in culture (Getz et al., 1976,

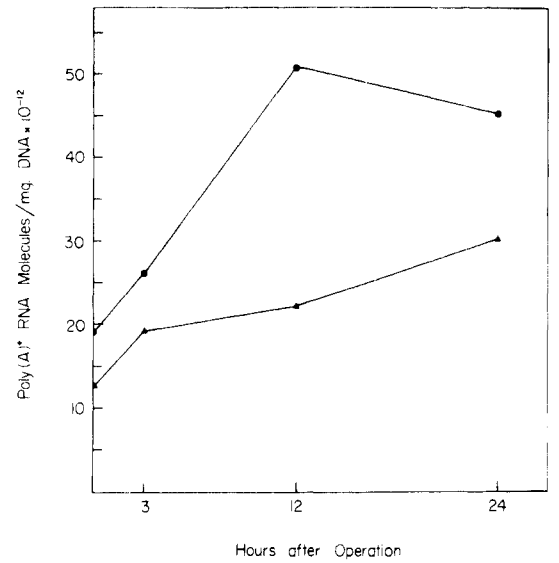

FIGURE 6: Content of liver polysomal (●) and subpolysomal (▲) poly(A)$^+$-RNA/mg of DNA at various times during regeneration. RNA was extracted from subpolysomal and polysomal fractions (see Figure 5) and poly(A) content determined as in Materials and Methods.

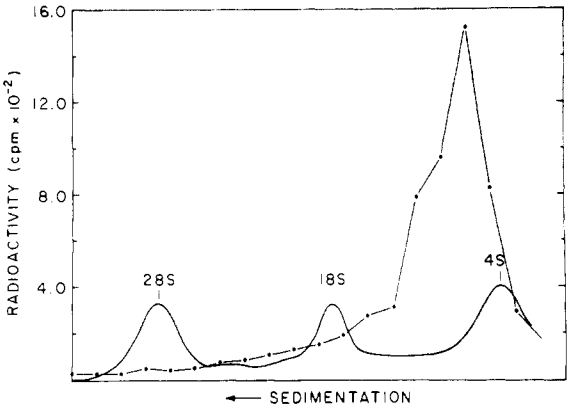

FIGURE 7: Size distribution of subpolysomal poly(A)$^+$-RNA from normal liver. One hundred micrograms of RNA obtained from the subpolysomal fraction (Figure 5 and 6) was centrifuged through nondenaturing sucrose density gradients. Fractions (0.965 mL) were collected and the RNA was precipatated with ethanol using tRNA as carrier. The RNA in each fraction was hybridized with [^{3}H]poly(U) to determine the size distribution of polyadenylated mRNA, A_{260} (—); cpm (- - -).

1977; Johnson et al., 1974, 1975; Rolton et al., 1977; Williams & Penman, 1975; Williams et al., 1977), little information is available on mRNA synthesis and accumulation in growing or regenerating mammalian organs in vivo. Since hepatocytes of adult animals do not divide under normal circumstances, it might be expected that the reprogramming necessary to initiate liver cell replication in vivo may differ considerably from the prereplicative changes which take place in cultured cells following stimulation by serum or mitogens.

We have found that in the first 12 h following partial hepatectomy, cytoplasmic and polysomal polyadenylated mRNA increased by 120%. These changes were not preceded by a shift of poly(A)$^+$-RNA from subpolysomal particles into polysomes. Despite the large accumulation of cytoplasmic mRNA, polyadenylated nuclear RNA rose by only 15–20%, resulting in an increase in the ratio of cytoplasmic to nuclear poly(A)$^+$-RNA. Neither the average size of nuclear poly(A) tracts nor that of cytoplasmic changed during liver regeneration, in agreement with similar observations in quiescent and growing cells in culture (Brawerman & Diez, 1975; Johnson et al., 1976). Glazer (1976) reported that the rate of incorporation of [^{3}H]orotic acid into nuclear poly(A)$^+$-RNA is elevated 2 h after partial hepatectomy and that the proportion of the total HnRNA that is polyadenylated does not change. Taken together, these data suggest that the observed rise in mRNA during liver regeneration is due to an increase in the efficiency of HnRNA transcription and conversion into cytoplasmic poly(A)$^+$-RNA.

Although the complexities of nuclear and polysomal poly(A)$^+$-RNA do not change during the first 12 h of regeneration (Colbert et al., 1977; Tedeschi et al., 1978), the amount of polysomal poly(A)$^+$-RNA approximately doubles, while nuclear polyadenylated RNA remains essentially constant. We have shown previously that during this stage of regeneration there appears to be no qualitative changes in nuclear RNA transcripts and only small qualitative alterations in polysomal poly(A)$^+$-mRNA. Thus, most of the newly synthesized poly(A)$^+$-RNA is similar to that of normal liver. Preferential transport of certain sequences of poly(A)$^+$-mRNA from nucleus to cytoplasm may take place during the regenerative process although a relatively small proportion of genes might be transcribed only in hypertrophic livers (Colbert et al., 1977; Tedeschi et al., 1978).

Cytoplasmic poly(A)$^+$-RNA increased proportionately more and at a faster rate than total RNA. This was also indicated by an increase in the proportion of poly(A) tracts in polysomal RNA from 0.08% in livers of unoperated rats to 0.15% in 12-h regenerating livers. Simiar increases in the ratio of polysomal poly(A)$^+$-RNA to polysomal RNA have been previously observed in regenerating liver (Fausto, unpublished observations; Glazer, 1977) and in mammalian cells stimulated to divide in vitro (Johnson et al., 1974; Getz et al., 1976). A disproportionate increase in polysomal polyadenylated mRNA relative to rRNA during liver regeneration would occur if the number of ribosomes bound per mRNA decreased. Paradoxically, polysome size increased very early during the regenerative process without a detectable change in the size of poly(A)$^+$-mRNA. This may suggest that nonadenylated mRNA molecules present in polysomes of normal liver are replaced by poly(A)$^+$-mRNA during regeneration.

Tsukada et al. (1968) have reported a large increase in protein synthesis during the first 3-h following partial hepatectomy in rats. This change in protein synthesis takes place prior to detectable increases in messenger and ribosomal RNA content and could be explained by (a) an increase in the number of ribosomes bound per mRNA or (b) recruitment of preexisting mRNA into polysomes. Only the first explanation appears valid. The second mechanism is apparently not operative since the percentage of poly(A)$^+$-RNA unattached to polysomes did not change throughout regeneration.

In the liver, as in certain mammalian cells in vitro (Spohr et al., 1970; Bandman & Gurney, 1975; McLeod, 1975), a substantial proportion of cytoplasmic poly(A)$^+$-RNA was not bound to polysomes. However, in our experiments, subpolysomal poly(A)$^+$-RNA was on the average smaller than total cytoplasmic poly(A)$^+$-RNA and may thus contain mRNA degraded in vivo or during isolation. It is also possible that the proportion of polyadenylated mRNA not bound to polysomes is high because the animals were starved overnight before the experiments (Yap et al., 1978).

It should be stressed that our conclusions regarding mRNA accumulation during liver regeneration refer exclusively to adenylated molecules since we made no estimations of the amounts of nonadenylated mRNA present in the various

preparations. A second source of uncertainty is that the cytoplasmic RNA used in these experiments may not represent the true intracellular population because the RNA was obtained from postmitochondrial supernatants without prior detergent treatment of the homogenates. Thus, a preferential loss of membrane-bound polysomes may have occurred. However, RNA from postmitochondrial supernatants of normal and 12- and 24-h regenerating livers have the same proportion of polyadenylated mRNA whether or not the original homogenates are treated with detergents (unpublished observations).

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References

Adelman, M. R., Blobel, G., & Sabatini, D. D. (1973) *J. Cell Biol. 56*, 191–205.

Bandman, E., & Gurney, T., Jr. (1975) *Exp. Cell Res. 90*, 159–168.

Bantle, J. A., Maxwell, I. H., & Hahn, W. E. (1976) *Anal. Biochem. 72*, 413–427.

Brawerman, G. (1974) *Annu. Rev. Biochem. 43*, 621–642.

Brawerman, G., & Diez, J. (1975) *Cell 5*, 271–280.

Brownhill, T. J., Jones, A. S., & Stacey, M. (1959) *Biochem. J. 73*, 434–438.

Bucher, N. L. R., & Swaffield, M. N. (1966) *Biochim. Biophys. Acta 129*, 445–459.

Bucher, N. L. R., & Malt, R. A. (1971) *Regeneration of Liver and Kidney*, Little, Brown and Co., Boston, MA.

Burton, K. (1956) *Biochem. J. 62*, 315–322.

Busch, H., Choi, Y. C., Kaskal, I., Inagaki, A., Olson, M. O. J., Reddy, R., Ro-Choi, T. S., Shibata, H., & Yeoman, L. C. (1972) *Gene Transcription Reprod. Tissue, Trans. Karolinska Symp. Res. Methods Reprod. Endocrinol., 5th*, 35–63.

Cammarano, P., Giudice, G., & Lukes, B. (1965) *Biochem. Biophys. Res. Commun. 19*, 487–493.

Colbert, D. A., Tedeschi, M. V., Atryzek, V., & Fausto, N. (1977) *Dev. Biol. 59*, 111–123.

Dahlberg, A. E., Dingman, C. W., & Peacock, A. (1969) *J. Mol. Biol. 41*, 139–147.

Fausto, N., & Butcher, F. R. (1976) *Biochim. Biphys. Acta 428*, 702–706.

Getz, M. J., Elder, P. K., Benz, E. W., Jr., Stephens, R. E., & Moses, H. L. (1976) *Cell 7*, 255–265.

Getz, M. J., Reiman, H. M., Jr., Siegal, G. P., Quinlan, T. J., Proper, J., Elder, P. K., & Moses, H. L. (1977) *Cell 11*, 909–921.

Glazer, R. I. (1976) *Biochim. Biophys. Acta 418*, 160–166.

Glazer, R. I. (1977) *Biochim. Biophys. Acta 475*, 492–500.

Greene, R. F., & Fausto, N. (1974) *Biochim. Biophys. Acta 366*, 23–34.

Higgins, G. M., & Anderson, R. M. (1931) *Arch. Pathol. 12*, 186–202.

Johnson, L. F., Abelson, H. T., Green, H., & Penman, S. (1974) *Cell 1*, 95–100.

Johnson, L. F., Williams, J. G., Abelson, H. T., Green, H., & Penman, S. (1975) *Cell 4*, 69–75.

MacLeod, M. C. (1975) *Biochemistry 14*, 4011–4018.

Moore, R. E., Cardelli, J. A., & Pitot, H. C. (1977) *Biochemistry 16*, 3037–3044.

Munro, H. N., & Fleck, A. (1966) *Methods Biochem. Anal. 14*, 113–176.

Peacock, A. C., & Dingman, C. W. (1968) *Biochemistry 7*, 668–674.

Price, J. M., & Laird, A. K. (1950) *Cancer Res. 10*, 650–658.

Rolton, H. A., Birnie, G. D., & Paul, J. (1977) *Cell Differ. 6*, 25–39.

Rosbash, M., & Ford, P. J. (1974) *J. Mol. Biol. 85*, 87–101.

Sippel, A. E., Hynes, N., Groner, B., & Shutz, G. (1977) *Eur. J. Biochem. 77*, 141–151.

Spirin, A. S. (1963) *Prog. Nucleic Acid Res. 1*, 301–346.

Spohr, G., Granboulan, N., Morel, C., & Scherrer, K. (1970) *Eur. J. Biochem. 17*, 296–318.

Tedeschi, M. V., Colbert, D. A., & Fausto, N. (1978) *Biochim. Biophys. Acta 521*, 641–649.

Tsukada, K., Moriyama, T., Umeda, T., & Lieberman, I. (1968) *J. Biol. Chem. 243*, 1160–1165.

Van Lancker, J. L., & Sempoux, D. G. (1958) *Arch. Biochem. Biophys. 77*, 129–137.

Webb, T. E., Blobel, G., & Potter, V. R. (1966) *Cancer Res. 26*, 253–257.

Williams, J. G., & Penman, S. (1975) *Cell 6*, 197–206.

Williams, J. G., Hoffman, R., & Penman, S. (1977) *Cell 11*, 901–907.

Yap, H. Y., Strair, R. K., & Shafritz, D. A. (1978) *J. Biol. Chem. 253*, 4944–4950.

Zimmerman, S. B., & Sandeen, G. (1966) *Anal. Biochem. 14*, 269–277.

Zweig, M., & Grisham, J. W. (1971) *Biochim. Biophys. Acta 246*, 70–80.