Volume 10 Number 3 1982 Nucleic Acids Research

Coordinate regulation of ribosomal protein mRNA level in regenerating rat liver. Study with the corresponding mouse cloned cDNAs

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Received 11 December 1981; Accepted 15 January 1982

ABSTRACT

Cloned mouse ribosomal protein (rp) cDNAs exhibit extensive homology with the corresponding rat sequences. The size of the rp-mRNAs and complexity of the rp-genes are very similar in the two species. Using the mouse rp-recombinant DNAs we find that the relative abundance of rat L7, L13, L18, L30, L32/33 and S16 mRNAs increases after partial hepatectomy. Their maximal level is about twice that of normal rat liver, and is achieved 12-18 h after the operation, while the relative abundance of albumin mRNA decreases to half the normal values 12 h after partial hepatectomy. This concomitant increase in the relative content of these rp-mRNAs indicates coordinate regulation of their level in the rat. The dissimilar behavior of L10 and L19 rp-mRNA suggests additional control mechanisms of rp-mRNA levels in the regenerating rat liver.

INTRODUCTION

The mammalian ribosome is composed of two subunits which together contain four molecules of RNA and more than seventy different species of protein, all of which appear in equimolar amounts (1). The genes coding for the ribosomal components are located on different chromosomes and are present in multiple copies (2,3,4). Thus to maintain the proper stoichiometry of the ribosomal components, the expression of these genes must be regulated and coordinated in a precise manner.

The prokaryotic ribosome structure and biogenesis have been studied in great detail in E. coli (for review see 5,6). The r-proteins are synthesized coordinately and stoichiometrically under a variety of conditions of cellular growth rate and metabolism (7,8). In E. coli, coordinated expression is facilitated by the presence of several polycistronic transcriptional units (6), controlled autogeneously at both the transcriptional and translational level (9). Although much less is known about the details or mechanisms of eukaryotic r-protein synthesis, it has been shown that most r-protein formation in yeast (10,11,12), mouse L-cells (13) and regenerating rat liver (14), is coordinately controlled.

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In mammals the liver responds to partial hepatectomy by a dramatic increase in macromolecular synthesis, lasting for approximately 12-16 hours after the operation. This hypertrophic phase is followed by hyperplasia characterized by a peak in DNA synthesis at about 24 hours and mitosis 6-8 hours later (for review see 15). The content of the ribosomal components in regenerating hepatocytes increases during the hypertrophic phase (16,17). Using a wheat germ cell free system, a parallel increase in the template activities of poly(A)$^+$ mRNA for r-protein has been shown (14).

Recently we isolated recombinant DNA plasmids containing mRNA sequences coding for several mouse r-protein mRNAs (18). In the study described here we have used these plasmids to demonstrate the homology between mouse and rat r-protein sequences, and to monitor the changes in the corresponding r-protein mRNAs in the regenerating rat liver. Our results indicate coordinate changes in the relative abundance of the r-protein mRNAs during the hypertrophic phase.

MATERIALS AND METHODS

Animals: Adult male Sabra HUS mice and 150 to 200 g male Sabra HUS rats were obtained from the Hebrew University breeding center. Partial hepatectomy resulting in the removal of 70% of the liver mass was performed on rats as described by Higgins and Anderson (19). Sham operated rats were laprotomized and their livers manipulated but not excised. All animals were sacrificed between 12 to 48 hours post operation but in all cases at 8 to 10 A.M. Poly(A)$^+$ mRNA was extracted from the same liver lobes in both partially hepatectomized and sham operated rats. For sequence homology studies, animals were fasted for 24 hours prior to sacrifice.

Extraction of poly adenylated mRNA from liver: Cytoplasmic RNA from mouse or rat liver was extracted as described by Schibler et al. (20). Poly(A)$^+$ mRNA was isolated according to Schibler et al. (21).

Isolation of liver DNA: DNA was prepared from isolated nuclei by phenol extraction and digestion with pronase and ribonuclease (22).

Gel electrophoresis of RNA and DNA: The poly(A)$^+$ mRNA isolated from rat and mouse livers was denatured (23) and size fractionated by electrophoresis on an agarose gel containing 2.2 M formaldehyde (24).

Mouse or rat liver DNAs were digested with 4 units EcoR1 per μg of DNA and restriction fragments were separated on 0.6% horizontal agarose slab gels (25).

Blotting and hybridization of nucleic acids: Poly(A)$^+$ mRNA and EcoR1 frag-

ments of DNA were transferred to nitrocellulose filters according to the procedures of Thomas (26) and Southern (27), respectively.

Direct immobilization of RNA on nitrocellulose paper: Poly(A)$^+$ mRNA was dot blotted on 1 cm^2 nitrocellulose filters according to Thomas (26). The relative abundance of r-protein mRNA was determined as described previously (28).

Hybridization procedures: After transfer the filters were heated to 80°C under vacuum for 2 h, and prehybridized overnight at 42°C in 50% Formamide (deionized), 5 x SSC (1 x SSC is 15 mM sodium citrate, 150 mM NaCl), 0.04% Ficoll, 0.04% Polyvinylpyrrolidone, 0.04% bovine serum albumin, 50 mM sodium phosphate pH 7.0, and 250 µg/ml denatured salmon sperm DNA. This prehybridization solution was removed and replaced with a fresh aliquot of the same solution containing 0.1% SDS, 1 mM EDTA, 10 µg/ml poly(A) and 2-5 x 10^6 cpm/ml of ^{32}P nick translated r-protein plasmid DNA (29). Hybridization was allowed to occur at 42°C for 24-48 hours. After incubation, filters were washed four times in 2 x SSC at room temperature, followed by one wash (DNA in 0.1 x SSC, 0.1% SDS; RNA in 0.5 x SSC, 0.1% SDS) at 50°C for 1 h.

RESULTS

a) Homology between mouse and r-protein sequences

Study of the r-protein gene expression in the regenerating rat liver requires a suitable set of sequence probes to identify r-protein genes and to monitor the abundance of the corresponding mRNAs. We had previously reported the construction of several mouse rp-cDNA clones (18), and their use in demonstrating substantial cross reactivity with both human (4) and hamster DNAs (3). Since the r-proteins from different mammalian species seem to be indistinguishable by two dimensional gel electrophoresis (30) the corresponding mRNAs most probably contain homologous sequences preserved during evolution.

The presence of sequence homology between rat liver and mouse liver rp-mRNAs was demonstrated by hybridizing electrophoretically size-fractionated poly(A)$^+$ mRNA with eight cloned mouse rp-cDNAs. Fig. 1 shows that for each r-protein probe a single hybridizing band is detected in poly(A)$^+$ mRNA isolated from livers of either species. Sizes of rat and mouse liver mRNA species recognized by a particular r-protein probe were very similar (Table 1). The size of mouse liver rp-mRNAs separated on formaldehyde agarose gels agrees with the corresponding mouse L-cell mRNAs, size fractionated on an agarose gel containing methyl mercury hydroxide (Table 1 and ref. 18). The apparent discrepancy in electrophoretic mobility of liver and L-cell L32/33, L30 and S16 mRNAs is unaccounted for, but may be an artifact of different de-

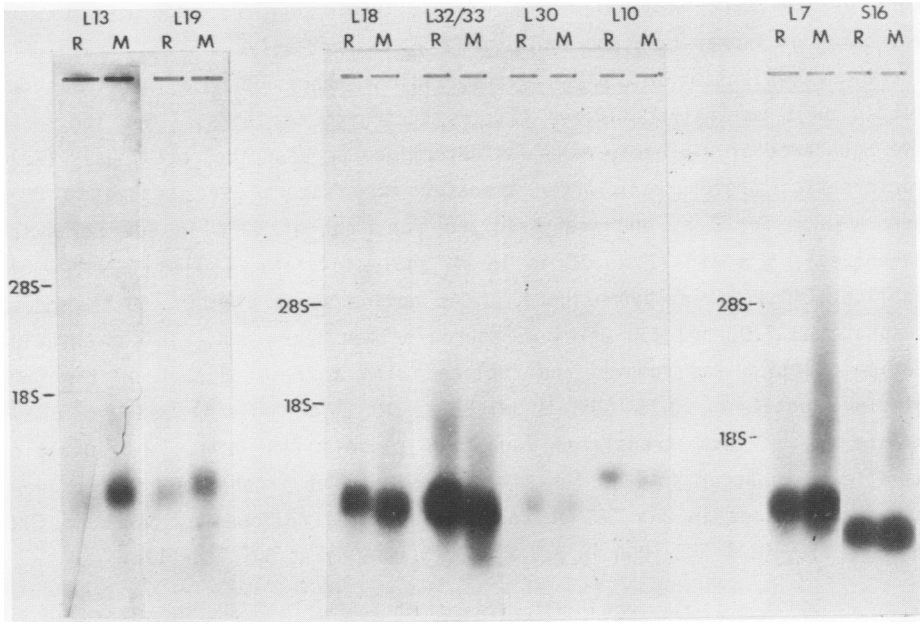

Fig. 1. Electrophoretic analysis of mouse and rat rp-mRNAs on denaturing agarose gels. 5 µg of mouse liver poly(A)$^+$ mRNA (M) and 5-10 µg, size fractionated (≤ 18 S) rat liver poly(A)$^+$ mRNA (R), were separated by electrophoresis in alternating tracks of 0.8% agarose slab gels containing 2.2 M formaldehyde, and transferred to nitrocellulose filters. Strips containing one "M" and one "R" sample were hybridized with various nick translated rp-plasmid DNAs (spec. act. 200-500 cpm/pg). The strips were washed, dried and exposed to x-ray film at -80°C. The positions of mouse 28 S and 18 S rRNA were determined on parallel tracks after staining with 2 µg/ml ethidium bromide.

naturing conditions (24,31).

b) Thermal stability of hybrids between rp-plasmid DNA and poly(A)$^+$ mRNA from rat and mouse liver

The degree of sequence homology between rp-mRNAs from mouse and rat livers, was estimated by measuring the thermal stability of their hybrids with cloned rp-cDNAs from mouse L-cells. Poly(A)$^+$ mRNA from liver of these two rodents were immobilized on nitrocellulose filters and hybridized to various nick translated rp-plasmid DNAs. Filters were washed as described in "Material and Methods" and then in 50% formamide, 0.1 x SSC, 0.1% SDS at increasing temperatures, and the radioactivity released at each temperature was determined (Fig. 2). We found differences of 0-7°C between the melting temperature (ΔTm) of rat rp-mRNA/mouse rp-cDNA hybrid and that of the homo-

Table 1. Sizes of r-protein mRNAs

Ribosomal protein	mRNA size (bases)	
	Rat	Mouse
L13	790	830
L19	830	890
L18	830	820
L32/33	780	750
L30	800	780
L10	1020	930
L7	1160	1160
S16	960	960

Values are based on data presented in Fig. 1. The estimated mRNA sizes were obtained using mouse 28 S and 18 S rRNA as molecular weight standards (21).

logous mouse rp-mRNA/mouse rp-cDNA hybrid (Table 2). Assuming that a ΔTm of 1°C results from about 1.5% mismatched base pairs (32), a ΔTm of 7°C corresponds to about 10% sequence heterogeneity within the hybridized region of mouse rp-cDNA and rat rp-mRNA. This region cannot span more than about 1/3 of the mouse mRNA sequence due to the proportion of the rp-plasmid insert relative to the size of the corresponding mRNAs (Tables 1 and 2). The Tm of mouse liver L18 mRNA/mouse L-cells L18 cDNA hybrid is about 2°C lower than the heterologous rat liver L18 mRNA/mouse L-cells cDNA hybrid (Fig. 2). This relationship was found in two independent experiments and might reflect drift in mouse L18 mRNA sequence during the propagation of mouse L-cells in culture. Variation in the pattern of rp-DNA fragments, among strains was observed in Southern blot analysis of mouse genomic DNA (4, and Meyuhas, unpublished data).

c) Southern blot analysis of mouse and rat genes encoding r-proteins

When we used the cloned rp-cDNAs in blot analysis of EcoR1 restricted mouse DNA, we observed numerous bands, indicating, together with other findings, that each individual r-protein species is represented by multiple genes in the mouse (Fig. 3 and ref. 4). Similar multiplicity of r-protein corresponding bands was obtained in Southern blot analysis of human (4) and hamster (3) genomic DNA. We observed complex fragment patterns when rat DNA

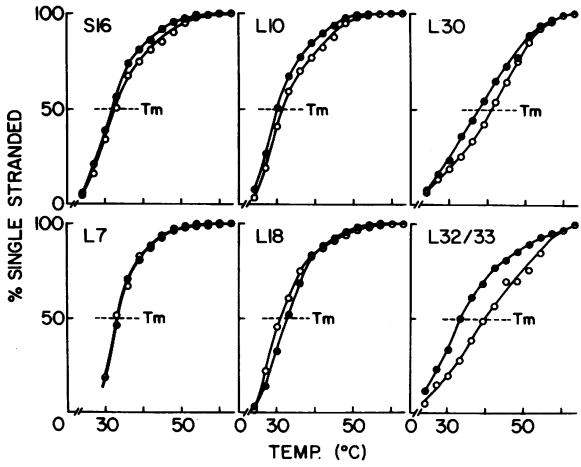

Fig. 2. Temperature stability of hybrids of rat or mouse liver rp-mRNAs and cloned mouse rp-cDNAs. 15 µg of rat (●—●) or mouse (o—o) liver cystolic poly(A)$^+$ mRNA were immobilized on nitrocellulose squares (1 cm^2) and hybridized with the indicated nick translated rp-plasmid DNA. Radioactivity not hybridized was washed as described in "Material and Methods". The filters were then incubated for 5 min in 50% formamide 0.1 x SSC, 0.1% SDS at 24°C, and subsequently the temperature was raised to 63°C in increments of 3°C. The trichloroacetic acid precipitable radioactivity, released at each temperature, was measured and plotted cumulatively. 15 µg E. coli tRNA immobilized on nitrocellulose squares served as a control for nonspecific binding and release of radioactivity throughout the experiment. Each point represents the average of two measurements.

Table 2. Homology between mouse and rat r-protein mRNAs sequences.

r-protein	Amount of mRNA sequence in probe* (bases)	Tm** (°C)	Apparent homology*** (percent)
S16	250	1	98.5
L10	350	2	97
L30	230	4	94
L7	520	0	100
L18	350	2	97
L32/33	320	7	90

* From estimates of plasmid insert (18).
** Values derived from Fig. 2.
***The homology was calculated assuming that a difference in Tm (ΔTm) of 1°C corresponds to about 1.5% mismatched base pairs (32).

was digested with EcoR1, blotted onto nitrocellulose and hybridized with various r-protein probes (Fig. 3). These results indicate that r-protein genes also exist in multiple copies in the rat.

d) The relative abundance of r-protein mRNAs in regenerating rat liver

Previous studies have shown that in regenerating rat liver the r-protein content followed the increase in the template activities of poly(A)$^+$ mRNA for r-proteins (14,16). To determine whether these increases resulted from elevation of the relative abundance of liver r-protein mRNAs following partial hepatectomy, we used the mouse rp-cDNA probes.

Increasing amounts of rat liver poly(A)$^+$ mRNA were spotted directly on nitrocellulose squares, heated to 80°C and hybridized with nick trans-

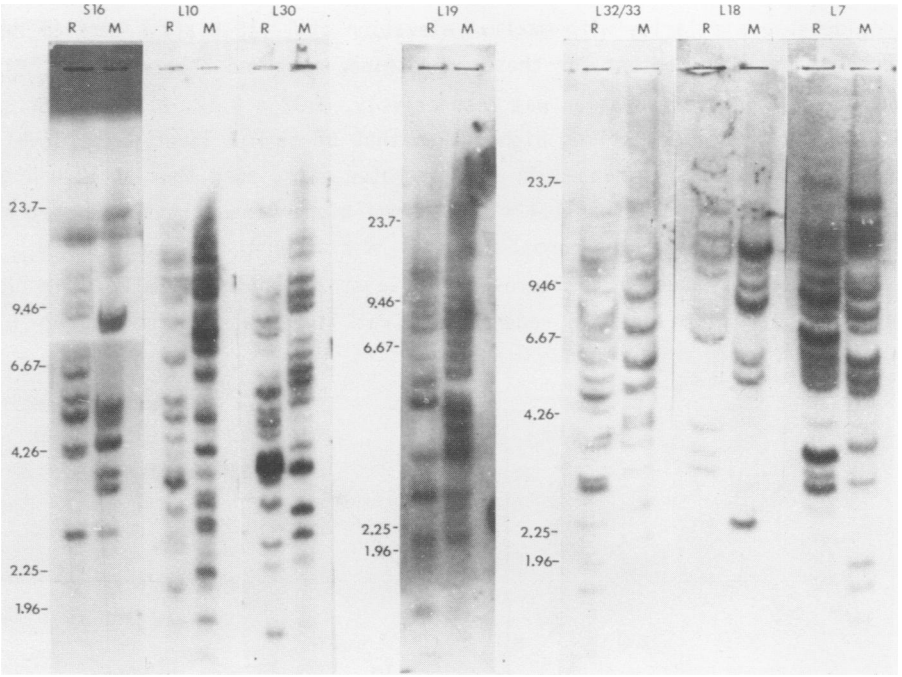

Fig. 3. Southern blot analysis of r-protein genes in liver DNA from rat and mouse. 30 µg of rat (R) and mouse (M) liver DNAs were restricted with EcoR1, size fractionated by electrophoresis in alternating tracks of 0.6% agarose slab gels and transferred to nitrocellulose filter. Strips containing one "R" and one "M" sample were analyzed for the presence of r-protein sequences by hybridization to various ^{32}P-labeled rp-plasmid DNAs, as indicated. λ DNA cleaved with Hind III was used as a molecular weight marker. The sizes of the resulting λ fragments (in kilobases) are indicated on the left of each gel.

lated L18 plasmid DNA (Fig. 4). Measurement of the radioactivity retained on the filters after extensive washes showed a linear relationship between the amounts of L18 hybridized and the amount of poly(A)$^+$ mRNA applied to the filter up to 4 μg. Similarly, we found a linear relationship using seven different r-protein probes (data not shown).

To quantitate rp-mRNA content in regenerating liver, poly(A)$^+$ mRNA was extracted from livers of either normal rats or partially hepatectomized and sham operated rats at various times after the operation. 1 μg from each poly(A)$^+$ mRNA preparation was immobilized on 1 cm^2 nitrocellulose square, and hybridized with the various nick translated rp-plasmid DNAs. The radioactivity retained in hybrid on filters was counted and plotted, against time to follow the relative abundance of rp-mRNA after the operation (Fig. 5). Comparison of the mRNA levels of L30, L7, S16, L18, L13 and L32/33 indicates considerable similarity with maximal elevation at 12-18 h after partial hepatectomy. The mRNA content for these r-proteins, measured at 12 h, 18 h, 24 h, and 48 h after the operation was respectively, 2.32 ± 0.12, 2.24 ± 0.18, 1.75 ± 0.09 and 1.67 ± 0.10 times higher than that of normal liver. The level of these rp-mRNAs also increased in sham operated rats, such that at 12 h after the operation its value was 1.4 ± 0.03 times higher than that of normal rat liver, and returned to the normal value 18-24 h after the sham operation.

L10 and L19 seemed to represent at least one unique family of r-proteins. Their mRNA level in regenerating rat liver, was maximal at 12-18 h

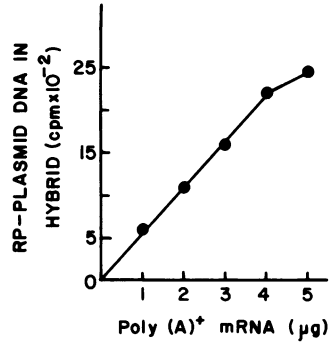

Fig. 4. Hybridization of L18 plasmid DNA with poly(A)$^+$ mRNA immobilized on nitrocellulose squares. Various amounts of rat liver poly(A)$^+$ mRNA were dot blotted onto 1 cm^2 nitrocellulose filters and hybridized with ^{32}P nick translated pL18 DNA. The filters were washed and retained radioactivity was counted to determine the extent of hybridization. Radioactivity bound nonspecifically to 5 μg E. coli tRNA, immobilized similarly, was subtracted. Each point is an average of two measurements.

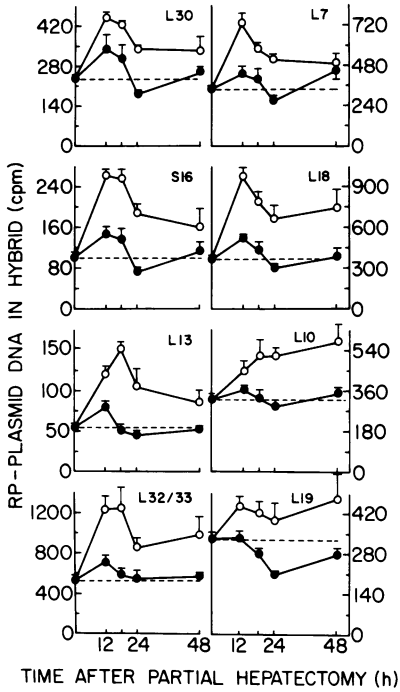

Fig. 5. Time course of the effect of partial hepatectomy on the level of rp-mRNAs in rat liver. Poly(A)$^+$ mRNA was extracted from livers of sham operated (●——●) and partially hepatectomized (o——o) rats at different times after the operation and from intact rats (●---). 1 µg of poly(A)$^+$ mRNA from each preparation was dot blotted onto 1 cm^2 nitrocellulose filters and hybridized to the indicated ^{32}P nick translated rp-plasmid DNA. The filters were washed and retained radioactivity was counted. Correction was made by subtracting the radioactivity bound nonspecifically to 5 µg E. coli tRNA. Each point represents the average cpm (± S.E.M.) obtained from 4 rat livers.

and did not decrease even 48 h after the operation. In sham operated, the levels of L10 and L19 mRNAs increased very slightly (14 and 2 percent respectively) above normal level at 12 h.

e) Albumin mRNA level in partially hepatectomized rat

The specificity of the changes in the relative abundance of rp-mRNA was assessed by comparing the alterations in their level to that of albumin mRNA. Albumin was chosen as a reference sequence since it is a major liver product and its synthesis represents a highly differentiated cell function that occurs exclusively in liver (for review see 33). The relative synthesis rate of liver albumin decreases after partial hepatectomy and subse-

quently its blood concentration drops (34). To elucidate whether this decrease results from a decrease in the albumin mRNA, its level was monitored during rat liver regeneration. The albumin plasmid (pralb-1) described by Kioussis et al. (35) and generously supplied by S.M. Tilghman was nick translated and hybridized with rat liver poly(A)$^+$ mRNA immobilized on nitrocellulose (Fig. 6). The amount of pralb-1 DNA hybridized with liver poly(A)$^+$ mRNA increased linearly with the amount of poly(A)$^+$ mRNA up to 5 µg (Fig. 6a). However, contrary to the rp-mRNAs, the relative abundance of liver albumin mRNA decreased after partial hepatectomy to half the level of normal liver at 12 h after the operation. A similar decrease was demonstrated in sham operated rats, but 12 h later (Fig. 6b).

DISCUSSION

a) Changes in rp-mRNA level during rat liver regeneration

The relative abundance of rat liver rp-mRNA increases to approximately twice the normal level within 18 h after partial hepatectomy and then decreases. A similar effect of lesser magnitude is characteristic after sham operation. Thus, the increased level of rp-mRNAs in regenerating liver may be, in part, a direct consequence of the operative trauma, rather than regenerative stimuli. However, comparison of the time courses suggests that the mRNAs of L7, L13, L18, L30, L32/33, and S16 belong to a family of mRNA exhibiting coordinate control. L10 and L19 seem to differ in that their re-

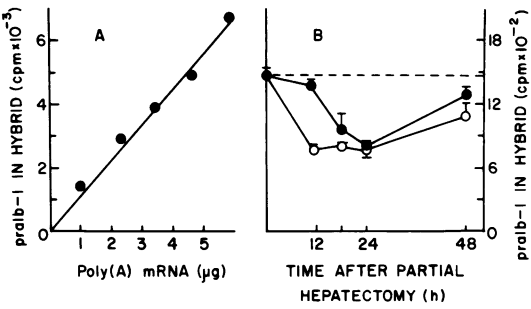

Fig. 6. Hybridization of albumin plasmid DNA with rat liver poly(A)$^+$ mRNA immobilized on nitrocellulose filters. (A) ^{32}P nick translated pralb-1 was hybridized with increasing amounts of immobilized rat liver poly(A)$^+$ mRNA. For further details see Fig. 4. (B) Time course of albumin mRNA levels in regenerating rat livers. Poly(A)$^+$ mRNA preparations from livers of partially hepatectomized (o——o), sham operated (•——•), and intact rats (•---), used also for hybridization with rp-plasmid DNAs (Fig. 5), were hybridized with ^{32}P-labeled pralb-1. For further details see Fig. 5.

lative initial elevation is considerably lower and does not decrease even 48 h after the operation (Fig. 5). We have previously shown that the multiple copies of mouse rp-genes are dispersed throughout the genome, precluding the possibility of an obligatory clustering of all rp-genes (3). Thus the functional linkage suggested here may involve only certain subsets of rp-genes as appears to be the case in E. coli (6).

The increase of relative abundance of rp-mRNA durng regeneration is of interest since similar experiments with growing mouse 3T6 fibroblasts showed no change of relative content of rp-mRNAs when compared to resting cells (36). Since hepatocytes of adult animals do not divide under normal circumstances, the prereplicative changes occuring in cultured fibroblasts following stimulation by serum, can be expected to differ considerably from changes connected with the initiation of liver cell proliferation in vivo.

Our studies strongly imply that the response of rp-mRNAs to partial hepatectomy is highly specific, since all measurements were of relative abundance using constant amounts (1 µg) of poly(A)$^+$ mRNA. Nevertheless, we sought to confirm this specificity by comparing changes in the relative abundance of rp-mRNA with that of rat liver albumin mRNA. This mRNA is highly abundant and constitutes 10% of the total poly(A)$^+$ mRNA of the normal liver (38). Contrary to the rp-mRNAs, the relative abundance of albumin mRNA decreases to half the normal value after partial hepatectomy.

The increase in relative abundance of rp-mRNAs most likely represents a net increase in the rp-mRNA content during liver regeneration since the amount of poly(A)$^+$ mRNA per gram of rat liver is similar for normal and regenerating rat liver at various times after partial hepatectomy (14). This increase follows a time course similar to that reported for the template activity for rp-mRNA (14). Thus, the stimulation of r-protein biosynthesis in regenerating liver observed previously (14,16) is caused by an increase in the content of rp-mRNAs. Variation in the rp-mRNA level reported here for rat liver may be a consequence of alterations in the rate of transcription, in processing efficiency, or in mRNA stability. Alternatively, this increase may result from activation of inactive member(s) of the rat r-protein mutigene family (Fig. 3). Investigation of the structure and organization of isolated cloned rat rp-genes, currently in progress in our laboratory, should shed more light on the regulatory mechanism(s) controlling rp-mRNAs levels in this species.

b) Homology between mouse and r-protein sequences

In previous papers we described the cloning of several mouse rp-cDNAs

and the use of these recombinant DNAs in study of the mouse rp-genes and their transcripts (3,4,18,36). In the present paper we report the use of these cloned mouse rp-cDNAs in monitoring the changes in the relative abundance of rp-mRNA in the regenerating rat liver. Initially we demonstrate the homology between the r-protein sequences in the two closely related rodents.

Electrophoretic analysis of mRNAs from mouse and rat on denaturing gels with the corresponding mouse rp-probes reveal a single and similar size class for each rp-mRNA in both species. Melting curves of mRNA/cDNA hybrids indicate a high degree of homology between mouse and rat rp-RNAs. This cross reactivity ranges between 90-100 percent (96% ± 1.5 average) with respect to the six rp-mRNAs examined. The results of our comparative study of rp-mRNAs in these species is consistent with previous reports on the similarity of patterns of mouse and rat r-proteins, separated electrophoretically on two dimensional gels (30,37). Southern blot analysis of rat genomic DNA demonstrates a pattern of hybridizing fragments of the same or greater complexity than those observed with mouse DNA, indicating that the r-protein genes exist in multiple copies in the rat as shown for the mouse (4).

Acknowledgement

We are grateful to Dr. S.M. Tilghman for the albumin plasmid. This research was supported by grants from the Israel Academy of Sciences and Humanities - Basic Research Foundation and from the United States - Israel Binational Science Foundation (BSF), Jerusalem, Israel.

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