PROCEEDINGS OF THE SOCIETY FOR EXPERIMENTAL BIOLOGY AND MEDICINE **181**, 312–317 (1986)

Effects of Starvation on Rat Liver mRNA Translation Products (42259)

GEORGETTE HOWARD, S. TIM CHITPATIMA,[1] RICHARD C. FELDHOFF, AND THOMAS E. GEOGHEGAN

Department of Biochemistry, University of Louisville School of Medicine, Louisville, Kentucky 40292

Abstract. The synthesis of rat liver protein and RNA decreases with starvation. It is not yet known whether such decreases are regulated strictly at a transcriptional level, or if post-transcriptional controls are also involved. In this study we investigate the effects of 0, 2, or 4 days starvation on the levels of specific, abundant mRNAs in total and polysomal RNA populations. The mRNAs were analyzed by translation *in vitro* in mRNA-dependent, cell-free, protein synthesizing systems. The resulting polypeptide products were separated by gel electrophoresis and visualized with fluorography. The amount of albumin translated from both polysomal and total cellular mRNA decreased 20–40% with fasting. In contrast, a specific peptide having a molecular mass of approximately 30 kDa increased two- to three-fold in total cellular RNA with a smaller increase observed in polysomal RNA. These changes were maximal at 2 days of starvation. Since starvation is known to cause alterations in liver metabolism the 30-kDa polypeptide may be related to enzymes or other proteins involved in this homeostatic response.

Total protein synthesis in the liver of many mammals decreases in response to starvation, and this is accompanied by a characteristic disaggregation of polyribosomes (1–4). Within 2 days of starvation one can detect as much as a 40% decrease in liver protein content in the rat. Also, total RNA levels decrease due to both a decrease in RNA synthesis and an increase in degradation (5, 6). Other results from starvation experiments have shown that amino acid concentrations in liver cytosol are unchanged (7). These, along with glucose refeeding studies, suggest that liver protein synthesis may be regulated by transcriptional or post-transcriptional mechanisms during starvation rather than strictly by the availability of amino acids (8).

Since total protein synthesis decreases in the starving rat, it is important to consider synthesis of albumin, the major protein produced by the liver. The rate of albumin synthesis can be influenced by a variety of hormonal and nutritional perturbations (9, 10). It has been reported that there is a specific decrease in albumin synthesis in rats subjected to starvation. This corresponds to a decrease in the levels of albumin mRNA in the cytoplasm (11). However, it has also been reported that a substantial amount of albumin RNA accumulates in an untranslated ribonucleoprotein (mRNP) form during the fasting state (3). Aside from albumin, the effects of starvation on other translatable mRNAs for liver specific proteins have not been widely investigated.

In this study we examined whether starvation caused any decrease in translatable albumin mRNA levels in both polysomal and total cellular RNA populations. Since total cellular RNA reflects both RNA in polysomes and in mRNP particles, starvation-induced accumulation of substantial albumin mRNA as untranslated mRNP would not result in a change of albumin mRNA levels. We have used mRNA-dependent cell-free protein synthesizing systems to compare total cellular and polysomal RNA from rat liver after different periods of starvation. Gel electrophoresis of the polypeptide products directed by these RNA fractions *in vitro* and analysis by autoradiography or fluorography allows us to quantitate not only the levels of translatable albumin mRNA, but also other changes which occur in the levels of specific rat liver mRNAs upon starvation.

Materials and Methods. *Animals.* Male Sprague–Dawley rats weighing 190–210 g were obtained from Charles River Breeding Laboratories, Boston, Massachusetts, and main-

[1] Current address: Department of Biochemistry, Tufts University School of Medicine, Boston, Mass. 02111.

0037-9727/86 $1.50

tained in our laboratory on a 12-hr light/12-hr dark cycle. The control animals were given Purina rat chow and water *ad libitum*. For starvation, animals were deprived of food in the morning 2 or 4 days prior to sacrifice. Because rats are nocturnal feeders, this treatment resulted in starvation periods of at least 24 and 48 hr, but which could be as much as 36 and 60 hr.

Chemicals and biochemicals. Standard chemicals and biochemicals were of the purest grade available (Sigma Chemical Co., St. Louis, Mo. or Fisher Scientific, Cincinnati, Ohio); guanidinium thiocyanate (Fluka Chemical Corp., Guappaye, N.Y.), guanidine hydrochloride and cesium chloride (Bethesda Research Laboratory, Inc., Gaithersburg, Md.), rabbit reticulocyte lysate translation kit, [3,4,5-^{3}H]leucine (sp act 158 Ci/mmole), and [^{35}S]methionine (1000 Ci/mmole) (New England Nuclear Corp., Boston, Mass.). Untoasted wheat germ was a gift of W. C. Mailhot, General Mills, Inc., Minneapolis, Minnesota.

Tissue preparation. All rats were sacrificed between 9:00 and 11:00 AM. Each liver was perfused with cold Tris-NaCl-$MgCl_2$ (TNM) buffer (50 m*M* Tris, pH 7.1, at 23°C, 25 m*M* NaCl, 5 m*M* $MgCl_2$), quickly excised, and weighed.

Polysomal preparations. Polysomes were prepared by modification of a previously described Mg(II) precipitation procedure (12). A 14% (w/v) homogenate was prepared at 4°C in a glass-Teflon homogenizer using 6 vol of 0.25 *M* sucrose containing TNM buffer and 500 μg/ml heparin. The homogenate was adjusted to 1% sodium-deoxycholate–Triton X-100, and centrifuged at 27,000*g* for 10 min at 4°C. The resultant postmitochondrial supernatant was adjusted to 0.2 *M* $MgCl_2$ and allowed to stand for 90 min at 4°C. The polysomes were collected by centrifugation at 12,000*g* for 30 min through a 1 *M* sucrose cushion containing TNM buffer, 0.1 *M* $MgCl_2$ and heparin. The polysomes were routinely monitored and shown to be undegraded by zone centrifugation through 10–40% sucrose gradients.

RNA preparation. Total RNA was prepared by extraction with guanidinium thiocyanate and centrifugation through CsCl (13). The RNA pellet was dissolved in 7.5 *M* guanidinium HCl containing 25 m*M* sodium citrate, pH 7.0, and 50 m*M* 2-mercaptoethanol. Total RNA was precipitated overnight at 33% ethanol and 16 m*M* acetic acid at −20°C. The pellet was washed twice with ethanol:0.1 *M* NaCl (2:1), once with ether, and solubilized in distilled water. RNA was stored frozen at −20°C. With these storage conditions template activity of the RNA *in vitro* was maintained for up to 6 months. Polysomal RNA was prepared from magnesium precipitated polyribosomes by alkaline-phenol extraction (14).

Translation in vitro. Total RNA was translated with a rabbit reticulocyte lysate translation kit (NEN) containing [^{3}H]leucine (15). Six micrograms of RNA was found to be optimal and well below the level at which the system was saturated. An equal amount of radioactivity from each translation reaction was prepared for gel electrophoresis by adjusting to 10% glycerol, 50 m*M* Tris, pH 6.8, 2% sodium dodecyl sulfate (SDS), and 50 m*M* 2-mercaptoethanol. The samples were loaded on a 9–20% gradient polyacrylamide–SDS slab gel. After electrophoresis at 70 V for 16 hr, the gel was stained with Coomassie blue R-250, destained, and soaked in 22.5% PPO in dimethyl sulfoxide to amplify the radioactivity for exposure to Kodak X-AR-5 film.

Polysomal RNA was translated in a wheat germ cell-free system containing [^{35}S]methionine (16). Prior to addition of RNA, the wheat germ extract was incubated with micrococcal nuclease (20 μg/ml) for 10 min at 23°C and then the nuclease was inactivated with 2 m*M* EGTA. Approximately 4 μg of polysomal RNA was used for each assay. This was also well below the saturation level for this system. Samples were prepared for gel electrophoresis as described above for the reticulocyte lysate translation products. The same amounts of the translation mixtures were loaded onto each lane of the gel.

Results. When male Sprague–Dawley rats were starved for 2 days, there was a small decrease in both the yield of RNA, and in the template activity of mRNA per microgram of RNA. Template activity was decreased in both the polysomal and total RNA populations (Table I).

The translation products from translation *in vitro* experiments were separated by polyacrylamide gel electrophoresis in the presence

TABLE I. RNA CONTENT AND TEMPLATE ACTIVITIES FROM CONTROL, 2-DAY AND 4-DAY STARVED RAT LIVER

	Polysomal		Total cellular	
	[RNA] (mg/g)[a]	Template activity (cpm/μg RNA)[b]	[RNA] (mg/g)	Template activity (cpm/μg RNA)[c]
Control, fed	5.6 (4)[d]	34,400 (4)	3.6 (2)	25,700 (2)
Starvation:				
2 days	4.8 (4)	27,600 (4)	2.1 (1)	18,900 (2)
4 days			1.9 (2)	22,700 (2)

[a] Values expressed as mg of RNA per g of liver wet weight.
[b] Translated in the wheat germ cell-free translation system. Endogenous incorporation was 5200 cpm.
[c] Translated in the rabbit reticulocyte cell-free translation system. Endogenous incorporation was 2200 cpm.
[d] The number of animals is in parentheses.

of SDS. The resulting autoradiogram for polysomal RNA was scanned with a densitometer and is presented in Fig. 1. The arrow at 4 cm corresponds to albumin having a molecular mass of 66 kDa. The arrow at 6.5 cm indicates the position of a peptide with an approximate molecular mass of 30 kDa. The amounts of these and several other translation products were estimated by both peak height and peak area measurements. In this experiment, equal aliquots of the translation products (representing equal amounts of translated RNAs) were analyzed, thus values from densitometer scans represent actual mRNA activity rather than relative activities corrected for total incorporation of [^{35}S]methionine. After 2 days of starvation, there was a 50% decrease in the amount of translatable albumin mRNA in polysomes. Some other translation products also decreased but to a lesser extent (less than 20%). The sum of these decreased translation products probably accounts for the overall decrease in total template activity of mRNA from starved animals.

It has been suggested that while albumin synthesis decreases during starvation, the levels of albumin mRNA remains relatively constant, but with an accumulation as nonpolysomal and untranslated mRNP. Since total RNA extracted with guanidinium thiocyanate would include both the polysomal and nonpolysomal components, levels of albumin mRNA might not be expected to change with starvation if indeed it accumulated as nonpolysomal mRNP. To examine this question, we included an analysis of total RNA preparations from livers of 2- or 4-day starved and control rats. The RNA was analyzed with an mRNA-dependent reticulocyte cell-free translation system. The overall specific template activity was marginally lower for RNA from starved animals consistent with what had been found for polysomal RNA (Table I). The translation products were examined by polyacrylamide gel electrophoresis and the resulting fluorogram scanned with a densitometer. The data are presented in Fig. 2. In this experiment, an equal amount of radioactive products was analyzed, so that changes observed in any individual bands are relative to changes in overall template activity. The scans demonstrate that a marked decrease in translatable albumin mRNA occurred with starvation. Other translatable mRNAs did not decrease significantly. The absolute amount of the decreased albumin mRNA activity paralleled that in the polysomal RNA population and this would support the hypothesis that albumin synthesis decreases during starvation primarily because of a decrease in translationally active albumin mRNA levels.

Starvation also resulted in markedly increased levels of the mRNA for the 30-kDa polypeptide. The relative amounts of this translation product and that of albumin were quantitated from densitometry scans, and are presented in Fig. 3. The levels of translatable albumin mRNA relative to other mRNAs decreased similarly in both polysomal and total RNA populations. The mRNA for the 30-kDa polypeptide increased by about 15% in polysomal RNA, and two- to three-fold in the total RNA from starved animals. The data indicate that starvation causes a rapid increase in the levels of this mRNA which is maximal after 2 days, decreases slightly after 4 days, but re-

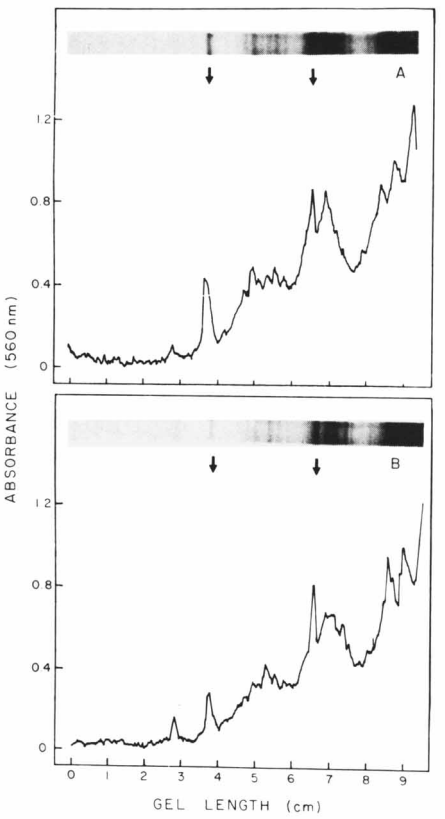

FIG. 1. Autoradiographs and densitometric scans of translation products from polysomal RNA. RNA isolated from livers of (A) fed or (B) 2-day starved rats were translated in the wheat germ cell-free translation system in the presence of [^{35}S]methionine. The peptide products were subjected to (9–20%) polyacrylamide gradient gel electrophoresis in the presence of SDS. The autoradiograph of the dried gel was scanned at 560 nm. As indicated by the arrows, albumin is the major peak at 4 cm and the 30-kDa peptide is the major peak at 6.5 cm.

mains two to three times higher than that found in control animals.

Discussion. This study was undertaken to investigate the effects of starvation on the levels of specific translatable rat liver mRNAs. There are several reports of changes in the levels and distribution of albumin mRNA during starvation or amino acid deprivation (3, 7, 8, 11, 17). While all the reports agree that albumin synthesis is decreased in response to nutritional deprivation, there is disagreement as to whether this is a transcriptional or post-transcriptional effect. Pain *et al.* (11) reported that rats on a protein-deficient diet for 2–9 days had decreased levels of total translatable al-

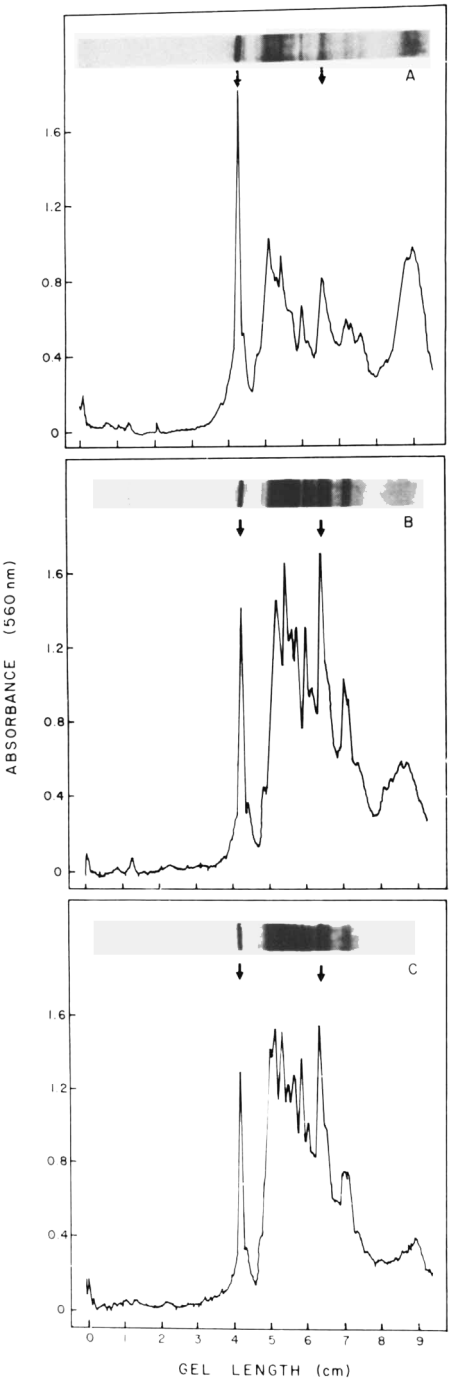

FIG. 2. Autoradiographs and densitometric scans of translation products from total cellular RNA. RNA isolated from livers of (A) fed, (B) 2-day starved, or (C) 4-day starved rats was translated in the rabbit reticulocyte lysate cell-free translation system in the presence of [^{3}H]leucine. The peptide products were treated by the same procedure as described in Fig. 1. The albumin peak and the 30-kDa peptide peak are at 4 and 6.5 cm, respectively.

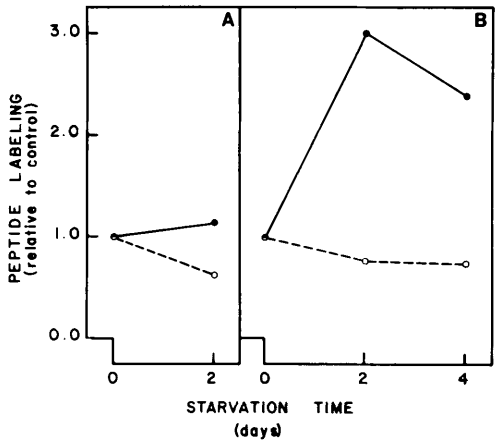

FIG. 3. Differences in albumin and 30-kDa peptide translation with starvation. Amounts are expressed as relative to fed control values. (A) Polysomal RNA products determined from Fig. 1. (B) Total cellular RNA products determined from Fig. 2. In both (A) and (B): albumin (○); 30-kDa peptide (●).

bumin mRNA and albumin synthesis. Yap *et al.* (3) using short-term starvations (24–30 hr) reported that while albumin synthesis decreased, albumin mRNA levels remained constant but accumulated in the postpolysomal fraction. This would suggest that the response of albumin synthesis to starvation is regulated by some specific translational control mechanism.

Our experiments analyzed translatable activities of mRNA derived from rat liver polysomal and total RNA populations after several days of starvation. Analysis of mRNA populations by translation *in vitro* requires that the levels of mRNA used be below saturation for the systems. In our studies care was taken to assure that this was true. The results showed a 20–40% decrease in translatable albumin mRNA upon starvation in both types of RNA preparations. These data verify the results obtained by Pain *et al.* (11). If we assume that starvation does not cause a change in the translational efficiency of deproteinized albumin mRNA *in vitro,* then the data suggest that nutritional deprivation results in an overall decrease in levels of albumin mRNA rather than its redistribution to a nonpolysomal compartment. It should be noted that while our data would seem to contradict results obtained by Yap *et al.* (3), there were substantial differences between the two studies. First, we have used a longer starvation time (2–4 days as opposed to 24–30 hr). It is possible that an initial short-term response to starvation may be the dissaggregation of polyribosomes, and subsequent storage of mRNAs in a ribonucleoprotein form, as has been shown in other systems (17, 18). However, continued long-term starvation could result in mRNA degradation which along with decreased synthesis would lead to decreased levels of the message as we found. Also Yap *et al.* (3) have used hybridization analysis which detects RNA sequences (including partially degraded ones) while our experiments would detect only functional, albumin mRNA.

One advantage of using translational analysis to examine a mRNA population is that changes in specific mRNAs other than the ones primarily of interest can be seen. In these studies we found that starvation induced a two- to three-fold increase in the mRNA directing the synthesis of a polypeptide with a molecular mass of 30 kDa (Figs. 1 and 2). This increase is much greater in the total RNA preparations but also occurs in polysomal RNA. The identity of this polypeptide is still unknown, although possible candidates include gluconeogenic or other enzymes involved in the homeostatic response to starvation (19), or perhaps a structural protein involved in changes which the liver undergoes in response to fasting. Since liver is the major site of plasma protein biosynthesis, the peptide could be the precursor of a protein which is not glycosylated in the cell-free translation system we used and so does not correspond in mobility on polyacrylamide gels to any known plasma protein. It is also possible that this protein is related to cellular stress proteins synthesized in mammalian cells exposed to environmental stress (20).

This research was supported in part by grants from the University of Louisville, American Heart Association–Kentucky Affiliate and the NIH (Grant 29434). The authors thank Dr. J. L. Hoffman, Dr. G. S. Schultz, Dr. Calvin A. Lang, and Dr. Kenneth P. McConnell for reviewing this manuscript. The skillful secretarial assistance of Ms. Glenda Hagan and Ms. Ellen Ford is greatly appreciated.

1. McNurlan MA, Tomkins AM, Garlick PJ. The effect of starvation on the rate of protein synthesis in rat liver and small intestine. Biochem J **178**:373–379, 1979.
2. Reid IM, Verney E, Sidransky H. Influence of acute

nutritional stress on polyribosomes and protein synthesis in brain and liver of young rats. J Nutr **100:** 1149–1156, 1970.
3. Yap SH, Strair RK, Shafritz DA. Effect of a short term fast on the distribution of cytoplasmic albumin messenger ribonucleic acid in rat liver. J Biol Chem **253:**4944–4950, 1978.
4. Henshaw EC, Hirsch CA, Morton BE, Hiatt HH. Control of protein synthesis in mammalian tissues through changes in ribosome activity. J Biol Chem **246:**436–446, 1971.
5. Enwonwu CO, Munro HN. Rate of RNA turnover in rat liver in relation to intake of protein. Arch Biochem Biophys **138:**532–539, 1970.
6. Ramsey JC, Steele WJ. Effect of starvation on the distribution of free and membrane-bound ribosomes in rat liver and on the content of phospholipid and glycogen in purified ribosomes. Biochim Biophys Acta **447:**312–318, 1976.
7. Yap SH, Hafkenscheid JCM. Effect of starvation on the synthesis rate of albumin *in vivo* and its relation to the concentrations of amino acids in the peripheral blood, the portal circulation and in the liver cytosolic fraction. Ann Nutr Metab **25:**158–164, 1981.
8. Princen JMG, Mol-Backx GPBM, Yap SH. Restoration effects of glucose refeeding on reduced synthesis of albumin and total protein and on disaggregrated polyribosomes in liver of starved rats: Evidence of a post-transcriptional control mechanism. Ann Nutr Metab **27:**182–193, 1983.
9. Feldhoff RC, Taylor JM, Jefferson LS. Synthesis and secretion of rat albumin *in vivo,* in perfused liver, and in isolated hepatocytes. J Biol Chem **252:**3611–3616, 1977.
10. Jefferson LS, Liao WSL, Peavy DE, Miller TB, Appel MC, Taylor JM. Diabetes-induced alterations in liver protein synthesis. J Biol Chem **258:**1369–1375, 1983.
11. Pain VM, Clemens MJ, Garlick PJ. The effect of dietary protein deficiency on albumin synthesis and on the concentration of active albumin messenger ribonucleic acid in rat liver. Biochem J **172:**129–135, 1978.
12. Chitpatima ST. Techniques for the purification and translation assay of rat liver mRNA: Effects of cell type and nutritional status. M.S. Thesis, University of Louisville, Ky. 1982.
13. Payvar F, Schimke RT. Improvements in immunoprecipitation of specific messenger RNA. Eur J Biochem **101:**271–282, 1979.
14. Geoghegan TE, Sonenshein GE, Brawerman G. Characteristics and polyadenylate content of the actin messenger RNA of mouse sarcoma-180 ascites cells. Biochemistry **17:**4200–4207, 1978.
15. Pelham HRB, Jackson RJ. An efficient mRNA-dependent translation system from reticulocyte lysates. Eur J Biochem **67:**247–256, 1976.
16. Roberts BE, Paterson BM. Efficient translation of tobacco mosaic virus RNA and rabbit globin 9S RNA in a cell-free system from commercial wheat germ. Proc Natl Acad Sci USA **70:**2330–2334, 1973.
17. Sonenshein GE, Brawerman G. Entry of mRNA into polyribosomes during recovery from starvation in mouse sarcoma 180 cells. Eur J Biochem **73:**307–312, 1977.
18. Geoghegan TE, Cereghini S, Brawerman G. Inactive mRNA-protein complexes form mouse sarcoma-180 ascites cells. Proc Natl Acad Sci USA **76:**5587–5591, 1979.
19. Lawson JWR, Guynn RW, Cornell N, Veech RL. In: Hanson RW, Mehlman MA, eds. Gluconeogenesis—Its Regulation in Mammalian Species. New York, Wiley, pp481–512, 1976.
20. Welch WJ, Garrels JI, Feramisco JR. The mammalian stress proteins. In: Schlesinger MJ, Ashburner M, Tissieres A, eds. Heat Shock from Bacteria to Man. Cold Spring Harbor, N.Y. Cold Spring Harbor Laboratory, pp369–377, 1982.

Received June 3, 1985. P.S.E.B.M. 1986, Vol. 181.
Accepted October 28, 1985.