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Regulation of albumin mRNA in H4 rat hepatoma cells by the availability of essential amino acids

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Deprivation of cultured H4 rat hepatoma cells for an essential amino acid (leucine, methionine, tryptophan or phenylalanine) under conditions in which the cells remain highly viable leads to a decrease in cytoplasmic albumin mRNA. The magnitude of this decrease is greatest in tryptophan-deprived and phenylalanine-deprived cells. In the tryptophan-deprived cells there is approximately a 15–17-fold decrease in albumin mRNA relative to total cytoplasmic RNA, and a 7–8-fold specific decrease in albumin mRNA relative to α-tubulin mRNA. Deprivation of the H4 cells for leucine or tryptophan causes approximately a 40–45% decrease in albumin gene transcription; however, this effect does not account for the 15–17-fold decrease in albumin mRNA abundancy caused by tryptophan limitation, or the greater effect of tryptophan limitation as compared to leucine limitation on albumin mRNA. Therefore, the decrease in albumin mRNA caused by tryptophan limitation is caused primarily by a post-transcriptional regulatory mechanism.

Nutrition is a major regulator of the synthesis of serum albumin in the liver [1–5]. In humans with kwashiorkor (protein-energy malnutrition), a reduction in plasma albumin is the most consistently observed biochemical feature [1]. A number of studies have indicated that dietary intake of essential amino acids is the major nutritional regulator of albumin synthesis [2–5]. In rats, lowering of protein in the diet causes a decrease in albumin synthesis, which is restored toward normal following administration of amino acids [2]. The role of amino acids in regulation of albumin synthesis has been further demonstrated from other feeding studies with rats, in which hypoalbuminemia is associated with a diet deficient in protein but adequate in energy [3]. Among the essential amino acids, tryptophan appears to be the limiting substrate and/or exert a specific regulatory effect on albumin synthesis in the liver [4].

Very little is known about the molecular mechanism(s) that cause the reduction in albumin synthesis under conditions of protein malnutrition. A recent report has suggested that the reduction in albumin synthesis observed in young growing rats fed a protein-deficient diet is associated with a reduction in albumin mRNA [5]. However, in this study the level of albumin mRNA was not compared to the level of any other control mRNA; thus, it was not clear whether albumin mRNA was specifically decreased relative to other mRNAs. In addition, albumin gene transcription was not measured, so that the effects of protein limitation on albumin mRNA may have been mediated either by a transcriptional or post-transcriptional mechanism.

Abbreviations: MEM, minimal essential medium; DME, Dulbecco's modified Eagle's medium.

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In a recent study of the effects of insulin on albumin mRNA levels in cultured H4 rat hepatoma cells, we obtained indirect evidence suggesting that essential amino acids exerted a regulatory effect on albumin mRNA levels in these cells [6]. Other cell culture studies have also suggested a regulatory effect of amino acids on albumin synthesis in hepatocytes [7] and mouse hepatoma cells [8]. However, the molecular mechanism for this regulation is unknown. In the present study we directly examined the effect of amino acid limitation on albumin and α-tubulin mRNA levels in the H4 rat hepatoma cells. Nuclear transcription assays were also performed to determine whether the specific changes observed in albumin mRNA levels reflect specific changes in gene transcription.

For amino acid deprivation experiments, the H4 cells were plated at a density of $2.0 \cdot 10^6$ cells per 75 cm^2 culture flask in minimal essential medium (MEM) plus 10% fetal bovine serum. After 3 days at 37 °C, the medium was aspirated and the cells were washed once with serum-free MEM. Serum-free F12/DME (a 1:1 mixture of Dulbecco's modified Eagle's medium (DME) and Ham's F12 medium, Ref. 6) was then added to each flask, and the incubation was continued for an additional 3 days. At this time, the cells had grown to form densely confluent monolayers. The medium was then changed to 10 ml serum-free MEM supplemented with 0.1 mM nonessential amino acids, or the same medium minus an essential amino acid, and incubation was continued for 24 h. Cytoplasmic RNA was extracted in the presence of 10 mM vanadyl ribonucleoside complex as described previously [6]. For Northern blot analysis, total cytoplasmic RNA (10 μg) was electrophoresed in 1% agarose gels containing 2.2 M formaldehyde. RNA was transferred to nitrocellulose filters, and the filters were hybridized overnight with ^{32}P-labeled pRSA13 serum albumin cDNA or ^{32}P-labeled kα1 α-tubulin cDNA probe, as described previously [6]. Autoradiograms were scanned with an LKB UltroScan laser densitometer. Image density units represented integrated peak area in absorption units × mm. Nuclear transcription elongation assays were performed as described previously [6], except that the hybridization results were quantified by liquid scintillation counting rather than by autoradiography.

To analyze the effect of amino acid limitation on serum albumin mRNA levels, H4 cells were cultured in serum-free MEM or MEM minus a single essential amino acid (leucine, methionine, tryptophan or phenylalanine) for a period of 24 h. A Northern blot showing albumin mRNA levels in cells cultured in complete MEM or amino

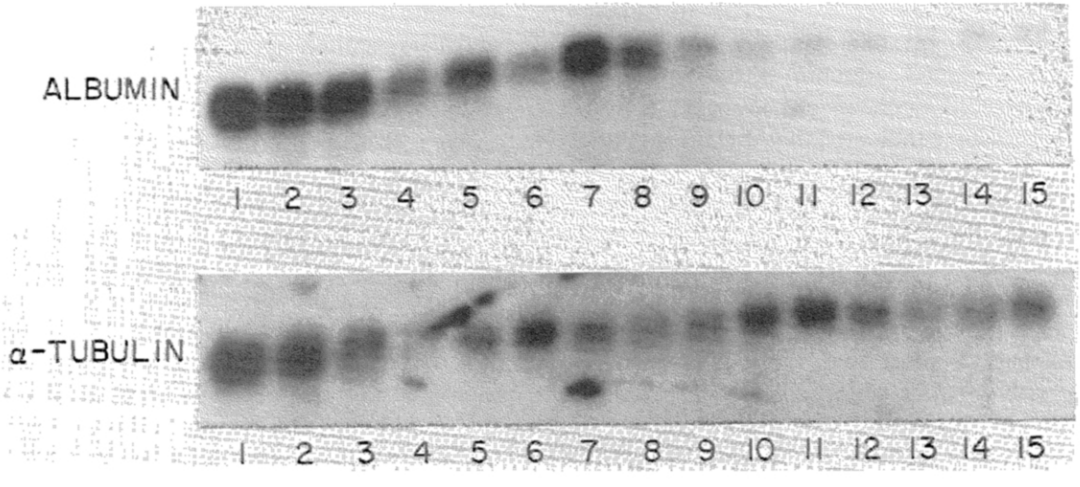

Fig. 1. Northern blot of cytoplasmic RNA from control and amino acid-deprived cultures. Cells were cultured for 24 h in serum-free MEM (lanes 1–3), MEM minus leucine (lanes 4–6), MEM minus methionine (lanes 7–9), MEM minus tryptophan (lanes 10–2), or MEM minus phenylalanine (lanes 13–15), as described in the text. Blots were hybridized with [32P]-labeled pRSA13 rat serum albumin cDNA (upper) or kα1 α-tubulin cDNA (lower). Cell survival relative to control cells cultured in complete MEM (determined from counts of cells remaining on monolayers) was 89% (−Leu cultures), 84% (−Met cultures), 98% (−Trp cultures) and 95% (−Phe cultures).

acid-deficient medium is shown in Fig. 1. A summary of densitometric scans of this gel is shown in Table I. Limitation of the H4 cells for tryptophan or phenylalanine caused a 15–18-fold decrease in albumin mRNA, whereas limitation for methionine or leucine caused only a 2–3-fold decrease. The effect of amino acid limitation on the mRNA for an unrelated 'housekeeping' gene, the gene encoding α-tubulin, was also studied. In contrast with the results obtained with albumin mRNA, limitation for any of the four amino acids caused a 2–3.5-fold decrease in α-tubulin mRNA (Fig. 1, Table I). Normalizing the changes in albumin mRNA levels to changes in α-tubulin mRNA levels, limitation for leucine or methionine did not decrease albumin mRNA relative to α-tubulin mRNA. In contrast, limitation for tryptophan caused an 8-fold decrease, and limitation for phenylalanine caused a 5-fold decrease in albumin mRNA relative to α-tubulin mRNA (Table I). These results suggest a specific regulatory effect of tryptophan and phenylalanine availability on the abundance of albumin mRNA. Cellular viability remained high during the 24 h amino acid limitation experiments (Fig. 1 legend); thus, the effects on mRNA were not simply a nonspecific result of cell death.

The effects of limitation for different amino acids on albumin mRNA levels were very reproducible. For example, in another experiment in which cells were limited for tryptophan or leucine, limitation for tryptophan caused a 17-fold decrease in albumin mRNA relative to total cytoplasmic RNA and a 7-fold decrease in albumin mRNA relative to α-tubulin mRNA. In contrast, limitation for leucine caused only a 3-fold decrease in albumin mRNA relative to total cytoplasmic RNA and essentially no decrease in albumin mRNA relative to α-tubulin mRNA (results not shown).

To determine whether the negative effect of amino acid limitation on albumin mRNA was caused by a decrease in albumin gene transcription, the effect of 24 h deprivation for leucine or tryptophan was examined in nuclear transcription elongation assays (Fig. 2). Limitation for leucine or tryptophan caused a 40–45% inhibition of albumin gene transcription but had little or no effect on α-tubulin gene transcription, which served as an internal control. The inhibition of albumin transcription was thus specific, in that α-tubulin gene transcription was not affected. However, the magnitude of the decrease in gene

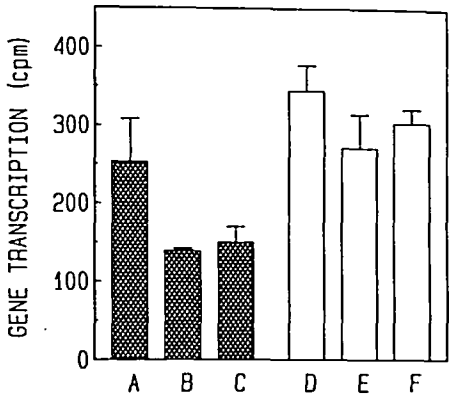

Fig. 2. Effect of amino acid deprivation on transcription of the albumin and α-tubulin genes. H4 cells were cultured in medium containing 10% serum for 3 days and then transferred to serum-free medium for an additional 3 days. The medium was then changed to serum-free MEM (bars A and D), MEM minus leucine (bars B and E), or MEM minus tryptophan (bars C and F), and incubation was continued for an additional 24 h. Nuclei were then isolated, and nuclear transcription assays were performed as described in the text. Bars A–C, albumin gene transcription; bars D–F, α-tubulin gene transcription. Each bar represents the average of determinations performed with nuclei from three different cultures, mean ± S.E. The radioactivity from pUC18 control hybridizations has been subtracted from each set of data.

TABLE I

SUMMARY OF DENSITOMETRIC SCANS OF NORTHERN BLOT SHOWN IN FIGURE 1.

Results are expressed as integrated image density units. All results represent the mean of three different RNA preparations ± S.E. The albumin/α-tubulin ratios shown in the third column of the table represent the mean of the albumin/tubulin mRNA ratios calculated for the three different RNA preparations ± S.E.

	mRNA species		$\frac{\text{Albumin}}{\alpha\text{-tubulin}}$ ratio
	albumin	α-tubulin	
Control	13.00 ± 1.09	8.25 ± 1.60	1.67 ± 0.24
−Leu	4.85 ± 1.13	3.25 ± 1.17	2.13 ± 0.90
−Met	5.55 ± 1.91	3.13 ± 0.16	1.78 ± 0.59
−Trp	0.87 ± 0.04	4.03 ± 0.49	0.22 ± 0.03
−Phe	0.73 ± 0.15	2.30 ± 0.39	0.33 ± 0.08

transcription did not early account for the 15–17-fold decrease in albumin mRNA caused by limitation for tryptophan, nor the specificity of the effect of tryptophan limitation as compared with leucine limitation on albumin mRNA levels. Therefore, the decrease in albumin mRNA caused by tryptophan limitation was produced primarily by a post-transcriptional mechanism.

The present study sheds new light on the regulation of albumin mRNA by essential amino acids. We demonstrate here that deprivation of the H4 cells for different amino acids has different effects on albumin and α-tubulin mRNA levels. In particular, limitation for tryptophan or phenylalanine but not methionine or leucine causes a specific reduction in albumin mRNA relative to α-tubulin mRNA. The magnitude of the effect of the different amino acids on albumin mRNA was not correlated in an obvious way with their representation in the translated sequence of rat pre-pro-albumin [9]. For example, the pre-pro-albumin sequence [9] contains 59 leucine residues but only two tryptophan residues. It is of interest that limitation for tryptophan had the greatest specific effect on albumin mRNA in view of previous observations suggesting that tryptophan is the limiting substrate for, or exerts a specific regulatory effect on albumin synthesis in the liver of fasting animals [4]. The observation that tryptophan limitation had the greatest specific effect on albumin mRNA levels relative to α-tubulin mRNA levels in the present study suggests that the cell culture model system may mimic events occurring in the liver in vivo under conditions of dietary protein restriction.

The reduction in the level of albumin mRNA produced by limitation for tryptophan results primarily from regulation at a post-transcriptional step. Post-transcriptional regulation of albumin mRNA levels has also been observed previously in normal rat hepatocytes cultured in medium with serum as compared with serum-free medium [10], and in the liver of *Xenopus* treated with estrogen [11]. Albumin mRNA is known to be relatively stable in vivo [12]. The post-transcriptional regulation of albumin mRNA observed in the present study, in *Xenopus* liver [11], and in cultured hepatocytes [10], might involve changes in albumin mRNA stability. In regard to the effects of nutrition on albumin mRNA, fasting has been shown previously to cause a shift of liver mRNA from polysomes to the free messenger ribonucleoprotein (mRNP) pool [13]. It is possible that this shift could result in a specific destabilization of albumin mRNA.

Limitation for amino acid has two well-known regulatory effects on gene expression in bacteria: attenuation [14] and the stringent response [15]. Amino acid limitation also regulates the expression of a number of genes in yeast [16]. In the present study, we report a novel finding that limitation of cultured rat heptoma cells for certain amino acids, especially tryptophan, exerts a specific post-transcriptional regulatory effect on albumin mRNA. The molecular mechanism by which this regulation is accomplished remains to be elucidated.

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References

1 Coward, W.A. and Lunn, P.G. (1981) Br. Med. Bul. 37, 19–24.
2 Morgan, E.H. and Peters, T., Jr. (1971) J. Biol. Chem. 246, 3500–3507.
3 Lunn, P.G. and Austin, S. (1983) J. Nutr. 113, 1791–1802.
4 Rothschild, M.A., Oratz, M., Mongelli, J., Fishman, L. and Schreiber, S.S. (1969) J. Nutr. 98, 395–403.
5 Sakuma, K., Ohyama, T., Sogawa, K., Fujii-Kuriyama, Y. and Matsumura, Y. (1987) J. Nutr. 117, 1141–1148.
6 Straus, D.S. and Takemoto, C.D. (1987) J. Biol. Chem. 262, 1955–1960.
7 Hutson, S.M., Stinson-Fisher, C., Shiman, R. and Jefferson, L.S. (1987) Am. J. Physiol. 252, E291–E298.
8 Ledford, B.E. and Jacobs, D.F. (1985) Eur. J. Biochem. 152, 611–618.
9 Sargent, T.D., Yang, M. and Bonner, J. (1981) Proc. Natl. Acad. Sci. USA 78, 243–246.
10 Jefferson, D.M., Clayton, D.F., Darnell, J.E., Jr. and Reid, L.M. (1984) Mol. Cell. Biol. 4, 1929–1934.
11 Kazmaier, M., Bruning, E. and Ryffel, G.U. (1985) EMBO J. 4, 1261–1266.
12 Wilson, S.H. and Hoagland, M.B. (1967) Biochem. J. 103, 556–566.
13 Yap, S.H., Strair, R.K. and Shafritz, D.A. (1978) J. Biol. Chem. 253, 4944–4950.
14 Kolter, R. and Yanofsky, C. (1982) Annu. Rev. Genet. 16, 113–134.
15 Gallant, J. (1979) Annu. Rev. Genet. 13, 393–415.
16 Fink, G.R. (1986) Cell 45, 155–156.