Characterization and metabolic regulation of a liver-specific 5.4-kilobase mRNA whose synthesis is transcriptionally induced by carbohydrates and repressed by glucagon and cyclic AMP

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Four clones derived from a carbohydrate-induced rat liver cDNA library were found to hybridize with a 5.4-kilobase mRNA species encoding a 36kDa protein. This mRNA was abundant in the liver, barely detectable in adipocytes and kidney, and absent from the other tissues tested. In the liver, the mRNA was fully induced by a carbohydrate-rich diet, but was undetectable during both starvation and feeding with a protein-rich or lipid-rich diet. Adrenalectomized, thyroidectomized and diabetic animals did not express the mRNA in their liver when re-fed with the carbohydrate-rich diet. When these animals were given the missing hormone, the amount of hybridizable RNA returned to normal values, but administration of the hormone alone failed to induce mRNA synthesis in starved animals. Both glucagon and its second messenger, cyclic AMP, abolished the induction of the mRNA in re-fed animals. Exogenous insulin, whatever the dose, did not reverse the inhibitory action of glucagon. In an isolated nuclei transcription system, no detectable RNA transcripts were found in starved animals, whereas feeding the animals with the carbohydrate-rich diet led to a maximum rate of gene transcription. Although unidentified, this mRNA proves to be a remarkable marker of dietary and hormonal control of gene expression *in vivo*. It will provide a useful model for further analysis of the role of cyclic AMP in regulating the transcription of eukaryotic genes.

Feeding starved animals with a carbohydrate-rich diet is known to increase the amounts of several enzyme mRNA species in the liver, including those for L-type pyruvate kinase, aldolase B (Weber *et al.*, 1984), 6-phosphogluconate dehydrogenase (Hutchinson & Holton, 1978), malic enzyme (Towle *et al.*, 1980) and fatty acid synthetase (Morris *et al.*, 1982). During the differential screening of a carbohydrate-induced rat liver cDNA library for L-type pyruvate kinase and aldolase B molecular cloning (Simon *et al.*, 1983), we selected several cDNA clones complementary to a 5.4kb mRNA species, present in the liver of carbohydrate-fed animals only. This mRNA is a very large one (5.4kb) which paradoxically encodes a small protein of only 36kDa. We show here that this mRNA species is a remarkable

marker for hormonal and dietary control of gene expression in the liver, since three conditions are required for its induction *in vivo*: (a) the presence of carbohydrates as glycolytic substrates, (b) a decrease in cyclic AMP and (c) the presence of several permissive hormones, including insulin.

Materials and methods

Animals and materials

Male Wistar rats, weighing approx 250g, were used throughout. Animals starved for 48 h were re-fed with either a maltose-rich or a fructose-rich diet for 18 h and given water containing 200g of the carbohydrate/*ad libitum*. Several animals were fed on either a lipid-rich or a protein-rich diet as described by Weber *et al.* (1984). Some animals underwent bilateral adrenalectomy or thyro-parathyroidectomy 8 days and 5 weeks before starvation respectively. Adrenalectomized rats were

Abbreviations used: kb, kilobases; SDS, sodium dodecyl sulphate; SSC, 0.15M-NaCl/0.015M-sodium citrate, pH 7.

maintained on 1% NaCl as the drinking water. Some of them were treated with cortisone acetate (10mg) intraperitoneally, twice a day, 24h before re-feeding. Thyro-parathyroidectomized animals were maintained on 1% CaCl₂ as the drinking water. Treated animals received one single dose of tri-iodo-L-thyronine (50µg) intraperitoneally, 1h before re-feeding. Diabetes was induced by intravenous injection of alloxan (30mg/rat) 4 days before re-feeding after starvation for 24h. Treated animals received rapid and long-acting insulin (5units each) subcutaneously once a day, 48h before and during re-feeding. All sets of animals were checked for their hormonal status, in order to confirm the success of both surgical and hormonal treatment (Munnich *et al.*, 1984).

Long-acting glucagon (0.4–1.5mg/kg per h), a gift from Novo Industry, was administered both intraperitoneally (zinc-glucagon) and subcutaneously (zinc-protamine-glucagon) 1h before re-feeding so as to obtain a rapid and sustained hormonal impregnation throughout the 18h period of re-feeding. Regular glucagon, theophylline, dibutyryl cyclic AMP (0.5mg/kg per h each) and arginine (10mM), either alone or in association, were administered at a continuous flow rate (0.5ml/h) through the femoral vein cannulated as described by Tabata & Chang (1982). For each experiment at least three animals were treated and their mRNA was studied separately.

Differential screening of the recombinant plasmids

The carbohydrate-induced rat liver cDNA library previously described (Simon *et al.*, 1983) was screened by using two [³²P]cDNA probes synthesized from rat liver 22–24S mRNA species. The latter were purified by sedimentation on a denaturing sucrose gradient and on a h.p.l.c. column. The probes were derived from the liver of either 48h-starved (probe '–') or 18h-carbohydrate-fed animals (probe '+'). Colonies containing sequences of interest were those scoring positive with the '+' probe and negative with the '–' probe. The selected colonies were cultured (Norgard *et al.*, 1979) and their plasmids purified (Wahl *et al.*, 1979). The recombinant plasmids were treated with the restriction enzyme *Pst*I, and the inserts sized by electrophoresis on 1.5%-agarose gel.

Characterization of the clones by positive hybridization-selection

The plasmid DNA species isolated were fixed on to nitrocellulose filters and used to select the specific polyadenylated RNA from the liver of fed animals (Ricciardi *et al.*, 1979; Ploegh *et al.*, 1980). The selected RNA species were translated in a rabbit reticulocyte cell-free lysate system (Pelham & Jackson, 1976) and the newly synthesized

polypeptide chains analysed by SDS/polyacrylamide-gel electrophoresis (Laemmli, 1970).

Size of the mRNA hybridizing with selected cDNA species ('Northern' blot)

Total RNA, extracted as described (Besmond *et al.*, 1981; Kahn *et al.*, 1981; Simon *et al.*, 1983), were subjected to electrophoresis in either methyl-mercury hydroxide-agarose (Bailey & Davidson, 1976) or formaldehyde-agarose gel (Hoffman *et al.*, 1981), then blotted on to nitrocellulose filters. Hybridization was carried out at 65°C in 0.5ml of 10% dextran sulphate, 1% SDS, 0.1% Ficoll, 1% glycine, poly(A) (10µg/ml), salmon DNA (100µg/ml) and nick-translated cDNA inserts (10⁶c.p.m./ml; sp. radioactivity 2 × 10⁸c.p.m./µg)/10cm². After 18h the filters were washed extensively with 1 × SSC/0.1% SDS solution, followed by a wash in 0.5 × SSC/0.1% SDS.

Southern-blot analysis

Total high-*M*_r DNA was extracted from rat liver (Gross-Bellard *et al.*, 1973; Blin & Stafford, 1976), then treated with various restriction enzymes and analysed by Southern blotting, with nick-translated inserts as probes (Southern, 1975). Hybridization conditions were similar to those described for the 'Northern' blot.

Characterization of the selected RNA species in different polyribosome fractions

Polyribosomes, isolated as described (Schackelford & Lebherz, 1979), were sedimented through a sucrose gradient (10–40%), and the fractions of similar sedimentation coefficient, derived from different gradient sets, were pooled for RNA extraction and 'Northern'-blot analysis.

Run-off transcription on isolated nuclei

This was done as described by Schibler *et al.* (1983): 8 × 10⁶ nuclei were used in each assay. Under those conditions (50–100) × 10⁶c.p.m. were incorporated into RNA during the 30 min incubation. Then the hybridization experiment was performed in a 200µl mixture containing 10 × 10⁶c.p.m.

Results

Characterization of the 5.4kb mRNA species

Four colonies, which were neither aldolase B nor L-type pyruvate kinase clones, gave a strong signal during the differential screening of the rat liver cDNA library (Fig. 1). These clones, complementary to carbohydrate-induced mRNA species, were therefore selected for further analysis. The sizes of the inserts were 440, 700, 420 and 640 base-pairs

Fig. 1. Filter hybridization of DNA from bacterial colonies. Replicate sets of filters containing lysed bacterial colonies were hybridized with two [32 P]cDNA probes, synthesized from purified rat liver mRNA. The probes were derived from the liver of either 48 h-starved (probe '-') or 18 h-carbohydrate-fed animals (probe '+'). The arrows indicate the position of a colony (3H6) yielding a strong signal with the '+' probe and no specific hybridization with the '-' probe.

long for clones 6H3, 3H6, 2B4 and 7D3 respectively. Cross-hybridization of the clones by Southern-blot analysis enabled determination of their relative positions. The four probes were overlapping in pairs. They hybridized with the same DNA restriction fragment, the signal obtained with 10 μ g of total cellular DNA being similar to that obtained with 5–10 pg of the linearized recombinant plasmid. This result suggests that the gene is not present in a large number of multiple copies (Fig. 2).

The four cDNA probes were complementary to one single RNA species, 5.4 kb long, found in the liver of re-fed animals only (Fig. 3). The RNA was bound to polyribosomes, which is a good indication that this RNA does direct the synthesis of a polypeptide. In addition, the 5.4 kb RNA was present in the same polyribosomal fraction as that containing aldolase B mRNA (1.8 kb long) and was found in fractions lighter than that containing L-type pyruvate kinase mRNA (3.5 kb). This is an indication that, despite its length, this RNA

Fig. 2. Southern-blot analysis of rat liver DNA restriction fragments with the 7D3, 3H6, 6H3 and 2B4 cDNA clones. Total DNA from rat liver was extracted, and 10 μ g samples were digested with restriction enzymes *Eco*RI (lane 1), *Hind*III (lane 2), *Bgl*II (lane 3), *Bgl*I (lane 4) and *Bam*HI (lane 5), and then resolved by agarose-gel electrophoresis and transferred to a nitrocellulose filter. The filter was hybridized with pooled nick-translated probes (10^6 c.p.m./ml of hybridization mixture).

species has a relatively short coding sequence and therefore carries only relatively few ribosomes (results not shown). In fact, the 5.4 kb RNA species selected by hybridization–translation directed the cell-free synthesis of a polypeptide of only 36 kDa (Fig. 4). Finally, when polyribosomes were prepared so as to separate free from bound, the 5.4 kb RNA was mainly detected in the free-polyribosome fraction. This result suggests that the 36 kDa unidentified protein is probably not secreted.

Large amounts of the 5.4 kb mRNA were found in the liver of fed animals, whereas only trace amounts were present in the kidney and in adipocytes (Fig. 3). In the liver of starved animals and in the other tissues tested, no specific hybridization was detected (Fig. 3). Nor was hybridization found in foetal liver (results not shown).

Dietary regulation of the 5.4 kb mRNA species in the liver

The 5.4 kb mRNA was undetectable in the liver of 48 h-starved animals, and was fully induced by the carbohydrate-rich diet. The kinetics of the mRNA induction and de-induction are shown in Fig. 5 (results of a typical experiment). No specific hybridization was found in the liver after starva-

Fig. 3. Size and tissue specificity of the mRNAs: determination by Northern-blot analysis

(a) Total RNA (10 µg) from 18 h-carbohydrate-fed (lanes 1–7) or starved animals (lane 8) was denatured in 10 mM-methylmercury hydroxide for 10 min at 20°C, then applied to a 1.5% agarose gel containing 10 mM-methylmercury hydroxide. The gel was treated with 1.1 M-formaldehyde before transfer on to a nitrocellulose filter. Hybridization was performed in the presence of 10^6 c.p.m. of labelled purified DNA insert (100 ng/ml). Tissues: 1, fed liver; 2, adipose tissue; 3, small intestine; 4, brain; 5, muscle; 6, kidney; 7, lung; 8, starved liver. (b) Calibration curve determined by migration of RNA standards. The arrow indicates the position of the sized RNA.

tion for 12 h and the signal remained undetectable as long as the animals were starved. The signal reappeared at 12 h of re-feeding. Maximum induction occurred at 18 h, and then the extent of specific hybridization in carbohydrate-fed animals decreased slowly. Both the maltose-rich and the fructose-rich diet induced maximum expression of the mRNA, results being similar to those obtained with the sucrose-rich diet (Table 1). In animals re-fed with the protein-rich or the lipid-rich diet, the 5.4 kb mRNA was undetectable, as it was in starved rats. In order to determine the extent of dietary regulation of the 5.4 kb mRNA, further experiments were performed in which the synthesis of the RNA sequences *de novo* was measured. In most isolated nuclei transcription systems, RNA production is due to elongation of RNA sequences, with no initiation of new chains. Thus, when nuclei are allowed to continue RNA synthesis *in vitro*, the amount of transcripts produced accurately reflects the extent of RNA synthesis at the time of death (Schibler *et al.*, 1983). No detectable 5.4 kb RNA transcripts were found in isolated nuclei from animals either 48 h-starved or re-fed with the lipid-rich or the protein-rich diet for 12 h. The rate of gene transcription dramatically increased in the liver of animals re-fed with the carbohydrate-rich diet for 12 h, then markedly decreased if the high-carbohydrate diet was continued (Fig. 6; results of a typical experiment). At 12 h labelled hybridized

RNA represented about 1/10000 of the total radioactivity of synthesized nuclear RNA. Since we do not know the total length of the primary transcript, we cannot deduce from this result the maximal percentage of the 5.4 kb mRNA.

Hormonal regulation of the 5.4 kb mRNA species in the liver

The 5.4 kb mRNA was undetectable in the liver of starved animals, even when treated with pharmacological doses of cortisone, tri-iodo-L-thyronine or insulin as described in the Materials and methods section (Fig. 7). When 48 h-starved adrenalectomized, thyroidectomized or diabetic animals were re-fed with either the maltose-rich or the fructose-rich diet, no hybridizable material was found in their liver, although their liver weight and food consumption matched those of fed controls (Fig. 7). However, when carbohydrate-fed adrenalectomized, thyroidectomized or diabetic animals were given the missing hormone, as described in the Materials and methods section, the amount of the 5.4 kb mRNA in their liver returned to fed control values (Fig. 7).

Long-acting glucagon resulted in a dramatic decrease in the 5.4 kb mRNA, which decreased to starvation values in the liver of animals re-fed with either of the carbohydrate-rich diets (Fig. 7). Regular glucagon had the same effects when administered intravenously at a constant flow rate,

Fig. 4. Characterization of the polypeptide chain synthesized under direction of the 5.4 kb mRNA species (a) Hybridization-selection experiment. Denatured plasmid DNA was bound to filters ($\sim 12 \text{ mm}^2$), which were then hybridized to polyadenylated RNA from carbohydrate-re-fed rat liver. RNA that hybridized was eluted and translated in a reticulocyte cell-free system. Lanes: 1, no DNA bound to the filter; 2, DNA from non-recombinant pBR322 plasmid bound to the filter; 3, DNA from clone 7D3 bound to the filter. (b) Cell-free translation of h.p.l.c.-purified carbohydrate-re-fed rat liver mRNA. RNA species were fractionated by sucrose-gradient centrifugation, then submitted to h.p.l.c. gel-filtration chromatography and translated in a reticulocyte cell-free system. Tracks 18 to 23 show different h.p.l.c. fractions. Abbreviation: PK, newly synthesized L-type pyruvate kinase. The lower arrow shows the location of the polypeptide synthesized under direction of the 5.4 kb mRNA species.

over the 18 h period of the re-feeding, through the cannulated femoral vein of conscious rats. Cyclic AMP mimicked the effects of glucagon when administered intravenously as dibutyl cyclic AMP. The effect of a physiological agent of glucagon release, arginine, was also tested. When the rats were infused with arginine (10 mM) over the 18 h period of re-feeding, the amount of hybridizable material was decreased to only 20% of that in fed controls. Finally, theophylline, an inhibitor of phosphodiesterase activity, decreased the amount of hybridizable RNA to the same extent (Fig. 7).

Very high amounts of immunoreactive insulin were found in the plasma of glucagon-treated animals (mean $\pm$ S.D. = $148 \pm 84 \mu\text{units/ml}$, range 77–355; fed controls, $50 \pm 10 \mu\text{units/ml}$). Nevertheless, despite very high contents of immunoreactive insulin in their plasma, neither endogenous nor

Fig. 5. Northern-blot hybridization analysis of the liver mRNA from starved and carbohydrate-fed rats. Electrophoresis was performed in agarose/formaldehyde gel: $2 \mu\text{g}$ of polyadenylated RNA was deposited in each slot. Starved-rat liver mRNA: 7 h starved (lane 2), 12 h starved (lane 3), 24 h starved (lane 4), 5 days starved (lane 5). Re-fed-rat liver mRNA: 12 h re-fed (lane 6), 18 h re-fed (lanes 7 and 8), 24 h re-fed (lane 8), 48 h re-fed (lane 9). The filters were hybridized with the 7D3 recombinant plasmid as described in Fig. 3. Essentially the same results were found in at least three independent experiments. mRNA was always undetectable in the liver of starved animals.

Table 1. Quantification of the 5.4 kb hepatic mRNA in starved, carbohydrate-fed and protein-fed animals

Male Wistar rats were starved for 48 h, then re-fed with either a carbohydrate-rich diet or a protein-rich diet for 18 h. The high-protein diet consisted of 90% proteins (casein) and 4% lipids and was devoid of carbohydrate. Animals were killed by decapitation, and the blood was collected for hormone determination. Specific mRNAs were quantified by dot-blot analysis: $4 \mu\text{g}$ of total RNA and five 1:2 dilutions in 10 mM-phosphate buffer/2.2 M-formaldehyde were dotted on nitrocellulose filters after a 10 min denaturation at 65°C . Hybridization was performed in the presence of 10^6 c.p.m. of labelled purified DNA insert/ml (0.5 ml of hybridization mixture per 10 cm^2 of filter surface). After autoradiographic exposure, the dots were cut out and their radioactivity was measured by liquid-scintillation counting. Results are expressed in c.p.m. of radio-labelled probe per $4 \mu\text{g}$ of total RNA (means $\pm$ S.D. for three different experiments performed in different animals). The blank represents non-specific hybridization to muscle non-polyadenylated RNA.

	Specific hybridization (c.p.m./ $4 \mu\text{g}$ of total RNA)
Starved 48 h	40 ± 10
Maltose diet	1940 ± 190
Fructose diet	1720 ± 150
Sucrose diet	1800 ± 120
Protein diet	40 ± 10
Blank	38 ± 12

Fig. 6. Run-off transcription on isolated liver nuclei: transcriptional regulation of the 5.4 kb mRNA species
Liver nuclei were isolated from rats either fed on a high-protein diet for 48 h (proteins) or re-fed with the sucrose-rich diet for 7, 12 or 72 h (carbohydrates). Transcripts were hybridized to immobilized 3H6, 6H3 and 7D3 probes in excess (about 2 µg each). We also used a rat serum albumin cDNA probe (Alb; gift from Dr. José Sala Trepas) as internal standard: the extent of hybridization of albumin transcripts was the same in all the dietary conditions, similar to that shown in the Figure for the protein diet. pBR: blanks with non-recombinant pBR322-plasmid DNA bound to the filters. In three independent experiments radioactivity bound to the specific filters was zero in protein-fed rats and around 350–500 c.p.m. after 12 h of carbohydrate re-feeding.

exogenous insulin administered together with glucagon could reverse the effects of glucagon (Fig. 7).

Discussion

We describe in this paper the properties of a liver 5.4 kb mRNA species which is regulated by diet and hormones and seems to direct synthesis of a 36 kDa polypeptide.

Since the number of ribosomes bound to a mRNA molecule increases with the length of the coding sequence, finding the 5.4 kb species in the polyribosomal fraction encoding aldolase B (39 kDa) is consistent with a relatively short coding sequence of the mRNA. Indeed, this very long mRNA encoded a protein of only 36 kDa. In order to encode a 36 kDa polypeptide, 1000 bases would be sufficient. This suggests that the 5.4 kb mRNA is made up of a 4000-base-pair-long non-coding sequence. Several examples of mRNA species with very long, generally 3', non-coding extensions have previously been reported, namely interferon mRNA (Sehgal *et al.*, 1982), α -crystallin mRNA (King *et al.*, 1982), L-type pyruvate kinase mRNA (Simon *et al.*, 1983), glycerol phosphate dehydrogenase mRNA (Spiegelman *et al.*, 1983) and fatty acid synthetase mRNA (Morris *et al.*, 1982). The

Fig. 7. Northern-blot analysis of the liver 5.4 kb mRNA derived from normal, adrenalectomized, thyroidectomized, diabetic, and glucagon- or dibutyryl cyclic AMP-treated animals, either starved or re-fed with the maltose-rich diet for 18 h

Total RNA (10 µg) was deposited in each slot, and electrophoresis was performed in methylmercury hydroxide-agarose gel. After transfer, the filters were hybridized with the nick-translated 6H3+7D3 probes. A similar pattern was obtained in animals re-fed with the fructose-rich diet. Normal rats starved for 48 h, starved for 18 h further (lanes 1–4) were given cortisone (lane 2), or tri-iodo-L-thyronine (lane 3), or no hormone (lane 1). Insulin (lane 4) was given for only 6 h to a 24 h-starved animal. Other animals (lanes 5–14) were starved for 48 h, then re-fed with the maltose-rich diet for 18 h. Lane 5, normal rat, fed; lane 6, adrenalectomized rat, fed; lane 7, as lane 6 + cortisone; lane 8, thyroidectomized rat, fed; lane 9, as lane 8, + tri-iodo-L-thyronine; lane 10, diabetic rat, fed; lane 11, as lane 10 + insulin (10 units); lane 12, fed rat, treated with long-acting glucagon (100 µg); lane 13, fed rat, treated with dibutyryl cyclic AMP (0.2 mg/kg per h); lane 14, fed rat, treated with long-acting glucagon (100 µg) + insulin (5 units). Essentially the same result was found in at least three independent experiments.

significance and the physiological role of these extensive 3' non-coding terminal stretches, if any, remain unclear.

To date, the 36 kDa protein is still unidentified. Considering that most of the non-coding extensions of the eukaryotic mRNA molecules are 3', and that our probes are complementary to the 3' end of the RNA (Simon *et al.*, 1983), sequencing our cDNA clones would not be informative for protein identification. Therefore, in order to sequence the exons and determine the amino acid composition of the 36 kDa protein, it is necessary to isolate genomic clones complementary to the cDNA probes. Nevertheless, a putative function for this protein can already be deduced from the dietary conditions of the mRNA induction. Since the synthesis of the hepatic mRNA is transcriptionally induced by feeding the rats with the

carbohydrate-rich diet, and repressed by both the lipid-rich and the protein-rich diets, the hypothesis of the liver-specific enzyme involved in glycolysis, glycogenesis or lipogenesis can be reasonably considered. Unfortunately, the structural and regulatory characteristics reported here fit no known protein.

Whatever the protein encoded, the 5.4kb mRNA species seems to be a remarkable marker of both hepatic differentiation and dietary carbohydrate-status. Indeed, the mRNA was present in the liver of carbohydrate-fed rats only, whereas no hybridizable material was found in the liver of starved, lipid-fed or protein-fed animals. Although we have not yet tested the stability of the mRNA, run-off transcription experiments on isolated nuclei suggest that regulation of the mRNA occurs at the transcriptional level.

Induction of the mRNA by carbohydrates required the presence of corticosteroids, thyroid hormones and insulin. Adrenalectomized, thyroidectomized and diabetic animals did not express the mRNA in their liver when re-fed with the carbohydrate-rich diets. When the animals were given the missing hormone, the extent of specific hybridization returned to normal values, but the administration of the hormone alone failed to induce mRNA synthesis in starved animals. Therefore corticosteroids, thyroid hormones and insulin exert a 'permissive effect' on the induction of the mRNA by carbohydrates in the liver.

Both exogenous glucagon and its second messenger, cyclic AMP, abolished the induction of the 5.4kb mRNA in re-fed animals. Exogenous insulin, whatever the dose, did not reverse the inhibitory action of glucagon. The effect of glucagon on the gene expression should be physiologically relevant, especially as no hybridizable material was found in the liver of animals re-fed with the protein-rich diet (which causes a release of both insulin and glucagon). Moreover, arginine, a potent amino acid stimulator of glucagon release (Gerich *et al.*, 1974), decreased the extent of specific hybridization to less than 20% of that for carbohydrate-fed controls. Thus, despite opposite effects on insulin release, both starvation and the protein-rich diet appear to alter the mRNA content in the same way. Indeed, these two very different nutritional situations share a major hormonal feature, namely hyperglucagonaemia.

Glucagon and its second messenger, cyclic AMP, have been shown to alter the transcription of several higher-eukaryotic genes (Lamers *et al.*, 1982; Lewis *et al.*, 1983; Beale *et al.*, 1982), but all the reported modifications consisted of an activation of the gene transcription. We show here that glucagon can also decrease a liver-specific cytoplasmic RNA. Similar observations have been

made for mRNA for two glycolytic enzymes, namely liver L-type pyruvate kinase and aldolase B (Munnich *et al.*, 1984). If, as is the case during starvation, the mechanism involved is the inhibition of the gene transcription, the present mRNA species will provide a useful model for further analysis of the role of cyclic AMP in regulating the transcription of eukaryotic genes, as a function of the dietary status *in vivo*.

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