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Biochemistry

Induction of two genes by glucose starvation in hamster fibroblasts

(mammalian cDNA clones/mRNA blot hybridization/culture cells/coordinate gene expression)

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ABSTRACT **The coordinated expression of two genes specifically induced by glucose starvation is demonstrated in a hamster fibroblast cell line, K12. Using two cDNA plasmids, p4A3 and p3C5, as hybridization probes, we examine the kinetics of induction of these genes when the cells are grown in medium deprived of glucose. The results show that (*i*) after a lag period of about 8 hr, there is a rapid and simultaneous increase of the p4A3 and p3C5 mRNA levels and (*ii*) the elevation of the mRNA levels for p4A3 and p3C5 is largely due to new transcription. In addition, we compare the mRNA transcripts encoded by these glucose-regulated genes in culture cells and phospho*enol*pyruvate carboxykinase, the enzyme that catalyzes the rate-limiting step in gluconeogenesis in fasted rats. Our results indicate that the expression of phospho*enol*pyruvate carboxykinase is not inducible by glucose starvation in our culture cells.**

Animal cells in culture respond to glucose starvation by increased synthesis of a set of proteins generally known as the glucose-regulated proteins (GRPs). The two major and most commonly observed GRPs in chicken, hamster, rat, mouse, and human cells have M_rs of 90,000–100,000 and 73,000–79,000 (1–6) and are different from the major heat shock proteins reported for animal cells (7). These proteins can also be induced in chicken embryo and rat fibroblasts after transformation by Rous sarcoma virus (8, 9), as glucose is rapidly depleted from the growth medium by transformed cells (1). Although the functions of the GRPs are still unknown, previous studies have established that these proteins are highly conserved in animal cells (10), implying that they may be important for cell survival when nutrients other than glucose have to be utilized as a source for carbon and energy.

A temperature-sensitive mutant, K12, derived from Chinese hamster fibroblasts has been shown to overproduce these specific proteins when incubated at the nonpermissive temperature (40.5°C) (5, 6). In an earlier report, we described the isolation of cDNA clones derived from poly(A)$^+$ RNA of K12 cells incubated at 40.5°C (10). Using the cDNA clones p4A3 and p3C5 prepared from the mutant hamster cells as hybridization probes, we have previously reported that (*i*) the corresponding genes were highly inducible in K12 mutant cells by incubation at 40.5°C, (*ii*) the level of mRNA complementary to p4A3 and p3C5 was increased severalfold when the cells were starved for glucose, and (*iii*) p4A3 and p3C5 are likely to contain sequences coding for the M_r 94,000 and M_r 78,000 GRPs, respectively, observed in hamster fibroblast cells (11). To understand the molecular control mechanisms leading to the specific expression of these genes during glucose starvation, we examined the kinetics of induction of these two genes when the cells were deprived of glucose and under other nutritional starvation conditions. Our results indicated that there was a coordinate induction of both p3C5 and p4A3 in glucose-starved cells and that the elevation in the mRNA levels for p3C5 and p4A3 was largely due to new transcription. Neither selective amplification nor rearrangement of these two genes was apparently involved in their increased expression.

Recently, the gene encoding for cytosolic phospho*enol*pyruvate carboxykinase (GTP) (EC 4.1.1.32) (*P-enol*pyruvate carboxykinase) has been isolated from the rat (12). Two observations prompted us to explore the relationship between *P-enol*pyruvate carboxykinase gene expression and the expression of K12 GRPs. First, the synthesis of the cytosolic form of *P-enol*pyruvate carboxykinase in rat liver is markedly altered by hormonal and dietary stimuli—e.g., glucose administration to a starved rat will cause de-induction of its mRNA (13). Second, the mRNA for *P-enol*pyruvate carboxykinase in rat liver cells is identical in size to the hamster transcript complementary to p3C5. Therefore, we compared the expression of these two genes in fibroblasts and hepatoma cells under conditions of glucose starvation or cAMP stimulation. From RNA blot hybridization analysis, we show that *P-enol*pyruvate carboxykinase mRNA and p3C5 mRNA are distinct molecular species.

MATERIALS AND METHODS

Cell Lines, Media, and Culture Conditions. The Chinese hamster lung fibroblast cell line K12 has been described (5). The rat liver hepatoma cell line (Fao-1) was supplied to us by R. E. K. Fournier (University of Southern California). Fao-1 is a ouabain-resistant, HPRT$^-$ derivative of Faca 967 (14).

To obtain glucose-starved cells, K12 cells were seeded at a density of 10^4 cells per cm^2 in 150-mm (diameter) culture dishes in complete Dulbecco's modified Eagle's medium (DME medium) containing 4.5 mg of glucose per ml and 10% calf serum. The cells were incubated at 35°C with a change of fresh medium every 2–3 days until the cells reached about 90% confluency. At that point (time 0), the regular medium was replaced by glucose-free DME medium supplemented with 10% calf serum previously dialyzed overnight at 4°C against 10 vol of phosphate-buffered saline (P_i/NaCl). The cells were incubated at 35°C and, at various times after the removal of glucose from the medium, RNA samples were prepared from the cells as described (11). To test the time course effect of N^6,$O^{2'}$-dibutyryladenosine 3',5'-cyclic monophosphate (Bt_2cAMP) and dexamethasone on the relative mRNA levels, the procedure of Gunn *et al.* (15) was used.

DNA Blot Hybridization Conditions. High molecular weight nuclear DNA was prepared by the method of Pellicer *et al.* (16). After restriction enzyme digestion, the DNA samples were size-separated on 1% agarose gels and blotted onto nitrocellulose filters. The filters were preincubated in the hybridization buffer containing 4× concentrated SET (SET = 0.15 M NaCl/0.03 M Tris·HCl, pH 8/2 mM EDTA), 5× concentrated Denhardt solution [Denhardt solution = 0.02% bo-

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Abbreviations: GRP, glucose-regulated protein; *P-enol*pyruvate carboxykinase, phospho*enol*pyruvate carboxykinase (GTP); Bt_2cAMP, N^6,$O^{2'}$-dibutyryladenosine 3',5'-cyclic monophosphate; PVP, polyvinylpyrrolidone; NP-40, Nonidet P-40; nt, nucleotide(s); kb, kilobase(s).

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vine serum albumin/0.02% polyvinylpyrrolidone (PVP)/0.02% Ficoll], 0.1% $NaDodSO_4$, 0.1% sodium pyrophosphate, 25 mM sodium phosphate buffer (pH 6.8), 50 μg each of poly (rA) and poly (rC) per ml, and 25 μg each of denatured salmon sperm and *Escherichia coli* DNA as carrier per ml. Prehybridization was carried out at 68°C for 2 hr. The annealing reaction was carried out in hybridization buffer containing about 3×10^7 cpm of heat-denatured probe in a volume of 10 ml at 68°C for 16 hr. After hybridization, the filters were washed once at room temperature in 0.60 M NaCl/0.060 M sodium citrate/0.1% $NaDodSO_4$/0.1% sodium pyrophosphate/0.025 M sodium phosphate buffer and three times at 68°C for 45 min each in 0.015 M NaCl/1.5 mM sodium citrate in the same buffer. The filters were dried and autoradiographed.

Measurement of *in Vivo* Transcription Rates. Conditions for the preparation of pulse-labeled nuclear RNA and the hybridization to excess plasmid DNA bound to nitrocellulose filters have been described (11). Essentially, at various times after the change to glucose-free medium, the cells were labeled for 10 min with 0.625 mCi (1 Ci = 37 GBq) of [^{3}H]uridine (New England Nuclear, 50 Ci/ml) per ml in a total volume of 4 ml per 150-mm (diameter) culture dish. After rinsing with ice-cold P_i/NaCl, the cell pellet from each dish was resuspended in 2.75 ml of ice-cold, isotonic high-pH buffer (0.14 M NaCl/0.01 M Tris·HCl, pH 8.4/1.5 mM $MgCl_2$), and 0.125 ml of a 10% (vol/vol) Nonidet P-40 (NP-40) solution was added. The nuclear pellet was collected by centrifugation at 800 × *g* for 3 min and resuspended in 1 ml of isotonic high-pH buffer. After addition of 0.05 ml of 10% NP-40, the nuclear pellet was again collected by centrifugation. For the extraction of nuclear RNA, the nuclear pellet was resuspended in 1.5 ml of a high-salt buffer containing 0.5 M NaCl, 0.01 M Tris·HCl (pH 7.4), 0.05 M $MgCl_2$, and 0.002 M $CaCl_2$. After addition of 0.003 ml of DNase I solution (10 mg/ml), the suspension was triturated until the suspension was fluid, and 0.03 ml (each) of 20% $NaDodSO_4$, 0.5 M EDTA, and 3 M sodium acetate (pH 5.4) was added. After addition of 2.5 ml of 60 mM sodium acetate, pH 5.4/10 mM EDTA and 200 μg of yeast RNA, the mixture was extracted twice with phenol/chloroform/isoamyl alcohol (25:24:1) pre-equilibrated with 60 mM sodium acetate, pH 5.4/10 mM EDTA at 65°C. The aqueous phase was extracted once with chloroform before the RNA was precipitated in 0.3 M sodium acetate (pH 5.4) and 2 vol of 95% alcohol at −20°C. The RNA was collected by centrifugation at 220,000 × *g* for 40 min and resuspended in 0.05 ml of 10 mM 2-{[2-hydroxy-1,1-bis(hydroxymethyl)ethyl]amino}ethanesulfonic acid (pH 7.4) (Sigma). The yield of RNA ranged from 2×10^6 cpm per dish for glucose-starved cells to about 2×10^7 cpm per dish for nonstarved cells.

RESULTS

cDNA Clones Used for Hybridization Probes. The cDNA clones designated p3C5, p4A3, and p3A10 were derived from a cDNA library constructed by using poly(A)$^+$ RNA extracted from hamster K12 cells incubated at 40.5°C. Though p3C5 and p4A3 have previously been shown to encode RNA specifically induced by glucose starvation (11), p3A10 was isolated from the same cDNA library but encodes an RNA species that is constitutively expressed at similar levels in K12 cells grown at either the permissive (35°C) or the nonpermissive (40.5°C) temperature. Therefore, p3A10 was used as an internal control in all of the RNA hybridization measurements reported here. pPCK10 is a near full-length cDNA clone derived from a rat liver cDNA library that contains gene sequences encoding rat cytosolic *P-enol*pyruvate carboxykinase (12). The insert size of these cDNA clones and size of the mRNA transcripts encoded by these genes are summarized in Table 1.

Table 1. Summary of cDNA clones

cDNA clone	Insert size, nt	mRNA size, nt*	Refs.
p3C5	2,550	2,700	10, 11
p4A3	1,400	3,200	11
p3A10	750	1,300	
pPCK10	2,600	2,800	12, 13

nt, Nucleotides.
*Values for p3C5, p4A3, and p3A10 were derived from RNA blot hybridization to hamster fibroblast cells; the value for pPCK10 was obtained from hybridization to rat liver cytosolic RNA.

Accumulation of p3C5 and p4A3 Transcripts During Glucose Starvation. K12 cells were grown to about 90% confluency in complete DME medium at 35°C. At this point, the cells were changed to glucose-free medium containing dialyzed serum and incubated at 35°C. At various times after the removal of glucose, total cytoplasmic RNA was extracted from the cells. The purified RNA samples were size-separated on denaturing formaldehyde-formamide gels, blotted onto nitrocellulose filters, and hybridized with nick-translated plasmid DNA. The result of an RNA blot hybridization experiment with mixed p3C5 and p3A10 probes is shown in Fig. 1. As seen from the autoradiogram, p3C5 hybridized predominantly to an RNA of 2,700 nt, whereas p3A10 hybridized to an RNA of 1,300 nt. The level of hybridization at each time point was quantitated by scanning the autoradiogram, and the relative peak area of the two major hybridization bands as a function of incubation time in the glucose-

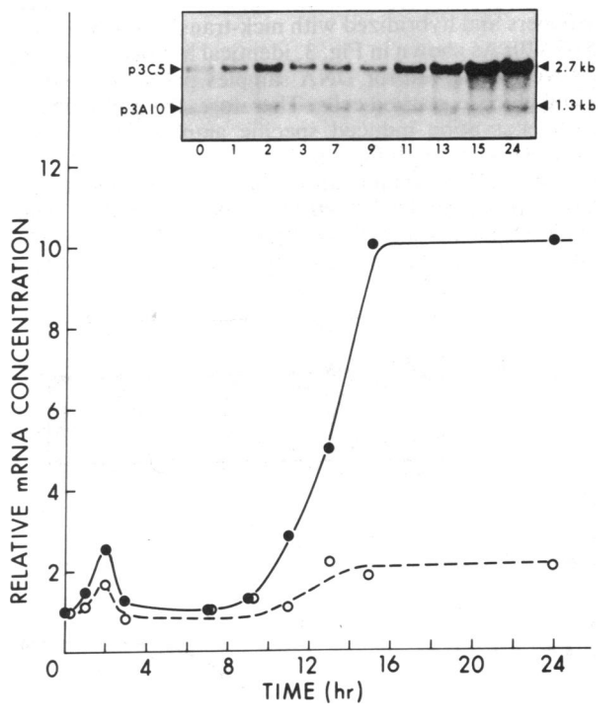

FIG. 1. Accumulation of p3C5 transcripts during glucose starvation. K12 cells were grown to about 90% confluency in complete DME medium. At time 0, the cells were changed to glucose-free medium. Total cytoplasmic RNA was isolated from K12 cells at various times after removal of glucose from the culture medium as described (11). Ten micrograms of each RNA sample was applied on a denaturing formaldehyde-formamide RNA gel and, after electrophoresis, was blotted onto nitrocellulose filters (11). The RNA blot was hybridized with ^{32}P-labeled plasmid DNA p3C5 and p3A10, prepared by nick-translation to a specific activity of about 5×10^7 cpm/μg DNA. The autoradiogram was quantitated by densitometry to obtain the relative levels of p3C5 (● — ●) and p3A10 (○ --- ○) transcripts at various times after incubation in the glucose-free medium. kb, Kilobases.

free medium is presented in Fig. 1. Within 1 hr of glucose removal, we consistently observed a 2-fold increase in the mRNA levels for both p3C5 and p3A10. In the case of p3A10, its mRNA returned to the initial basal level within 4 hr and was enhanced only 2-fold after 24 hr. However, after a lag period of about 8 hr, we observed a rapid increase in the steady-state concentration of p3C5 mRNA. Within 16 hr, the mRNA level had reached a plateau level. At that point, there was a 10- to 11-fold increase in the p3C5 mRNA level.

Next, we analyzed the kinetics of accumulation of the p4A3 transcripts during glucose starvation conditions. RNA blots identical to those described for Fig. 1 were hybridized with mixed p4A3 and p3A10 nick-translated probes. The results are shown in Fig. 2. As reported previously, p4A3 hybridized to an RNA size of 3,200 nt. In these experiments, we again observed a slight increase in mRNA levels for both p4A3 and p3A10 within several hours of glucose removal. Though the level of p3A10 remained constant within a factor of 1.5 throughout the 24-hr incubation period, the level of p4A3 RNA gradually increased, starting about 9 hr. By 16-hr, the level of p4A3 mRNA reached a plateau representing a 4- to 5-fold increase over the basal level.

Are Increases in mRNA Levels Due to Gene Amplification? To determine whether the cultured cells responded to glucose starvation by amplifying the genes encoding p3C5 and p4A3, we extracted nuclear DNA from K12 cells grown in complete DME medium and from cells grown in glucose-free medium for 24 hr. Equal amounts of DNA were digested with the restriction endonuclease *Eco*RI and size-separated on 1% agarose gels. The DNA was blotted onto nitrocellulose papers and hybridized with nick-translated p3C5, p4A3, and p3A10. As shown in Fig. 3, identical hybridization intensities were observed for DNA samples prepared from control and glucose-starved cells. Therefore, the possibility that glucose starvation induced specific amplification of these three genes can be eliminated.

Because p3C5 contains an *Eco*RI site in its insert, it hybridized to two bands of 4,300 and 2,500 nt. A middle band of about 3,500 nt was sometimes observed with varying intensity. It is interesting to note that, although p3C5 and p4A3 are

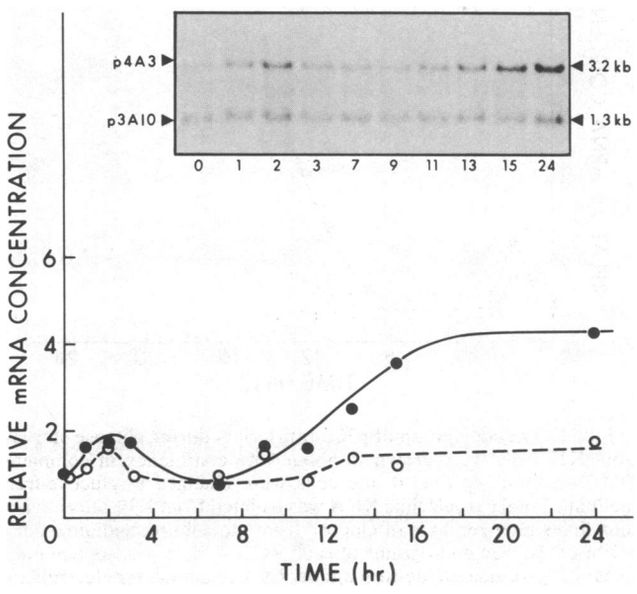

FIG. 2. Accumulation of p4A3 transcripts during glucose-starvation. Cytoplasmic RNA samples prepared from glucose-starved K12 cells as described in the legend of Fig. 1 were hybridized with nick-translated p4A3 and p3A10. The autoradiograms were quantitated to obtain the relative levels of p4A3 (● — ●) and p3A10 (○ --- ○) transcripts at various times after incubation in the glucose-free medium.

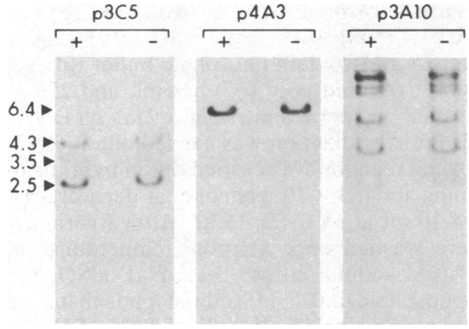

FIG. 3. Relative gene copy number of p3C5, p4A3, and p3A10. Nuclear DNA was extracted from K12 cells grown in complete DME medium or in glucose-free medium for 24 hr. Ten micrograms of each DNA sample was digested with *Eco*RI and applied to 1% agarose gels. As a size marker, λ DNA digested with *Hin*dIII was run in parallel. The DNA was blotted by the method of Southern (17) and hybridized separately with p3C5, p4A3, and p3A10 nick-translated to a specific activity of about 3×10^7 cpm/μg. Hybridization and washing conditions are described in the text. +, DNA from cells grown in complete DME medium; −, DNA from cells grown in glucose-free medium. Values are given in kb.

probably single-copy genes in the hamster genome, p3A10 hybridized to a series of discrete *Eco*RI fragments, implying that it may belong to a multigene family.

Relative *in Vivo* Transcriptional Rates During Glucose Starvation. To determine the nuclear transcription rates of p3C5, p4A3, and p3A10 in K12 cells subjected to glucose starvation, we pulse-labeled the cells with [^{3}H]uridine for 10 min at various times after glucose removal and prepared nuclear RNA from each of the cell samples. For the determination of the basal nuclear transcription rate for each of these genes, one batch of cells was pulse-labeled just prior to the medium change. In three different sets of experiments, we consistently observed that the total amount of [^{3}H]uridine incorporated per culture dish for control cells was about 10 times higher than those incubated in glucose-free medium for 10–16 hr. Although this difference can be explained (partly) by the arrest of cell division for those cells that were shifted to glucose-free medium, the large difference in uridine incorporation may actually be the result of a general shutdown of RNA synthesis triggered by glucose starvation or the result of expansion of the uridine nucleotide pool.

We next hybridized the labeled nuclear RNA with excess amounts of p3C5, p4A3, and p3A10 plasmid DNA bound to nitrocellulose filters. The percent of total pulse-labeled nuclear RNA that hybridized to each plasmid was calculated and compared with the value obtained for the basal level prior to the onset of glucose starvation. The fold increase in transcription rates at 10 and 12 hr after glucose starvation is summarized in Table 2. In the case of p3C5 and p4A3, we detected severalfold increases in their nuclear transcription rates during this time period. These rates were maintained at least to 16 hr after removal of glucose from the medium. In contrast, the transcription rate for p3A10 remained unchanged at 10 hr and was slightly less than the basal rate at 12–16 hr.

Comparison with Cytosolic *P-enol*pyruvate Carboxykinase Gene Transcript. The hybridization patterns of p3C5, p4A3, and pPCK10 with total cytoplasmic RNA extracted from hamster K12 cells and rat hepatoma Fao-1 cells are shown in Fig. 4. pPCK10 hybridized to an RNA species of 4,000 nt in hamster lung fibroblasts; in contrast, it hybridized to an RNA size of 2,700 nt in rat hepatoma cells. Consistent with previous reports (13), the level of cytosolic *P-enol*pyruvate carboxykinase gene transcripts was markedly increased by the administration of Bt_2cAMP to rat hepatoma Fao-1 cells. However, in the hamster lung fibroblast cells, we did not

Table 2. Relative transcription rates during glucose starvation

Time of pulse, hr†	Fold increases over $t = 0$*		
	p3C5	p4A3	p3A10
0	1	1	1
10	3.8	1.9	1
12	7.4	2.5	0.7

*The fold increase in the transcription rate above the basal level was calculated by dividing the cpm bound at various pulse-label times with the value obtained for the nonstarved cells. The amount of cpm used for each hybridization experiment ranges from 2×10^6 to 2×10^7 total. The background hybridization to filters with no DNA was about 20 cpm; the experimental samples were from 10 to 150 cpm above the background.

†Time of pulses were as follows: 0 hr, subconfluent K12 cells grown in complete DME medium just prior to change to glucose-free medium; 10 hr, cells incubated in a glucose-free medium for 10 hr; 12 hr, cells incubated in glucose-free medium for 12 hr.

detect any increase in *P-enol*pyruvate carboxykinase mRNA levels during 24 hr of glucose starvation nor was there any effect by treatment of these cells with Bt_2cAMP and dexamethasone. Identical results were obtained when we eliminated the pretreatment with serum-free medium prior to addition of Bt_2cAMP. Interestingly, there appeared to be a 2-fold increase in the 4,000-nt *P-enol*pyruvate carboxykinase transcript level when the K12 cells were incubated at 40.5°C, suggesting that the level of *P-enol*pyruvate carboxykinase transcripts can be slightly affected by the elevated temperature.

In contrast, p3C5 and p4A3 mRNA levels were inducible by glucose starvation (Fig. 4) as well as by the temperature shift to 40.5°C, the nonpermissive temperature for the K12 *ts* mutation. However, p3C5 and p4A3 gene transcript levels in both fibroblasts and liver cells were unaffected by the addition of Bt_2cAMP or dexamethasone. Based on the difference in transcript sizes in the hamster fibroblast cells as well as the difference in response to hormonal stimulation and nutritional starvation conditions, we conclude that p3C5 and pPCK10 encode different gene transcripts.

DISCUSSION

When hamster fibroblast cells are starved of glucose, the most obvious consequence is arrested cell division for about 2 days. Apparently, during this period the cells are adapting to the loss of glucose as the primary carbon and energy source, and new sets of genes may be turned on or the expression of other genes may be modulated (or both) in response to this nutritional stress. From 10 to 16 hr after the removal of glucose from the medium, the cells incorporate 1/10th as much [^{3}H]uridine into the nuclear RNA, suggesting either an inhibition of transcription or a dramatic expansion of the *in vivo* uridine pool. The latter could be brought about by the degradation of nucleotides during glucose starvation. Thus, it is interesting to note that against this background of general growth arrest in glucose-starved cells, two genes encoding the hamster cDNA clones p3C5 and p4A3 are induced. In addition, the kinetics of their induction follows a nearly identical pattern. Essentially, after a lag period of 8 hr, there is a parallel increase in their mRNA levels, and the nuclear transcription rates of these two genes are simultaneously increased. The maximal steady-state of these mRNAs is achieved within 16–24 hr, reaching a plateau level representing a 10-fold increase for p3C5 and a 4-fold increase for p4A3. If the mRNA levels for p3C5 and p4A3 are measured after 3 days of incubation in glucose-free medium, the increase for both genes is about 3- to 4-fold (11). By restoring the glucose concentration of the medium to 4.5 mg/ml, the expression of these two genes can readily be repressed. We generally observe a drop of these two mRNA levels within 16 hr of glucose addition (data not shown).

We have also tested whether other nutritional manipulations can induce the expression of these genes. From our experience, the transcriptional activities of these genes are specifically controlled by the availability of glucose in the culture medium. The removal of pyruvate or glutamine from the DME medium showed no effect in a 24-hr incubation period (unpublished data), and we previously reported that for cells grown at 35°C without any medium change for 4 days but supplemented with fresh glucose daily p3C5 and p4A3 remained at basal levels (11).

Furthermore, we asked the question whether these genes are conserved in other mammalian cells and whether their expression is similarly regulated. Using these hamster cDNA clones as hybridization probes in genomic DNA blot analysis, we have been able to detect hybridization bands in DNA extracted from human, mouse, and rat. The assign-

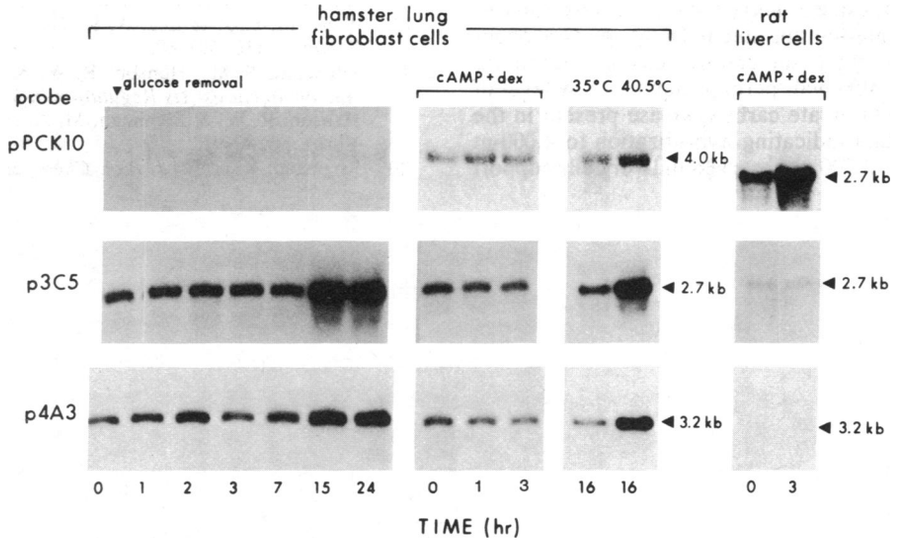

FIG. 4. Comparison of pPCK10, p3C5, and p4A3 mRNAs in hamster lung fibroblast and rat hepatoma cells. Hamster K12 or rat hepatoma Fao-1 cells were grown to 90% confluency in complete DME medium. After various treatments (described in detail in the text), total cytoplasmic RNA was isolated from cells incubated in various times in (*i*) glucose-free medium, (*ii*) medium supplemented with 0.5 mM Bt_2cAMP, 1 mM theophylline, and 0.2 mM dexamethasone (dex), and (*iii*) complete DME medium at either 35°C or 40.5°C. Equal amounts of each RNA sample (10 μg) were applied to each gel lane and, after electrophoresis, were blotted onto nitrocellulose filters. The RNA samples were hybridized, as indicated, with nick-translated pPCK10, p3C5, and p4A3. The sizes (in kb) of the major hybridization bands are indicated by the arrows.

ment of the p3C5 locus on human chromosome 9 has recently been established by somatic cell genetic techniques (unpublished data). We have also isolated from rat genomic libraries overlapping clones corresponding to p3C5 gene sequences. When normal rat kidney fibroblast cells were starved of glucose, the level of a gene transcript identical in size to that of p3C5 (2,700 nt) was significantly increased. In view of the general occurrence of these two genes in mammalian cells and their specific response to glucose, they may encode essential functions that allow the cells to overcome the loss of glucose as a primary carbon and energy source. The existence of a temperature-sensitive cell line such as K12 that activates these genes when shifted to the nonpermissive temperature also constitutes a useful tool for the elucidation of the molecular control mechanism involved in the coordinated expression of these genes. Because we have already shown that K12 hamster cells are highly competent for DNA-mediated gene transfer studies (18) and because genomic clones containing the flanking sequences of these genes have been isolated, specific DNA sequence(s) in these genes required for their regulated expression can be examined.

The fact that the mRNAs encoding by p3C5 and cytosolic *P-enol*pyruvate carboxykinase were nearly identical in size and both were repressed by the addition of glucose led us to test the hypothesis that the two genes may be related. Our results clearly indicate that although p3C5 transcripts are inducible by glucose starvation in fibroblasts, the cytosolic *P-enol*pyruvate carboxykinase gene expression is not. It is notable that although pPCK10 hybridized to mRNA of 2,700 nt in rat liver and rat hepatoma cells, it hybridized to an mRNA species of 4,000 nt in the hamster fibroblasts. In addition, although *P-enol*pyruvate carboxykinase is highly induced by cAMP and dexamethasone, neither p3C5 mRNA nor p4A3 mRNA is affected in liver or fibroblasts. Thus, based on these observations, we conclude that p3C5 does not code for cytosolic *P-enol*pyruvate carboxykinase.

Because the expression of the *P-enol*pyruvate carboxykinase gene is tissue specific, being produced predominantly in liver and kidney but not in fibroblasts, our results reaffirm this view. It is known that in vertebrates, glucose is critically needed during starvation for tissues such as the brain, kidney medulla, and erythrocytes (ref. 19, for a review). If glucose is eliminated from the diet, both the liver and the kidney will rapidly synthesize *P-enol*pyruvate carboxykinase, which catalyzes the rate-limiting step in lactate gluconeogenesis (20). Apparently, our tissue culture cells do not require glucose synthesis for survival, perhaps due to a low level of mitochondrial *P-enol*pyruvate carboxykinase present in the mitochondria. Our data indicating hybridization to 4,000-nt mRNA instead of the 2,700 nt observed in liver cells support this hypothesis. Therefore, the genes that are induced during glucose starvation may be involved in different metabolic processes, such as converting other available nutrients as sources for carbon and energy.

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