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Genetics

Trans regulation of the phospho*enol*pyruvate carboxykinase (GTP) gene, identified by deletions in chromosome 7 of the mouse

(gluconeogenic enzymes/mRNA concentration/transcription rate/regulatory genes)

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ABSTRACT **Livers from newborn mice homozygous for either one of the lethal deletions c^{14CoS} or c^{3H} in chromosome 7 have drastically reduced levels of cytosolic phospho*enol*pyruvate carboxykinase (GTP) [GTP:oxaloacetate carboxy-lyase (transphosphorylating), EC 4.1.1.32] activity when compared with normal littermates. The structural gene for the enzyme maps on chromosome 2 and appears intact and not grossly rearranged in deletion homozygotes. These mice also have negligible levels of hepatic mRNA encoding this enzyme. Studies of the transcription rate of the gene showed that it was reduced to 25–50% of normal in hepatic nuclei obtained from mice homozygous for either deletion. We suggest that, in addition to the reduction in the level of transcription, the deletions in chromosome 7 may also cause alterations in messenger stability, processing, or transport from the nucleus.**

Several radiation-induced overlapping deletions near the albino locus on chromosome 7 of the mouse cause deficiencies of certain hepatic enzymes in newborn homozygotes (1). The structural genes encoding two of these enzymes—glucose-6-phosphatase and tyrosine aminotransferase—were shown to map on chromosomes other than that carrying the deletions (2, 3). Further analysis suggested that the chromosomal region included by the deletions encoded a *trans*-acting factor(s), normally required for expression of the relevant genes (4). The role of phospho*enol*pyruvate carboxykinase (GTP) [PEPCK; GTP:oxaloacetate carboxy-lyase (transphosphorylating), EC 4.1.1.32] in hepatic gluconeogenesis and the similarity of its developmental regulation to that of tyrosine aminotransferase (5) suggested that the deletions might also affect PEPCK. Although previous studies indicated normal PEPCK activity (6), a more sensitive assay revealed drastically reduced enzyme activity in livers of newborn deletion homozygotes. This paper reports the results of a detailed evaluation of the specific steps in the expression of the PEPCK gene affected in deletion homozygotes.

MATERIALS AND METHODS

Materials. Restriction enzymes, bacteriophage T7 DNA ligase, and DNA polymerase were purchased from Boehringer Mannheim and used according to the supplier's recommendations. T7 RNA polymerase and pT7 cloning vectors were purchased from United States Biochemical (Cleveland, OH). Nitrocellulose (BA-85) was obtained from Schleicher & Schuell.

Animals. Mice carrying the lethal albino deletions c^{3H} and c^{14CoS} are maintained at the Albert Einstein College of Medicine. Since deletion homozygotes die perinatally, the deletions must be bred as heterozygotes with chinchilla (c^{ch}) as the normal allele. Deletion homozygotes are albino. Normal littermates, either homozygous (c^{ch}/c^{ch}) or heterozygous (c^{ch}/c^{3H} or c^{ch}/c^{14CoS}), have pigmented eyes and served as controls. Livers were removed and either used immediately or stored in liquid nitrogen and analyzed as described below.

PEPCK Enzyme Activity. PEPCK activity was determined on at least two concentrations of protein for each sample, using the $^{14}CO_2$-fixation assay of Chang and Lane (7), and was calculated in nmol/min per gram (wet weight) of liver by linear regression analysis of kinetic data.

Plasmid DNA. The plasmid pPCK10, which contains a nearly full-length 2.6-kilobase (kb) cDNA copy of rat cytosolic PEPCK mRNA (8), was used in most of the present studies. Some employed a plasmid (pT7-PCK10) constructed by ligating a 1.4-kb *Bgl* II–*Pst* I fragment from the 5′ end of pPCK10 into pT7-2. This plasmid contains a single T7 RNA polymerase promoter, which allows synthesis of very high specific activity (4×10^8 cpm/μg) [^{32}P]RNA probes complementary to PEPCK mRNA (9).

Analysis of Mouse Genomic DNA. Frozen mouse carcasses were powdered in liquid nitrogen and digested for 3 hr with proteinase K. DNA was subsequently purified by RNase treatment and phenol extraction (10). The DNA was digested with specific restriction enzymes, separated by electrophoresis in 0.7% agarose gels, transferred to nitrocellulose (11), and hybridized to ^{32}P-labeled pPCK10 (see legend, Fig. 1).

RNA Analysis. RNA was isolated from frozen mouse livers (12), denatured in 15 mM methylmercuric hydroxide, separated by electrophoresis in a 0.8% agarose gel in 2.2 M formaldehyde, transferred to nitrocellulose, and hybridized to nick-translated [^{32}P]pPCK10 or [^{32}P]pT7-PCK10 RNA (8). The levels of RNA from the various animals were quantitated by dot-blot analysis (13). Four concentrations of RNA (2–12 μg) were applied to nitrocellulose and hybridized to [^{32}P]pT7-PCK10 RNA under stringent conditions (see legend, Fig. 2). Samples were cut from the nitrocellulose, and the radioactivity was determined.

RNA Transcription by Isolated Nuclei. Livers were removed from normal or mutant animals, homogenized in 30 volumes of buffer A (0.3 M sucrose/60 mM KCl/15 mM NaCl/0.5 mM spermidine/15 mM Tris Cl, pH 7.5/0.15 mM spermine/2 mM EDTA/0.5 mM EGTA/14 mM 2-mercaptoethanol), and centrifuged at 800 × *g* for 5 min. The nuclei were resuspended in buffer A containing 0.1% Triton X-100,

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Abbreviations: PEPCK, phospho*enol*pyruvate carboxykinase (GTP); Bt_2cAMP, N^6,O^2-dibutyryladenosine 3′,5′-cyclic monophosphate; kb, kilobase(s).

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centrifuged at 800 × *g* for 5 min, and resuspended in buffer B [50% (vol/vol) glycerol/4 mM $MnCl_2$/1 mM $MgCl_2$/20 mM Tris Cl, pH 7.4/5 mM dithiothreitol]. The transcription assay was carried out as described previously (14), except that the RNase treatment was omitted because of the use of a heterologous cDNA probe. [^{32}P]RNA synthesis was initiated by addition of 100 μCi (1 Ci = 37 GBq) of [α-^{32}P]UTP and allowed to continue for 20 min at 25°C. [^{32}P]RNA was prepared by DNase and proteinase K treatment, followed by phenol/chloroform (1:1, vol/vol) extraction and ethanol precipitation. Transcription rates were determined by hybridization of labeled nuclear RNA for 36 hr to nitrocellulose-immobilized pPCK10 or rat albumin [pALB, (15)], mouse α_1-antitrypsin (16), or β-actin (17) cDNA clones. Filters were washed and the bound radioactivity was determined either by liquid scintillation counting or by autoradiography.

RESULTS

The activity of hepatic PEPCK in fetal and newborn mice is presented in Table 1 and shows the perinatal rise expected in the normal newborn (18). Differences in enzyme activities between normal fetuses of the two strains c^{3H} and c^{14CoS} may reflect differences in fetal age at the time of dissection. Cytosolic PEPCK activity was markedly reduced in mutant newborn livers compared with normal littermates. In both c^{14CoS} and c^{3H} deletion homozygotes, activity amounted to ≈14% of normal. Newborn mice from another strain, carrying the c^{112K} deletion (not included in Table 1), showed PEPCK activity of 23.4 nmol/min per gram of liver in deletion homozygotes as compared with 108.9 nmol/min per gram in normal littermates. A small percentage of total PEPCK activity could have been due to the release of the mitochondrial form of this enzyme during liver homogenization. However, this is known to represent a maximum of 5–10% of the total activity of the enzyme in rodent species (19). Since a gentle homogenization method was used for the solubilization of the enzyme, the overall contribution of the mitochondrial enzyme to the observed activity is not likely to be significant.

In order to confirm that the low levels of PEPCK activity in deletion homozygotes were not due to absence or gross alterations of the structural gene for the enzyme, genomic DNA from homozygous deletion mutants and normal littermates was analyzed by Southern blotting (Fig. 1). Genomic DNA was digested with *Eco*RI or *Xba* I and then hybridized to nick-translated pPCK10. Rat genomic DNA was also digested with these two restriction enzymes to determine the efficiency of hybridization of the rat cDNA probe to mouse genomic DNA. The rat PEPCK gene is contained in a single *Eco*RI fragment (8), and digestion of rat genomic DNA produced the expected 5.4-kb fragment (Fig. 1*A*, lane R). In the mouse, the entire gene is contained in an ≈12-kb fragment of DNA from both normal (Fig. 1*A*, lanes 1, 2, 5, and 6) and homozygous mutant (lanes 3, 4, 7, and 8) newborn animals. In addition, a 2.2-kb band was observed in some of the c^{3H}

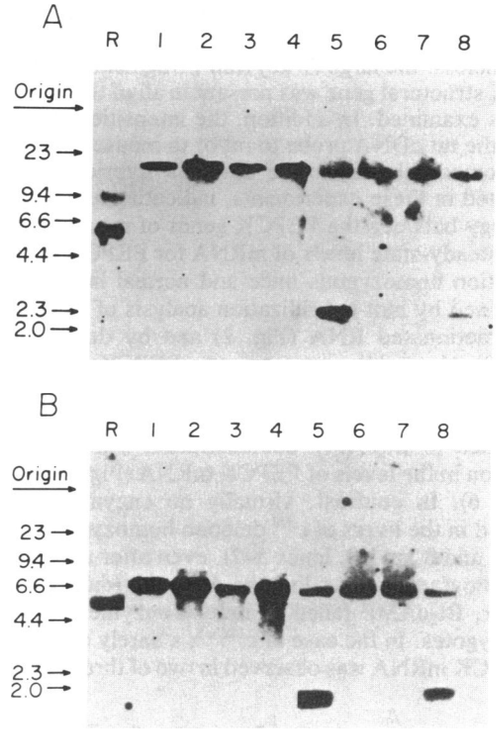

FIG. 1. Southern analysis of genomic DNA prepared from newborn normal and homozygous mutant c^{14CoS} and c^{3H} mice. DNA (12 μg) was digested with restriction enzyme *Eco*RI (*A*) or *Xba* I (*B*), electrophoresed in a 0.7% agarose gel, and transferred to nitrocellulose (12). Membranes were prehybridized in 5× SSC/0.1% $Na_4P_2O_7$/1% NaDodSO$_4$/0.2% Ficoll/0.2% polyvinylpyrrolidone/5 mM Na_2EDTA/50 mM Tris Cl, pH 7.5/10% dextran sulfate with denatured salmon sperm DNA (150 μg/ml) for 4 hr at 65°C. (1× SSC is 0.15 M NaCl/15 mM trisodium citrate). ^{32}P-labeled pPCK10 was added (2 × 10^6 cpm/ml, specific activity ≈ 250 × 10^6 cpm/μg of DNA) and hybridized to the filters at 62°C. Filters were washed three times (30 min each) in 2× SSC/0.1% NaDodSO$_4$ at room temperature and twice in 0.1× SSC/0.1% NaDodSO$_4$ at 65°C and exposed to x-ray film. Lanes: 1 and 2, DNA from c^{14CoS} normal littermates; 3 and 4, DNA from c^{14CoS} homozygous deletion mutants; 5 and 6, DNA from c^{3H} normal littermates; 7 and 8, DNA from c^{3H} homozygous deletion mutants; R, rat genomic DNA (12 μg). Numbers at left refer to the position of size markers (*Hin*dIII-digested λ phage DNA) in kb.

animals, both normal and mutant (lanes 5 and 8). No smaller bands of this type were present in the preparations from either normal or mutant mice of the c^{14CoS} strain. When mouse genomic DNA was digested with *Xba* I, a 7-kb fragment was found to hybridize to the rat PEPCK cDNA probe (Fig. 1*B*). Again, certain normal as well as mutant animals of the c^{3H} strain contained a smaller, 1.9-kb fragment. These smaller DNA fragments were noted in four separate analyses, so that it is unlikely that they are due to artifacts of the Southern blotting procedure. However, their

Table 1. Activity of cytosolic PEPCK in the livers of fetal and newborn deletion homozygotes and normal littermates

Mice	Enzyme activity, nmol/min per g of liver	
	Fetal	Newborn
c^{14CoS}/c^{14CoS}	27.2 (1)	26.4 ± 7.5 (9) [14.2% of normal]
Normal littermates	78.3 ± 8.5 (4)	185.7 ± 5.7 (7)
c^{3H}/c^{3H}	17.3 ± 3.3 (4)	16.9 ± 2.8 (5) [13.6% of normal]
Normal littermates	18.5 ± 1.5 (5)	124.0 ± 31.7 (6)

Livers were isolated from fetuses (18–19 days of gestation) or newborn mice, homogenized in 0.25 M sucrose (1:10, wt/vol) with a Potter–Elvejhem homogenizer, and centrifuged at 30,000 × *g* for 30 min at 4°C. The supernatant fraction was assayed for PEPCK activity at 30°C (9). Data were calculated as the mean ± SD for the number of animals shown in parentheses.

significance remains unknown. Any possible interpretation must keep in mind the highly inbred state of the c^{3H} strain. Nevertheless, the large (7-kb) *Xba* I fragment containing the PEPCK structural gene was present in all of the mouse DNA samples examined. In addition, the intensities of hybridization of the rat cDNA probe to rat or to mouse genomic DNA were comparable under the stringent hybridization conditions used in these experiments, indicating a high degree of homology between the PEPCK genes of rat and mouse.

The steady-state levels of mRNA for PEPCK in the livers of deletion homozygous mice and normal littermates were determined by blot-hybridization analysis of electrophoretically fractionated RNA (Fig. 2) and by dot-blot analysis (Table 2). Normal livers contained a PEPCK mRNA of 3 kb, slightly larger than the 2.8-kb mRNA for the rat enzyme (20). Normal newborn mice of both the c^{3H} and c^{14CoS} strains responded to Bt_2cAMP administration with a 2- to 3-fold induction in the levels of PEPCK mRNA (Fig. 2*A*, lanes 1, 2, 5, and 6). In contrast, virtually no enzyme mRNA was detected in the livers of c^{3H} deletion homozygotes (Fig. 2 *A*, lanes 7 and 8, and *B*, lanes 5–7), even after exposure of the autoradiograms for as long as 6 weeks (data not shown). Further, Bt_2cAMP failed to induce enzyme mRNA in c^{3H} homozygotes. In the case of c^{14CoS}, a barely detectable level of PEPCK mRNA was observed in two of three homozygotes

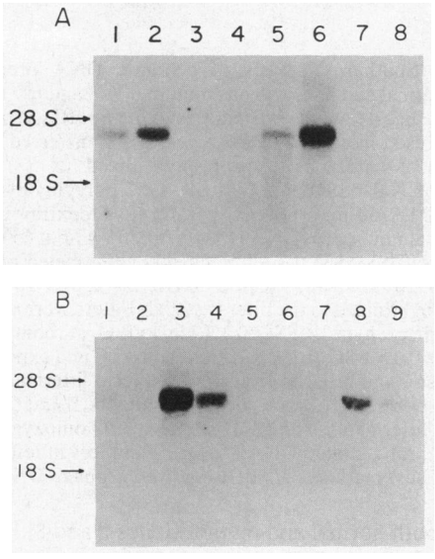

FIG. 2. Hybridization analysis of RNA prepared from livers of c^{14CoS} and c^{3H} normal and homozygous deletion newborn mice. Total RNA (20 μg) was denatured in 15 mM methylmercuric hydroxide, electrophoresed in a 0.8% agarose gel in 2.2 M formaldehyde, transferred to nitrocellulose (12), and hybridized to either [^{32}P]pPCK10 DNA (*A*) or [^{32}P]PCK10 RNA (*B*). (*A*) Filters were prehybridized for 4 hr and then hybridized to nick-translated pPCK10 in 50% formamide/0.8 M NaCl/20 mM Pipes/5 mM EDTA/0.5% $NaDodSO_4$ at 42°C. Membranes were subsequently washed three times in 2× SSC/0.1% $NaDodSO_4$ at room temperature and twice in 0.1× SSC/0.1% $NaDodSO_4$ at 55°C and exposed to x-ray film. Lanes: 1 and 2, normal c^{14CoS} littermates; 3 and 4, c^{14CoS} deletion homozygotes; 5 and 6, c^{3H} normal littermates; 7 and 8, c^{3H} deletion homozygotes. Samples in lanes 2, 4, 6, and 8 are from animals injected with Bt_2cAMP (125 mg/kg of body weight). (*B*) Filters were hybridized to [^{32}P]RNA synthesized with T7 polymerase using the 5′ portion of pPCK10 as the template. Membranes were prehybridized for 4 hr and hybridized at 65°C as described (8), washed three times at room temperature in 2× SSC/0.1% $NaDodSO_4$ and twice in 0.1× SSC/0.1% $NaDodSO_4$ at 65°C. Lanes: 1 and 2, c^{14CoS} deletion homozygotes; 3 and 4, c^{14CoS} normal littermates; 5–7, c^{3H} deletion homozygotes; 8 and 9, c^{3H} normal littermates. Lanes 1, 3, 5, and 7 contain RNA from newborn mice injected with Bt_2cAMP. Positions of 28S and 18S rRNA markers are shown at left.

Table 2. Levels of PEPCK mRNA in the livers of newborn deletion homozygotes and normal littermates

Mice	Bt_2cAMP	cpm/μg of RNA	% of normal
c^{14CoS}/c^{14CoS}	−	10.6 ± 2.3 (3)	11
	+	33.3 ± 11 (2)	15
Normal littermates	−	95.8 ± 23.4 (3)	
	+	222.9 ± 105 (2)	
c^{3H}/c^{3H}	−	9.2 ± 2.9 (4)	5.7
	+	6.0 ± 1.1 (2)	2.3
Normal littermates	−	161 ± 15 (3)	
	+	252 ± 69 (2)	

Where indicated, animals were injected with 125 mg of N^6, O^2-dibutyryladenosine 3′,5′-cyclic monophosphate (Bt_2cAMP) per kg of body weight and killed within 2 hr. PEPCK mRNA was measured in livers of all mice by a dot-blot assay using a [^{32}P]RNA probe prepared with T7 polymerase. Details of hybridization and washing procedures are given in the legend to Fig. 2*B* and in ref. 7. Each sample was assayed at four RNA concentrations (2–12 μg), and the relative concentration of PEPCK mRNA was determined from the slope of the calculated regression curve ($r = 0.99 \pm 0.02$). Values are means ± SEM for the number of samples shown in parentheses.

(Fig. 2 *A*, lane 3, and *B*, lane 2), and Bt_2cAMP caused some induction of enzyme mRNA, though at a level far below that found in Bt_2cAMP-treated normal mice (Fig. 2 *A*, lane 4 vs. lane 2, and *B*, lane 1 vs. lane 3).

The relative levels of PEPCK mRNA were also determined by dot-blot analysis (13) using ^{32}P-labeled pT7-PCK10 RNA (Table 2). This procedure, more sensitive than the DNA·RNA hybridization technique (Fig. 2*A*), made it possible to evaluate the extremely low levels of PEPCK mRNA in the livers of deletion homozygotes. In c^{14CoS} homozygotes, the hepatic PEPCK mRNA was 11% of normal. Even after induction with Bt_2cAMP, the final levels were only 15% of normal. In c^{3H} homozygous mutants, the levels of hepatic PEPCK mRNA amounted to 2–6% of normal and were not inducible by Bt_2cAMP.

In order to determine whether the reduction in PEPCK mRNA might be due to a decrease in RNA synthesis, the relative transcription rate of the PEPCK gene was measured, using nuclei from the livers of mutant and normal mice (Table 3, Fig. 3). All animals received Bt_2cAMP to maximize transcription rates. In general, total RNA synthesis by isolated nuclei was similar for all animals studied. Moreover, liver nuclei of c^{14CoS} and c^{3H} homozygotes actively transcribed the PEPCK gene, as measured by the *in vitro* nuclear transcription assay. In both cases, however, the level of gene

Table 3. Transcription of the gene for PEPCK in nuclei isolated from livers of mutants and normal littermates

Mice	Total RNA synthesis, cpm × 10^{-6}	% (× 10^2) PEPCK	% (× 10^2) albumin
c^{14CoS}/c^{14CoS}	2.37 ± 0.46	0.77 ± 0.25 (5)	0.22 (2)
Normal littermates	2.37 ± 0.66	1.50 ± 0.18 (5)	0.25 (2)
c^{3H}/c^{3H}	2.83 ± 0.05	0.66 ± 0.26 (3)	0.33 (2)
Normal littermates	3.31 ± 0.73	1.74 ± 0.48 (3)	0.35 (2)

Mice were injected with Bt_2cAMP (125 mg/kg of body weight); after 20–45 min, hepatic nuclei were isolated and RNA synthesis was determined as outlined in *Materials and Methods*. As a control for nonspecific binding of newly synthesized [^{32}P]RNA, a nitrocellulose filter containing pBR322 was included. Approximately 100 cpm of nonspecific binding was noted in these experiments. Synthesis of RNA specific for PEPCK or albumin was calculated as follows: % specific RNA synthesis = [(cpm, specific RNA − cpm, pBR-322)/cpm in total RNA] × 100. Values are means ± SEM for the number of animals shown in parentheses.

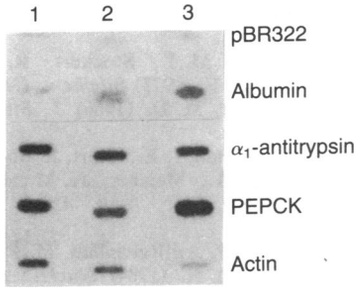

FIG. 3. [^{32}P]UTP-labeled RNA prepared from the nuclei of four individual animals was hybridized to 5 μg of the indicated cDNAs immobilized on nitrocellulose. Hybridizations were carried out in 5× SSC at 65°C for 24 hr. Each reaction mixture contained 10^7 cpm of total input RNA. Lanes: 1, four pooled livers from newborn c^{14CoS} deletion homozygotes; 2, four pooled livers from c^{14CoS} deletion homozygotes in an independent experiment; 3, four pooled livers from normal littermates. The relative rates of transcription were calculated using autoradiography at multiple exposures followed by densitometric scanning of autoradiographs. All animals were injected with Bt_2cAMP (125 mg/kg) prior to isolation of nuclei.

transcription was reduced in comparison to that of normal littermates. In one series of experiments (Table 3), the rate of albumin mRNA synthesis was measured in the same nuclei in order to compare the transcription rate of this liver-specific gene with that of the PEPCK gene in mutant and normal littermates. Albumin mRNA synthesis by liver nuclei from c^{14CoS} and c^{3H} homozygotes was approximately the same as in normal littermates, confirming the results of earlier studies of the levels of albumin mRNA in mutant and normal newborn mice (21).

In another series of experiments (Fig. 3), the rate of PEPCK gene transcription relative to those of the albumin, α_1-antitrypsin, and β-actin genes was measured in c^{14CoS} deletion homozygotes and control littermates. The transcription rate for PEPCK in the deletion mutants (Fig. 3, lanes 1 and 2) was found to be reduced to ≈25% of normal, relative to actin and α_1-antitrypsin, and 50% of normal relative to albumin (Fig. 3). Similar results were obtained in several additional experiments with two pools of five c^{14CoS} deletion homozygous newborns each and one pool of four normal littermates. Finally, the results were also similar in yet another experiment, which used two pools of three and four, respectively, uninduced livers from normal and two pools of three and four, respectively, uninduced livers from deletion homozygous newborn mice. It is interesting to note that, at birth, the rates of transcription of the albumin and actin genes are changing rapidly in mice while α_1-antitrypsin remains constant (17). Therefore, the relative rates of transcription of these genes will vary somewhat with respect to PEPCK from one experiment to another. However, as expected, the transcription rate of α_1-antitrypsin appeared constant (Fig. 3).

The results of both series of experiments demonstrate that the relative rate of transcription of the PEPCK gene in deletion homozygotes is measurable, though at a level significantly below that of induced normal littermates. Nevertheless, the relative transcription rate of the PEPCK gene in the deletion homozygotes (i.e., 25–45% of normal) is higher than the relative levels of mRNA for PEPCK (2–15% of normal).

DISCUSSION

This study demonstrates that the activity of hepatic PEPCK is reduced to about 14% of normal in the livers of newborn mice homozygous for either the c^{14CoS} or the c^{3H} deletion in chromosome 7. PEPCK thus joins other enzymes in the gluconeogenic pathway or in the metabolic sequence from amino acids to glucose precursors, including glucose-6-phosphatase (1), tyrosine aminotransferase (1), and fructose-1,6-bisphosphatase (R.W.H. and D.S.L., unpublished observations), that are either absent or present at very low levels in the livers of deletion homozygotes. These four enzymes form a neonatal family, normally not active in mammals during early fetal development, but appearing perinatally in parallel with the induction of hepatic gluconeogenesis (20, 22).

Southern blot analysis of the PEPCK gene, mapping on chromosome 2 in the mouse (23), indicated identical restriction fragment patterns for its DNA in deletion homozygotes and normal littermates. Nevertheless, deletion homozygotes present a markedly different pattern of PEPCK expression than do their normal littermates. Both enzyme activity and steady-state levels of its mRNA are at most 15% of normal in the livers of homozygous mutants and cannot be significantly induced by Bt_2cAMP administration. However, the reductions in enzyme activity and in concentration of PEPCK mRNA are greater than the decrease in the rate of transcription of the PEPCK gene in deletion homozygotes. This might indicate the existence of defects at both transcriptional and posttranscriptional levels in the regulation of the PEPCK gene expression in the mutants. In general, hormonally induced changes in the levels of PEPCK mRNA parallel alterations in gene transcription (24). However, recent studies, comparing these two parameters in various tissues (25) or after thyroid hormone treatment (13), have suggested a possible posttranscriptional regulation of PEPCK gene expression. In addition, the fungal toxin ochratoxin reduces PEPCK mRNA levels but has little effect on the rate of mRNA synthesis (5).

Recently, Schmid *et al.* (4) reported that only marginal levels of tyrosine aminotransferase steady-state mRNA could be detected in the livers of albino deletion homozygotes, even though no difference from normal was found in the structural gene for the enzyme, which maps on chromosome 8. The residual levels of tyrosine aminotransferase mRNA could not be modulated by either cAMP or glucocorticoids, agents that markedly induce this enzyme in normal animals (26). The authors suggest that a *trans*-acting regulatory factor(s) required for expression and inducibility of tyrosine aminotransferase in mouse liver appears to be encoded within the deleted region of chromosome 7. The same suggestion had been made as the result of earlier studies using somatic-cell-hybridization techniques to produce cell hybrids between rat hepatoma cells and liver cells of mice homozygous for the deletion. In these experiments, mouse glucose-6-phosphatase (2) and mouse tyrosine aminotransferase (3), both of them deficient in deletion homozygotes, were expressed by the hybrid cells. This indicated that factors present in the rat hepatoma parental cells had complemented the missing regulatory functions of the deleted mouse genes and caused normal expression of the intact mouse structural genes in cell hybrids.

The results of the present study strongly indicate that the structural gene encoding PEPCK on chromosome 2 is also subject to regulation by the *trans*-acting factor(s) encoded within sequences mapping in the deleted region of chromosome 7. At this time, it is not possible to decide whether the same factor(s) controls both transcription of the PEPCK gene as well as a possible, as yet unknown posttranscriptional step.

On the other hand, it is possible that the basal levels of PEPCK enzyme activity and mRNA accumulation in the livers of deletion homozygotes, amounting to ≈15% of normal, reflect the existence of regulatory factors different from those encoded within the deletions and operating prior to birth (27). Actually, PEPCK activity is identical in normal

and deletion homozygous fetuses at about 18 days of gestation (Table 1). Systems of gene regulation, as identified in yeast (28), include both positive and negative regulatory factors, and there is no doubt that similar complexities exist in higher eukaryotes. In fact, a negative regulatory factor, Tse-1, that extinguishes tyrosine aminotransferase activity in fibroblasts has been identified in the mouse (29). Such negative regulatory factors must interact with other, positive regulatory factors, such as that encoded within the deletion, in the realization of cell-type-specific gene expression.

The present data demonstrate a decrease in the relative transcription rate of PEPCK in c^{14CoS} and c^{3H} deletion homozygotes. The residual rate of PEPCK gene transcription may be controlled by different regulatory mechanisms, operating also in the late fetus; these might account for the basal levels of mRNA accumulation and enzyme activity found perinatally in the livers of deletion homozygotes.

The nature of the regulatory factor(s) encoded in the deleted sequences near the albino locus on chromosome 7 of the mouse, as well as the mechanisms by which these factors coordinately regulate the expression of structural genes mapping elsewhere in the genome, remains to be determined. The affected functions include those of certain enzymes instrumental in hepatic gluconeogenesis. However, a large number of other liver-specific enzymes remain normal, and total liver protein synthesis is reduced only 15–20% (1). The coordinately regulated differentiation of a cluster of genes by a *trans*-acting factor is reminiscent of the mating-type system in yeast and its regulation by a master regulatory gene complex (28). The analysis of the deletions in chromosome 7 of the mouse has identified a system of control of hepatocyte-specific differentiation by a regulatory gene(s) that might serve as a model for the genetic regulation of differentiation of other cell types. Its further study may reveal some of the complexities in the regulatory pathways of cell-specific gene expression in higher eukaryotes (30).

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