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Genetics

Developmental regulation of constitutive and inducible expression of hepatocyte-specific genes in the mouse

(tyrosine aminotransferase/phospho*enol*pyruvate carboxykinase/inducible gene expression)

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ABSTRACT **Deletions in chromosome 7 of the mouse have been shown to cause failure of expression of certain liver-specific enzymes in newborn deletion homozygotes. Among these enzymes are L-tyrosine:2-oxoglutarate aminotransferase (EC 2.6.1.5) and phospho*enol*pyruvate carboxykinase (GTP) [GTP:oxaloacetate carboxy-lase (transphosphorylating); EC 4.1.1.32]. The studies reported here show that in fetal stages constitutive expression of the relevant genes on the level of steady-state mRNA is identical in the livers of homozygous deletion mutants and normal littermates. Furthermore, prenatally these enzymes are expressed also in cell types other than hepatocytes. Thus, the putative trans-acting regulatory factors encoded in the deleted region of chromosome 7 of the mouse appear to be concerned specifically with the regulation of cell type-specific inducible expression of various hepatocyte-specific genes, whereas constitutive expression of the same genes is not affected.**

Previous studies of the effects of several radiation-induced lethal deletions at and around the albino (*c*) locus in chromosome 7 of the mouse have implicated a gene or genes, included in the deleted region, in the trans-regulation of liver-specific expression of a cluster of structural genes. These structural genes encode various hepatocyte-specific enzymes and map on different chromosomes; they have in common their inducibility by hormones—e.g., cAMP and glucocorticoids (1). Activity of the respective enzymes is either absent or very low in newborn mice homozygous for the deletions, whereas heterozygotes show normal enzyme activities. Somatic cell-hybridization experiments excluded possible deletions or mutations of the structural genes as causes of the enzyme defects by demonstrating normal gene expression in hybrids between mutant mouse hepatocytes and rat hepatoma cells (2). Subsequently, Southern blot analysis proved the structural genes for two of the enzymes, tyrosine aminotransferase [TAT; L-tyrosine:2-oxoglutarate aminotransferase (EC 2.6.1.5)] and phospho*enol*pyruvate carboxykinase (GTP) [PEPCK; GTP:oxaloacetate carboxy-lase (transphosphorylating); EC 4.1.1.32], to be identical in normal and deletion homozygous newborns (3, 4). Whereas enzyme activities served as measures of gene expression in earlier studies (5), the availability of DNA probes for TAT as well as for PEPCK made it possible to examine transcription of the respective genes. Steady-state mRNA and rates of transcription were found to be reduced for both TAT and PEPCK in deletion homozygotes (3, 4). The identification of measurable, though decreased, enzyme activities, as well as mRNA levels, in newborn deletion homozygotes (3–5) suggested an investigation of prenatal expression of the structural genes for TAT and PEPCK in the same genotypes. This report describes the results of these studies and offers the suggestion that constitutive expression of the affected structural genes is not altered in the deletion homozygotes in contrast to the failure of their hormone-inducible expression, which normally develops around the time of birth. The nature of possible regulatory factors missing in the lethal deletion homozygotes and their role in the normal mechanism of hormone inducible gene expression is discussed.

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MATERIALS AND METHODS

Animals. Mice carrying the radiation-induced lethal albino deletion c^{14CoS} are bred and maintained in our laboratory. Newborn deletion homozygotes (c^{14CoS}/c^{14CoS}) are albino and die a few hours after birth, whereas heterozygotes (c^{ch}/c^{14CoS}) are completely viable and not distinguishable from normal littermates (c^{ch}/c^{ch}) until about 10 days of age when coat color can be identified (1).

Fetuses were obtained after timed matings and dissections of the mother on the appropriate days after detection of a vaginal plug on day 0. Absence or presence of eye pigmentation serves to distinguish deletion homozygotes (c^{14CoS}/c^{14CoS}) from normal littermates (c^{ch}/c^{14CoS} or c^{ch}/c^{ch}). Livers, brains, and other tissues—e.g., heart and lung, of fetal and newborn deletion homozygotes and normal littermates were removed without pretreatment, frozen immediately, and stored in liquid nitrogen.

Recombinant Plasmids Containing DNA Sequences for TAT, PEPCK, and β-Actin. The following recombinant plasmids were used in this study: (*i*) pSP6-TAT, containing a 1.4-kilobase (kb) insert of genomic DNA, representing the 3′ end of the mouse TAT gene (3). The specific activities of the ^{32}P-labeled complementary SP6-RNA probes were (1.0–5.2) $\times 10^8$ cpm/μg. (The plasmid was provided by W. Schmid, German Cancer Research Center, Heidelberg.) (*ii*) pT7-PCK10, containing a 1.4-kb insert of rat PEPCK cDNA, corresponding to the 5′ end of the PEPCK gene (6). The specific activities of the ^{32}P-labeled complementary T7-RNA probes were (1.0–7.0) $\times 10^9$ cpm/μg. (The plasmid was provided by R. Hanson, Case Western Reserve University, Cleveland, OH.) (*iii*) β-actin, containing a 1-kb insert of mouse β-actin cDNA. The specific activity of the ^{32}P-labeled nick-translated probe was 3.2×10^8 cpm/μg. (The plasmid was provided by K. Krauter, Albert Einstein College of Medicine, NY.)

RNA Purification. Frozen organs were homogenized with a Polytron at medium speed (Brinkmann), and the total RNA was extracted with guanidinium thiocyanate (7). Poly(A)$^+$ RNA was isolated with messenger affinity paper (Amersham) according to the manufacturer's recommendations.

RNA Blots. One microgram of poly(A)$^+$ RNA for each sample was electrophoresed on 0.8% agarose/2.2 M formaldehyde gel (40 V, 16–20 hr). The RNA was transferred to

Abbreviations: TAT, tyrosine aminotransferase; PEPCK, phospho*enol*pyruvate carboxykinase (GTP).

Nytran filters (Schleicher & Schuell). The filters were baked for 2 hr at 80°C in a vacuum oven.

Hybridization. Filters were prehybridized for 16 hr or more at 42°C in 50% formamide/5× Denhardt's solution (1× Denhardt's solution = 0.02% polyvinylpyrrolidone/0.02% Ficoll/0.02% bovine serum albumin)/5× SSC (1× SSC = 0.15 M sodium chloride/0.015 M sodium citrate, pH 7)/0.1% $NaDodSO_4$ containing salmon sperm DNA at 200 μg/ml and yeast RNA at 50 μg/ml. Hybridization was done for 16–20 hr at 60°C in the same solution as for prehybridization but with 2× Denhardt's solution and probe concentration of approximately 2×10^7 cpm/ml. Filters were washed with 2× SSC/0.1% $NaDodSO_4$ for 30 min at 65°C and with 0.1× SSC/0.1% $NaDodSO_4$ for 1 hr at 65°C.

Autoradiography was conducted at −70°C with two intensifying screens for 1 day, 4 days, and 1 week, and mRNA levels were quantified from densitometric scans.

RESULTS

The levels of TAT and PEPCK steady-state poly(A)$^+$ mRNA obtained from single livers or brains of c^{14CoS} deletion homozygotes and their normal littermates at 17, 18, and 19 days of gestation as well as newborns immediately after delivery were compared on RNA blots as shown in Figs. 1 and 2. These results were reproduced without significant variations in five different experiments. β-actin served as an internal control to assure the loading of equivalent or comparable amounts of mRNA. The sizes of the single bands, ≈2.3 kb for TAT mRNA, ≈3 kb for PEPCK mRNA, and ≈2 kb for actin mRNA, were estimated from the migration of 18S and 28S RNA in the same gel and correspond to the known specific sizes of the respective mRNAs. Densitometry scanning detected no significant differences between steady-state mRNA levels for TAT and PEPCK in livers or brains from normal and deletion homozygous fetuses 1 or 2 days before birth (Figs. 1 and 2). At the time of birth, TAT as well as PEPCK steady-state mRNA levels are significantly higher in the normal liver than in the mutant liver, the normal brain, and the mutant brain (Figs. 1 and 2). Actin hybridized to all samples to the same extent, indicating no differences between samples in loading or transfer.

These results were confirmed using normal and mutant fetal heart/lung preparations (data not shown), which also expressed the same steady-state mRNA levels for TAT and PEPCK as did the livers of normal and deletion homozygous fetuses 1 or 2 days before birth.

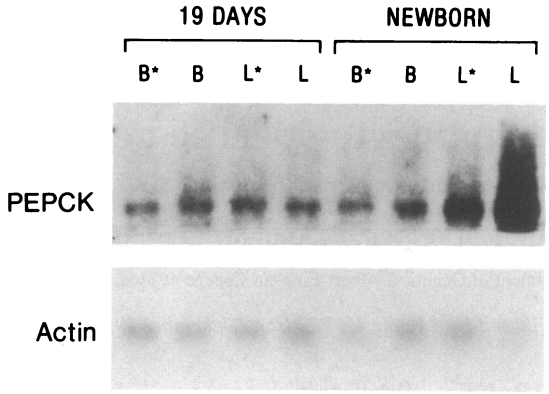

FIG. 2. RNA blot analysis of PEPCK and actin poly(A)$^+$ RNA derived from liver and brain of normal (c^{ch}/c^{ch} and c^{ch}/c^{14CoS}) and deletion homozygous (c^{14CoS}/c^{14CoS}) fetal (19 days of gestation) and newborn mice. L, normal liver; L*, homozygous deletion liver; B, normal brain; and B*, homozygous deletion brain.

DISCUSSION

The developmental profile of TAT and PEPCK gene expression identified by the studies reported here demonstrates the existence of prenatal constitutive gene expression in a variety of cell types including those in which postnatally these same genes are not expressed. No differences of TAT and PEPCK on the level of steady-state mRNA could be detected between fetal liver, brain, heart, and lung cells of normal fetuses or those homozygous for the lethal albino deletion. These findings must be considered in the light of the previous demonstration that in the newborn, enzyme activities, mRNA accumulations, and transcription rates for TAT and PEPCK, though drastically reduced, are not totally absent in the livers of mice homozygous for a lethal albino deletion (3–5). In the normal postnatal mouse, TAT and PEPCK enzyme activities are characterized by their cell-type specificity and their inducibility, which is limited to hepatocytes and restricted regions of the kidney. Furthermore, transcription studies of genes expressed specifically in the liver of mature normal mice demonstrated failure of brain cell nuclei to transcribe liver-specific mRNAs, at least not above a level of 1/10 to 1/50 of that of liver cell nuclei (8). However, in prenatal mice the present studies show the levels of steady-state TAT mRNA to be identical though low in all tissues—i.e., livers, brain, heart (data not shown), and lung (data not shown), of normal as well as of deletion-homozygous fetuses. These findings indicate that constitutive prenatal and perinatal expression of the genes

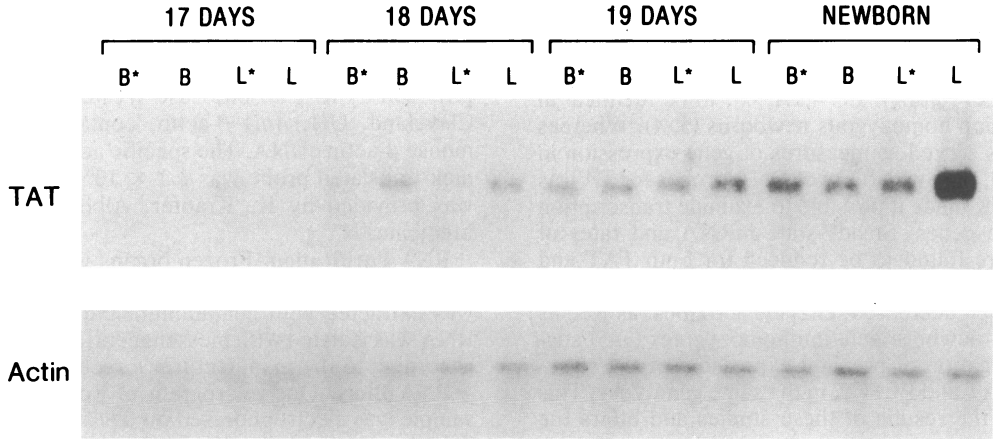

FIG. 1. RNA blot analysis of TAT and actin poly(A)$^+$ RNA derived from liver and brain of normal (c^{ch}/c^{ch} and c^{ch}/c^{14CoS}) and deletion homozygous (c^{14CoS}/c^{14CoS}) fetal (17, 18, and 19 days of gestation) and newborn mice. L, normal liver; L*, homozygous deletion liver; B, normal brain; and B*, homozygous deletion brain.

for TAT and PEPCK may not be affected by the deletions in chromosome 7 and that this constitutive expression is not restricted to specific cells—i.e., hepatocytes and kidney cells, but may be found also in other cell types.

Studies by Killary and Fournier (9) have identified a gene in the mouse (*Tse-1*) mapping on chromosome 11 and mediating tissue-specific extinction of TAT expression in cell types other than hepatocytes and certain kidney cells. Our data reported here indicate that such an extinguishing effect does not appear to be active before birth. Thus, *Tse-1* itself may be developmentally regulated, and its negative control of TAT expression in nonspecific cell types may be targeted at terminally differentiated postnatal stages.

From our studies we propose that, in contrast to constitutive expression, inducible expression of these cell-type specific genes in the liver requires one or more trans-acting factor(s) encoded in chromosome 7 and missing in the lethal albino deletion homozygotes. The precise nature of these factors is not known at present, but they are, no doubt, normally involved in the hormonal induction mechanism. It is significant that studies of hormonal induction in normal rat fetuses (10) have shown that in fetal stages glucocorticoids failed to induce TAT mRNA levels in the liver—indicating a possible lack of competence on the part of the TAT structural gene to react to inducing stimuli in prenatal stages. It therefore appears conceivable that the regulatory factor(s) missing in the deletion homozygotes may be essential in conferring in the course of differentiation the required competence on the *reacting system*—i.e., the structural gene and its regulatory sequences, perhaps by affecting chromatin conformation.

An alternative interpretation—i.e., that the lack of inducibility of TAT and PEPCK in the deletion homozygotes by glucocorticoid hormones and by cAMP may be due to a major defect in a component of the induction pathway does not appear likely, as discussed before (11, 12). It can, however, not be excluded that in the case of glucocorticoids the trans-acting factor(s) might be implicated in the processing, maturation, or transport of the receptor–hormone complex before the latter's binding to the enhancer sequences. This argument would, however, not apply to the failure of cAMP to induce gene expression because of the different induction mechanism of cAMP.

In a discussion of the mechanism of glucocorticoid induction, Yamamoto (13) stresses the role of enhancer elements in the mediation of transcriptional gene regulation and, in particular, that of the glucocorticoid response element to which the receptor hormone complex must bind for induction to be accomplished. It is possible that action of a regulatory protein — e.g., that believed to be encoded in the deleted genes, is required in order to confer on the glucocorticoid response element enhancer sequence the necessary competence to bind the complex (14).

A correlation of a gene's expression, its glucocorticoid inducibility and modification of chromatin structure, has been reported for two liver genes, TAT and tryptophan oxygenase (15). Whether these hormone-specific chromatin structural changes are prerequisites for or perhaps effects of receptor binding is, however, not yet known. The various liver genes regulated by the trans-acting factor in chromosome 7 conceivably have identical specific chromatin sites in their enhancer regions that must be recognized by the regulatory gene product in preparation for the structural gene's hormone-inducible expression.

The most likely interpretation of the data obtained thus far suggests a primary effect of the deletions on the regulation of inducible gene transcription. Nevertheless, the data do not exclude a possible additional posttranscriptional effect—e.g., on mRNA stability. In the case of PEPCK, earlier studies (4) had shown that rates of transcription as measured by run-on experiments decreased to about 25–45% of normal in deletion homozygotes, whereas the amount of accumulated steady-state mRNA was 2–15% of normal, suggesting an effect on message stability.

The next step in the analysis of the nature and possible mechanism of action of the proposed trans-acting regulatory factor(s) must obviously be to attempt its isolation and identification. This would also facilitate the eventual cloning of the gene(s) deleted in the lethal albino mutants and normally responsible for the trans-regulation of the cluster of hepatocyte-specific structural genes that fail to show hormone-inducible expression in the deletion homozygotes.

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