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Genetics

Regulatory genes linked to the albino locus in the mouse confer competence for inducible expression on the structural gene encoding serine dehydratase

(hormone inducibility/lethal chromosome 7 deletions)

MARIE LIA, DEEKSHA BALI, AND SALOME GLUECKSOHN-WAELSCH

Department of Molecular Genetics, Albert Einstein College of Medicine, Bronx, NY 10461

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ABSTRACT **A cluster of unlinked genes encoding gluconeogenic enzymes in the mouse is characterized by the failure of normal hormone-inducible expression in animals homozygous for one of several overlapping deletions mapping on chromosome 7 near the albino locus. Previous investigations have shown hormones and their receptors to be normal in the mutants and therefore not responsible for the abnormalities of inducibility. Instead, these studies have implicated a possible failure of the affected structural enzyme genes themselves to attain during prenatal development the competence for inducible gene expression. The results reported here add serine dehydratase (EC 4.2.1.13) and its structural gene to the affected group of gluconeogenic enzymes and their genes. Even though, in deletion homozygotes, serine dehydratase is expressed normally on the constitutive level, hormone-inducible expression fails to develop. The abnormality appears to reside in a defect of prenatal differentiation of cis-acting regulatory elements of the structural gene essential in the pathway of inducible gene expression.**

The widespread developmental effects of a series of chromosomal deletions at and around the albino locus in chromosome 7 of the mouse have been interpreted to result from the absence of one or more regulatory genes normally mapping within the deleted region (1, 2). These genes, referred to as hepatocyte-specific developmental regulators (*hsdr*), appear to act during late fetal stages and to be concerned primarily with the essential developmental regulation of a particular group of unlinked structural genes encoding gluconeogenic enzymes and proteins, expressed specifically in hepatocytes and normally inducible by hormones. In deletion homozygotes, these structural genes lack the ability of hormone-inducible expression. Among the genes in this cluster identified and studied in detail are those encoding tyrosine aminotransferase (TAT) (3), phospho*enol*pyruvate carboxykinase (PEPCK) (4), and metallothionein (Mt-1) (5). Glucocorticoid hormones and receptors were shown to be normal in deletion homozygotes; they therefore cannot be made responsible for the absence of inducible gene expression (6). Further analysis of the mode of action of the deleted regulatory genes, the mechanisms of regulation normally controlled by them, and their sphere of activity profits from the identification of more targets of their action. We report here the results of investigations of an additional structural gene that is affected by the deletions and lacks the ability of hormone inducibility; it encodes the enzyme serine dehydratase (SDH; EC 4.2.1.13). Our studies concern, in particular, the prenatal and perinatal development of mRNA expression of the SDH gene and its hormone inducibility in normal and deletion homozygous mice.

MATERIALS AND METHODS

Animals. Mice carrying the radiation-induced lethal albino deletions c^{3H} and c^{14CoS} and used in the present studies were maintained and bred in our laboratory. Matings of pigmented heterozygotes c^{3H}/c^{ch} and c^{14CoS}/c^{ch} *inter se* provide albino homozygotes—i.e., c^{3H}/c^{3H} and c^{14CoS}/c^{14CoS}, which die within a few hours after birth.

For hormonal induction studies, newborn albinos (c^{3H}/c^{3H}, c^{14CoS}/c^{14CoS}) and their pigmented normal littermates (c^{3H}/c^{ch}, c^{ch}/c^{ch} and c^{14CoS}/c^{ch}, c^{ch}/c^{ch}) were injected intraperitoneally with a combination of N^6,$O^{2'}$-dibutyryladenosine 3',5'-cyclic monophosphate (100 mg/kg of body weight) and dexamethasone (100 μg/kg of body weight) in 0.85% saline or with 0.85% saline only (control). Livers and brains were removed from albino deletion homozygous and pigmented normal littermates 2 hr after injection. All tissues were frozen in dry ice immediately and subsequently stored at −80°C. Individual livers and brains were used for mRNA determinations.

Timed matings of heterozygotes *inter se* provided the material for studies of SDH mRNA expression in fetuses. These were obtained from mothers dissected 18 or 19 days after the detection of a vaginal plug. Livers and brains were removed from albino deletion homozygous and pigmented normal littermate fetuses and frozen immediately. Tissues of individual fetuses were used for mRNA determinations.

Recombinant DNA Probes. (*i*) The plasmid pBR322 containing 1.4-kilobase rat SDH cDNA inserted into the *Eco*RI site and with an open reading frame of 1089 base pairs was kindly provided by Henry Pitot (McArdle Laboratory for Cancer Research, The Medical School, University of Wisconsin, Madison, WI) (7). It was digested with *Eco*RI and the ^{32}P-labeled DNA probe was prepared by the oligonucleotide random primer technique (Boehringer Mannheim). (*ii*) A chicken β-actin probe (obtained from D. DeFranco, University of Pittsburgh, PA) served as an internal control of the amount of mRNA loaded per lane.

The specific activities of the ^{32}P-labeled probes were 1×10^8 to 1×10^9 cpm/μg.

Isolation of mRNA. Total RNA was extracted from newborn and fetal frozen tissues using the LiCl/urea technique (8). Poly(A)$^+$ RNA was isolated with Poly(A) Quik mRNA purification kit columns (Stratagene). Two to 5 μg of mRNA was electrophoresed on 1% agarose/2.2 M formaldehyde gels (30 V, 16–20 hr) and transferred to nitrocellulose membranes (BA-S85, Schleicher & Schuell). The membranes were baked for 2 hr at 80°C in a vacuum oven.

Hybridization. Prehybridization was performed for 2–3 hr at 42°C in 50% formamide/5× Denhardt's solution/5× SSCPE (1× SSCPE = 0.15 M NaCl/0.015 M sodium citrate/10

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Abbreviations: SDH, serine dehydratase; TAT, tyrosine aminotransferase; PEPCK, phospho*enol*pyruvate carboxykinase (GTP); Mt-1, metallothionein.

mM phosphate/1 mM EDTA)/500 μg of salmon sperm DNA per ml. Hybridization was carried out for 16–20 hr at 42°C in a similar medium as that used for prehybridization but with 1× Denhardt's solution, 10% dextran sulfate, and 100 μg of salmon sperm DNA per ml (9). Filters were cleaned in two succeeding washes with 2× SSC (1× SSC = 0.15 M NaCl/0.015 M sodium citrate)/0.1% SDS at 65°C; the first wash was for 30 min and the second was for 15 min.

Autoradiography was done at −80°C for varying periods of time. SDH mRNA levels were quantified from densitometric scans of autoradiograms.

RESULTS

SDH mRNA Levels Are Reduced in Livers of Newborn Albino Deletion Homozygotes. Postnatal SDH mRNA expression in the liver was determined in c^{3H} and c^{14CoS} deletion homozygous albino newborns and their pigmented heterozygous and wild-type homozygous normal littermates.

The livers of c^{14CoS} deletion homozygous newborns (c^{14CoS}/c^{14CoS}) express much lower levels of SDH mRNA than uninduced or hormone-induced livers of normal heterozygous (c^{ch}/c^{14CoS}) or homozygous (c^{ch}/c^{ch}) littermates. The examples illustrated in Fig. 1 express SDH mRNA levels in albino deletion homozygotes (c^{14CoS}/c^{14CoS}, lanes 1 and 2) reduced to 15% of those of uninduced (lane 3) and 9% of those of hormone-induced (lane 4) normal c^{14CoS}/c^{ch} or c^{ch}/c^{ch} newborn littermates. Furthermore, livers of c^{14CoS}/c^{14CoS} newborns express no increase in SDH mRNA levels after hormone injection (compare lanes 1 and 2), whereas those of hormone-injected c^{14CoS}/c^{ch} and c^{ch}/c^{ch} normal littermates show mRNA levels approximately one and a half times higher than those determined in uninduced livers (compare lanes 3 and 4). These results were confirmed in animals from 12 litters with SDH mRNA expression in c^{14CoS} deletion homozygous livers ranging from 15% to 40% of uninduced normal littermates and total absence of inducible expression; in contrast, normal heterozygous and wild-type homozygous littermates showed hormone inducibility of SDH mRNA in the liver that amounted to 2- to 4-fold over uninduced levels.

Similar results were obtained with livers of newborn mice carrying the c^{3H} deletion. SDH mRNA levels in the livers of c^{3H} albino homozygotes (c^{3H}/c^{3H}) ranged from 15% to 38% of those determined in normal heterozygous (c^{3H}/c^{ch}) and wild-type homozygous (c^{ch}/c^{ch}) littermates. After hormone administration, the livers of normal newborns expressed SDH mRNA levels approximately 2- to 4-fold higher than those of their uninduced littermates, whereas livers of induced c^{3H} deletion homozygotes failed to express any increase of SDH mRNA beyond the uninduced level. These results were confirmed in animals derived from 5 litters.

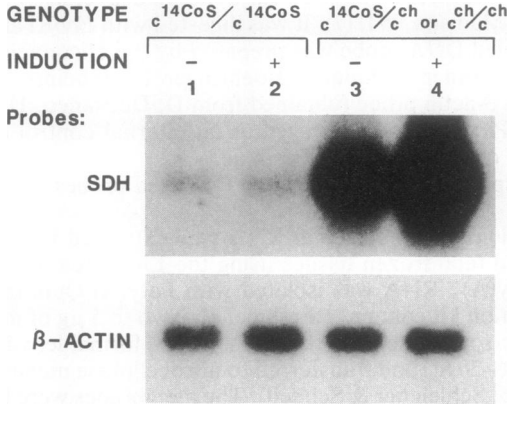

FIG. 1. Effect of $N^6,O^{2'}$-dibutyryladenosine 3',5'-cyclic monophosphate and dexamethasone on the SDH mRNA levels in livers of deletion homozygous (c^{14CoS}/c^{14CoS}) and normal (c^{14CoS}/c^{ch} or c^{ch}/c^{ch}) newborn mice. Northern blot analysis of poly(A)$^+$ RNA derived from livers of noninduced (lane 1) and hormone-induced (lane 2) newborn deletion homozygous (c^{14CoS}/c^{14CoS}) and noninduced (lane 3) and hormone-induced (lane 4) normal (c^{14CoS}/c^{ch} or c^{ch}/c^{ch}) littermates.

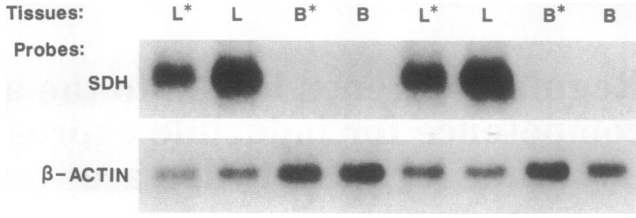

FIG. 2. Northern blot analysis of SDH and β-actin poly(A)$^+$ RNA derived from livers and brains of two deletion homozygous (c^{14CoS}/c^{14CoS}) and two normal (c^{14CoS}/c^{ch} or c^{ch}/c^{ch}) littermate fetuses at 19 days of gestation. L*, c^{14CoS}/c^{14CoS} liver; L, c^{14CoS}/c^{ch} or c^{ch}/c^{ch} liver; B*, c^{14CoS}/c^{14CoS} brain; B, c^{14CoS}/c^{ch} or c^{ch}/c^{ch} brain.

No SDH mRNA signal whatsoever was obtained in the brains of any of the newborn albino homozygotes or in those of their heterozygous and wild-type homozygous normal littermates (data not shown).

SDH mRNA Levels in Fetal Albino Deletion Homozygotes and Their Heterozygous and Wild-Type Homozygous Normal Littermates. Measurements of SDH mRNA expression in the livers of fetuses at 19 days of postconceptual age (12 litters) demonstrated levels of SDH mRNA in c^{14CoS}/c^{14CoS} albino homozygotes that resembled those of newborn deletion homozygotes and ranged from 80% (1 litter, Fig. 2) to 50% (11 litters) of their normal c^{14CoS}/c^{ch} and c^{ch}/c^{ch} littermates (data not shown). SDH mRNA levels in c^{14CoS}/c^{ch} and c^{ch}/c^{ch} normal fetuses are about 45% of those determined in the normal newborn. Similar results were obtained in fetuses at 18 days of postconceptional age (5 litters).

Livers of fetuses homozygous for the c^{3H} deletion and their heterozygous c^{ch}/c^{3H} and wild-type homozygous c^{ch}/c^{ch} normal littermates at 18 (6 litters) and 19 (4 litters) days of fetal life gave results similar to those described for c^{14CoS}.

As in newborns, no SDH mRNA expression could be detected in the brains of c^{3H} or c^{14CoS} deletion homozygous, heterozygous, and wild-type homozygous fetuses (Fig. 2).

DISCUSSION

The structural gene encoding the gluconeogenic enzyme SDH can now be added to the group of genes whose expression is affected in a similar way by the *hsdr* gene or genes included in the lethal deletions around the albino locus in mouse chromosome 7 (10). In analogy to the genes encoding glucose-6-phosphatase, TAT, and PEPCK, the SDH gene normally starts responding to the administration of hormones with increased gene expression around the time of birth (11). Also, in analogy to the earlier mentioned enzyme-encoding genes (12), the normal constitutive expression of the SDH gene in deletion homozygotes eliminates the possibility of a mutational change in its DNA sequence. Analysis of the effects of the albino deletions on the hormone-inducible expression of certain genes encoding gluconeogenic enzymes is beginning to reveal the normal developmental pattern of hormone inducibility that is shared by the affected genes. At the same time, the mutationally caused abnormalities provide the potential for the eventual identification of the respective normal mechanisms responsible for the differentiation of the structural genes during prenatal development. In particular, as mentioned before, the deletions appear to include regulatory genes encoding factors normally required by the relevant cluster of structural enzyme genes during prenatal develop-

ment in the process of achieving their eventual competence for inducible gene expression in response to hormones.

Even though the effects of the albino deletions on the expression of SDH mRNA are in principle the same as those described previously for TAT and PEPCK (12), certain differences between the reactions of the genes have been identified by the present studies. In analogy to TAT and PEPCK, the expression of SDH is refractory to hormone induction in deletion homozygotes. Also, deletion homozygous fetuses express constitutive levels of SDH mRNA, even though these are below those of normals, in contrast to TAT and PEPCK mRNA levels, which are the same in all genotypes. Finally, in contrast to TAT and PEPCK, both of which were shown to be expressed prenatally also in tissues other than liver—e.g., the brain (12)—SDH mRNA could not be identified in extrahepatic tissues in normal or deletion homozygous fetuses. Relatively minor differences between the patterns of developmental and hormonal regulation of expression of different structural genes may be the reason for the observed prenatal expression differences between SDH and the two other genes TAT and PEPCK. Alternatively, it is possible that the lower than normal level of prenatal constitutive SDH mRNA expression in the liver of deletion homozygotes and its total absence in brain of any genotypes might result from differences in sensitivity of the respective probes.

Nonetheless, these minor differences do not detract from the primary common phenomenon—namely, the effect of the albino lethal deletions on the competence of a particular group of hormone-inducible genes to react to hormonal stimulation. The expression analysis of the SDH gene serves to add support to the interpretation that the genome deleted in the lethal albino mutants normally includes one or more developmental regulatory genes (*hsdr*) with trans-acting effects targeted at a particular cluster of structural genes during prenatal stages and their differentiation of competence for hormone inducibility. The absence of the trans-acting factors encoded in the deleted genome may result in defects of chromatin differentiation of the affected genes that depend on these factors for their normal differentiation. At present, comparative studies of chromatin structure of these genes in deletion homozygotes and their normal littermates are necessary to investigate this question further.

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