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Metallothionein mRNA expression in mice homozygous for chromosomal deletions around the albino locus

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ABSTRACT **Deletions in chromosome 7 of the mouse affect the expression of the metallothionein gene *Mt-1*, which maps on chromosome 8, and steady-state levels of *Mt-1* mRNA are reduced to 15–40% of normal in livers of newborn mice homozygous for either the c^{3H} or c^{14CoS} deletion. Glucocorticoids fail to induce hepatic *Mt-1* mRNA levels in deletion homozygotes in contrast to normal littermates. However, zinc chloride is effective in inducing *Mt-1* mRNA levels in livers of deletion homozygotes as well as of their normal littermates. Other tissues (e.g., kidney and intestine) of deletion homozygotes express basal levels of *Mt-1* mRNA higher than those of normal littermates. In the intestine these are furthermore inducible by both hormonal and metal agents. Thus, loss of inducibility of the *Mt-1* gene in deletion homozygotes concerns glucocorticoids only and is furthermore restricted to specific cell types (i.e., hepatocytes). The trans-acting factor(s) normally encoded in the deleted region of chromosome 7 appears to be instrumental in conferring on the metallothionein gene in hepatocytes the essential competence to respond to hormonal inducing signals.**

A series of radiation-induced lethal deletions at and around the albino locus on chromosome 7 in the mouse have been identified that, when homozygous, cause pre- or neonatal death. Ultrastructural and biochemical analyses revealed severe defects in the livers of deletion homozygotes, and some abnormalities were also observed in the newborn kidney (1). Subsequent molecular studies revealed reduced mRNA levels for certain inducible liver-specific genes [including those encoding tyrosine aminotransferase (TAT) (2), aldolase (3), and phospho*enol*pyruvate carboxykinase (PEPCK) (4)] mapping on various chromosomes other than chromosome 7. Hormonal agents such as cAMP and glucocorticoids failed to induce expression of glucose-6-phosphatase, TAT, and PEPCK in livers of newborn deletion homozygotes, in contrast to normal littermates. These observations led to the hypothesis that the DNA sequences deleted from chromosome 7 normally encoded regulatory factor(s) that act in trans to regulate the expression of a number of unlinked liver-specific genes (5). TAT and PEPCK mRNA levels as well as PEPCK enzyme activity were found to be identical in the 18-day fetal liver of mutant and normal littermates, suggesting that the principal defect within these mutants was the inability of the respective genes to respond to hormonal or metabolic signals at birth (ref. 4; M. E. Donner, C.M.L., and S.G.-W., unpublished data).

In the course of perinatal hepatocyte differentiation the expression of specific genes (e.g., those encoding TAT and PEPCK) becomes activated whereas that of others [e.g., those encoding metallothioneins (7)] decreases. Metallothioneins are low molecular weight, cysteine-rich proteins with various physiological functions including the regulation of Zn^{2+} and Cu^{2+} homeostasis and heavy-metal detoxification. They are ubiquitous among eukaryotes and are expressed in various adult tissues, although the predominant site of synthesis is the liver (8). In the mouse, two metallothionein genes, *Mt-1* and *Mt-2*, have been identified that map within a 10-kilobase region on chromosome 8 (9). Metallothionein gene transcription is responsive to various inducers including glucocorticoids and heavy metals both in adult tissues and in fetal liver (7, 10, 11). In the mouse, metallothionein mRNA levels, still relatively high in neonatal liver, gradually decline to normal adult levels within a few weeks postpartum (7).

In contrast to other genes whose expression has been studied in the lethal deletion homozygotes, metallothionein gene expression is not restricted to liver but takes place also to a significant extent in various other fetal tissues (7). The developmental pattern of metallothionein gene expression, its tissue distribution, and its inducibility by various agents suggested the analysis reported here. In particular, a comparison of the patterns of perinatal expression of the metallothionein genes with those for TAT and PEPCK seemed indicated. The results of these studies provide an expanded analysis of tissue-specific effects of the albino deletions in homozygotes and add *Mt-1* to the list of genes that have lost their normal glucocorticoid inducibility. Further, they show that different transcription-inducing agents act through independent regulatory mechanisms that are affected differentially by the deletions in chromosome 7.

MATERIAL AND METHODS

Animals. Mice carrying the c^{3H} and c^{14CoS} lethal albino deletions are bred at the Albert Einstein College of Medicine. These deletions must be maintained in the heterozygous state, since homozygotes die within a few hours of birth. Newborn deletion homozygotes are distinguished from heterozygotes and normal homozygotes by the absence of eye pigment. Tissues were removed shortly after birth from untreated animals. For studies of inducibility, newborn mice were injected subcutaneously with dexamethasone (100 μg/kg of body weight) or $ZnCl_2$ (10 μg/g of body weight) in isotonic saline. Control littermates were injected with saline alone. Tissues were removed 45–90 min following injections and frozen immediately in liquid nitrogen. Frozen tissues were shipped on dry ice to Pittsburgh for further analysis.

Isolation and Analysis of RNA. Total RNA was isolated from frozen tissue (12), subjected to electrophoresis in agarose gels containing formaldehyde (13), and then blot-transferred to GeneScreen membranes (New England Nuclear). For dot blot analysis, total RNA was spotted directly onto the membranes. RNA was fixed to the filters by UV

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

crosslinking (14). The plasmid pMT-1 (kindly provided by R. D. Palmiter, Department of Biochemistry, University of Washington, Seattle), containing mouse *Mt-1* cDNA sequence, was used to prepare ^{32}P-labeled DNA probes by the oligonucleotide random-primer technique (Pharmacia). Specific activities of 1–2 × 10^9 cpm/μg of DNA were routinely obtained. Hybridizations and posthybridization washes were performed at 43°C and 50°C, respectively (15). *Mt-1* mRNA levels were quantified from densitometric scans of autoradiographs. Several exposures were analyzed to assure that measurements were made within the linear response range of the film. Hybridization of the original blots with a β-actin probe provided an internal standard of the amount of RNA loaded per lane, which varied by <20%.

RESULTS

Steady-State Levels of *Mt-1* mRNA Are Reduced in Liver of Newborn Deletion Homozygotes. Steady-state levels of *Mt-1* mRNA were determined by RNA gel blot analysis and densitometric scanning. As shown in Fig. 1, *Mt-1* mRNA levels in the liver of newborn c^{3H} deletion homozygotes were reduced to 15–40% of those of wild-type littermates as determined in six separate experiments. β-Actin mRNA, serving as control, was expressed to the same extent in mutant and normal littermates (data not shown). The *Mt-1* cDNA clone hybridizes specifically to transcripts generated from the *Mt-1* gene but does not cross-hybridize to the *Mt-2* gene transcripts. Therefore the status of *Mt-2* gene expression in liver of newborn deletion homozygotes is unknown.

***Mt-1* mRNA Levels Are Elevated in Some Tissues of Newborn Deletion Homozygotes.** Deleterious effects of the homozygous deletions are not confined to hepatocytes. Morphological abnormalities have been observed also in proximal convoluted tubules of the kidney of c^{3H} and c^{14CoS} homozygotes. Glucose-6-phosphatase and PEPCK are expressed in both neonatal liver and kidney, and enzyme activities as well as PEPCK mRNA levels have been found to be reduced in these organs in deletion homozygotes. Since the effects of the lethal deletions on the expression of different genes may vary in different tissues, *Mt-1* mRNA levels were analyzed in various tissues of four different litters of newborn c^{3H} and c^{14CoS} homozygotes. The reduction in *Mt-1* mRNA levels in the liver of deletion homozygotes as demonstrated on the gel blots (Fig. 1) was confirmed by the dot blot analysis (Fig. 2). In normal animals different tissues express different basal levels of *Mt-1* mRNA, as reported previously (10). The difference in the tissue profile of *Mt-1* mRNA in the normal littermates of c^{3H} and c^{14CoS} deletion homozygotes probably reflects the different genetic backgrounds of the two strains. *Mt-1* mRNA levels are identical in newborn brain of normal c^{14CoS} homozygotes but are elevated ≈1.5-fold in c^{3H} homozygotes. Interestingly, *Mt-1* mRNA levels are elevated ≈2.5-fold over wild-type littermates in both kidney and intestine of newborn c^{3H} deletion homozygotes (Fig. 2). In the case of c^{14CoS}, *Mt-1* mRNA levels are elevated ≈2-fold in kidney and ≈1.5-fold in intestine.

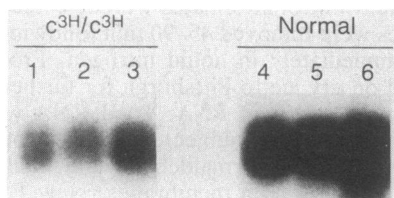

FIG. 1. *Mt-1* mRNA levels in uninduced livers of newborn c^{3H} homozygotes (lanes 1–3) and normal littermates (lanes 4–6). Total RNA (5 μg per lane) was electrophoresed, blotted, and hybridized to a ^{32}P-labeled mouse *Mt-1* cDNA probe. RNA samples from paired littermates were loaded on lanes 1 and 4, 2 and 5, and 3 and 6.

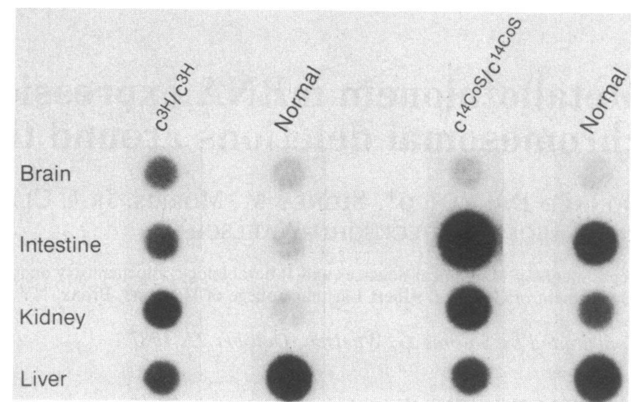

FIG. 2. *Mt-1* mRNA levels in various tissues of newborn c^{3H} and c^{14CoS} homozygotes and normal littermates. Five micrograms of total RNA was fixed and hybridized as described in the legend to Fig. 1.

Failure of Glucocorticoids, but Not Heavy Metals, to Induce *Mt-1* Expression in Liver of c^{3H} Homozygotes. In the liver of newborn c^{3H} and c^{14CoS} homozygotes, TAT and PEPCK mRNAs not only are drastically reduced but, in contrast to normal littermates, also fail to respond to two hormonal inducing agents—i.e., glucocorticoids and cAMP (2, 4). The effects of glucocorticoids and heavy metals, normally inducers of hepatic metallothionein expression, on *Mt-1* mRNA levels in livers of newborn c^{3H} deletion homozygotes were therefore determined.

In adult liver, the induction of metallothionein transcription by heavy metals and glucocorticoids is rapid, and increases in mRNA levels become apparent within 2 hr (10, 11). Since mice homozygous for the c^{3H} deletion die soon after birth, we were not able to perform induction experiments for more than 90 min at the most. The 2-fold increase in *Mt-1* mRNA levels observed in livers of newborn normal animals treated for 60 min with dexamethasone (Fig. 3*A*, lanes 5–8) and $ZnCl_2$ (Fig. 3*B*, lanes 5 and 6) agrees with that expected from extrapolations of previously published time-course experiments (11). *Mt-1* mRNA levels in livers of homozygous deletion littermates were not increased by dexamethasone treatment (Fig. 3*A*, lanes 1–4). In contrast, *Mt-1* mRNA levels were increased 2-fold in the livers of newborn deletion homozygotes induced for 60 min with $ZnCl_2$ (Fig. 3*B*, lanes 1–4). β-Actin mRNA levels were not affected by either dexamethasone or $ZnCl_2$ in mutant and normal littermates (data not shown). Thus the defect in the c^{3H} homozygotes does not result in a complete loss of inducibility of hepatic *Mt-1* expression in neonates but specifically affects glucocorticoid inducibility.

Normal Induction of *Mt-1* Expression by Dexamethasone in Intestine and by $ZnCl_2$ in Kidney of c^{3H} Homozygotes. The effects of the deletions on inducibility by glucocorticoids but not by heavy metals in the liver and the finding that newborn c^{3H} homozygotes expressed higher than normal basal levels of *Mt-1* mRNA in kidney and intestine suggested a study of inducibility by these agents in these tissues. Dexamethasone treatment failed to increase *Mt-1* mRNA levels in kidneys of normal as well as mutant newborns (data not shown). In the adult mouse kidney only a slight induction of this mRNA has been reported (11) following hormone treatments more prolonged than possible in our experiments. As shown in Fig. 4, *Mt-1* mRNA levels were elevated 2-fold in the kidney of newborn c^{3H} homozygotes (lanes 1–4) and 5-fold in normal animals (lanes 5–8) treated with $ZnCl_2$ for 1 hr. The induction of *Mt-1* mRNA levels by $ZnCl_2$ in the c^{3H} homozygotes

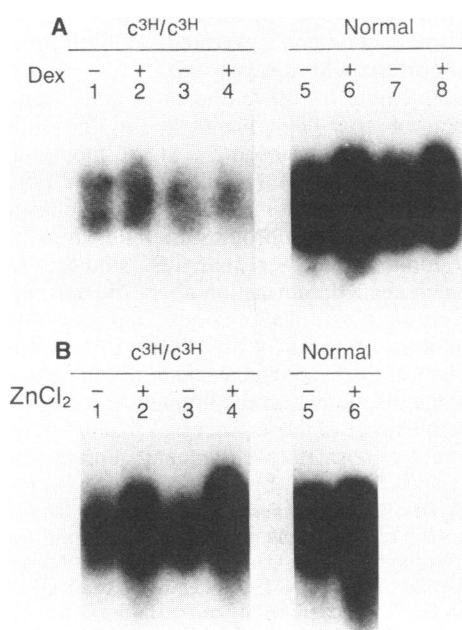

FIG. 3. (*A*) Effects of dexamethasone on *Mt-1* mRNA in livers of newborn c^{3H} homozygotes (lanes 1–4) and normal littermates (lanes 5–8). Total RNA was prepared from induced (+) and uninduced (−) livers and processed as described in the legend to Fig. 1. RNA samples loaded on lanes 1, 2, 5, and 6 and those on lanes 3, 4, 7, and 8 were derived from separate litters. (*B*) Effects of $ZnCl_2$ on *Mt-1* mRNA in livers of newborn c^{3H} homozygotes (lanes 1–4) and normal littermates (lanes 5 and 6). Total RNA was prepared from induced (+) and uninduced (−) livers and processed as described in the legend to Fig. 1. RNA samples loaded on lanes 1, 2, 5, and 6 and those on lanes 3 and 4 were derived from separate litters.

is superimposed upon a basal level elevated 2.5-fold over that of normal animals; this may account for the lower induction ratio observed in the mutants. However, it is apparent that heavy metals are able to induce *Mt-1* mRNA levels in liver as well as kidney of deletion homozygotes, despite the opposite effects of the deletions on the basal levels of *Mt-1* mRNA in these tissues.

The results of glucocorticoid induction of *Mt-1* mRNA levels in the intestine of newborn c^{3H} homozygotes are shown in Fig. 5. As demonstrated in Fig. 2, the basal levels of *Mt-1* mRNA in the intestine of newborn mutants are 2.5 times higher than normal. In contrast to the lack of glucocorticoid inducibility in the livers of newborn mutants, induction of *Mt-1* mRNA levels is observed in both the mutant and the normal intestine. The level of induction is lower in the mutants (≈2-fold) than in the controls (≈4-fold).

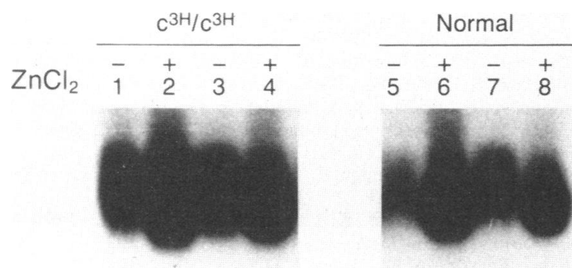

FIG. 4. Effects of $ZnCl_2$ on *Mt-1* mRNA in kidneys of newborn c^{3H} homozygotes (lanes 1–4) and normal littermates (lanes 5–8). Total RNA was prepared from induced (+) and uninduced (−) livers and processed as described in the legend to Fig. 1. RNA samples loaded on lanes 1, 2, 5, and 6 and those on 3, 4, 7, and 8 were derived from separate litters.

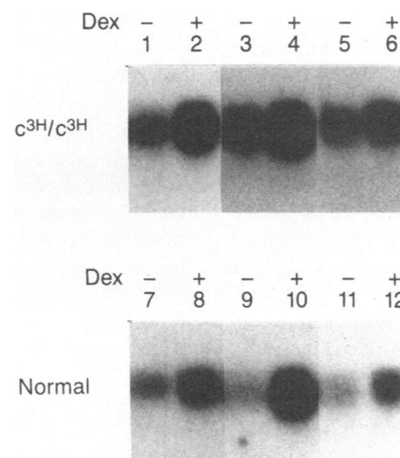

FIG. 5. Effects of dexamethasone on *Mt-1* mRNA in the intestine of newborn c^{3H} homozygotes (lanes 1–6) and their normal littermates (lanes 7–12). Total RNA was prepared from induced (+) and uninduced (−) livers and processed as described in the legend to Fig. 1. RNA samples loaded on lanes 1, 2, 7, and 8 and on lanes 3, 4, 9, and 10 and lanes 5, 6, 11, and 12 were derived from separate litters.

Mt-1 mRNA levels were also induced by $ZnCl_2$ in the intestine of both newborn mutants and their normal littermates (data not shown). Thus the complete loss of glucocorticoid responsiveness of the *Mt-1* gene is confined to the liver of deletion homozygotes.

DISCUSSION

The results of this study demonstrate several specific defects of *Mt-1* expression in neonatal tissues of c^{3H} and c^{14CoS} deletion homozygotes. Thus the *Mt-1* gene can be added to the list of genes whose expression is affected in the liver of newborn deletion homozygotes. However, whereas *Mt-1* gene expression in the normal animal decreases perinatally, other liver genes affected by the deletions (e.g., those for glucose-6-phosphatase, TAT, and PEPCK) normally express a significant increase of enzyme levels during this period. Although this fundamental difference in perinatal expression is also observed on the mRNA level, it is not known whether the alterations of *Mt-1* mRNA levels in the deletion homozygotes reflect synthesis, processing, transport, or stability of the message. A direct analysis of transcription rates by nuclear run-on assays would be required to answer this question.

Among the most striking effects of the chromosome 7 deletions is the lack of hormonal inducibility of various normally inducible enzymes in the neonatal liver. Thus, the normal perinatal induction of glucose-6-phosphatase, TAT, and PEPCK expression by glucocorticoids and cAMP is not observed in livers of deletion homozygotes (2, 4, 16). Even though the two hormonal inducers operate through different mechanisms (17), both are subject to the effects of the deletions. In the case of cAMP induction, protein kinase activities, cAMP binding, and the ability to catalyze the phosphotransferase reaction were found to be normal in deletion homozygotes (18). It cannot be excluded at this time that the failure of glucocorticoid responsivenes in the newborn mutants might perhaps reflect the reduction of receptor levels, which, however, were shown to be decreased also in tissues other than liver (19). Interestingly, our experiments show that even though *Mt-1* mRNA levels could not be induced by glucocorticoids in the livers of c^{3H} homozygotes, induction of *Mt-1* by heavy metals was unaffected. Thus the defect in the c^{3H} deletions does not abolish the inducibility of

Mt-1 gene expression in the liver but affects specifically glucocorticoid responsiveness.

The normal inducibility of the *Mt-1* gene in deletion homozygotes by $ZnCl_2$ but not by glucocorticoids is reminiscent of earlier experiments (20) that reported transcriptional regulation by $CdCl_2$ but not by glucocorticoids of mouse *Mt-1* genes transfected into mouse cell cultures, indicating differences between the two regulatory mechanisms. The specific regulatory genes included in the deletions of chromosome 7 appear to be concerned primarily with the hormone-inducible expression of liver genes. In addition, this defect in glucocorticoid inducibility of *Mt-1* in c^{3H} homozygotes is actually restricted to liver, since hormonal induction was observed in the intestine of the same animals. Thus, the analysis of *Mt-1* expression in the chromosome 7 deletion homozygotes has uncovered two features of the deletions not previously observed: (*i*) the deletion does not abolish inducible expression altogether, but only specific responses of the affected gene, and (*ii*) the deletion has tissue-specific effects interfering with the inducibility of the gene in some but not all tissues.

Induction of *Mt-1* expression by heavy metals and by glucocorticoids proceeds through different pathways even though both inducing agents evoke primary responses with similar kinetics (20, 21). This is indicated also by the results of chromatin-structure studies of endogenous mouse and rat *Mt-1* genes (22, 23), both of which were found to contain nuclease-hypersensitive sites in their 5′ flanking regions in the absence of any exogenous induction. In both mouse and rat genes, increased nuclease sensitivity around the transcription start site becomes apparent upon heavy-metal induction of transcription, whereas no change in chromatin structure is observed upon glucocorticoid stimulation of transcription. It would be of interest to analyze the chromatin structure of the mouse *Mt-1* gene in the liver of newborn deletion homozygotes, where basal *Mt-1* mRNA levels are reduced and inducibility by glucocorticoids but not by heavy metals is affected.

Palmiter and coworkers (7) argued that the relatively high basal *Mt-1* mRNA levels in fetal and newborn liver are the result of induction by endogenous glucocorticoids. According to this view, the reduced basal levels of *Mt-1* mRNA in the liver of newborn deletion homozygotes would be due to the failure of induction of *Mt-1* expression by glucocorticoids. Its constitutive expression, however, would remain unaffected, in analogy to TAT and PEPCK gene expression in deletion homozygotes (ref. 4; M. E. Donner, C.M.L., and S.G.-W., unpublished data).

Metallothionein expression during mouse development shares certain features with α-fetoprotein both with respect to sites of synthesis and with respect to chromatin structure in fetal liver (24); however, the two genes are affected in opposite ways in the deletion homozygotes. Whereas α-fetoprotein mRNA levels were found to be elevated in newborn livers of deletion homozygotes in comparison with normal (3), *Mt-1* mRNA levels were reduced. Since, at least in the rat, α-fetoprotein gene expression has been shown to be negatively regulated by glucocorticoids in fetal and neonatal liver (25), the increased α-fetoprotein mRNA levels in the livers of deletion homozygotes might be explained by a defect in glucocorticoid responsiveness. However, the same explanation cannot be applied to the elevated *Mt-1* mRNA levels in kidney and intestine of newborn deletion homozygotes, since in these tissues the *Mt-1* gene is normally under positive regulation by glucocorticoids. Curiously, tissue-specific effects on metallothionein mRNA expression analogous to those reported here are also caused by another genetic system in the mouse, the recessive X chromosome-linked mottled mutations (6, 26, 27), emphasizing the significance of the genetic and developmental control of heavy-metal homeostasis and metabolism.

The complexities of developmental gene regulation in higher eukaryotes are being highlighted by the studies of the albino deletions in chromosome 7 of the mouse. Increased emphasis on the role of metallothionein in normal fetal development lends special interest to genetic changes affecting this protein—e.g., the chromosome 7 deletions, which will be useful tools for further analytical studies. These will provide much needed information about the normal developmental expression of metallothionein and its regulation, as well as the normal function of this protein in development. As further details of the biochemical and molecular abnormalities caused by the deletions become known, a clearer perception may begin to emerge of those processes that are of fundamental importance in normal developmental gene regulation.

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