Proc. Natl. Acad. Sci. USA
Vol. 82, pp. 2866–2869, May 1985
Genetics

Deletions near the albino locus on chromosome 7 of the mouse affect the level of tyrosine aminotransferase mRNA

(liver differentiation/glucocorticoid control/cAMP control/regulatory genes)

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Contributed by Salome G. Waelsch, December 27, 1984

ABSTRACT **Overlapping chromosomal deletions at the albino locus on chromosome 7 of the mouse affect the expression of several liver enzymes, including tyrosine aminotransferase (TAT; L-tyrosine:2-oxoglutarate aminotransferase, EC 2.6.1.5). With cloned TAT DNA the integrity of the TAT structural gene and its expression and inducibility by glucocorticoids and cAMP were examined in deletion homozygous mice. No difference in the structure of the gene between normal and mutant mice was detected by Southern blotting. Severely reduced amounts of TAT mRNA were detected in homozygous mutants. The residual mRNA levels could not be modulated by glucocorticoids or cAMP. We conclude that a *trans*-acting control function required for expression and inducibility of mouse TAT can be assigned to the chromosomal region near the albino locus.**

The analysis of several overlapping radiation-induced chromosomal deletions at and around the albino locus on chromosome 7 in the mouse has given strong indications for the existence of one or more regulatory factors encoded within the deleted region and concerned specifically with the control of various genes that map on other chromosomes and express liver-specific enzymes and proteins (1).

One such enzyme with dramatically reduced activity in deletion homozygous newborn mice is tyrosine aminotransferase (TAT; L-tyrosine:2-oxoglutarate aminotransferase, EC 2.6.1.5) (2). The absence of a dosage effect in deletion heterozygotes made it appear unlikely that the lack of enzyme activity was due to the inclusion in the deleted region of the structural gene for TAT. This suspicion was confirmed in subsequent experiments in which somatic cell hybrids between mouse liver cells homozygous for the deletion and hypertetraploid rat hepatoma cells were shown to express normal mouse TAT enzyme activity (3). With the use of an enzyme marker, glucose-6-phosphate isomerase, the TAT structural gene was shown not to map on chromosome 7, which carries the deletion. A similar result was subsequently obtained for glucose-6-phosphatase, also a liver-specific enzyme with virtually no activity in deletion homozygotes but expressed normally in somatic cell hybrids (4). The conclusion was therefore reached that, in individuals homozygous for the deletion in chromosome 7, several liver functions encoded elsewhere in the genome failed to differentiate. Furthermore, the equivalent of the deleted DNA was assumed to include one or more regulatory gene(s) encoding factors essential for the expression of a distinct set of liver-specific functions during the course of differentiation (5).

The availability of DNA clones of the rat as well as the mouse TAT gene (refs. 6 and 7; unpublished results) provided the necessary material for attempts to identify the level of this regulatory defect. Therefore, experiments were designed to examine the integrity of the TAT structural gene in deletion homozygous mice as well as the expression and inducibility of this gene by dexamethasone and cAMP. The results of these studies clearly show the TAT structural gene to be intact in deletion homozygotes and allow us to ascribe their failure to express TAT enzyme activity to defects at the level of mRNA production. These in turn are assumed to be due to the absence of a regulatory gene mapping within the deleted region.

MATERIALS AND METHODS

Mouse strains carrying various albino deletion mutations are bred at the Albert Einstein College of Medicine. For the present experiments, the lethal deletions c^{3H} and c^{14CoS} were used. Since mice homozygous for these deletions die within a few hours after birth, it is necessary to maintain them in the heterozygous state. Newborn deletion homozygotes are recognized as albinos, and heterozygotes as well as normal homozygotes by the presence of eye pigment. For certain experiments, livers were removed shortly after birth without any previous treatment. In the experiments involving hormonal induction, the following procedures were used. Newborn mice were injected subcutaneously with $N^6,O^{2'}$-dibutyryladenosine 3',5'-cyclic monophosphate (Bt_2cAMP) alone (50 or 125 mg/kg of body weight in 0.85% saline), dexamethasone alone (100 μg/kg of body weight in 0.85% saline), or a combination of the two in the same concentrations as above. Livers were removed from deletion homozygotes and normal littermates 45–60 min after Bt_2cAMP and 2 hr after dexamethasone or Bt_2cAMP plus dexamethasone injections. All livers were frozen in liquid nitrogen and subsequently shipped to Heidelberg for further analysis.

The mouse TAT gene was isolated from a λ phage mouse genomic DNA library. The isolated clone, λmTAT-1, was characterized with the help of subclones of the rat TAT gene and rat TAT cDNA clones (6, 7). Southern blot analysis of the mouse TAT gene was performed as described (8). The preparation of poly(A)$^+$ RNA and RNA blot analysis were carried out as described (9) except for the use of messenger affinity paper (mAP) instead of poly(U)-Sepharose for the preparation of poly(A)$^+$ RNA (10). Blots were probed with uniformly ^{32}P-labeled complementary SP6 RNA probes. The plasmids used for synthesis of the SP6 RNA probes were constructed by inserting the fragment ES1.4 (Fig. 1, line b), TAT cDNA (6), or albumin cDNA (11) into the vector SP62-PL (12, 13). Specific activity of the probe was approximately 5×10^8 dpm/μg of RNA, and probe concentration was 25×10^6 dpm/ml. Hybridization and washing were carried out as described (12), including a final wash of the filters in 15 mM NaCl/1.5 mM sodium citrate at 70°C for 1 hr.

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Abbreviations: TAT, tyrosine aminotransferase; Bt_2cAMP, $N^6,O^{2'}$-dibutyryladenosine 3',5'-cyclic monophosphate; kb, kilobase(s).

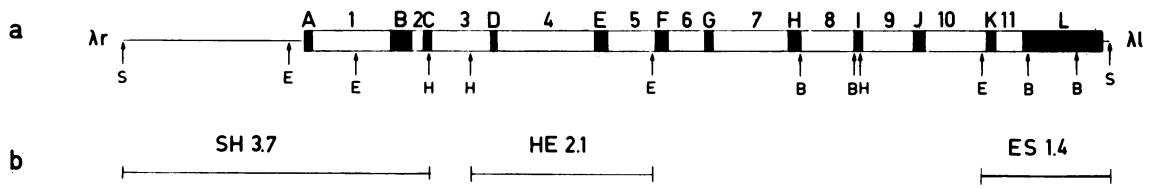

FIG. 1. Organization of the mouse TAT gene. The exon/intron structure of the mouse TAT gene was determined by analysis of heteroduplexes between the DNA of λmTAT-1, a λ phage recombinant containing the mouse TAT gene and mouse poly(A)$^+$ RNA. In line a, black boxes A–L indicate exons, open boxes 1–11 indicate introns, and the line indicates flanking sequences. λl and λr indicate the junction to the left and right arm of λ DNA. The letters below indicate the restriction enzyme cleavage sites *Bam*HI (B), *Eco*RI (E), *Hin*dIII (H), and *Sal* I (S). Fragments used for DNA and RNA analysis are shown in line b. The numbers above the restriction fragments indicate the size in kilobases (kb).

RESULTS

Integrity of the TAT Structural Gene Is Not Affected by the Lethal Albino Deletions. To analyze the TAT gene in homozygous wild-type and homozygous mutant mice, the mouse TAT gene was isolated and characterized. The organization of the isolated mouse TAT gene is shown in Fig. 1, line a, which presents the exon/intron structure and a restriction enzyme cleavage map. The structure was determined by heteroduplex analysis between mouse TAT mRNA and cloned TAT DNA (unpublished data). The 2.3-kb TAT mRNA is encoded by a gene that extends over 9.2 kb and is interrupted by 11 introns. Fig. 1, line b shows the fragments used to probe the structure of the TAT gene (SH3.7 and HE2.1) and to determine the concentration of TAT mRNA (ES1.4) in normal and mutant mice.

Previous studies involving somatic cell hybridization had shown the TAT structural gene to be expressed normally and therefore presumably to be intact in newborn mice homozygous for the deletions c^{14CoS} and c^{112K} as well as c^{3H} (3). To establish definitively the physical integrity of the TAT structural gene in deletion homozygotes, DNAs from c^{3H}/c^{3H} and normal littermate (c^{ch}/c^{ch}) newborn mice were digested with *Bam*HI and *Eco*RI, and the fragments were separated on agarose gels, transferred to nitrocellulose filters, and probed with the ^{32}P-labeled fragments SH3.7 and HE2.1 as indicated in Fig. 1, line b. As shown in Fig. 2, no differences can be detected between c^{3H}/c^{3H} and normal mice in the number and size of hybridizing fragments. Thus, no gross rearrangements appear to have occurred in the structural TAT gene of deletion homozygotes. Support for these results has been obtained by mapping the TAT gene by restriction fragment length polymorphisms to the distal part of chromosome 8 (unpublished data), unlinked to the albino deletions in chromosome 7 of the mouse.

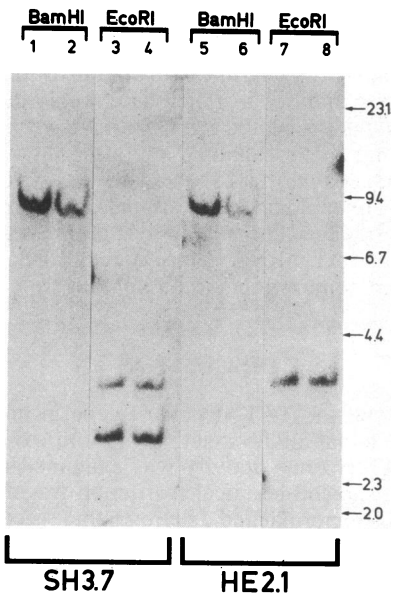

FIG. 2. Southern blot analysis of the TAT gene of normal and mutant mice. DNA from c^{3H}/c^{3H} (lanes 1, 3, 5, and 7) and c^{ch}/c^{ch} (lanes 2, 4, 6, and 8) mice was digested with *Bam*HI and *Eco*RI and transferred to nitrocellulose after separation on a 0.8% agarose gel. As visualized from the ethidium bromide-stained agarose gel, the concentration of DNA of c^{ch}/c^{ch} mice after *Bam*HI digestion (lanes 2 and 6) is about half. The filters were probed with fragment SH3.7, a single-copy DNA fragment located on the 5′ end, and with fragment HE2.1, a single-copy fragment derived from the middle part of the gene. The 0.8-kb *Eco*RI fragment containing the first exon has run out of the gel. The arrows indicate the positions of *Hin*dIII fragments of λ DNA used as size markers (kb).

TAT mRNA Levels in Livers of Deletion Homozygotes Are Severely Reduced and Cannot Be Induced by Glucocorticoids or cAMP. To determine whether the TAT enzyme deficiency in homozygous mutants results from defective TAT gene expression, TAT mRNA concentrations were analyzed by RNA blot analysis. Initial determinations of TAT mRNA concentrations in livers of maximally induced newborn mutant and normal littermates were carried out with the complementary-strand SP6 RNA probe of the rat TAT cDNA clone pcTAT-3 (6). Fig. 3*a* shows the presence of TAT mRNA in the livers of normal heterozygous (c^{3H}/c^{ch}) and homozygous (c^{ch}/c^{ch}) hormone-induced newborn mice. In contrast, no TAT mRNA can be detected in the RNA from livers of similarly induced deletion homozygotes (c^{3H}/c^{3H}), even after prolonged exposure of the autoradiograms. Albumin mRNA, a liver-cell-specific gene product not inducible by cAMP or dexamethasone (11), served as an internal stan-

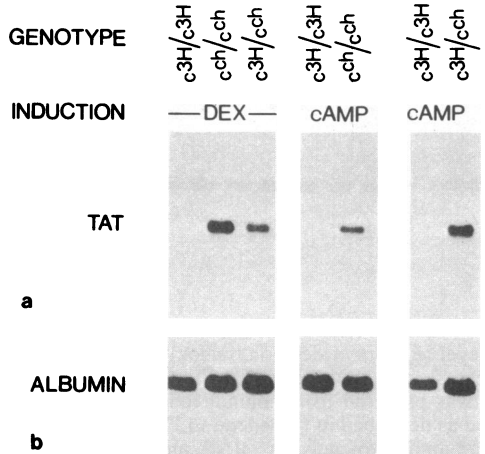

FIG. 3. RNA blot analysis of liver poly(A)$^+$ RNA derived from deletion homozygous (c^{3H}/c^{3H}), heterozygous (c^{3H}/c^{ch}), and wild-type (c^{ch}/c^{ch}) newborn mice. Animals were induced with either dexamethasone or Bt_2cAMP. Two micrograms of poly(A)$^+$ RNA was electrophoresed, blotted, and hybridized to ^{32}P-labeled antisense RNA transcribed from SP6 plasmids containing rat TAT cDNA (*a*) and albumin cDNA (*b*). The filters were finally washed twice for 30 min at 70°C in 15 mM NaCl/1.5 mM sodium citrate.

dard for normalization of amounts of TAT mRNA. Albumin mRNA is expressed to the same degree in mutant and normal littermates (Fig. 3*b*).

Since under stringent conditions the rat cDNA probe was found to hybridize to a considerably lesser degree to mouse TAT mRNA than to the rat message, some heterogeneity between the rat and the mouse genes appeared to exist. Therefore, the single-copy fragment ES1.4 (Fig. 1, line b) representing the 3′ end of the mouse TAT gene was cloned in the appropriate orientation, in SP62-PL, and the ^{32}P-labeled complementary SP6 RNA transcribed from the inserted mouse TAT DNA was used as a probe for further analysis (12). The availability of the homologous TAT probe permitted a more stringent washing of filters and resulted in a reduced filter background. The increased sensitivity in the RNA blot analysis made possible detection of low residual TAT mRNA levels in homozygous mutants.

Using this homologous probe, we determined levels of TAT mRNA in heterozygous (c^{3H}/c^{ch}) and homozygous

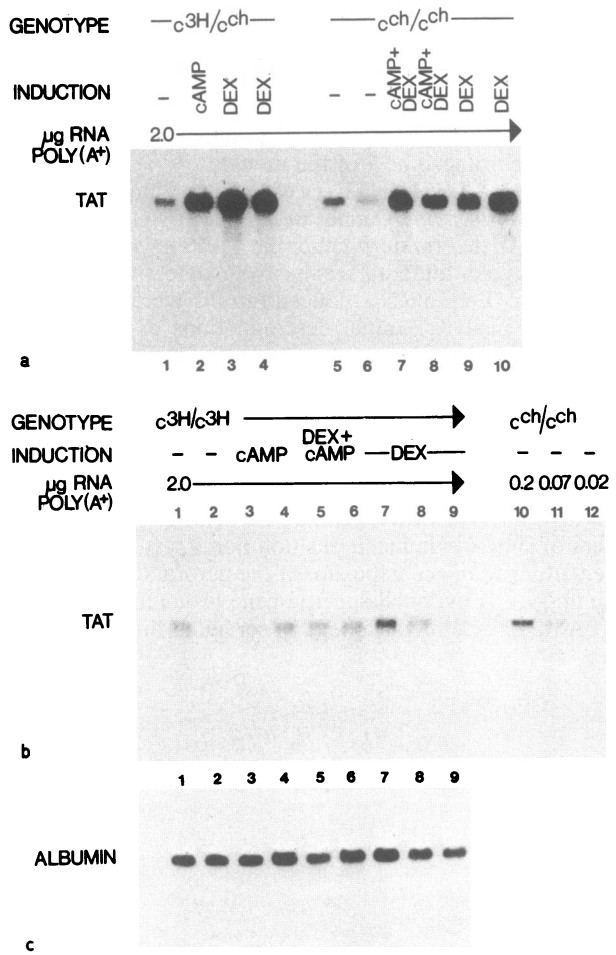

FIG. 4. Effect of Bt_2cAMP and dexamethasone on the TAT mRNA level in livers of deletion homozygous (c^{3H}/c^{3H}), heterozygous (c^{3H}/c^{ch}), and wild-type (c^{ch}/c^{ch}) newborn mice. Poly(A)$^+$ liver RNA was prepared from noninduced and induced animals and processed as described in the legend to Fig. 3. Genotypes, inducing agent, and amount of poly(A)$^+$ RNA analyzed are indicated. The filters were probed with ^{32}P-labeled antisense RNA transcribed from a SP6 plasmid containing the mouse genomic TAT DNA fragment ES1.4 (*a* and *b*) and albumin cDNA (*c*). To estimate the amount of TAT mRNA present in mutant livers, a dilution series of poly(A)$^+$ RNA from an uninduced wild-type mouse (*a*, lane 6) was run on the same gel (lanes 10–12). The filters were exposed for 3.5 hr (*a*) and 30 hr (*b*). The filter used for hybridization with TAT complementary-strand SP6 RNA (*b*) was rehybridized with albumin SP6 RNA (*c*).

normal (c^{ch}/c^{ch}) newborn mice as well as homozygous mutants (c^{3H}/c^{3H}), both with and without hormonal induction. Fig. 4*a* shows uninduced levels of TAT mRNA as well as those induced by cAMP, dexamethasone, or both in livers from heterozygous and homozygous normal mice. The extent of induction, when normalized with respect to albumin mRNA, varies approximately 3- to 7-fold. This variation is not surprising since TAT enzyme activity rises sharply after birth, and the animals used in this experiment were not littermates. Therefore, slight differences in postnatal age would be expected to be reflected in the state of activity of the TAT gene. Hybridization of the mouse TAT probe to liver poly(A)$^+$ RNA of homozygous mutant mice is shown in Fig. 4*b*. In lanes 1–9, 2 µg of poly(A)$^+$ RNA prepared from individual mutant livers and, in lanes 10–12, 0.2, 0.07, and 0.02 µg of uninduced liver poly(A)$^+$ RNA from a homozygous normal newborn mouse (Fig. 4*a*, lane 6) were applied to the gel to make possible a quantitative estimate of TAT mRNA reduction in mutant liver cells. Hybridization of the same filter to the complementary-strand SP6 probe of an albumin cDNA clone (Fig. 4*c*) was used for normalization of amounts of TAT mRNA. With the homologous probe, low levels of TAT mRNA were detected in all homozygous mutant mice. However, in comparison to the dilution series of liver mRNA from a homozygous normal mouse (lanes 10–12), the amounts of TAT mRNA in the mutants appear drastically reduced, to values between 2% and 8% of the noninduced normal newborns. It is significant that similar variations were observed even between members of pairs of mutant newborns originating from the same litter and treated identically (in Fig. 4*b*, compare lanes 1 and 2, lanes 3 and 4, and lanes 7 and 8). Since these differences in TAT mRNA amounts were of the same order of magnitude as those between induced and noninduced mutants, the conclusion appears justified that deletion homozygotes are not inducible by cAMP, dexamethasone, or a combination of the two. The amounts of albumin mRNA show only minimal variability (Fig. 4*c*); therefore, inappropriate recovery of the poly(A)$^+$ RNA is excluded as a possible explanation for the results. To minimize age variations as much as possible, the induction experiments were repeated with six matched pairs each of normal and mutant (c^{3H} and c^{14CoS}) littermates. In the normal pairs, induced partners showed a 3- to 4-fold increase of TAT mRNA over noninduced animals. In contrast, in the mutant pairs, induced and noninduced partners showed similar amounts of TAT mRNA, confirming the lack of inducibility in deletion homozygotes (data not shown).

DISCUSSION

The integrity of the TAT structural gene in mice homozygous for the lethal albino deletion c^{3H} and expressing only marginal TAT enzyme activity was examined on Southern blots, which showed identical restriction fragment patterns for TAT DNA in normal and deletion homozygous mice. The steady-state levels of TAT mRNA, however, are reduced drastically in c^{3H} and c^{14CoS} deletion homozygotes, and they cannot be modulated by glucocorticoids and cAMP as is the case in normal newborn animals. It is not possible to decide at this time whether the mRNA deficiencies result from defective transcription or changes in message processing and/or stability. Measurements of the actual transcription rate of the TAT gene will allow a distinction between these possibilities. The TAT structural gene had been shown in earlier experiments to be present in deletion homozygotes, to be expressed normally in somatic cell hybrids between homozygous deletion mouse liver cells and rat hepatoma cells, and therefore to map on a chromosome other than number 7 (3). The Southern blot data reported here give strong support for

these conclusions but cannot exclude the existence of small deletions or point mutations in regulatory regions of the TAT gene that may be responsible for the observed defects in TAT mRNA expression and induction. However, this is not likely to be the case since the mouse TAT gene was mapped definitively to chromosome 8 (unpublished results), while the albino deletions are located on chromosome 7. Furthermore, one additional liver enzyme encoded elsewhere in the genome (4) is similarly affected by the same deletions (1), suggesting that a genetic defect mapping in a location other than that of the respective structural genes is responsible for the different enzyme deficiencies.

In the homozygous state the deletions cause a reduction of steady-state TAT mRNA to levels less than 10% of normal and a loss of induction by hormones. The variability of the residual mRNA levels among different individuals homozygous for the deletions cannot be explained at this time; it is, however, consistent with previous data on TAT enzyme activity (2). It is noteworthy that the marginal levels of TAT mRNA observed in deletion homozygous newborns are equivalent to those found in normal fetuses just before birth.

The deficiency of TAT mRNA amounts might be caused by the absence in deletion homozygotes of an essential regulatory factor normally encoded within the deleted region and responsible for transcriptional modulation of the TAT gene. Such a factor must be assumed to be acting in the *trans* position. It is interesting that absence of this factor in deletion homozygotes interferes with induction of TAT mRNA by glucocorticoids as well as by cAMP, particularly since these two inducers are known to act by distinct mechanisms (14, 15). It thus appears that the regulatory factor absent from the albino deletion homozygotes plays a fundamental role in the basic expression as well as the hormonal modulation of this gene. It may be analogous to the diffusible gene product of rat hepatoma cells that is presumed to be responsible for the activation of the mouse TAT structural gene in somatic cell hybrids derived by fusion of rat hepatoma cells with deletion homozygous liver cells (3).

The effects of the deletions in chromosome 7 of the mouse provide a model system for the identification of regulatory factors controlling sets of genes expressed in a cell-specific manner, and for determining their genetic basis as well as their role in cell differentiation.

We thank Craig Robinson and Felicia Forma for most valuable assistance in providing the mutant material. We are grateful to R. Miksicek, I. Scheffler, S. Ruppert, and Eva and Mark Dworkin for critical comments and M. Cole, M. Lancellotti, and I. Difigola for preparation of the manuscript. This work was supported by grants from the National Institutes of Health (GM 27250) and the American Cancer Society (CD-38) to S.G.-W. and by grants to G.S. from the Deutsche Forschungsgemeinschaft (Schu 51/4-1 and Schu 51/5-3) and the Fonds der Chemischen Industrie.

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