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mRNA levels and methylation patterns of the tyrosine aminotransferase gene in aging inbred rats

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We have examined the mRNA levels and methylation patterns of the liver-specific tyrosine aminotransferase (TAT) gene in inbred female rats aged 6, 24 and 36 months. Northern hybridization analysis of total RNA showed a 65% decrease in the steady state transcript level of TAT in the liver of 24- and 36-month old rats as compared to 6-month old animals. The TAT gene as studied by Southern hybridization analysis using the isoschizomers Hpa II and Msp I was found to be hypomethylated in the liver as compared to spleen and brain at six CpG sites within the gene. Methylation at these sites remained unchanged during aging.

Tyrosine aminotransferase; Aging; DNA methylation; mRNA

1. INTRODUCTION

Aging of metazoa has been attributed to the loss of epigenetic control [1]. There is strong evidence that DNA methylation of specific CpG sites is associated with the control of tissue and stage specific gene transcription [2], cell differentiation [3] and X-chromosome inactivation [4]. Alterations in DNA methylation have also been associated with the aging process. A progressive decline of the 5-methylcytosine (5mC) level in total genomic DNA was observed with age in various systems, including human peripheral blood lymphocytes [5], various organs and tissues of the mouse [6], and in cultured fibroblasts during replicative senescence [7]. However, it is not clear whether this loss of 5mC from the genome involves the methylation patterns of individual genes and how it affects gene transcription. Both de- and repression of gene transcription has been associated with aging. In only a limited number of studies, however, a relationship has been found between such changes and alterations in the methylation status of specific CpG sites (for a review, see [8]).

We have studied the mRNA levels and methylation patterns of the liver-specific tyrosine aminotransferase (TAT) gene in various tissues of the aging rat. The TAT mRNA level showed a 65% decrease in the 24- and 36-month-old rats as compared to 6-month-old animals. We found a liver-specific hypomethylation of six CpG sites within the TAT gene. The methylation pattern at these or other sites did not alter during aging.

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Abbreviations: kb, kilobasepairs; OTC, ornithine transcarbamoylase (EC 2.1.3.3); TAT, tyrosine aminotransferase (EC 2.6.1.5); 5mC, 5-methylcytosine

2. MATERIALS AND METHODS

2.1. *Animals*

Organs were dissected from female inbred Wistar-derived WAG/Rij rats aged 6, 24 and 36 months. Animals were fed ad libitum and maintained under clean conventional conditions at the colony of the TNO Institute for Experimental Gerontology [9]. Under these circumstances the animals have a median lifespan of 33 months. All animals sacrificed for this study were subjected to complete gross and microscope examination. Tissues affected by a well defined disease process, e.g. tumors etc., were excluded from this study.

2.2. *DNA probes*

For the rat TAT gene, three probes, derived from the lambda genomic clone rTAT1 [10] were used. Their inserts correspond to exons B and C (pUTAT0.94), exons F, G, H and part of exon I (pUTAT2.45) and exons K and L (pUTAT1.05). Plasmid pHR28.1 [11] and pR021 [12], both used as a control, contain genomic fragments from the human 5.8S and 28S rRNA genes and the rat OTC cDNA respectively. Only the 2 kb *BglI-EcoRI* fragment corresponding to 28S rDNA was used as a probe. All hybridization experiments were performed with purified insert fragments.

2.3. *Northern, Southern and dot blot hybridization analysis*

Total RNA was extracted from whole liver, brain and spleen using the frozen tissue/LiCl procedure [13]. The amount of total RNA isolated per gram liver, brain or spleen did not change with age. Dot blotting was performed as described [14]. For Northern analysis equal amounts of total RNA (20 μg) were size-fractionated by electrophoresis for 16 h at 25 V/15 mA in 1.5% agarose gels containing formaldehyde. The gels were then subjected to Northern blotting [15]. Total genomic DNA was isolated as described earlier [16]. Genomic DNA was digested for 3 h at 37°C with a 5-fold excess of *MspI* or *HpaII* restriction enzyme according to the manufacturer's specifications (BRL). Then, the samples were chloroform-extracted, ethanol-precipitated, solubilized and re-digested under the aforemen-

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tioned conditions. After quantification of the digested samples equal amounts of DNA (5 μg) were electrophoresed in 0.8% agarose gels and subjected to Southern blotting [16]. Hybridization of Northern, Southern and dot blots was carried out as described by Church and Gilbert [17], using random primed ^{32}P-labeled probes with a specific activity of 5×10^8 cpm/μg [18]. Autoradiography was performed at −80°C using Kodak X-omat AR2 films and X-omatic intensifying screens. The hybridization intensity of the dots and bands was measured by densitometric scanning, using a Model 620 Video Densitometer (Bio-Rad). For rehybridization the membranes were stripped for 10 min at 100°C in 1% SDS, 1.8 mM NaCl, 0.1 mM NaH_2PO_4, 0.01 mM EDTA.

3. RESULTS AND DISCUSSION

3.1. *mRNA levels*

Northern analysis of total rat liver RNA revealed a 2.4 kb TAT mRNA in all three age groups with no additional bands present (Fig. 1). As a control for the presence of comparable mRNA concentrations on the Northern blot, the same filter was rehybridized with a cDNA probe for the liver-specific rat OTC gene (Fig. 1B). The OTC mRNA levels vary interindividually but not with age.

For quantitative comparison of the TAT mRNA level in the three age groups, the hybridization signals obtained after autoradiography of the Northern blot were measured by densitometric scanning (Fig. 2A) and normalized to the hybridization signals obtained after dot-blot analysis of the same RNA samples using a 28S rRNA probe (Fig. 2B) as described earlier [14]. The TAT mRNA level in the rat liver showed a 65% decline when the 24- and 36-month-old rats were compared with the 6-month-old animals. Using the Northern hybridization analysis we found no expression of TAT mRNA in tissues other than the liver (results not shown).

A decrease in TAT enzyme activity combined with a 35% decline in mRNA level has been previously observed in the liver of 25-month-old Sprague-Dawley rats as compared to 10-month-old adults [19]. The results obtained in our present study on WAG/Rij rats confirm this and indicate that no further changes in the TAT mRNA level occur at old age, that is, between 24 and 36 months. It is important to note that results from Horbach et al. [20] as well as our own unpublished data indicate that the mRNA level of another liver-specific gene, albumin, was elevated 80% in 24-month-old WAG/Rij rats as compared to 6-month-old animals. Such early age-associated alterations in the expression of inducible genes are likely to reflect regulatory changes rather than an age-related loss of transcriptional control.

3.2. *Methylation pattern*

In order to establish whether the age-related alteration in TAT mRNA level is associated with methylation changes, a number of CpG sites within the TAT gene were examined in liver, spleen and brain of 6-, 24- and 36-month-old rats by Southern hybridization analysis (Fig. 3A) using the isoschizomers *Hpa*II and *Msp*I [21]. As a control for the presence of equal DNA concentrations in each lane, the same filter was rehybridized with the OTC cDNA probe (Fig. 3B). A liver-specific methylation pattern was observed for 6 CpG sites within the TAT gene. Among the 5 hybridizing *Msp*I restriction fragments, three bands, representing 1.5 kb, 1.2 kb and 0.8 kb fragments were also generated

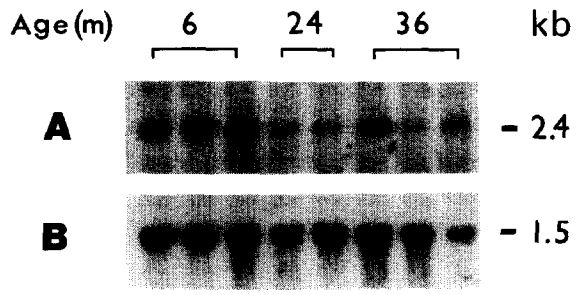

Fig. 1. Northern hybridization patterns of TAT (A) and OTC (B) mRNAs in 20 μg of total RNA isolated from the liver of female WAG/Rij rats aged 6, 24 and 36 months. The same filter was sequentially hybridized, stripped and rehybridized with a mixture of pUTAT0.94, pUTAT2.45 and pUTAT1.05 (A) and pR021 (B). Size markers are presented in kb.

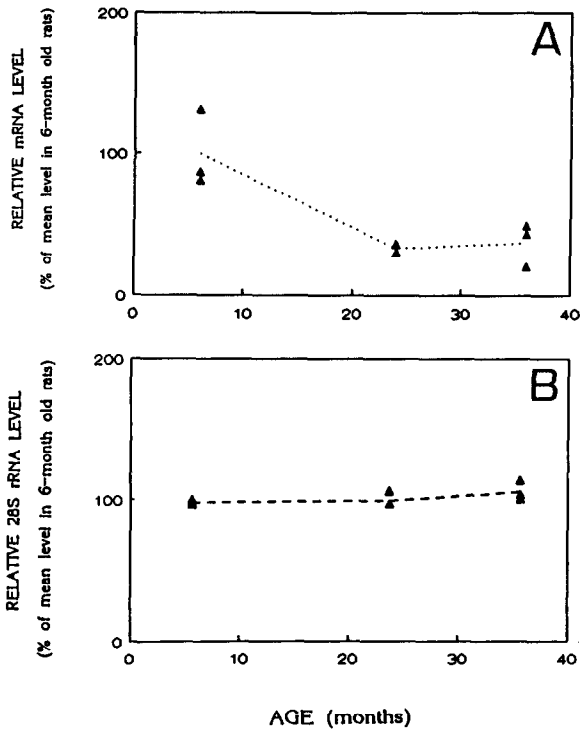

Fig. 2. Expression level of TAT mRNA in rat liver in relation to aging (A). The data, obtained by densitometric scanning of the specific bands shown in Fig. 1, were normalized to the relative 28S rRNA concentration of the samples as measured by dot blot analysis (B). Each point in both figures represents the result of a determination on one individual animal and is expressed as the percentage of the average value in the 6-month-old rats (100%). Repeated determinations on one sample indicated an experimental variation of less than 10%.

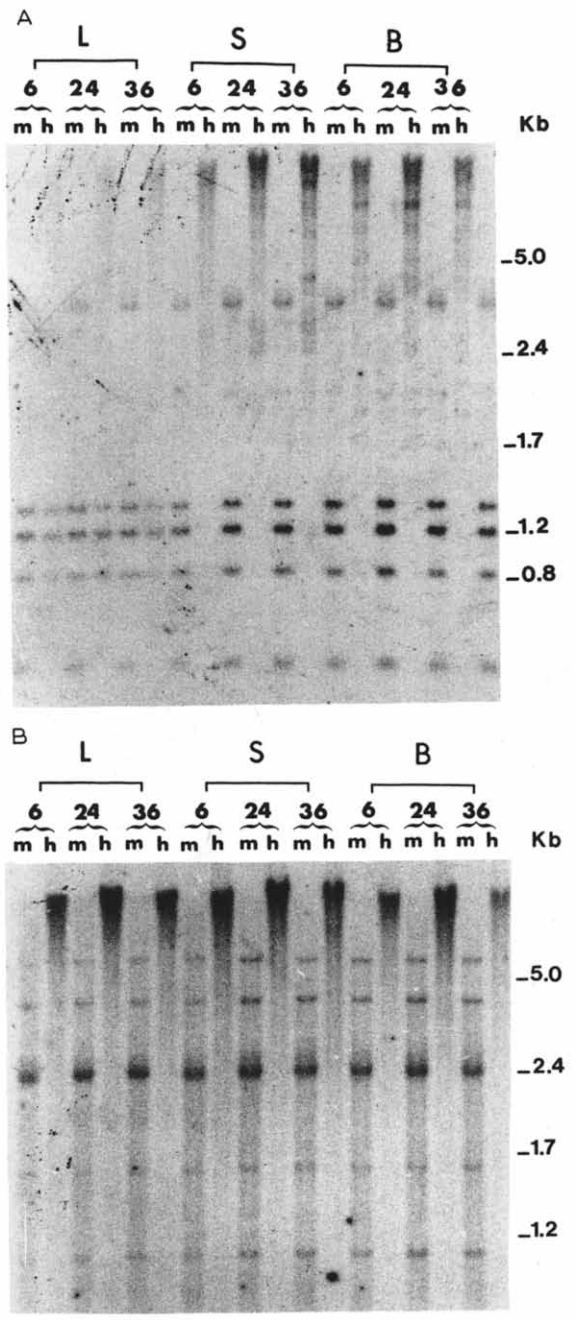

Fig. 3. Southern hybridization analysis of the TAT (A) and OTC (B) genes in genomic DNA isolated from liver (L), spleen (S) and brain (B) of rats of different ages (6, 24 and 36 months) and digested with the isoschizomeric restriction enzymes *Msp*I (m) and *Hpa*II (h). The probes used were the genomic (A) and cDNA (B and C) probes mentioned in Fig. 1. For each age group results obtained with only one animal are shown; among the animals studied per age group (at least 3) no differences were observed. Fragment sizes were estimated from lambda × *Hin*dIII and ϕX174 × *Hae*III markers (kb).

following *Hpa*II digestion of liver DNA; in spleen and brain DNA these fragments were absent (Fig. 3A). This indicates that in most liver cells the *Hpa*II/*Msp*I recognition sites generating these fragments are hypomethylated. To our knowledge this has not been reported before.

It can be concluded that the age-related decrease of the TAT mRNA level is not associated with methylation changes of any of the sites examined. In spite of the previously reported random loss of 5mC with age and the age-related methylation changes at CpG sites for which tissue-specificity was observed (e.g. the c-myc gene [22]), such alterations were not detected within the TAT gene.

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