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Brain-specific expression of transthyretin mRNA as revealed by cDNA cloning from brain

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cDNAs for rat transthyretin mRNA were cloned from a brain cDNA library. Sequencing analyses showed the presence of an additional 5′ sequence that had not been reported for the liver mRNA corresponding to the flanking promoter region of the gene. This additional sequence was expressed only in the brain, suggesting the presence of a brain-specific promoter.

Transthyretin; Prealbumin; Nucleotide sequence; Brain-specific expression; Alternative promoter; (Rat brain)

1. INTRODUCTION

Transthyretin (prealbumin) binds thyroid hormones and also binds vitamin A via the retinol binding protein, playing an important role in their transport processes [1]. In addition to its well-characterized three-dimensional structure, both cDNA and the genome structures for transthyretin of several species have been studied [2–7]. The molecular and genetic basis for accumulation of a variant transthyretin in a heredofamilial amyloidotic polyneuropathy has also been studied [8,9].

In rodents, transthyretin mRNA was detected in the liver and in extremely high levels in the choroid plexus of the brain [10]. The mRNA was described as being expressed from the same starting site of the same gene in both tissues [5,11]. Multiple nuclear factors and the proximal regulatory regions of the mouse gene functioning in liver-specific expression have been reported [11]. However, the mechanism by which the choroid plexus expresses this gene but not other liver-specific genes has not been characterized at all.

To begin the study of brain-specific expression of transthyretin mRNA, we re-examined whether the mRNA is indeed transcribed from the same starting site in both the liver and the brain by cloning rat transthyretin cDNAs from a brain cDNA library.

2. MATERIALS AND METHODS

A brain cDNA library (Clonetech, CA, USA) was screened under high-stringency hybridization conditions with the ^{32}P-labeled cDNA synthesized from the total poly(A) RNA isolated from adult rat hepatocytes. Hepatocytes were isolated by in situ perfusion of the liver with collagenase [12], and isolation of poly(A) RNA and synthesis of cDNA were done by the standard methods described [13]. Phages which gave strong signals were randomly selected and their DNAs were purified. The cDNA inserts were subcloned into pUC18. Sequence analyses were done by the dideoxy chain termination method using the denatured plasmid DNAs as templates. Transthyretin cDNA clones were identified by computer-assisted alignments of the sequences with the published data [7] using the DNASIS program of Hitachi Software Engineering (Yokohama, Japan).

Slot blot hybridization using the total extracts from various tissues of rat was carried out according to [14] and the DNA concentrations of the samples for normalization were determined as in [15]. The hybridization probes were prepared by nick translation of the plasmid DNAs by a standard method [13]. Plasmid DNA for probe A in fig.1 was constructed by ligation of a *Sau*3A fragment (nucleotide 11–76) of clone B1 DNA into *Bam*HI site of pUC18, followed by sequencing analysis for confirmation.

Restriction enzymes, DNA polymerase I, T4 DNA ligase, universal and reverse sequence primers were obtained from Takara Shuzo (Kyoto, Japan). Reverse transcriptase was from Seikagaku-kogyo (Tokyo, Japan). [α-^{32}P]dCTP (>700 Ci/mmol) was from ICN (CA, USA).

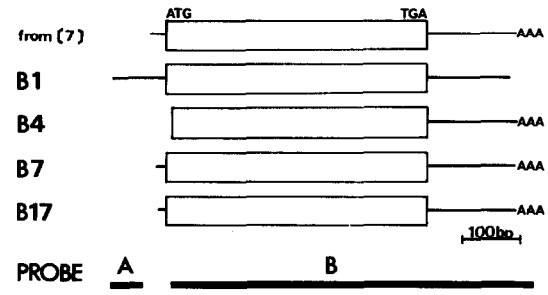

Fig.1. Comparison of the structures of the 4 brain transthyretin cDNAs with the published sequence [7]. Solid lines indicate non-coding regions and boxes indicate translated region of the mRNA.

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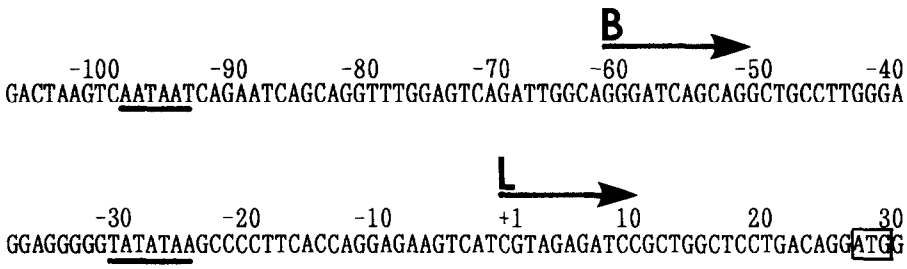

Fig.2. Comparison of the 5′-noncoding sequence of the brain transthyretin cDNA (B1) with the published 5′ flanking region of the gene [7]. L represents the major RNA starting site (number 1) in the liver; B represents the 5′-end of clone B1. Putative TATA boxes are underlined.

3. RESULTS

Four out of 8 randomly selected clones were identified as transthyretin cDNA clones by sequencing analyses. Such a high proportion of transthyretin clones would reflect unusual cell-specific and abundant expression of the mRNA [10]. Among other clones, two contained the sequences for mitochondrial cytochrome *c* oxidase for unknown reasons and two contained the sequences with no significant homologies to

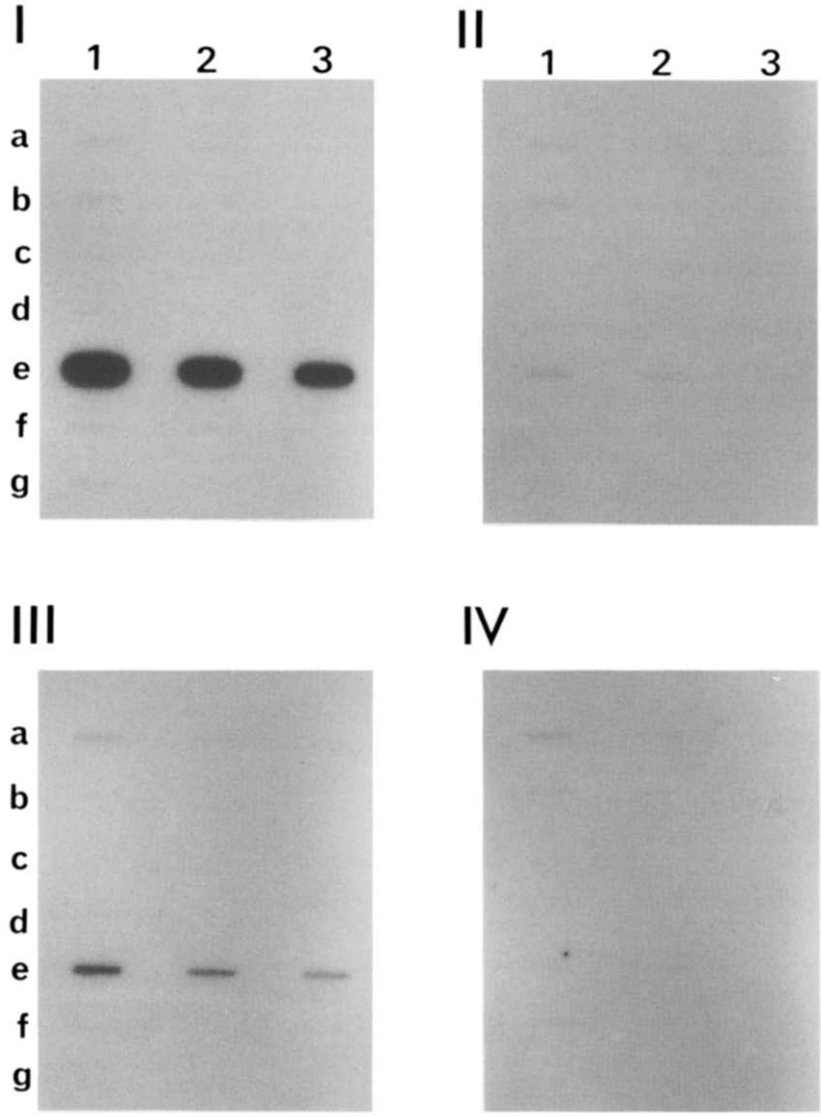

Fig.3. Slot blot analyses of albumin (I), apoE (II), transthyretin (probe B) (III), and transthyretin (probe A) (IV) mRNA in various tissues. Total extracts were normalized by DNA contents and columns 1–3 show extracts containing 0.4, 0.2 and 0.1 μg of DNA. Columns a–g show extracts from brain, heart, thymus, spleen, liver, kidney and lung, respectively.

any known sequences in the data base (GenBank R55.0 and EMBL R15.0).

The sequences of 4 transthyretin cDNA clones from the brain library were determined and compared with the published rat sequence [7] (fig.1). Though the 5′-end of the published sequence was determined by primer extension analysis, clone B1 has an additional 61 bp at the 5′-end. The 5′-noncoding sequence of the brain transthyretin cDNA (B1) was then compared with the published 5′-flanking region of the gene [7] and is shown in fig.2. The additional sequence of clone B1 matched perfectly with the flanking region including a TATA-like sequence.

To examine the possibilities that the 5′ additional sequence of B1 clone is expressed in both the brain and the liver, or that the sequence is produced by a cloning artifact, total RNA from the brain and the liver was analyzed by dot hybridization using specific cDNA probes. As shown in fig.3, under conditions where apoE [16] and albumin [17] mRNAs were detected as expected, the transthyretin mRNA sequence was detected in both the liver and the brain when the sequence of clone B7 (probe B in fig.1) was used as a probe, but it was detected only in the brain when the additional 5′ sequence of clone B1 (probe A in fig.1) was used. These results show that the 5′ additional sequence of B1 clone is not an artifact and is expressed in the brain at a significant level but little or not at all in the liver.

4. DISCUSSION

Transthyretin mRNA has been reported to be expressed in both the liver and the brain from the same RNA start site in both tissues [5,11]. The RNA start sites in the liver of mouse [5], rat [7], and human [6] were reported at homologous positions, but no direct data have been reported on the start sites in the brain.

Our present data show that the brain has an additional transcriptional start site for the mRNA. This site is located at least 65 bp upstream of the published one passing over a TATA-like sequence and it is used significantly in the brain but little in the liver. Another TATA-like sequence, 5′-AATAAT-3′ was found in the upstream region. Though our present results do not exclude the possibility that the brain uses both RNA start sites and quantitative studies to determine which is the major one in the brain are obviously necessary, the choroid plexus may have its specific promoter distinct from the liver promoter. As the liver, the choroid plexus should transcribe the gene efficiently using several activator proteins and the regulatory regions of the gene. The simplest mechanism would be accomplished by two distinct sets of transcriptional factors: one set for the liver and another for the choroid plexus. This type of regulation was shown α-amylase in the liver and in the salivary gland accompanied by alternative splicing [18]. The possibility that alternative promoters are used in a tissue-specific manner without alternative splicing deserves further investigation.

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