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Cloning and characterization of a putative mouse acetyl-CoA transporter cDNA

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Abstract

A mouse acetyl-CoA transporter (*Acatn*) cDNA was isolated by PCR cloning. Mouse Acatn exhibited 92% homology with human sequence on the basis of amino-acid sequence. The predicted gene product of *Acatn* is a 61 kDa hydrophobic protein with six to 10 transmembrane domains. Transfection of mouse *Acatn* cDNA into HeLa/GT3$^+$ cells resulted in significant increase in the amount of 9-*O*-acetylated gangliosides, suggesting that Acatn does play an important role in the acetylation of gangliosides. Northern blot analysis of *Acatn* mRNA suggested that transcript of *Acatn* is widely distributed in various adult tissues. Expression of *Acatn* was found to be developmentally regulated, with high expression levels during early embryonic stages, and then there was a subsequent decrease in expression levels in the later embryonic stages. © 1999 Elsevier Science B.V. All rights reserved.

Keywords: Dorsal root ganglia; Embryonic development; *O*-Acetylated gangliosides

1. Introduction

Gangliosides are a diverse series of sialic acid-containing glycosphingolipids present on the plasma membrane of most vertebrate cells. They are particularly abundant in the central nervous system. Sialic acid residues on gangliosides are sometimes modified by *O*-acetylation at the 9-position. Some biological properties are found to be associated with the modification of sialic acids by *O*-acetylation. The expression of 9-*O*-acetylated gangliosides is apparently associated with neural cell differentiation and migration (Mendez-Otero and Cavalcante, 1996; Constantine Paton et al., 1986). Expression of *O*-acetylated sialic acids on cell surfaces can also cause significant effects on the action of bacterial sialidases (Corfield et al., 1986; Varki and Diaz, 1983). It also affects virus binding, cell adhesion and the immunogenicity of sialic acid residues of gangliosides (Varki, 1992). Expression of *O*-acetylated gangliosides on the cell surface can alter the binding of pathogenic viruses. In most cases, it confers protection to the host from the corresponding pathogen. Interestingly, Influenza C and Corona viruses bind specifically to 9-*O*-acetylated sialic acids; however, these are relatively benign pathogens compared to the Influenza A and B viruses, whose binding to sialic acids is abrogated by *O*-acetylation (Higa et al., 1985; Rogers et al., 1986). *O*-Acetylation of sialic acids on murine erythrocytes appears to inhibit binding of the malarial parasites (Reuter et al., 1991). These data are supportive that *O*-acetylation of sialic acids provides protection from pathogens.

O-Acetylation of sialic acids shows remarkable tissue-specific and developmentally regulated expression in a variety of systems, suggesting highly specific roles for this modification in tissue development and organization (Varki, 1992; Schlosshauer et al., 1988; Zhang et al., 1997). Expression of sialic-acid-specific 9-*O*-acetylesterase in the fertilized egg consistently arrested development at the two-cell stage, suggesting that *O*-acetylated sialic acids might be involved in segmentation of the embryo,

Abbreviations: cDNA, DNA complementary to mRNA; PCR, polymerase chain reaction; Acatn, acetyl-CoA transporter; *Acatn*, gene encoding Acatn; mAb, monoclonal antibody; nt, nucleotide(s); ED, embryonic day; DIG, digoxigenin; GAPDH, glyceraldehyde-3-phosphate dehydrogenase; GD3, NeuAcα(2-8)NeuAcα(2-3)-Galβ(1-4)Glcβ(1-1′)Cer; GT3, NeuAcα(2-8)NeuAcα(2-8)NeuAcα-(2-3)Galβ(1-4) Glcβ(1-1′)cer.

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and the late expression in specific organs caused developmental abnormalities (Varki et al., 1991).

In spite of its importance, the *O*-acetylation mechanism is poorly understood at the molecular and genetic levels. The molecular cloning of genes encoding for various factors that take part in the biosynthesis of acetylated gangliosides is necessary to understand the biological functions of *O*-acetylated gangliosides during development. Previously, we have reported the isolation of a novel cDNA encoding for a putative acetyl-CoA transporter that is required for the formation of *O*-acetylated gangliosides (Kanamori et al., 1997). Since expression of *O*-acetylated gangliosides is developmentally regulated, it is necessary to analyze the expression of acetyl-CoA transporter protein during embryonic development. As mouse is a model experimental system, the study of mouse genes is becoming increasingly important with the advent of gene-targeting technology. However, mouse acetyl-CoA transporter cDNA has not been isolated so far. In this paper we report, for the first time, cDNA cloning of putative mouse acetyl-CoA transporter, and study its expression during embryonic development.

2. Materials and methods

2.1. PCR cloning and DNA sequencing

PCR cloning was performed to isolate mouse acetyl-CoA transporter cDNA. A mouse melanoma cDNA library in Uni-ZAP™XR vector (Stratagene, La Jolla, CA, USA) was used directly as a template. Forward primer, 5′-ATGTCACCCACCATCTCCCACAAG-3′ corresponding to nucleotide position 388–412 and reverse primer, 5′-TTAATTGTTCCTTTTGCATTTCCAC-3′, corresponding to nucleotide position 2012–2037 of human *Acatn* cDNA sequence, were used for DNA amplification. Expand High Fidelity PCR system (Boehringer Mannheim, Germany) was used according to the manufacturer's instructions. Thirty PCR cycles were carried out on 1 μl of mouse melanoma cDNA library (1.5×10^{10} pfu/ml). Each PCR cycle consisted of denaturation at 94°C for 1 min, annealing at 55°C for 1 min and extension at 72°C for 2 min. The amplified fragment of 1.65 kb was subcloned into pZErO-1 vector (Invitrogen CA, USA) after digestion with *Eco*RV. Nucleotide sequence was determined in both directions using cycle sequencing kit (Amersham Life Technologies, USA) based on the dideoxy chain termination method (Sanger et al., 1977). A LI-COR 4000L Sequencer (LI-COR, NE, USA) was used for the analysis.

2.2. DNA transfection and immunocytochemical analysis

A *Bam*HI–*Xba*I fragment of mouse *Acatn* cDNA containing the entire coding sequence was subcloned into *Bam*HI and *Xba*I sites of mammalian expression vector pcDNA3.1 (Invitrogen, CA, USA) and the resultant plasmid was designated as pcDNA3.1-Acatn. Stable transfectant of HeLa cells expressing gangliosides GD3 and GT3, named HeLa/GT3$^+$ was isolated as reported (Kanamori et al., 1997) and used as recipient cells for transient expression of *Acatn* cDNA. Cells were cultured on coverslips in Dulbecco's modified Eagle medium (DMEM) containing 10% fetal-bovine serum (FBS) and grown in a humidified 5% CO_2 incubator at 37°C. HeLa/GT3$^+$ cells were transfected with 10 μg of plasmid DNA using Super Fect Transfection reagent (QIAGEN, Germany) following the manufacturer's instructions. Immunocytochemical analysis was carried out as described previously (Kanamori et al., 1997). Briefly, 40 h after transfection, the cells were fixed with 2% paraformaldehyde for 20 min at room temperature and incubated with mAb493D4 (obtained from S. Fujita, Mitsubishi Kasei Institute of Life Sciences) for 2 h at room temperature, followed by incubation with Alexa-conjugated anti mouse IgG (Molecular probes, Eugene, USA) for 1 h at room temperature. Fluorescence labeling was detected by using a Zeiss Axioplan Fluorescence microscope.

2.3. Northern blot analysis

Normal adult tissues were obtained from 8–10 week old male Balb/c mice. mRNA was isolated from the adult tissues using Poly A Tract mRNA isolation kit (Promega, WI, USA) following the manufacturer's instructions. Approximately 2 μg of mRNA was electrophoresed through 1% agarose-formaldehyde gel and transferred to nylon membrane according to Sambrook et al. (1989). Mouse Embryo Multiple Tissue blot (Clontech, CA, USA) was used for analysis of *Acatn* expression during developmental stages. Digoxigenin-labeled *Acatn* antisense RNA probe corresponding to nucleotide position between 1 and 688 was generated by SP6 RNA polymerase, using DIG-RNA labeling kit (Boehringer Mannheim, Germany). Hybridization was carried out at 68°C for 14 h. The membrane was washed with $2 \times$SSC/0.5% SDS and $0.1 \times$SSC/0.5% SDS each for 30 min at 68°C. Bound RNA probes were revealed by incubation with anti-digoxigenin-alkaline phosphatase conjugate, followed by chemiluminescence detection according to the manufacturer's instructions (Boehringer Mannheim, Germany).

2.4. In situ hybridization

Rat Hybrid-Ready tissues (Novagen, WI, USA) were used to detect the *Acatn* mRNA expression during

developmental stages, since expression of *O*-acetylated gangliosides is well characterized in rat system (Varki, 1992). Rat embryos, cut into sagittal sections, were from strain Sprague–Dawley. Digoxigenin-labeled *Acatn* antisense RNA probe corresponding to nucleotide position between 1 and 688 was generated by SP6 RNA polymerase, using DIG-RNA labeling kit. In situ hybridization was carried out using modified protocol of Mutter and Wolgemuth (1987). Hybridization was carried out at 42°C in the presence of 50% formamide for 14 h, followed by washing in 2 × SSC at 42°C. Sections were then incubated with anti-DIG-alkaline phosphatase conjugate for 2 h at room temperature, and the bound antibody was detected by a standard immuno-alkaline phosphatase reaction using nitroblue tetrazolium chloride/5-bromo-4-chloro-3-indolyl phosphate as substrate.

3. Results and discussion

3.1. Cloning and characterization of mouse acetyl-CoA transporter cDNA

Mouse acetyl-CoA transporter (*Acatn*) cDNA was isolated by PCR cloning using mouse melanoma cDNA library in Uni-ZAP™XR vector as a template. A full-length coding region (nt 1–1653) was amplified using forward and reverse primers specific to human *Acatn* cDNA. The amplified fragment was cloned into pZErO-1 vector and the nucleotide sequence was determined in both directions. The nucleotide sequence and deduced amino-acid sequence of mouse *Acatn* cDNA are shown in Fig. 1. The nucleotide sequence of mouse *Acatn* cDNA was further confirmed by cloning and sequencing the exon sequences of mouse *Acatn* gene (unpublished data). Mouse *Acatn* cDNA encodes for a protein of 550 amino acids, with a predicted molecular mass of 61 kDa. The mouse *Acatn* cDNA sequence was highly homologous with the human cDNA, and it exhibited 87% homology at nucleotide sequence level and 92% homology on the basis of amino acid sequence. Hydropathy analysis revealed a highly hydrophobic, transmembrane protein with approximately 6 to 10 transmembrane domains, similar to human Acatn protein (data not shown). Mouse Acatn protein was also found to contain a leucine zipper motif in the transmembrane domain III. This motif is often found in the transporter proteins (Eckhardt et al., 1996; Abeijon et al., 1996).

Homology searches of mouse Acatn against currently available nucleotide and protein databases revealed two hypothetical proteins with high degree of homology: a putative transmembrane protein of *Saccharomyces cerevisiae* (EMBL, accession No. Z36088) that has 560 amino acids with 34% homology, and a protein from

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   1 ATGTCACCGACCATCTCCCACAAGGACAGCAGTCGGCAGCGGCGGTCAGGCATGTTTAGC     60
   1  M  S  P  T  I  S  H  K  D  S  S  R  Q  R  R  S  G  M  F  S     20

  61 CACGCTCTGGATATGAAGAGTGGCCCGCTGCCACCGGGCGGCTGGGATGACAGTCGCCGA    120
  21  H  A  L  D  M  K  S  G  P  L  P  P  G  G  W  D  D  S  R  R     40

 121 GACTCGGTGGGCGGGGAGGGGGACCGGGAAGTTCTTCTAGGTGATGCCGGCCCTGGTGAC    180
  41  D  S  V  G  G  E  G  D  R  E  V  L  L  G  D  A  G  P  G  D     60

 181 TTACCTAAAGCCCCGCGGAGCTATAGATCCGAACTAAGTAGCATTTTGTTACTACTCTTT    240
  61  L  P  K  A  P  R  S  Y  R  S  E  L  S  S  I  L  L  L  L  F     80

 241 CTTTACGTGCTTCAGGGCATTCCCTTAGGCCTGGCGGGGAGCATCCCCCTCATTTTGCAA    300
  81  L  Y  V  L  Q  G  I  P  L  G  L  A  G  S  I  P  L  I  L  Q    100

 301 AGCAAAAATGTTAGCTATACAGATCAGGCTTTCTTCAGTTTTGTCTTCTGGCCATTCAGT    360
 101  S  K  N  V  S  Y  T  D  Q  A  F  F  S  F  V  F  W  P  F  S    120

 361 CTTAAGTTGCTCTGGGCTCCCTTGGTTGATGCCGTCTACTTTAAGAACTTTGGTCGTCGC    420
 121  L  K  L  L  W  A  P  L  V  D  A  V  Y  F  K  N  F  G  R  R    140

 421 AAGTCCTGGCTTGTCCCTACTCAGTATACACTAGGAATCTTCATGATATATTTATCCACT    480
 141  K  S  W  L  V  P  T  Q  Y  T  L  G  I  F  M  I  Y  L  S  T    160

 481 CAAGTGGACCGTTTGCTCGGAAATATTGATGGCAGAACACCGGATGTAGTTGCTCTCACT    540
 161  Q  V  D  R  L  L  G  N  I  D  G  R  T  P  D  V  V  A  L  T    180

 541 GTGACATTCTTCTTGTTTGAATTCCTGGCTGCCACACAGGACATCGCTGTGGATGGTTGG    600
 181  V  T  F  F  L  F  E  F  L  A  A  T  Q  D  I  A  V  D  G  W    200

 601 GCATTAACTATGTTATCCCGGGAAAACGTGGGCTATGCTTCGACATGCAATTCTGTGGGC    660
 201  A  L  T  M  L  S  R  E  N  V  G  Y  A  S  T  C  N  S  V  G    220

 661 CAAACAGCTGGCTACTTTTTGGGCAATGTTTTGTTTTTGGCTCTTGAATCTGCTGACTTT    720
 221  Q  T  A  G  Y  F  L  G  N  V  L  F  L  A  L  E  S  A  D  F    240

 721 TGTAACAAATACTTGCGGTTTCAGCCTCAACCCAGGGGAATCGGTAACCCTTCAGATTTT    780
 241  C  N  K  Y  L  R  F  Q  P  Q  P  R  G  I  G  N  P  S  D  F    260

 781 CTATTTTTCTGGGGAACTGTATTTTTAATAACAACAACATCACTAGCCCTTCTGAAAAAA    840
 261  L  F  F  W  G  T  V  F  L  I  T  T  T  S  L  A  L  L  K  K    280

 841 GAAAACAGAGAAGCGTCAATAGTAAAAGAAGAGACCCCAGGGATCACAGACACTTACAAG    900
 281  E  N  R  E  A  S  I  V  K  E  E  T  P  G  I  T  D  T  Y  K    300

 901 CTGCTATTCTCAATTATAAAAATGCCAGCAGTTCTGGCCTTTTGCCTTCTGATTCTAACG    960
 301  L  L  F  S  I  I  K  M  P  A  V  L  A  F  C  L  L  I  L  T    320

 961 TCAAAGATTGGCTTCTCAGCAGCTGATGCTGTGACAGGCCTGAAGCTGGAAGAAGAAGGG   1020
 321  S  K  I  G  F  S  A  A  D  A  V  T  G  L  K  L  E  E  E  G    340

1021 GTGCCTAAAGAGCACCTGGCCTTACTAGCTGTCCCAATGGTCCCTCTGCAGATAATCCTG   1080
 341  V  P  K  E  H  L  A  L  L  A  V  P  M  V  P  L  Q  I  I  L    360

1081 CCACTCCTCATCAGCAAGTATACTGCAGGTCCCCAGCCTCTGAACATATTTTACAAAGCC   1140
 361  P  L  L  I  S  K  Y  T  A  G  P  Q  P  L  N  I  F  Y  K  A    380

1141 ATGCCCTACAGATTATTGCTTGGATTAGAATATGCTCTACTTGTTTGGTGGACTCCTAAA   1200
 381  M  P  Y  R  L  L  L  G  L  E  Y  A  L  L  V  W  W  T  P  K    400

1201 GTAGAGCATCAAGGAGGATTCCCTCTATATTACTATATTATAGTGCTGCTGAGTTATGCA   1260
 401  V  E  H  Q  G  G  F  P  L  Y  Y  Y  I  I  V  L  L  S  Y  A    420

1261 TTACATCAGGTCACTCTGTACAGCATGTATGTGTCGATAATGGCTTTCAATGCCAAGGTC   1320
 421  L  H  Q  V  T  L  Y  S  M  Y  V  S  I  M  A  F  N  A  K  V    440

1321 AGTGATCCTCTTATTGGGGGAACATACATGACCCTTTTAAACACTGTGTCTAATCTGGGA   1380
 441  S  D  P  L  I  G  G  T  Y  M  T  L  L  N  T  V  S  N  L  G    460

1381 GGAAACTGGCCTTCTACAGTGGCTCTTTGGCTAGTGGATCCCCTCACAGTGAAGGAATGT   1440
 461  G  N  W  P  S  T  V  A  L  W  L  V  D  P  L  T  V  K  E  C    480

1441 GTAGGAGCTTCAAACCAGAATTGTCGGACACCTGATGCTATCGAACTTTGCAAAAAACTT   1500
 481  V  G  A  S  N  Q  N  C  R  T  P  D  A  I  E  L  C  K  K  L    500

1501 GGAGGCTCGTGTGTTACAGCTCTGGATGGTTACTATGTGGAATCCATTATCTGTGTTCTC   1560
 501  G  G  S  C  V  T  A  L  D  G  Y  Y  V  E  S  I  I  C  V  L    520

1561 ATTGGATTTGGTTGGTGGTTCTTTCTTGGTCCAAAATTTAAAAAGTTACAGGATGAAGGA   1620
 521  I  G  F  G  W  W  F  F  L  G  P  K  F  K  K  L  Q  D  E  G    540

1621 CCATCCTCATGGAAGTGCAAAAGGAACAACTGA                               1653
 541  P  S  S  W  K  C  K  R  N  N  *                               551
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Fig. 1. Nucleotide sequence and predicted amino-acid sequence of mouse *Acatn* cDNA. The potential N-linked glycosylation sites are enclosed in the boxes. The putative leucine zipper motif is underlined. The nucleotide sequence data will appear in the DDBJ/EMBL/GenBank nucleotide sequence databases with the accession number AB016795.

Caenorhabditis elegans T26C5.3 (EMBL, accession No. Z50859) that has 632 amino acids with 49% homology on the basis of amino-acid sequence. As shown in Fig. 2,

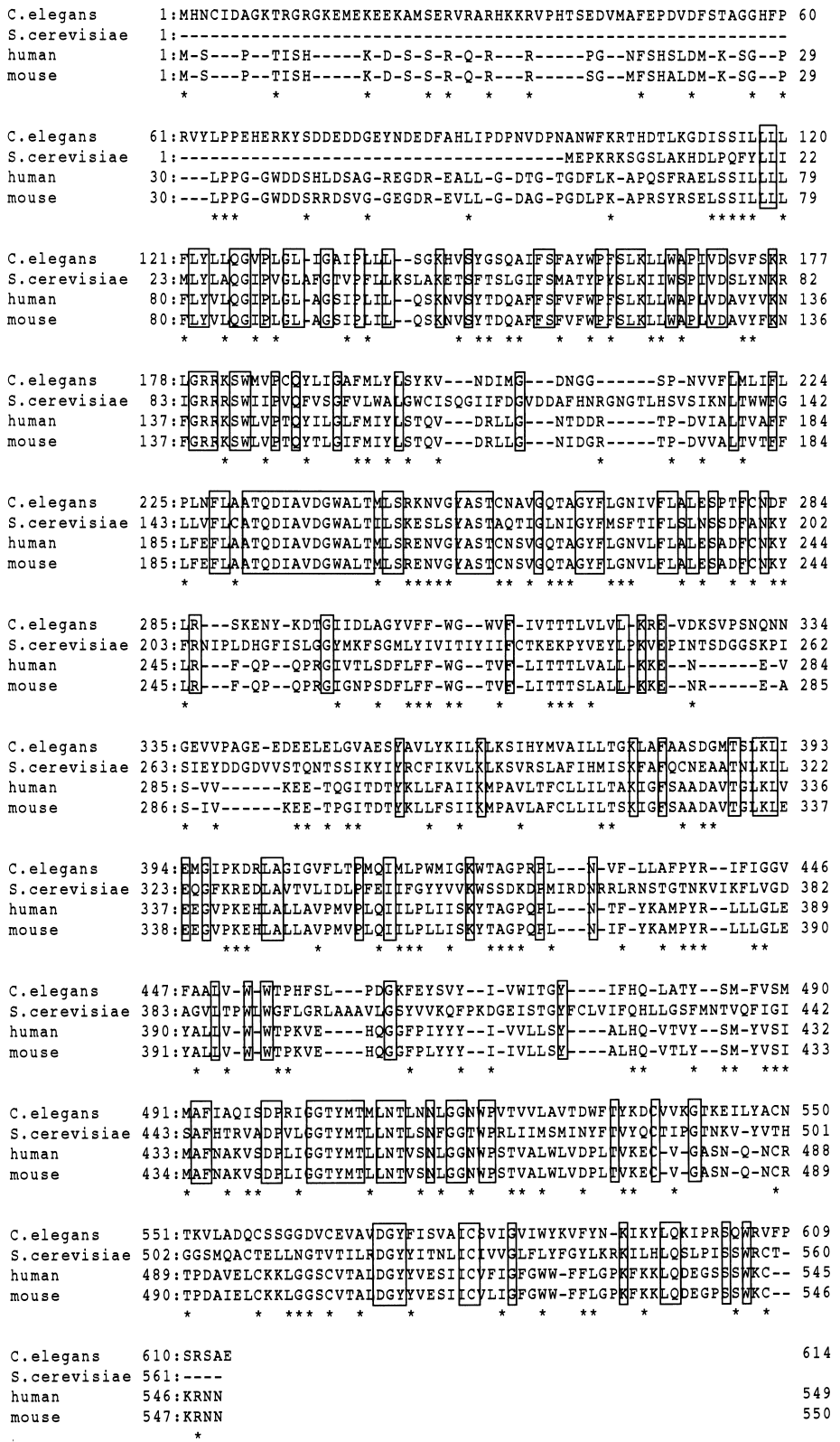

Fig. 2. Amino-acid sequence alignment of mouse, human Acatn and the homologs. Gaps have been introduced to maximize alignments. The identical amino-acid residues among all the species are enclosed in boxes, and the asterisks indicate conserved residues in three species.

there are highly conserved segments among acetyl-CoA transporter and its homologs from different organisms. In particular, maximum homology is seen in the regions corresponding to amino acid residues 72–246 and 431–482 of mouse Acatn, suggesting that these might be functionally more significant domains of Acatn protein. The function of these homologous proteins in *C. elegans* and *S. cerevisiae* is not yet known. Since these organisms lack both sialic acids and gangliosides, it is unlikely that Acatn is involved solely in acetylation of sialic acids. Thus Acatn (and its homologs) might be involved in other acetylation processes as well. In addition to these two proteins, mouse Acatn protein also exhibited low similarity to Amp G protein of *Escherichia coli* (EMBL, accession No. X82158) with 22% identity on the basis of amino acid sequence (data not shown), indicating an evolutionary relationship between these two proteins. Interestingly, like other homologs, there are several conserved amino-acid residues in the region corresponding to amino-acid position between 66 and 237 of mouse Acatn. The gene product of *ampG* is a 53 kDa hydrophobic protein with 7 to10 transmembrane domains, and is found to be involved in the transport of muropeptides of bacterial cell wall inside the cell and also in the regulation of beta-lactamase induction (Lindquist et al., 1993; Jacobs et al., 1994). At present, the role of AmpG protein in acetylation process is not known.

3.2. Expression of 9-*O*-acetylated gangliosides after introduction of mouse Acatn cDNA into HeLa/ GT3$^+$ cells

Mouse *Acatn* cDNA was introduced into HeLa/GT3$^+$ recipient cells, that express precursor gangliosides GT3 but lack 9-*O*-acetylated GT3. A *Bam*HI–*Xba* I fragment of mouse *Acatn* cDNA (nt 1–1653) containing the entire coding sequence was subcloned into *Bam*HI and *Xba*I sites of mammalian expression vector pcDNA3.1. The resultant plasmid, designated as pcDNA3.1-Acatn, was transfected into HeLa/GT3$^+$ cells. Expression of 9-*O*-acetylated gangliosides in HeLa/GT3$^+$ cells was detected by immunostaining with mAb 493D4, specific for 9-*O*-acetylated GT3. As shown in Fig. 3, expression of 9-*O*-acetylated gangliosides was strongly detected with mAb 493D4 on *Acatn* transfected cells as compared with the cells transfected with vector pcDNA3.1 alone, indicating that Acatn protein does play an important role in acetylation of gangliosides. Our previous studies on the expression of human *Acatn* cDNA in COS-1/GD3$^+$ and HeLa/GT3$^+$ cells also showed a high level expression of 9-*O*-acetylated GT3 and GD3 in the transfected cells (Kanamori et al., 1997). Based on these results and also earlier studies on in vitro transport activity for acetyl-CoA (Kanamori et al., 1997), the protein encoded by *Acatn* is suggested to be an acetyl-CoA transporter that is involved in the process of *O*-acetylation.

3.3. Tissue distribution of Acatn mRNA

The expression of *Acatn* mRNA in various mouse tissues was examined by Northern blot analysis, using mRNA purified from adult mouse tissues. A major transcript of 3.0 kb was detected in all the tissues examined, including brain, heart, kidney, liver and spleen, as shown in Fig. 4A. The maximum expression of *Acatn* was observed in kidney and liver. In contrast to human, which expressed two species of the mRNA corresponding to 3.3 and 4.3 kb respectively (Kanamori et al., 1997), mouse expressed only a single transcript of 3.0 kb.

3.4. Tissue-specific and developmentally regulated expression of mouse Acatn gene

Expression of *Acatn* mRNA was examined during embryonic development by Northern blot analysis. High level of expression was observed in early embryonic

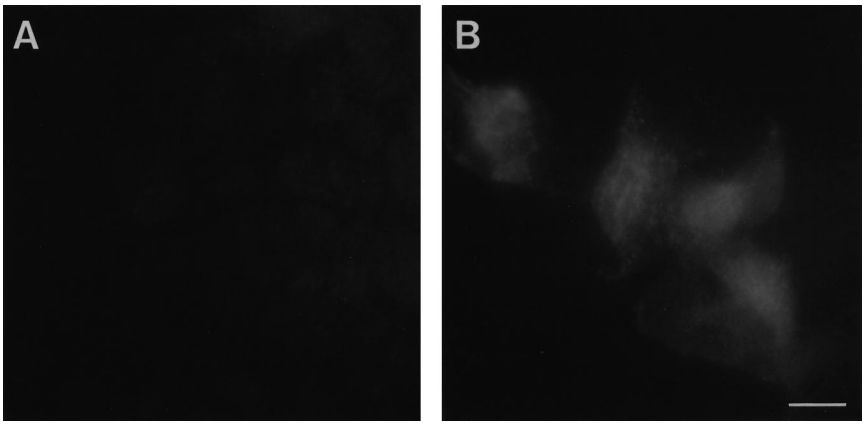

Fig. 3. Immunocytochemical analysis to study the expression of *O*-acetylated gangliosides in the transfected cells. HeLa/GT3$^+$ cells were transfected with vector pcDNA3.1 (A) or pcDNA3.1-Acatn (B). Bar = 20 μm.

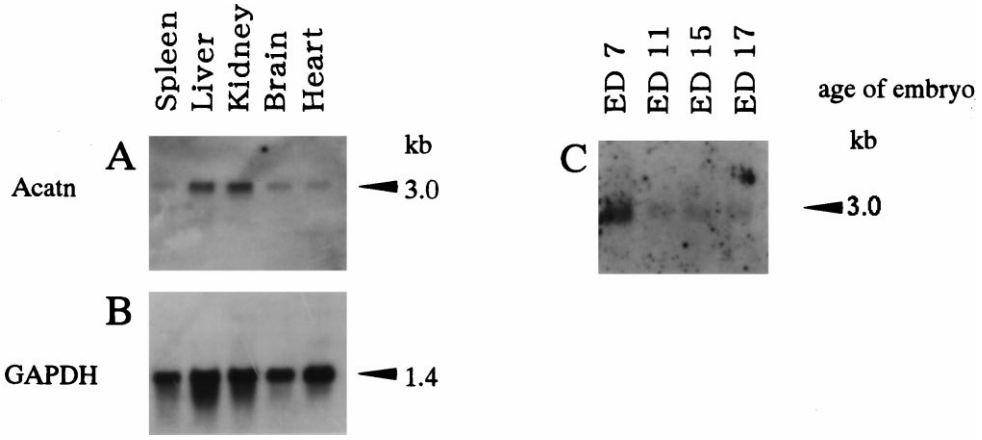

Fig. 4. Northern blot analysis of *Acatn* mRNA in adult mouse tissues and embryos. Northern blot analysis of mRNA from mouse adult tissues hybridized with *Acatn* antisense RNA probe (A) or hybridized with GAPDH antisense RNA probe as control experiment (B) and mRNA from mouse embryos (2 μg per lane) hybridized with *Acatn* antisense RNA probe (C).

stage ED7, and then there was a subsequent decrease in the expression level up to embryonic day 17, as shown in Fig. 4C, suggesting a highly specific role for Acatn protein during early embryonic development. A major transcript of 3.0 kb was detected in all the embryonic stages. Expression of *Acatn* mRNA during developmental stages was also examined by in situ hybridization using rat embryos, because expression of acetylated gangliosides is very well characterized in rat system (Varki, 1992; Schlosshauer et al., 1988). In the 10 day old embryo, high level of mRNA expression was detected in the neural tube and neural crest cells using *Acatn* antisense RNA probe (data not shown). In the 13 day old embryo, maximum expression was detected in the dorsal root ganglia (derived from neural crest) with *Acatn* antisense RNA probe (Fig. 5A). In the 16 day old embryo, expression of *Acatn* mRNA was also detected in dorsal root ganglia, but the expression level was low as compared with that in the 13 day old embryo (Fig. 5C). With the control sense probe, no signal was detected in the rat embryos (Fig. 5B, D). In the 18 day old embryo, *Acatn* expression was barely detected in dorsal root ganglia (data not shown), suggesting that there is a decrease in *Acatn* expression levels at later stages of embryonic development, as also observed by Northern blot analysis. Earlier studies have indicated the developmentally regulated expression of 9-*O*-acetylated gangliosides in rat. In early embryonic stages, ED8–11, expression of 9-*O*-acetylated gangliosides was observed in neural tube and neural crest cells. In embryonic stages from ED13–18, expression of 9-*O*-acetylated gangliosides was detected in dorsal root ganglia and germinal cells in the ventricular zones (Varki, 1992). Hence, the expression of *Acatn* mRNA is in accordance with the expression of *O*-acetylated gangliosides during embryonic development.

4. Conclusion

We have isolated a mouse cDNA encoding for an acetyl-CoA transporter which exhibited 87% homology with the human cDNA at nucleotide sequence level. Acatn is found to be evolutionarily conserved as its homologs are detected in various organisms including *C. elegans*, *S. cerevisiae* and *E. coli*, exhibiting 49%, 34% and 22% homology, respectively, with mouse Acatn protein. In particular, maximum homology is seen in the regions corresponding to amino acid residues 72–246 and 431–482 of mouse Acatn protein. Transfection of *Acatn* cDNA into HeLa/GT3$^+$ cells resulted in high expression of 9-*O*-acetylated gangliosides, indicating that it plays an important role in the acetylation of gangliosides. During embryonic development, *Acatn* expression levels were high during early embryonic stages such as ED7 and there was a subsequent decrease in expression levels in later stages, suggesting that *Acatn* expression is developmentally regulated. Expression of *Acatn* was also found to be tissue-specific, as it was detected in neural tube and neural crest cells in early embryonic stages of development and, in later stages, the expression was detected in dorsal root ganglia. In adult stages, transcript of *Acatn* was detected in all the tissues, with higher expression levels in kidney and liver. Although transcript of *Acatn* is more widely distributed in adult stages, until now, acetylated gangliosides have been reported only in adult kidney, brain and adrenal medulla (Reivinen et al., 1992; Leclerc et al., 1992; Schlosshauer et al., 1988), suggesting that some other factors also might be involved in regulation of the expression of acetylated gangliosides. In fact, another cDNA clone that is involved in *O*-acetylation of gangliosides had been isolated from rat brain (Ogura et al., 1996). The predicted sequence of the protein does not

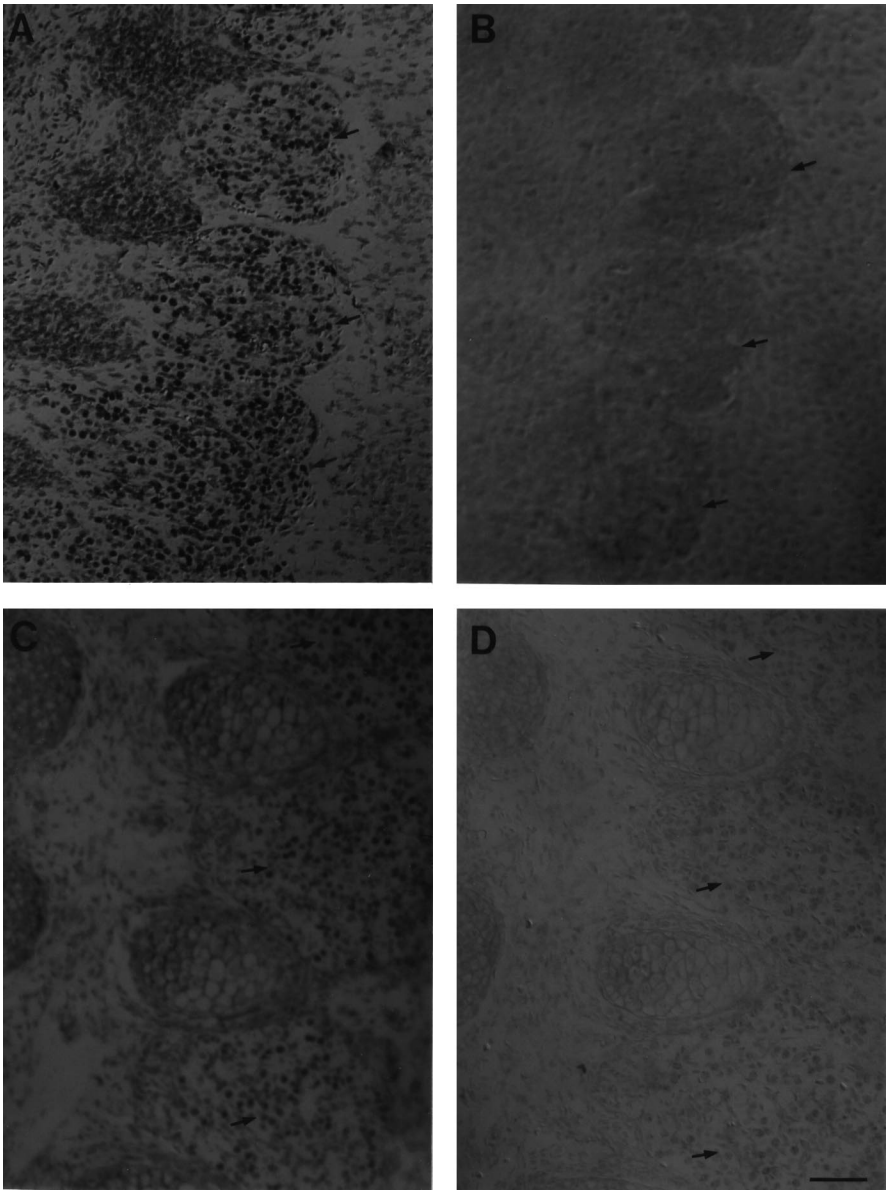

Fig. 5. In situ hybridization of rat embryo sections with *Acatn*-specific probe. Embryonic sections were hybridized with digoxigenin-labeled *Acatn* antisense RNA probe (A, C) and sense probe (B, D), corresponding to nucleotide position between 1 and 688. (A, B) A 13 day old embryo hybridized with antisense and sense probe, respectively. (C, D) A 16 day old embryo hybridized with antisense and sense probe, respectively. Dorsal root ganglia region is marked by arrows. Bar = 20 μm.

share any similarity with Acatn protein. The function of this protein as an *O*-acetyltransferase is not yet clear. Homologs of Acatn protein have been identified in *C. elegans* and *S. cerevisiae*. Since these organisms have neither gangliosides nor sialic acids, it is unlikely that Acatn is involved solely in acetylation of sialic acids. Thus Acatn protein might be involved in other acetylation processes in addition to the acetylation of sialic acids in gangliosides. Our earlier studies have suggested that Acatn protein functions as an acetyl-CoA transporter (Kanamori et al., 1997); development of transgenic mice with both the alleles of *Acatn* disrupted will be required to further elucidate the biological functions of Acatn during embryonic development.

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