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The acetyl-CoA transporter family SLC33

Received: 21 January 2003 / Accepted: 23 March 2003 / Published online: 9 May 2003
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Abstract The acetyl-CoA (Ac-CoA) transporter (AT-1) is a multiple transmembrane protein in the endoplasmic reticulum. Ac-CoA is transported to the lumen of the Golgi apparatus, where it serves as the substrate of acetyltransferases that modify the sialyl residues of gangliosides and glycoproteins. The AT-1 gene, originally named ACATN (acetyl-CoA transporter), was cloned from human melanoma cells. Although homologs of this family of proteins have been identified in lower organisms, such as *Escherichia coli*, *Drosophila melanogaster*, and *Caenorhabditis elegans*, currently only one member of this SLC33A1 family has been identified in humans. Thus, SLC33A1 proteins should be re-named ACATN1 or AT-1. Although acetylated gangliosides show a highly tissue-specific distribution, AT-1 is ubiquitously expressed. Phylogenetically, the AT-1 gene is highly conserved, suggesting that it is particularly significant. The precise physiological roles of this transporter protein, however, remain to be elucidated.

Keywords Acetyl-CoA transporter · Endoplasmic reticulum

Introduction

Elongation of glycan chains is often terminated by sialyltransferases. The terminal sialic acids of glycoproteins and gangliosides are sometimes further modified by O-acetylation, frequently at the 9-position [1]. O-Acetylation of sialic acids is remarkably tissue specific and developmentally regulated in a variety of biological systems. In fertilized eggs, ectopic expression of sialic

acid-specific 9-O-acetyltransferase consistently arrests development at the two-cell stage, suggesting that the O-acetylated sialoglycan chains may be involved in segmentation of the embryos [2]. In embryos, the late expression of 9-O-acetyltransferase in specific organs causes morphological abnormalities [2]. O-Acetylation of ganglioside GD3 suppresses the proapoptotic activity of mitochondrial GD3 [3]. Despite its importance, however, the O-acetylation mechanism is poorly understood at the molecular and gene levels.

Acetylation of sialic acid occurs in the lumen of the Golgi apparatus (Fig. 1) [4]. The acetate donor, acetyl-CoA (Ac-CoA), is synthesized in the cytosol. Therefore, Ac-CoA must be transported into the lumen of the Golgi apparatus/endoplasmic reticulum to drive the acetylation reaction [4]. The recent cloning of the gene encoding the Ac-CoA transporter protein provides new clues for understanding the roles of Ac-CoA transport and metabolism during development and differentiation.

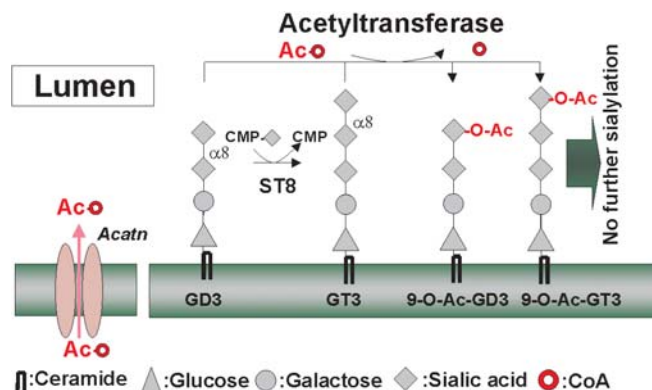


Fig. 1 Roles of ACATN (acetyl-CoA transporter). Gangliosides GD3 and GT3 are synthesized in the lumen of the Golgi apparatus via a reaction catalyzed by the sialyltransferase STSia8-I. Terminal sialic acids are further modified by acetylation at the 9-position. The addition of an acetyl group to sialic acid prevents subsequent sialylation. GD3 NeuAca2–8NeuAca2–3Galβ1–4GlcβCer; GT3 NeuAca2–8NeuAca2–8NeuAca2–3Galβ1–4GlcβCer

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Table 1 SLC33 acetyl-CoA transporter family

Human gene name	Protein name	Aliases	Predominant substrates	Transport type/couplings	Tissue distribution and cellular/subcellular expression	Link to disease	Human gene locus	Sequence accession ID	Splice variants and their specific features
SLC33A1	ACATN	AT-1	Acetyl-CoA	F	Brain, spinal cord, prostate, placenta, uterus, testis, thymus, trachea/endoplasmic reticulum	Not known	3q26.1	NM_004733	Not known

Identification of an Ac-CoA transporter

In 1997, an Ac-CoA transporter cDNA (AT-1) was isolated using the expression cloning method and COS-1 and HeLa cells possessing the precursor gangliosides GD3 and GT3 [5]. Expression of human Ac-CoA transporter in HeLa cells induces production of 9-O-acetylated GD3 and GT3 [4]. Rat and mouse Ac-CoA transporter cDNAs were subsequently cloned [6, 7] and the genomic structure and promoter regions of the mouse Ac-CoA transporter gene (*slc33a1*) were elucidated [8].

Because the transporter protein is tightly membrane bound and is unstable, the homogeneous protein has never been purified. Nonetheless, transmembrane hidden Markov model (TMHMM) analysis (www.cbs.dtu.dk/services/TMHMM-2.0/) [9] predicts that the amino acid sequence of human ACATN protein contains approximately 11 transmembrane-spanning domains. Mammalian ACATN proteins also contain a leucine-zipper motif. Immunofluorescence analysis shows that the 54-kDa protein localizes to the cytoplasm, presumably in endoplasmic reticulum. Incorporation assay of radiolabeled Ac-CoA into ganglioside fraction using semi-intact cells of stable ACATN transfectants indicates that the ACATN protein functions as an Ac-CoA transporter (Fig. 1).

Two major transcripts (3.3 kb and 4.3 kb) are present in all tissues (Table 1). In mouse, a single 3.0-kb transcript is detected [7] and ubiquitously expressed in adult tissues, including brain. The expression of *Acatn* is developmentally regulated; increased expression occurs during early embryonic stages.

The human and mouse genes have been mapped to chromosome bands 3q21.1 and 3E1-E3, respectively [10].

Phylogenetic features

Mouse and rat *Acatn* cDNAs are highly homologous to human ACATN cDNA, exhibiting 87% and 86% similarity, respectively, in their amino acid sequences. Homology searches of currently available nucleotide and protein databases for human ACATN identify three homologous proteins: (1) a putative transmembrane protein from *Saccharomyces cerevisia* (EMBL, accession no. Z36088)

that has 560 amino acids and 31% shared identity; (2) a protein from *Caenorhabditis elegans* T26C5.3 (EMBL, accession no. Z50859) that has 632 amino acids and 48% shared identity; and (3) a protein from *Drosophila melanogaster* that has 525 amino acids and 48% shared identity. In the nematode *C. elegans*, two alternative spliced forms of the *Acatn* gene (T26C5.3a and T26C5.3b) are predicted. *Escherichia coli* and *Haemophilus influenzae* also express a homologous protein that bears little similarity to human ACATN (22% identity). AmpG, the homolog in *E. coli*, is a 53-kDa multitransmembrane protein required for recycling of murein tripeptide [11]. The presence of a *N*-acetylglucosaminyl- β -1,4-anhydro-*N*-acetylmuramic acid is critical, because it is the substrate for AmpG permease [12]. This muramic acid is also involved in regulating β -lactamase induction [11]. There are several conserved amino acid residues in the *N*-terminal region of AmpG and mammalian ACATN proteins. At present, the role of AmpG protein in Ac-CoA metabolism is unknown.

Genetic manipulation of SLC33 proteins will enable us to identify their functional roles in vivo. For example, using *C. elegans* as a model animal, we have examined the effect of RNAi (RNA-mediated interference) on *Acatn* gene expression. Knocking out *Acatn* reduces brood size and shortens body length due to insufficient maturation of the gonadal system. It is also important to note that embryos bearing this *Acatn* knock-out occasionally die (unpublished observation).

Diseases and mutants

To date, no human diseases involving ACATN have been described. In *C. elegans*, we have isolated a deletion mutant allele of the *Acatn* gene (unpublished observation). Worms bearing this deletion mutant allele are viable, but their development is severely compromised. The gonadal system of these worms appears to be also severely compromised. Using mutant worms, we expect to identify new biological systems in which the Ac-CoA transporter plays a key role.

References

1. Butor C, Diaz S, Varki A (1993) High level O-acetylation of sialic acids on N-linked oligosaccharides of rat liver membranes. Differential subcellular distribution of 7- and 9-O-acetyl groups and of enzymes involved in their regulation. *J Biol Chem* 268:10197–10206
2. Varki A, Hooshmand F, Diaz S, Varki NM, Hedrick SM (1991) Developmental abnormalities in transgenic mice expressing a sialic acid-specific 9-O-acetyltransferase. *Cell* 65:65–74
3. Malisan F, Franchi L, Tomassini B, Ventura N, Condo I, Rippo MR, Rufini A, Liberati L, Nachtigall C, Kniep B, Testi R (2002) Acetylation suppresses the proapoptotic activity of GD3 ganglioside. *J Exp Med* 196:1535–1541
4. Varki A, Diaz S (1985) The transport and utilization of acetyl coenzyme A by rat liver Golgi vesicles. O-acetylated sialic acids are a major product. *J Biol Chem* 260:6600–6608
5. Kanamori A, Nakayama J, Fukuda MN, Stallcup WB, Sasaki K, Fukuda M, Hirabayashi Y (1997) Expression cloning and characterization of a cDNA encoding a novel membrane protein required for the formation of O-acetylated ganglioside: a putative acetyl-CoA transporter. *Proc Natl Acad Sci USA* 94:2897–2902
6. Bora RS, Kanamori A, Hirabayashi Y (1999) Cloning and characterization of a putative mouse acetyl-CoA transporter cDNA. *Gene* 238:455–462
7. Bora RS, Ichikawa S, Kanamori A, Hirabayashi Y (2000) cDNA cloning of putative rat acetyl-CoA transporter and its expression pattern in brain. *Cytogenet Cell Genet* 89:204–208
8. Bora RS, Ichikawa S, Kanamori A, Hirabayashi Y (2000) Genomic structure and promoter analysis of putative mouse acetyl-CoA transporter gene. *FEBS Lett* 473:169–172
9. Krogh A, Larsson B, Heijne G von, Sonnhammer EL (2001) Predicting transmembrane protein topology with a hidden Markov model: application to complete genomes. *J Mol Biol* 305:567–580
10. Bora RS, Kanamori A, Hirabayashi Y (1998) Assignment of a putative acetyl-CoA transporter gene (*Acatn*) to mouse chromosome band 3E1-E3 by in situ hybridization. *Cytogenet Cell Genet* 83:78–79
11. Jacobs C, Huang LJ, Bartowsky E, Normark S, Park JT (1994) Bacterial cell wall recycling provides cytosolic muropeptides as effectors for beta-lactamase induction. *EMBO J* 13:4684–4694
12. Cheng Q, Park JT (2002) Substrate specificity of the AmpG permease required for recycling of cell wall anhydro-muropeptides. *J Bacteriol* 184:6434–6436