
Solute Carrier Family 35 (CMP-Sialic Acid Transporter), Member A1 (SLC35A1)

121

Shoko Nishihara

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Introduction

CMP-sialic acid is a donor substrate of sialyltransferases. CMP-sialic acid transporter (CST) couples with the antiport of CMP and transports CMP-sialic acid from the cytosol into the lumen of the medial and trans cisternae of the Golgi apparatus (Capasso and Hirschberg 1984; Tiralongo et al. 2006; Zhao et al. 2006), where sialylation is mediated by a variety of sialyltransferases (Fig. 121.1). Therefore, expression of CST regulates the sialylation of glycans on the cell surface.

CST is categorized as the first member of subgroup A of solute carrier family 35 (SLC35A1) (Ishida and Kawakita 2004). A phylogenetic tree of SLC35A nucleotide sugar transporters is shown in Fig. 121.2. To date, three types of mammalian

S. Nishihara

Department of Bioinformatics, Faculty of Engineering, Soka University, Hachioji, Tokyo, Japan
e-mail: shoko@soka.ac.jp

Fig. 121.1 Schematic representation of the CMP-sialic acid transporter (CST/SLC35A1) in the glycosylation pathway. CMP-sialic acid transporter transports CMP-sialic acid from the cytosol into the lumen of the Golgi compartment by coupling with the antiport of CMP, which is produced as the result of a sialyltransferase reaction

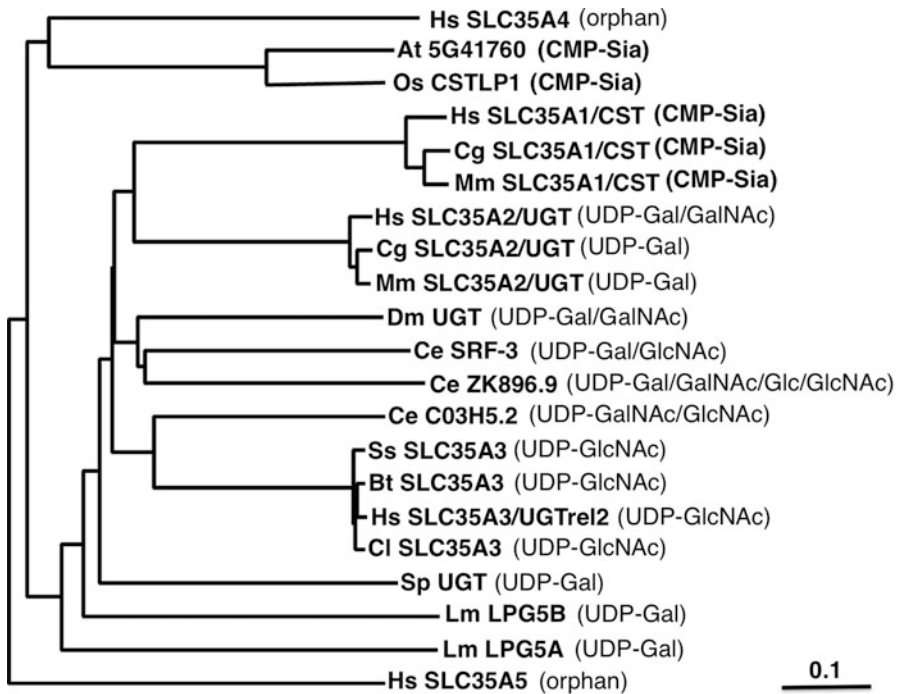
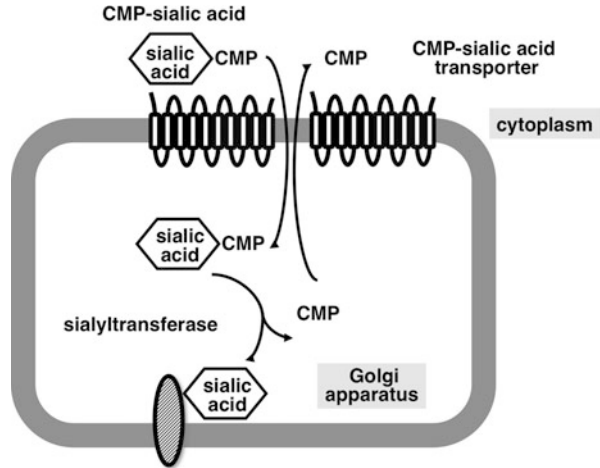


Fig. 121.2 Phylogenetic tree of nucleotide sugar transporters of solute carrier family 35 member A (SLC35A) including CMP-sialic acid transporters (CST/SLC35A1). Parentheses indicate transport substrates that have been identified by in vitro activity analysis or complementation analysis. *At Arabidopsis thaliana*, *Bt Bos taurus*, *Ce Caenorhabditis elegans*, *Cg Cricetus griseus*, *Cl Canis lupus familiaris*, *Dm Drosophila melanogaster*, *Hs Homo sapiens*, *Lm Leishmania major*, *Mm Mus musculus*, *Os Oryza sativa Japonica Group*, *Sp Saccharomyces pombe*, *Ss Sus scrofa*

CST have been identified based on their transporter activity, namely, *Cricetulus griseus*, *Homo sapiens*, and *Mus musculus*, and two types of plant CST have been identified, namely, *Arabidopsis thaliana* and *Oryza sativa* (Fig. 121.2 and see section “[Databanks](#)”). The plant CSTs cluster on the phylogenetic tree with human SLC35A4 rather than human SLC35A1. Thus, the substrate specificity of a nucleotide sugar transporter cannot easily be predicted from its shared phylogenetic homologies.

CST activity was initially identified in microsomal membrane vesicles prepared from mouse liver (Carey et al. 1980). To date, *CST* genes from the mouse (Eckhardt et al. 1996), human (Eckhardt and Gerardy-Schahn 1997; Ishida et al. 1998) and two plant species (Bakker et al. 2008; Takashima et al. 2009) have been expressed in a yeast expression system and their CST activities proved by an in vitro CMP-sialic acid transport assay using yeast Golgi-enriched vesicles (Aoki et al. 2001, 2003; Bakker et al. 2008; Berninsone et al. 1997; Takashima et al. 2009).

In humans, inactivation of both *CST* alleles has been found to cause congenital glycosylation type IIf disorder in which the affected patient showed macrothrombocytopenia and neutropenia that probably arose from the lack of sialyl Lewis X structures (Martinez-Duncker et al. 2005).

Databanks

In the case of CMP-sialic acid transporter (CST), solute carrier family 35 member A1 (SLC35A1)

No EC number has been allocated.

CMP-Sialic Acid Transporter (CST) (SLC35A1)

Species	Gene symbol	GenBank accession number	Uniprot ID	PDB accession number
<i>Arabidopsis thaliana</i>	AT5G41760	NM_001036915 NM_123541	Q8LGE9	N/A
<i>Cricetulus griseus</i> (Chinese hamster)	Slc35a1	NM_001246755	O08520	N/A
<i>Homo sapiens</i> (Human)	SLC35A1	NM_001168398 NM_006416	P78382	N/A
<i>Mus musculus</i> (Mouse)	Slc35a1	NM_011895	Q61420	N/A
<i>Oryza sativa Japonica</i> group	Os06g0523400	NM_001064288	Q654D9	N/A

Name and History

Almost all vertebrate CMP-sialic acids are synthesized in the nucleus by CMP-sialic acid synthetase (Kean et al. 2004) and then moved to the cytosol. However, sialylation reactions occur in the lumen of the Golgi apparatus where they are mediated by a variety of sialyltransferases. SLC35A1/CST is responsible for the transport of CMP-sialic acid from the cytosol into the lumen of the Golgi

apparatus (Ishida and Kawakita 2004) (Fig. 121.2). This transporter activity was initially demonstrated in microsomal membrane vesicles prepared from mouse liver (Carey et al. 1980), and subsequent biochemical characterization of Golgi apparatus vesicles and reconstituted proteoliposomes confirmed its exclusive localization to the Golgi apparatus (Capasso and Hirschberg 1984; Milla and Hirschberg 1989).

Lec2 mutant cells, derived from a Chinese hamster ovary (CHO) cell line, lack sialylated oligosaccharides, and their Golgi membrane vesicles are deficient in CST activity (Deutscher et al. 1984). The mouse *CST* gene was first cloned by complementation of a defect in Lec2 cells (Eckhardt et al. 1996), and, subsequently, the hamster *CST* gene was identified by the same approach (Eckhardt and Gerardy-Schahn 1997). The human *CST* gene was cloned using its high homology to the murine *CST* gene, and its CMP-sialic acid transport activity was analyzed using microsomal vesicles prepared from Lec2 cells that overexpressed the human *CST* gene (Ishida et al. 1998). These CSTs are localized in the Golgi apparatus. Recently two *CST* genes, *Arabidopsis thaliana* and *Oryza sativa Japonica group*, have also been identified in plants and their activities have been characterized (Bakker et al. 2008; Takashima et al. 2009).

Structure

Mouse and Chinese hamster CSTs consist of 336 amino acids; analyses of the predicted structures suggest that they are multi-pass membrane proteins that have ten membrane-spanning domains. The amino and carboxyl termini are oriented toward the cytosol (Eckhardt et al. 1999). Human CST has two isoforms that consist of 337 amino acids and 278 amino acids, respectively. The longer isoform has the ten membrane-spanning domains and its C-terminus is exposed to the cytosol (Ishida et al. 1998).

Transporter Activity Assay and Substrate Specificity

In the initial assays of CST activity, microsomal membrane vesicles from mouse liver (Capasso and Hirschberg 1984) or reconstituted proteoliposomes from Golgi protein extracts (Milla and Hirschberg 1989) were incubated with CMP-[¹⁴C] sialic acid or CMP-[³H] sialic acid and then separated from the incubation mixture by centrifugation or by gel filtration using a Sephadex G-50 column. The amount of incorporated radiolabeled sialic acids in the vesicles or proteoliposomes was measured with a liquid scintillation counter to determine CMP-sialic acid transport activity.

In vitro assays of CST activity are most commonly carried out using a yeast expression system. As yeast (*Saccharomyces cerevisiae*) microsomal vesicles do not show any strong intrinsic nucleotide sugar transport activity except for GDP-mannose, then incorporation of radiolabeled sialic acids into the vesicles

can be used to measure in vitro activity (Abeijon et al. 1989). This in vitro assay system has been used for functional identification and characterization of mouse CST (Berninsone et al. 1997) and plant CSTs (Bakker et al. 2008; Takashima et al. 2009) and for determination of the substrate recognition site in human CST (Aoki et al. 2001, 2003). The first step in the assay is to subclone a cDNA encoding a CST, or a CST tagged with an HA-epitope at the C-terminus, into a yeast expression vector, and the CST is then expressed. Golgi-enriched vesicles are prepared from the yeast incubated with CMP-[³H] sialic acid or CMP-[¹⁴C] sialic acid for the appropriate time and separated from the incubation mixture by centrifugation or filtration using a nitrocellulose filter (0.45 μm) (Abeijon et al. 1989). CST activity is measured using a liquid scintillation counter to determine the amount of radiolabeled CMP-sialic acids incorporated into the vesicles. Golgi-enriched vesicles prepared from *S. cerevisiae* transformed with an empty vector are used as a control. The apparent Km of mouse CST was determined as 2.9 μM by this system (Berninsone et al. 1997).

The *E. coli* expression system has also been used as an in vitro CST activity assay under zero background conditions (Tiralongo et al. 2006). A CST cDNA tagged with HA-epitopes is first subcloned into the *E. coli* expression vector, which has an isopropyl-β-D-thiogalactopyranoside- (IPTG) inducible trp/lac promoter, and expression of the CST is induced by adding IPTG to the culture medium. The recombinant HA-tagged CST is expressed in the inclusion body pellet. Washed inclusion body pellets are solubilized in buffer containing 8 M urea, and the CST is renatured by dialysis against a renaturation buffer. The renatured CST is reconstituted into proteoliposomes using the freeze-thawing method (Kasahara and Hinkle 1976) and used for the CST activity assay. The proteoliposomes are incubated with CMP-[³H] sialic acid for the appropriate time and then separated from the incubation mixture using a spin column. A liquid scintillation counter is used to determine CST activity in the collected void volume. Through use of this system, mouse CST has been shown to specifically transport CMP-sialic acid by coupling with the antiport of CMP; there is evidence that mouse CST could not transport any UDP-sugars (Tiralongo et al. 2006).

Preparation

Golgi-enriched vesicles containing CSTs have been prepared from mouse liver (Capasso and Hirschberg 1984), and proteoliposomes containing CSTs have been reconstructed from Golgi protein extracts and egg yolk phosphatidylcholine (Milla and Hirschberg 1989). The CST or HA-tagged CST of mouse (Berninsone et al. 1997), human (Aoki et al. 2001, 2003), and the two plant species (Bakker et al. 2008; Takashima et al. 2009) has been expressed in *S. cerevisiae*, and Golgi-enriched vesicles including CST have been prepared from the *S. cerevisiae*. These vesicles still include other nucleotide sugar transporters, although overexpressed CST might be a main component of the vesicles. Functional

proteoliposomes have been successfully reconstituted from phosphatidylcholine and renatured mouse CST expressed in *E. coli* (Tiralongo et al. 2006). The CST was renatured from washed inclusion body pellets that contain unfolded proteins induced or overexpressed in *E. coli*. Almost all proteins in the inclusion body pellet are induced proteins; thus the purity of the renatured CST is regarded as being high. Therefore, reconstituted proteoliposomes effectively include no other nucleotide sugar transporters.

Biological Aspects

Human CST shows high homology (43 %) to human SLC35A2/UGT that transports UDP-galactose and UDP-*N*-acetyl galactosamine (Fig. 121.2) (Aoki et al. 2001; Segawa et al. 2002). Functional analyses using chimeric proteins showed that different regions of each transporter provide a critical contribution to recognition of each substrate and that some chimeric proteins could transport both substrates (Aoki et al. 2001, 2003). These findings suggest two possible developmental mechanisms for *CSTs*: (1) *CST* and *UGT* evolved from a common ancestral gene, or (2) *CST* is derived from *UGT*. Analyses of chimeric proteins and the substitution of amino acids showed that the Tyr214 and Ser216 residues of the seventh transmembrane region and the Lys272 residue of the fourth cytosolic loop are essential for *CST* activity (Aoki et al. 2003; Chan et al. 2010; Takeshima-Futagami et al. 2012).

CST is expressed in various tissues where it localizes to the medial and trans cisternae of the Golgi apparatus (Zhao et al. 2006) and supplies a donor substrate, CMP-sialic acid, to sialyltransferases. To date, twenty sialyltransferases have been identified in humans. Defects in *CSTs* result in abnormal sialylation patterns. Thus, for example, *Lec2* CHO mutant cells have no sialylated *N*-glycans (including either type of sialic acid, *N*-acetylneuraminic acid, or *N*-glycolylneuraminic acid) and no sialylated *O*-glycans (North et al. 2010), because the Gly189Glu mutation in the *CTS* of *Lec2* cells abolishes *CTS* activity (Eckhardt et al. 1998). Another CHO mutant cell line, MAR-11, shows a decrease in the levels of cell surface sialic acid because of a C to T mutation in the *CTS* gene that results in a premature stop codon (Lim et al. 2008). In humans, a patient with a congenital disorder of glycosylation type IIf and who had inactivation of both *CST* alleles (Martinez-Duncker et al. 2005) was found to exhibit marked macrothrombocytopenia and neutropenia caused by the lack of sialyl Lewis X structures (Willig et al. 2001). In the plant *Arabidopsis thaliana*, T-DNA insertions that disrupt the *CST* gene cause a lethal phenotype, indicating that *CST* is essential for plant development (Takashima et al. 2009).

Knockout and Transgenic Mice

To date, no *CST* knockout or transgenic mice have been reported.

Human Diseases

A patient with congenital disorder of glycosylation type II_f, whose polymorphonuclear cells completely lacked sialyl Lewis X antigen (CD15_s, NeuAc α 2,3Gal β 1,4(Fuc α 1,3)GlcNAc-R), showed macrothrombocytopenia and neutropenia, and died at age 37 months (Martinez-Duncker et al. 2005). The paternally inherited allele of the *CST* gene contained 2 microdeletions (G277 and C281) that produced a frame shift and premature stop codon at position 327, in addition to a T147C substitution. The maternally inherited allele had a 130 bp deletion in exon 6 that generated a frame shift and premature stop codon at position 684. A common 4 bp insertion in intron 6 found in the mother was responsible for the splice mutation. Neither allele of the patient was able to complement Lec2 mutant cells, demonstrating that they were inactive.

Future Perspectives

Sialic acids are nine carbon sugars that contain an α -keto acid group (2-keto-3-deoxynononic acid) and consist of a large family with more than 50 derivatives. The principal members of this family are *N*-acetylneuraminic acid, *N*-glycolylneuraminic acid, and deaminoneuraminic acid. Sialic acids show diversity in their structures, unlike other nucleotide sugars. Currently, the type of sialic acid in many species is unclear. Indeed, it is yet certain whether all species have a sialic acid. To date, sialylated structures have been identified in vertebrates, echinoderms, plants, fungi, *Drosophila*, and squid; however, *CST* genes have not yet been identified in fungi, *Drosophila*, or squid. These genes will undoubtedly be determined in the near future. Such information will provide further insight into the evolution of sialylation.

The structural basis of substrate specificity for CSTs has not yet been clarified, although essential amino acids, Tyr214, Ser 216, and Lys272, have been determined using chimeric proteins and amino acid substitution analyses (Aoki et al. 2003; Chan et al. 2010; Takeshima-Futagami et al. 2012). Three-dimensional structural analyses using X-ray diffraction should provide more precise information on the mechanism for recognition of a specific nucleotide sugar substrate and for its transport.

Cross-References

- [Solute Carrier Family 35 \(UDP-Galactose Transporter\), Member A2 \(SLC35A2\)](#)
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