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Introduction

ST3Gal-III is a member of the N-acetyllactosaminide α -2,3-sialyltransferase family, transferring sialic acid from CMP-NeuAc in α -2,3 linkage preferentially to Gal β 1-3GlcNAc, but also to Gal β 1-4GlcNAc and Gal β 1-3GalNAc termini on glycoproteins and glycolipids. It was among the first sialyltransferases purified and cloned (Wen et al. 1992), leading to the discovery of sialyltransferase sialyl motifs.

Although thought to act primarily on Gal β 1-3(4)GlcNAc termini of N-linked glycans, mouse knockouts reveal that ST3Gal-III also sialylates the Gal β 1-3GalNAc terminus of gangliosides (sialylated glycosphingolipids), the major sialoglycans in the brain (Sturgill et al. 2012). *St3gal3*-null mice display nervous system deficits and

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altered regulation of inflammatory signals, presumably due to loss of distinct tissue-specific functional sialoglycans. Mutations in the *ST3GAL3* gene in humans are associated with rare severe familial intellectual disability (Hu et al. 2011). These recent findings have opened new avenues for research on ST3Gal-III and the functional roles of its sialoglycan products.

Databanks

ST3Gal-III

NC-IUBMB nomenclature: E.C. 2.4.99.6

ST3 beta-galactoside alpha-2,3-sialyltransferase 3 (ST3GAL3)

Species	Gene	GenBank	Uniprot	PDB
<i>Homo sapiens</i>	<i>ST3GAL3</i>	NM_174963.3 ^a	Q11203	N/A
<i>Mus musculus</i>	<i>St3gal3</i>	NM_009176.4 ^a	P97325	N/A
<i>Pan troglodytes</i>	<i>ST3GAL3</i>	NM_001037299.1 ^b	P61132	N/A
<i>Macaca mulatta</i>	<i>ST3GAL3</i>	XM_001091910.2 ^c	N/A	N/A
<i>Canis lupus</i>	<i>ST3GAL3</i>	XM_818282.2 ^c	N/A	N/A
<i>Bos taurus</i>	<i>ST3GAL3</i>	NM_001002882.1 ^b	N/A	N/A
<i>Rattus norvegicus</i>	<i>St3gal3</i>	NM_031697.1 ^b	Q02734	N/A
<i>Gallus gallus</i>	<i>St3gal3</i>	XM_00123359.2 ^c	N/A	N/A

^aTranscript variant 1

^bProvisional

^cPredicted

Name and History

ST3Gal-III is officially denoted CMP-N-acetylneuraminate- β -1,4-galactoside α -2,3-sialyltransferase (<http://www.ncbi.nlm.nih.gov/protein>, <http://www.uniprot.org>). It is a member of the N-acetylglucosaminide α -2,3-sialyltransferase family (E.C. 2.4.99.6, <http://www.chem.qmul.ac.uk/iubmb>). Its official names do not reflect the broader specificity of this enzyme (see below). ST3Gal-III was first named Gal β 1,3(4)GlcNAc α -2,3-sialyltransferase when the enzyme, purified nearly 10⁶-fold from rat liver (Weinstein et al. 1982b), was found to preferentially transfer NeuAc from CMP-NeuAc to Gal β 1,3GlcNAc and (less so) to Gal β 1,4GlcNAc, either as free saccharides or termini of N-linked glycans. These findings led the enzyme to be referred to as ST3N until it was redesignated as ST3Gal-III upon unification of the abbreviated sialyltransferase nomenclature (Tsuji et al. 1996). Sequence data derived by mass spectrometry of trypsin fragments from the purified enzyme allowed cloning from a liver cDNA library (Wen et al. 1992), and the gene name *ST3GAL3* was subsequently adopted (<http://www.genenames.org>).

Structure

Like other eukaryotic sialyltransferases (CAZY GT-29 family, <http://www.cazy.org>), ST3Gal-III is a type II membrane protein with a short cytoplasmic amino-terminal domain, a single transmembrane domain, and a large intra-Golgi catalytic carboxy-terminal domain. There are over 25 alternatively spliced isoforms (Grahn et al. 2002), of which isoform B1 is expressed most broadly and has been chosen as the “canonical” sequence (<http://www.uniprot.org>). Although the three-dimensional structure of ST3Gal-III has not been reported, it is expected to share features with other members of the eukaryotic sialyltransferase family and especially with its sister enzyme, ST3Gal-I, which has had the structure of its catalytic domain solved (Rao et al. 2009). By analogy and sequence analysis, ST3Gal-III has a very short (eight amino acid) cytoplasmic domain, a standard 20-amino acid transmembrane alpha helix, a stem region of ~50 amino acids, and a globular catalytic domain of ~300 amino acids. The ST3Gal-III sequence contains four conserved sialyl motifs (large, small, very small, and motif three) (Audry et al. 2011), which by analogy to ST3Gal-I form and surround the active site in a single Rossmann-like fold, GT-A variant glycosyltransferase topology (Rao et al. 2009).

Enzyme Activity Assay and Substrate Specificity

ST3Gal-III transfers sialic acids (and sialic acid analogs) from CMP-sialic acid to galactose-terminated glycans on glycoproteins, glycolipids, and oligosaccharides. A typical reaction contains buffer (50 mM sodium cacodylate, pH 6.0), detergent (0.5 % Triton CF-54), bovine serum albumin (1 mg/ml), CMP-sialic acid (0.15 mM CMP-[¹⁴C]NeuAc), acceptor, and enzyme (Weinstein et al. 1982b). A convenient assay takes advantage of the activity of ST3Gal-III for the free tetrasaccharide acceptor lacto-N-tetraose (Gal β 1-3GlcNAc β 1-3Gal β 1-4Glc). After incubation, the reaction is diluted with water or low-ionic-strength buffer (5 mM sodium phosphate, pH 6.8) and loaded onto a strong ion exchange resin (Dowex-1 PO₄⁻²). The donor (CMP-NeuAc) is captured on the resin, whereas the product, sialyllacto-N-tetraose, elutes efficiently and is quantified. In this assay the K_D for CMP-NeuAc is 30 μ M and for lacto-N-tetraose is 70 μ M.

Many assay variations have been reported. A solid-state assay of ST3Gal-III was performed on a glycan array containing hundreds of covalently bound glycans (Blixt et al. 2008). In this assay the enzyme (50 mU), a CMP-NeuAc analog with a leashed biotin linked to the NeuAc 9-hydroxyl (1 mM), and bovine serum albumin (30 mg/ml) in Tris-HCl buffer (100 mM, pH 7.0) were incubated on the glass slide microarray, washed, and transfer of the sialic acid determined semiquantitatively using fluorescent streptavidin. In this way, the comparative efficiencies of hundreds of potential acceptors were determined simultaneously.

Acceptor specificity studies of purified ST3Gal-III (Weinstein et al. 1982a) identified it as “Gal β 1-3(4)GlcNAc α 2-3 sialyltransferase” based on its robust ability to transfer sialic acid to Gal β 1-3GlcNAc or lacto-N-tetraose (Gal β 1-3GlcNAc β 1-3Gal β 1-4Glc, $K_m < 0.1$ mM) and its reduced ability to transfer to Gal β 1-4GlcNAc or lacto-N-neotetraose (Gal β 1-4GlcNAc β 1-3Gal β 1-4Glc, $K_m = 4$ mM). In those studies low activity was also detected using Gal β 1-3GalNAc as the acceptor. Similar side-by-side in vitro enzyme activity comparisons (Kono et al. 1997) confirmed the disaccharide acceptor specificity as Gal β 1-3GlcNAc > Gal β 1-4GlcNAc, which has been contrasted to ST3Gal-IV that has the opposite preference.

Solid-phase acceptor specificity studies (Blixt et al. 2008) indicate that ST3Gal-III transfers sialic acid to many glycosides with Gal β 1-3GlcNAc, Gal β 1-4GlcNAc, or Gal β 1-3GalNAc termini. The top three acceptor glycans in this assay were Gal β 1-3GalNAc β 1-R, Gal β 1-3(NeuAc α 2-6)GlcNAc β 1-4Gal β 1-4Glc β 1-R, and Gal β 1-4GlcNAc β 1-6GalNAc α 1-R (where “R” is a short amino-alkyl leash), indicating transfer to terminal β -galactosides similar to those found on N-linked glycoprotein glycans, O-linked glycoprotein glycans, and glycolipids. Whereas Gal β 1-3GalNAc β 1-R was the best acceptor in this assay, Gal β 1-3GalNAc α 1-R was a weak acceptor. This may indicate more robust activity toward the common gangliotetraose terminus (Gal β 1-3GalNAc β 1-4Gal) compared to core 1 O-linked glycans (Gal β 1-3GalNAc α 1-Ser/Thr). In contrast, a core 6 O-linked glycan (Gal β 1-4GlcNAc β 1-6GalNAc α 1-R) was one of the best acceptors. A fair conclusion from these studies is that ST3Gal-III has the ability to transfer NeuAc to β -galactosides in a variety of linkages in vitro and that in vivo studies will be required to more accurately define its biosynthetic functions (see “[Knockout Mouse and Transgenic Mice](#),” below).

ST3Gal-III uses CMP-NeuAc as its preferred donor but will also use CMP-NeuGc, CMP-KDN, or derivatives of NeuAc (e.g., 9-biotinamido-9-deoxy-CMP-NeuAc) with reduced efficiency (Ivannikova et al. 2003; Blixt et al. 2008).

Preparation

Native ST3Gal-III was solubilized from rat liver membranes using Triton CF-54, then purified to homogeneity (>800,000-fold) by sequential affinity chromatography on CDP-hexanolamine-agarose and asialoprothrombin-agarose (Weinstein et al. 1982b). The resulting enzyme, purified in 7 % yield, had a specific activity of 28 U/mg using lacto-N-tetraose (Gal β 1-3GlcNAc β 1-3Gal β 1-4Glc) as acceptor. The purified protein provided material to perform mass spectrometry of tryptic fragments, which was the basis for cloning the *St3gal3* gene from rat liver (Wen et al. 1992). Soluble chimeric enzyme lacking the membrane-spanning region and fused with protein A was expressed in COS cells and then readily purified by binding to IgG-Sepharose beads (Kono et al. 1997). The resulting beads were used as a source of enzyme to determine the relative kinetics of different disaccharide acceptors including Gal β 1-3GlcNAc ($K_m = 0.3$ mM), Gal β 1-4GlcNAc ($K_m = 3.0$ mM), and Gal β 1-3GalNAc ($K_m = 2.2$ mM).

For use in chemoenzymatic syntheses, soluble ST3Gal-III was fused with a His₆ N-terminal tag, incorporated in recombinant baculovirus, expressed in *Sf9* cells, and purified using nickel-agarose beads (Ivannikova et al. 2003). The immobilized enzyme was used sequentially to synthesize a disialylated derivative of a branched synthetic di-LacNAc and a dansyl glycoside of Gal β 1-3GlcNAc, both at high yield.

Biological Aspects

The *ST3GAL3* gene is widely expressed in human tissues, with higher mRNA levels in the brain, skeletal muscle, adrenal gland, uterus, and lymphocytes (Kitagawa and Paulson 1994; Grahn et al. 2002). In mouse, *St3gal3* gene expression was detected in all tissues tested, with highest expression in the brain, liver, heart, kidney, colon, skeletal muscle, and ovary (Kono et al. 1997; Ellies et al. 2002). Phylogenetic analysis reveals that the genes coding ST3Gal-III in vertebrates, from fish to man, share closest links to the ST3Gal-IV and ST3Gal-V families (Harduin-Lepers 2010).

Based on its relatively broad substrate specificity and tissue distribution, ST3Gal-III is likely to participate in biosynthesis of a range of glycoproteins and glycolipids. Its preference for Gal β 1-3(4)GlcNAc has led to suggestions that it is involved in the generation of the tumor-associated carbohydrate antigens sialyl-Le^a and sialyl-Le^x (Harduin-Lepers et al. 2012) and ST3Gal-III overexpression in pancreatic cancer cells enhanced their metastasis in an animal model (Perez-Garay et al. 2010).

Knockout Mouse and Transgenic Mice

Mice null for expression of ST3Gal-III were generated by targeting exon 2 (the intracellular, transmembrane, and part of the stalk region) with a *loxP*-flanked vector (Ellies et al. 2002). ES cells with the exon deleted by transient Cre expression were used to generate *St3gal3*-null mice, which were backcrossed onto a C57Bl/6 strain background. The mice are viable and fertile and displayed no significant defects in hematology or in E- or P-selectin binding. However, they were smaller than wild-type mice at weaning and displayed hindlimb dysreflexia characteristic of deficits in nerve conduction (Sturgill et al. 2012).

ST3Gal-III, in conjunction with ST3Gal-II, plays major roles in brain sialylation and function. In the mature brain, most sialic acid is on gangliosides – sialylated glycosphingolipids (Fig. 59.1) – with the rest on N- and O-linked glycoproteins (Schnaar 2004). Studies of brain sialoglycans in *St3gal3*-null mice (Sturgill et al. 2012) revealed a 33 % decrease in brain protein sialic acid (N- and O-linked combined) and no change in brain gangliosides. Further studies, however, implicated *St3gal3* in brain ganglioside biosynthesis as well.

In *St3gal2*-null mice, terminal ganglioside sialylation (GD1a and GT1b expression, Fig. 59.1) was reduced by half. In *St3gal2/St3gal3*-double-null mice, GD1a and GT1b were essentially absent, with GM1 and GD1b concomitantly increased (Sturgill et al. 2012). The double-null mice were half the size of their wild-type

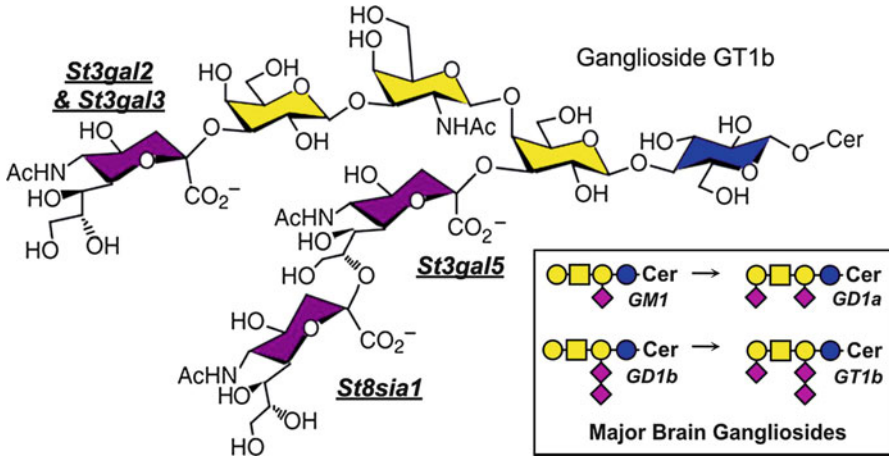


Fig. 59.1 The structure of the major brain ganglioside GT1b is shown, with the sialyltransferase genes responsible for addition of each of its three sialic acids indicated. *Inset*: The four major brain gangliosides that constitute the majority of sialic acid in the brains of all mammals are shown. Studies using sialyltransferase knockout mice indicate that ST3Gal-II and ST3Gal-III are responsible for adding the terminal sialic acid to GM1 and GD1b to form GD1a and GT1b, respectively (arrows)

siblings, short-lived (~ 8 weeks), and displayed profound dysreflexia at weaning. These data indicate that *St3gal3*, unexpectedly, is the major enzyme sialylating the Gal β 1-3GalNAc terminus on brain gangliosides in the absence of *St3gal2* and implicating *St3gal3* as a ganglioside biosynthetic enzyme.

St3gal3-null mice also had deficits in immune cell regulation, most probably due to changes in Siglec function. Siglecs are sialic acid-binding Ig-like lectins that are often expressed on immune cells where they regulate the immune response when they engage their sialoglycan counter-receptors on tissues (Crocker et al. 2007). In mice, Siglec-F (equivalent to Siglec-8 in humans) is expressed on allergic inflammatory cells (eosinophils), where it acts to downregulate and control allergic immune responses (Zhang et al. 2007). Siglec-F binds to sialoglycans on wild-type mouse airways on lung tissue sections, whereas it fails to bind to airways of *St3gal3*-null mice (Guo et al. 2011). These data implicate ST3Gal-III as the sialyltransferase responsible for biosynthesis of the sialoglycan counter-receptor for Siglec-F in the mouse lung and implicate this enzyme in the expression of immune regulatory glycans.

Human Disease

Intellectual disability is a disorder of cognitive impairment (intelligence quotient below 70) characterized by delayed acquisition of language and motor skills and significantly diminished intellectual abilities in adulthood, often due to

genetic aberrations (Ropers 2008). A search for genetic linkage in familial intellectual disability without other congenital anomalies (nonsyndromic autosomal recessive form, NSARID) in two families revealed two distinct single-nucleotide mutations in the *ST3GAL3* gene, one in the N-terminal transmembrane domain and one near the C-terminal (Hu et al. 2011). Whereas wild-type ST3Gal-III expressed in mammalian cells was localized in the Golgi, both single-nucleotide mutations (resulting in amino acid substitutions A13D and D370Y) resulted in mislocation of ST3Gal-III to the endoplasmic reticulum. Isolation of the mutant proteins revealed wild-type enzyme activity in the A13D form and profoundly diminished enzyme activity in the D370Y mutation. Since both failed to reach the Golgi, neither would be effective in sialylation of glycoproteins or glycolipids and affected individuals in the two families had similar severe disabilities (intelligence quotient 25–55, with 100 being average). This recent study implicates ST3Gal-III in brain development and function at the highest organizational levels.

Although increased or decreased expression of *ST3GAL3* has been related to various human cancers, and overexpression of ST3Gal-III resulted in increased metastasis of human pancreatic cancer cells injected in nude mice (Perez-Garay et al. 2010), ST3Gal-III expression has not been identified as an independent cancer marker for clinical use (Harduin-Lepers et al. 2012).

Future Perspectives

ST3Gal-III is involved in brain development and function based on its relatively high expression in the brain (Kitagawa and Paulson 1994; Kono et al. 1997; Grahn et al. 2002) and the recent discoveries of its role in human cognition and mouse nervous system function (Hu et al. 2011; Sturgill et al. 2012). Further study of brain sialoglycans, ST3Gal-III expression patterns, and nervous system development appear to be fruitful areas for future research.

The gene for ST3Gal-III is expressed at relatively high levels in many tissues. As specific functions of sialoglycans in cell-cell recognition emerge, as they did for Siglec-F counter-receptors, it will be fruitful to use the *St3gal3*-null mice, as well as clinical information from the rare individuals with ST3Gal-III deficiency, to link its expression to its functions in health and disease.

Cross-References

- ▶ [ST3 Beta-Galactoside Alpha-2,3-Sialyltransferase 2 \(ST3GAL2\)](#)
- ▶ [ST3 Beta-Galactoside Alpha-2,3-Sialyltransferase 4 \(ST3GAL4\)](#)
- ▶ [ST3 Beta-Galactoside Alpha-2,3-Sialyltransferase 5 \(ST3GAL5\)](#)
- ▶ [ST8 Alpha-N-Acetyl-Neuraminide Alpha-2,8-Sialyltransferase 1 \(ST8SIA1\)](#)

Further Reading

- Wen et al. (1992): Purification and cloning of ST3Gal-III and discovery of the first “sialyl motif”.
- Hu et al (2011): Loss of ST3Gal-III in humans is associated with severe intellectual disability.
- Sturgill et al. (2012): ST3Gal-III and ST3Gal-II are responsible for terminal sialylation of gangliosides in the brain.

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