Two Naturally Occurring \( \alpha_{2,6} \)-Sialyltransferase Forms with a Single Amino Acid Change in the Catalytic Domain Differ in Their Catalytic Activity and Proteolytic Processing

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The \( \alpha_{2,6} \)-sialyltransferase (ST) is a Golgi glycosyltransferase that adds sialic acid residues to glycoprotein N-linked oligosaccharides. Here we show that two forms of \( \alpha_{2,6} \)-sialyltransferase are expressed by the liver and are encoded by two different RNAs that differ by a single nucleotide. The ST tyr possesses a Tyr at amino acid 123, whereas the ST cys possesses a Cys at this position. The ST tyr is more catalytically active than the ST cys; however, both are functional when introduced into tissue culture cells. The proteolytic processing and turnover of the ST tyr and ST cys proteins differ dramatically. The ST cys is retained intact in COS-1 cells, whereas the ST tyr is rapidly cleaved and secreted. Analysis of the N-linked oligosaccharides of these proteins demonstrates that both proteins enter the late Golgi. However, differences in ST tyr and ST cys proteolytic processing may be related to differences in their localization, because ST tyr but not ST cys is expressed at low levels on the cell surface. The possibility that the ST tyr is cleaved in a post-Golgi compartment is supported by the observation that a \( 20 \) °C temperature block, which stops protein transport in the trans Golgi network, blocks both cleavage and secretion of the ST tyr.

It is now more widely appreciated that oligosaccharide structures of glycoproteins and glycolipids play pivotal roles in a number of biological processes (reviewed in Ref. 1). Sialylated terminal oligosaccharide structures are receptors for bacterial toxins, parasites, and viruses. In addition, these structures both mediate and modulate cell-cell and cell-matrix interactions (2–4). For example, sialylated and fucosylated terminal polylactosamine structures interact with selectin proteins to mediate a series of cell-cell interactions including those involved in inflammation and lymphocyte homing (4). In addition, increases in sialylation and branching of oligosaccharide structures expressed by cancer cells has been correlated with metastatic potential (4). In addition, several glycosyltransferases are involved in inflammation and lymphocyte homing (4). In addition, increases in sialylation and branching of oligosaccharide structures expressed by cancer cells has been correlated with metastatic potential (4).

In this work we describe a third mechanism that could control the sialylation of glycoproteins by different cell types. We have identified two forms of the ST, which differ in catalytic activity, proteolytic processing, and turnover. The high activity ST tyr form has a Tyr residue at amino acid 123 in the catalytic domain, whereas the low activity ST cys form has a Cys residue at this position. Only the ST tyr form is encoded by genomic DNA, whereas both forms are encoded by liver RNA. In addition, the ST cys form is retained in the Golgi for long periods of time, whereas the ST tyr form is cleaved and secreted from the cell. Initial experiments suggest that ST tyr cleavage is occurring in a post-Golgi compartment and that the difference in proteolytic processing and secretion of the ST tyr and ST cys may be related to differences in the localization of these two proteins.

EXPERIMENTAL PROCEDURES

Materials

Tissue culture media and reagents, including minimal essential medium, Dulbecco’s modified Eagle’s medium (DMEM), Lipofectin, Lipofectamine, and Geneticin (G418) and the SuperScript® II preamplification system were purchased from Life Technologies, Inc. Fetal bovine serum was obtained from Atlanta Biologicals (Norcross, GA). Male Sprague-Dawley rats were obtained from Harlan (Indianapolis, IN).
Sequencing enzyme and DNA was obtained from U. S. Biochemical Corp. (Cleveland, OH). RNAzol® and DNA STAT-60 were purchased from TelTest, Inc. (Friendswood, TX). QIAquick PCR Purification Kit was purchased from Qiagen Inc. (Chatsworth, CA). Vent DNA polymerase was purchased from New England Biolabs (Beverly, MA). Fluorescein isothiocyanate (FITC)-conjugated goat anti-rabbit IgG, all immobilized lectins (concanavalin A, wheat germ agglutinin, Erythrina cristagalli agglutinin, Limulus polyphemus agglutinin (LPA), and Limax flavus agglutinin) were purchased from EY Laboratories (San Mateo, CA). FITC-conjugated Sambucus nigra agglutinin (SNA) lectin was purchased from Vector Laboratories (Burlingame, CA). Protein A-Sepharose Fast Flow was purchased from Pharmacia Biotech, Inc. Protein molecular weight standards were purchased from Bio-Rad. Endo-ß-acteylgalactosaminidase H (Endo H) was purchased from Boehringer Mannheim.

**Methods**

**Genomic DNA and RNA Isolation, PCR Amplification, and Nucleic Acid Sequencing** Genomic DNA and tissue RNA isolations were performed using RNAzol® and STAT-60 according to the protocols provided by Tel-Test Inc. Reverse transcription-PCR was performed essentially according to the protocol provided in the SuperScript® II preamplification system (Life Technologies, Inc.). The oligonucleotides used for amplification of reverse transcribed RNA were 94 sense (TAT-GAGGCCCCTAATCAGT) and 943A antisense (GCCGGAGGATGGGGAATGGTGG). The oligonucleotides used for genomic DNA amplification were 94 sense (TATGAGGCCCTTACACTG) and 573A (GAATCTCCT-GACCAAACTCGGAGTT). An aliquot of each genomic PCR and reverse transcription-PCR products was analyzed by agarose gel electrophoresis. The rest of the reverse transcription-PCR products were incubated at 37 °C in the presence of RNase A for 3 h and purified by using QIAquick PCR Purification Kit (Qiagen) to remove oligonucleotide primers prior to sequencing. DNA sequencing was performed using the Sequenase, version 2.0 DNA Sequencing Kit (U. S. Biochemical Corp.) and sequencing primer 315 (TCTGCTGAGATCTCGGAGAAC).

**Transfections and Generation of Stably Expressing Cell Lines** COS-1 and Chinese hamster ovary (CHO) cells maintained in DMEM, 10% fetal bovine serum (COS-1), or minimal essential medium, 10% fetal bovine serum (CHO) were plated on 100-mm tissue culture dishes and grown in a 37 °C, 5% CO2 incubator until 50–70% confluent. Lipofectamine and 200 ml of lentin were incubated with Tris-buffered saline, 10 mM CaCl2 for 1 h at room temperature followed by four washes. Cells were visualized and photographed using a Nikon Axiophot microscope equipped with epifluorescence illumination and a 60X oil emersion Plan Apochromat objective.

**Sialyltransferase Forms Differing in Activity and Processing** We used internal sequencing as described previously (18). ST proteins were immunoprecipitated from both cell lysates and medium using the anti-ST antibody and protein A-Sepharose, and immunoprecipitated proteins were analyzed by SDS-polyacrylamide gel electrophoresis and fluorography (18). Bio-Rad prestained broad range gel standards were used to estimate molecular mass. G418-resistant colonies, 118 kDa; bovine serum albumin, 86 kDa; ovalbumin, 50.3–53.1 kDa; carbonic anhydrase, 33.3–34.1 kDa; soybean trypsin inhibitor, 29 kDa; lysozyme, 19.2 kDa; and aprotinin, 7.5 kDa.

Endo-ß-acteylgalactosaminidase H Digestions—Endo H digestions were performed, and Endo H-digested samples and untreated controls were analyzed by SDS-polyacrylamide gel electrophoresis and fluorography as described previously (16, 17).

**Lectin Affinity Chromatography**—Metabolically labeled ST proteins were immunoprecipitated as described above and analyzed by lectin chromatography according to the method of Low et al. (19). Immunoprecipitated proteins were eluted from protein A-Sepharose beads by boiling in 5 min in 100 µl of Tris-HCl, pH 7.5, 0.1% SDS followed by dilution to 200 µl with a 1:100 dilution of FITC-conjugated SNA in Tris-buffered saline, 10 mM CaCl2, 10 µg/ml of lentin, 10 µg/ml of lectin-conjugated agarose was incubated with above diluted immunoprecipitates for 2 h at room temperature. The agarose beads were washed three times with buffer A (25 mM Tris-HCl, pH 7.8, 500 mM NaCl, 0.5% Triton X-100, 1 mM MgCl2, and 1 mM MnCl2). 100 µl of a 50% slurry of lectin-conjugated agarose was incubated with above diluted immunoprecipitates for 2 h at room temperature. The agarose beads were washed three times with buffer A (25 mM Tris-HCl, pH 7.8, 500 mM NaCl, 0.5% Triton X-100, and 1 mM phenylmethylsulfonyl fluoride) and three times with buffer B (10 mM Tris-HCl, pH 7.8, and 150 mM NaCl). After washing, the bound material was eluted by boiling in 100 µl of SDS sample buffer (10% glycerol, 65 mM Tris, pH 6.8, 2% SDS, 0.15% bromphenol blue, 10% ß-mercaptoethanol) and analyzed by SDS-polyacrylamide gel electrophoresis and fluorography.

**RESULTS**

**Genomic DNA Encodes One ST Form, whereas Liver RNA Encodes Two ST Forms Differing by a Single Nucleotide**—Weinstein et al. (20) originally cloned two forms of the rat liver ST that differed in a single nucleotide and thus a single amino acid in the catalytic domain. The ST tyrosine possesses a Tyr (nucleotide sequence, TAC) at amino acid 123, whereas the ST cytos possesses a Cys (nucleotide sequence, TGC) at this position (Fig. 1). The sequencing of 10 random clones suggested that the ST tyrosine was the most predominant enzyme form (7 out of 10 clones) (20). To rule out the possibility that the ST cytos was an artifact generated during the amplification of the agt11 cdna library used for cloning, we analyzed both rat genomic DNA and rat liver RNA for the presence of these two ST forms. Genomic DNA and liver RNA were isolated from several rats, the RNA was reverse transcribed, and the ST sequences surrounding the altered nucleotide were amplified using the primers shown in Fig. 1. Direct sequencing of amplified DNA and reverse transcribed cDNA showed that only the ST tyros form was encoded by rat genomic DNA, whereas both the ST tyros and ST cytos forms were encoded by rat liver RNA (Fig. 2). In all PCR amplifications, no DNA bands were observed in control reactions lacking template DNA, suggesting that the PCR reagents were not contaminated with laboratory ST plasmids. In addition, we obtained similar results when PCR amplified fragments were subcloned into the Bluescript® (KS) plasmid (Stratagene) prior to sequencing (data not shown). In this case we used internal EcoRI and PstI sites for the subcloning (Fig. 1). The EcoRI site was originally mutated in all of the plasmid forms used in order to simplify subcloning. This insured that only amplified fragments from reverse transcribed RNA or genomic DNA could be cut with EcoRI and subcloned into the Bluescript® (KS) plasmid for sequencing.

2 J. Weinstein and E. U. Lee, personal communication.
Sialyltransferase Forms Differing in Activity and Processing

FIG. 1. ST tyr and ST cys sequences and primers used in analysis of genomic DNA and reverse transcribed RNA. The a2.6-ST gene consists of several exons (shown here as exons II–VI (EXII–EXVI)) with the enzyme’s coding sequence being found in exons II–VI. The single nucleotide change converting ST tyr (TAC) to ST cys (TGC) is found in the catalytic domain sequences encoded in exon II. The 94 sense and 943A antisense primers were used for amplification of reverse transcribed RNA. The 94 sense and 573A antisense primers were used for amplification of genomic DNA. DNA sequencing was performed with the 315 sense primer. In some experiments, amplified DNA was cloned into the Bluescript + (RS) plasmid using the EcoRI and PstI sites shown and DNA sequencing performed using the T7 primer.

FIG. 2. Rat genomic DNA encodes only ST tyr, whereas rat liver RNA encodes both ST tyr and ST cys. Following isolation from rat liver, genomic DNA in the area of interest was PCR amplified using the 94 sense and 573A antisense primers and directly sequenced using the 315 sense primer and the Sequenase enzyme (U. S. Biochemical). Rat liver RNA was reverse transcribed, and the resulting DNA was amplified using the 94 sense and 943A antisense primers prior to sequencing.

These results demonstrate that both the ST tyr and ST cys forms are naturally found in liver RNA and suggest that the ST cys form may be generated from the ST tyr form by a post-transcriptional event.

The ST tyr Protein Has a Higher Catalytic Activity than Does the ST cys Protein; however, Both Are Functional when Expressed in CHO Cells—To test whether this amino acid change altered enzyme activity, we analyzed the catalytic activity of both ST forms following expression in COS-1 cells. Lysates from cells transiently expressing either ST tyr or ST cys and mock transfected controls were assayed for ST activity using asialo-α1 acid glycoprotein as a substrate. We found that the cellular ST tyr form was approximately 5–6-fold more active than the ST cys form in two separate experiments (Table I). In each experiment, transfection efficiencies for the two forms were nearly identical ranging from 25 to 28%, eliminating the possibility that a low transfection efficiency for the ST cys form may be generated from the ST tyr form by a post-transcriptional event.

In addition, ST cys and ST tyr proteins that were partially purified from these expressing cells using CDP-hexanolamine agarose affinity chromatography also exhibited similar differences in catalytic activity (data not shown). We also observed similar differences in activity using asialofetuin, a more general substrate for ST tyr. Both ST tyr and ST cys proteins in CHO cells, and the expression of a2.6-sialyltransferases (21) (data not shown). This suggested that while being somewhat less active than the ST tyr form, the ST cys form did not differ dramatically from the ST tyr form in general substrate specificity. To determine whether both ST forms were functional in vivo, we stably expressed both the ST tyr and ST cys proteins in CHO cells, and the expression of a2.6-sialylated glycoconjugates was examined by staining cells with a FITC-conjugated SNA lectin that specifically recognizes a2.6-linked sialic acid (22, 23). We found that while wild type CHO cells that survived the G418 selection exhibited no staining with the SNA lectin, cells stably expressing ST tyr and ST cys both exhibited robust SNA staining, suggesting that both forms of the enzyme are catalytically active and functional in these cells (Fig. 3).

The ST cys Protein Is Retained in the Cell, whereas the ST tyr Is Rapidly Cleaved and Secreted—To investigate the possibility that this single amino acid change leads to differences in the transport and intracellular localization of the two ST forms, we expressed both proteins in COS-1 cells and analyzed their biosynthesis by pulse-chase analysis. COS-1 cells expressing either ST tyr or ST cys were metabolically labeled for 30 min and chased for up to 12 h with medium containing unlabeled amino acids. ST proteins were immunoprecipitated from both cell lysate and medium fractions and analyzed by SDS-polyacrylamide gel electrophoresis and fluorography. We found that the ST cys protein remains cell associated for up to 12 h of chase and even longer in other experiments (17), whereas the ST tyr protein is rapidly cleaved to a 42-kDa form and secreted into the cell medium with a half-time of 6 h in COS-1 cells and 3–4 h in CHO and HeLa cells (Fig. 4 and data not shown). We cannot rule out that the single amino acid change in the ST cys protein leads to a conformational change that prevents proteolytic cleavage; however, it is also possible that this single amino acid change leads to differences in the intracellular localization of these two forms and thus a difference in their access to proteases.

The ST tyr and ST cys Proteins Are Differently Localized in COS-1 Cells—To determine whether the ST tyr and ST cys are localized differently in cells, we analyzed their localization in COS-1 cells by indirect immunofluorescence microscopy using an affinity purified polyclonal anti-ST antibody (Fig. 5). Initial examination of ST expressing cells suggested that both ST forms were predominantly localized in the perinuclear Golgi region (Fig. 5, top panels, ST tyr and ST cys). However, closer examination of expressing cells demonstrated differences in the localization of these two forms. In overexpressing cells, the ST cys protein was also found in the endoplasmic reticulum as has been observed for other overexpressed glycosyltransferases (Fig. 5, bottom panel, ST cys) (24, 25). In contrast, the
ST tyr form exhibited light cell surface staining, suggesting that a portion of this ST form may be transported past the Golgi to the cell surface (Fig. 5). Previous experiments have demonstrated that the Asn-linked oligosaccharides of the ST expressed in rat liver or in COS-1 cells are never completely processed to Endo H resistant, complex forms (15, 17). Therefore, the low level of Endo H-resistant oligosaccharides observed on these proteins expressed in COS-1 cells was not surprising. These results suggested that ST cys was not greatly delayed in its endoplasmic reticulum to Golgi transport relative to ST tyr and that very slow movement though the secretory pathway or retention in the cis Golgi could not account for its lack of proteolytic processing.

The Processing of the Complex Oligosaccharides of the ST tyr Form Is Similar to Those of the ST cys Form and Suggests That Both Enzyme Forms Are Transported to the Late Golgi—To determine whether differences in the middle to late Golgi localization of the ST cys and ST tyr forms can explain differences observed in their proteolytic processing, we further examined the terminal processing of the oligosaccharides present

**Fig. 3.** Both ST tyr and ST cys catalyze the addition of α2,6-linked sialic acid when expressed in CHO cells. CHO cells were transfected with ST tyr-pcDNA3neo or ST cys-pcDNA3neo using the Lipofectamine method. Stably expressing cells were selected in minimal essential medium, 10% fetal bovine serum containing 1 mg/ml G418. These mixed populations of cells were stained for α2,6-sialylated glycoconjugate expression using the FITC-conjugated SNA lectin and immunofluorescence staining visualized as described under “Experimental Procedures.” The top panels represent the SNA staining of ST tyr and ST cys expressing cells. The bottom panels represent the corresponding phase contrast micrographs of these cells. CHO cells surviving the selection procedure but not expressing either ST form are not stained with SNA-FITC. Magnification, ×750.

**Fig. 4.** The ST cys is retained intracellularly, whereas the ST tyr is rapidly cleaved and secreted. COS-1 cells transiently expressing either ST tyr-pSVL or ST cys-pSVL were labeled for 30 min with 100 μCi/ml 35S-Express protein labeling mix in methionine- and cysteine-free DMEM and chased for 1–12 h with DMEM, 10% fetal bovine serum. ST proteins were immunoprecipitated from both medium and cell lysates, and immunoprecipitated proteins were analyzed by SDS-polyacrylamide gel electrophoresis and fluorography (18). Protein molecular mass markers (Bio-Rad): 50.3 kDa, ovalbumin; 33.3 kDa, carbonic anhydrase.

**Fig. 5.** Localization of ST cys and ST tyr expressed in COS-1 cells. COS-1 cells transiently expressing either ST tyr or ST cys were for immunofluorescence microscopy as described previously (18). After 16 h of expression, cells were fixed with either −20 °C methanol (internal staining, panels A, C, and D) or with 3% paraformaldehyde (surface staining, panel B) prior to incubations with primary and secondary antibodies. No cell surface expression of the ST cys form was detected in overexpressing COS-1 cells (data not shown). Magnification, ×750.
on both ST forms using lectin chromatography. ST proteins were expressed in COS-1 cells, metabolically labeled for 1 h, and chased for 6 h as described above, and their binding to a series of agarose-conjugated lectins was analyzed (Fig. 7). We found that both the ST tyr and ST cys bound similarly to concanavalin A, which recognizes mannose residues in the chitobiose core of high mannose, hybrid, and complex oligosaccharides (Fig. 7, ConA) (36). A lesser amount of both ST forms bound to wheat germ agglutinin-agarose demonstrating that less than half of the ST proteins were modified by complex oligosaccharides containing terminal GlcNAc residues (37, 38). This was consistent with the results of the Endo H analysis (Fig. 6). Interestingly, a small amount of both ST forms bound to E. cristagalli agglutinin-agarose. This lectin specifically recognizes terminal Galβ1, 4GlcNAc (39), and suggests that some of each protein possesses complex oligosaccharides modified by the β1,4-galactosyltransferase, which is generally found in the trans Golgi and/or trans Golgi network of cells (40, 41). The secreted form of ST tyr bound only E. cristagalli agglutinin, and there was no detectable wheat germ agglutinin binding, suggesting that the ST tyr protein’s complex oligosaccharides were completely galactosylated prior to secretion. Interestingly, little to no terminally sialylated complex oligosaccharides were detected on either the ST tyr or ST cys forms expressed in COS-1 cells using either LPA or L. flavus agglutinin lectin affinity chromatography (Fig. 7, LPA, and data not shown) (42, 43). A similar analysis performed with the ST proteins synthesized in HeLa cells demonstrated that the complex oligosaccharides of both the ST cys and ST tyr proteins are sialylated to the same extent in these cells (data not shown). Taken together, these data suggest that both forms of the ST are capable of being transported to the late Golgi, where they are equally terminally glycosylated. These data eliminate the possibility that the ST cys form is retained in an earlier Golgi compartment and that this prevents its proteolytic cleavage.

The ST tyr Is Proteolytically Processed and Secreted from a Post-Golgi Compartment—The similar terminal processing of the complex oligosaccharides of the ST tyr and ST cys proteins demonstrated that they are not significantly different in their intra-Golgi localization. However, the presence of some ST tyr at the cell surface suggests that this protein is transported past the trans Golgi and trans Golgi network and may be cleaved in a post-Golgi compartment. To test whether the cleavage of the ST tyr takes place in a post-Golgi compartment, we used a 20 °C temperature block that has been shown to stop protein transport at the level of the trans Golgi network and associated vesicles (44–47). We labeled cells expressing either ST tyr or sp-ST, a soluble, secreted form of the enzyme (16), for 1 h at 37 °C and chased for 6 h at either 37 or 20 °C. After a 6-h chase at 37 °C, one-half of the ST tyr form was found in the medium, whereas nearly all of the sp-ST was found in the medium, consistent with previous results (Ref. 16 and Fig. 4). However, after a 20 °C chase, no ST tyr was observed in the medium, and notably no cleavage had taken place. In addition, sp-ST secretion was essentially blocked at 20 °C, demonstrating that secretory pathway transport was blocked at this temperature. These results suggest that ST tyr cleavage takes place in a post-Golgi compartment. Taken together with the observation that the ST tyr but not the ST cys is observed in small amounts on the cell surface, these results also suggest that the difference in proteolytic processing of the two ST forms is related to their differences in their intracellular localization and access to proteases.

DISCUSSION

Weinstein et al. (20) originally cloned two forms of the ST from rat liver α1t11 cDNA libraries. In this study we charac-
terize these two forms and demonstrate that they do exist in vivo and that they differ in catalytic activity, proteolytic processing, and secretion. Direct sequencing of rat liver genomic DNA and reverse transcription-PCR products demonstrate that both ST cys and ST tyr forms are encoded by rat liver RNA in vivo but that only the ST tyr form is detected in rat genomic DNA (Fig. 2). Analysis of the biosynthesis of the ST tyr and ST cys proteins revealed that the ST cys form remains intact within the cell for as long as 12–24 h of chase, whereas the ST tyr form is cleaved and secreted with a half-time of 6 h (Fig. 4). Analysis of the N-linked carbohydrate structures of the ST tyr and ST cys proteins demonstrate that the carbohydrate structures of both proteins are processed similarly and that some proportion of both proteins is transported to the late Golgi (Figs. 6 and 7). However, immunofluorescence microscopic localization of the two ST forms showed that the ST tyr but not the ST cys moves beyond the Golgi and is observed at low levels on the cell surface (Fig. 5). These subtle differences in the localization of the ST tyr and ST cys proteins suggest that differences in their localization may lead to the observed differences in their proteolytic processing. This idea is supported by the observation that the cleavage and secretion of the ST tyr is totally blocked at 20 °C, a temperature that has been shown to halt protein transport in the trans Golgi network and associated vesicles in several cell types (44–47) (Fig. 8). These results suggest that the ST tyr is cleaved in a post-Golgi compartment and are consistent with a model that predicts that the ST cys is completely retained in the Golgi whereas the ST tyr is able to move beyond the Golgi where it is proteolytically cleaved and secreted.

There are several ways the ST tyr and ST cys forms could be generated. First, these ST forms could be encoded by two different genes with a single nucleotide change. This possibility has been ruled out by our data (Fig. 2) and the data of Lau and colleagues who have demonstrated by Southern analysis that the ST is encoded by a single gene in mouse, rabbit, and humans (12). Second, the splicing of an alternative exon encoding the ST cys change could generate this form. This possibility has also been ruled out by the genomic mapping experiments of Svensson et al. (8) and O’Hanlon et al. (11). Third, allelic variation of a single gene could lead to the expression of two forms of the enzyme. This type of mechanism has been shown to determine the blood group ABO antigen(s) expressed by an individual (48). A fourth possibility is that the A → G change in the nucleotide sequence could be the result of a post-transcriptional event such as RNA editing (49).

RNA editing has been observed in plants, protozoa, and mammals (49). Mammalian substitution or modification editing falls into two categories: C to U and U to C editing as found in the apolipoprotein B mRNA (CAA → UAA) and the Wilms’ tumor susceptibility protein (WT1) mRNA (CUC → CCC), and A to I editing as found in the GluR-B subunit of the α-amin-3-hydroxy-5-methyl-4-isoxazolopropionate receptor, which mediates neurotransmission in the central synapses (49, 50). The replacement of a Gln (CAG) in one of the transmembrane regions of the GluR-B subunit with a Arg (CGG) leads to lower calcium ion permeabilities and ion conductance in receptor channels formed with this subunit. This change occurs as the result of modification of the A in the Gln codon to an I by an adenosine deaminase-like activity that requires specific intron sequences and secondary structure as well as specific sequences around the edited site (50, 51). Ultimately, the I is translated as a G leading to the amino acid change to an Arg.

The single amino acid change that differentiates the ST tyr from the ST cys form is the result of a single A to G change in the nucleotide sequence (TAC → TGC) and may result from a similar editing event.

Interestingly, Schneikert and Herscovics (52) cloned two naturally occurring forms of the mouse α1,2-mannosidase IB cDNA that differ at three nucleotide positions in their coding sequences and thus three amino acids in their catalytic domains. In each case, a T(U) in clone 16 is replaced by a C in clone 4. The change from a Phe at position 468 (TTC) in clone 16 to a Ser (TCC) at this position in clone 4 is sufficient to inactivate the enzyme (52). The other two amino acid changes do not inactivate the enzyme but do appear to alter its folding and transport since the corresponding soluble, protein A fusion proteins are not secreted well. Both clone 4 and clone 16 forms are observed in the RNA of inbred and outbred mouse strains (52), whereas only clone 16 appears to be encoded by genomic DNA. These changes in the mannosidase IB coding sequence could also be the result of a U to C RNA editing event, and if so, this would be the first example of this type of editing occurring at multiple locations in an RNA.

Soluble circulating forms of glycosyltransferases, as well as those found at the cell surface, are presumably catalytically inactive due to the lack of sugar nucleotide donors in the extracellular space. Several years ago, soluble forms of glycosyltransferases were detected in and purified from body fluids (53–59). More recently, expression of recombinant glycosyltransferases in tissue culture cells has again demonstrated that some of these enzymes are proteolytically cleaved and secreted (20, 60–65). How and why integral membrane glycosyltransferases are proteolytically processed to soluble forms is largely unstudied and it is unclear whether cleavage and secretion reflect enzyme turnover or actually create proteins that function as lectins in the circulation (58, 66). Our data demonstrate that the ST tyr protein is rapidly cleaved and secreted, whereas the ST cys protein is retained for longer times in the Golgi. Although the proteolytic processing of the ST tyr protein could control the release of sialic acid-specific lectin activity into the circulation, it is more likely that it regulates the Golgi residence time of this enzyme and has a greater impact on the efficiency of protein sialylation.

Work by Lammers and Jamieson (26) and Weinstein et al. 3 J. Lau, personal communication.

4 A. Herscovics, personal communication.
Asn63 and Ser64. The secreted form of the ST tyr is identical in age. They showed that prosomatostatin transport and proteolytic pathway (44, 46, 47, 71). Xu and Shields (71) used 20°C have lowered temperatures to block transport along the secretory pathway of the oligosaccharides of these proteins. Differences in the structural change induced by the single Tyr to Cys change in the catalytic domain of ST not only results in a small decrease in catalytic activity but also leads to differences in the intracellular localization of the ST cys and ST tyr proteins. This is consistent with previous work from our laboratory that showed that although the transmembrane domain plus flanking sequences and stem region are independent Golgi retention sequences, other luminal sequences, although not sufficient for retention, did appear to influence the efficiency of this retention process and the transport of proteins through the secretory pathway (17, 18).

Our results suggest that the ST tyr and ST cys proteins are both transported to a late Golgi galactosyltransferase containing compartment and that the ST tyr protein is capable of moving past this compartment and is found in small amounts on the cell surface. These observations suggest at least two possibilities. First, the ST cys could move only as far as the trans cisternae of the Golgi, whereas the ST tyr moves to the trans Golgi network where it is cleaved. Second, the ST cys could move as far as the trans Golgi network, whereas the ST tyr moves past the Golgi into late endosomes or prelysosomal compartments where it is cleaved. Although the appearance of small amounts of the ST tyr on the cell surface could indicate a surface cleavage event, this is unlikely because most proteins that are cleaved at the cell surface are found at much higher levels in this location (28). Consequently, the small amount of the ST tyr observed at the cell surface would reflect material that escaped cleavage and secretion. Because the terminal glycosyltransferases are found both in the trans Golgi and trans Golgi network of tissue culture cells (34, 35, 67–70), we cannot distinguish between these two possibilities by analysis of the oligosaccharides of these proteins. Differences in the intra-Golgi localization of these two ST forms will require future analysis by immunoelectron microscopy coupled with strategies to distinguish intact and cleaved ST tyr forms.

The proteolytic processing of the ST tyr form in a very late Golgi or post-Golgi compartment is supported by the results of a 20°C transport block experiment in Fig. 8. Several groups have lowered temperatures to block transport along the secretory pathway (44, 46, 47, 71). Xu and Shields (71) used 20°C block to determine the site of prosomatostatin proteolytic cleavage. They showed that prosomatostatin transport and proteolytic processing is blocked in the trans Golgi network of GH3 cells at 20°C. Here we used 20°C block to determine whether the ST tyr is cleaved in the Golgi. We found that the cleavage and secretion of ST tyr is totally blocked when COS-1 cells expressing this protein are chased for 6 h at 20°C. Because both the ST tyr and ST cys appear to reach the late Golgi and it is unlikely that a specific protease would be completely inhibited at 20°C, these results strongly suggest that the ST tyr ultimately moves beyond the late Golgi to a post-Golgi compartment where it is cleaved.

What are the roles of the ST tyr and ST cys proteins in cells? Sialyltransferase assays using conventional glycoprotein substrates (asialo-a1 acid glycoprotein and asialofetuin) and analysis of α2,6-sialylated glycoconjugate expression in CHO cells by SNA immunofluorescence analysis demonstrate that both the ST tyr and ST cys proteins are functional sialyltransferases and that the 5–6-fold lower catalytic activity of the ST cys form does not appear to dramatically limit its activity (Table I and Fig. 3). It is possible that differences in the catalytic activity of these two forms are equalized by differences in their residence times in the Golgi. However, it is equally possible that these two enzymes sialylate different glycoproteins and also differ in their efficiency leading to differences in the levels of sialylated glycoconjugates expressed by the cell. Another possibility is that the coexpression of the ST tyr and ST cys proteins in the same cell could lead to a longer residence time for the ST tyr in that cell. Oligomerization and kin recognition are lawful potential mechanisms for the retention of glycosyltransferases in the Golgi (72, 73). Preliminary experiments in our laboratory suggest that the ST does form insoluble oligomers in the low pH (6.4) of the late Golgi. In addition, coexpression of the ST cys and ST tyr forms in COS-1 cells does lead to decreased cleavage and an increased retention time for the ST tyr protein. The co-expression of the ST tyr and ST cys proteins in specific tissues may lead to the formation of mixed ST oligomers and result in an extended retention of the ST tyr in the late Golgi with the ST cys protein, thus preventing its rapid cleavage and secretion. We predict that coexpression of these two ST forms as well as their individual expression in different tissues and cell types could lead to a variety of expression/localization patterns and thus subtly control the ST activity levels in a cell.

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Sialyltransferase Forms Differing in Activity and Processing 679

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Two Naturally Occurring α2,6-Sialyltransferase Forms with a Single Amino Acid Change in the Catalytic Domain Differ in Their Catalytic Activity and Proteolytic Processing

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