Caveolin-1 Regulates the Functional Localization of N-Acetylgalcosaminyltransferase III within the Golgi Apparatus*

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In an investigation of the mechanism underlying the functional sublocalization of glycosyltransferases within the Golgi apparatus, caveolin-1 was identified as a possible cellular factor. Caveolin-1 appears to regulate the localization of N-acetylgalcosaminyltransferase III (GnT-III) in the intra-Golgi subcompartment. Structural analyses of total cellular N-glycans indicated that the overexpression of GnT-III in human hepatoma cells, in which caveolin-1 is not expressed, failed to reduce branch formation, whereas expression of caveolin-1 led to a dramatic decrease in the extent of branching with no enhancement in GnT-III activity. Because the addition of a bisecting GlcNAc by GnT-III to the core β-Man in N-glycans prevents the action of GnT-IV and GnT-V, both of which are involved in branch formation, this result suggests that caveolin-1 facilitates the prior action of GnT-III, relative to the other GnTs, on the nascent sugar chains in the Golgi apparatus and that GnT-III is redistributed in the earlier Golgi subcompartment by caveolin-1. Indeed, when caveolin-1 was expressed in human hepatoma cells, it was found to be co-localized with GnT-III, as evidenced by the fractionation of Triton X-100-insoluble cellular membranes by density gradient ultracentrifugation. Caveolin-1 may modify the biosynthetic pathway of sugar chains via the regulation of the intra-Golgi subcompartment localization of this key glycosyltransferase.

N-Acetylgalcosaminyltransferase III (GnT-III) is one of the Golgi-resident glycosyltransferases that catalyzes the transfer of GlcNAc from an UDP-GlcNAc to a core β-mannosyl residue of asparagine-linked glycans (N-glycans) via a β1,4-linkage, resulting in the formation of a unique structure, a bisecting GlcNAc (1). Oligosaccharides containing a bisecting GlcNAc (bisected oligosaccharides) do not serve as substrates for GnT-IV and GnT-V, which catalyze the formation of a β1,4-branched at an α1,3Man and a β1,6-branched at an α1,6Man, respectively (2, 3). We previously reported that the action of GnT-V was strictly inhibited by the addition of a bisecting GlcNAc at the catalytic step (4). Therefore, the action of GnT-III prior to these enzymes in the Golgi leads to an inhibition of the biosynthesis of multiantennary oligosaccharides. Indeed, the ectopic expression of GnT-III caused an effective reduction in multiantennary oligosaccharides in various types of cells (5, 6). For example, in the case of swine endothelial cells, the expression of GnT-III led to a dramatic decrease in multiantennary oligosaccharides, accompanied by a marked increase in bisected biantennary ones (6). However, there are exceptions, in which a high GnT-III activity did not lead to a decrease in branching. In the case of human hepatoma cells, the overexpression of GnT-III resulted in a dramatic increase in bisected oligosaccharides but did not cause a decrease of the number of antennae (7). It would be expected that GnT-IV and GnT-V act prior to GnT-III in HepG2 cells, because the activities of GnT-III, GnT-IV, and GnT-V are essentially the same level in both types of cells and because GnT-IV and GnT-V are unable to act on the bisected sugar chains, a product of GnT-III. Namely, if there are fundamental differences in the order of GnT-III action between both types of cells, then a regulating mechanism must be operative that is responsible for the prior action of GnT-III in swine endothelial cells.

As long as the final products of oligosaccharide structures are mediated by the sequential action of glycosyltransferases, the order of their action should be, at least in part, regulated by their sublocalization within the Golgi apparatus. It has been suggested that the “CTS” region (cytoplasmic tail, transmembrane domain, and stem region) of the glycosyltransferases plays a role in its intra-Golgi functional localization (8). However, the cell type-specific regulation of oligosaccharide biosynthesis, observed in swine endothelial cells and hepatoma cells, indicates that the functional localization of glycosyltransferases cannot be explained only by the CTS theory. It is possible that this regulation involves cellular factors other than glycosyltransferases.

Here, we hypothesize that caveolin-1 is a candidate molecule that plays a role in the functional localization of GnT-III within the subcompartment of the Golgi apparatus, because it is well known that normal liver expresses a negligible amount of caveolin-1 mRNA (9) and that most hepatoma cells do not express caveolin-1 (10), in contrast to its high expression in many endothelial cells, including swine endothelial cells. It is also known that caveolin-1 is localized not only on the cytoplasmic surface of the plasma membrane (11) but also at the Golgi apparatus (12), where many glycosyltransferases function. Therefore, it is likely that caveolin-1 may function as a regulating factor for the functional localization of Golgi-resident enzymes.

To evaluate this hypothesis, we prepared stable transferrants that express GnT-III and caveolin-1, and the oligosaccharide structures produced were analyzed. As a result, we...
found that the expression of caveolin-1 leads to a dramatic decrease in the extent of branching of N-glycans because of the addition of a bisecting GlcNAc, a product of GnT-III, without the activation of GnT-III activity, suggesting that caveolin-1 regulates the functional localization of GnT-III, thereby modifying N-glycan biosynthesis. The present findings provide new information concerning the mechanism that controls the intracellular functional sublocalization of glycosyltransferases and the organization of their actions in the oligosaccharide biosynthetic pathway.

EXPERIMENTAL PROCEDURES

MATERIALS—Glycosidases were obtained from the following sources: sialidase (Arthrobacter ureafacies) from Nacarai tesque, β-galactosidase (jack bean) from Seikagaku Co., and α-fucosidase (bovine kidney) from Sigma. UDP-GlcNAc and GlcNAc were obtained from Sigma. Pyridylaminated (PA) oligosaccharides standards were purchased from Takara. Antibodies were obtained from the following sources: anti-caveolin-1 polyclonal antibody from Santa Cruz, anti-GnT-III monoclonal antibody from FUJIREBIO Inc., horseradish peroxidase-conjugated anti-rabbit IgG antibody from ICN Biomedicals Inc. Biotin-labeled lectins were obtained from Seikagaku Co. Restriction endonucleases and DNA modifying enzymes were purchased from Takara, Toyobo, or New England Biolabs. Oligonucleotide primers were synthesized by Greiner. Other common chemicals were obtained from Wako Pure Chemicals.

Plasmid Construction—A full length of human caveolin-1 DNA (9) was amplified by reverse transcription-PCR from human umbilical vein endothelial cells. PCR was performed with the primers 5'-ACACGAA-TTCTGTCGAGGGCAACAAA-3' and 5'-ACCGGAAATCTTATATTTCTT-TCTGC-3' to introduce the EcoRI site at both terminal ends. The amplified fragment was subcloned into the EcoRI site of a pCXN-II expression vector (13), a generous gift from J. Miyazaki (Osaka University), and the resulting plasmid is referred to as pCXN-Cav. The absence of nucleotide misincorporation during PCR was verified by analysis of the nucleotide sequence.

Cell Culture—Huh6 cells were grown and maintained at 37 °C in Dulbecco's modified Eagle's medium containing 4.5 g/liter of glucose (Nikkon) supplemented with 10% fetal calf serum (Invitrogen), 50 units/ml penicillin G, and 50 μg/ml streptomycin under a humidified atmosphere of 95% air and 5% CO2.

Establishment of Stable Cell Lines—Huh6 cells were transfected with pCXN-Cav and pCXN-GnT-III, an expression plasmid for hGnT-III (14), by LipofectAMINE Plus (Invitrogen) according to the manufacturer's instructions. Stable transfectedants were selected in a complete medium containing 300 μg/ml G418. The surviving cell colonies were isolated after 10 days.

Transient Expression—Expression plasmids were transfected into Huh6 cells by electroporation (15) using a Gene Pulser (Bio-Rad). The cells were washed with HEPES-buffered saline and resuspended. Plasmids (20 μg) purified by CsCl gradient ultracentrifugation were added to the cell suspension (4 × 107), followed by electroporation. The transfected cells were subjected to biochemical analyses 48 h after transfection.

SDS-PAGE, Immunoblotting, and Lectin Blot Analysis—Cell monolayers were washed twice in ice-cold phosphate-buffered saline and then scraped into ice-cold lysis buffer (50 mM HEPES, pH 7.5, 150 mM NaCl, 10% (v/v) glycerol, 1% Triton X-100). SDS-PAGE was carried out according to Laemmli (16). The separated proteins were electrophoretically transferred to a PROTRAN (Schleicher & Schuell), followed by blocking with 5% skim milk for immunoblotting or 2% bovine serum albumin for lectin blot analysis. The resulting membrane was incubated with the first antibody or biotin-labeled lectin. After washing, the membrane was reacted with the appropriate second antibody for immunoblotting or with a horseradish peroxidase-avidin complex (Vector Co.) for the lectin blot. The reactive signals were visualized by chemiluminescence using an ECL system (Amersham Biosciences).

Immunoprecipitation—Equivalent amounts of lysate were pre-cleared with protein A-Sepharose (Amersham Biosciences) for 1 h at 4 °C and then immunoprecipitated with protein A-Sepharose conjugated to an anti-caveolin-1 antibody for 3 h at 4 °C. The immunoprecipitates were then washed five times with ice-cold lysis buffer. The resulting samples were subjected to Western blotting as described above.

Glycosyltransferases Activity Assay—GnT-III, -IV, and -V activities were assayed using fluorescence-labeled oligosaccharide acceptors, as described previously (17). As acceptors, the PA-agalacto-biantennary oligosaccharide was used for the reactions of GnT-III and GnT-V, and the PA-agalacto-triantennary oligosaccharide, a product of the reaction of GnT-V, was used for the assay of GnT-IV. Cell homogenates (5 μg of proteins) were incubated at 37 °C for 6 h in the appropriate reaction buffer (17) containing 10 μM acceptor and 40 μM UDP-GlcNAc as a donor. The reaction was terminated by boiling, and the sample was then centrifuged at 15,000 rpm for 5 min in a microcentrifuge. The resulting supernatant was analyzed by reverse phase HPLC (Shimadzu) using TSKgel ODS-80 (4.6 × 150; Tosoh). The solvent used was a 20 mM pH 4.0 ammonium acetate buffer, and the substrate and the product were separated isocratically. Fluorescence was detected with a fluorescence detector (RF-10AXL; Shimadzu) at excitation and emission wavelength of 320 and 400 nm, respectively.

Isolation, Labeling, and Characterization of the N-Glycans—The crude cellular membrane fraction was isolated by ultracentrifugation. Briefly, cells (2 × 108) were homogenized in phosphate-buffered saline and centrifuged at 10,000 × g for 10 min at 4 °C to remove cellular debris and nuclei. The resulting supernatants were further centrifuged at 100,000 × g for 1 h at 4 °C. The pellets were resuspended and sonicated in 1 ml of phosphate-buffered saline. N-Glycans were isolated from the resulting membrane fraction, then labeled, and characterized as described by Ihara et al. (7). Briefly, the free oligosaccharides were labeled with [1-14C]NaBD4 for 10 h as described by Hase et al. (18). The resulting oligosaccharides were reductively aminated with the fluorescent reagent, 2-aminoypyridine, and the PA-derivatives were separated by gel filtration on an HW-40 (Toyopearl; Tosoh) column equilibrated with 10 mM NH4HCO3 (19). The PA sugar chains were treated with sialidase, β-galactosidase, and α-fucosidase. The digested PA sugar chains were analyzed by reversed phase HPLC (Shimadzu) using a TSKgel ODS-80TM column (4.6 × 150 mm; Tosoh). The solvent used was a 20 mM ammonium acetate buffer (pH 4.0) containing 0.1–0.25% butanol. Various PA sugar chains were used as standards.

Incorporation of Caveolin-1 into a High Molar Weight Complex—The cells were removed by scraping in the presence of a 20 mM Tris, 150 mM NaCl buffer (pH 9.0) supplemented with 1% Triton X-100 and 80 μM octylglucoside and incubated on ice for 30 min. After removal of cellular debris and nuclei by centrifugation at 15,000 rpm for 10 min at 4 °C, the supernatant material was loaded on the top of a linear 5–30% sucrose gradient and centrifuged for 16 h at 34,000 × g in a Beckman TL100 rotor. The fractions were collected from the top of the gradient, and the protein was precipitated by the addition of trichloroacetic acid. The precipitates were resuspended in SDS-PAGE sample buffer.

Fraction of Triton X-100-insoluble Membranes—Each dish of cells was washed in ice-cold MES buffer (25 mM MES, pH 6.5, 150 mM NaCl) and then scraped from the dish into 150 μl of MES buffer supplemented with 1% Triton X-100. The cells were further incubated on ice for 20 min before homogenizing the sample with a homogenizer. The homogenates were transferred to ultracentrifuge tubes and mixed with an equal volume of 2.5 mM sucrose. The sample was then overlaid with a 10–30% linear sucrose gradient and centrifuged for 16 h at 30,000 × g in a Beckmann TL100 rotor. The fractions were collected from the top of the gradient, and the total protein in each fraction was precipitated with trichloroacetic acid. The precipitates were then resuspended in SDS-PAGE sample buffer.

Protease Protection Assay—A protease protection assay was performed as previously described (20, 21). Briefly, cells from a 100-mm cell culture dish were collected in 1 ml of buffer consisting of 250 mM sucrose, 20 mM Tricine, and 1 mM EDTA (pH 7.0) and homogenized. The nuclear cell debris were removed by centrifugation at 1,000 × g for 10 min at 4 °C. The supernatant fraction was centrifuged by 1 h at 100,000 × g in a Beckmann TLA-45 rotor. The pellet was resuspended in 100 μl of a buffer consisting of 100 mM sodium phosphate (pH 7.4), 150 mM NaCl, 4 mM KCl, 2 mM MgCl2, and 0.02% sodium azide and transferred to microtubes. Each sample was then incubated for 15 min on ice in buffer alone, 100 μg/ml trypsin, or trypsin plus 1% Triton X-100. Each sample was resuspended in SDS-PAGE buffer and analyzed.

Protein Determination—The protein concentrations were determined according to the method of Bradford using bovine serum albumin as a standard (22).

RESULTS

To evaluate the effect of caveolin-1 on GnT-III activity and on N-linked oligosaccharide biosynthesis, several lines of transfor-
mants were established using human hepatoma Huh6 cells, which do not endogenously express caveolin-1 (10). The absence of caveolin-1 was confirmed by an immunoblot analysis (Fig. 1A) and reverse transcription-PCR (data not shown). On the other hand, Huh6 cells have GnT-III activity, albeit at low level (Fig. 1B), but GnT-III protein could not be detected by an immunoblot analysis probably because of its low expression (Fig. 1A). In Huh(GIII) cells, which were established by transfection with the GnT-III expression plasmid, the GnT-III activity was 60 times higher than that in parental Huh6 cells, whereas the activities of other GnT enzymes examined were nearly the same (Fig. 1). In conjunction with the increased activity, the GnT-III protein was increased to a detectable level in the immunoblot analysis. By the transfection of Huh6 cells with a caveolin-1 expression plasmid, three clones of transfants, Huh(cav) cells, were also established, as shown in Fig. 1A. The GnT-III activity in the Huh(cav) cells was decreased only slightly, compared with its activity in the parental cells, and the activities of GnT-IV and GnT-V were variable regardless of the expression of caveolin-1 (Fig. 1B), probably because of clonal selection. This suggests that caveolin-1 does not have great effect on the expression and activities of these glycosyltransferases.

Using these stable transformants, the functional consequence of caveolin-1 expression on oligosaccharide structures was investigated. Lectin blot analyses were first carried out using erythroagglutinating phytohemagglutinin (E4-PHA), leukoagglutinating phytohemagglutinin (L4-PHA), and wheat germ agglutinin (WGA) lectins. As shown in Fig. 2A, all three Huh(cav) cell clones reacted with E4-PHA, which preferentially binds to bisected oligosaccharides, to a level comparable with that of Huh(GIII) in which GnT-III is overexpressed, despite no increase in GnT-III activity. In the case of lectin blotting using wheat germ agglutinin, however, the staining pattern in the Huh(cav) cells differed from that in the Huh(GIII) cells (Fig. 2B). This clearly shows that caveolin-1 causes structural alterations of oligosaccharides that are distinct from the case of the increased activity of GnT-III, as found in the Huh(GIII) cells, although the levels of bisecting GlcNAc are increased in both type of transfectants. As shown by the L4-PHA blotting results (Fig. 2C), no significant difference was detected among the parental Huh6 cells, Huh(cav) and Huh(GIII), although GnT-V activities were varied (Fig. 1B).

For further evaluation, pyridylaminated forms of total N-glycans were also prepared from the cells and then analyzed with respect to core structures by reversed phase HPLC. The elution profiles of the pyridylaminated oligosaccharides from parental Huh6, Huh(cav), and Huh(GIII) cells are shown in Fig. 3. In the case of Huh(cav) cells, clone 3 was selected, because the enzyme activities of GnT-IV and GnT-V were nearly equal to those of parental Huh6 and Huh(GIII). Because biantennary and tetra-antennary nonbisected sugar chains were not separated under the chromatographic conditions used, the overlapping peaks were collected and reseparated under different conditions, i.e. isocratically (data not shown). The relative amounts of the eight possible structures of the core are summarized in Fig. 4. The overexpression of GnT-III increased the amount of whole bisected oligosaccharides to al-
most two times as much, consistent with the increase in enzyme activity (Fig. 4A), but the ratio of the oligosaccharides with multiantennae, triantennae, and tetra-antennae relative to the total sugar chains remained unchanged (Fig. 4B). These multiantennary structures suggest that GnT-III acted after GnT-IV and GnT-V. On the other hand, caveolin-1 expression led to an increase in the levels of the bisected oligosaccharides (Fig. 4A), although GnT-III activity was not increased (Fig. 1B). In contrast to the case of GnT-III overexpression, moreover, the formation of multiantennary structures with the bisecting GlcNAc was significantly inhibited (Fig. 4C). In the Huh(cav) cells, the bisected tetra-antennary structure was not detected (Figs. 3B and 4C), and more than 75% of the bisected oligosaccharides were biantennary sugar chains (Fig. 4C). As shown in earlier substrate specificity studies (2, 3) or kinetic analysis (4), it has been reported that GnT-IV and GnT-V are unable to act on bisected sugar chains \textit{in vitro}, whereas GnT-III is capable of reacting with any types of chain, \textit{i.e.} bi-, tri-, or tetra-antennary. Therefore, these marked alterations in the structural profiles provide reasonable evidence to indicate that caveolin-1 allows the prior action of GnT-III on the nascent oligosaccharides relative to other GnTs, which serve as common acceptor substrates in the Golgi apparatus. It is likely that caveolin-1 regulates the subcompartment distribution of GnT-III within the Golgi apparatus.

As described above, it was found that caveolin-1 affects the ultimate oligosaccharide structure without any significant alteration in the enzyme activities of glycosyltransferases. To assess a mechanism underlying the regulation of the oligosaccharide biosynthesis by caveolin-1, the interaction between GnT-III and caveolin-1 was examined. Huh cells endogenously expressed GnT-III activity, but the protein level was much lower than the detectable level in the immunoblot analysis, as shown in Fig. 1. Probably because of this low protein level, co-immunoprecipitation of endogenous GnT-III protein with caveolin-1 was not successful (Fig. 5A, lane 1). Hence, GnT-III was transiently transfected in Huh(cav) cells to analyze their association.

As a result, GnT-III was found to be co-immunoprecipitated with caveolin-1. Although a small amount of GnT-III was also precipitated with a control antibody (Fig. 5A, lane 4) or beads alone (data not shown), probably because of nonspecific binding, the amount of GnT-III in the precipitate with the anti-caveolin-1 antibody was clearly increased (Fig. 5A, lane 3), indicating that GnT-III is potentially associated with caveolin-1 \textit{in vivo}. Furthermore, the interaction of GnT-III with caveolin-1 was examined using Chinese hamster ovary cells, which endogenously express caveolin-1. In the Chinese hamster ovary cells transiently transfected with GnT-III, essentially the same result was obtained as in the GnT-III/caveolin
double transfected Huh cells (data not shown). These results further support the suggestion that GnT-III is associated with caveolin-1 within cells.

Caveolins are thought to be associated with membranes via a central 33-amino acid hydrophobic domain. This allows both the N- and C-terminal domains to remain entirely on the cytoplasmic side (23). On the other hand, the large catalytic domain of GnT-III is retained in the luminal region, and therefore, it would be expected that complex formation between GnT-III and caveolin-1 is due to an indirect association. However, it was reported that caveolin-1 protein moved into the Golgi lumen by cholesterol oxidation (20) and that GnT-III contains three binding motifs for caveolin-1 in its luminal region (24). This suggests the possibility that caveolin-1 could bind directly to caveolin-1 within the Golgi lumen. To determine the topology of GnT-III and caveolin-1 and to examine the issue of whether GnT-III is associated with caveolin-1 directly or indirectly, GnT-III was transiently expressed in Huh(cav) cells.

The transfected cells were then gently homogenized in an isotonic buffer to preserve the integrity of intracellular organelles and treated with trypsin as described under “Experimental Procedures.” As a result, caveolin-1 was digested with trypsin without the addition of detergents, although Golgi lumen-resident GnT-III was resistant to protease treatment, indicating that they are in a different topology (Fig. 5B). This finding suggests that caveolin-1 and GnT-III are associated indirectly over the Golgi membranes.

To investigate how the complex between GnT-III and caveolin-1 is formed over the Golgi membrane, fractionation by sucrose density gradient ultracentrifugation of the Triton X-100-insoluble membranes was carried out. As shown in Fig. 6A, in the case of mock transfectant of Huh(cav) cells, caveolin-1 was detected only in the Triton X-100-insoluble light membrane fraction. On the other hand, in the case of Huh(cav) cells transiently expressing GnT-III, a substantial fraction of caveolin-1 was not incorporated into the Triton X-100-insoluble light membranes in the GnT-III-transfected Huh(cav) cells but was found to be co-localized with GnT-III. These results suggest that caveolin-1 is capable of forming a complex with GnT-III in the Golgi fraction. On the other hand, such an incorporation of caveolin-1 into the Golgi fraction was not observed in GnT-V-overexpressing Huh(cav) cells, although another GnT, GnT-V, was detected in Golgi fraction (data not shown). Therefore, the complex formation with caveolin-1 seems to be specific to GnT-III.

**Fig. 4. Comparison of structural profiles of sugar chains.** Relative amounts of core structures of complex type sugar chains are shown for parental Huh6, Huh(GIII), and Huh(cav) cells. A, the ratio of bisected (striped bars) and nonbisected oligosaccharides (open bars) is indicated. The total amount of oligosaccharide is 100%. B, relative amounts of total bi- (closed bar), total tri- (striped bar), and total tetra-antennary (open bars) oligosaccharides are shown. The total amount of oligosaccharide is 100%. C, relative amounts of bi- (closed bars), tri- (striped bars), and tetra-antennary (open bars) oligosaccharides with the bisecting GlcNAc are shown. The rate of total bisected oligosaccharides (not including nonbisected oligosaccharides) is 100%.

**Fig. 5. Association and topology of GnT-III and caveolin-1.** A, GnT-III was transiently expressed in Huh6 cells (lane 2) and Huh(cav) cells (lanes 3 and 4). The lysates from resulting cells were immunoprecipitated (IP) with an anti-caveolin-1 antibody (lanes 1–3) or control antibody (lane 4). Co-precipitating proteins were detected by immunoblotting with anti-GnT-III antibody (middle panel) and anti-caveolin-1 antibody (lower panel). The initial amount of GnT-III was also indicated (upper panel). B, protease protection assay was performed as described under “Experimental Procedures.” The samples were separated by SDS-PAGE and immunoblotted (IB) with the indicated antibodies.
Although multiantennary sugar chains may be synthesized via several different pathways, the bisecting GlcNAc structure, a reaction product of GnT-III, is capable of inhibiting certain steps during their assembly and of reducing branching (4, 28–30). Therefore, the predominance of multiantennary oligosaccharides in the bisected species in parental and GnT-III-overexpressing Huh6 cells suggests that GnT-IV and GnT-V are localized at the earlier subcompartment in the Golgi apparatus relative to GnT-III. On the other hand, the increased levels of the bisected biantennary sugar chain in caveolin-1-transfected cells indicate that caveolin-1 facilitates the prior action of GnT-III on nascent oligosaccharides during their assembly, and therefore, it is reasonable that GnT-III may well be relocated to an earlier subcompartment in the Golgi, as the result of caveolin-1 expression. Thus, the structural characterization of oligosaccharides would be useful for determining the relative positions of glycosyltransferases in a biosynthetic pathway in cells, whereas immunohistochemical methods do not provide any information concerning the in vivo functional activity of glycosyltransferases.

Because deletion mutants, which contain CTS regions alone, could be localized at the Golgi apparatus (31–33), the CTS regions function as Golgi retention signals for various glycosyltransferases (34). Furthermore, because the substitution of CTS regions for those of other glycosyltransferases resulted in different ultimate oligosaccharide structures because of an alteration in subcompartment distribution (8), the CTS regions of glycosyltransferases play important roles as intrinsic signals for regulating their distribution in the Golgi subcompartment. However, cell type-specific regulation of oligosaccharide biosynthesis cannot be explained only by the CTS theory. It is most likely that the cellular factors other than glycosyltransferases are responsible for this regulation, and in the present study, it is demonstrated that caveolin-1 represents one such regulating factor in the case of GnT-III action.

Oligosaccharides on glycoproteins are assembled by the sequential action of various glycosyltransferases during their transport from the endoplasmic reticulum to the trans-Golgi network (35, 36). Thus, the order of action of glycosyltransferases, which may be associated with their sublocalization in the Golgi, as well as their expression levels and activities, would be a significant factor in determining the ultimate structures of cellular oligosaccharides. Therefore, the identification of cellular factors, which are involved in the functional localization of individual glycosyltransferases, is an important issue for understanding oligosaccharide biosynthesis. Such factors would probably function to organize the actions of various glycosyltransferases and contribute to the formation of cell type-specific oligosaccharide structures.

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