The cytoplasmic tail of α1,3galactosyltransferase inhibits Golgi localization of the full length enzyme

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Running Title: Mechanisms of Golgi localization of Glycosyltransferases

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It is currently under debate whether the mechanism of Golgi retention of different glycosyltransferases is determined by sequences in the transmembrane, luminal, or cytoplasmic domains or a combination of these domains. We have shown that the cytoplasmic domains of $\alpha$1,3galactosyl- (GT) and $\alpha$1,2fucosyl- (FT) transferases are involved in Golgi localization. Here we show that the cytoplasmic tails of GT and FT are sufficient to confer specific Golgi localization. Further, we show that the expression of only the cytoplasmic tail of GT can lead to displacement or inhibition of binding of the whole transferase and that cells expressing the cytoplasmic tail of GT were not able to express full length GT or its product, Gal$\alpha$(1,3)Gal. Thus, the presence of the cytoplasmic tail prevented the localization and function of full length GT, suggesting a possible specific Golgi binding site for GT. The effect was not altered by the inclusion of the transmembrane domain. Although the transmembrane domain may act as an anchor, these data show that, for GT, only the cytoplasmic tail is involved in specific localization to the Golgi.

Proteins and lipids are transported through the endoplasmic reticulum (ER) and Golgi where carbohydrates are sequentially added by glycosyltransferase enzymes. The glycosyltransferases are considered to reside in the Golgi in a spatial configuration which allows the sequential specific addition of carbohydrates (1), although the mechanisms governing their ordered localization and retention is not clear. Glycosyltransferases are type II integral membrane proteins, with a C-terminal catalytic domain in the lumen of the Golgi and an N-terminal cytoplasmic tail (2). Two mechanisms of glycosyltransferase localization in the Golgi have been suggested: - kin-recognition (3), where like proteins form aggregates within the Golgi and the lipid bilayer model (4), where the length of the hydrophobic transmembrane domain retains the
transferases. However, neither mechanism fully explains the localization of transferases to specific sites within the Golgi. Thus far, no amino acid consensus sequence for localization has been described, and there is difficulty in competitively saturating the glycosyltransferase retention mechanisms (5). We and others have proposed that a third mechanism of retention involving the cytoplasmic domain of proteins resident in the Golgi. Indeed, there is a growing body of evidence implicating the cytoplasmic domain of Type I (6) and Type II resident Golgi proteins (7-11), coiled-coil proteins (12,13) and viral proteins (14-17). One of the most convincing studies showed that myc- and Flag- tags placed at the N-terminus of the cytoplasmic tails of α2,6-sialyltransferase and N-acetylglucosaminyltransferase I disrupted Golgi localization (18). A 20 amino acid spacer between the tag at the N-terminus and the cytoplasmic tail sequence was able to restore Golgi localisation suggesting the importance of the cytoplasmic tail in localization(18,19). However, the currently accepted view in the field is that the influence of the cytoplasmic domain is of minor importance compared with the influence of kin-recognition and the length of the transmembrane domain (20-22). We recently showed that the cytoplasmic tails of α1,3galactosyltransferase (GT) or α1,2fucosyltransferase (FT), determine the order of action and localization within the Golgi of the two enzymes (23,24) and have also shown that a specific sequence in the cytoplasmic tail of FT is important for localization of FT (24). We demonstrate that the cytoplasmic tails of GT and FT are sufficient to confer Golgi localization and that the cytoplasmic tail of GT can displace the full length transferase or inhibit localization, suggesting a specific Golgi binding site for the GT cytoplasmic tail.
EXPERIMENTAL PROCEDURES

DNA Constructs

GT tail-Ly9-Flag (GLF) and FT tail-Ly9-Flag (FLF) was produced by annealing two partially overlapping oligonucleotides and completing the strands using Pwo polymerase (Boehringer-Mannheim/Roche). The oligonucleotides for GLF were: 5’-

GCGGATCCATGAATGTCAAAGGAAGATTTTGCTCTGCTCCTCCTGTTTTGCTCTTGTTGATGCTCATTGGCGGTTAC-3’ and 5’-

CGTCTAGATCACTTGTGCTATCGTCCTTGTAGTCCCTCAAATGAGTAACCAGCAATGAGCATCAACAAG-3’. The oligonucleotides for FLF were 5’-

GCGGATCCATGTGGGTCCCCAGCCGCCTTTTGGCTCCTGCTCCTCCTGTTTTGCTCTTGTTGATGCTCATTGGCGGTTAC-3’ and 5’-

CGTCTAGATCACTTGTGCTATCGTCCTTGTAGTCCCTCAAATGAGTAACCAGCAATGAGCATCAACAAG-3’. Untagged GT (25), FT (26) and the chimeric gtFT (23) were as described. The GT-myc construct contains the myc tag at the C-terminus and was produced by PCR using the untagged pig GT (25) as template. The oligonucleotides were 5’-

GCGGATCCATGAATGTCAAAGGAAGATGTGGTTCTG-3’ and 5’-

CGTCTAGATCACTTGTGCTATCGTCCTTGTAGTCCCTCAAATGAGTAACCAGCAATGAGCATCAACAAG-3’. Tagged DNA was digested with BamHI and XbaI and ligated into pcDNA1.

Peptide encoding constructs were produced by annealing sense and antisense oligonucleotides containing BamHI and Xba I sites at their ends and ligated into pCDNA 1 (In Vitrogen). The sense and antisense oligonucleotides for the constructs encoding GT-tail were 5’-

GATCCATGAATGTCAAAGGAAGATGAT-3’ and 5’-
CTAGCTCATCTTCCCCCTTTGACATTCATG-3'; for GT-tail/TM were 5'-
GATCCATGAATGTCAAAGGAAGAGTGGTTCTGTCAATGCTGCTTGTCTCAACTGT
AATGGTTGTGTTTGGGAATGAT-3' and 5'-
CTAGATCATCCACACAAACCATTACAGTTGAGACAAGCAGCATTGACAGAAAC
CACTCTTCCCTTGACATTCATG-3'; for GT random-tail were 5'-
GATCCATGGGAAGAAATAAAGTCTGAT-3' and 5'-CTAGATCAGACTTTATTT
CTTCCCATG-3'.

Cell Culture and Transfection

DNA was purified using Qiagen products and transfected into COS-7 cells using DEAE-dextran
(27) or LipofectAMINE Plus (Gibco) as recommended by the manufacturer. COS-7 cells were
grown in DMEM (CSL) supplemented with 10% FCS (CSL).

Immunofluorescence and Confocal Microscopy

Cells were surface stained 24-48 hours after transfection by incubation in antibody or lectin
diluted in 0.5% BSA/PBS. Counting was performed blind and the experiments were repeated at
least 3 times. For confocal microscopy, cells were passaged into chamber slides and cultured
overnight prior to incubation in cycloheximide (100 µg/ml for 2 hours). Cycloheximide
prevents the synthesis of protein and eliminates this as a cause of Golgi localization. Brefeldin
A (5 µg/ml) which disrupts Golgi structure (28) was also used to confirm Golgi localization.

Cells were fixed in 2% paraformaldehyde/PBS followed by permeabilisation and incubation in
0.5% saponin/PBS containing diluted antibodies or lectins. The cells were mounted in Prolong
(Molecular Probes). The lectins IB4 and UEA 1 (Sigma) and the anti-myc monoclonal antibody
9E10 (29) were conjugated to FITC. Sheep anti mouse Ig -FITC and -biotin were from Silenus
and streptavidin-Alexa 594 was from Molecular Probes. The confocal laser-scanning
microscope was by Optiscan.
RESULTS

The cytoplasmic tail of GT and FT is sufficient to confer Golgi localization- DNA constructs encoding tagged wild type GT and FT and chimeric proteins (Fig. 1 A-J), were used to determine if cytoplasmic tail sequences could affect the detection, and therefore localization, of the full length transferase. To test whether the GT or FT tail alone could localize to the Golgi without contributions from transmembrane or luminal domains of the transferase, expression constructs were made encoding each cytoplasmic domain fused to a hydrophobic sequence from an unrelated plasma membrane protein, mouse Ly9 (30), and a Flag-tag at the C-terminus (sequence DYKDDDDK). The GLF chimera (Fig. 1A) contained the 6 amino acid cytoplasmic tail (MNVKGR) of GT, the FLF chimera (Fig. 1B) contained the 8 amino acid cytoplasmic tail of FT (MWVPSRRH) and the ranGLF chimera (Fig. 1C) contained the 6 amino acid cytoplasmic tail of GT in random order (MGRNKV). In each case, the expression of the construct could be detected with biotinylated mAb anti-Flag M2 and streptavidin-Alexa 594. The DNA for full length GT was also used and was constructed to be myc-tagged at the C-terminus (Fig. 1D, myc epitope EQKLISEEDL, detected by the mAb 9E10 -FITC); the myc tag was used as there are no reagents to directly stain for GT in the Golgi.

Three lines of evidence demonstrated that GT-myc, GLF and FLF were all localized to the Golgi (Figs. 2A-2F): (a) The intracellular juxtanuclear staining pattern of wild type GT-myc, GLF and FLF after transfection of COS-7 cells was typical of Golgi staining after cycloheximide treatment (dense non-granular perinuclear appearance, Figs. 2A, 2C and 2E, respectively). (b) Brefeldin A treatment entirely disrupted the staining of the GT-myc, GLF and FLF (Figs. 2B, 2D and 2F respectively). (c) GT-myc colocalized with the trans Golgi network (TGN) marker, γ-adapting (data not shown). Furthermore, full length Ly9 localized to the cell surface but not the Golgi (data not shown) and ranGLF (GT cytoplasmic tail in random order)
did not localize to the Golgi but was distributed throughout the cytoplasm (Fig. 2G). Clearly, the 6 and 8 amino acid cytoplasmic tails of GT and FT are sufficient to localize the enzymes to the Golgi.

*The cytoplasmic tail of GT displaces/inhibits the full length enzyme from localizing in the Golgi*- It was considered that if GT is localized to a specific site in the Golgi by a saturable mechanism, high level expression of the cytoplasmic tail should affect the level of expression of the full length transferase by displacing the transferase and inhibiting its binding to a specific localization site. Fluorescent microscopy was used to determine whether full length GT could be inhibited from binding or displaced by coexpression of the GT cytoplasmic tail. COS-7 cells were transfected with full length GT-myc and with GFL or FLF (Fig. 3A-I). Expression of GT-myc correlates with Gal\(\alpha_1,3\)Gal as measured by IB4-FITC binding. As expected, transfection of GT-myc alone resulted in ~50% of cells staining positive for Gal\(\alpha_1,3\)Gal (data not shown). It was noted that cells expressing high levels of the GLF protein (red, ~10% of cells, short white arrows, Fig. 3A) did not have concurrent staining for full length GT-myc protein (short white arrows, Fig. 3C) whereas cells expressing lower levels of GLF protein (red, ~40% of cells, Fig. 3A, no white arrows) had GT-myc staining (green, Fig. 3B) and when the images were superimposed costaining was yellow (long white arrows, Fig. 3C). Thus, in the presence of high levels of GLF, GT-myc was not detectable.

To confirm the findings using a more sensitive technique, measurements of both GT function (using intracellular IB4 staining for Gal\(\alpha_1,3\)Gal) and localization of GLF protein were performed. Detection of Gal\(\alpha_1,3\)Gal intracellular IB4 staining is more sensitive than staining for the GT-myc protein as GT products, ie Gal\(\alpha_1,3\)Gal, can be detected with IB4 even when cells are expressing undetectable levels of GT-myc protein (data not shown). Again, cells expressing high levels of the GLF protein (red) (as described above) (short white arrows, Fig.
3D) did not display detectable IB4 staining (short white arrows, Fig. 3F), whereas cells expressing low levels of the GLF protein (red) (Fig. 3D, cells without white arrows) also stained with IB4 (Fig. 3E). Thus the presence of the inhibitory chimeric protein containing only the cytoplasmic tail of GT displaced full length GT from the Golgi.

These effects were specific, as when GLF protein was coexpressed with full length FT, cells containing high levels of GLF protein, (Fig. 3G) did not displace FT (detected by UEA 1 lectin which measures H, the product of FT, Fig. 3H). All cells stained for both intracellular UEA 1 lectin (green) and GLF (red) and when the images are superimposed, costaining was yellow (Fig. 3I). Thus, methods detecting either the transferases, or their products, demonstrate that full length GT can be displaced or inhibited from binding by the expression of the GT cytoplasmic tail and suggests a specific Golgi binding site for the GT cytoplasmic tail. *The inhibitory binding of the GT cytoplasmic tail is specific and is not affected by the transmembrane domain* - The specificity of the binding of the GT cytoplasmic tail and the impact of the transmembrane domain on displacement was further tested and quantified. The ability of the GT cytoplasmic tail alone (GT tail, Fig. 1E), the cytoplasmic tail and transmembrane domain (GT tail/TM, Fig. 1F) or the GT cytoplasmic tail in random order (GT random tail, Fig. 1G) to inhibit the localization of full length GT (Fig. 1H), was examined by measuring the enzymatic function of GT by cell surface staining with IB4-FITC (Fig. 4 A-C).

The full length GT DNA was transfected at different concentrations: 1.5 µg, 0.75 µg and 0.15 µg DNA/well together with DNA for either the GT tail, GT tail/TM or GT random tail or vector alone (1.5µg DNA/well). The amount of DNA transfected was progressively reduced as DNA transfected at very high levels affects the distribution of glycosyltransferases in the cell (31). There was a reduction in the number of cells expressing Galα1,3Gal (by approximately
50%) at all concentrations when GT tail and GT tail/TM were cotransfected with full length GT (Fig. 4A) showing that the glycosyltransferase was not overexpressed.

The presence of the transmembrane domain did not augment the level of inhibition conferred by the GT cytoplasmic tail. The presence of either the GT tail or GT tail/TM inhibited the function of the full length GT similarly, as there was a 50% reduction in the number of cells expressing Galα1,3Gal, whereas the GT random tail and the vector alone did not inhibit expression of Galα1,3Gal (Fig. 4A). In preliminary experiments, this effect was not caused by transfection efficiency as the level of GT mRNA expressed in each sample was similar (data not shown).

Again, the inhibition caused by expression of the GT cytoplasmic tail was specific, as the presence of the 6 amino acid GT tail protein did not decrease the function of a different glycosyltransferase with a different cytoplasmic tail sequence (FT, Fig. 1I). There was no alteration in the function of full length wildtype FT (1.5 µg, 0.75 µg and 0.15 µg DNA transfected/well) as measured by cell surface UEA 1 staining when expressed with any of the proteins (1.5 µg DNA transfected/well, Fig. 4B) showing that the expression of GT tail proteins specifically inhibited GT but not FT.

To exclude the possibility that overexpression of chimeric proteins effects the enzymatic function of GT rather than its position in the Golgi GT tail, GT tail/TM or GT random tail proteins were coexpressed with a full length chimeric transferase, gtFT which contains the 6 amino acid cytoplasmic tail of GT followed by the FT transmembrane and catalytic domains (Fig. 1J). Thus, the chimera has the GT localizing sequence in the cytoplasmic tail, but as it contained the FT catalytic domain, it could be measured by staining the cells with UEA 1. When the gtFT chimera (1.5 µg, 0.75 µg and 0.15 µg DNA/well, Fig. 4C) was coexpressed with GT tail, GT tail/TM or GT random tail proteins (1.5 µg DNA/well) and tested in the inhibition
assay, it behaved like the GT rather than the FT. Thus, the GT tail and GT tail/TM peptides did inhibit the function of the gtFT chimera as cell surface staining was reduced at least by 50% at all concentrations (Fig. 4C) similar to the reduction shown for the full length GT (Fig. 4A).

The data show that: (a) specific localization conferred by the cytoplasmic tail does not require the transmembrane domain, and (b) the localization conferred by the cytoplasmic tail is specific and is not dependent on the catalytic function of the enzyme. Thus, the expression of the 6 amino acid cytoplasmic tail inhibits the function of the full-length enzyme, presumably by affecting the ability of the enzyme to bind to a specific localizing site on the cytoplasmic surface of the Golgi.

DISCUSSION

The data described show that the cytoplasmic tails of GT and FT are sufficient to direct both localization and retention of these transferases in the Golgi. The 6 amino acids of the GT cytoplasmic tail, MNVKGR, must contain Golgi retention and localization sequences as (a) when this 6 amino acid sequence is attached to an irrelevant transmembrane domain the chimeric protein was expressed in the Golgi; (b) the GT cytoplasmic tail is able to displace full length GT; and (c) the GT cytoplasmic tail specifically inhibited the localization of full length GT or gtFT. These effects did not require the presence of the GT transmembrane domain as there was no improvement when the transmembrane domain was included. Thus for GT, the 6 amino acid GT cytoplasmic tail specifically inhibited the binding of full length transferase to specific sites in the Golgi and the transmembrane domain did not have a role in the inhibition of the GT binding site. For FT, the localization and function in COS-7 cells relies mainly on one amino acid residue in the cytoplasmic tail (24) and here we show that the 8 amino acids of the FT cytoplasmic tail, MWVPSRRH, were sufficient to localize to the Golgi when attached to an irrelevant transmembrane domain. In addition to the kin-recognition and lipid bilayer models,
the findings provide further evidence for the third mechanism of Golgi localization determined by the cytoplasmic tails of GT and FT (24).

The concept of a specific binding site or receptor is supported by the ability of the GT cytoplasmic tail to inhibit localization. Previously, it was suggested that the retention mechanism for glycosyltransferases would be (a) difficult to saturate because enzyme activity was already increased 25 fold in transfected cells from a different species and (b) is not species specific (5,31). However, the assay of GT and FT competition for substrate enables specific changes in localization to be quantified by a readout of the function of the enzyme (23,24).

Firstly, it is possible for glycosylation to occur when a transferase does not have a cytoplasmic tail, as FT without its cytoplasmic tail remained in the Golgi and TGN but was still able to fucosylate even though it was not localized in its specific functional subcompartment (24). Presumably this is due to the fact that the transmembrane domain holds the transferase in the Golgi/TGN and it still has access to acceptor substrate and donor sugar. This would account for a certain level of glycosylation but our assay of competition for substrate is more sensitive and can detect saturability (Figs. 3 and 4). Secondly, the cytoplasmic tail sequences of many glycosyltransferases are highly conserved across species and it is possible that the specific Golgi binding site would be compatible with transferases from other species. Therefore, the nature of the retention afforded by the transmembrane domain could contribute to a high level of expression in transfected cells, but the more specific localization site to which the cytoplasmic tail binds can be blocked.

The role of the transmembrane domain is not entirely clear. Our studies show that it has no specific localization role as the GT transmembrane domain can be exchanged with that of a cell surface molecule (Ly-9) and still localize to the Golgi. Further, the cytoplasmic tail without the transmembrane domain could still inhibit GT localization (Fig 4A), indicating that the
transmembrane domain does not play a major role in specific localization. In the kin recognition model, the transmembrane domain can play an important role in Golgi localization of transferases in the TGN (32), whereas for medial Golgi enzymes mannosidase II and N-acetylglucosaminyltransferase I, amino acids in the luminal stem domain affect aggregation (33,34). It is not clear in this model how such aggregation would lead to localization in the Golgi at a specific site. Our data for the GT and FT cytoplasmic tails can be reconciled with the kin-recognition theory as the cytoplasmic tail of certain transferases could interact with a binding site in Golgi and thus localize aggregates to a specific compartment. The lipid bilayer model which has been suggested for α2,6sialyltransferase (4), is not reliant upon specific sequences in the transmembrane domain but the general property of hydrophobicity and length. This model proposed that a 17 amino acid transmembrane domain of the α2,6sialyltransferase could not span the more cholesterol rich membranes beyond the Golgi/TGN. Our data showing that the GLF and FLF proteins, containing a longer transmembrane domain, successfully localized to the Golgi (Fig. 2C,D,E & F) indicates that the transmembrane domain is not the major means by which specificity is conferred on the localization of these transferases to the site in the Golgi where they perform their function.

Based on these models, we propose that the transmembrane domain acts as a general anchor or retention sequence not unlike that which occurs for cell surface molecules where the transmembrane domain acts as a hydrophobic sequence holding them in the membranes. The difference in the Golgi is that transferases are required to be in distinct sites so that sequential glycosylation can occur. For GT and FT that site is determined by the cytoplasmic tail, although this does not exclude other interactions of the transmembrane domain within the Golgi membrane. We believe that for GT and FT, the cytoplasmic tail confers specific localization in the Golgi, possibly by binding to a specific site or receptor(s) on the cytoplasmic side of the
Golgi. The approximately 12 amino acid cytoplasmic tails of p24 family proteins in the Golgi are involved in complex and specific interactions with cytoplasmic proteins COPI and COPII (35,36) providing another example of cytoplasmic tail involvement in protein-protein interactions in the Golgi. Receptor-mediated retention of Golgi enzymes is compatible with the current models for Golgi formation and maintenance of cisternal maturation (37-39) and vesicular transport (40-42), for review see (22), as a receptor could facilitate recycling and/or retention of transferases in an appropriate compartment. A receptor-mediated model involving Golgi localisation is also consistent with other examples of localisation in the cell such as endoplasmic reticulum localisation via the KDEL sequence (43-45). Thus, we propose that, although the two currently accepted means of localization are likely to be important in retaining these proteins in the Golgi, sequences within the cytoplasmic domain confer the key determinants for specific localization within the Golgi by interacting with a cytoplasmic binding site or receptor.

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FOOTNOTES
The abbreviations used are NacLac, N-acetyllactosamine; GT, α1,3galactosyltransferase; FT,
α1,2fucosyltransferase; IB4, Griffonia (Bandeiraea) Simplicifolia Lectin 1; UEA 1, Ulex
Europaeus Agglutinin 1; FITC, fluorescein isothiocyanate; PBS, phosphate buffered saline;
TGN, trans Golgi network.
FIGURE LEGENDS

FIG. 1. DNA constructs used and shown in domains.

FIG. 2. Full length GT (A and B) and chimeric peptides containing the GT (C and D) and FT (E and F) cytoplasmic domain localize to the Golgi. GT-myc, GLF, (MNVKGRFWLLLLVLLLLMLRRGYFILRDYKDDDK) (A and B), FLF, (MWVSRRHFWLLLLVLLLLMLRRGYFILRDYKDDDK) and ranGLF, (MGRNKVFWLLLLVLLLLMLRRGYFILRDYKDDDK) were transfected into COS-7 cells which were permeabilised and stained with anti-myc antibody (A and B) anti-Flag M2-biotinylated followed by streptavidin-Alexa-594 or anti-Flag M2 followed by sheep anti-mouse-FITC (C-G). GT-myc, GLF and FLF all localized to the Golgi in the presence of cycloheximide (A, C and E respectively) and the localization was disrupted in the presence of cycloheximide and Brefeldin A (B, D and F respectively). ranGLF did not localize to the Golgi (G).

FIG. 3 Full-length GT is displaced by inhibitory peptides corresponding to the GT cytoplasmic tail. Cells were cotransfected with DNA encoding GT-myc and GLF (panels A-F) and stained with anti-Flag M2 and anti-myc-FITC (A-C), showing that concurrent staining of full length transferase and GLF protein cannot be observed when expression of GLF is high (short white arrows). Similarly, staining cells with anti-Flag M2 and IB4-FITC (D-F) shows that concurrent staining of the product of GT enzyme (Galα1,3Gal, detected by IB4) and GLF protein cannot be observed when expression of GLF is high (short white arrows). However, cells cotransfected with FT and GLF (panel G-I) and stained with anti-Flag M2 and UEA 1-FITC (G-I) shows that virtually every cell contains both GLF and UEA 1 staining to give a
yellow staining pattern. This shows that the GT enzyme specifically displaces full length GT, but not full length FT.

FIG. 4. **Specificity of the inhibition of GT function by inhibitory peptides corresponding to the GT cytoplasmic tail.** COS-7 cells were cotransfected with either DNA encoding A, GT; B, FT and C, gtFT and DNA encoding GT random tail peptide (MGRNKV), the pcDNA vector alone, the GT tail peptide (MNVKGR) or the GT tail/TM peptide (MNVKGRVLSMLVSTVMVVFWE) (symbols are as indicated). Cells were surface stained with IB4-FITC (a) or UEA 1-FITC (b and c), fields were counted blind and results are the percentage of stained cells.
Fig 1
Fig 2
Fig 3
Fig 4

A

Percent positive cells

µg GT

0.15 0.75 1.5

B

Percent positive cells

µg FT DNA

0.15 0.75 1.5

C

Percent positive cells

µg gtFT

0.15 0.75 1.5

- GT+pcdna
- GT+GT tail
- GT+GT tail/TM
- GT+GT random tail

- FT+pcdna
- FT+GT tail
- FT+GT tail/TM
- FT+GT random tail

- gtFT+pcdna
- gtFT+GT tail
- gtFT+GT tail/TM
- gtFT+GT random tail
The cytoplasmic tail of α1,3galactosyltransferase inhibits Golgi localization of the full length enzyme

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