Molecular Cloning and Enzymatic Characterization of a UDP-GalNAc:GlcNAcβ-R β1,4-N-Acetylgalactosaminytransf erase from Caenorhabditis elegans*

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Ziad S. Kawar‡, Irma Van Die§, and Richard D. Cummings¶

From the ‡Department of Biochemistry and Molecular Biology, Oklahoma Center for Medical Glycobiology, University of Oklahoma Health Sciences Center, Oklahoma City, Oklahoma 73104 and the §Department of Molecular Cell Biology and §VU University Medical Center, van der Boechorststraat 7, 1081 BT Amsterdam, The Netherlands

A common terminal structure in glycans from animal glycoproteins and glycolipids is the lactosamine sequence Galβ4GlcNAc-R (LacNAc or LN). An alternative sequence that occurs in vertebrate as well as in invertebrate glycoconjugates is GalNAcβ4GlcNAc-R (LacldNAc or LDN). Whereas genes encoding β4GalTs responsible for LN synthesis have been reported, the β4GalNAcTs responsible for LDN synthesis has not been identified. Here we report the identification of a gene from Caenorhabditis elegans encoding a UDP-GalNAc:GlcNAcβ-R β1,4-N-acetylgalactosaminytransferase (Ceβ4GalNAcT) that synthesizes the LDN structure. Ceβ4GalNAcT is a member of the β4GalT family, and its cDNA is predicted to encode a 383-amino acid type 2 membrane glycoprotein. A soluble, epitope-tagged recombinant form of Ceβ4GalNAcT expressed in CHO-Lec8 cells was active using UDP-GalNAc, but not UDP-Gal, as a donor toward a variety of acceptor substrates containing terminal β-linked GlcNAc in both N- and O-glycan type structures. The LDN structure of the product was verified by co-chromatography with authentic standards and 1H NMR spectroscopy. Moreover, Chinese hamster ovary CHO-Lec8 and CHO-Lec2 cells expressing Ceβ4GalNAcT acquired LDN determinants on endogenous glycoprotein N-glycans, demonstrating that the enzyme is active in mammalian cells as an authentic β4GalNAcT. The identification and availability of this novel enzyme should enhance our understanding of the structure and function of LDN-containing glycoconjugates.

Many of the functional moieties of complex glycoconjugates are in the terminal sequences of N- and O-glycans of glycoproteins and in glycolipids, which are recognized by a growing number of carbohydrate binding proteins (1–4). A common terminal motif that is modified in a variety of ways by the additions of other sugars and sulfate groups is the lactosamine sequence Galβ4GlcNAc-R (LacNAc or LN), which is generated by a large family of UDP-Gal:GlcNAcβ-R β1,4-galactosyltransferases (β4GalTs) acting on terminal GlcNAc residues (5). However, another common terminal motif found in vertebrate and invertebrate glycoconjugates is the GalNAcβ4GlcNAc-R (LacldNAc or LDN) sequence. The LDN motif occurs in mammalian pituitary glycoprotein hormones, where the terminal GalNAc residues are 4-O-sulfated (6) and function as a recognition marker for clearance by the endothelial cell Man/4GlcNAc-R receptor (7). However, nonpituitary mammalian glycoproteins also contain LDN determinants (8–11), indicating that expression of LDN determinants in vertebrate glycoconjugates is more widespread than once thought. In addition, LDN and modifications of LDN sequences are common antigenic determinants in many parasitic nematodes and trematodes (12–17).

The LDN structure can be considered a variant of the more typical LN structure generated by a family of β4GalTs that includes the best characterized of all glycosyltransferases, the β4GalT I or lactose synthase (18–26). As more members of this family have been studied and the cDNAs encoding them have been cloned, it is evident that they share highly homologous regions within their amino acid sequences (27–36). Interestingly, those regions of homology are also found within the amino acid sequence of a snail UDP-GlcNAc:GlcNAcβ-R β1,4-N-acetylgalactosaminytransferase (37–39). This latter finding raised the possibility that the β4GalNAcT enzyme(s) might also have amino acid sequence homology to members of the β4GalT family. However, despite many studies reporting on the activity of a putative β4GalNAcT capable of generating LDN sequences (11, 40–46), the gene(s) encoding the putative β4GalNAcT responsible for LDN synthesis has not been identified.

In searching for the putative β4GalNAcT required for LDN synthesis, we examined genes in Caenorhabditis elegans. The C elegans genome contains three open reading frames that encode proteins with sequence homology to the β4GalT family. One of these open reading frames (ORF R10E11.4; sqv-3) is predicted to encode a protein involved in vulval invagination (47) and is likely to be a UDP-Gal:xylose β-R β1,4-galactosyltransferase (33, 48). Another of these open reading frames (ORF W02B12.11) encodes a protein for which no enzymatic activity has yet been reported. The third open reading frame (ORF Y73ETA.7) was identified more recently than the two mentioned above and therefore had not been reported in pre-

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The nucleotide sequence(s) reported in this paper has been submitted to the GenBank™/EBI Data Bank with accession number(s) AJ130767.

† To whom correspondence should be addressed: Dept. of Biochemistry and Molecular Biology, University of Oklahoma Health Sciences Center, 975 NE 10th St., BRC Rm. 417, Oklahoma City, OK 73104. Tel.: 405-271-2481; Fax: 405-271-3910; E-mail: richard-cummings@ouhsc.edu.

‡ The abbreviations used are: LN or LacNAc, Galβ4GlcNAc; β4GalT,
vious studies (27, 31). In this study, we have cloned a cDNA corresponding to the latter open reading frame and demonstrate that it encodes a β4GalNAcT, which we have termed Ceβ4GalNAcT. Ceβ4GalNAcT is active when expressed in mammalian cells in generating LDN determinants on N-glycans of glycoproteins.

**EXPERIMENTAL PROCEDURES**

**Materials**—All chemicals and reagents used in this study, unless otherwise indicated, were from Sigma. The C. elegans cDNA library was a gift from Dr. Robert Barstead (Oklahoma Medical Research Foundation, Oklahoma City, OK). The QIAQuick gel extraction kit was from Qiagen (Valencia, CA). Restriction enzymes were from New England Biolabs (Beverly, MA). The PCR 2.1 vector was from Invitrogen. The pcDNA3.1(-)+TH was a gift from Dr. Alineza R. Rezaie (Department of Biochemistry and Molecular Biology, St. Louis University School of Medicine, St. Louis, MO). FuGENE 6 and Complete protease inhibitor mixture were from Roche Molecular Biochemicals. Acceptor compounds (see Table I) 1, 3, 5, 9, and 12 were purchased from Sigma, 4 was obtained from Toronto Research Chemicals, and 6 were from Toronto Research Chemicals. Compounds 10 and 11 were a kind gift from Dr. L. Anderson (University of Wisconsin, Madison, WI), and 14 and 17 were from Dr. J. Lüngren (University of Stockholm). Compounds 13 (39) and 18–21 (32) were synthesized as described previously. Radiolabeled nucleotide sugars were obtained from PerkinElmer Life Sciences and were diluted with unlabeled nucleotide sugars (Sigma) to give the desired specific radioactivity.

Cloning and Sequencing of the Ceβ4GalNAcT cDNA—A BlastP search of the NCBI nonredundant protein data base for homologues of the human βGalT I (accession number CAA39074) identified a hypothetical protein encoded by an open reading frame in the C. elegans genome designated Y73E7A.7. A cDNA was amplified by PCR from a mixed stage C. elegans cDNA library using primers corresponding to the 5′- and 3′-ends of this open reading frame (5′-CCACATGGCCTTGTCCTATTGTCG-3′; 5′-CTAAAAACACGGTTGGAAAGTC-3′). Amplification was carried out at 95 °C for 2:30 min followed by 35 cycles at 95 °C for 50 s, 53 °C for 50 s, and 72 °C for 1:50 min and then at 72 °C for 10 min. The PCR product was purified from an agarose gel slice using a QIA Quick gel extraction kit, cloned into the pcR 2.1 vector, and sequenced on both strands at the Sequencing Facility of the Oklahoma Medical Research Foundation (Oklahoma City, OK).

Construction of an Expression Vector Encoding a Soluble, Epitope-tagged Form of Cε4GalNAcT—A PstI (partial/PvuII DNA fragment starting at bp 87 of the Ceβ4GalNAcT open reading frame and extending beyond the stop codon was subcloned into the EcoRV site of the pcDNA 3.1(+)-TH vector. The resulting vector (pcMV-SH-Ceβ4GalNAcT) encodes a fusion protein, designated SH-Ceβ4GalNAcT, which consists of a signal peptide at the N terminus followed by an HPC4 epitope and then the catalytic domain of the Ceβ4GalNAcT (beginning at Lys49, the first amino acid after the transmembrane domain). The HPC4 epitope is recognized by the Ca++-dependent monclonal antibody HPC4 (49, 50). SH-Ceβ4GalNAcT is under the transcriptional control of the cytomegalovirus promoter, which is present in the vector.

Expression of SH-Ceβ4GalNAcT—CHO-Lec8 and CHO-Lec2 cells were transfected with pcMV-SH-Ceβ4GalNAcT using FuGENE 6, according to the manufacturer’s instructions, and cultured in Dulbecco’s modified Eagle’s medium containing 10% fetal calf serum and 600 μg/ml Geneticin to select for stably transformed cells. After 4 weeks of culturing in medium containing Geneticin, the cells were cultured in the same medium without Geneticin, and the culture medium was harvested every 3 days and used to purify SH-Ceβ4GalNAcT. To assay intracellular β4GalNAcT activity and for Western blots, cells were washed with 75 ml sodium cacodylate, pH 7.0, and lysed in a buffer of 50 mM sodium cacodylate, pH 7.0, 20 mM MnCl2, 1% Triton X-100, 1× Complete protease inhibitor mixture (EDTA-free). The lysates were centrifuged at 15,000 × g for 5 min, and the supernatant was used for further analyses.

Purification of SH-Ceβ4GalNAcT—Medium containing SH-Ceβ4GalNAcT was centrifuged at 1,500 × g for 5 min to remove cellular debris and then incubated with HPC4-UltraLink beads (5 mg of HPC4 antibody/ml of beads; 0.1 μl of beads/ml of medium) for 1 h at room temperature on a rotating platform. The beads were collected by cen-
AY130767) was amplified by PCR from a mixed stage C. elegans cDNA library using primers corresponding to the 5'- and 3'-ends of this open reading frame, establishing that the gene is expressed in vivo. The cDNA of Y73E7A.7 encodes a predicted 383-amino acid protein, which is a common topological motif in glycosyltransferases. The protein encoded by Y73E7A.7 is predicted to contain six potential N-glycosylation sites and two DVD motifs, which are thought to be involved in metal ion binding (53) (Fig. 1). Curiously, the last N-glycosylation site of the predicted protein participates in metal ion binding (53) (Fig. 1). The acceptor substrate specificity of SH-Ce4GalNAcT efficiently utilizes UDP-GalNAc, but did not significantly utilize UDP-Gal, UDP-GlcNAc, or UDP-Glc. To define the acceptor specificity of Ce4GalNAcT, the enzyme was tested with a wide variety of acceptors (Table II). SH-Ce4GalNAcT efficiently utilizes free GlcNAc and all substrates containing terminal β-linked GlcNAc in both N- and O-glycan type structures. SH-Ce4GalNAcT less effectively utilizes α-linked GlcNAc or 6-sulfated GlcNAc and does not utilize acceptors with terminal β-linked Gal, Glc, or GalNAc. The acceptor substrate specificity of SH-Ce4GalNAcT is therefore similar to the broad specificity reported for human 4GalT I (32). In contrast, the snail β4-GlcNAcT has a marked preference for acceptors with β1,6-linked terminal GlcNAc (39) (see Table II for a side-by-side comparison).

In view of the sequence homology between Ceβ4GalNAcT and the β4GalT family, we examined whether the modifier protein α-lactalbumin would affect the acceptor specificity of SH-Ceβ4GalNAcT. α-Lactalbumin, which is expressed in lactating mammary glands, associates with β4GalT I and switches its acceptor specificity from GlcNAc-R to free Glc, thus forming lactose synthase (58). However, unlike its effect on β4GalT I, α-lactalbumin does not induce SH-Ceβ4GalNAcT to utilize Glc as an acceptor instead of GlcNAc (Table III). α-Lactalbumin does appear to slightly depress activity of
Ceβ4GalNAcT toward the free GlcNAc acceptor, suggesting a possible weak interaction between the enzyme and α-lactalbumin.

**Product Characterization by HPAEC-PAD and ¹H NMR**—The product generated by SH-Ceβ4GalNAcT using GlcNAc1-O-pNP as acceptor was analyzed by HPAEC-PAD (Fig. 4). The product co-eluted with the authentic GalNAcβ1–4GlcNAcβ1-O-pNP standard but not with two other disaccharide-O-pNP standards (GlcNAcβ1–3GalNAcα1-O-pNP and GlcNAcβ1–6GalNAcα1-O-pNP).

To further establish the structure of the product generated by SH-Ceβ4GalNAcT using GlcNAcβ1-S-pNP as acceptor, the product was analyzed by ¹H NMR spectroscopy (Fig. 5). The spectrum shows two H-1 doublets at δ = 5.146 ppm and 4.540 ppm. The coupling constants of the H-1 doublets (10.5 and 8.5 Hz, respectively) indicate that both C-1 atoms are in β-anomeric conformation (59). The doublet at 5.146 ppm and the signal at δ = 2.013 ppm can be assigned to the H-1 and the CH₃-Nac of GlcNAcβ1-S-pNP by analogy to the resonance po-
sitions in GlcNAcβ1-4GlcNAcβ1-S-pNP (38). The doublet at δ = 4.540 ppm and the signal at δ = 2.077 ppm have shifts that are close to those reported for a β4-linked GalNAc residue (44, 45). The NMR spectrum therefore confirms that the analyzed product is GalNAcβ1-4GlcNAcβ1-S-pNP.

**In Vivo Synthesis of LDN Structures on N-Glycans by SH-**

**Ceβ4GalNAcT**—Since SH-Ceβ4GalNAcT was active in cell extracts when expressed in CHO-Lec8 cells (Fig. 3A), we examined whether it can generate LDN structures on endogenous glycan acceptors in animal cells. Cell lysates from nontransfected CHO-Lec8 and CHO-Lec2 cells and transfected CHO-Lec8 and CHO-Lec2 cells expressing SH-Ceβ4GalNAcT were examined for the presence of LDN determinants by a Western blot analysis using a monoclonal antibody SMLDN1.1 against LDN (16) (Fig. 6A). As indicated above the CHO-Lec8 cells are deficient in UDP-Gal transport into the Golgi and hence generate nonsialylated glycans terminating in Gal residues (60). Nontransfected CHO-Lec8 and CHO-Lec2 cells did not express detectable levels of LDN determinants as detected by SMLDN1.1 (Fig. 6A). By contrast, both cell lines expressing SH-Ceβ4GalNAcT expressed the LDN epitope on several glycoproteins. It would be predicted that the Ceβ4GalNAcT might only add GalNAc to N-glycans in CHO cells, since CHO cells produce O-glycans of the core 1 structure (Galβ3GalNAcα1 Ser/Thr) lacking in GlcNAc residues (61, 62). Cell extracts derived from CHO cell lines expressing SH-Ceβ4GalNAcT were treated with N-glycanase to determine whether LDN determinants were present in N-glycans. N-Glycanase treatment quantitatively removed the LDN-reactive epitopes from glycoproteins, demonstrating that LDN was expressed exclusively on N-glycans by the SH-Ceβ4GalNAcT. Transfected CHO-Lec2 cells expressed lower levels of LDN determinants than transfected CHO-Lec8, possibly due to competition from endogenous β4GalTs, since the cells expressed equivalent amounts of SH-Ceβ4GalNAcT as detected by a Western blot using the HPC4 antibody (Fig. 6B). The latter experiment also confirmed the molecular weight of SH-Ceβ4GalNAcT, demonstrating that N-glycanase treatment shifted the 59.4-kDa protein to 43.1 kDa, the predicted peptide size of SH-Ceβ4GalNAcT.

**DISCUSSION**

The results presented here provide several new insights into the biosynthesis of animal cell glycoproteins. We have identified a specific N-acetylgalactosaminyltransferase Ceβ4GalNAcT from *C. elegans* capable of utilizing UDP-GalNAc as the donor for the transfer of GalNAc residues to terminal GlcNAc residues in a wide variety of acceptors to generate the LaediNac (LDN) sequence GalNAcβ4GlcNAc-R. The enzyme is a member of the β4-galactosyltransferase family, although Ceβ4GalNAcT is unable to utilize UDP-Gal as the donor. In vertebrate cells, the recombinant form of Ceβ4GalNAcT is fully functional and capable of generating the LDN structure in complex-type N-glycans of glycoproteins. This represents the first identification of a β4GalNAcT capable of generating the LDN sequence in animal glycoconjugates.

Although the LacNac (LN) sequence Galβ4GlcNAc-R is a more general terminal modification in vertebrate glycoconjugates, the LN sequence also occurs in several vertebrate glycoproteins and glycolipids, including pituitary glycoprotein hormones (63) and other glycoconjugates (8, 11, 64–66). A hormone-specific β4GalNAcT enzyme, active in the pituitary gland and other tissues, acts preferentially on glycoproteins containing a specific peptide motif (46, 63, 67–70). The GalNAc residue added to these hormones is subsequently 4-O-sulfated (71–73), and the resulting terminal GalNAc-4-SO4 acts as a clearance signal that regulates their circulatory half-lives (6, 74–76). The addition of the LDN motif to other glycoproteins, such as glycoalbumin (9, 66) and protein C (8), may also be cell- and protein-specific and may be important to the functional activities of these glycoproteins. In addition to the hormone-specific β4GalNAcT, a motif-independent β4GalNAcT activity has been detected in extracts from many cells (69), including human 293 cells (11), bovine mammary gland (43), snails (40, 41), insect cells (45), and schistosomes (42, 44). The LDN motif is also a more common structural feature in invertebrate glycoconjugates compared with the LN motif, especially as seen in many parasitic nematodes and trematodes (12–17, 77). However, neither the enzyme(s) nor gene(s) encoding the enzyme

![Graph](image-url)
and UDP-GalNAc, and in the presence of GalT I or lactose synthase is capable of utilizing both UDP-Gal with a UDPGalNAc concentration of 0.5 mM. For comparison, the 100% activity corresponds to 2.1 nmol/min/ml beads suspension.

Responsible for LDN synthesis in invertebrates have previously species ranging from enzymes with homology to the other members found in various

Therefore, it is especially interesting that the Ceβ4GalNAcT family of

assays were carried out in duplicate as described under “Experimental Procedures” using SH-Ceβ4GalNAcT attached to HPC4 beads with a donor concentration of 0.5 mM and an acceptor concentration of 1 mM terminal GlcNAc. For comparison, 100% activity (using free GlcNAc as acceptor) corresponds to 2.1 nmol/min/ml bead suspension.

As assays were carried out in duplicate as described under “Experimental Procedures” using SH-Ceβ4GalNAcT attached to HPC4 beads with a UDPGalNAc concentration of 0.5 mM. For comparison, the 100% activity corresponds to 2.1 nmol/min/ml beads suspension.

| Acceptor Specificity of Ceβ4GalNAcT and Comparison to Other Members of the β4GalT Family |
|-----------------------------------------------|-------------------------------|-------------------------------|
| Acceptor | Ceβ4GalNAcT | Human β4GalT | Lymanea stagnalis β4GlcNAcT |
|-----------|--------------|--------------|-----------------------------|
| %         | %            | %            |
| 1. GlcNAcβ-S-pNP | 285 | 232 | 5380 |
| 2. GlcNAcα1-pNP | 14 | 39 | 95 |
| 3. Galβ-pNP | 1 | | |
| 4. Glcβ1-methyl-umbelliferone | 0.5 | | |
| 5. GalNAcβ-pNP | 0.5 | | |
| 6. SO$_4$-6-GlcNAcβ1-pNP | 6 | 25 | |
| 7. GlcNAcβ1-6GlcNAc-pNP | 145 | 197 | 250 |
| 8. GlcNAcβ1-6Galβ1-3GalNAc-pNP | 159 | 195 | 5570 |
| 9. GlcNAc | 100 | 100 | 100 |
| 10. GlcNAcβ1-3Gal | 121 | 176 | |
| 11. GlcNAcβ1-6Gal | 328 | 1590 | |
| 12. GlcNAcβ1-4GlcNAcβ1-4GlcNAc | 115 | 24 | |
| 13. GlcNAcβ1-6GlcNAc | 109 | 467 | |
| 14. GlcNAcβ1-2Man | 132 | 34 | |
| 15. GlcNAcβ1-4Man | 156 | 425 | |
| 16. GlcNAcβ1-6\ Man | 115 | 176 | |
| 17. GlcNAcβ1-4\ Man | 112 | 58 | |
| 18. GlcNAcβ1-2Manα1-6\ Manβ1-4GlcNAcβ1-4GlcNAc | 71 | 360 | |
| 19. GlcNAcβ1-2Manα1-6\ Manβ1-4GlcNAcβ1-4GlcNAc | 122 | 381 | |
| 20. GlcNAcβ1-6\ Manβ1-4GlcNAcβ1-4GlcNAc | 111 | 372 | |
| 21. GlcNAcβ1-2Manα1-6\ Manβ1-4GlcNAcβ1-4GlcNAc-Asn-glycopeptide | 48 | 365 | |

* Assays were carried out in duplicate as described under “Experimental Procedures” using SH-Ceβ4GalNAcT attached to HPC4 beads with a donor concentration of 0.5 mM and an acceptor concentration of 1 mM terminal GlcNAc. For comparison, 100% activity (using free GlcNAc as acceptor) corresponds to 2.1 nmol/min/ml bead suspension.

* Also for comparison, relative activities with the same acceptors for human β4GalT I (32) and L. stagnalis β4GlcNAcT (39) are taken from previous publications.

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**Table II**

**Table II**

Effect of α-lactalbumin on activity of the Ceβ4GalNAcT

| Acceptor | α-Lactalbumin (5 mg/ml) | Relative activity $^a$ |
|-----------|------------------------|------------------------|
| %         | %                      |
| GlcNAc (1 mM) | – | 100 |
| GlcNAc (1 mM) | + | 40 |
| Glc (30 mM) | – | 3 |
| Glc (30 mM) | + | 6 |

* Assays were carried out in duplicate as described under “Experimental Procedures” using SH-Ceβ4GalNAcT attached to HPC4 beads with a UDPGalNAc concentration of 0.5 mM. For comparison, the 100% activity corresponds to 2.1 nmol/min/ml beads suspension.

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**Figure 4.** HPAEC-PAD analysis of the reaction product catalyzed by SH-Ceβ4GalNAcT using GlcNAcβ1-O-pNP as acceptor. A, analysis of the reaction products obtained in the absence of SH-Ceβ4GalNAcT. The arrows indicate the elution positions of reference compounds. a, GalNAcβ1-4GlcNAcβ1-O-pNP; b, GalNAcβ1-6GalNAcα1-O-pNP; c, GalNAcβ1-3GalNAcα1-O-pNP; d, GalNAcβ1-O-pNP. The peak corresponding to GalNAcβ1-4GlcNAcβ1-O-pNP is shaded.
although a member of the β4GalT family, does not utilize UDP-Gal. Two recent crystallographic studies on β4GalT I have shed light on the amino acid residues that are important in donor and acceptor recognition by the enzymes of the β4GalT family. The first study demonstrated that changing a tyrosine residue (Tyr289) in the bovine β4GalT I to isoleucine altered its donor specificity from UDP-Gal to UDP-GalNAc (21). It is noteworthy that the C. elegans β1,4-N-Acetylgalactosaminyltransferase contains an isoleucine residue (Ile257) at the corresponding position. The second study identified 12 amino acids in the bovine β4GalT I that constitute its acceptor binding site (79). These amino acids vary considerably among members of the β4GalT family, and Ceβ4GalNAcT has between 2 and 4 of these residues in common with each of the other members of this family. The specific amino acids in Ceβ4GalNAcT responsible for its sugar nucleotide and acceptor specificity await identification.

It is noteworthy that the soluble form of the Ceβ4GalNAcT, when expressed in CHO-Lec8 or CHO-Lec2 cells, is capable of generating LDN epitopes on cellular glycoproteins. Interestingly, a significant amount of the total Ceβ4GalNAcT was
present in cell extracts compared with extracellular media (Fig. 3A). This implies that the soluble enzyme is sufficiently retained in the cell to allow productive interactions with intracellular acceptor glycoproteins. The mode of retention of the soluble Ce4GalNAcT in CHO cells is not known. Targeting and retention in the Golgi apparatus for many glycosyltransferases requires membrane anchoring, although other domains of the enzymes are also important (80, 81). Similarly, we previously observed that the soluble form of the α1,3-galactosyltransferase is also functional within cells (82). However, soluble forms of other glycosyltransferases inefficiently glycosylate intracellular acceptors (83, 84). It is conceivable that the high concentration of potential terminal GlcNAc-R acceptors in CHO-Lec6 and CHO-Lec2 cells could cause the retention of Ce4GalNAcT in appropriate Golgi compartments, based on the observation that many glycosyltransferases show affinity for their acceptor substrates and can be purified by affinity chromatography on immobilized acceptors (85). The Ce4GalNAcT could also interact with some other Golgi-resident protein, such as another glycosyltransferase, as proposed in the kin recognition hypothesis (86). Overall, our results support the possibility that Golgi retention of glycosyltransferases is likely to be a complex event mediated in part by multiple domains of the enzymes and not necessarily by the transmembrane domains.

Although Ce4GalNAcT is able to act on most of the common types of mammalian N- and O-glycans, there is only a limited knowledge of the glycan structures produced in C. elegans. It has been reported that the LDN motif appears at the reducing end of unusual O-glycans of C. elegans with the predicted sequence R-GlcNAc4GalNAc-Ser/Thr (87). Whether Ce4GalNAcT is responsible for synthesis of this type of structure is currently unknown, as are the enzymes that can potentially act to extend a glycan from the LDN motif.

The availability of a recombinant, well characterized 4GalNAcT active in mammalian cells should help advance our understanding of this type of glycosyltransferase and the structures and functions of LDN-containing glycans. The enzyme can be a valuable tool for both the in vitro and in vivo synthesis of LDN-based glycan structures, which may be used for further studies on their function in both vertebrates and invertebrates, as well as for studying LDN-containing antigenic glycans and pharmaceutical or commercial products.

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