Biochemical and Biophysical Research Communications 454 (2014) 486–492

Contents lists available at ScienceDirect

Biochemical and Biophysical Research Communications

journal homepage: www.elsevier.com/locate/ybbrc

Short N-terminal region of UDP-galactose transporter (SLC35A2) is crucial for galactosylation of *N*-glycans

Paulina Sosicka [a], Piotr Jakimowicz [b], Teresa Olczak [a], Mariusz Olczak [a,*]

[a] Laboratory of Biochemistry, Faculty of Biotechnology, University of Wroclaw, 14A F. Joliot-Curie St., 50-383 Wroclaw, Poland
[b] Laboratory of Biotechnology, Faculty of Biotechnology, University of Wroclaw, 14A F. Joliot-Curie St., 50-383 Wroclaw, Poland

ARTICLE INFO

Article history:
Received 14 October 2014
Available online 28 October 2014

Keywords:
Chimeric protein
Golgi apparatus
Glycosylation
UDP-galactose transporter
UDP-*N*-acetylglucosamine transporter

ABSTRACT

UDP-galactose transporter (UGT) and UDP-*N*-acetylglucosamine transporter (NGT) form heterologous complexes in the Golgi apparatus (GA) membrane. We aimed to identify UGT region responsible for galactosylation of *N*-glycans. Chimeric proteins composed of human UGT and either NGT or CMP-sialic acid transporter (CST) localized to the GA, and all but UGT/CST chimera corrected galactosylation defect in UGT-deficient cell lines, although at different efficiency. Importantly, short N-terminal region composed of 35 N-terminal amino-acid residues of UGT was crucial for galactosylation of *N*-glycans. The remaining molecule must be derived from NGT not CST, confirming that the role played by UGT and NGT is coupled.

1. Introduction

Glycosylation of macromolecules is one of the most important posttranslational modifications of proteins [1]. The substrates required for glycosylation are sugars activated by the addition of mono- or diphosphonucleotides (UDP, GDP or CMP). To be delivered into the endoplasmic reticulum (ER) and the Golgi apparatus (GA), they need to be transported across their membranes by nucleotide sugar transporters (NSTs). NSTs are highly conserved type III multitransmembrane proteins with molecular weight of 30–45 kDa that function as antiporters delivering nucleotide sugars into the ER or GA and transferring corresponding nucleoside monophosphate into the cytosol [1–4].

One of the best characterized NSTs is UDP-galactose transporter (UGT; SLC35A2). Two splice variants of UGT (UGT1 and UGT2) have been identified in human tissues, the Chinese hamster ovary (CHO) and Madin–Darby canine kidney II (MDCK) cell lines [5–8]. MDCK cells also possess an additional, shorter UGT isoform [9]. Characterization of UGT was possible after mammalian cell lines deficient in UGT activity, such as CHO-Lec8, MDCK cells resistant to *Ricinus communis* agglutinin (MDCK-RCA^r) and murine Had-1 cells, have been identified [6,10,11]. Glycans produced in these cells are enriched in terminal *N*-acetylglucosamine (GlcNAc) and exhibit significantly reduced terminal galactose and sialic acid. Although UGT is not active in mutant cells, some galactosylation still occurs [8,10–14]. Recently, it has been shown that *SLC35A2* gene mutation can result in early-onset epileptic encephalopathy and developmental delay [15,16].

In contrast to UDP-galactose transporter, little is known about mammalian UDP-*N*-acetylglucosamine transporter (NGT; SLC35A2), which is assumed to play the main role in UDP-GlcNAc transport. Multi-specific transporters SLC35D1, SLC35D2 and SLC35B4 appear to be involved in glycosylation of other macromolecules and seem to be rather tissue specific [17–20]. It is worth noting that silencing of the gene encoding NGT results in significant decrease of highly branched *N*-glycans as well as keratan sulfate, but do not affect mono- and diantennary oligosaccharide structures [21]. Recently, it has been reported that impaired UDP-GlcNAc transport into the Golgi vesicles leads to serious disorders. Point mutations in the gene encoding NGT result in complex vertebral malformation in cattle [22] and autism spectrum disorders, epilepsy as well as arthrogryposis in humans [23].

We hypothesize that the role played by NGT and UGT in galactosylation is coupled. We have shown that overexpression of NGT may partially restore galactosylation of *N*-glycans in the mutant cells defective in UGT activity [24]. Moreover, using immunoprecipitation and FLIM-FRET technique we proved that NGT, UGT1 and UGT2 form heterologous complexes in the GA membrane [25]. Our recently published [21] and unpublished data also

Abbreviations: UGT, UDP-galactose transporter; NGT, UDP-*N*-acetylglucosamine transporter; NST, nucleotide sugar transporter; ER, endoplasmic reticulum; GA, Golgi apparatus.

* Corresponding author. Fax: +48 71 3752 608.
E-mail address: Mariusz.Olczak@biotech.uni.wroc.pl (M. Olczak).

http://dx.doi.org/10.1016/j.bbrc.2014.10.098
0006-291X/

suggest that NGT and UGT1 are in the close proximity to *N*-acetylglucosamine glycosyltransferases (Mgats).

In our previous study we have demonstrated that UGT/NGT chimeric transporter is able to correct galactosylation defect in UGT-deficient mammalian cells at better degree compared with the NGT overexpression [26]. The chimeric protein was composed of 224 N-terminal amino-acid residues of UGT fused to C-terminal region of NGT. Even though almost half of the protein was derived from the different NST, the chimera localized to the GA membrane, was fully functional in respect to UDP-galactose transport and restored galactosylation in UGT-deficient cells. This phenomenon convinced us to study the role of UGT N-terminal region in galactosylation in more details. Therefore, we constructed six novel chimeric proteins and analyzed their involvement in galactosylation in UGT-deficient mammalian cell lines.

2. Materials and methods

2.1. Construction of chimeric transporters

Chimeric constructs 2–4 were prepared by introducing unique AflII restriction site into plasmids containing sequences encoding UGT and NGT, without changing of protein sequences (Fig. 1), as previously described for UGT/NGT chimera [27]. Chimeric construct 5 was prepared using primers which amplify N-terminal region of UGT and C-terminal region of NGT. After purification, the blunt-ends of DNA fragments were phosphorylated using T4 kinase (Thermo Scientific) and ligated (Fast Ligation Kit, ThermoScientific). The ligation reaction mixture was used as a template to perform PCR using primers specific for 5′ sequence of UGT and 3′ sequence of NGT, respectively. Finally, the PCR product was cloned to polilinker #1 of the modified pVitro1 vector, containing 6His-HA N-terminal tag, as previously described [26]. The same strategy was used in the case of UGT/CST chimera, with appropriate primers designed to amplify N-terminal region of UGT and C-terminal region of CST. Sequence encoding NGT/UGT1 chimera was amplified using forward primer containing additional, short sequence encoding 5 N-terminal amino-acid residues derived from NGT. The cDNA was synthesized using total RNA purified from HeLa cells as a template (Superscript III, Invitrogen). All mutagenesis primers and primers used in PCR are listed in Supplementary Table 1.

2.2. Cell maintenance and transfection

CHO, CHO-Lec8, MDCK and MDCK-RCA^r cells were grown and transfected with expression plasmids as described previously [7]. Stable transfectants expressing chimeric proteins were selected in complete media containing either 500 μg/ml (CHO-Lec8) or 600 μg/ml (MDCK-RCA^r) G-418 sulfate (InvivoGen).

2.3. Immunofluorescence microscopy

Immunofluorescence microscopy was performed as described previously [26] using MDCK-RCA^r cells overexpressing all analyzed chimeric proteins except that GA was visualized using a 1:200 dilution of rabbit polyclonal antibody against giantin (Abcam) followed by incubation with a 1:100 dilution of goat anti-rabbit Cy5-conjugated antibody (Abcam).

2.4. Analysis of glycoproteins with lectins

Proteins present in cell lysates were subjected to SDS–PAGE using 10% polyacrylamide gels and transferred onto nitrocellulose membranes (Whatman) [7]. Reactivity of glycoproteins with lectins was performed as described previously [7,24].

2.5. Isolation and separation of fluorescently labeled N-glycans and MALDI-TOF MS analysis

N-glycans were isolated, purified, fluorescently labeled with 2-aminobenzamide (2-AB) and separated on a GlycoSep N column (Glyko) as described previously [24]. Prior mass spectrometry (MS) analysis, *N*-glycans were subjected to neuraminidase treatment. MALDI-TOF MS analysis was carried out in positive ion mode with Na^+ excess as previously reported [24].

3. Results

3.1. Localization of chimeric proteins

We constructed six novel chimeric transporters (Fig. 1) and overexpressed them stably in UGT-deficient mammalian cell lines. All proteins were successfully overexpressed in several clones and localized in the GA membrane as shown by immunofluorescence microscopic analysis using MDCK-RCA^r cells (Supplementary Fig. 1). The expression levels of chimeras 2–5 and UGT/CST chimera were significantly higher compared to NGT/UGT1 chimera (Supplementary Fig. 2).

3.2. Reactivity of glycoproteins with lectins

To determine whether and to what extent all analyzed chimeric proteins would be able to restore galactosylation of CHO-Lec8 and MDCK-RCA^r cells, analysis of lectin reactivity was employed using GSLII (*Griffonia simplicifolia* lectin II) and VVL (*Vicia villosa* lectin) lectins. Glycoproteins produced in CHO-Lec8 and MDCK-RCA^r cells are significantly enriched in terminal GlcNAc in *N*-glycans and *N*-acetylgalactosamine (GalNAc) in *O*-glycans, resulting in increased reactivity with GSLII and VVL lectins, respectively. Here we demonstrate that only UGT/CST chimera did not correct the mutant phenotype of the cells examined (Supplementary Figs. 3 and 4). Overexpression of the other five transporters (chimeras 2–5 and NGT/UGT1 chimera) both in CHO-Lec8 and MDCK-RCA^r resulted in decreased reactivity with GSLII and VVL lectins to the level similar to the wild-type cells. These data show that in contrast to UGT/CST chimera, correction of galactosylation defect, in terms of both *N*- and *O*-glycosylation was clearly visible.

MALI (*Maackia amurensis* lectin I) lectin is specific for terminal galactose and alpha 2,3-sialic acid-galactose, structures characteristic for the wild-type cells. An increase of galactose-specific MALI lectin reactivity with *N*-glycosylated proteins synthesized by MDCK-RCA^r cells expressing chimeras 2–5 as well as NGT/UGT1 chimera confirms correction of galactosylation defect. This effect was not visible in the case of UGT/CST chimera (Supplementary Fig. 3). In contrast to MDCK and MDCK-RCA^r cells, no difference in MALI reactivity was observed between CHO and CHO-Lec8 cells.

3.3. Identification of glycan structures

To identify more specific structural changes, *N*-glycans isolated from glycoproteins derived from the cells overexpressing chimeric transporters were characterized. Although correction of the mutant phenotype was visible for MDCK-RCA^r and CHO-Lec8 cells, we demonstrate detailed data for CHO and CHO-Lec8 cells analysis only, because MDCK and MDCK-RCA^r cells are not the best model for glycosylation analysis since they produce mostly *N*-glycans of high-mannose type [24]. Correction of the mutant phenotype was clearly visible for CHO-Lec8 cells overexpressing chimera 2

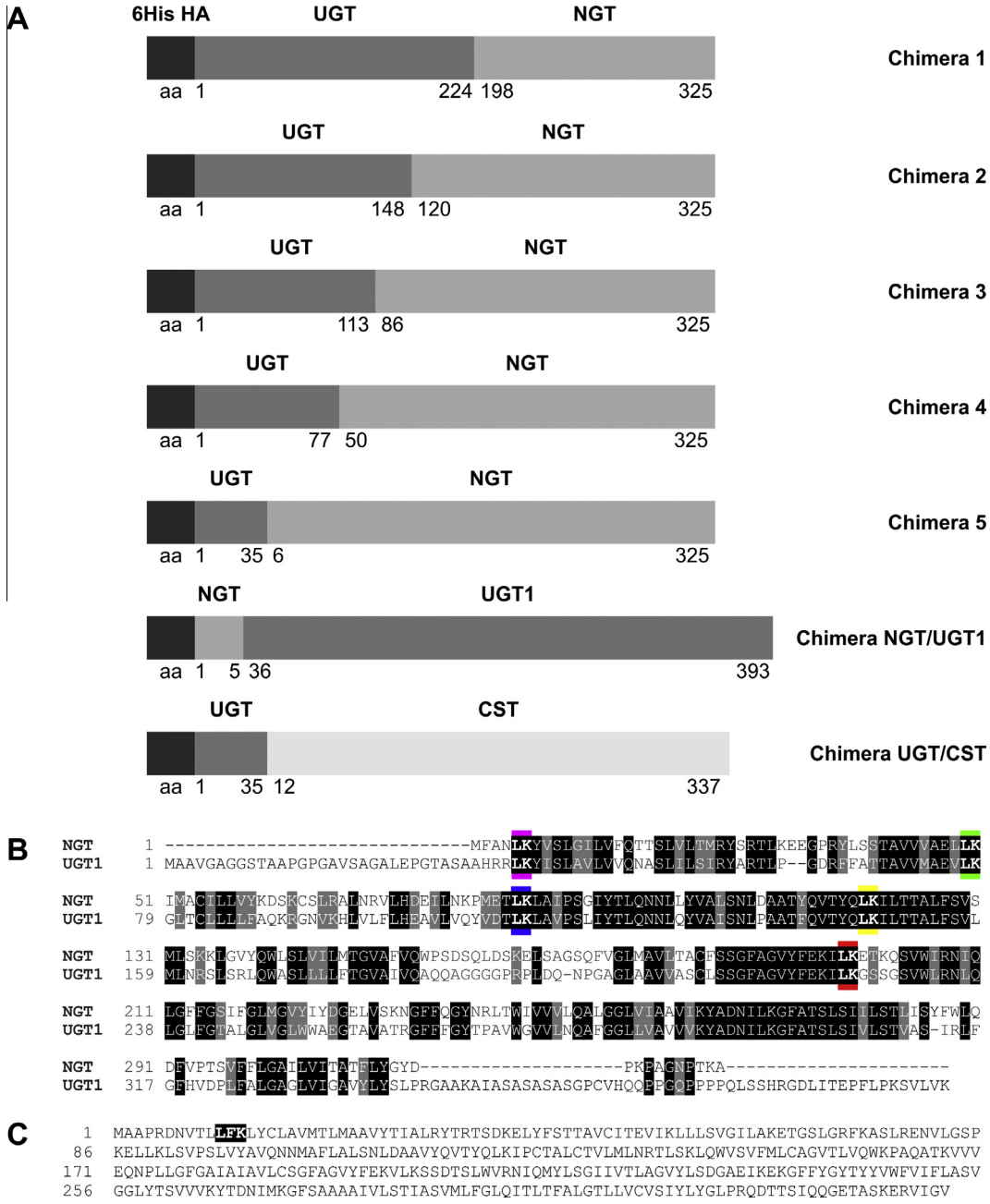

Fig. 1. Schematic representation of chimeric transporters. Chimeras 1–5 were constructed by the fusion of the respective C-terminal portions of human NGT and the respective N-terminal portions of UGT. NGT/UGT1 chimera consists of five N-terminal amino-acid residues of NGT and C-terminal portion of UGT1. UGT/CST chimera was constructed by combination of the same UGT N-terminal portion as in chimera 5, but instead of NGT C-terminal portion of CST was fused (A). Protein sequence alignment of NGT and UGT1 (B). Novel chimeric transporters constructed in this study are mark with yellow (2), blue (3), green (4) and purple (both 5 and NGT/UGT1) boxes (B). The chimeric protein described previously [26] is indicated with the red box. Protein sequence of CST (C). The site of CST fusion with UGT was indicated with black box. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

in *N*-glycan profiles obtained after separation on a GlycoSep N column, whereas in the case of chimeras 3–5 and NGT/UGT1 chimera galactosylation was only partially corrected (Fig. 2 and Supplementary Fig. 5). UGT/CST chimera did not show any effect on galactosylation in the mutant cells examined.

In attempt to determine the extent of phenotypic correction more precisely, MALDI-TOF MS analysis of desialylated *N*-glycans was carried out. Obtained results corroborated correction of galactosylation by chimera 2 in MDCK-RCA^{r} (data not shown) and CHO-Lec8 mutant cells (Fig. 3A and B). Surprisingly, this analysis showed that also NGT/UGT1 chimera was able to correct galactosylation defect. As expected, chimeras 3–5 restored galactosylation only to some extent. In contrast, UGT/CST chimeric transporter was not able to correct the mutant phenotype. The only effect observed was tri-antennary galactosylated *N*-glycan structure produced after overexpression of this chimera in the mutant cells (Fig. 3B).

4. Discussion

It has been demonstrated by others that some regions of UGT could be replaced with CST counterparts or even deleted without loss of the UDP-galactose transporting activity [28–30]. However,

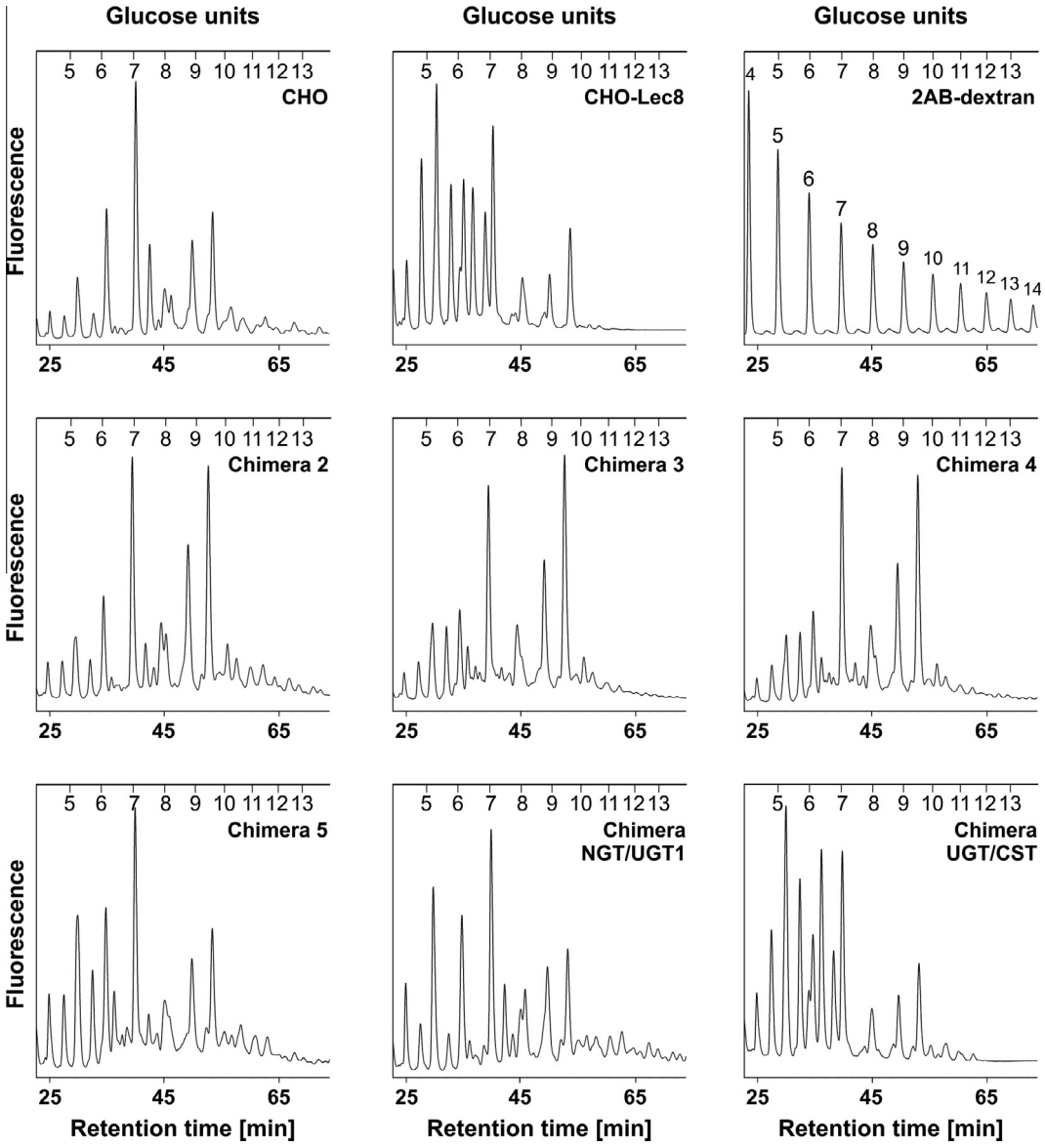

Fig. 2. Analysis of *N*-glycan profiles. *N*-glycans were enzymatically released from glycoproteins produced by the cells, fluorescently labeled with 2-AB, purified and separated on the normal phase GlycoSep N column using HPLC. Representative data out of two independent measurements are shown. Respective glycans are expressed in glucose units (GU).

according to those studies the majority of chimeric transporter sequence must be derived from UGT to preserve its activity. In contrast, our previous study showed that chimeric transporter constructed by the replacement of 169 C-terminal amino-acid residues of UGT with NGT is able to transport UDP-galactose with efficiency similar to the wild-type UGT [26]. It is also worth noting that NGT, when overexpressed in UGT-deficient cell lines, can partially restore galactosylation. This may indicate that the amount of endogenous NGT is insufficient to correct mutation defect, whilst overexpression of this protein can facilitate galactosylation in the mutant cells examined [24]. We hypothesize that NGT forms functional complexes with UGT, an therefore it is likely that excess of NGT may replace UGT in such complexes.

The main aim of this work was to determine the region within the N-terminal portion of UGT essential for galactosylation of *N*-glycans. We have demonstrated that chimeric protein composed of 113 amino-acid residues derived from the N-terminal portion of UGT in fusion with NGT was able to partially correct the mutant phenotype of CHO-Lec8 and MDCK-RCA^r cells. The chimeric transporter containing 148 N-terminal amino-acid residues of UGT fused to NGT was able to fully correct galactosylation defect. Although we were able to create protein where NGT was replaced with CST and fused with 35 N-terminal amino-acid residues of UGT, such chimeric transporter did not influence galactosylation reinstatement in these cells, with the exception of the production of one *N*-glycan structure absent in the mutant cells. To determine whether N-terminus is essential for UGT function, we constructed additional chimeric protein composed of five N-terminal amino-acid residues derived from NGT and fused with almost the entire UGT1 protein sequence (NGT/UGT1 chimera). Surprisingly, correction effect was significantly lower compared to chimeras 1 or 2, where longer N-terminal regions derived from UGT were analyzed. These data strongly suggest that N terminus of UGT is extremely important for its activity.

NGT, UGT1 and CST are localized in the GA membrane. Detailed studies showed that CST contains very strong motifs that strictly determine its localization in the medial and trans cisterns of the GA. Localization of NGT and UGT1 has not been studied in detail,

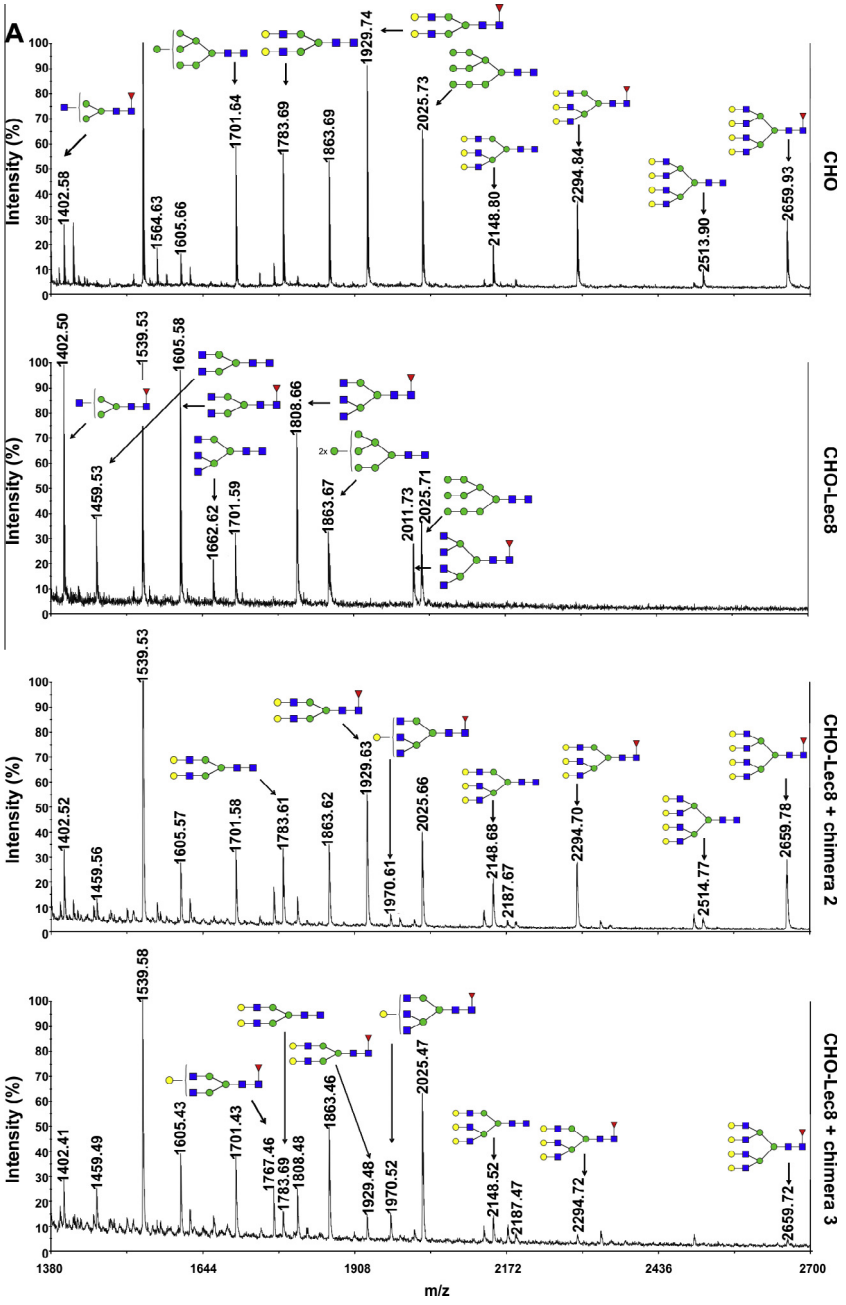

Fig. 3. Structural analysis of *N*-glycans. 2-AB labeled *N*-glycans derived from the appropriate cells were treated with neuraminidase and subjected to MALDI-TOF MS analysis carried out in positive ion mode with Na+ excess. Wild-type CHO, mutant CHO-Lec8 cells defective in UGT activity and mutant cells overexpressing chimera 2 or 3 (A) and mutant cells overexpressing chimera 4, 5, NGT/UGT1 or UGT/CST (B). Glycans were identified by comparing experimental data expressed in GU resulting from separation on a GlycoSep N column with data deposited in GlycoBase 3.0 (The National Institute for Bioprocessing Research and Training, Dublin, Ireland), followed by comparing these data with molecular weights of respective structures, experimentally determined by MALDI-TOF MS. *N*-Glycan composition was subsequently estimated using the GlycoMod tool. Representative data out of 2 independent measurements with a similar tendency are shown at the mass region exhibiting the most profound differences from selected clones. Blue squares, *N*-acetylglucosamine (GlcNAc); green circles, mannose (Man); yellow circles, galactose (Gal); red triangles, fucose (Fuc). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

but it is assumed that these proteins are located in the cis and medial cisterns of the GA [27,31–33]. Because UGT and NGT form heterologous complexes in the GA membrane [25], we hypothesize that effect exhibited by chimeric transporters overexpressed in the mutant cells lacking UGT activity might be induced by complex formation between endogenic NGT and UGT/NGT chimera or between endogenic NGT and NGT/UGT1 chimera. We also presume that these chimeras may be able to recreate functional complexes formed between NGT, UGT and *N*-acetylglucosamine glycosyltransferases. Such a complex would be fully functional and able to correct galactosylation defect. The subcellular localization of CST is different compared to NGT and UGT1, which is why we believe that CST does not belong to the same glycosylation complex. Although there is high amino-acid sequence similarity between UGT and NGT (approximately 59%) as well as between UGT and CST (approximately 54%) [34], chimeric protein composed of the two latter transporters did not correct galactosylation defect in analyzed cells. This strongly suggests that amino-acid sequence similarity is not decisive factor in the NSTs complex formation. We hypothesize that if CST was a part of the same complex, it would be

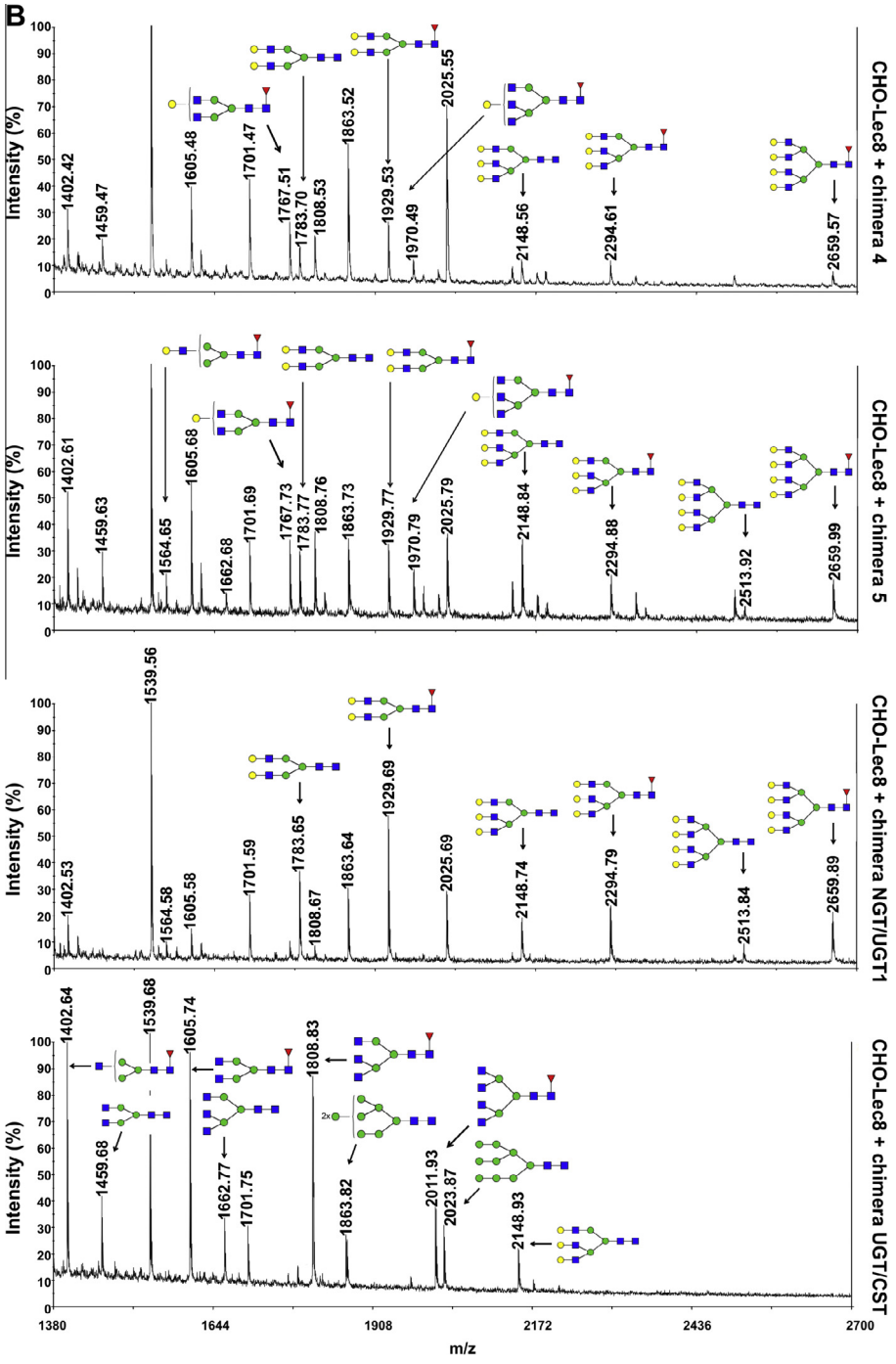

Fig. 3 (*continued*)

able, at least in part, to restore galactosylation in CHO-Lec8 and MDCK-RCA^r cells as it was observed for NGT [24]. Altogether, this might explain why the UGT/CST chimera did not have significant impact on galactosylation in UGT-deficient cells.

Surprisingly, such an extreme replacement of UGT transmembrane domains in the case of chimeric transporters did not have a negative impact on the protein activity, and chimeras 3, 4 and 5 corrected galactosylation defect to some extent in UGT deficient cell lines. Moreover, observed effect was much more visible compared to overexpression of NGT in these cells. In contrast, it was reported that point mutation in human UGT could be the reason of early-onset epileptic encephalopathy and developmental delay [15,16]. Similar observations have been made for human NGT, where point mutation in the gene encoding SLC35A3 results in autism spectrum disorders [23].

In conclusion, results gained in this study supports our hypothesis that biological function of UGT and NGT is coupled. It seems that UGT and NGT may replace function played by the partner when appropriate regions of these transporters are preserved, with the exception of short N-terminal region of UGT, which is crucial for UDP-Gal transport activity. In our previous study we demonstrated that NGT interacts with β-1,3-*N*-acetylglucosaminyltransferase 7 (β3GnT7), Mgat5 [21]. Our unpublished data show that this transporter forms heterologous complexes also with other

Mgats. Interestingly, all these glycosyltransferases are also in the close proximity to UGT1. This observation would be an additional corroboration, which may confirm our hypothesis concerning NGT and UGT functional relationship. Based on our findings, we suspect that UGT and NGT could be not simple nucleotide sugar transporters, but might be involved in proper positioning of glycosyltransferases, allowing specific substrate binding.

Acknowledgments

This work was supported by Grant No. 2011/03/B/NZ1/02084 from the National Science Center (NCN), Krakow, Poland. The funder had no role in study design, data collection and analysis, or preparation of the manuscript.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bbrc.2014.10.098.

References

[1] B. Hadley, A. Maggioni, A. Ashikov, C.J. Day, T. Haselhorst, J. Tiralongo, Structure and function of nucleotide sugar transporters: current progress, Comput. Struct. Biotechnol. J. 10 (2014) 23–32.
[2] J.M. Capasso, C.B. Hirschberg, Mechanism of glycosylation and sulfation in the Golgi apparatus: evidence for nucleotide sugar/nucleoside monophosphate and nucleotide sulfate/nucleoside monophosphate antiports in the Golgi apparatus membrane, Proc. Natl. Acad. Sci. U.S.A. 81 (1984) 7051–7055.
[3] M.E. Milla, C.A. Clairmont, C.B. Hirschberg, Reconstruction into proteoliposomes and partial purification of the Golgi apparatus membrane UDP-galactose, UDP-xylose, and UDP-glucuronic acid transporter activities, J. Biol. Chem. 267 (1992) 103–107.
[4] J. Tiralongo, A. Ashikov, F. Routier, M. Eckhardt, H. Bakker, R. Gerardy-Schahn, M. von Itzstein, Functional expression of the CMP-sialic acid transporter in *Escherichia coli* and its identification as a simple mobile carrier, Glycobiology 16 (2006) 73–81.
[5] N. Miura, N. Ishida, M. Hoshino, M. Yamauchi, T. Hara, D. Ayusawa, M. Kawakita, Human UDP-galactose translocator: molecular cloning of a complementary DNA that complements the genetic defect of a mutant cell line deficient in UDP-galactose translocator, J. Biochem. 120 (1996) 236–241.
[6] N. Ishida, N. Miura, S. Yoshioka, M. Kawakita, Molecular cloning and characterization of a novel isoform of the human UDP-galactose transporter, and of related complementary DNAs belonging to the nucleotide-sugar transporter gene family, J. Biochem. 120 (1996) 1074–1078.
[7] D. Maszczak-Seneczko, T. Olczak, L. Wunderlich, M. Olczak, Comparative analysis of involvement of UGT1 and UGT2 splice variants of UDP-galactose transporter in glycosylation of macromolecules in MDCK and CHO cell lines, Glycoconj. J. 28 (2011) 481–492.
[8] S. Oelmann, P. Stanley, R. Gerardy-Schahn, Point mutations identified in Lec8 Chinese hamster ovary glycosylation mutants that inactivate both the UDP-galactose and CMP-sialic acid transporters, J. Biol. Chem. 276 (2001) 26291–26300.
[9] M. Olczak, E. Guillen, Characterization of a mutation and an alternative splicing of UDP-galactose transporter in MDCK-RCAr mutant cell line, Biochim. Biophys. Acta 1763 (2006) 82–92.
[10] P. Stanley, Lectin-resistant CHO cells: selection of new mutant phenotypes, Somat. Cell Genet. 9 (1983) 593–608.
[11] A.W. Brandli, G.C. Hansson, E. Rodriguez-Boulan, K. Simons, A polarized epithelial cell mutant deficient in translocation of UDP-galactose into the Golgi complex, J. Biol. Chem. 263 (1988) 16283–16290.
[12] L. Toma, M.A. Pinhal, C.P. Dietrich, H.B. Nader, C.B. Hirschberg, Transport of UDP-galactose into the Golgi lumen regulates the biosynthesis of proteoglycans, J. Biol. Chem. 271 (1996) 3897–3901.
[13] N. Ishida, S. Yoshioka, M. Iida, K. Sudo, N. Miura, K. Aoki, M. Kawakita, Indispensability of transmembrane domains of Golgi UDP-galactose transporter as revealed by analysis of genetic defects in UDP-galactose transporter-deficient murine Had-1 mutant cell lines and construction of deletion mutants, J. Biochem. (Tokyo) 126 (1999) 1107–1117.
[14] E.B. Briles, E. Li, S. Kornfeld, Isolation of wheat germ agglutinin-resistant clones of Chinese hamster ovary cells deficient in membrane sialic acid and galactose, J. Biol. Chem. 252 (1977) 1107–1116.
[15] B.G. Ng, K.J. Buckingham, K. Raymond, M. Kircher, E.H. Turner, et al., Mosaicism of the UDP-galactose transporter SLC35A2 causes a congenital disorder of glycosylation, Am. J. Hum. Genet. 92 (2013) 632–636.
[16] H. Kodera, K. Nakamura, H. Osaka, Y. Maegaki, K. Haginoya, S. Mizumoto, et al., De novo mutations in SLC35A2 encoding UDP-galactose transporter cause early-onset epileptic encephalopathy, Hum. Mutat. 34 (2013) 1708–1714.
[17] N. Ishida, T. Kuba, K. Aoki, S. Miyatake, M. Kawakita, Y. Sanai, Identification and characterization of human Golgi nucleotide sugar transporter SLC35D2, a novel member of the SLC35 nucleotide sugar transporter family, Genomics 85 (2005) 106–116.
[18] T. Suda, S. Kamiyama, M. Suzuki, N. Kikuchi, K. Nakayama, et al., Molecular cloning and characterization of a human multisubstrate specific nucleotide-sugar transporter homologous to Drosophila fringe connection, J. Biol. Chem. 279 (2004) 26469–26474.
[19] A. Ashikov, F. Routier, J. Fuhlrott, Y. Helmus, M. Wild, et al., The human solute carrier gene SLC35B4 encodes a bifunctional nucleotide sugar transporter with specificity for UDP-xylose and UDP-*N*-acetylglucosamine, J. Biol. Chem. 280 (2005) 27230–27235.
[20] M. Muraoka, M. Kawakita, N. Ishida, Molecular characterization of human UDP-glucuronic acid/UDP-*N*-acetylgalactosamine transporter, a novel nucleotide sugar transporter with dual substrate specificity, FEBS Lett. 495 (2001) 87–93.
[21] D. Maszczak-Seneczko, P. Sosicka, T. Olczak, P. Jakimowicz, M. Majkowski, M. Olczak, UDP-*N*-acetylglucosamine transporter (SLC35A3) regulates biosynthesis of highly branched N-glycans and keratan sulfate, J. Biol. Chem. 288 (2013) 21850–21860.
[22] B. Thomsen, P. Horn, F. Panitz, E. Bendixen, A.H. Petersen, et al., A missense mutation in the bovine SLC35A3 gene, encoding a UDP-*N*-acetylglucosamine transporter, causes complex vertebral malformation, Genome Res. 16 (2006) 97–105.
[23] S. Edvardson, A. Ashikov, C. Jalas, L. Sturiale, A. Shaag, et al., Mutations in SLC35A3 cause autism spectrum disorder, epilepsy and arthrogryposis, J. Med. Genet. 50 (2013) 733–973.
[24] D. Maszczak-Seneczko, T. Olczak, P. Jakimowicz, M. Olczak, Overexpression of UDP-GlcNAc transporter partially corrects galactosylation defect caused by UDP-Gal transporter mutation, FEBS Lett. 585 (2011) 3090–3094.
[25] D. Maszczak-Seneczko, P. Sosicka, M. Majkowski, T. Olczak, M. Olczak, UDP-*N*-acetylglucosamine transporter and UDP-galactose transporter form heterologous complexes in the Golgi membrane, FEBS Lett. 586 (2012) 4082–4087.
[26] M. Olczak, D. Maszczak-Seneczko, P. Sosicka, P. Jakimowicz, T. Olczak, UDP-Gal/UDP-GlcNAc chimeric transporter complements mutation defect in mammalian cells deficient in UDP-Gal transporter, Biochem. Biophys. Res. Commun. 434 (2013) 473–478.
[27] D. Maszczak-Seneczko, T. Olczak, M. Olczak, Subcellular localization of UDP-GlcNAc, UDP-Gal and SLC35B4 transporters, Acta Biochim. Pol. 58 (2011) 413–419.
[28] K. Aoki, G.H. Sun-Wada, H. Segawa, S. Yoshioka, N. Ishida, M. Kawakita, Expression and activity of chimeric molecules between human UDP-galactose transporter and CMP-sialic acid transporter, J. Biochem. (Tokyo) 126 (1999) 940–950.
[29] K. Aoki, N. Ishida, M. Kawakita, Substrate recognition by UDP-galactose and CMP-sialic acid transporters. Different sets of transmembrane helices are utilized for the specific recognition of UDP-galactose and CMP-sialic acid, J. Biol. Chem. 276 (2001) 21555–21561.
[30] K. Aoki, N. Ishida, M. Kawakita, Substrate recognition by nucleotide sugar transporters. Further characterization of substrate recognition regions by analyses of UDP-galactose/CMP-sialic acid transporter chimeras and biochemical analysis of the substrate specificity of parental and chimeric transporters, Biol. Chem. 278 (2003) 22887–22893.
[31] N. Ishida, M. Ito, S. Yoshioka, G.H. Sun-Wada, M. Kawakita, Functional expression of human Golgi CMP-sialic acid transporter in the Golgi complex of a transporter-deficient Chinese hamster ovary cell mutant, J. Biochem. 124 (1998) 171–178.
[32] W. Zhao, T.L. Chen, B.M. Vertel, K.J. Colley, The CMP-sialic acid transporter is localized in the medial-trans Golgi and possesses two specific endoplasmic reticulum export motifs in its carboxyl-terminal cytoplasmic tail, J. Biol. Chem. 281 (2006) 31106–31118.
[33] S. Yoshioka, G.H. Sun-Wada, N. Ishida, M. Kawakita, Expression of the human UDP-galactose transporter in the Golgi membranes of murine Had-1 cells that lack the endogenous transporter, J. Biochem. 122 (1997) 691–695.
[34] H. Segawa, M. Kawakita, N. Ishida, Human and *Drosophila* UDP-galactose transporters transport UDP-*N*-acetylgalactosamine in addition to UDP-galactose, Eur. J. Biochem. 269 (2002) 128–138.