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Reduction in Golgi apparatus dimension in the absence of a residential protein, *N*-acetylglucosaminyltransferase V

Zhizhong Dong,
Division of Cell and Molecular Pathology, Department of Pathology, University of Zürich, 8091 Zürich, Switzerland

Christian Zuber,
Division of Cell and Molecular Pathology, Department of Pathology, University of Zürich, 8091 Zürich, Switzerland

Michael Pierce,
Complex Carbohydrate Research Center, Department of Biochemistry and Molecular Biology, University of Georgia, Athens, GA 30602, USA

Pamela Stanley, and
Department of Cell Biology, Albert Einstein College of Medicine, New York, NY 10461, USA

Jürgen Roth
Division of Cell and Molecular Pathology, Department of Pathology, University of Zürich, 8091 Zürich, Switzerland

Jürgen Roth: jurgen.roth@bluewin.ch

Abstract

Various proteins are involved in the generation and maintenance of the membrane complex known as the Golgi apparatus. We have used mutant Chinese hamster ovary (CHO) cell lines Lec4 and Lec4A lacking *N*-acetylglucosaminyltransferase V (GlcNAcT-V, MGAT5) activity and protein in the Golgi apparatus to study the effects of the absence of a single glycosyltransferase on the Golgi apparatus dimension. Quantification of immunofluorescence in serial confocal sections for Golgi α-mannosidase II and electron microscopic morphometry revealed a reduction in Golgi volume density up to 49 % in CHO Lec4 and CHO Lec4A cells compared to parental CHO cells. This reduction in Golgi volume density could be reversed by stable transfection of Lec4 cells with a cDNA encoding *Mgat5*. Inhibition of the synthesis of β1,6-branched *N*-glycans by swainsonine had no effect on Golgi volume density. In addition, no effect on Golgi volume density was observed in CHO Lec1 cells that contain enzymatically active Glc-NAcT-V, but cannot synthesize β1,6-branched glycans due to an inactive GlcNAcT-I in their Golgi apparatus. These results indicate that it may be the absence of the GlcNAcTV protein that is the determining factor in reducing Golgi volume density. No dimensional differences existed in cross-sectioned cisternal stacks between Lec4 and control CHO cells, but significantly reduced Golgi stack hits were

Correspondence to: Jürgen Roth, jurgen.roth@bluewin.ch.

observed in cross-sectioned Lec4 cells. Therefore, the Golgi apparatus dimensional change in Lec4 and Lec4A cells may be due to a compaction of the organelle.

Keywords

Golgi apparatus; *N*-acetylglucosaminyltransferase V; *N*-acetylglucosaminyltransferase I; CHO Lec4 cells; CHO Lec1 cells; β1,6-branched *N*-glycans; Golgi volume density; Golgi α-mannosidase II

Introduction

In higher eukaryotic cells, the Golgi apparatus forms a continuous branching and anastomosing ribbon consisting of stacks of cisternae bridged by non-compact, fenestrated regions, and tubular networks at its *cis* and *trans* face (Day et al. 2013; Klumperman 2011; Martínez-Alonso et al. 2013). Depending on cell-type, the number of cisternae making up the stack and the area comprising the *cis* and *trans* Golgi networks may vary (Han et al. 2013; Rambourg and Clermont 1997; Sengupta and Linstedt 2011; Uemura and Nakano 2013). While structurally complex, the Golgi apparatus is a highly dynamic organelle (Boncompain and Perez 2013; Colanzi et al. 1997; Cole et al. 1996; Polishchuk and Lutsenko 2013; Presley et al. 1998, 2002; Sciaky et al. 1997; Tillmann et al. 2013; Willett et al. 2013) and most important for cellular traffic (Boncompain and Perez 2013; Chia et al. 2013; Farquhar and Hauri 1997; Machamer 2013; Polishchuk and Lutsenko 2013; Sandvig et al. 2013; Tillmann et al. 2013; Warren 2013; Willett et al. 2013). Not surprisingly therefore, variations in the size of the Golgi apparatus or parts thereof have been reported to depend on the functional state of the cell and to become altered under disease conditions (Clermont et al. 1995; Fujita et al. 2002; Griffiths et al. 1989; Maeda et al. 2008; Noske et al. 2008; Rambourg et al. 1993; Sengupta and Linstedt 2011; Stieber et al. 1998).

There is much interest in understanding the molecular mechanisms responsible for generating and maintaining the integrity of the Golgi apparatus, and various types of proteins involved in this process have been identified. While microtubules and associated proteins are important for positioning the Golgi apparatus (Kreis et al. 1997; Presley et al. 1997; Zhu and Kaverina 2013), microtubule disassembly results in Golgi apparatus vesiculation (Thyberg and Moskalewski 1999). Cytoplasmic dynein and probably other motor proteins as well as actin filaments seem to be additionally involved in the formation and maintenance of Golgi apparatus structure (Allan 1996; Burkhardt 1998; Dippold et al. 2009; Egea et al. 2006, 2013; Harada et al. 1998; Yadav et al. 2012). Much information about the Golgi stack reassembly has been obtained through studies on the Golgi apparatus during mitosis (Acharya and Malhotra 1996; Barr and Warren 1996; Rabouille and Kondylis 2007; Shorter and Warren 2002). Golgi reassembly stacking proteins (Barr et al. 1997; Feinstein and Linstedt 2008; Puthenveedu et al. 2006; Sengupta et al. 2009; Shorter et al. 1999; Xiang and Wang 2010), a *cis* Golgi matrix protein, GM130 (Lowe et al. 1998; Marra et al. 2007; Nakamura et al. 1995, 1997), an NSF-like ATPase, p97, and NSF together with SNAPs and p115, a vesicle docking protein (Nelson et al. 1998; Rabouille et al. 1995b, 1998), seem to be important for the formation of the cisternal stack. In interphase cells,

proteins cycling between the endoplasmic reticulum and the Golgi apparatus, such as Rab1b (Haas et al. 2007; Monetta et al. 2007; Romero et al. 2013; Tomas et al. 2012; Wilson et al. 1994), Arf1 (Boal et al. 2010; Lin et al. 2011; Manolea et al. 2008; Zhang et al. 1994) and TAP/p115 (Nelson et al. 1998; Puthenveedu and Linstedt 2001; Radulescu et al. 2011), are involved in maintaining Golgi apparatus morphology. Furthermore, the spectrin membrane skeleton (Nelson et al. 1998) is required for Golgi apparatus architecture.

Glycosyltransferases are Golgi residential proteins (Dunphy and Rothman 1983; Goldberg and Kornfeld 1983; Roth and Berger 1982; Roth et al. 1985), and the activity of a particular subset results in the synthesis of complex *N*-glycans (Kornfeld and Kornfeld 1985; Zuber and Roth 2009). Together with other proteins, glycosyltransferases seem to be involved in the formation and maintenance of the Golgi apparatus architecture. An illustrative example for this function was provided by studies on the parasitic protozoan *Giardia lamblia* (Lujan et al. 1995). During differentiation from throphozoites to cysts, the developmental induction of Golgi enzyme activities correlated with the appearance of a morphologically identifiable Golgi apparatus, which was absent in non-encysting cells. There are also data that *N*-acetylglucosaminyltransferase I (GlcNAcT-I) is involved in maintaining the structure of the cisternal stack (Nilsson et al. 1996). Replacement of part or the entire membrane-spanning domain of this type II membrane protein (Kumar et al. 1990) with leucine residues transformed the part of the Golgi stack housing the mutated GlcNAcT-I from flat cisternae into tubulo-vesicular membranes.

A number of Chinese hamster ovary (CHO) cells defective in glycosylation, due to mutated glycosyltransferases, have been isolated and characterized (Stanley and Ioffe 1995). In the CHO Lec4 and Lec4A cell mutants, *N*-acetylglucosaminyltransferase V (GlcNAcT-V) activity is affected (Chaney et al. 1989; Weinstein et al. 1996). Glc NAcT-V adds a β1,6 *N*-acetylglucosamine (GlcNAc) branch to the core α1,6-mannose (Man) in complex *N*-linked glycans attached to proteins. Lec4 cells lack GlcNAcT-V activity, due to a base insertion at nucleotide 822 of the *Magt*5 gene that shifts the open reading frame. A 155 amino acid truncated GlcNAcT-V (instead of a full length 740 amino acid enzyme) may be synthesized, which consists of the cytosolic and transmembrane domains and a short piece of the stem region. The fate of this truncated GlcNAcT-V is not known, but recognition by the protein quality control and subsequent proteolysis by ER-associated protein degradation may occur, as shown for other proteins (Roth et al. 2010). By contrast, CHO Lec4A cells have GlcNAcT-V activity equivalent to that of parental CHO cells in cellular homogenates. However, a single-point mutation, changing leucine to arginine at position 188, causes GlcNAcT-V to mislocalize to the endoplasmic reticulum and consequently to be functionally inactive in vivo. In both CHO Lec4 and Lec4A cells, complex *N*-glycans lack a β1,6 GlcNAc branch. In addition, the Golgi apparatus in Lec4A, and probably also in Lec4 mutant cells, lacks the residential protein GlcNAcT-V.

The structure of the Golgi apparatus in cells stably transfected and therefore overexpressing different glycosyltransferases appears normal (Lee et al. 1989; Nilsson et al. 1993; Rabouille et al. 1995a). However, more recent quantitative studies (Guo and Linstedt 2006) provided evidence that overexpression of the Golgi glycosyltransferase GlcNAcT-II results in a 1.3-

fold increase in the size of the Golgi apparatus and that this change is sustained depending on interaction of GlcNAcT-II with COPII-coated transport carriers.

Currently, there is no information on the effects on Golgi apparatus structure and size of eliminating a Golgi residential protein such as a glycosyltransferase. Here, we demonstrate that the absence of the Golgi apparatus residential protein GlcNAcT-V causes a reduction in the dimensional size of the Golgi apparatus without affecting its general architecture.

Materials and methods

Cell lines

Chinese hamster ovary mutant cell lines Pro^{-}Lec4.7b, Pro^{-}Lec4A.12.2, Pro^{-}5, and Pro^{-}5 Lec1.3c were previously characterized (Chaney et al. 1989; Puthalakath et al. 1996; Stanley et al. 1982; Weinstein et al. 1996). Cells were maintained in α-MEM supplemented with 10 % fetal calf serum, glutamine and DNA, and RNA precursors.

Transfection of Lec4 cells with *Mgat5* cDNA

Rat *Mgat5* cDNA (Shoreibah et al. 1993) was subcloned into the *Eco*RI site of the pcDNA3 expression vector (Invitrogen, San Diego, CA). Lec4 cells were plated on 35 mm cell culture dishes and grown in α-MEM medium supplemented with 10 % fetal calf serum. Plasmid DNA (1 μg per 35 mm culture dish) was transfected into Lec4 cells, using Fugene™ 6 transfection reagent (Boehringer Mannheim, Germany) according to the manufacturer's protocol. Following transfection, clonal cell lines were established by selection for G418 resistance and tested for cell surface expression of β1,6 GlcNAc-branched *N*-glycans using digoxigenin-conjugated leukoagglutinating *Phaseolus vulgaris* lectin (dig L-PHA; Boehringer Mannheim, Germany) as described below. The positive clonal cell lines were designated Lec4 GnTV-N5, Lec4 GnTV-N10, and Lec4GnTVN30. Lec4 cells were mock-transfected with the pcDNA3 expression vector as described above, and clonal cell lines were established and designated Lec4 pcDNA3.

GlcNAcT-V assay

Cells were grown to confluence, harvested in 50 mM PBS, and concentrated to a pellet by centrifugation in microfuge tubes. Cell pellets were frozen on dry ice and shipped to Michael Pierce (Athens, GA, USA). For the assay, an approximately equal volume of ice-cold buffer (0.1 M MES, pH 6.5) was added to each pellet, followed by rapid thawing and sonication as described (Palcic et al. 1990). Each assay tube contained 10^6 cpm of UDP-[^{3}H]-GlcNAc (25 cpm/pmol) and 10 nmol of synthetic trisaccharide acceptor (octyl 6–O–[2–O–(2-acetamido-2-deoxy-β-D-glucosyl-pyranosyl)-α-d-mannopyranosyl[-β-D-glucopyranoside) that were dried under vacuum in a 1.5-ml tube. The dried contents of each tube were resuspended in 0.05 ml of assay buffer (0.05 mM MES, pH 6.5, 2.0 % Triton X-100). Next, 5 μl of cell lysate was added to the tube, and the contents gently mixed by pipetting. Tubes were incubated for 6 h at 37 °C. Reactions were quenched by adding 0.5 ml water. Radiolabeled product was isolated using a C18 Sep-Pak (Waters) cartridge, eluted with 2 ml of methanol, and subjected to scintillation counting. Assay values for each extract were conducted in duplicate or triplicate and averaged. Aliquots of cell lysates were

removed prior to enzymatic assay for protein determination in triplicate using the Bio-Rad protein assay for calculation of GlcNAcT-V specific activities.

Detection of β1,6-branched *N*-glycans with L-PHA

For lectin blots (Zuber et al. 1998), cells grown to 50–80 % confluence were removed by EDTA treatment (0.1 % EDTA-PBS at 37 °C for 10 min), pelleted by centrifu gation (1,500×*g*, 5 min at 4 °C) and lysed in three vol umes of 1 % Triton X-100 containing 1 mM AEBSF and 1 % aprotinin on ice for 30–40 min. After centrifugation (8,000×*g*, 10 min at 4 °C), the supernatant was denatured by boiling in Laemmli buffer. Samples were electrophoretically resolved in 3–10 % gradient polyacrylamide gels (120 μg protein per lane) and transferred to nitrocellu-lose. After blocking in 0.05 % Tween 20 and 1 % BSA in PBS, nitrocellulose strips were incubated with dig L-PHA (2 μg/ml for 2 h), rinsed, and incubated in alkaline phosphatase-conjugated polyclonal sheep anti-dig $(Fab)_2$ fragments (5,000-fold diluted, Boehringer Mannheim, Germany) for 1 h. Alkaline phosphatase activity was revealed by the NBT-BCIP color reaction according to the manufacturer's instructions.

For light microscopic cell surface staining, cells grown on coverslips were fixed in 2 % paraformaldehyde in Earle's balanced salt solution (EBSS) (pH 7.2–7.4) for 5 min at 37 °C, and then at ambient temperature for 25 min, and the fixation was stopped by immersing the cover slips in 50 mM NH_4Cl in PBS for 30 min (Roth et al. 1989). The coverslips were blocked in 2 % BSA in PBS for 30 min, incubated with digoxigenated (dig) L-PHA (2 μg/ml) for 2 h, followed by three rinses in PBS containing 0.1 % BSA (5 min each) and rhodamine-conjugated sheep anti-digoxigenin $(Fab)_2$ fragments (200-fold diluted in 2 % BSA in PBS) for 1 h. After rinses, coverslips were embedded in Mowiol and mounted on glass slides. Cells were observed with a Leica TCS4D confocal laser scanning microscope.

For scanning electron microscopy, cells grown on glass coverslips were fixed in 2 % paraformaldehyde–0.1 % glutaraldehyde in EBSS as described above. Cells were incubated with directly 8 nm gold particle-labeled L-PHA (absorbance at 525 nm = 0.12) for 1 h. Following rinses and critical point drying, samples were coated and observed in a scanning electron microscope under standard and backscattering mode (Hermann et al. 1996).

Golgi α-mannosidase II immunostaining and morphometric analysis

Cells grown on coverslips were fixed as described above and permeabilized with PBS containing 0.15 % saponin and 0.1 % BSA for 15 min. After rinses with buffer, the cover-slips were incubated with rabbit anti-Golgi α-mannosidase II (1,000-fold diluted in PBS containing 0.5 % saponin and 0.1 % BSA; antibody kindly provided by Dr. Kelley More-men, University of Georgia, Athens, GA) for 2 h, rinsed in PBS containing 0.1 % BSA, and incubated in rhodamineconjugated affinity-purified goat anti-rabbit IgG (1,000-fold diluted in 0.1 % BSA-PBS; Jackson ImmunoResearch Laboratories Inc., West Grove, PA) for 1 h, rinsed with 0.1 % BSA in PBS and distilled water and embedding in Mowiol. After storage overnight at 4 °C, the cells were observed under a Leica TCS4D confocal laser scanning microscope. Cells grown in the presence of swainsonine (see below) were processed in the same way.

We estimated Golgi apparatus volume density by confocal laser scanning microscopy based on differential voxel counting of consecutive serial optical sections. As per each experimental set, three different views containing 30–40 cells were recorded randomly with a 40× objective and consecutive serial optical sections (0.4-μm optical resolution and optical section distance) were taken from the top to the bottom of each cell. The digitized images were analyzed using Photoshop®. Since Golgi α-mannosidase II has a broad Golgi distribution in CHO cells (Velasco et al. 1993), it was used to delineate the Golgi apparatus by immunofluorescence. The entire cells could be discerned by the presence of a faint cytoplasmic fluorescence. Using a histogram program, the sum of pixels for Golgi α-mannosidase II immunofluorescence and for the cytoplasmic fluorescence was obtained separately. From the ratio of the sum of pixels for Golgi α-mannosidase II immunofluorescence and the sum of pixels for the remaining cytoplasm, the volume density of the Golgi apparatus was calculated using the following equation:

$$v/V(\mathrm{G/C}) = \frac{\sum_{i=\mathrm{m}}^{i=255} g_i}{\sum_{i=\mathrm{n}}^{i=255} g_i}$$

In this equation, v/V (G/C) represents the volume density of the Golgi apparatus; m and n represent the initial gray level for the Golgi apparatus (G) and cytoplasm (C), respectively; and g_i represents the number of pixels at each gray level *i*.

Transmission electron microscopy and morphometric analysis

Cells grown on Thermanox coverslips (Nalge Nunc. International, Roskilde, Denmark) were fixed in 2 % paraformaldehyde–1 % glutaraldehyde in EBSS (pH 7.2–7.4) for 5 min at 37 °C and then at ambient temperature for 25 min. After rinses with buffer and immersion in 50 mM NH_4Cl in PBS for 30 min (Roth et al. 1989), cells were postfixed in 1 % reduced osmium tetroxide (Karnovsky 1971) for 30 min, rinsed in distilled water and stained with 2 % aqueous uranyl acetate for 15 min. The cells were embedded in Epon according to standard protocol. Ultrathin sections were contrasted with uranyl acetate and lead citrate. From each cell line, photographs were taken randomly from 30 to 40 cross-sectioned cells. In addition, photographs at the original magnification of 25,000 were taken from each cell containing the Golgi apparatus in the cross section. The number of cisternae per Golgi stack, the length and thickness of cisternal stacks, as well as the area occupied by the Golgi stacks and the size of the cell profile in the cross sections were measured on digitized photographic negatives. The volume density of the Golgi apparatus was calculated by the ratio of the surface area of the Golgi stacks, and the surface size of the cell profile in the cross sections (Weibel 1979).

Swainsonine treatment of cell lines and lectin spot plots

Swainsonine (1 μg/ml; Sigma Chemical Co, Ltd., St. Louis, MO) was added to the culture medium of cells growing at 30–40 % confluence, and the cells were analyzed 12 and 24 h after addition of the drug. For L-PHA spot blot analysis, cells were harvested and lysed as described above. About 1 μl aliquots of serially diluted cell lysates were pipetted onto nitrocellulose strips and air-dried, and β1,6-branched *N*-glycans detected with dig L-PHA as

described above. The quantitative analysis was performed with Wincam® software (Camtek Oy, Vantaa, Finland). Using the gray levels of the serial dilutions of the zero time-point homogenates as reference, the gray levels (relative percentage) of the spots from the homogenates of the cells treated with swainsonine for 12 and 24 h were estimated.

Results

Expression of β1,6-branched *N*-glycans in various CHO cell lines

Parent and mutant CHO cell lines were studied by confocal laser scanning microscopy and lectin-gold scanning electron microscopy as well as by lectin blotting using the leukoagglutinating *Phaseolus vulgaris* lectin (L-PHA) to detect β1,6-branched complex *N*-glycans (Cummings and Kornfeld 1982), the product of GlcNAcT-V. While wild-type CHO Pro^{-}5 cells were reactive with L-PHA, CHO Lec4 and Lec4A mutant cells lacking GlcNAcT-V and mock-transfected Lec4pcDNA3 cells were unreactive (Fig. 1a, b, e, f). The three clonal cell lines stably expressing GlcNAcT-V exhibited cell surface L-PHA staining undistinguishable from that observed for CHO Pro^{-}5 cells (Fig. 1c, g). In L-PHA blots, two major bands at 140 and 85 kDa and several minor bands were evident in parent CHO and Lec4 GlcNAcT-V cells lines (Fig. 1d), but CHO Lec4, CHO Lec4A, and mock-transfected CHO Lec4 cell glycoproteins did not bind L-PHA (data not shown). Different GlcNAcT-V activity levels were found in the transfected cell lines (data not shown). The Lec4 GnTV-N5 transfectants had enzyme activity similar to CHO Pro^{-}5 cells and, based on densitometric evaluation of spot blots, synthesized similar amounts of β1,6-branched *N*-glycans. Therefore, CHO Lec4 GnTV-N5 cells were used in most comparative analyses.

Golgi apparatus dimension is reduced in the absence of GlcNAcT-V

To measure the dimension of the Golgi apparatus at the light microscopic level, immunofluorescence for Golgi α-mannosidase II, a *cis*/medial Golgi resident protein in CHO cells (Velasco et al. 1993), was quantified by confocal laser scanning microscopy (Fig. 2). As detailed in Table 1, the volume density of the Golgi apparatus of CHO Lec4 and CHO Lec4A cells, as well as of CHO Lec4 pcDNA3 cells, was significantly smaller to that of CHO parental cells and of the three clonal CHO Lec4 GnTV cell lines. By electron microscopy, no differences in the structure of the Golgi apparatus were apparent between CHO Pro^{-}5 (Fig. 3a), CHO Lec4 (Fig. 3b), and CHO Lec4 GnTV (Fig. 3c). The results of the electron microscopic morpho-metric analyses are presented in Table 2 and confirm the subjective impression. The mean number of Golgi stack hits per cross-sectioned CHO Lec4 cells was reduced by 31–42 % as compared to CHO Pro^{-}5 and CHO Lec4 GnTV cells. Therefore, the volume density of the Golgi apparatus was significantly smaller in CHO Lec4 cells (reduced by 37–49 %) as compared to CHO Pro^{-}5 and CHO Lec4 GnTV cells. Interestingly, the mean number of cisternae per stack, as well as the horizontal and vertical dimension of the Golgi cisternal stacks, was similar in all cell lines studied (Table 2). Therefore, the most important contribution for the reduced volume density of the Golgi apparatus in CHO Lec4 cells came from the reduced Golgi stack hits in cross-sectioned cells. In order to obtain more insight into this phenomenon, we analyzed the distribution pattern of several other parameters. We found that the number of cisternae per stack, as well as the horizontal and vertical dimension of the Golgi stacks, had a Poisson distribution for

each cell line (not shown). However, the distribution of the Golgi apparatus volume density was considerably different between CHO Lec4 cells and CHO Pro^{-}5 as well as CHO Lec4 GnTV cells (Fig. 4). The number of cross-sectioned cells not exhibiting Golgi stacks was much higher in Lec4 cells than in Pro^{-}5 and Lec4 GnTV. N5 cells. This parameter was the main contributor to differences in the mean value of Golgi apparatus volume density (Table 3).

Golgi apparatus dimension is not influenced by the absence of GlcNAcT-V glycosylation product

At this point of the study, the question arose: Is the physical absence of the GlcNAcT-V protein in the Golgi apparatus, and/or the lack of its enzymatic activity and glycosylation products, the basis for the changes in Golgi apparatus dimension in Lec4 cells? Unfortunately, no specific GlcNAcT-V inhibitor was available for in vivo studies with intact cells. Thus, to address this question indirectly, we investigated CHO Lec1 cells, which contain an enzymatically inactive GlcNAcT-I protein in the Golgi apparatus (Kumar et al. 1990; Puthalakath et al. 1996; Stanley et al. 1975). As a consequence, no acceptor substrate for GlcNAcT-V is synthesized in Lec1 cells. Thus, although they contain enzymatically active GlcNAcT-V in their Golgi apparatus, β1,6-branched *N*-glycans cannot be synthesized. We found that the volume density of the Golgi apparatus in CHO Lec1 cells, based on the evaluation of Golgi α-mannosidase II immunofluorescence, was not different from that of Pro^{-}5 or Lec4 GnTV cells (Table 4). Second, we used swainsonine, an inhibitor of Golgi α-mannosidase II activity, to block the synthesis of acceptor substrate for GlcNAcT-V. Both CHO Pro^{-}5 cells and CHO Lec4 GnTV cells treated for 24 h with 1 μg/ml of swainsonine contained less than 10 % L-PHA reactivity, as compared to control or swainsonine-treated CHO Lec4 cells (Fig. 5). However, the swainsonine treatment had no influence on the dimension of the Golgi apparatus in CHO Pro^{-}5 and CHO Lec4 GnTV cells, or in CHO Lec4 cells (Table 4).

Discussion

The present studies were undertaken to investigate whether the absence of a single glycosyltransferase protein, and/or the product of its transferase activity, has an influence on the morphology of the Golgi apparatus. For this, we have made use of CHO cells lines with mutations in glycosyltransferase genes (Patnaik and Stanley 2006) and have focused on GlcNAcT-V. GlcNAcT-V with a molecular mass of 95 kDa is a comparatively large glycosyltransferase of the Golgi apparatus (Shoreibah et al. 1993). It initiates the synthesis of tri- and tetra-antennary complex *N*-glycans, whose expression is associated with malignancy and the metastatic potential of tumor cells (Dennis 1999; Dennis et al. 1987; Granovsky et al. 2000; Partridge et al. 2004; Seelentag et al. 1998). The absence of tri- and tetraantennary, β1,6-branched *N*-glycans has no influence on the viability of CHO Lec4 and CHO Lec4A cells although they differ in gross morphology from the parental cells (Stanley and Sudo 1981). The present study demonstrates that the defect in CHO Lec4 and CHO Lec4A cells is associated with a significant decrease in the volume density of their respective Golgi apparatus. This defect could be fully compensated by stable transfection of a cDNA encoding GlcNAcT-V into CHO Lec4 cells. The analysis of Golgi apparatus

dimension in cell lines treated with swainso-nine to block the synthesis of β1,6-branched *N*-glycans, or in CHO Lec1 cells, which cannot synthesize the acceptor substrate for GlcNAcT-V, provides strong evidence that the observed change of volume density in CHO Lec4 and CHO Lec4A cells does not simply result from the synthesis of truncated *N*-glycans. Rather it seems that the absence of the GlcNAcT-V protein in these cell lines leads to the change in Golgi apparatus volume density. This assumption is also supported by our analysis of CHO Lec1 cells, which contain enzymatically inactive Golgi apparatus located Glc-NAcT-I (Puthalakath et al. 1996) and which have the same Golgi apparatus dimension as the parental CHO Pro^{-}5 cell line. In this context, it is interesting to note that the Golgi apparatus in wild-type yeast *Saccharomyces cerevisiae* lacks clearly defined stacks of cisternae and appears in the form of single cisternae or individual networks of tubules (MorinGanet et al. 1998; Preuss et al. 1992; Rambourg et al. 1995). It is tempting to speculate that the structurally simple Golgi apparatus of yeast cells may be related to the low number of Golgi glycosyltransferases, essentially five mannosyltransferases (Herscovics and Orlean 1993; Lehle and Tanner 1995), as compared to the large families of glycosyltransferases existing in the Golgi apparatus of higher eukaryotic cells.

The reduction in volume density of the Golgi apparatus by up to 49 % in the absence of GlcNAcT-V is surprising even if one considers its molecular mass of 95 kDa (Shoreibah et al. 1993). We noticed from electron microscopic analyses that the number of cisternae per Golgi stacks, as well as the length and the thickness of the Golgi cisternal stacks, was the same in parental and the CHO Lec1 cell mutant. Thus, in contrast to the replacement of part or the entire membrane-spanning domain of GlcNAcT-I by leucine, which disrupted part of cisternal structure (Nilsson et al. 1996), the absence of GlcNAcTV protein was compatible with normal cisternal structure. However, our quantitative analysis revealed that the mean number of Golgi stack hits per cell cross section was clearly reduced in CHO Lec4 cells as compared to CHO Pro^{-}5 cells and CHO Lec4 cells stably expressing GlcNAcT-V.

How could these data be related to the measured reduction in the volume density of the Golgi apparatus in CHO Lec4 cells? All current evidence indicates that the inter-phase Golgi apparatus in higher eukaryotic cells can be regarded as a single organelle composed of numerous dynamic and interrelated units (Hermo and Smith 1998; Rambourg and Clermont 1997). Non-compact regions composed of tubules link these units, which correspond to compact cisternal regions, to each other laterally, and therefore forming a twisting ribbon that bifurcates and rejoins. Although there can be variability in the compact cisternal regions, the mean number of cisternae in one stack, and the size of the units, can be considered to be cell-type specific and relatively constant under steady-state conditions. One possible explanation for the present results could be that the unit size of the Golgi apparatus is reduced in the absence of GlcNAcT-V. Although a reduction in the number of Golgi apparatus units cannot be ruled out, this seems rather unlikely. A more likely explanation, which would account for the reduction in Golgi apparatus volume density, could be a change in the overall three-dimensional shape of the organelle, resulting in a more compact structure in CHO Lec4 and CHO Lec4A cells. The absence of GlcNAcT-V may result in secondary effects on, for example, interactions with intercisternal matrix proteins and other

protein– protein interactions, which have been proposed to be of importance for Golgi apparatus structure (Sengupta and Linstedt 2011).

In summary, our results indicate that the absence of the Golgi residential protein GlcNAcT-V results in the adaptive reduction in volume density of the Golgi apparatus without altering its overall architecture. Although the mechanism leading to this phenomenon remains to be clarified, this finding adds a novel aspect to the structural flexibility of this dynamic steady-state organelle.

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Abbreviations

GlcNAcT-I	*N*-acetylglucosaminyltransferase I
GlcNAcT-V, MGAT5	*N*-acetylglucosaminyltransferase V
CHO	Chinese hamster ovary cells
GlcNAc	*N*-acetylglucosamine
Man	Mannose
L-PHA	Leukoagglutinating *Phaseolus vulgaris* lectin
Dig	Digoxigenin

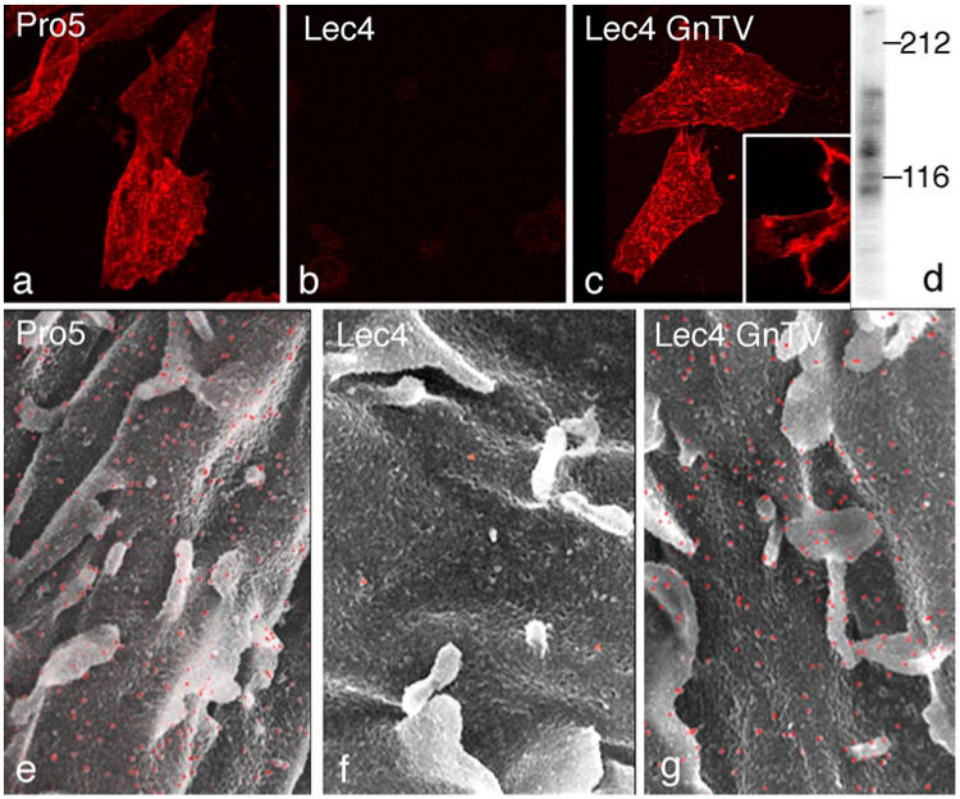

Fig. 1.

Demonstration of β1,6-branched *N*-glycans on the CHO cell surface using dig L-PHA and confocal laser scanning microscopy (**a**–**c**) and lectin-gold scanning electron microscopy (**e**–**g**). The CHO Pro^{-}5 cells (**a**, **e**) and CHO Lec4 GnTV-N5 cells (**c**, **g**) showed positive cell surface staining with microvilli being strongly positive. In contrast, CHO Lec4 cells (**b**, **f**) showed no specific cell surface labeling. ×1,100 (**a**–**c**), ×9,000 (**e**–**g**). Dig L-PHA—blot of homogenates from CHO Lec4 GnTV cells revealed two major bands at 140 and 85 kDa and several minor bands (**d**)

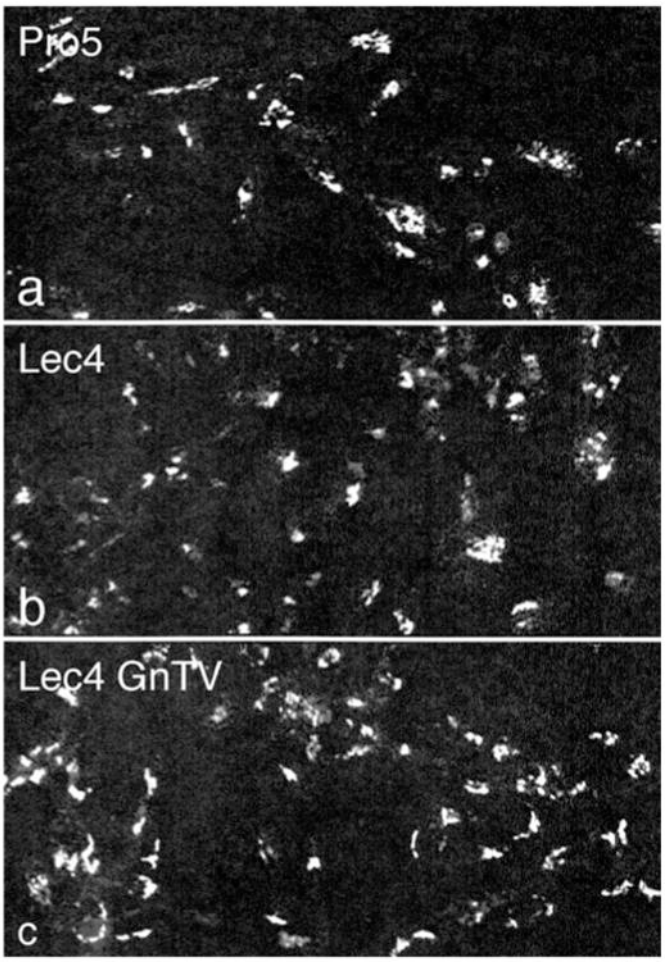

Fig. 2.

Confocal laser scanning immunofluorescence for Golgi α-mannosidase II. CHO Pro⁻5 cells (**a**), CHO Lec4 cells (**b**), and CHO Lec4 GnTV-N5 cells (**c**) exhibit a typical perinuclear Golgi staining pattern in a single confocal optical section (×560)

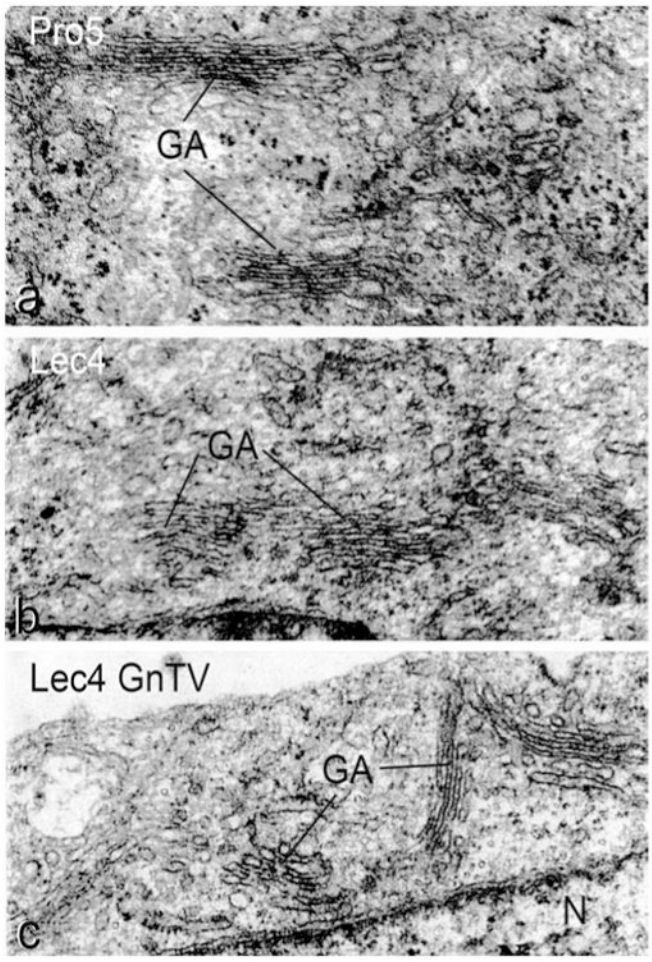

Fig. 3.

Transmission electron micrographs showing the morphology of the Golgi apparatus (GA) in CHO Pro^{-}5 cells (**a**), CHO Lec4 cells (**b**), and CHO Lec4 GnTV-N5 cells (**c**). Note the structural similarity in appearance of the Golgi apparatus in the different cells lines. *N* part of the nucleus (×41,300)

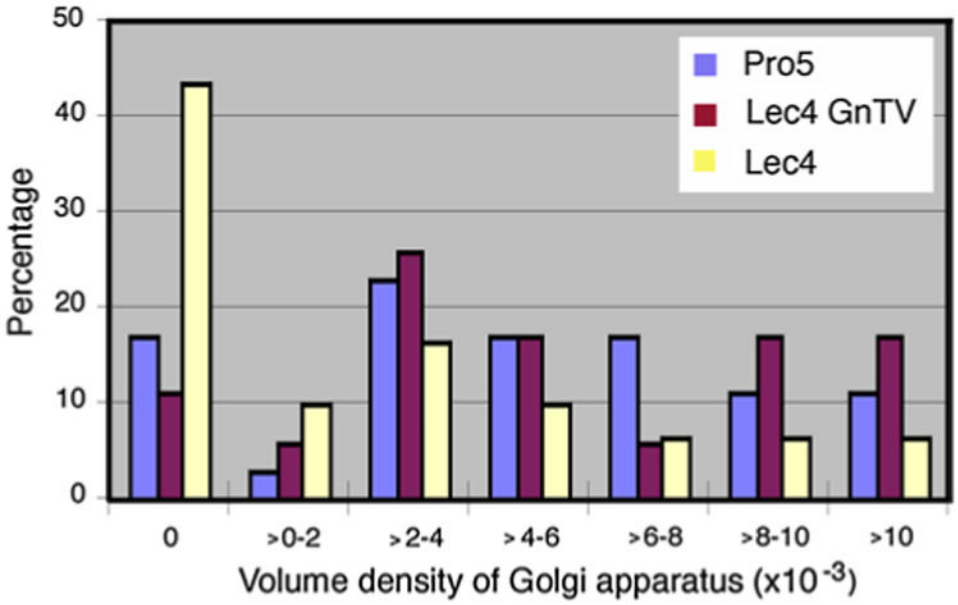

Fig. 4.

The distribution pattern of volume density of the Golgi apparatus in the various CHO cell lines. The number of cross-sectioned cells containing no cisternal stacks was much higher in CHO Lec4 cells than in CHO Pro^{-}5 as well as CHO Lec4 GnTV-N5 cells

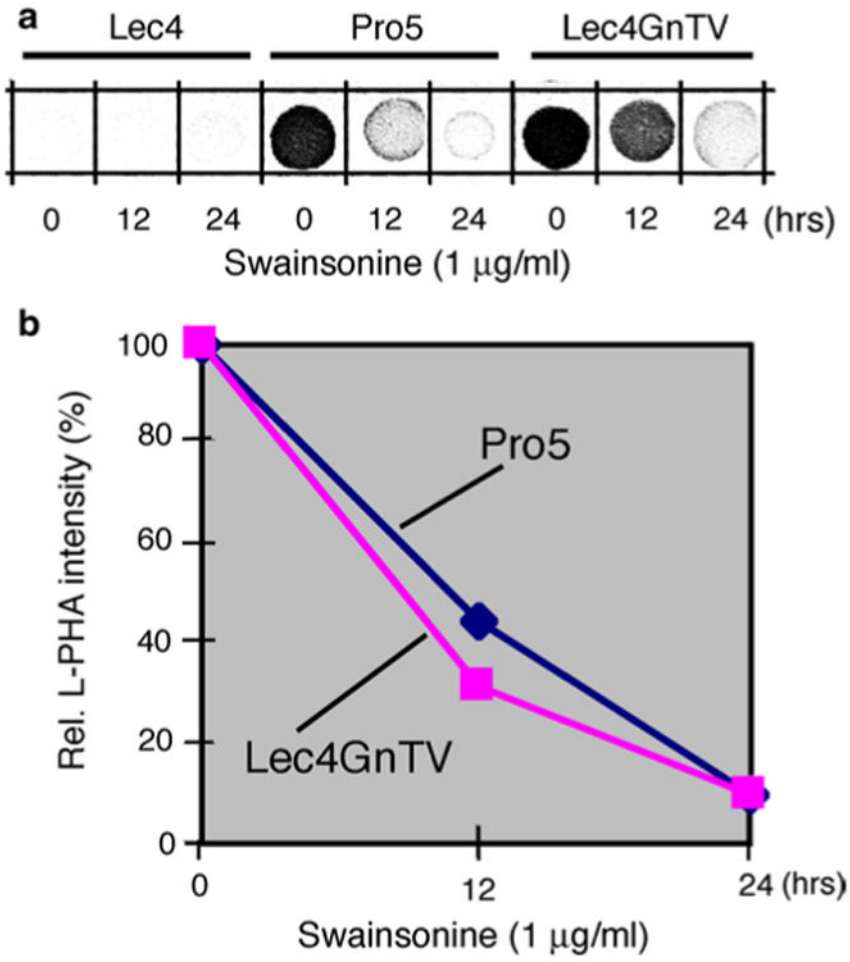

Fig. 5.

Reduction in the β1,6-branched *N*-glycans in CHO cells after swainsonine treatment. Swainsonine (1 μg/ml) was added to the cell culture medium, and cells were collected after 0, 12, and 24 h of treatment. Homogenates were processed for L-PHA spot blots, and the results densitometrically analyzed. The CHO Lec4 cells served as control

Table 1

Morphometric analysis of the Golgi mannosidase II immunofuorescence

Cells	GlcNAcT-V protein	Exp. 1		Exp. 2		Exp. 3	
		v/*V*[a]	*R* (%)[b]	*v*/*V*[a]	*R* (%)[b]	*v*/*V*[a]	*R* (%)[b]
Pro^{-}5	+	30 ± 1[c]	43	27 ± 4[c]	44	28 ± 1[c]	36
Lec4	−	17 ± 1	–	15 ± 1	–	18 ± 1	–
Lec4 GnT-N5	+	25 ± 1[c]	32	26 ± 1[c]	42	27 ± 3[c]	33
Lec4 GnT V-N10	+	25 ± 5[d]	32	–		–	
Lec4 GnT V-N30	+	30 ± 5[c]	43	–		–	
Lec4 PcDNA3	−	17 ± 4	–	–		–	
Lec4A	+[e]	18 ± 1	6	–		–	

[a] Volume density of Golgi apparatus, data are expressed as mean ± SD. Three different views were evaluated in each experiment

[b] Reduction rate of the volume density of the Golgi apparatus in Lec4 cells as compared to other cell lines

[c] Indicates significant difference as compared with Lec4, Lec4 PcDNA3, or Lec4A cells ($p < 0.01$)

[d] Indicates significant difference as compared with Lec4, Lec4 PcDNA3, or Lec4A cells ($p < 0.05$)

[e] GlcNAcT-V protein mislocated in the endoplasmic reticulum

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Table 2

Morphometric analysis of the Golgi apparatus by electron microscopy

Cells	GlcNAcT-V protein	Golgi cisternal stack[a]			Number of stacks/cell cross section	Size of cell profle in cross sections[a] (μm^2)	Volume density of GA[a] (×10^{-3})
		Number of cisternae	Length (μm)	Thickness (μm)			
Pro$^-$5							
Exp. 1 (n = 35)	+	5.1 ± 0.1	0.71 ± 0.03	0.19 ± 0.01	1.7	54.7 ± 4.0[b]	5.1 ± 0.6[c]
Exp. 2 (n = 67)	+	4.7 ± 0.1	0.68 ± 0.03	0.21 ± 0.01	1.6	61.9 ± 4.5[b]	4.1 ± 0.5[c]
Lec4							
Exp. 1 (n = 30)	-	5.0 ± 0.1	0.64 ± 0.04	0.18 ± 0.01	1.1	54.9 ± 3.4	3.2 ± 0.7
Exp. 2 (n = 50)	-	4.5 ± 0.1	0.61 ± 0.03	0.20 ± 0.01	1.1	65.4 ± 2.9	2.4 ± 0.3
Lec4 GnTV-N5							
Exp. 1 (n = 36)	+	5.0 ± 0.1	0.61 ± 0.03	0.17 ± 0.01	1.9	43.1 ± 2.4[b]	5.5 ± 0.7[c]
Exp. 2 (n = 71)	+	4.7 ± 0.1	0.57 ± 0.02	0.22 ± 0.01	1.7	49.3 ± 3.4[b]	4.7 ± 0.5[c]

[a] Data are expressed as mean ± SEM

[b] Indicates significant difference as compared with Lec4 cells ($p < 0.01$, Wilcoxon test) within the same experiment

[c] Indicates significant difference as compared with Lec4 cells ($p < 0.05$, Wilcoxon test) within the same experiment

Table 3

Mean values of Golgi apparatus volume density with or without Golgi stack hits in cross-sectioned cells

Cells	Mean volume density of Golgi apparatus[a] × 10^{-3}	Mean volume density of Golgi apparatus[b] × 10^{-3}
Pro^{-}5	5.1	6.1
Lec4	3.2	5.6
Lec4 GnTV	5.5	6.4

[a] Calculations were performed by considering only cross sections containing Golgi apparatus

[b] Calculations were performed by including cross sections with and without Golgi apparatus hits

Table 4

The volume density of the Golgi apparatus in various CHO cell lines following swainsonine treatment

Cells	GlcNAcT-V protein	Volume density ($\times 10^{-3}$)[a]	
		Control	Swainsonine treatment[b]
Pro^{-}5	+	27 ± 4[c]	26 ± 4[c]
Lec4	–	15 ± 3	16 ± 3
Lec4 GnTV-N5	+	27 ± 3[c]	26 ± 3[c]
Lec1	+	26 ± 3[c]	–

[a] Data are expressed as mean ± SD and based on three different evaluations each comprising three different randomly taken views containing 30–40 cells

[b] Cells were treated for 24 h with 1 μg/ml swainsonine

[c] Indicates significant difference as compared with Lec4 or Lec4A cells ($p < 0.01$, x^2 test, *t* test)