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Dictyostelium gnt15 encodes a protein with similarity to LARGE and plays an essential role in development

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Abstract

LARGE is a putative glycosyltransferase found to be mutated in mice with myodystrophy or patients with congenital muscular dystrophy. By homology searches, we identified in the *Dictyostelium discoideum* genome four open reading frames, i.e. *gnt12–15*, encoding proteins with sequence similarity to LARGE. Semi-quantitative RT-PCR analysis revealed distinct temporal expression patterns of the four *gnt* genes throughout *Dictyostelium* development. To explore the gene function, we performed targeted disruptions of *gnt14* and *gnt15*. The *gnt14*$^-$ strains showed no obvious phenotypes. However, *gnt15*$^-$ cells grew slowly, changed in morphology, and displayed a developmental phenotype arresting at early stages. Compared with the wild type, *gnt15*$^-$ cells were more adhesive and exhibited altered levels of some surface adhesion molecules. Moreover, lectin-binding analysis demonstrated that *gnt15* disruption affected profiles of membrane glycoproteins. Taken together, our data suggest that Gnt15 is essential for *Dictyostelium* development and may have a role in modulating cell adhesion and glycosylation.

Keywords: LARGE; Glycosyltransferase; *Dictyostelium*; Development; Cell adhesion; Glycoprotein; Lectin-binding

Glycosylation is a ubiquitous form of post-translational modification that profoundly affects expression and function of many proteins. Processing of oligosaccharides in cells requires the participation of many different enzymes, including glycosyltransferases that add sugar moieties to nascent polypeptides, and glycosidases that remove sugar moieties from glycoproteins [1]. It is noted that protein glycosylation changes during differentiation and development, under different physiological conditions, and in various diseases [2,3], indicating that glycosylation enzymes are highly regulated. An increasing number of genes encoding for putative or demonstrated glycosyltransferases have been associated with different forms of congenital muscular dystrophies which exhibit defective processing of α-dystroglycan (α-DG) [4,5]. Among these is *LARGE* [6] which encodes a predicted transmembrane protein containing two putative catalytic domains with the conserved DXD (Asp-any-Asp) motif typical of many glycosyltransferases [7]. The proximal catalytic domain of LARGE is most homologous to a bacterial glycosyltransferase family 8 (GT8) member [8] while the distal domain most resembles the human UDP-GlcNAc:Gal-β1,3-*N*-acetytglucosaminyltransferase (iGnT) [9], a member of GT49 family [8]. LARGE is required for the generation of functional, properly glycosylated forms of α-DG [10,11]. Recently a highly homologous gene *LARGE2* has been found and its product also supports the maturation of α-DG [12]. Despite the sequence feature and evidence suggesting their function in protein glycosylation, LARGE proteins have not been directly demonstrated to be bona fide glycosyltransferases.

To find targets for future comparative structure-function studies, we searched in lower eukaryotic model organisms for LARGE-like sequences. Here we report our discovery in *Dictyostelium discoideum*, an organism that undergoes a starvation-induced developmental program in which many glycoproteins serve essential functions [13]. We found four putative glycosyltransferase genes

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(*gnt12–15*) encoding products with significant similarity to mammalian LARGE proteins. Our initial characterization of *gnt14–15* revealed that *gnt15* (but not *gnt14*) is essential for development. We provide evidence for the involvement of Gnt15 in regulating growth, morphology, and adhesion of cells. Our results are also consistent with a role of Gnt15 in modulating glycosylation.

Materials and methods

Dictyostelium growth, development, and transformation. *Dictyostelium* cells were grown at 22 °C on SM plates with *Klebsiella aerogenes* or in HL5 medium [14]. For development, cells were washed and plated onto DB plates at 1.5×10^6 cells/cm^2 or developed in DB buffer with gentle shaking as described [14,15]. Transformation of cells with DNA was performed by electroporation [16]. Transformants were grown in HL5 with appropriate selection, i.e. 5 μg/ml blasticidin S (Cayla, France) or 10 μg/ml G418 (Sigma).

RT-PCR analysis of gene expression. Total RNA from wild-type (Ax2) cells at different developmental stages was isolated using Trizol (Gibco-BRL) according to the manufacturer's protocol. First strand cDNA was prepared by reverse transcription using an oligo-dT primer, and gene-specific primer sets (Supplementary Table S1) were used in subsequent PCR amplifications.

Plasmids and construction of *gnt14*$^-$ and *gnt15*$^-$ strains. See Supplementary materials and methods.

Spore assay. Cells developed on DB agar for 48 h were collected and assayed for spore formation as described [17]. Detergent-resistant spores were counted under microscopes and plated clonally on SM plates with bacteria to estimate the number of viable spores in each sample.

Adhesion assay. Cell–cell adhesion was assayed as described [18] with modifications. Cells were harvested, washed twice, and resuspended in PB (50 mM KH_2PO_4, 50 mM Na_2HPO_4 pH 6.1) with or without 10 mM EDTA to 5×10^6 cells/ml. After complete disperse of cells by vortexing, 1-ml aliquots of cell suspension were placed in 10-ml beakers and gently shaken at 100 rpm on a gyratory shaker for 20 min. Triplicate samples were taken and cells not in "clumps" (i.e. ⩾3 cells aggregated) were counted on a hemocytometer and % Adhesion (i.e. [total numbers of cells − numbers of cells not in clumps] ÷ total numbers of cells) was determined.

Western and lectin blot analyses on membrane proteins. Cells were harvested and washed twice in 20 mM sodium phosphate buffer, pH 6.5, resuspended to 1×10^8 cells/ml, and lysed by passing through 5 μm Nucleopore filters (Whatman). Crude extracts were centrifuged at 20000*g* for 30 m in at 4 °C. Pellets (i.e. membrane fractions) were dissolved in 1× sample buffer. Samples of equal protein amount were separated using 10% SDS–PAGE and transferred onto PVDF membranes (Millipore). For Western analysis, blots were blocked in 5% bovine serum albumin (BSA) for 1 h, incubated first with primary antibodies in TBS buffer containing 0.1% Tween 20 (TBST) at 4 °C overnight and subsequently with horse raddish peroxidase (HRP)-conjugated anti-rabbit immunoglobulins (1:5000 in TBST) for 1 h, and detected by enhanced chemiluminescence (ECL). Primary antibodies used include anti-gp24 (1:2000), anti-gp80 (1:200), and anti-gp150 (1:2000) kindly provided by Dr. C.-H. Siu, at University of Toronto, and anti-gp130 (10 μg/ml) kindly provided by Dr. Catherine Chia, at University of Nebraska-Lincoln). For lectin-blot analysis, membranes were blocked in 5% BSA for 1 h, incubated first with 10 μg/ml biotin-lectins (including concanavalin A, winged bean agglutinin, and wheat germ agglutinin; in PBS buffer containing 1% BSA and 0.1% Tween 20) for 1 h, and subsequently with streptavidin-HRP (1:2000 in PBST) for 1 h, and detected by ECL.

Results and discussion

Dictyostelium gnt12–15 encode LARGE-like proteins

BLASTp searches in the *D. discoideum* genome database, i.e. the dictyBase (http://dictybase.org) [19] using human LARGE protein sequence as the query found four *Dictyostelium* open reading frames with sequence similarity to LARGE, i.e. *gnt14*, *gnt15*, *gnt13*, and *gnt12* in descending order of scores (Table 1). The four sequences are annotated as putative glycosyltransferases, possibly *N*-acetylglucosaminyltransferases (GlcNAc Transferases), hence named *gnt* genes.

We examined the sequences for shared motifs or domains. MEME (Multiple Expectation-maximization for Motif Elicitation, version 3.5.4; San Diego Supercomputer Center, UCSD; http://meme.sdsc.edu/meme) analysis identified conserved sequence blocks I–IV among all analyzed proteins; these blocks cluster in the C-terminal part of LARGE proteins (Supplementary Fig. S1). Gnt12–15 each possesses a DXD motif in block II, and ClustalW analysis revealed that these *Dictyostelium* DXD motifs are most similar to the third DXD motif in LARGE proteins (data not shown). All the analyzed proteins contain a predicted transmembrane domain (Fig. 1A), consistent with the possibility that they are ER- or Golgi-residing glycosyltransferases. Mammalian LARGE proteins contain two putative catalytic domains, the N-terminal GT8 and the C-terminal GT49 domains [8]. Only the GT49 domain is shared by *Dictyostelium* Gnt12–15; the degrees of similarity calculated over this region ranged from 44% to 59% (Table 1). Consistently, in a report describing results of a genome-wide survey of *Dictyostelium* sequences similar to glycosyltransferases classified in the CAZy (Carbohydrate-Active enZymes) database (http://afmb.cnrs-mrs.fr/CAZY), Gnt12–15 were also listed as GT49 β3GlcNAcT-like sequences [20]. Among Gnt12–15, only Gnt14 has the DXD motif located in the GT49-like region; Pfam HMM searches (The Sanger Institute; http://www.sanger.ac.uk/Software/Pfam/search.shtml) found that Gnt13 and Gnt15 have their DXD located in a region weakly matching the GT2 domain [8].

Dictyostelium gnt12–15 exhibit distinct temporal expression patterns during development

To determine if *Dictyostelium* indeed express *gnt12–15*, we performed RT-PCR analysis on RNA samples prepared from wild-type cells developed to different stages. Distinct temporal expression patterns throughout development were noted (Fig. 1B). Levels of *gnt14* and *gnt13* expression were relatively steady during vegetative growth and development. Conversely, *gnt15* showed highest expression at vegetative and pre-aggregation stages while *gnt12* expression peaked at mid- to late-stages of development. These results suggest that Gnt12–15 may function at distinct developmental stages.

Disruption of gnt15 but not gnt14 affects growth, morphology, and development

To explore their function, we further characterized the two *Dictyostelium* genes with highest LARGE-BLASTp

Table 1
Dictyostelium LARGE-like genes identified by BLAST searches[a]

Gene name	dictyBase ID	Chr[b]	Product (aa)	GT[c]-domain	Score (bits)[d]	*E*-value[d]	Identity (%)[d]	Similarity (%)[d]
gnt14	DDB0231852	4	640	431–587	94.4	2e−18	30	55
gnt15	DDB0231849	2	516	335–457	89.0	1e−16	32	58
gnt13	DDB0231851	3	635	460–583	88.6	1e−16	33	59
gnt12	DDB0231850	1	550	359–484	38.5	0.15	21	44

[a] Genes identified by BLASTp searches in dictyBase (http://dictybase.org/db/cgi-bin/blast.pl) using human LARGE sequence (NP_004728) as the query are listed in descending order of BLASTp scores.
[b] Chromosome location of the *gnt* genes.
[c] Amino acid positions of the GT49 glycosyltransferase-like domain in the Gnt proteins.
[d] Obtained by comparing the GT-domain in each Gnt protein with the GT49 domain of human LARGE (aa 556–715) using BLAST-2-sequences analysis (http://www.ncbi.nlm.nih.gov/blast/bl2seq/wblast2.cgi).

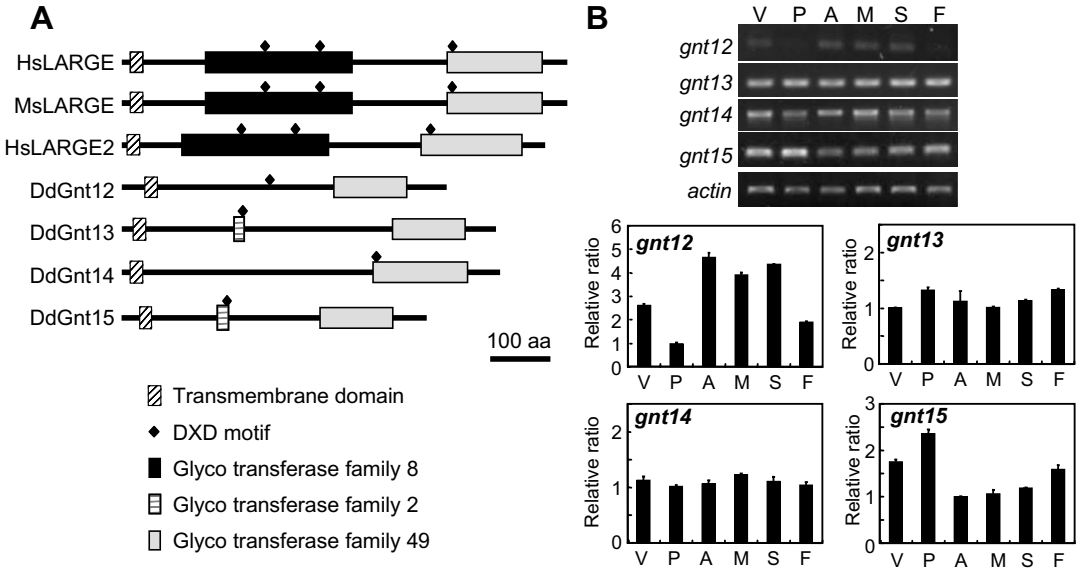

Fig. 1. *Dictyostelium gnt12–15* encode LARGE-like proteins. (A) A schematic alignment of *Dictyostelium* Gnt12–15 with mammalian LARGE proteins. Regions with similarity to known functional domains are indicated. Accession Numbers for sequences analyzed are: NP_004728.1 (HsLARGE), NP_034817.1 (MmLARGE), NP_689525.2 (HsLARGE2), DDB0231850 (DdGnt12), DDB0231851 (DdGnt13), DDB0231852 (DdGnt14), and DDB0231849 (DdGnt15). (B) Expression of *gnt12–15* during *Dictyostelium* development. Samples of total RNA prepared from wild-type Ax2 cells at vegetative (V) or different developmental stages, including pre-aggregation (P), aggregation (A), mound (M), slug (S), and fruiting body (F) stages, were subjected to RT-PCR analysis using gene-specific primer sets. For quantitative representation of results, *gnt* signals were normalized to the *actin* signal in densiotometric analysis. Expression levels for each *gnt* gene are presented as relative ratios to the value of lowest expression time-point. Shown are averages from three independent experiments.

scores. Constructs for targeting *gnt14* or *gnt15* by homologous recombination were prepared and transformed into the wild-type Ax2 strain. Knockout clones were identified by genomic PCR and subsequently confirmed by Southern analysis (Supplementary Fig. S2). Analyses of knockout strains revealed no definitive phenotypes of *gnt14*$^-$ cells and they exhibited normal developmental morphology (Supplementary Fig. S3A). However, *gnt15* disruption affected cell growth, morphology and development (Fig. 2).

By using two *gnt15*-targeting constructs (Supplementary Fig. S2B), we obtained *gnt15*$^-$ cells of two different genotypes, represented by clones B5–8 and B12, with coding regions for amino acids 157–273 (including the DXD motif) or the carboxyl-terminal 103 amino acids replaced by the blasticidin resistance expression cassette, respectively (Fig. 2A). Both B5–8 and B12 grew significantly more slowly compared with wild-type cells, while growth rates of the strains rescued by expressing a Flag-Gnt15 fusion protein were comparable to those of wild-type cells. The approximate doubling times in axenic cultures were 9.8 h for the wild-type cells, 14.2 h for both B5–8/Vec and B12/Vec, 10.8 h for B5–8/Gnt15, and 12.1 h for B12/Gnt15. These data suggest a role of Gnt15 in supporting optimal cell growth.

We noticed heterogeneous cell sizes in B5–8 and B12 *gnt15*$^-$ mutants; some cells were larger than the average wild-type size while some appearing broken into debris-like fragments (Fig. 2B). This abnormal morphology could be corrected by expressing Flag-Gnt15 in mutants, suggesting that Gnt15 somehow participates in regulating cell morphology.

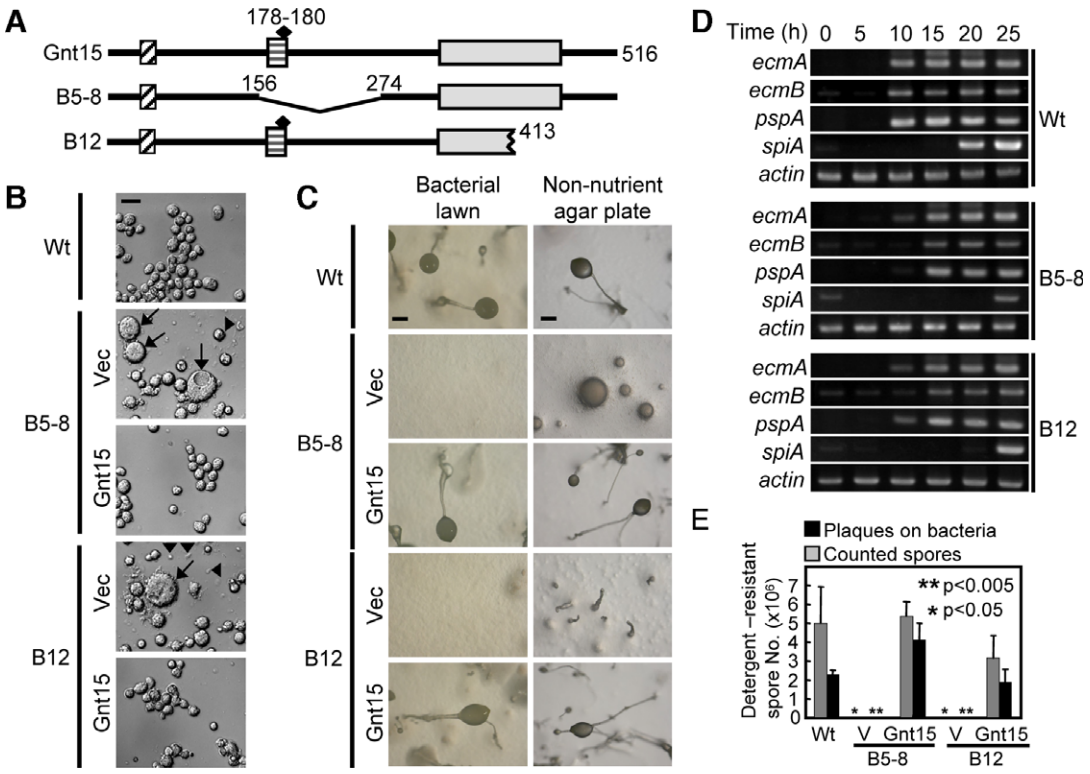

Fig. 2. Disruption of *gnt15* affects morphology and development. (A) Schematic representation of *gnt15* disruption mutants. Numbers are amino acid positions. (B) Morphology of *gnt15*$^-$ cells. Axenically cultured wild type (Wt) and *gnt15*$^-$ cells (B5–8 and B12) transformed with pTX-FLAG (Vec) or pCJ11 (Gnt15) were photographed using Nomarski DIC. Bar = 20 μm. Arrows, abnormally large cells. Arrowheads, examples of cell "debris". (C) Development of *gnt15*$^-$ clones. Cells developed on bacteria or DB plates were photographed at 5 days and 36 h, respectively. Bar = 200 μm. (D) Cell differentiation in *gnt15*$^-$ mutants. Expression of cell-type specific markers was examined by RT-PCR analysis on total RNA isolated from cells at various developmental time-points as indicated. (E) Spore formation in *gnt15*$^-$ clones. Cells (5×10^7) were developed on DB for 48 h and collected. Numbers of detergent-resistant spores were determined by counting under microscopes or plating clonally on bacterial lawns. Shown are averages from three independent experiments.

Disruption of *gnt15* resulted in aberrant development (Fig. 2C). When plated on bacterial lawns, B5–8 and B12 mainly produced smooth plaques without forming fruiting bodies; occasionally loose aggregates were seen in B12 plaques. When developed on non-nutrient agar, B5–8 formed mounds or finger-like structures while B12 produced small finger-like structures. Expression of Flag-Gnt15 restored the ability of *gnt15*$^-$ cells to form fruiting bodies on bacterial lawns and non-nutrient plates, confirming that the abnormal development was caused by loss of Gnt15 function. The basis for the slight difference between the developmental morphology of B5–8 and B12 remains to be determined; possible explanations include positional effects of disruption or expression in the mutants of different truncated Gnt15 forms with structure/function differences. Discrepancy in the developmental morphology on different plates observed in *gnt15*$^-$ strains is not unprecedented; other examples, including *csA*$^-$ and *dtfA*$^-$ mutants, demonstrate influences of substratum types on developmental outcomes [21,22]. Interestingly, like *gnt15*$^-$ cells (see below), both *csA*$^-$ and *dtfA*$^-$ mutants are defective in adhesion; perhaps differential cell–cell or cell–substratum adhesion on different substrata can affect phenotype manifestation.

As *gnt15*$^-$ mutants did form some multi-cellular structures during development, we examined cell-type differentiation by surveying the expression of several marker genes, including prestalk genes *ecmA* and *ecmB*, a prespore gene *pspA* and a spore coat protein gene *spiA*. Results of RT-PCR analysis demonstrated that *gnt15*$^-$ cells indeed expressed these genes, although with a slight delay (Fig. 2D), suggesting that *gnt15* disruption did not abolish cell differentiation. We further tested if spores formed in mutants, by counting numbers of detergent-resistant spores. Our data revealed a severe spore formation defect which can be rescued by expressing Flag-Gnt15 (Fig. 2E), suggesting a role of Gnt15 in modulating spore formation.

Gnt15 may participate in modulating cell adhesion

With the speculation that Gnt15 may be a glycosyltransferase and given that cell surface glycoproteins participate in adhesion [23–26], we tested cell–cell adhesion in *gnt15*$^-$ cells. Since both EDTA-sensitive and EDTA-resistant adhesion systems exist in *Dictyostelium* [27], we performed adhesion assays with or without EDTA. In vegetative (0 h) or aggregative (5 h) cells, we found similar adhesion in the absence of EDTA among the wild-type, *gnt15*$^-$ and

gnt15$^-$/Gnt15 cells (Fig. 3A). However, *gnt15*$^-$ mutants showed significantly stronger EDTA-resistant cell–cell adhesion than the wild type at both stages; expression of Flag-Gnt15 reversed the adhesion to wild-type-like levels, suggesting the involvement of Gnt15 in modulating adhesion.

We next examined several surface adhesion molecules in *gnt15*$^-$ cells, including the 24-kDa EDTA-sensitive adhesion molecule DdCad1 (gp24) [23], the 80-kDa glycoprotein (gp80) mediating EDTA-resistant adhesion at aggregative stage [24], the heavily glycosylated 130-kDa membrane protein (gp130) [25], and the 150-kDa *lagC* product (gp150) mediating post-aggregation EDTA-resistant adhesion [26]. While gp24 and gp80 levels were similar in wild-type and *gnt15*$^-$ cells, decreased gp130 and gp150 levels were noted at their respective expression time in *gnt15*$^-$ mutants compared with the wild type (Fig. 3B). Expressing Flag-Gnt15 reversed gp130 and gp150 amount in mutants to wild-type levels, indicating that the observed difference was an effect of *gnt15* disruption.

It is possible that changes in gp130 and gp150 levels resulted from altered glycosylation in *gnt15*$^-$ cells, as others have reported decreased stability of specific *Dictyostelium* proteins caused by glycosylation changes [28]. Decreased gp130 levels in *gnt15*$^-$ cells may explain the increased vegetative-stage EDTA-resistant adhesion since *gp130*$^-$ mutants also display increased cell–cell adhesion [25]. How changes in gp150 levels affect cell adhesion is not clear as adhesion assays were done when little gp150 was expressed. Glycosyltransferases may act on multiple cellular proteins; if Gnt15 was indeed a glycosyltransferase, there might be involvement of other glycoproteins in causing adhesion changes or aberrant development in *gnt15*$^-$ mutants.

Disruption of gnt15 alters membrane glycoproteins

Given the DXD motifs and homology to LARGE C-terminal putative glycosyltransferase domain in Gnt proteins, we tested if *gnt14* or *gnt15* disruption affects protein glycosylation by performing lectin-blot analysis on membrane proteins. When comparing wild-type and *gnt14*$^-$ samples, the distinct distribution patterns of lectin-binding protein bands on SDS–PAGE gels detected by concanavalin A (Con A, which binds α-Man), winged bean agglutinin (WBA, which binds Gal and GalNAc), or wheat germ agglutinin (WGA, which binds GlcNAc) were essentially the same (Supplementary Fig. S3B). Perhaps the lack of detectable phenotypes in *gnt14*$^-$ cells is due to functional redundancy provided by other protein(s). Gnt13 represents a good candidate protein for compensating Gnt14 function, given the similarity in sequences and temporal expression patterns (Fig. 1B).

Conversely, lectin-labeled membrane proteins in *gnt15*$^-$ samples appeared different from wild-type ones on the blots (Fig. 4). In our experiments, Con A interacted with many membrane proteins while WBA and WGA interacted with a relatively smaller group. The three lectin-binding

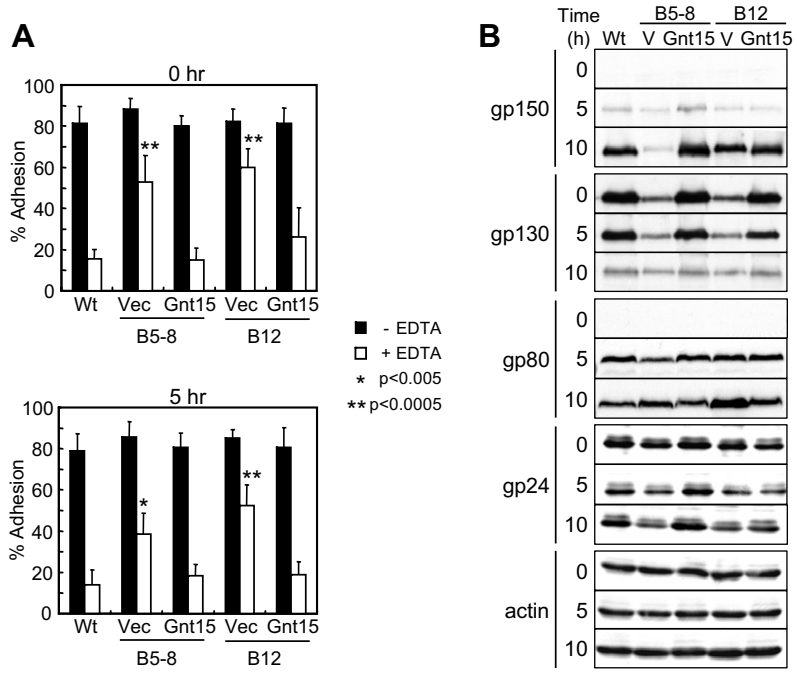

Fig. 3. Disruption of *gnt15* affects cell adhesion. (A) Cell–cell adhesion. Aliquots (1 ml) of completely dissociated vegetative or 5 h-developed cells were placed in 10-ml beakers with or without 10 mM EDTA and incubated for 20 min with gentle rotation at 100 rpm. Number of aggregated cells was determined by microscopy and % adhesion was calculated. The assay was done five times with triplicate samples. (B) Western analysis of surface adhesion molecules. Lysates of equal protein amount prepared from cells at different developmental time-points were separated using 8% SDS–PAGE and subjected to analysis.

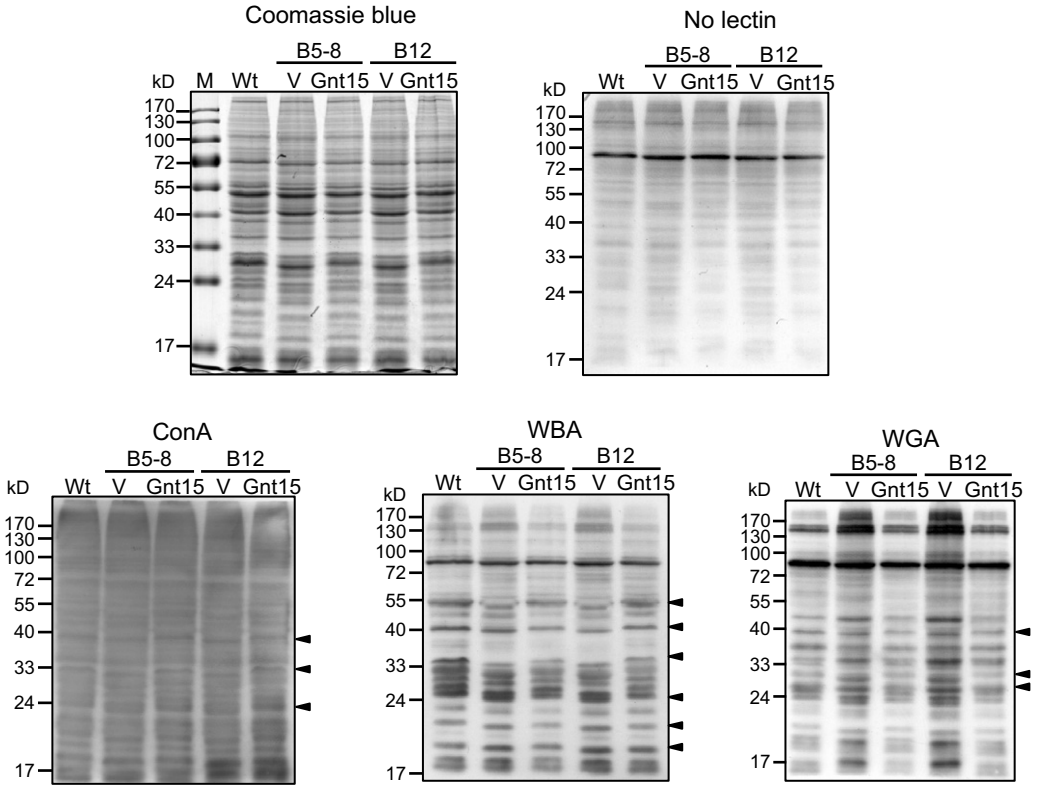

Fig. 4. Disruption of *gnt15* changes profiles of membrane glycoproteins. Membrane proteins were prepared from vegetative cells and subjected to lectin blot analysis. Samples of equal protein amount were separated on 10% SDS–PAGE gels (as shown by Coomassie blue staining). Proteins were transferred onto PVDF membranes, and incubated without lectin or with 10 μg/ml of biotin-labeled concanavalin A (ConA), winged bean agglutinin (WBA) or wheat germ agglutinin (WGA), respectively. Detection was done by ECL after incubating the blots with streptavidin-HRP. Arrowheads point to examples of shifted bands.

patterns were distinct, and different from the pattern of signals in the "no lectin" control, suggesting that lectin-labeling was probably a result of interaction between lectins and glycans on proteins. When compared with wild-type samples, some lectin-labeled bands in *gnt15*$^{-}$ samples were noticeably shifted in positions and some appeared stronger in WGA binding. Expression of Flag-Gnt15 in mutants eliminated these differences. Together, our data suggest the existence of alterations in glycosylation on membrane proteins of *gnt15*$^{-}$ cells, which is consistent with the notion that Gnt15 may function as a glycosyltransferase.

In conclusion, our bioinformatics approach towards LARGE-like sequences turned out to be fruitful in *Dictyostelium*; a group of four putative glycosyltransferase genes was discovered. The regulation of protein glycosylation in response to extracellular signals remains hardly known in *Dictyostelium*. This group of *gnt* genes with differential temporal expression patterns provides an opportunity for studying the regulation of glycosyltransferases in response to dynamic cellular environment, as well as for elucidating the roles of glycosylation in cell differentiation and morphogenesis. Characterization demonstrated an important role of Gnt15 in *Dictyostelium* development and its possible involvement in affecting glycosylation of a spectrum of cellular proteins. The cloned *gnt15* gene and the traceable phenotypes of mutants present a new model system for structure-function studies of LARGE-like proteins or glycosyltransferases in general.

Acknowledgments

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bbrc.2007.06.016.

References

[1] J.B. Lowe, J.D. Marth, A genetic approach to mammalian glycan function, Annu. Rev. Biochem. 72 (2003) 643–691.

[2] R.G. Spiro, Protein glycosylation: nature, distribution, enzymatic formation, and disease implications of glycopeptide bonds, Glycobiology 12 (2002) 43R–56R.
[3] R.S. Haltiwanger, J.B. Lowe, Role of glycosylation in development, Annu. Rev. Biochem. 73 (2004) 491–537.
[4] F. Muntoni, M. Brockington, S. Torelli, S.C. Brown, Defective glycosylation in congenital muscular dystrophies, Curr. Opin. Neurol. 17 (2004) 205–209.
[5] P.K. Grewal, J.E. Hewitt, Glycosylation defects: a new mechanism for muscular dystrophy, Hum. Mol. Genet. 12 (2003) R259–R264.
[6] C. Longman, M. Brockington, S. Torelli, C. Jimenez-Mallebrera, C. Kennedy, N. Khalil, L. Feng, R.K. Saran, T. Voit, L. Merlini, C.A. Sewry, S.C. Brown, F. Muntoni, Mutations in the human *LARGE* gene cause MDC1D, a novel form of congenital muscular dystrophy with severe mental retardation and abnormal glycosylation of alpha-dystroglycan, Hum. Mol. Genet. 12 (2003) 2853–2861.
[7] M. Peyrard, E. Seroussi, A.C. Sandberg-Nordqvist, Y.G. Xie, F.Y. Han, I. Fransson, J. Collins, I. Dunham, M. Kost-Alimova, S. Imreh, J.P. Dumanski, The human LARGE gene from 22q12.3-q13.1 is a new, distinct member of the glycosyltransferase gene family, Proc. Natl. Acad. Sci. USA 96 (1999) 598–603.
[8] P.M. Coutinho, E. Deleury, G.J. Davies, B. Henrissat, An evolving hierarchical family classification for glycosyltransferases, J. Mol. Biol. 328 (2003) 307–317.
[9] K. Sasaki, K. Kurata-Miura, M. Ujita, K. Angata, S. Nakagawa, S. Sekine, T. Nishi, M. Fukuda, Expression cloning of cDNA encoding a human beta-1,3-N-acetylglucosaminyltransferase that is essential for poly-N-acetyllactosamine synthesis, Proc. Natl. Acad. Sci. USA 94 (1997) 14294–14299.
[10] M. Kanagawa, F. Saito, S. Kunz, T. Yoshida-Moriguchi, R. Barresi, Y.M. Kobayashi, J. Muschler, J.P. Dumanski, D.E. Michele, M.B. Oldstone, K.P. Campbell, Molecular recognition by LARGE is essential for expression of functional dystroglycan, Cell 117 (2004) 953–964.
[11] R. Barresi, D.E. Michele, M. Kanagawa, H.A. Harper, S.A. Dovico, J.S. Satz, S.A. Moore, W. Zhang, H. Schachter, J.P. Dumanski, R.D. Cohn, I. Nishino, K.P. Campbell, LARGE can functionally bypass alpha-dystroglycan glycosylation defects in distinct congenital muscular dystrophies, Nat. Med. 10 (2004) 696–703.
[12] K. Fujimura, H. Sawaki, T. Sakai, T. Hiruma, N. Nakanishi, T. Sato, T. Ohkura, H. Narimatsu, LARGE2 facilitates the maturation of alpha-dystroglycan more effectively than LARGE, Biochem. Biophys. Res. Commun. 329 (2005) 1162–1171.
[13] S. Alexander, Developmental regulation and function of glycoproteins in *Dictyostelium discoideum*, in: Y. Maeda, K. Inouye, I. Takeuchi (Eds.), *Dictyostelium*—A Model System for Cell and Developmental Biology, Universal Academy Press, Tokyo, 1997, pp. 349–377.
[14] M. Sussman, Cultivation and synchronous morphogenesis of *Dictyostelium* under controlled experimental conditions, Methods Cell Biol. 28 (1987) 9–29.
[15] P. Devreotes, D. Fontana, P. Klein, J. Sherring, A. Theibert, Transmembrane signaling in *Dictyostelium*, Methods Cell Biol. 28 (1987) 299–331.
[16] D. Knecht, K.M. Pang, Electroporation of *Dictyostelium discoideum*, Methods Mol. Biol. 47 (1995) 321–330.
[17] B. Wang, A. Kuspa, CulB, a putative ubiquitin ligase subunit, regulates prestalk cell differentiation and morphogenesis in Dictyostelium spp, Eukaryot. Cell 1 (2002) 126–136.
[18] L. Desbarats, S.K. Brar, C.H. Siu, Involvement of cell–cell adhesion in the expression of the cell cohesion molecule gp80 in *Dictyostelium discoideum*, J. Cell Sci. 107 (1994) 1705–1712.
[19] R.L. Chisholm, P. Gaudet, E.M. Just, K.E. Pilcher, P. Fey, S.N. Merchant, W.A. Kibbe, dictyBase, the model organism database for *Dictyostelium discoideum*, Nucleic Acids Res. 34 (2006) D423–D427.
[20] C.M. West, H. van der Wel, P.M. Coutinho, B. Henrissat, Glycosyltransferase genomics in *Dictyostelium discoideum*, in: W.F. Loomis, A. Kuspa (Eds.), *Dictyostelium* Genomics, Horizon Scientific Press, Norfolk, 2005, pp. 235–264.
[21] E. Ponte, E. Bracco, J. Faix, S. Bozzaro, Detection of subtle phenotypes: the case of the cell adhesion molecule csA in *Dictyostelium*, Proc. Natl. Acad. Sci. USA 95 (1998) 9360–9365.
[22] R.S. Ginger, L. Drury, C. Baader, N.V. Zhukovskaya, J.G. Williams, A novel *Dictyostelium* cell surface protein important for both cell adhesion and cell sorting, Development 125 (1998) 3343–3352.
[23] D.A. Knecht, D.L. Fuller, W.F. Loomis, Surface glycoprotein, gp24, involved in early adhesion of *Dictyostelium discoideum*, Dev. Biol. 121 (1987) 277–283.
[24] L.M. Wong, C.H. Siu, Cloning of cDNA for the contact site A glycoprotein of *Dictyostelium discoideum*, Proc. Natl. Acad. Sci. USA 83 (1986) 4248–4252.
[25] C.P. Chia, S. Gomathinayagam, R.J. Schmaltz, L.K. Smoyer, Glycoprotein gp130 of *Dictyostelium discoideum* influences macropinocytosis and adhesion, Mol. Biol. Cell 16 (2005) 2681–2693.
[26] E.N. Gao, P. Shier, C.H. Siu, Purification and partial characterization of a cell adhesion molecule (gp150) involved in postaggregation stage cell–cell binding in *Dictyostelium discoideum*, J. Biol. Chem. 267 (1992) 9409–9415.
[27] C.H. Siu, T.J. Harris, J. Wang, E. Wong, Regulation of cell–cell adhesion during *Dictyostelium* development, Semin. Cell Dev. Biol. 15 (2004) 633–641.
[28] H.P. Hohmann, S. Bozzaro, R. Merkl, E. Wallraff, M. Yoshida, U. Weinhart, G. Gerisch, Post-translational glycosylation of the contact site A protein of *Dictyostelium discoideum* is important for stability but not for its function in cell adhesion, EMBO J. 6 (1987) 3663–3671.