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Transgenic Note

Modification of the surface carbohydrate composition of tobacco protoplasts transformed with the human UDP-galactose transporter gene *hUGT1*

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Abstract Tobacco BY-2 cells were transformed with the human UDP-galactose transporter 1 gene (*hUGT1*) under the control of a 35S promoter. Accumulation of mRNA and protein derived from *hUGT1* was detected in the *hUGT1*-transformed BY-2 cells. To identify the cell surface carbohydrates, BY-2 protoplasts were treated with 21 kinds of biotinylated lectin, stained with fluorescein isothiocyanate (FITC)-conjugated streptavidin and observed by fluorescence microscopy. FITC-ConA and -RCA_{120} staining exhibited strong fluorescence on the surface of BY-2 protoplasts, whereas staining with other FITC-lectin conjugates showed faint or no fluorescence. Regarding FITC-ConA and -RCA_{120} staining, the fluorescence of *hUGT*-expressing BY-2 protoplasts was weaker than that of control cells. Decreased FITC fluorescence of *hUGT*-expressing BY-2 protoplasts was also detected by fluorescence-activated cell sorting (FACS) analysis, suggesting that the surface carbohydrate composition was modified.

Key words: BY-2, FACS, lectin, tobacco, UDP-galactose transporter.

Glycosylation of glycoproteins and glycolipids is variously carried out by sequential addition of sugar residues in the Golgi apparatus. Nucleotide sugar transporters (NSTs) are indispensable components of glycosylation, since they translocate the substrates for glycosylation reactions into the lumen of the Golgi apparatus (Berninsone and Hirschberg 2000). Moreover, NSTs are considered to act as partners for glycosyltransferases in the biosynthesis of oligosaccharides, polysaccharides and glycoconjugates (Kawakita et al. 1998). Genes encoding NST family proteins have been isolated from animal cells, *Drosophila*, Nematoda, yeast, *Arabidopsis* and so on (Berninsone and Hirschberg 2000), while the development of numerous genome projects has increased the number of genes predicted to encode NST family proteins. Using a phylogenetic approach, the NST proteins have been classified into three main clades (designated NST families 1, 2 and 3), each of which is further classified into several subfamilies (Martinez-Duncker et al. 2003).

The UDP-galactose transporter is a member of the NST protein family and involved in the transport of UDP-galactose into the Golgi lumen (Berninsone and Hirschberg 2000). UDP-galactose transporters have been described in humans (Miura et al. 1996), mice (Ishida et al. 1999), *Drosophila* (Sagawa et al. 2002), yeast (Sagawa et al. 1999; Kainuma et al. 2001) and *Arabidopsis* (Norambuena et al. 2002; Bakker et al. 2005; Rollwitz et al. 2006), and are located in the membrane of the Golgi apparatus with 10 membrane-spanning domains. Molecular characterization of the human UDP-galactose transporter has revealed its isoforms (Ishida et al. 1996), functions and substrate specificity (Sun-Wada et al. 1998), and subcellular location (Yoshioka et al. 1997).

Similar to the case in animal cells, glycoproteins and glycolipids are synthesized in the Golgi apparatus in plant cells. Furthermore, cell wall polysaccharides, such as pectin and xyloglucan, are also biosynthesized in the Golgi apparatus (Carpita and Gibeaut 1993). Therefore, plant NSTs must play crucial roles in the import of nucleotide-sugars into the Golgi apparatus, similar to the case for animal NSTs. The GDP-mannose transporter gene GONST1 and its homologues have been isolated from *Arabidopsis thaliana* (Baldwin et al. 2001; Handford et al. 2004). The *Arabidopsis* UDP-galactose/UDP-glucose transporter AtUTr1 has also been isolated

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and characterized (Norambuena et al. 2002), while functional characterization of AtNST-KT1 led to its identification as a UDP-galactose transporter (Rollwitz et al. 2006). Based on their homologies to typical NSTs originating from other animals and yeasts and their Golgi localizations, these plant NSTs must contribute to the production of carbohydrates on the surface of plant cells. However, the machinery involved in this process is not yet fully understood.

Owing to its tractability and proliferation, the tobacco cultured cell line BY-2 is an excellent model for many types of investigations, such as analyses of gene expression, cell cycle, cellular dynamics and so on (Nagata et al. 2004). Several studies on the production of human-type carbohydrates using plant cells, particularly BY-2 cells, have been carried out. Human β-1,4-galactosyltransferase (βGalT), which is involved in the catalysis of galactose transfer from UDP-galactose to N-acetylglucosamine of N-linked complex oligosaccharides, was introduced into tobacco BY-2 suspension-cultured cells (Palacpac et al. 1999a), which originally lack this enzyme (Palacpac et al. 1999b). As a result, terminal-galactosylated N-glycans were obtained (Palacpac et al. 1999a).

Recently, Fujiyama et al. (2006, 2007) reported that a mouse monoclonal antibody produced in BY-2 cells coexpressing human βGalT was glycosylated with galactose-extended sugar chains, while expression of the mouse monoclonal antibody alone in BY-2 cells led to recombinant antibodies carrying the plant-specific residues β-(1,2)-xylose and α-(1,3)-fucose. Although exogenous glycosylation-related enzymes seem to play roles in improving the polysaccharide structures of plant cell surfaces, the contribution of the UDP-galactose transporter to the modification of plant cell surfaces remains unknown. The introduction of both human βGalT and human UDP-galactose transporter into plant cells may cause more effective modification of glycoconjugates on human glycoproteins, because this combination must occur naturally in the human cell system. As a preliminary step, we examined whether the expression of human UDP-galactose transporter alone in tobacco BY-2 cells resulted in possible modification of the surface carbohydrate structures.

A complementary DNA (cDNA) encoding the human UDP-galactose transporter 1 gene (*hUGT1*) (Miura et al. 1996) was used to transform tobacco BY-2 cells. After digestion of the expression vector pMKIT-neo containing *hUGT1* (Miura et al. 1996) with *Eco*RI, the site was filled in and a *Sal*I linker was added. The resulting plasmid was double-digested with *Sal*I and *Not*I to isolate the *hUGT1* cDNA together with a nucleotide sequence coding 9 amino acids (YPYDVPDYA) of hemagglutinin (HA) as an epitope tag attached to its C-terminus. The *Sal*I–*Not*I fragment containing the *hUGT1* cDNA was inserted into the identical restriction sites between a CaMV 35S promoter and a nopaline synthase (*nos*) terminator in the binary vector pBIN19, resulting in pBIN-*hUGT1* (Figure 1A). The binary vectors were amplified in *Escherichia coli* strain DH5α, and then introduced into *Agrobacterium tumefaciens* strain LBA4404. To obtain transformed BY-2 cells, BY-2 cells were cultured for 3 days in liquid Linsmaier and Skoog's medium (Linsmaier and Skoog 1965) containing 0.2 mg l^{-1} 2,4-dichlorophenoxyacetic acid (LSD) and then inoculated with an overnight culture of *A. tumefaciens* containing a binary vector treated with 10 μM acetosyringone. After a 2-day coculture, the BY-2 cells

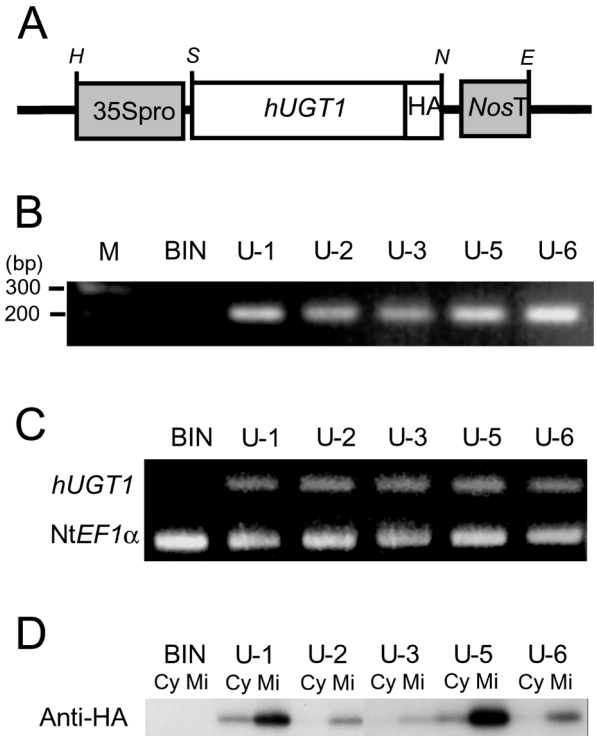

Figure 1. The pBIN-*hUGT1* construct and results of integration and expression of *hUGT1* in BY-2 cells. (A) The pBIN-*hUGT1* construct in the binary vector pBIN19. The cDNA of *hUGT1* with hemagglutinin (HA) was inserted between the CaMV 35S promoter (35Spro) and nopaline synthase terminator (*Nos*T), and the construct was cloned into the binary vector pBIN19 at the *Hin*dIII and *Eco*RI sites. *H*, *Hin*dIII; *S*, *Sal*I; *N*, *Not*I; *E*, *Eco*RI. (B) PCR analysis of the *hUGT1*-transformed BY-2 cells. Aliquots (10 mg) of the cells were taken from BY-2 cells transformed by the empty pBIN19 vector (BIN) or pBIN-*hUGT1* (U-1, U-2, U-3, U-5 and U-6). Small aliquots (0.5 μl) of the cell lysates containing genomic DNA were used as a template for PCR. (C) RT-PCR analysis of pBIN19-transformed (BIN) or pBIN-*hUGT1*-transformed (U-1, U-2, U-3, U-5 and U-6) BY-2 cells. An aliquot (1 μg) of total RNA isolated from the protoplasts of each transformed line was used as a template. After incubation for 30 min for 42°C for reverse transcription, 35 cycles for detection of *hUGT1* or 30 cycles for detection of Nt*EF1-α* were performed for PCR amplification. (D) Protein gel blot analysis of pBIN19-transformed (BIN) or *hUGT1*-transformed (U-1, U-2, U-3, U-5 and U-6) BY-2 cells. Aliquots (5 μg of protein) of the cytosolic fraction (Cy) and Golgi-rich fraction (Mi) were subjected to SDS-PAGE. To detect the hUGT1 protein, a rat anti-HA antibody was used as the primary antibody.

were washed with LSD liquid medium and then cultured in the dark on LSD medium supplemented with 500 mg l^{-1} carbenicillin and 200 mg l^{-1} kanamycin solidified with 0.2% Gelrite. After complete elimination of *A. tumefaciens*, the transformed BY-2 cells were maintained in LSD liquid medium containing 100-mg l^{-1} kanamycin by weekly transfer. Empty vector pBIN19-transformed BY-2 cell lines (pBIN19-transformed cell lines) were established in the same way and used as controls.

Eighteen cell lines were randomly selected from calluses transformed with pBIN-*hUGT1* (*hUGT1*-transformed cell lines). The *hUGT1*-transformed cell lines displayed no alterations in growth activity, although the cells showed a tendency toward longitudinal elongation compared with the control pBIN19-transformed cell lines (Figure 2A, B).

To detect the *hUGT1* cDNA in the *hUGT1*-transformed cell lines by polymerase chain reaction (PCR), 3-day cultures of pBIN19- and *hUGT1*-transformed cells (*ca.* 10 mg) were lysed in 100 μl of lysis buffer (20 mM Tris-HCl pH 7.0, 5 mM EDTA, 400 mM NaCl, 0.3% SDS, 200 μg ml^{-1} proteinase K) at 55°C for 3 h. A portion of the *hUGT1* cDNA (205 bp) was amplified from the genomic DNA in small aliquots of the lysates (0.5 μl) using Ampdirect® Plus (Shimadzu Biotech, Kyoto, Japan) and a set of primers (5′-GCAA-CCTGCAACTGGGCCTCCT-3′ and 5′-GAGGTG-GCAAAGCCCTTGAGGA-3′) according to the manufacturer's instructions. As an internal control, the Nt*EF1-α* gene was amplified from the same genomic DNA extracts using a set of primers (5′-AGGGTG-CTGCCAGCTTTACCTC-3′ and 5′-ATGGGCTTGG-TGGGAATCATCT-3′). DNA fragments were amplified from the genomic DNA extracts of all the *hUGT1*-transformed cell lines, but not from those of the pBIN19-transformed cell lines (Figure 1B), indicating the *hUGT1* cDNA was inserted into the genome of *hUGT1*-transformed cells. Based on the results of the PCR analyses, we mainly used five *hUGT1*-transformed cell lines (U-1, U-2, U-3, U-5 and U-6) for subsequent experiments.

To detect *hUGT1* transcripts by reverse transcription (RT)-PCR, total RNA was isolated from 3-day cultures of *hUGT1*-transformed cells using an RNeasy Plant Mini Kit (Qiagen Inc., MD) according to the manufacturer's instructions. Total RNA (1 μg) was used as a template for RT-PCR using Ready-To-Go RT-PCR Beads (GE Healthcare UK Ltd., Buckinghamshire, England) and a set of primers identical to those used for the PCR analyses described above. mRNA accumulation was detected by RT-PCR (Figure 1C), thereby confirming the transcription of the inserted *hUGT1* gene in all the *hUGT1*-transformed cell lines examined.

To detect hUGT1 protein, we performed protein gel blot analyses. Protein samples were prepared according to the following method. A 3-day culture of *hUGT1*-transformed cells was treated with 0.4 M mannitol solution (pH 5.8) containing 1% Cellulase ONOZUKA RS (Yakult Pharmaceutical Ind. Co. Ltd., Tokyo, Japan) and 0.1% Pectolyase Y-23 (Kyowa Chemical Products Co. Ltd., Osaka, Japan) for 3 h at 25°C. After washing with 0.4 M mannitol, the isolated protoplasts were ruptured in a buffer (10 mM Tris-10 mM boric acid pH 8.3, 0.25 M sucrose) supplemented with protease inhibitors by sonication for 5–10 s. After centrifugation

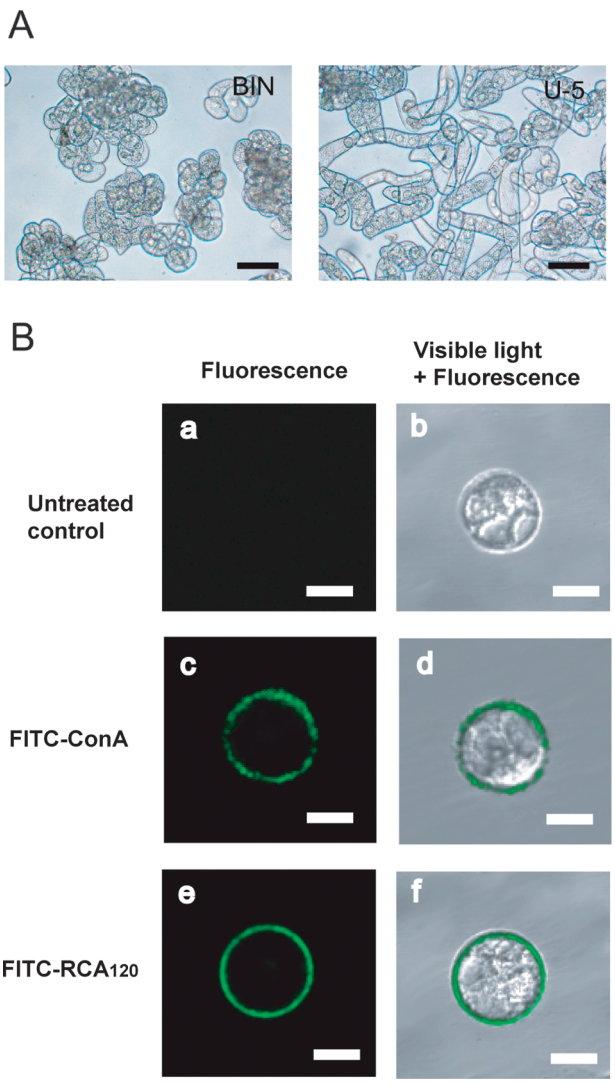

Figure 2. Morphologies and FITC-lectin staining of *hUGT1*-transformed cells. (A) Cell morphologies of 3-day cultures of a pBIN19-transformed cell line (BIN) and a *hUGT1*-transformed cell line (U-5). Bars indicate 100 μm. (B) FITC-lectin-stained BY-2 protoplasts detected by confocal laser-scanning fluorescence microscopy (excitation at 488 nm, left side) and merged visible light (Nomarski optics) and fluorescence images (right side). BY-2 protoplasts without FITC-lectin staining (upper, a and b). BY-2 protoplasts with FITC-Con A staining (middle, c and d) and FITC-RCA_{120} staining (lower, e and f). Bars indicate 20 μm. The fluorescence images were processed with the Photoshop software (Adobe Systems Inc., Mountain View, CA).

at 1,000×*g* for 5 min, each supernatant was transferred to a new tube and kept on ice. The pellet was resuspended in the same buffer and the centrifugation was repeated. After the second centrifugation, the supernatant fractions (S1) were combined and centrifuged at 6,000×*g* for 10 min. After discarding the resulting pellet (cell walls and unbroken cells), the supernatant (S2) was centrifuged at 45,000×*g* for 30 min and the supernatant (cytosolic fraction) was collected. The pellet (microsomal fraction) was resuspended in urea/Triton/DTT solution (9 M urea, 2% Triton X-100 and 1% dithiothreitol) and homogenized by a brief sonication. After addition of a one-fourth volume of 10% lithium lauryl sulfate to the homogenate, the pH was adjusted to neutral using 1 M Tris and the mixture was homogenized again by a brief sonication. Next, the samples were separated by SDS-polyacrylamide gel electrophoresis (PAGE), followed by protein gel blot analysis to detect hUGT protein. A rat anti-HA monoclonal antibody (Roche Diagnostics GmbH, Mannheim, Germany) and an horseradish peroxidase (HRP)-conjugated goat anti-rat IgG antibody (Roche Diagnostics GmbH) were used as the primary and secondary antibodies, respectively. Chemiluminescent signals developed with an ECL detection reagent (Amersham Biosciences, Buckinghamshire, UK) were detected using RX-U X-ray film (Fuji Photo Film Co. Ltd., Tokyo, Japan).

The protein gel blot analysis revealed that hUGT1 protein accumulated in the cells of all the *hUGT1*-transformed cell lines examined. A 42-kD HA-tagged hUGT1 protein was mainly detected in the microsomal fractions isolated from all the *hUGT1*-transformed cell lines, although some was also detected in their cytosolic fractions, whereas no signals were detected in the fractions isolated from pBIN19-transformed cell lines (Figure 1D). In our experiments, hUGT1-RFP accompanied by GFP fused with either sialyltransferase (a *trans*-Golgi marker) or AtRerB1 (a *cis*-Golgi marker) was transiently expressed in BY-2 protoplasts. Indeed, we found that the subcellular localization of hUGT1 was in the *trans*-Golgi, probably the *medial*-Golgi, and the endoplasmic reticulum but not in the *cis*-Golgi (Khalil et al., unpublished data). Therefore, it is suggested that hUGT1 protein was located in the Golgi vesicles of the transformed tobacco cells, similar to the case for human cells. However, the signal strengths differed among the *hUGT1*-transformed cell lines. The signals in U-1 and U-5 cells were strong, while those in the other cells were weak, suggesting that different amounts of hUGT1 protein accumulated in each cell line. These results indicate that the *hUGT1* gene was expressed in the transformed BY-2 cells.

It was possible that *hUGT1* expression in the transformed BY-2 cells caused alterations in the surface carbohydrate structure, such as the sugar chain compositions of glycoproteins or glycolipids, due to excessive import of UDP-galactose into the Golgi apparatus. In animal cells, the sugar chain compositions of glycoproteins and glycolipids on the surface of cells can be detected using fluorescein isothiocyanate (FITC)-lectin as a probe. To examine whether the surface carbohydrate structure could be detected by FITC-lectin staining, the binding of 21 kinds of lectin to BY-2 protoplasts was examined. An aliquot of protoplasts (1×10^5 cells) was suspended in 100 μl of 1% bovine serum albumin (Fraction V; Sigma Chemical Co., St. Louis, MO) in wash buffer (20 mM HEPES pH 7.4, 0.4 M mannitol) and incubated for 10 min to block the cell surface. Next, 1 μg of biotinylated lectin (Vector Laboratories Inc., Burlingame, CA) was added and incubated for 60 min at room temperature. After centrifugation to wash the protoplasts, 1 μg of FITC-labeled streptavidin (Vector Laboratories Inc.) was added to each protoplast suspension, and incubated for 60 min at room temperature. The FITC fluorescence of the resulting FITC-lectin-stained protoplasts was observed using a Leitz DMIRB fluorescence microscope (Leica Mikroskopie und Systeme GmbH, Wetzlar, Germany) and a TSC 4D confocal laser-scanning microscope (Leica Lazertechnik GmbH, Heidelberg, Germany).

Among the 21 kinds of lectin, concanavalin A (Con A) and RCA_{120} (*Ricinus communis* agglutinin 120 or RCA-I) strongly bound to the surface carbohydrate structures (Figure 2B), whereas the remainder lectins did not, although very faint fluorescence of FITC (outlines of cells with weak green fluorescence) was observed for samples treated with PSA, GSL-II, LEL and STL (Table 1). Figure 2B shows the FITC fluorescence of BY-2 protoplasts treated with Con A-FITC or RCA_{120}-FITC detected by confocal laser-scanning microscopy. Taken together, these results indicate that the surface carbohydrate structure of tobacco BY-2 protoplasts can be specifically determined by binding of FITC-labeled lectins such as ConA and RCA_{120}.

The surface carbohydrate modification of the *hUGT1*-transformed protoplasts was examined by FITC-lectin staining and fluorescence microscopy. Protoplasts were isolated from 3-day cultures of five *hUGT1*-transformed cell lines (U-1, U-2, U-3, U-5 and U-6) and a control pBIN19-transformed BY-2 cell line. The protoplasts were treated with the 21 kinds of lectin conjugated with biotin and then stained with FITC-conjugated streptavidin. Compared to the control pBIN19-transformed control line, all the *hUGT1*-transformed lines displayed decreased fluorescence of FITC-labeled ConA and RCA_{120}, while no new or enhanced fluorescence was observed on the surface of protoplasts after staining with other FITC-labeled lectins (data not shown).

To clarify the decreased fluorescence on the surface of protoplasts derived from the *hUGT1*-transfomed cell lines, fluorescence-activated cell sorting (FACS) analysis was employed. Protoplasts isolated from the pBIN19- and *hUGT1*-transformed cell lines were stained with FITC-conjugated ConA (FITC-ConA; Vector Laboratories Inc.) or FITC-conjugated RCA_{120} (FITC-RCA_{120}; Vector Laboratories Inc.), and their sizes and fluorescence were determined using a FACSCalibur (Becton, Dickinson and Company, Franklin Lakes, NJ). Using FACS analysis under the same conditions, the relative fluorescence of protoplasts derived from pBIN19-transfomed control cells was stronger for FITC-RCA_{120} staining than for FITC-ConA staining (Table 2), suggesting that RCA_{120}-binding sites were more abundant on the protoplast surface than ConA-binding sites. FACS analysis revealed a significant decrease in the surface fluorescence of protoplasts from all the *hUGT1*-transformed cell lines after FITC-ConA staining and their fluorescence was 20–45% lower than that of protoplasts from the pBIN19-transformed control line (Table 2). After FITC-RCA_{120} staining, the fluorescence levels of all the *hUGT1*-transformed cell lines except for U-6 were also decreased. Although their range of decreases (maximum of 35% reduction for line U-5, Table 2) was smaller than that for FITC-Con A (Table 2), the reduction in fluorescence was more impressive because of the original fluorescence intensity of FITC-RCA_{120}. The protoplasts of lines U-5 and U-6 were slightly expanded, while the protoplasts of the other lines were similar in size (Table 2). In the case of line U-6, the cell surface area exhibited a 9% increase compared with that of pBIN19-transformed control cells. However, the decreased fluorescence level of FITC-Con A was far larger than the degree of the increased surface area, indicating that the decreased fluorescence was not caused by the expansion of the protoplasts diluting the density of the surface carbohydrates. These results indicate that the surface carbohydrate structure was modified by expression of the *hUGT1* gene.

Table 1. FITC-lectin staining of BY-2 protoplasts.

Lectin	Specificity[1]	Fluorescence[2]
Con A	Manα, Glcα	+
DSL	$(GlcNAc)_n$, Galβ_{1-4}GlcNAc	−
DBA	GalNAcα	−
ECL	Galβ_{1-4}GlcNAc, Gal	−
GSL-I	Galα, GalNAcα	−
GSL-II	GlcNAcα, GlcNAcβ	±
Jacalin	Sialyl-Galβ_{1-3}GalNAc-*O*-	−
LCA	Manα	−
LEL	$(GlcNAc)_n$	±
PNA	Galβ_{1-3}GalNAc	−
PHA-E	N-linked bi-antennary sugar	−
PHA-L	N-linked tri/tetra-antennary sugar	−
PSA	Manα	±
RCA_{120}	Gal, GalNAc	+
STL	$(GlcNAc)_n$	±
SJA	Galβ, GalNAcβ	−
SBA	GalNAc	−
UEA I	Fucα	−
VVL	GalNAc	−
WGA	$(GlcNAc)_n$, sialic acid	−
S-WGA	$(GlcNAc)_n$	−

[1] Gal, D-galactose; GalNAc, N-acetyl-D-galactosamine; Glc, D-glucose; GlcNAc, N-acetylglucosamine; Fuc, L-fucose; Man, D-mannose.

[2] +, strong; ±, faint; -, not detected.

Table 2. Decreases in FITC-lectin fluorescence on protoplasts of the *hUGT1*-transformed cells.

		Relative fluorescence intensity (RFI; mean ± SD) RFI relative to the control RFI (%)					
Cell line		BIN	U-1	U-2	U-3	U-5	U-6
Con A	1	233.35 ± 5.25 100	139.48 ± 2.02* 59.8	131.68 ± 3.09* 56.4	151.61 ± 4.24* 65.0	160.88 ± 1.26* 68.9	185.82 ± 5.28* 79.6
	2	249.71 ± 2.60 100	145.91 ± 2.92* 58.4	145.84 ± 1.96* 58.4	168.82 ± 2.56* 67.6	163.19 ± 6.43* 65.3	185.66 ± 2.02* 74.3
	3	184.20 ± 8.34 100	97.71 ± 8.36* 53.0	112.52 ± 4.41* 61.1	95.26 ± 6.80* 51.7	85.77 ± 3.84* 46.6	128.75 ± 2.54* 69.9
RCA_{120}	1	717.57 ± 18.09 100	538.79 ± 10.60* 75.0	559.68 ± 8.80* 77.9	676.96 ± 9.81* 94.2	470.06 ± 6.37* 65.4	718.57 ± 4.50 100.0
	2	563.98 ± 10.40 100	501.18 ± 2.20* 88.9	505.90 ± 3.00* 89.7	576.10 ± 21.11 102.1	532.70 ± 10.60 94.5	662.84 ± 12.33 117.5
	3	666.55 ± 65.90 100	406.68 ± 56.70* 61.0	473.92 ± 36.02* 71.1	507.86 ± 56.39* 76.2	394.32 ± 33.15* 59.2	669.98 ± 44.94* 100.5
Diameter** (μm)		32.72 ± 0.43	32.96 ± 0.41	32.90 ± 0.43	32.96 ± 0.39	33.90 ± 0.83	34.11 ± 0.68
Surface area (μm^2) (%)***		13453 (100)	13651 (101)	13602 (101)	13651 (101)	14441 (107)	14620 (109)

Two independent FACS analyses using triplicate samples were performed. * $P<0.01$. ** Average diameter of protoplasts. *** Surface area of each type of protoplasts calculated from the average diameter of the protoplasts and the percentage of the surface area relative to the control (BIN) surface area.

In this article, we successfully transformed the tobacco suspension-cultured cell line BY-2 with the human nucleotide sugar transporter gene *hUGT1* and found that their protoplasts exhibited altered lectin-binding activities, as detected by FITC fluorescence, suggesting that the surface carbohydrate composition was modified by excessive nucleotide sugar transport into the Golgi apparatus. These findings indicate that this NST plays a crucial role in the biosynthesis of carbohydrates in plant cells.

Palacpac et al. (1999) reported that BY-2 cells originally lack β-1,4-galactosyltransferase, resulting in the absence of terminal-galactosylated N-glycans. On the other hand, membrane proteins of 58, 64 and 67 kDa with Galβ1,4GlcNAc at the terminal ends of their sugar chains were detected in the plasma membrane of BY-2 cells by lectin blot analysis using RCA_{120} and these sugar chains could be removed with N-glycanase (Kochibe N., Gunma University, personal communication), indicating the possible existence of glycoproteins with terminal-galactosylated N-glycans in the plasma membrane of BY-2 cells. Therefore, the sugar chains of these glycoproteins may be modified via the excessive transport of UDP-galactose by hUGT1 into the Golgi apparatus. However, the modification mechanism of the sugar chains of glycoproteins remains unknown.

The mouse cell line Had-1, which is derived from mouse FM3A cells, lacks the UDP-galactose transporter. FM3A cells show resistance to the lectin GSII (and GSLII), which binds to terminal N-acetylglucosamine (GlcNAc, Table 1), and sensitivity to the lectin WGA, which mainly binds to terminal sialic acid (Table 1), while Had-1 cells show the opposite lectin-sensitivity spectrum because of their immature carbohydrate structure (Kawakita et al. 1998). When Had-1 cells were transformed with a cDNA encoding hUGT1, they exhibited GSII-resistance and WGA-sensitivity. Furthermore, the *hUGT1*-transformants showed significantly higher WGA-sensitivity than the parental FM3A cells (Ishida et al. 1996). The overexpression of *hUGT1* therefore seemed to induce increased sialylation of the cell surface carbohydrates, resulting in increased binding of WGA. Although the mechanism of the increased sialylation remains unknown, this phenomenon in Had-1 cells must have been caused by the high concentration of UDP-galactose in the Golgi apparatus as a result of increased *hUGT1* expression. In the present study, the decrease in RCA_{120} may have been caused by hypergalactosylation of the surface carbohydrates owing to the high concentration of UDP-galactose in the Golgi apparatus. Furthermore, the carbohydrates detected by Con A also appeared to have been modified by expression of *hUGT1*.

In future studies, we need to clarify the reason why the carbohydrates of Con A-detectable Manα-glycoproteins were changed. To clarify the cause of the decreased fluorescence on *hUGT1*-transformed cells in detail, the glycoproteins on the plasma membrane need to be further investigated by lectin blot analysis using HRP-conjugated ConA and HRP-conjugated RCA_{120}. At present, the determination of glycoproteins with decreased amounts of carbohydrates is in progress.

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