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Complementation of an *Arabidopsis thaliana* mutant that lacks complex asparagine-linked glycans with the human cDNA encoding *N*-acetylglucosaminyltransferase I

(complex glycans/glycosyltransferase/protein glycosylation)

LUIS GOMEZ AND MAARTEN J. CHRISPEELS*

Department of Biology, University of California at San Diego, La Jolla, CA 92093-0116

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ABSTRACT **N-Acetylglucosaminyltransferase I (EC 2.4.1.101) initiates the conversion of high-mannose asparagine-linked glycans to complex asparagine-linked glycans in plant as well as in animal cells. This Golgi enzyme is missing in the *cgl* mutant of *Arabidopsis thaliana*, and the mutant cells are unable to synthesize complex glycans. Transformation of cells from the mutant plants with the cDNA encoding human *N*-acetylglucosaminyltransferase I restores the wild-type phenotype of the plant cells. Fractionation of the subcellular organelles on isopycnic sucrose gradients shows that the human enzyme in the complemented cells bands at the same density, 1.14 g/cm^3, typical of Golgi cisternae, as the enzyme in the wild-type plant cells. These results demonstrate that complementation results from the presence of the human enzyme in the plant Golgi apparatus, where it is functionally integrated into the biosynthetic machinery of the plant cell. In addition, given the evolutionary distance between plants and mammals and the great diversity of glycoproteins that are modified in each, there is probably no specific recognition between this Golgi enzyme and the polypeptide domains of the proteins it modifies.**

The integration of metabolic pathways and biosynthetic processes in specific organelles depends in part on the correct targeting of enzymes to these subcellular compartments. Recently a mutant, called *cgl*, of *Arabidopsis thaliana* was isolated in which a specific pathway carried out in the Golgi apparatus—the modification of asparagine-linked glycans on glycoproteins—is interrupted. The mutant cells lack *N*-acetylglucosaminyltransferase I (GnT I) activity, and it is presumed that this is the reason why they are unable to convert high-mannose glycans into complex glycans (1). To find out whether the mammalian enzyme that has the same catalytic activity could be expressed in plants and be integrated into this organelle and into the pathway, we have transformed the *cgl* mutant of *Arabidopsis* with the human gene encoding GnT I.

In plants, as in mammals, asparagine-linked glycans are transferred *en bloc* from lipid carriers to nascent polypeptide chains in the endoplasmic reticulum. Glycosidases and glycosyltransferases in the endoplasmic reticulum and in the Golgi compartment subsequently convert these $Glc_3Man_9GlcNAc_2$ glycans first into high-mannose glycans ($Man_{5-9}GlcNAc_2$) and then into complex glycans. The first step that leads to the formation of complex glycans in both plants and animals is catalyzed by the Golgi enzyme GnT I (2, 3). Its substrate is a $Man_5GlcNAc_2$ asparagine-linked glycan, and the enzyme transfers a single GlcNAc from UDPGlcNAc to the terminal α1→3 mannose that is linked to the β-linked mannose of the glycan core. In mutants that lack this enzyme, no complex glycans are synthesized (1). The glycoproteins in such mutant cells have an abundance of $Man_5GlcNAc_2$ glycans linked to asparagine. After the step catalyzed by GnT I and the removal of two additional mannose residues by mannosidase II, the pathways for complex glycan biosynthesis in plants and mammalian cells begin to diverge. The complex glycans of mammalian cells can have up to six branches, or antennae, that start with a mannose residue and often end with sialic acid (3). The complex glycans of plants, on the other hand, are much smaller and almost invariably contain a β1→2 xylose residue attached to the β-linked mannose residue of the core; they usually have an α1→3 fucose residue on the asparagine-linked GlcNAc, and sialic acid has never been found in these glycans (4).

The *cgl* mutant of *Arabidopsis*, which lacks GnT I, was obtained by screening a population of mutagenized plants with a serum that reacts specifically with the complex asparagine-linked glycans found on plant glycoproteins (1). The glycoproteins in the mutant plants do not react with this serum. Here we report that transformation of cells of this mutant with cDNA encoding human GnT I restores the wild-type phenotype. We chose the mammalian gene for this complementation experiment for two reasons. First, mammalian genes encoding GnT I have been cloned for several organisms, including human, mouse, rabbit, and hamster (5), but a plant gene has not yet been cloned; neither has the protein been purified. Second, we wished to find out whether a mammalian Golgi enzyme would be correctly targeted to the plant Golgi compartment and function there. The *Arabidopsis* cells complemented with the human enzyme synthesize glycoproteins that react with the antiserum and incorporate [^{3}H]fucose. These glycans do not bind to concanavalin A (Con A), indicating that they are not of the high-mannose type. The complementation of the mutant suggests that human GnT I must be localized in the plant Golgi compartment and this localization has been confirmed by the use of sucrose density gradients. Together, these results indicate the correct arrival of the human enzyme in the plant Golgi compartment and its integration in the normal pathway for biosynthesis of complex glycans.

MATERIALS AND METHODS

***Arabidopsis* Transformation and Tissue Culture Conditions.** To express human GnT I in *Arabidopsis* cells, the *Avr* II–*Apa* I fragment of plasmid pHG4.5 containing the human cDNA for this enzyme (6) was inserted into the *Xba* I site of pA35S (7), giving rise to pLHGT I (Fig. 1*A*). The resulting plant expression cassette was cloned into pBI121 (Clontech), giving rise to construct p35SGTI, which was subsequently used for *Agrobacterium*-mediated transformation of *Arabidopsis* root explants (9). Kanamycin-resistant calli were isolated and

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Abbreviation: GnT I, N-acetylglucosaminyltransferase I.
*To whom reprint requests should be addressed.

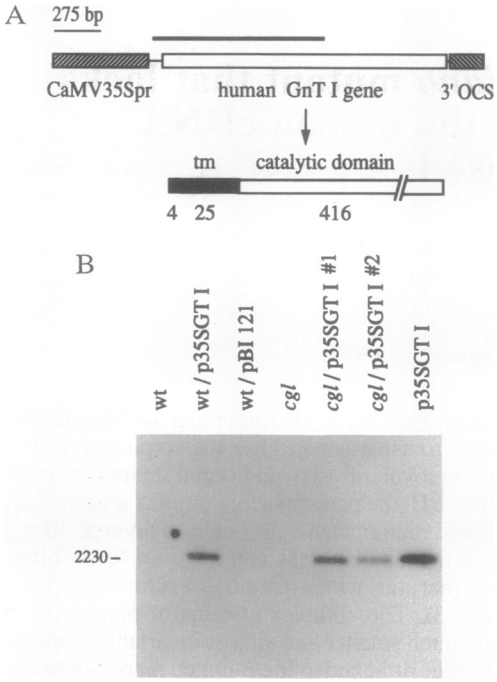

FIG. 1. Transformation of *Arabidopsis* with the gene encoding human GnT I. (*A*) Schematic representation of the plant expression cassette from pLHGTI containing the human gene for GnT I. This fragment (2230 bp) was cloned into the *Eco*RI–*Hin*dIII site of the binary vector pBI121 and used to transform *Arabidopsis* root explants. The human gene encodes a type II membrane protein whose large carboxyl-terminal catalytic domain (416 amino acids) is in the Golgi lumen (8). CaMV35Spr, cauliflower mosaic virus 35S promoter; 3'OCS, octopine synthase 3' untranslated sequence; tm, transmembrane region. (*B*) Total genomic DNA was purified from kanamycin-resistant regenerated calli, digested with *Eco*RI and *Hin*dIII, and analyzed by Southern blot hybridization using as a probe the *Bam*HI–*Msc* I fragment (942 bp) indicated in *A*. Size of the hybridizing fragment (2230 bp) is indicated. wt, Wild type.

grown under low-light conditions on callus-inducing agar medium (9).

Southern Blot Analysis. Total DNA from *Arabidopsis* calli was purified according to Dellaporta *et al.* (10). After digestion with *Eco*RI and *Hin*dIII, the DNA restriction fragments were separated in a 0.8% agarose gel and transferred to Hybond-N membranes by following the instructions of the manufacturer (Amersham). Membranes were prehybridized for 2 hr at 42°C in 6× SSPE (1× SSPE is 0.15 M NaCl/10 mM sodium phosphate, pH 7/1 mM EDTA)/5× Denhardt's solution/0.5% SDS with denatured salmon sperm DNA at 0.1 mg/ml and hybridized for an additional 16 hr in the same solution containing 50% (vol/vol) formamide with ^{32}P-labeled probe (see below) at 10^6 cpm/ml. After hybridization, membranes were washed twice in 2× SSC (1× is 0.15 M NaCl/15 mM sodium citrate, pH 7)/0.1% SDS at room temperature for 15 min, twice in 1× SSC/0.1% SDS at 42°C for 15 min, and once in 0.1× SSC/0.1% SDS at 65°C for 10 min. The 942-bp *Bam*HI–*Msc* I fragment from pLHGT I, including most of the coding region of the human gene (Fig. 1*A*), was purified by agarose gel electrophoresis and labeled with [α-^{32}P]dATP by priming denatured DNA with random hexamers from BRL.

Immunoblot Analysis. To analyze transformed calli for the presence of complex glycans, callus material (0.5 g) was homogenized in 50 mM Hepes (pH 7.5) containing 0.1% SDS, 2 mM sodium bisulfite and leupeptin, aprotinin, and pepstatin A each at 1 μg/ml. Insoluble material was eliminated by centrifugation and protein was quantitated by the Bio-Rad protein assay. For each sample analyzed, 25 μg of protein was fractionated by SDS/PAGE, transferred to nitrocellulose, and probed with an antiserum specific for complex glycans (11).

Metabolic Labeling with [^{3}H]Glucosamine and [^{3}H]Fucose. Callus material (2 g) was incubated in a Petri dish for 4 hr in 10 ml of Murashige–Skoog medium (12) without sucrose. Incubation was done at 27°C in the dark on a rotary shaker plate. Cell clumps were collected by low-speed centrifugation, resuspended in 2 ml of fresh medium containing 80 μCi of D-[6-^{3}H]glucosamine (0.96 TBq/mmol; Amersham; 1 μCi = 37 kBq) or 80 μCi of L-[5,6-^{3}H]fucose (1.85 TBq/mmol; Amersham) and further incubated for 24 hr as above.

Isolation and Analysis of ^{3}H-Labeled Glycopeptides. Prior to protein extraction, labeled cells were washed three times with 10 ml of Murashige–Skoog medium and homogenized in liquid nitrogen. The powder was then extracted (1) and proteins were precipitated by the addition of cold acetone (80%, vol/vol) followed by centrifugation at 17,000 × *g* for 20 min. The pellet was washed twice with 80% acetone in water and dried. For the analysis of [^{3}H]fucose-labeled glycopeptides, the dried pellet was suspended in 1 ml of 50 mM Hepes (pH 7.5) containing 0.1% SDS, 2 mM sodium bisulfite and protease inhibitors (1 μg/ml) as described above. Labeled glycopeptides were then precipitated with 7% (wt/vol) trichloroacetic acid and analyzed by SDS/PAGE and fluorography.

To analyze [^{3}H]glucosamine-labeled glycopeptides, the dried pellet was suspended in 100 mM Tris/HCl (pH 8.0) containing 5 mM $CaCl_2$ and proteins were digested for 24 hr with Pronase (13). For the separation of glycopeptides containing asparagine-linked high-mannose type glycans and complex-type glycans, the lyophilized glycopeptide mixture was dissolved in 20 mM Tris/HCl, pH 7.0, containing 0.5 M NaCl, 1 mM $CaCl_2$, 1 mM $MgCl_2$, 1 mM $MnCl_2$, and 0.05% sodium azide and chromatographed on a Con A-Sepharose 4B column (Sigma) equilibrated and eluted with the same buffer. Bound high-mannose oligosaccharides were eluted with 200 mM methyl α-D-mannopyranoside.

Isolation of Membranous Organelles and Subcellular Fractionation. Fresh calli (2 g) were homogenized in 4 ml of a buffer containing 12% (wt/wt) sucrose, 100 mM Tris/HCl (pH 7.8), 1 mM EDTA, and protease inhibitors (1 μg/ml; see above). Cell walls were eliminated by centrifugation at 1000 × *g* for 5 min. Supernatants were layered over a two-step discontinuous gradient of 16% sucrose (5 ml) on top of 48% sucrose (1 ml) in 100 mM Tris/HCl (pH 7.8) with 1 mM EDTA. The gradient was centrifuged at 150,000 × *g* for 2 hr and subcellular organelles were collected at the 16%/48% interface.

Separation of subcellular organelles was performed with isopycnic 18–50% sucrose gradients (14). After centrifugation, 0.6-ml fractions were collected and aliquots were assayed for GnT I and NADH–cytochrome-*c* reductase activity and for sucrose concentration by refractometry.

GnT I Assay. The assay mixture (300 μl) contained 100 mM Mes (pH 6.25), 10 mM $MnCl_2$, 0.1% Triton X-100, ribonuclease (0.3 μg/ml, type XII-B; Sigma), swainsonine (0.5 μg/ml), 0.1 mM UDP[^{3}H]GlcNAc (1.17 Bq/mmol; DuPont), proteinase inhibitors (1 μg/ml, see above), and the membrane fraction. The reaction was allowed to proceed at 30°C for 60 min and then stopped by the addition of trichloroacetic acid to 6%. Pellets were washed three times with 80% acetone at 37°C to extract contaminating ^{3}H-labeled glycolipids and then dried. This material was resuspended in 50 μl of 60 mM Tris/HCl (pH 6.8) containing 3 M urea, heated at 65°C for 5 min, and centrifuged. The supernatant was diluted 10-fold with water and centrifuged again. Finally, ^{3}H-labeled glycoproteins were precipitated with trichloroacetic acid and adsorbed onto Millipore HA discs (0.45-μm pore size) and radioactivity was determined.

NADH–Cytochrome-*c* Reductase Assay. The assay mixture (975 μl) contained 20 mM potassium phosphate (pH 7.2), 0.2 mM NADH, 0.02 mM cytochrome *c*, and 5 mM KCN. The reaction was started by adding 25 μl of each membrane fraction, and the reduction of cytochrome *c* was followed spectrophotometrically as the absorbance increase at 550 nm for 1 min by using an LKB Ultrospec II and LKB kinetics software.

RESULTS

Transformation of the *cgl* Mutant with the Human GnT I cDNA. Transformation of *Arabidopsis* (wild type or mutant) was carried out with vector p35SGT I, in which the coding sequence of the human GnT I cDNA is flanked at the 5′ end by a portion of the cauliflower mosaic virus 35S promoter and at the 3′ end by the octopine synthase 3′ untranslated sequence (Fig. 1*A*). A Southern blot of DNA extracted from the resulting kanamycin-resistant calli and probed with the fragment of the human GnT I gene indicated in Fig. 1*A* is shown in Fig. 1*B*. A positive control is shown in lane 7. Our results confirmed that transformation of the *Arabidopsis* cells with the human cDNA had been achieved.

Transformation Restores the Wild-Type Phenotype. To determine whether transformation of the plant cells resulted in functional complementation, we immunoblotted protein extracts of these cells with the antiserum directed at the asparagine-linked complex glycans of plant glycoproteins (11). The wild-type cells contained numerous glycoproteins that reacted with the serum, whereas the mutant cells did not contain crossreactive glycoproteins (Fig. 2*B*, compare lanes 3 and 1). The complemented mutant cells also contained glycoproteins that crossreacted with the serum, although the intensity of this reaction was less (compare lanes 4 and 5 with lane 2), even though the lanes contained equal amounts of protein (compare the same lanes in Fig. 2*A*). The lower intensity of staining in the complemented mutant cells may indicate lower levels of enzyme or a less efficient reaction of the human enzyme with the plant glycoproteins. Nevertheless, we concluded that the enzyme encoded by the human gene restored the plant phenotype.

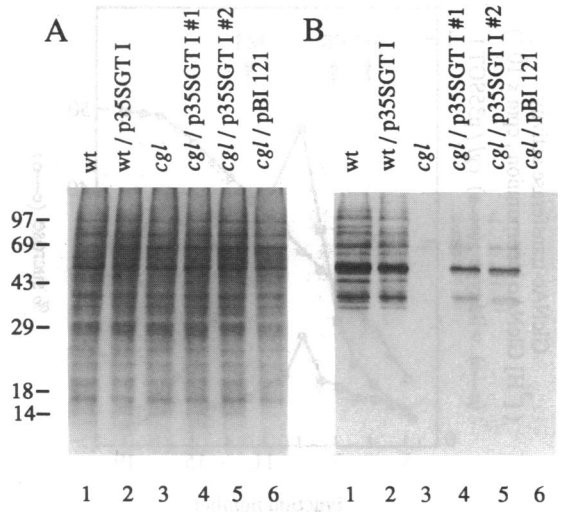

FIG. 2. Immunodetection of asparagine-linked complex glycans. Total protein extracts from cultured calli were fractionated by SDS/PAGE and stained with Coomassie blue (*A*) or transferred to nitrocellulose, and probed with an antiserum specific for complex glycans (*B*). The pattern of immunoreactive glycoproteins did not differ significantly among all transformants tested (extracts from two different calli, nos. 1 and 2, are shown in lanes 4 and 5). The positions of molecular size markers (kDa) are indicated at left.

To understand the extent to which high-mannose glycans had been converted to complex glycans in these complemented mutant cells, we separated these two types of glycans by affinity chromatography on Con A-Sepharose. In Fig. 3*A* (glycopeptides of wild-type cells) the first peak represents the glycopeptides with asparagine-linked complex glycans that do not bind to Con A and the second peak represents glycopeptides with asparagine-linked high-mannose glycans that bind to Con A but can be eluted with methyl α-D-mannoside. In these cells about 75% of the radioactivity was in complex glycans. The mutant cells contained no complex glycopeptides (Fig. 3*B*). In the cells of the complemented mutant, 35% of the radioactivity was in high-mannose glycopeptides and 65% was in complex glycopeptides. These data suggest that in these complemented mutant cells the human enzyme transforms the high-mannose glycans into complex glycans with a somewhat lower efficiency than the plant enzyme in the wild-type cells.

The serum used in these experiments is directed primarily at the β1→2 xylose residues on these glycoproteins (15), and reactivity implies that xylose residues are present. Glycoproteins without xylose react very poorly with this serum. To find out whether the glycans contained fucose, cells were incubated with [^{3}H]fucose, the proteins were separated by SDS/PAGE, and a fluorograph was prepared (Fig. 4). Fucose was incorporated into numerous glycoproteins in the wild-type cells as well as in the complemented mutant cells, whereas there was no incorporation of fucose in the glycoproteins of the mutant cells. The incorporation of fucose and xylose into plant glycoproteins is completely dependent on the prior action of GnT I (14). The possibility that these sugars are present in hybrid glycans is remote because hybrid glycans have not been found in plant cells. Rather, it seems more likely that the glycans are normally processed by the other enzymes present in the Golgi apparatus. Close examination of the fluorograph shows that the low molecular weight proteins were more heavily labeled in the complemented mutant than in the wild-type cells (squares in Fig. 4),

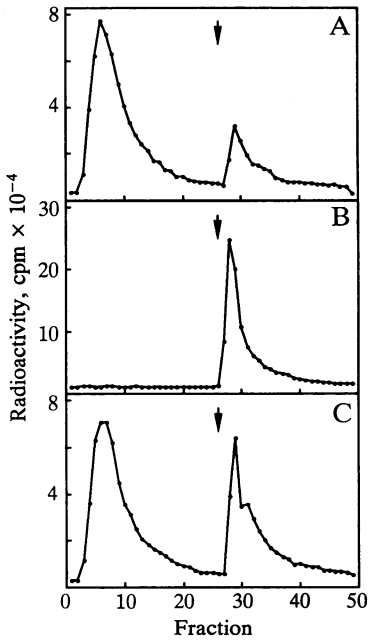

FIG. 3. Con A chromatography of [^{3}H]glucosamine-labeled glycopeptides. Metabolically labeled glycoproteins from wild-type (*A*), *cgl* (*B*), and *cgl*/p35SGT I (*C*) *Arabidopsis* calli were digested with Pronase, and the glycopeptides were purified by gel filtration and subsequently separated by affinity chromatography on Con A-Sepharose. The starting point of elution with methyl α-D-mannoside is indicated by an arrow.

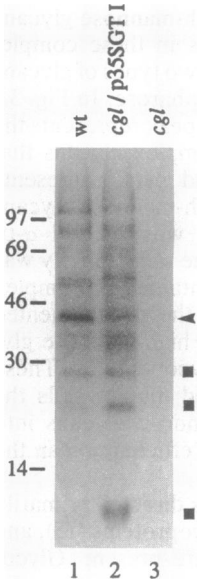

FIG. 4. Fluorograph of [^{3}H]fucose-labeled glycoproteins. Glycoproteins metabolically labeled with L-[5,6-^{3}H]fucose were fractionated by SDS/PAGE and visualized by fluorography. The arrowhead points to a 40-kDa glycoprotein that is less heavily labeled in the complemented mutant, whereas those glycoproteins more heavily labeled than in the wild type (wt) are marked by squares. Positions of molecular size markers (kDa) are indicated.

whereas at least one protein of 40 kDa was less heavily labeled (arrowhead). Such differential labeling of glycoproteins may result from the differential interaction of these glycoproteins with the human GnT I compared with the plant GnT I.

Subcellular Location of Human GnT I. The complementation results described above implied that the human GnT I enzyme was present in the plant Golgi apparatus and functionally integrated in the glycan modification pathway. To confirm this point we examined the distribution of GnT I on sucrose gradients after separating the organelles. Enzyme activity was measured with bovine ribonuclease as a substrate and in the presence of the glycan processing inhibitor swainsonine. This ribonuclease is a glycoprotein that contains mostly $Man_5GlcNAc_2$ glycans and is therefore a good substrate for GnT I. Swainsonine prevents the degradation of those glycans by the acid (vacuolar) mannosidase that may contaminate the organelle preparations and it also prevents the GnT II-catalyzed incorporation of UDP[^{3}H]GlcNAc into glycoproteins processed by GnT I, by blocking the action of mannosidase II (16). We ascertained that the level of swainsonine used here (0.5 μg/ml) completely inhibited all α-mannosidase activity (data not shown).

A comparison of the total GnT I activities in the organelle fractions of wild-type, mutant, and complemented mutant cells is shown in Fig. 5. The mutant cells lacked the enzyme activity, as previously demonstrated (1). GnT I activity in the complemented mutant was only 20–25% of that found in wild-type cells. This result is surprising, since the 35S promoter used to drive the expression of the GnT I gene is considered to be a strong promoter for expression in plant cells.

Fractionation of the organelles on isopycnic sucrose gradients resulted in a single peak of GnT I activity at 1.14 g/cm^3 in both the wild type and the complemented mutant (Fig. 6). The endoplasmic reticulum marker enzyme NADH–cytochrome-*c* reductase formed a peak around 1.11 g/cm^3. Tonoplast vesicles banded at 1.08 g/cm^3 and mitochondria at 1.17 g/cm^3. These densities are consistent with the densities of plant cell organelles observed in other laboratories (17, 18).

DISCUSSION

We show in this paper that the *cgl* mutant of *A. thaliana*, lacking asparagine-linked complex glycans and GnT I activity, can be complemented with the human cDNA encoding GnT I. The biosynthesis of complex asparagine-linked glycans involves a series of membrane-bound enzymes in the Golgi apparatus (3, 4). Our results demonstrate that human GnT I localizes to the plant Golgi apparatus and can function there as part of the glycan modification pathway.

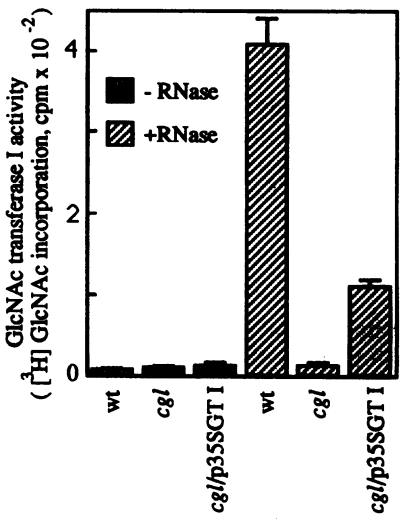

FIG. 5. Determination of GnT I activities in membranous organelles. Each organelle fraction was assayed for GnT I activity using UDP[^{3}H]GlcNAc as donor and ribonuclease (Sigma type XII-B) as exogenous acceptor, in the presence of swainsonine (0.5 μg/ml) to inhibit aryl mannosidase and processing mannosidase II. When ribonuclease was not added (first three bars), no significant activity was found, possibly due to the trimming of the glycans on the endogenous glycoprotein. Data are expressed as the mean ± SD of three independent experiments. wt, Wild type.

Subcellular Location of GnT I. In mammalian cells, GnT I is a type II membrane protein (N_{out}/C_{in}) with greatest abundance in the medial Golgi cisternae. The location of the enzyme is specified by its transmembrane domain and flanking sequences (19, 20). In the complemented plant cells, human GnT I was associated with membranes of density 1.14 g/cm^3; in the wild-type cells, the plant GnT I was found in membranes that had the same density (Fig. 6; see also ref. 4). This is the typical density at which Golgi membranes (vesic-

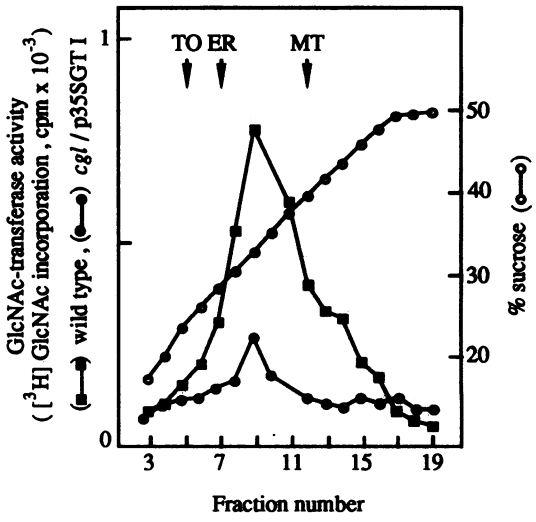

FIG. 6. Localization of human GnT I in plant Golgi membranes. Subcellular fractionation on isopycnic sucrose gradients and enzymatic assays were performed as described in *Materials and Methods*. As membrane markers we used NADH–cytochrome-*c* reductase activity (for endoplasmic reticulum, ER, and mitochondria, MT) and immunoblots with antibodies against the aquaporin γ-TIP (for tonoplast, TO).

ulated cisternae) are found in sucrose gradients, and other known Golgi enzymes also band at this density (13). Whether the enzyme is most abundant in the medial Golgi, as it is in mammalian cells, is not clear. There is at present no evidence for plant cells that the cis, medial, and trans Golgi compartments have different densities (13), although they may contain different enzymes (21). The finding that this human membrane protein is confined to the Golgi apparatus of plant cells is in contrast to results obtained with the G protein of vesicular stomatitis virus. Expression of this protein in plant cells resulted in its presence in several membrane systems, including the endoplasmic reticulum, the Golgi stack, and the plasma membrane (17).

We do not have evidence that the human GnT I was retained in the Golgi apparatus of the plant cells. The lower activity of the human GnT I compared to the plant GnT I could be a reflection of the continuous transport of the enzyme by bulk flow through the secretory system. The default destination of membrane proteins has not yet been determined for plant cells, but in *Saccharomyces cerevisiae* the vacuolar membrane has been shown to be the default destination for membrane proteins (22, 23). If the tonoplast is also the default destination in plant cells, as suggested by several indirect lines of evidence (7, 24), then we could expect the catalytic domain of the protein to be degraded by vacuolar proteases when the enzyme reaches the tonoplast. Lack of specific retention could explain the low enzyme activity. Alternatively, the low level of activity could be caused by the instability of this mammalian protein or its RNA in plant cells. Yet another possibility is that proteolytic processing between the catalytic domain and the transmembrane domain results in the detachment of the catalytic domain from the membrane and its subsequent secretion from the cells. Such proteolytic processing does occur for several glycosyltransferases, including GnT I, and results in the release of smaller catalytically active proteins (5). Deciding which of these three deficient interpretations is the correct one will require considerable further experimentation.

Efficiency of Glycan Conversion. GnT I catalyzes the first committed step in asparagine-linked glycan modification. Yet *et al.* (25) postulated that this first step should be regulated by the protein matrix, rather than by the glycan itself. However, the observed complementation implies that there is no specific recognition between this glycosyltransferase and the proteins it modifies. It is also possible, but seems less likely, that such recognition is conserved between plant and mammalian proteins. The GlcNAc 1-phosphotransferase of mammalian cells that modifies high-mannose glycans of lysosomal enzymes as a first step in the generation of the mannose 6-phosphate lysosomal targeting signal does recognize specific proteins. After recognition of specific peptide domains, it modifies the glycans of lysosomal enzymes but not those of secreted enzymes (26, 27).

Overall, the GnT I activity was substantially lower in the complemented cells than in the wild-type cells. Nevertheless, the radioactive labeling experiments indicate that nearly the same proportion of high-mannose glycans was converted in the complemented mutant as in the wild-type cells. This result supports the conclusion that the enzyme is not limiting in the wild-type cells, although there does not appear to be a great excess of enzyme.

The incorporation of radioactive fucose into the proteins indicates that lower molecular weight polypeptides incorporated relatively more fucose in the complemented cells than in the wild-type cells, suggesting that the mammalian GnT I may be more effective with low molecular weight proteins. This finding supports the conclusion reached by Yet *et al.* (25) that the protein matrix has an important influence on the efficiency of glycan processing. In the wild-type cells, the glycans on these small proteins may not have xylose, as shown by their lack of reactivity with the anti-complex glycan serum which is nearly specific for xylose (11, 15). Our data suggest, but do not prove, that the complex glycans on those small polypeptides in the complemented cells may be fucose-rich and xylose-poor. Small proteins may not be as good a substrate for the xylosyltransferase as for the fucosyltransferase.

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