Genetic defect in *N*-acetylglucosaminyltransferase I gene of a ricin-resistant baby hamster kidney mutant

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The analysis of mutations associated with glycosylation-defective cell lines has the potential for identifying critical residues associated with the activities of enzymes involved in the biosynthesis of glycoconjugates. A ricin-resistant (Ric^R) baby hamster kidney (BHK) cell mutant, clone Ric^R14, has a deficiency in *N*-acetylglucosaminyltransferase I (GlcNAc-TI) activity and as a consequence is unable to synthesize complex and hybrid N-glycans. Here we show that Ric^R14 cells transfected with wild-type GlcNAc-TI regained the ability to synthesize complex N-glycans, demonstrating that the glycosylation defect of Ric^R14 cells is due solely to the lack of GlcNAc-TI activity. With the use of specific antibodies to GlcNAc-TI, Ric^R14 cells were shown to synthesize an inactive GlcNAc-TI protein that is correctly localized to the Golgi apparatus. We have cloned and sequenced

the open reading frame of GlcNAc-TI from parental BHK and Ric^R14 cells. A comparison of several Ric^R14 cDNA clones with the parental BHK GlcNAc-TI sequence indicated the presence of two different Ric^R14 cDNA species. One contained a premature stop codon at position +81, whereas the second contained a point mutation in the catalytic domain of GlcNAc-TI resulting in the amino acid substitution Gly³²⁰ → Asp. The introduction of a Gly³²⁰ → Asp mutation into wild-type rabbit GlcNAc-TI resulted in a complete loss of activity; the GlcNAc-TI mutant was correctly localized to the Golgi, indicating that the inactive GlcNAc-TI protein was transport-competent. Gly³²⁰ is conserved in GlcNAc-TI from all species so far examined. Overall these results demonstrate that Gly³²⁰ is a critical residue for GlcNAc-TI activity.

INTRODUCTION

Plant lectins are a class of cytotoxic proteins that exhibit high affinity for specific oligosaccharides. These oligosaccharides are commonly found in glycoconjugates on the cell surface of eukaryotes. Over the past two decades, the cytotoxic activity of lectins has been used to generate lectin-resistant cell lines that exhibit altered carbohydrate structures. Lectin-resistant cell lines have been useful for the study of glycan biosynthesis [1] and as tools for membrane transport *in vitro* [2,3]. Lectin-resistant cell lines have also been used for the cloning of glycosyltransferase genes by complementation [4]. Despite their widespread use in glycobiology, the genetic basis of lectin resistance has been identified in only a few instances [5,6].

The ricin-resistant (Ric^R) group of baby hamster kidney (BHK) cell mutants was generated by chemical mutagenesis followed by selection in the presence of the galactose-specific lectin ricin. Twenty-two clones were isolated, and these were divided into four classes on the basis of their ability to bind ricin and other lectins [7]. Ric^R14 cells are resistant to ricin owing to the loss of cell-surface carbohydrate receptors for ricin. Ric^R14 cells lack β -*N*-acetylglucosaminyltransferase I activity (GlcNAc-TI) [8,9], the enzyme that catalyses the first step in the conversion of high-mannose N-glycans into complex and hybrid N-glycans. A structural analysis of the N-glycans of Ric^R14 cells revealed an absence of complex and hybrid N-glycans and the accumulation of oligosaccharides bearing Man₅-GlcNAc₂-Asn structures [10], the substrate of GlcNAc-TI *in vivo*. This block in N-glycan synthesis accounts for the decrease in binding sites for ricin and

for phytohaemagglutinin, which also binds to galactose-containing complex N-glycans. The Ric^R14 mutant has different morphological properties from those of the parental BHK cells: in particular it displays decreased cell-substratum adhesion and does not exhibit the typical alignment of cells at high density that is characteristic of wild-type BHK cells [11].

The analysis of mutations associated with glycosylation-defective cell lines has the potential for identifying critical residues associated with activities of glycosyltransferases. In the present study we have identified the genetic lesion of the Ric^R14 cell line. The glycosylation defect of Ric^R14 cells was shown to be due solely to the lack of GlcNAc-TI activity as the expression of functional GlcNAc-TI results in the synthesis of complex N-glycans. By immunofluorescence and immunoblotting we have demonstrated that Ric^R14 cells express a full-length, Golgi-localized GlcNAc-TI protein. Furthermore we have identified the Ric^R14 mutation as a point mutation in the GlcNAc-TI gene and demonstrated that Gly³²⁰ is a critical residue for GlcNAc-TI activity.

EXPERIMENTAL

Cell culture

Cells were maintained at 37 °C as monolayers in Dulbecco's modified Eagle's medium supplemented with 10 % (v/v) fetal-calf serum (FCS), 2 mM glutamine, 100 i.u./ml penicillin and 0.1 % streptomycin. BHK21 and Ric^R14 cells [7] were obtained

Abbreviations used: BHK, baby hamster kidney; FCS, fetal calf serum; Ric^R, ricin-resistant; GlcNAc-TI, *N*-acetylglucosaminyltransferase I; L-PHA, leuco-phytohaemagglutinin; RT, reverse transcriptase.

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The nucleotide sequence data reported will appear in DDBJ, EMBL and GenBank Nucleotide Sequence Databases under the accession numbers AF087456 and AF087457.

from Dr. Colin Hughes (National Institute for Medical Research, Mill Hill, London, U.K.).

Antibodies

Mouse anti-(rabbit GlcNAc-TI) antibodies were produced as described [12].

Immunofluorescence

Cells grown on 12-well glass microscope slides were rinsed in PBS, fixed and permeabilized with cold acetone (-20°C) for 30 s, then rinsed again in PBS before incubation with mouse anti-(rabbit GlcNAc-TI) antibodies [12] at a 1:200 dilution in PBS containing 5% (v/v) FCS. Bound antibodies were detected with FITC-conjugated sheep anti-mouse immunoglobulin (Silenus, Australia). Images were acquired on a Bio-Rad MRC 1024 confocal microscope.

Immunoblotting

Cells were harvested with a cell scraper and solubilized in 1% (v/v) Triton X-100 in PBS with Complete[™] protease inhibitors (Boehringer Mannheim) for 45 min on ice. Extracts were cleared by centrifugation for 15 min (15000 g). Cell extracts were separated by SDS/PAGE and transferred electrophoretically to nitrocellulose membranes. Membranes were blocked with 5% (w/v) non-fat skimmed milk in PBS and incubated for 1 h with mouse anti-(rabbit GlcNAc-TI) antibodies [12] diluted 1:200 in PBS containing 5% (w/v) non-fat skimmed milk, then washed with PBS containing 0.05% (v/v) Tween 20. Bound antibodies were detected with horseradish peroxidase-conjugated rabbit anti-mouse immunoglobulin (Dako), followed by enhanced chemiluminescence (Dupont).

Transfections

COS-7 cells were transfected as described previously [12] with GlcNAc-TI constructs cloned into pCI-neo (Promega). Mouse 3T3 cells were transfected with GlcNAc-TI constructs in pCI-neo by using Superfect (Qiagen) in accordance with the manufacturer's instructions. Ric^R14 cells were co-transfected with pSVTgpt-GlcNAc-TI [12] and pSV2-Neo [13] by the calcium phosphate method [13]. Positive transfectants were selected in 0.4 mg/ml geneticin (Life Technologies) and identified by indirect immunofluorescence on acetone-fixed cells with mouse anti-(rabbit GlcNAc-TI) antibodies. Clonal cell lines were obtained from the transfected populations by limiting dilution.

Lectin binding assay

Subconfluent monolayers were harvested with PBS containing 1 mM EDTA. Cells were washed in PBS/FCS/Na₃ [PBS containing 2% (v/v) FCS and 0.02% Na₃] then incubated for 45 min with FITC-conjugated leuco-phytohaemagglutinin (L-PHA) (Sigma) at 10 µg/ml in PBS/FCS/Na₃. Cells were washed twice in PBS/FCS/Na₃, then analysed by flow cytometry with a FACScan (Becton Dickinson).

Isolation and sequence analysis of parental BHK and Ric^R14 GlcNAc-TI clones

Genomic clones of GlcNAc-TI were isolated from parental BHK cells as described for Chinese hamster ovary cells [5]. As in GlcNAc-TI of other species, the coding region of the BHK GlcNAc-TI gene resides in a single exon. Ric^R14 cDNA clones

were obtained by PCR amplification of cDNA generated from RNA by reverse transcriptase (RT-PCR). RNA was isolated as described by Sambrook et al. [14] or by the use of an RNA isolation kit (Ambion). Total RNA (2.5 µg) was reverse-transcribed with 5 units of avian myeloblastosis virus reverse transcriptase (Life Technologies) and 50 pmol of oligo(dT) primer at 37 °C for 1 h. PCR was performed with 2.5 units of *Taq* polymerase (Life Technologies) and 0.016 unit of cloned *Pfu* DNA polymerase (Stratagene) with a 55 °C annealing temperature. Degenerate primers based on the sequence of rabbit, rat, mouse and human GlcNAc-TI were as follows: 5'-CCGAATTCAGGATGCTGAAGAAGCAG-3' and 5'-GGCAATTCTAA(T/A)TTCA(G/A)CTAGGATCATA-3'. A 1.3 kb product was isolated from agarose gels, digested with *Eco*RI and sub-cloned into the *Eco*RI site of pCI-neo (Promega).

The nucleotide sequence of clones was determined by using a Prism dye-deoxy terminator cycle kit and a 373A DNA sequencer (Applied Biosystems).

Site-directed mutagenesis

A Gly³²⁰ → Asp mutation was introduced into rabbit GlcNAc-TI by using a megaprimer mutagenesis protocol adapted from that of Ke and Madison [15]. The mutagenic primer, 5'-TGGCCGGAAGGATGTGAGTCATGGGCAGTTC-3', was based on the rabbit GlcNAc-TI sense strand with the two base substitutions underlined. These base substitutions convert the Gly residue at position +320 to Asp, and also eliminate a unique *Nco*I site to assist in screening for mutagenized clones. The 5' and 3' flanking primers were used as described above, and rabbit GlcNAc-TI was used as template. The PCR was initiated asymmetrically, starting with 10 pmol of mutagenic primer. After five rounds of synthesis, 100 pmol of anti-sense flanking primer was added, and amplification was continued for 25 cycles. The resulting product was purified from an agarose gel and used as a megaprimer in a second PCR amplification. This second PCR was also initiated asymmetrically, with only the megaprimer for the first five rounds. Sense flanking primer (100 pmol) was then added and the PCR was continued for a further 25 cycles. The final product was digested with *Eco*RI, purified from an agarose gel and cloned into pCI-Neo (Promega). The sequences of mutant clones were confirmed by DNA sequence analysis.

GlcNAc TI enzyme assays

COS-7 cells were transfected as described above; 48 h after transfection, cells were harvested and lysed in 50 mM Tris/maleate, pH 7.0, containing 1% (v/v) Triton X-100 and 250 mM NaCl. Extracts were cleared by centrifugation for 15 min at 15000 g, and GlcNAc-TI activity was assayed as described by Vischer and Hughes [8] by using 250 µg of extract protein in 100 µl reactions, with ovalbumin as an acceptor.

RESULTS

Expression of rabbit GlcNAc-TI in Ric^R14 cells

The ricin-resistant BHK cell line Ric^R14 is unable to synthesize hybrid and complex N-glycans. To determine whether active GlcNAc-TI could revert the Ric^R14 phenotype, Ric^R14 cells were transfected with rabbit GlcNAc-TI cDNA as described in the Experimental section. The synthesis of complex N-glycans by the transfected cells was assessed by cell-surface binding of the lectin L-PHA, which binds to galactose-containing complex N-glycans [16]. Flow cytometric analysis revealed that, whereas Ric^R14 cells did not bind FITC-L-PHA, transfected Ric^R14 cells bound FITC-L-PHA at a similar level to that of wild-type BHK cells

Figure 1 Detection of cell-surface complex N-glycans on GlcNAc-TI-transfected Ric^R14 cells

Parental BHK, Ric^R14 and rabbit GlcNAc-TI-transfected Ric^R14 cells, as indicated, were incubated for 30 min in the absence (A) or the presence (B) of FITC-conjugated L-PHA and analysed by flow cytometry.

(Figure 1). Therefore the inability of Ric^R14 cells to produce complex N-glycans is due solely to the absence of active GlcNAc-TI.

The expression of enzymically active rabbit GlcNAc-TI in Ric^R14 cells had no effect on the morphological features of this cell line. The transfected Ric^R14 cells displayed the same tendency to detach from the surface of tissue culture vessels and failed to display the typical alignment at high density that is characteristic of wild-type BHK cells. Thus it seems that the aberrant morphological features of the Ric^R14 cell line [11] are not due to the lack of complex N-glycans.

Ric^R14 cells express an inactive GlcNAc-TI protein

To investigate whether Ric^R14 cells express GlcNAc-TI protein, we used mouse antibodies that had been raised against rabbit GlcNAc-TI [12]. The antiserum cross-reacts effectively with BHK GlcNAc-TI, as demonstrated by the perinuclear staining pattern obtained with parental BHK cells (Figure 2A), a staining pattern typical of the Golgi apparatus. In addition, the antibody specifically immunoblotted a component of approx. 48 kDa, the expected size of GlcNAc-TI, from either parental BHK cells or from COS cells transfected with BHK GlcNAc-TI (Figure 3). A smaller, 47 kDa, component was also detected from transfected COS cells; this was presumably a breakdown product.

Ric^R14 cells also showed a perinuclear staining pattern with the anti-rabbit GlcNAc-TI antibody (Figure 2B). Furthermore

Figure 2 Detection of Golgi-localized GlcNAc-TI in BHK and Ric^R14 cells

Acetone-fixed BHK (A) and Ric^R14 (B) cells were incubated with mouse anti-(rabbit GlcNAc-TI) serum, followed by FITC-conjugated anti-mouse immunoglobulin. Scale bar, 10 μ m.

Figure 3 Immunoblotting of parental BHK, Ric^R14 and transfected COS cell extracts with anti-(GlcNAc-TI) antibodies

BHK cells, Ric^R14 cells and COS cells, either untransfected (COS/UT) or transfected with pCIneo containing the wild-type BHK GlcNAc-TI gene (COS/wt GlcNAc-TI), as indicated, were lysed in buffer containing 1% (v/v) Triton X-100 and aliquots were analysed by immunoblotting with mouse anti-(rabbit GlcNAc-TI) antibody. Bound antibody was detected by peroxidase-conjugated anti-mouse immunoglobulin and enhanced chemiluminescence. The position of the 48 kDa GlcNAc-TI protein is indicated.

an immunoreactive component of 48 kDa was detected in Ric^R14 cells (Figure 3). These results indicate that Ric^R14 cells expressed full-length GlcNAc-TI that was correctly localized to the Golgi apparatus.

Figure 4 Intracellular localization of wild-type and mutant GlcNAc-TI in transfected cells

(A) COS cells were transfected with pCI-neo containing the cDNA encoding wild-type BHK GlcNAc-TI, Ric^R14 GlcNAc-TI, wild-type rabbit GlcNAc-TI or rabbit GlcNAc-TI Gly³²⁰ → Asp mutant (rabbit G320D GlcNAc-TI), as indicated. (B) Murine 3T3 cells were transfected with pCI-neo containing the cDNA encoding wild-type BHK GlcNAc-TI or the truncated Ric^R14 GlcNAc-TI mutant (Ric^R14 Δ81–447 GlcNAc-TI). Cells were fixed in acetone 48 h after transfection, then stained by indirect immunofluorescence with mouse anti-(rabbit GlcNAc-TI) antiserum followed by FITC-conjugated anti-mouse immunoglobulin. Neither untransfected COS cells nor 3T3 cells were stained with the mouse anti-(GlcNAc-TI) antiserum. Scale bar, 20 μm.

Identification of a point mutation in Ric^R14

The presence of an inactive GlcNAc-TI correctly localized to the Golgi apparatus suggests that the genetic lesion responsible for the lectin-resistant phenotype in Ric^R14 cells is a point mutation or short deletion within the GlcNAc-TI gene. To identify the genetic lesion, GlcNAc-TI cDNA clones were isolated from Ric^R14 RNA by RT-PCR with degenerate primers based on the sequence of rabbit, rat, mouse and human GlcNAc-TI. A comparison of the parental BHK GlcNAc-TI gene sequence with several Ric^R14 cDNA clones indicated the presence of two different Ric^R14 transcripts. One Ric^R14 GlcNAc-TI cDNA contained a point mutation in the open reading frame resulting in the amino acid substitution of an Asp residue for Gly³²⁰; a second cDNA contained a stop codon at position +81, predicted to result in a truncated GlcNAc-TI protein of approx. 9 kDa. To establish whether these cDNA species were derived from transcripts from the same or different cells, the Ric^R14 cell line was re-cloned by limiting dilution and a single clone was selected. From this Ric^R14 clone three GlcNAc-TI clones were isolated from two independent RT-PCRs; all three cDNA species were identical and contained the Gly³²⁰ → Asp substitution. A full-length Ric^R14 GlcNAc-TI open reading frame is consistent with the immunoblotting data (Figure 3), which showed Ric^R14 to have a GlcNAc-TI protein of similar size to wild-type GlcNAc-TI. It is therefore likely that the two Ric^R14 GlcNAc-TI cDNA species were derived from independent cells.

Wild-type GlcNAc-TI is efficiently localized to the Golgi apparatus at high expression levels [12]. To determine whether the full-length Ric^R14 GlcNAc-TI behaves in a similar manner, the intracellular localization of Ric^R14 GlcNAc-TI was analysed with the transient COS cell transfection system, which results in

high expression levels. By using the mouse anti-(rabbit GlcNAc-TI) antibodies, Ric^R14 GlcNAc-TI was found predominantly localized to a juxtanuclear location in transfected COS cells (Figure 4A). A very similar staining pattern was also obtained for wild-type BHK GlcNAc-TI (Figure 4A); therefore, even at relatively high levels of expression, Ric^R14 GlcNAc-TI is effectively Golgi-localized.

Gly³²⁰ → Asp mutation results in loss of GlcNAc-TI activity

To confirm that the Gly³²⁰ → Asp mutation was responsible for the loss of GlcNAc-TI activity in Ric^R14, this substitution was introduced into the wild-type rabbit sequence. COS cells were transfected with wild-type rabbit GlcNAc-TI, the Gly³²⁰ → Asp mutant or with the pCI-neo vector alone. The Asp³²⁰ mutant was localized to the Golgi apparatus in transfected COS cells, with a staining pattern similar to that of wild-type rabbit GlcNAc-TI and that of Ric^R14 GlcNAc-TI (Figure 4A). The level of heterologous gene expression was determined by immunoblotting. A similar amount of rabbit GlcNAc-TI protein was detected in extracts from cells transfected with either the wild-type rabbit GlcNAc-TI or with the Gly³²⁰ → Asp mutant (Figure 5A). The total GlcNAc-TI activity in extracts of transfected COS cells was determined by using ovalbumin as an acceptor. COS cells transfected with wild-type GlcNAc-TI showed an approx. 2-fold increase in GlcNAc-TI activity compared with COS cells transfected with vector only, whereas cells transfected with the GlcNAc-TI Gly³²⁰ → Asp mutant showed no increase in GlcNAc-TI activity over endogenous levels (Figure 5B). The lack of activity of the GlcNAc-TI Gly³²⁰ → Asp mutant was also confirmed by transfection of Lec1 Chinese hamster

Figure 5 Gly³²⁰ → Asp mutation results in the loss of GlcNAc-TI activity

Extracts from COS cells transfected with pCI-neo containing cDNA encoding either wild-type or mutant rabbit GlcNAc-TI were lysed in buffer containing 1% (v/v) Triton X-100 and aliquots containing equal amounts of protein were analysed by immunoblotting with mouse anti-(rabbit GlcNAc-TI) antiserum (A) and assayed for GlcNAc-TI activity in quadruplicate in the presence or absence of the acceptor ovalbumin (B). The recovery of radiolabelled product is given as d.p.m.

ovary cells, a mutant also defective in GlcNAc-TI activity [17]. Whereas parental GlcNAc-TI restored the ability of Lec1 cells to synthesize complex N-glycans, as demonstrated by FITC-L-PHA binding, transfection of Lec1 cells with the GlcNAc-TI Gly³²⁰ → Asp mutant did not result in an increase in the number of L-PHA-binding sites compared with untransfected cells (results not shown).

Golgi localization of truncated Ric^R14 GlcNAc-TI

Because the original population of Ric^R14 cells showed positive Golgi staining with the mouse anti-(GlcNAc-TI) antibody, we also investigated the intracellular localization of the truncated Ric^R14 GlcNAc-TI species. Mouse 3T3 cells were transfected with either wild-type BHK GlcNAc-TI or Ric^R14 GlcNAc-TI containing a stop codon at position +81; the products were localized using the mouse anti-(rabbit GlcNAc-TI) antiserum. Both wild-type BHK GlcNAc-TI and the truncated Ric^R14 GlcNAc-TI were localized to a juxtanuclear location in transfected 3T3 cells (Figure 4B), whereas untransfected 3T3 cells showed no staining with this antiserum. Therefore the mouse anti-(GlcNAc-TI) antiserum cross-reacted with the truncated Ric^R14 GlcNAc-TI protein, which was Golgi-localized in these transfected cells.

DISCUSSION

GlcNAc-TI catalyses the first step in the conversion of high-mannose N-glycans into complex and hybrid structures [18]. Complex N-glycans are critical for the viability of the developing

	300	320	340
BHK (AF087456)	RKGRACIRPEISRT---MTFGRKGVSHGQFFDQHLKFIKLNQF		
CHO (U65791)			
RAT (Q09325)			
MOUSE (P27808)			
RABBIT (P27115)	V.....		
HUMAN (P26572)	Q.....		
C.elegans (Z46381)	..QRQ.....GM-.KY.KE.T.K....SD..EK..V.DLP		
C.elegans (U23516)	..DRQ.....GM-.SY.KE.A.K....SK..AK..V.DKY		
C.elegans (U28735)	..SRS.....SHN.KLAG..S.G.M.K.YLS.ISASSANI		

Figure 6 Gly³²⁰ is conserved in GlcNAc-TI sequences across species

Comparison of GlcNAc-TI coding sequences. The conserved Gly³²⁰ is boxed.

embryo, as mice lacking a functional GlcNAc-TI gene die before birth [19,20]. However, complex N-glycans are not essential for the viability of cells cultured *in vitro* because several mutants have been isolated that lack GlcNAc-TI activity.

Here we have identified the genetic basis for the glycosylation defect of the Ric^R14 mutant as point mutations in the GlcNAc-TI open reading frame. These mutations affect the primary structure of the enzyme. A sequence comparison of GlcNAc-TI cDNA species from Ric^R14 cells with the parental BHK GlcNAc-TI gene identified two point mutations. These mutations were found on different Ric^R14 GlcNAc-TI cDNA species. Isolation and sequencing of GlcNAc-TI cDNA species from a re-cloned population of Ric^R14 cells yielded only one of two mutations, indicating that the original Ric^R14 cell line might not have been clonal. One of the cDNA species isolated contained a stop codon after residue 80. As this cDNA was isolated from two independent PCR reactions it is likely to have been derived from a distinct mRNA species rather than being a PCR-induced mutation. GlcNAc-TI is a type II membrane protein with a short cytoplasmic tail, a transmembrane domain and a luminal catalytic domain separated from the transmembrane domain by a stem region [21]. The premature stop mutation of Ric^R14 GlcNAc-TI is located in the stem region of GlcNAc-TI and would therefore lead to a truncated protein lacking the catalytic domain. Interestingly, this truncated protein was immunoreactive and Golgi-localized when expressed in mouse 3T3 cells, a result consistent with the presence of Golgi retention signals in the transmembrane domain and the stem region of GlcNAc-TI [22–24]. The second Ric^R14 GlcNAc-TI cDNA contained a single mutation resulting in an amino acid substitution of an Asp residue for Gly³²⁰. The presence of this second cDNA is consistent with our observation that Ric^R14 cells express a GlcNAc-TI protein of the same size as active wild-type BHK enzyme, which is correctly localized to the Golgi apparatus.

Gly³²⁰ is located in the catalytic domain of GlcNAc-TI. An analysis of GlcNAc-TI sequences in GenBank revealed that Gly³²⁰ is conserved in GlcNAc-TI sequences from all species examined so far, including human, rabbit, mouse, rat, hamster and three GlcNAc-TI homologues from the nematode *Caenorhabditis elegans* (Figure 6).

It is unlikely that the Gly³²⁰ → Asp³²⁰ mutation markedly affects the folding of GlcNAc-TI, as the enzyme is correctly localized to the Golgi apparatus in Ric^R14 cells and in COS cells transfected with this Ric^R14 GlcNAc-TI cDNA. Therefore the Gly³²⁰ mutation might result in a subtle structural alteration of GlcNAc-TI or, alternatively, Gly³²⁰ might be a critical residue of the enzyme active site. Very little is known about the nature of the active site of N-acetylglucosaminyltransferases. This Ric^R14 mutant protein, along with the Lec1 GlcNAc-TI mutant in which Cys¹²³ was identified as a critical residue for activity [5], will be useful for the identification of substrate-binding sites and for

interpreting GlcNAc-TI structural data when this information becomes available.

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