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Decreased UDP-GlcNAc:Glycopeptide β-2-*N*-Acetylglucosaminyltransferase II Activity in a Ricin-resistant Mutant of Baby Hamster Kidney (BHK) Cells

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The ricin-resistant mutant baby hamster kidney (BHK) cell line RIC^{R}21 is unable to make the sialylated bi- or triantennary complex *N*-glycans found in wild type cells and accumulates instead non-bisected hybrid structures containing three Man residues and one or two sialylated antennae (Hughes *et al* 1983, Carbohydr Res 120:215-34). Specific assays for *N*-acetylglucosaminyltransferases I, II, III and IV were applied to Triton X-100 extracts of wild type BHK, RIC^{R}14 and RIC^{R}21 cells. It was shown that RIC^{R}21 cell extracts had a decreased *N*-acetylglucosaminyltransferase II specific activity (17 to 27% of wild type values). It is suggested that in wild type cells *N*-acetylglucosaminyltransferase II action proceeds quickly, leading to complex *N*-glycan synthesis, while in RIC^{R}21 cells potential substrates for *N*-acetylglucosaminyltransferase II move into the trans-Golgi compartment before the transferase can act, thereby leading to hybrid structures.

Several laboratories have used toxic lectins such as ricin, *Phaseolus vulgaris* phytohaemagglutinin (PHA) or wheat germ agglutinin (WGA) to select lectin-resistant mutant cell lines [1, 2]. Some of these mutant lines show a greatly reduced ability to bind lectin to the cell surface. The defect in these mutants is therefore believed to be in the structure of the cell surface carbohydrates. For example, Chinese hamster ovary and baby hamster kidney (BHK) mutants have been isolated in which there is an absence of the enzyme UDP-GlcNAc:glycopeptide β-2-*N*-acetylglucosaminyltransferase I (GlcNAc-transferase I), the key enzyme in the conversion of high mannose *N*-glycosyl oligosaccharides to hybrid and complex structures [3-6]. These cells show decreased binding of ricin, PHA,

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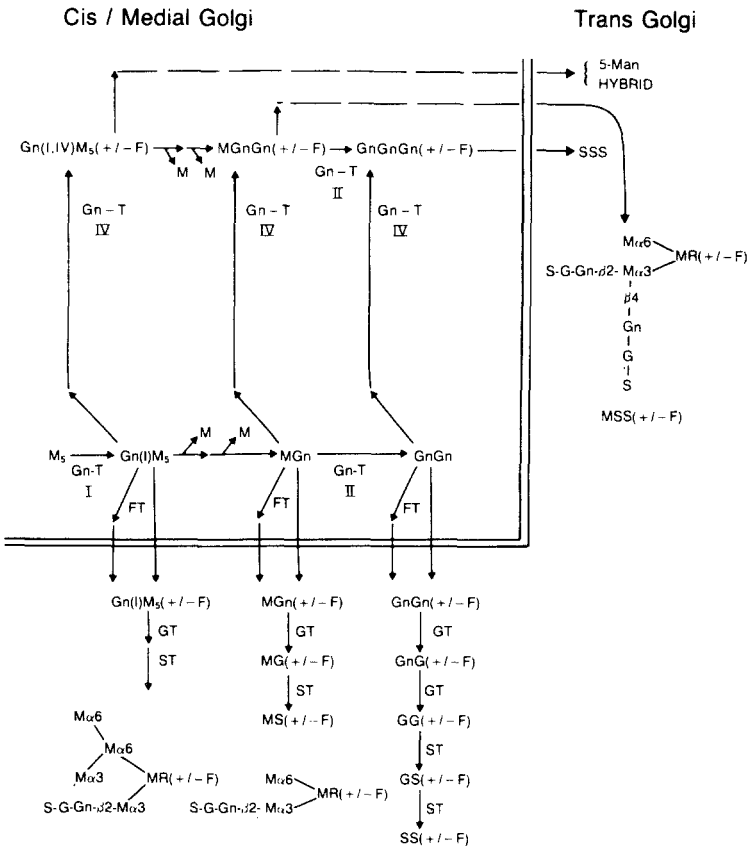

Figure 1. Biosynthetic pathway for the conversion of the high mannose intermediate M_5, i.e. Manα1-3{Manα 1-6(Manα1-3)Manα1-6}Manβ1-4R where R is GlcNAcβ1-4GlcNAc-Asn-polypeptide, to complex and hybrid *N*-glycans. Steps involving the formation of bisected glycans due to the action of GlcNAc-transferase III have been omitted since mutant and wild type BHK cells lack this enzyme. The structure $Gn(I)M_5$ is the product of GlcNAc-transferase I (Gn-T I), i.e. M_5 with a GlcNAc linked β1-2 to the Manα1-3Manα1-4- terminus. The structure $Gn(I, IV)M_5$ is formed by the action of GlcNAc-transferase IV on $Gn(I)M_5$ and contains a GlcNAc linked β1-4 to the Manα1-3Manβ1-4- terminus. All other structures are non-bisected *N*-glycans of the complex or hybrid type containing only three Man residues. These are named according to the sugar residues at the non-reducing termini, the residues on the Manα1-6- arm being named first [11].

Abbreviations: Gn, GlcNAc; M, Man; (+F), the structure contains a core α-6-linked fucose; G, Gal; S, sialyl residue; Gn-T, GlcNAc-transferase; FT, α-6-fucosyltransferase; GT, galactosyltransferase; ST, sialyltransferase.

The structures MS(+F) and MSS(+F) are shown in detail since these are the non-bisected hybrids which accumulate in RIC^R21 mutants. These structures are not found in wild type BHK cells which contain instead sialylated complex bi- and tri-antennary *N*-glycans, i.e. SS(+F) and SSS(+F). The decisive control points for the RIC^R21 mutant are therefore whether MGn(+F) and MGnGn(+F) are acted on by GlcNAc-transferase II (Gn-T II) or leave the cis/medial Golgi.

The assignment of enzymes to compartments is based on published data [17-20] and the assumption that GlcNAc-transferase IV is localized in the cis/medial Golgi compartment. The model may become more complex if it is shown that there is further sub-compartmentation of the enzymes in the cis/medial Golgi compartment. Some of the reactions shown in the scheme have not as yet been proved *in vitro*, e.g. action of mannosidase II on Gn(I, IV) M_5 or action of Gn-T II on MGnGn. Further, it has not been established that the Gn-T IV acting at the three-Man and five-Man stages are the same enzyme.

WGA and other lectins but increased binding of Concanavalin A due to the accumulation of high mannose structures on the cell surface. In another mutant line [7, 8], a large increase was noted in the α-3-fucosyltransferase acting on the antennae of complex *N*-glycosyl oligosaccharides. Although sialyltransferase levels were normal, increased fucosylation of antennae led to decreased sialylation and consequent resistance to WGA.

Thus, significant changes in cell surface carbohydrate can be caused by either a decreased or increased level of enzyme. However, in many lectin-resistant mutants, *in vitro* enzyme assays carried out on cell extracts have either failed to reveal any defect in glycosyltransferase or glycosidase activity, or have shown only relatively small changes in these enzymes. One possible explanation [9] is that glycosyltransferase assays are not sufficiently specific, i.e., they pick up several enzyme activities only one of which may be altered in the mutant. This defect may be missed because of the other interfering activities.

We have recently developed specific assays for four distinct GlcNAc-transferases that we have named I, II, III and IV [10-13]. We report here the application of these assays to a ricin-resistant BHK mutant cell line RIC^R21 previously isolated by Meager *et al* [14, 15]. Ricin, the toxic lectin of castor beans (*Ricinus communis*), binds to oligosaccharides with terminal β-D-galactosyl or β-*N*-acetyl-D-galactosaminyl residues. RIC^R21 cells bind ricin very poorly relative to wild type cells. In a previous study [4], Triton X-100 extracts of RIC^R21 cells were assayed for several glycosyltransferases and glycosidases. The only differences relative to wild type extracts were a 50% reduction in galactosyltransferase activity towards several GlcNAc-terminal acceptors, a 50% reduction in GlcNAc-transferase I and a 75% reduction in GlcNAc-transferase II.

More recently, Hughes *et al* [9] studied the glycopeptides released by Pronase® from wild type and RIC^R21 BHK cells. They found that the mutant cells lacked bi- and triantennary complex *N*-glycosyl oligosaccharides containing, respectively, two and three sialyl residues, and accumulated instead acidic hybrid *N*-glycans containing one or two residues of sialic acid (structures MS(+F) and MSS(+F), Fig. 1). They suggested that a relative deficiency in GlcNAc-transferase II might explain their findings if it is assumed that rapid galactosylation of the GlcNAcβ1-2Manα1-3-antenna prevents GlcNAc-transferase II action. The presence of Gal on the Galβ1-4GlcNAcβ1-2Manα1-3- antenna does indeed prevent GlcNAc-transferase II action, as well as the actions of GlcNAc-transferases III and IV, α-6-fucosyltransferase and mannosidase II [6, 10-13, 16].

However, the concept of competition between GlcNAc-transferase II and Gal-transferase does not take into account recent evidence suggesting that these two enzymes are in different compartments of the Golgi [17-20]. Further, the GlcNAc-transferase II assays carried out by Vischer and Hughes [4] were not specific for this enzyme but would also have picked up GlcNAc-transferases III [12] and IV [13].

We have therefore re-investigated the GlcNAc-transferase levels of RIC^R21 cell extracts using specific assays. This work has confirmed the original finding [4, 9] that RIC^R21 cells have a relative deficiency in GlcNAc-transferase II rather than a complete absence of this enzyme. The substrates for GlcNAc-transferase II, i.e., Manα1-6(GlcNAcβ1-2Man α1-3)Manβ1-4GlcNAcβ1-4GlcNAc-Asn-X (structure MGn, Fig. 1) and Manα1-6{GlcNAcβ1-2(GlcNAcβ1-4)Manα1-3}Manβ1-4GlcNAcβ1-4GlcNAc-Asn-X (structure MGnGn, Fig. 1), can either be acted on by GlcNAc-transferase II to form complex *N*-glycans or can enter the trans-Golgi to become hybrid *N*-glycans. The RIC^R21 phenotype is probably due to

the movement of these glycoproteins into the trans-Golgi compartment before GlcNAc-transferase II can act.

Materials and Methods

Cells

Baby hamster kidney (BHK) cells and ricin-resistant mutants [14, 15] were grown at 37°C in monolayer culture in alpha minimum essential medium (Medical Sciences Building Central Services, University of Toronto, Toronto, Canada) supplemented with 10% heat-inactivated fetal calf serum and glutamine (2 mM), in the presence of penicillin G (100 mg/l) and streptomycin sulphate (100 mg/l). Cells (0.5×10^6/10 ml) were added to 10 cm plastic Petri dishes. After 48 h, $40 - 50 \times 10^6$ cells were harvested, at or near confluency, from 10 dishes, by scraping with a rubber policeman. The cells were suspended in phosphate-buffered saline followed by three washes with 0.85% saline at 4°C. Cells were collected during these washes by centrifugation at 2 000 rpm for 10 min. Three separate batches of cells were grown for these studies. Washed cells were stored at -20°C for 7 - 10 days prior to extraction and enzyme assays.

Cell Extract

The washed cell pellets were suspended in 2 - 3 ml 0.2 M saline and subjected to 3 cycles of freezing (dry ice/ethanol) and thawing (at 37°C). The freeze-thawed cells were washed once (Expt. 1) or three times (Expts. 2 and 3) with 0.85% saline at 4°C using centrifugation at 2 000 rpm for 15 min to harvest the pellets. The pellets were extracted with 1.0% Triton X-100 in 0.85% saline and centrifuged at 2 000 rpm for 15 to 20 min at 4°C. The Triton X-100 supernatants contained from 2 to 6 mg protein per ml and were assayed for enzyme activity.

Materials

The following materials were purchased from commercial sources: UDP-*N*-acetyl-D-[U-^{14}C]glucosamine (306 mCi/mmole, New England Nuclear, U.S.A.), was diluted to a specific activity of 4 000 - 6 000 cpm/nmole with non-radioactive UDP-GlcNAc (Sigma, U.S.A.); Triton X-100 (Sigma); 2-(*N*-morpholino)ethanesulfonate (MES) (Calbiochem-Behring, U.S.A.); Concanavalin A/Sepharose (Pharmacia, Sweden) and Bio-Gel P-2 (100 - 200 mesh) (Bio-Rad, U.S.A.).

Glycopeptides

Glycopeptide GnGn(+F), GlcNAcβ1-2Manα1-6(GlcNAcβ1-2Manα1-3)Manβ1-4R where R is GlcNAcβ1-4(Fucα1-6)GlcNAc-Asn, was prepared from a human multiple myeloma IgG (Tem) as previously described [21-23]. GnGn free of fucose was prepared from transferrin [13]. Glycopeptides MGn(+F), Manα1-6(GlcNAcβ1-2Manα1-3)Manβ1-4R, and MM(+F), Manα1-6(Manα1-3)Manβ1-4R, were prepared from a human multiple myeloma IgG (Tem) as previously described [21-24]. These biantennary complex *N*-glycans are

named according to the sugars present at the non-reducing termini of the antennae, the Manα1-6- arm being named first; the abbreviations are M for Man, Gn for GlcNAc, F for fucose, G for Gal and S for sialic acid.

GlcNAc-transferase Assays

The standard incubation contained in a volume of 0.040 ml: glycopeptide substrate (18 - 22 nmoles, 0.45 - 0.55 mM); UDP-[^{14}C]GlcNAc (4 000 - 6 000 cpm/nmole, 0.58 - 0.65 mM); MES buffer, pH 6.3 (0.125 M); GlcNAc (0.2 M); Triton X-100 (0.25%, v/v); $MnCl_2$ (12.5 mM); 5 - 10 μl Triton extracts of BHK cells as enzyme source (10 - 60 μg protein). After 2 h at 37°C, the reaction was stopped by addition of 0.010 ml 2% sodium tetraborate - 0.25 M EDTA. Product formation was assessed, as previously described [12, 13, 24] by high voltage paper electrophoresis in 1% borate, washing the paper with 80% ethanol and counting strips of the dried paper in 15 ml OCS (Organic Counting Scintillant, Amersham, U.K.). Activity in the absence of exogenous acceptor was determined and subtracted in the calculation of enzyme activity. Product formation was proportional to enzyme content. All transferase assays are the averages of 2 to 4 separate determinations. The variation between duplicates was usually 10% or less.

Gal-transferase Assays

The standard incubation contained in a volume of 0.040 ml: glycopeptide GnGn (0.4 mM); UDP-[^{14}C]Gal (2 100 cpm/nmole, 2.7 mM); MES buffer, pH 5.7 (0.25 M); Triton X-100 (0.25%, v/v); $MnCl_2$ (12.5 mM); Triton extracts of BHK cells as enzyme source (20 - 60 μg protein). After 1 h at 37°C, the reaction was stopped and product formation was assayed as described above for GlcNAc-transferases.

GlcNAc-transferase Product Isolation

The substrate MGn(+F) can be acted on not only by GlcNAc-transferase II but also by GlcNAc-transferases III [12] and IV [13] (Fig. 2). It was therefore necessary to separate the products of these three enzymes. Radioactive product was recovered after scintillation counting by washing the papers with toluene (3 times), 1% acetic acid in methanol (6 times) and methanol (6 times), followed by extraction of the radioactivity by 8 washes with water [25]. The radioactive product was acetylated with non-radioactive acetic anhydride [12], purified by gel filtration on a Bio-Gel P-2 column (1.5 × 46 cm), eluted with water, and analyzed by chromatography on Con A/Sepharose columns (0.7 × 6 - 8 cm) as previously described [12, 13, 21]. The product of GlcNAc-transferase II adheres to the column whereas the products of the other two transferases do not (Fig. 2).

MGn(+F) can also be acted on by β-*N*-acetylglucosaminidase in the Triton extracts to produce glycopeptide MM(+F) which can in turn be converted to radioactive MGn(+F) by GlcNAc-transferase I in the cell extracts (Fig. 2). MGn(+F) and the product of GlcNAc-transferase II, GnGn(+F), both adhere to Con A/Sepharose. The Con A-positive product was therefore fractionated by high voltage paper electrophoresis in 1% borate to separate MGn(+F) and GnGn(+F) [22]. The radioactivity in the regions of markers of glycopeptides GnGn(+F) and MGn(+F) was determined.

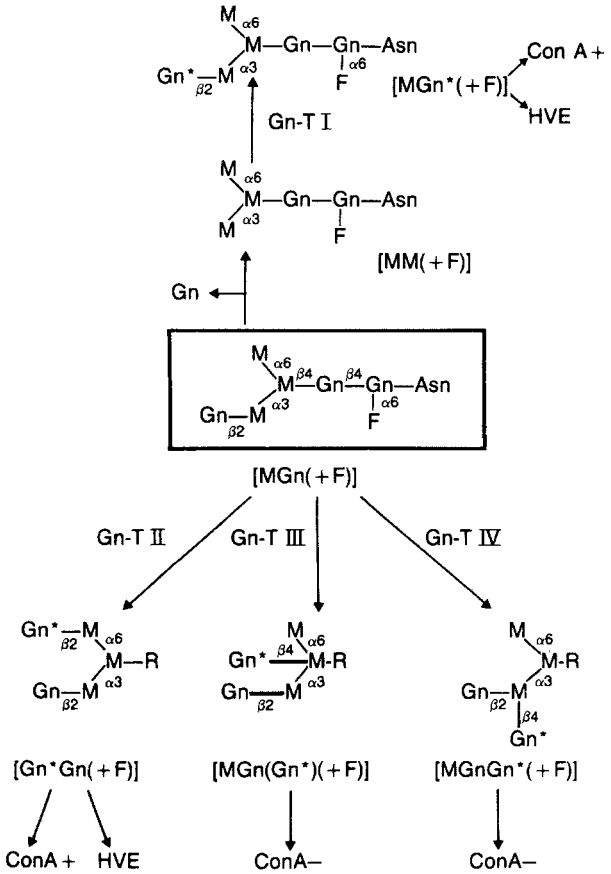

Figure 2. The possible fates of glycopeptide MGn when used as an acceptor in GlcNAc-transferase assays.

Abbreviations as for Fig. 1. The asterisk indicates a radioactive GlcNAc residue. Con A+ represents material that binds to Con A/Sepharose while Con A- represents material that passes through the column. GnGn and MGn can be separated by high voltage paper electrophoresis (HVE).

Protein Determinations

Protein was determined [26] using bovine serum albumin as standard.

Results

Table 1 summarizes the results on three separate batches of cells. For all three cell lines, the product formed when Triton extracts were incubated with glycopeptide MGn(+F) and radioactive UDP-GlcNAc was fractionated into a minor component non-adherent to Con A/Sepharose and a major Con A-adherent fraction (Fig. 3).

Table 1. Glycosyltransferase specific activities of triton extracts of BHK cells. Wild type and ricin-resistant mutants of BHK cells were grown either to confluency or near confluency. The cells were harvested and washed and Triton X-100 extracts were prepared as described in the text. These extracts were assayed for GlcNAc-transferase and Gal-transferase activities as described in the Methods section.

Cells	Expt No.	Cell confluence	Protein concentration of cell extract (mg/ml)	GlcNAc-transferase (Gn-T) specific activity (nmoles/mg/h)				Gal-transferase (GT) specific activity (nmoles/mg/h)	GT/Gn-T II ratio
				Con A-negative product[a]	Gn-T II		Gn-T I[d]		
					Con A assay[b]	HVE assay[c]			
Wild type	1	No	6.0	3.0	17.4	—	—	—	—
	1	Yes	1.7	2.6	7.6	—	—	—	—
	2	Yes	3.6	0.5 1.5[e]	8.5 8.3[f]	7.2	2.2	—	—
	3	No	2.9	0.5	18.1	18.1	8.5	62.9 75.9[g]	3.5
RIC^R14:									
	1	Yes	2.0	1.3	12.3	—	—	—	—
	2	Yes	2.6	1.3 3.4[e]	14.3 14.6[f]	15.6	0.6	—	—
	3	No	2.8	1.2	14.2	14.5	0.05	44.8 57.1[g]	3.2
RIC^R21:									
	1	Yes	2.0	1.4	1.5	—	—	—	—
	2	Yes	6.0	0.4 1.4[e]	2.8 3.3[f]	3.0	7.1	—	—
	3	No	3.1	0.4	3.0	3.2	7.5	35.9 64.4[g]	12.0

Most of the Con A-negative fraction was totally unretarded (Fig. 3) indicating that the product was Manα1-6{(GlcNAcβ1-2)([^{14}C]GlcNAcβ1-4)Manα1-3}Manβ1-4R, where R is GlcNAcβ1-4(Fucα1-6)GlcNAc-Asn, due to the action of GlcNAc-transferase IV [13]. Retarded Con A-negative radioactivity characteristic of GlcNAc-transferase III action [12] was not detected in appreciable amounts in any of the assays suggesting that all three BHK lines lack GlcNAc-transferase III. GlcNAc-transferase IV activities ranged from 0.4 to 3.0 nmoles/mg protein/h (Table 1) and did not show consistent variations between the three BHK lines.

The Con A-adherent fraction showed a skewed elution profile typical of GnGn when the Con A/Sepharose column was eluted with α-methyl glucoside (Fig. 3) [12, 13, 21, 24]. High voltage paper electrophoresis in borate [22] was carried out on this fraction to separate radioactive MGn(+F), formed due to β-*N*-acetylglucosaminidase and GlcNAc-transferase I action, from the GlcNAc-transferase II product, GnGn(+F) (Fig. 2). Over

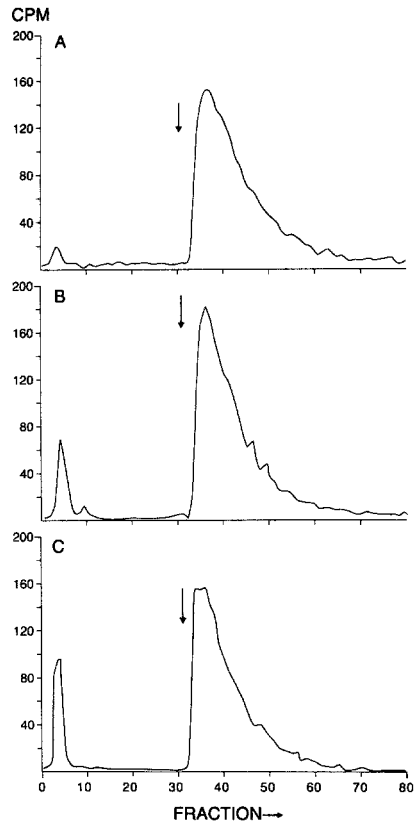

Figure 3. Chromatography of GlcNAc-transferase product formed with MGn(+F) as acceptor, on a Con A/Sepharose column (0.7 × 8 cm). The arrow shows the position of addition of α-methyl glucoside. The enzyme was a Triton extract of (A) wild type BHK cells, (B) RIC^R14 cells, and (C) RIC^R21 cells. About 2 000 cpm were usually applied to the column and recoveries were routinely over 90%. All assays gave a qualitatively similar profile although there were significant quantitative variations as shown in Table 1. Fraction size, 1.0 ml.

90% of product radioactivity was recovered in the GnGn region of the electrophoretogram (compare Con A and high voltage electrophoresis assays, Table 1). This indicates that our method of preparing cell extracts and the addition to the assay of GlcNAc to inhibit β-*N*-acetylglucosaminidase were effective in preventing significant breakdown of MGn by lysosomal hydrolases. The supernatant obtained after freeze-thaw lysis of cells and centrifugation at 2 000 rpm was centrifuged at 100 000 × g for 1 h and the resulting pellet was extracted with Triton X-100 and assayed for GlcNAc-transferase I as a Golgi marker; over 70% of the total transferase activity was recovered in the 2 000 rpm pellet indicating this fraction to be representative of the cell's Golgi apparatus. Transferase assays have also been carried out on total homogenates of these cells (ref. 4 and unpublished data); although substrate breakdown was appreciable under these conditions, the data for GlcNAc-transferases I and II was similar to that in Table 1.

Table 1 shows that the GlcNAc-transferase II specific activities of wild type BHK cells appear to vary with the state of confluency of the cells at the time of harvest. It is possible that the rate of glycoprotein synthesis decreases as cells approach confluency. However, neither mutant line appears to show this effect. The activity of GlcNAc-transferase II in RIC^R21 cells is 17 - 27% of that in wild type cells, confirming previous observations using a less specific assay [4]. It is also seen that GlcNAc-transferase I is nearly absent in RIC^R14, as previously reported [4]. Although Gal-transferase specific activity is decreased in RIC^R21 cells relative to wild type, the ratio of Gal-transferase activity to GlcNAc-transferase II activity increases from about 3 in wild type and RIC^R14 cells to 12 in RIC^R21 cells (Table 1).

Discussion

Many lectin-resistant mutant cell lines have been isolated [1, 2]. In only a few of these lines has the enzymatic basis for lectin resistance been clearly demonstrated. The first to be studied were those mutants in which there is an almost complete absence of GlcNAc-transferase I [3-6] with resultant accumulation of high mannose *N*-glycans containing five Man residues and lack of complex and hybrid structures. The mutant cells therefore show decreased ability to bind several lectins (*Phaseolus vulgaris* phytohemagglutinin, ricin, wheat germ agglutinin and *Lens culinaris* agglutinin) but bind increased amounts of Con A.

Another interesting example is a wheat germ agglutinin-resistant mutant of the B16 mouse melanoma cell line [7, 8]. This line has an increased level of GDP-Fuc:Galβ 1-4GlcNAc-R (Fuc to GlcNAc) α-3-fucosyltransferase. Increased fucosylation of antennae leads to decreased sialylation and consequent decreased ability of wheat germ agglutinin to bind to the cell surface. It is interesting that the sialyltransferase levels are completely normal in this mutant and yet there is a marked decrease in sialylation. It appears therefore that lectin resistance in some mutant lines may not be due to the complete deletion of an enzyme, but to the interplay of quantitative rather than qualitative changes in glycosyltransferase or glycosidase levels. The wheat germ agglutinin-resistant melanoma B16 line is such a mutant. The resistance of the RIC^R21 line to ricin is probably due to a similar process.

Vischer and Hughes [4] have suggested that GlcNAc-transferase II deficiency might be the cause of RIC^R21 lectin resistance. Since their GlcNAc-transferase II assay method was not specific, RIC^R21 extracts were re-examined with more specific assays for GlcNAc-transferases I, II, III and IV. We have, however, confirmed their finding that GlcNAc-transferase II is not absent in RIC^R21 cells but is decreased by about 73 - 83%. Hughes *et al* [9] have shown the accumulation in RIC^R21 cells of mono- and di-sialo nonbisected hybrids containing three Man residues (the structures shown as MS(+F) and MSS(+F) in Fig. 1). These are unusual hybrid structures since they contain three rather than the four or more Man residues commonly found in hybrids; in fact, MS(+F) and MSS(+F) can be considered incomplete complex rather than hybrid *N*-glycans.

Vischer and Hughes [4] suggested that there is competition between Gal-transferase and GlcNAc-transferase II for the common substrates MGn and MGnGn (Fig. 1) and that GlcNAc-transferase II predominates in wild type cells leading to complex *N*-glycans while Gal-transferase acts first in RIC^R21 cells thereby shutting off further GlcNAc-trans-

ferase II action with resultant formation of non-bisected hybrid structures. This hypothesis is supported by our finding that the ratio of Gal-transferase specific activity to GlcNAc-transferase II specific activity increased from 3.5 in wild type cells to 12 in RIC^R21 cells (Table 1). We have also shown that addition of Gal to the GlcNAcβ1-2Manα1-3- arm does indeed block further GlcNAc-transferase II action [10, 11]. It should be pointed out that both the wild type BHK and the RIC^R21 cells make large amounts of high mannose oligosaccharides [9] indicating that the accumulation of MS(+F) and MSS(+F) in the mutant is not due to an inability to add Man residues during oligosaccharide biosynthesis.

While the above competition model appears to explain the RIC^R21 phenotype, it does not take into account recent findings concerning the subcellular distribution of Golgi glycosyltransferases [17-20]. In particular, it appears that Gal-transferase is located in the trans-Golgi compartment whereas GlcNAc-transferases I and II, mannosidase II and α-6-fucosyltransferase are located earlier on in the assembly line. We have tentatively assigned these enzymes to the cis/medial Golgi compartment and have assumed that GlcNAc-transferase IV is also in this compartment although there is yet no data on this point (Fig. 1). GlcNAc-transferase III was omitted from the scheme since this enzyme is absent from BHK cells. The scheme shown in Fig. 1 indicates that there is no competition between Gal-transferase and GlcNAc-transferase II because they occur in different compartments. The RIC^R21 phenotype can be explained by assuming that movement of the potential GlcNAc-transferase II substrates MGn(+F) and MGnGn(+F) (Fig. 1) from the cis/medial- to the trans-Golgi compartment occurs before GlcNAc-transferase II can act. Hughes *et al* [9] found no trace of sialylated non-bisected hybrid *N*-glycans in wild type cells nor sialylated bi- and triantennary complex *N*-glycans in RIC^R21 cells. The presence of appreciable residual GlcNAc-transferase II activity in RIC^R21 cells would predict some overlap in structures between wild type and mutant cells and it is not clear why the biosynthetic controls are so stringent. The cores of the RIC^R21 hybrids were shown to be fully fucosylated. Fucose incorporation into the core must occur after GlcNAc-transferase I action and before Gal-transferase action [11, 16] and can therefore occur in RIC^R21 cells at any point between Gn(I)M_5 and just prior to the exit of glycoprotein from the cis/medial Golgi compartment (Fig. 1).

The action of Gal-transferase has been shown to prevent the further action of GlcNAc-transferases II, III and IV, mannosidase II and α-6-fucosyltransferase [11]. If Gal-transferase is in a different compartment from these latter enzymes, one can question whether this turn-off action of galactosylation plays a functional role in glycoprotein synthesis. The scheme in Fig. 1 ensures that initiation of antennae is complete before Gal or sialyl residues are added. It is possible that once the glycoprotein has entered the trans-Golgi compartment, the turn-off action of galactosylation ensures that no further initiation of antennae occurs due to low levels of GlcNAc-transferases in the trans-Golgi. It is clear that transferase substrate specificity and subcellular compartmentation must both be taken into consideration when attempting to explain lectin-resistant mutant phenotypes.

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