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MINI REVIEW

Ectopic localizations of Golgi glycosyltransferases

Eric G. Berger

Institute of Physiology, University of Zurich, Winterthurerstrasse 190, CH-8057 Zurich, Switzerland

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Glycosyltransferases involved in N- and O-glycan chain elongation and termination are localized in the Golgi apparatus. Early evidence in support of this rule was based on fractionation techniques and was corroborated by numerous immunocytochemical studies. Usually these studies were confined to cultured cell lines exhibiting little differentiation features, such as HeLa cells. However, localization studies conducted in primary cell cultures (e.g., human umbilical vein endothelial cells), cells obtained *ex vivo* (e.g., sperm cells), and tissue sections (e.g., intestinal, renal, or hepatic tissue) often reveal ectopic localizations of glycosyltransferases usually at post-Golgi sites, including the plasma membrane. Hence, extracellular cues resulting from specific adhesion sites may influence post-Golgi trafficking routes, which may be reflected by ectopic localization of Golgi enzymes.

Key words: cytoarchitecture/glycosyltransferases/Golgi apparatus/immunocytochemistry/post-Golgi traffic

Introduction

Several recent publications point to unusual subcellular localizations of glycosyltransferases, which normally are localized to the Golgi apparatus (GA). This review is intended to summarize the main classical findings on localization of endogenously expressed glycosyltransferases, describe their newly found localizations, and discuss how they may arise.

Since the advent of electron microscopy, fractionation techniques, cytochemical approaches, and immunocytochemical techniques between 1950 and 1980, the GA has been firmly established as a subcellular entity whose main function is associated with secretion, posttranslational modifications, packaging, and sorting (Berger, 1997; Farquhar and Palade, 1998). It was a key finding from several laboratories that subcellular fractions containing membranes resembling Golgi cisternal stacks exhibited high glycosyltransferase activities, in particular galactosyltransferase (β4GalT), which then became the classical marker enzyme of Golgi fractions. Immunocytochemical localizations at both the light- and electron-microscopic levels carried out in HeLa cells and fibroblasts established the unique localization of β4GalT in *trans*-Golgi cisternae (Berger *et al.*, 1981; Roth and Berger, 1982). These data delineated the shape of the GA in tissue cultured cells as a compact juxtanuclear organelle and gave support to a concept of subcompartmentation of Golgi glycosylating enzymes. Of note, there is little convincing evidence to show by immunocytochemical methods that endogenously expressed β4GalT occurs on the cell surface of undifferentiated cultured cells. These and data from other groups (Burke *et al.*, 1994; Chen *et al.*, 1995) supported the notion of exclusive Golgi localization of glycosyltransferases and imposed stringent criteria for the demonstration of any ectopic localization.

Golgi glycosyltransferases are usually classified according to the donor substrate, for example, in groups of sialyl-, fucosyl-, galactosyl-, N-acetylglucosaminyl-, and N-acetylgalactosaminyltransferases. The prefix refers to the linkage type they catalyze between the transferred sugar residue and the acceptor, which usually is not included in the trivial name (e.g., β4GalT). This classification, however, does not reflect the actual redundancy of enzymes catalyzing similar linkages and subtle differences in substrate specificity. The reader is referred to the following website for a current comprehensive overview (available online at *http://afmb.cnrs-mrs.fr/~cazy/CAZY/index.html*) that compares a glycosyltransferase to its E.C. number, primary structure, and subgroup of homologues. In the following material, the families of glycosyltransferases as listed on this website are given in the headings.

Golgi glycosyltransferases belong to class II membrane proteins sharing a domain structure consisting of cytoplasmic, transmembrane, stem, and catalytic portions (Paulson and Colley, 1989). They are synthesized by translation and translocation into the endoplasmic reticulum (ER), then they move forward to a more or less defined Golgi subcompartment, where they exert their function in glycosylation. The mechanism of this anterograde traffic has not been elucidated in detail but is generally assumed to occur by vesicular transport involving retention to the Golgi compartment. Retention is generally specified by the transmembrane domain and, in some cases, by the formation of complexes by dimerization. So far no specific recognition motifs have been described that would explain partitioning among Golgi subcompartments (Colley, 1997). In many cases, Golgi glycosyltransferases are released from the cell in a form processed by limited proteolysis in the stem region. The post-Golgi trafficking pathways and the site of processing are poorly characterized. The best investigated examples with respect to processing include β4GalT1 (Strous, 1986), ST6Gal (Kitazume *et al.*, 2001), FucT6 (Borsig *et al.*, 1998), and GM2 synthase (Jaskiewicz *et al.*, 1996). The function of soluble glycosyltransferases (if any), which are found in many body fluids (see, for example, Gerber *et al.*, 1979), is unknown.

What is convincing evidence for ectopic localization of Golgi glycosyltransferases?

As a general rule, different approaches providing independent support are usually required to unequivocally locate an enzyme: subcellular fractionation with specific activity measurements and immunoblotting, immunoprecipitation, as well as *in situ* immunostaining using antibodies with proven monospecificity. These antibodies should not contain specificities to glycotopes expressed on glycosyltransferases (Feizi and Childs, 1987), a requirement that is easily met by producing the antigen as a recombinant enzyme in a nonglycosylating host, such as *Escherichia coli* (Taatjes *et al.*, 1988). In any case, biochemical identification of the ectopically localized antigen by immunoblotting in lysates of the same tissue—or, preferably, immunoprecipitation of the antigen using the same antibody followed by peptide mapping or sequencing—may be the gold standard. The following description of ectopically localized glycosyltransferases will include the nature of the evidence provided as far as it is accessible by published data. This review does not include recombinant tagged glycosyltransferases expressed in a heterologous system (except for some special cases) because ectopic localizations of recombinant glycosyltransferases may not be physiological and can be due to overexpression or to tags used for immunolocalization (Yang *et al.*, 1996; Kobayashi *et al.*, 2000) or to fusion with fluorescent proteins (Sciaky *et al.*, 1997): Indeed, a β4GalT1-GFP fusion protein transfected in HeLa cells was well colocalized with endogenous β4GalT1 in *trans*-Golgi cisternae, but no evidence for constitutive secretion could be obtained by time-lapse imaging (Sciaky *et al.*, 1997), which is in contrast with the established secretion of this enzyme as processed enzyme (Strous, 1986). It is also important to mention that there are some immunohistochemical studies in which monoclonal antibodies (mAbs) to glycosyltransferases were used in which no evidence for ectopic localization was found. These include mAbs to histo-blood group A/B transferase (White *et al.*, 1990; Mandel *et al.*, 1992) and GalNAcT1 to -2 (Mandel *et al.*, 1999).

Ectopically localized glycosyltransferases

Among all endogenously expressed Golgi-associated glycosyltransferases, those that have been investigated by immunocytochemical approaches and found at ectopic sites are listed in Table I.

β1,4Galactosyltransferase 1 (GT family 7, E.C. 2.4.1.22/38, β4GalT1)

This enzyme catalyzes the incorporation of Gal from UDP-Gal to GlcNAc residues terminally located in nascent glycoproteins. The enzyme also forms lactose in the presence of α-lactalbumin. Its role in chain elongation of glycoproteins implicates its localization in the GA at the *trans* site, an assumption substantiated by strong immunocytochemical evidence (Roth and Berger, 1982; Slot and Geuze, 1983; Whitehouse *et al.*, 1997; Berger *et al.*, 2001). Out of the six members of this family, β4GalT1 is the enzyme whose subcellular localization has been most thoroughly investigated.

Since the early days of glycosyltransferase biology, β4GalT1 received great attention for its putative surface localization as an ectoenzyme with specific adhesive properties as first proposed by Roseman (Roseman, 1970; Shur and Roth, 1975). The enzyme has also been localized to the cell surface by Shur and associates (see following discussion) and Berger and associates (Pestalozzi *et al.*, 1982; Davis *et al.*, 1984; Roth *et al.*, 1985a) on the apical surface in various epithelial cells. However, these latter localization studies were performed using antibodies that reacted with glycotopes (Childs *et al.*, 1986), which explains the intense staining observed at brush borders and intestinal goblet cells. Though most of this staining must be considered nonspecific, some antibody binding may truly reflect the presence of ecto-β4GalT1, as recently suggested by flow cytometric analysis of T lymphocytes using a mAb to β4GalT1 (Mrkoci Felner *et al.*, 1997). In fact, surface expression of β4GalT1 is a trivial finding in transiently transfected cells, such as *Cercopithecus aethiops* cells, probably as a result of overexpression. In stably transfected Chinese hamster ovary cells, little evidence for surface expression at the light-microscopic level is available. In summary, in most cell types cultured *in vitro*, endogenously expressed β4GalT1 is a *trans*-Golgi enzyme.

The ambiguities of surface localization due to cross-reactivity of the used antisera to native β4GalT1 with carbohydrate epitopes prompted Taatjes *et al.* (1992) and Suganuma *et al.* (1991) to reevaluate surface expression. Mouse mAbs to purified soluble bovine β4GalT1 (Ulrich *et al.*, 1986) confirmed *trans*-Golgi localization using an immunogold technique on cryosections, but little evidence for surface expression in intestinal epithelial cells was found; conversely, staining was found on microvilli of tracheal epithelial cells. Suganuma *et al.* (1991) used rat mAbs to mouse β4GalT1, whose protein specificity was determined by showing binding to N-deglycosylated β4GalT1 and absence of reactivity to AB0 antigens (Taatjes *et al.*, 1992). In addition to strong Golgi staining, surface localization was confirmed in a variety of epithelial cells lining the gastrointestinal tract and rat testis. In this study, however, brush border staining was absent and cell surface staining of sperm was diffuse. Staining of the nuclear envelope was also recorded, an observation otherwise restricted to overexpressed β4GalT1 in cell lines.

Independently, Shaper *et al.* (1985) provided evidence of cell surface expression of β4GalT1 using affinity-purified polyclonal antibodies to bovine milk β4GalT1. However, positive evidence of exclusive protein specificity was not provided. Thus reactivity to carbohydrate epitopes cross-reactive with those possibly present on the immunogen was not excluded.

Shur and colleagues reported on many instances the localization of β4GalT1 on the cell surface. Here I review the most recent articles and refer the reader to citations given. Early evidence using a pulse-chase protocol in tunicamycin-treated HeLa cells as well as *in vitro* translation showed that unglycosylated β4GalT1 exists in two forms that differed by ~ 2 kDa (Strous *et al.*, 1988). The longer form contains an aminoterminal extension of 13 amino acids and was postulated to be expressed at the cell surface as indicated by immunofluorescence using a peptide antibody to the N-terminal extension (Youakim *et al.*, 1994). The short form, by contrast, resided only in the GA. This difference of intracellular trafficking of the short and long forms of β4GalT1 is an intriguing observation but still awaits confirmation by independent laboratories. The enzyme has been localized on the dorsal surface of human, bovine, equine,

Table I. Ectopic expression of Golgi glycosyltransferases

Enzyme	Localization	Cell type or tissue	Evidence	Putative function	Reference
βGalT1	PM, GA	Madin-Darby bovine kidney cells	affi-plc AB to native bovine milk β4GalT1	?	Shaper *et al.*, 1985
	PM, anterior cap	Bull sperm	plc AS to murine recombinant β4GalT1 (*E. coli*)	Facilitates sperm–oocyte binding	Tengowski *et al.*, 2001
	PM	HeLa cells transfected with murine 4GalT1	plc AS to murine rec. β4GalT1 (*E. coli*)	Cell adhesion	Nguyen *et al.*, 1994
	PM	3T3 fibroblasts	plc AS to murine rec. β4GalT1 (*E. coli*)	signaling involving focal adhesion kinase	Wassler and Shur, 2000
	PM, GA, nuclear envelope	F9 cells in culture, tissue cryostat sections, epithelial cells of stomach, intest. goblet cells; testicular cells, sperm	rat mAb to mouse native β4GalT1	?	Suganuma *et al.*, 1991
	PM, GA	Intestine, trachea	mouse mAb to bovine soluble milk β4GalT1	?	Taatjes *et al.*, 1992
	PM, GA	Parotid acinar cells	plc AB to bovine soluble milk β4GalT1	?	Marchase *et al.*, 1988
	Junctional complex	Pancreas tissue	mAB to human ovarian β4GalT	Adhesion?	Yamamoto *et al.*, 1999
ceramide-GalT	PM, myelin lamellae	Rat brain	plc AB to native ceramide-GalT	?	Roussel *et al.*, 1987
ST3GalV	Axons, neurites	Cerebellar white matter, spinal cord, hippocampal neurons, PC12 cells	plc AB to 52 aminoacids of catalytic portion (*E. coli*)	Biosynthetic? Lectin function?	Stern and Tiemeyer, 2001
ST6Gal	GA, TGN	Rat hepatocytes	plc AB to native rat liver enzyme	Biosynthetic	Roth *et al.*, 1985b
	PM, apical vesicles	Rat colon epithelial cells	protein-epitope purified plc AB to rat liver ST6Gal	?	Taatjes *et al.*, 1988
	Cytoplasmic vesicles	Primary culture of rat hepatocytes	plc AB to purified rat liver enzyme	?	Taatjes *et al.*, 1987
ST3Gal III	Subapical region	Epithelial cells of proximal rat kidney tubuli	plc AB to recombinant soluble ST3Gal III expressed in *E. coli* as fusion protein with β-galactosidase	?	Burger *et al.*, 1998
FucT6	Weibel Palade bodies	Human endothelial cells	plc AB to peptide specific for FucT6	?	Schnyder-Candrian *et al.*, 2000
FucT3	Apical region, GA	Colon epithelial cells	mAb to rec. FucT3 *(E. coli)*	?	Narimatsu *et al.*, 1996
FucT1	Cell surface	Prostatic epithelial cells	mAb 2103 to embryonic rat pancreatic ductal cell lines antigens		Marker *et al.*, 2001

PM: plasma membrane; GA: Golgi apparatus; affi-plc: affinity-purified antibodies; AS: antiserum; TGN: *trans*-Golgi network.

rat, mouse, guinea pig, and rabbit sperm cells, where β4GalT1 is believed to mediate gamete recognition (Nixon *et al.*, 2001). In mice, β4GalT1 binds to ZP3, a receptor located in the zona pellucida of the oocyte. This interaction is mediated by a lectin-like (not catalytic) property of β4GalT1 that leads to aggregation of β4GalT1 followed by an acrosomal reaction and lateral displacement of β4GalT1. The presence of β4GalT1 on the dorsal and anterior aspect of the sperm head in close proximity of the acrosome has been substantiated by use of the same antibody to bovine β4GalT1 (Scully *et al.*, 1987) as described above and in activity measurements.

To overcome the inherent limitations of polyclonal antisera to native β4GalT1, polyclonal rabbit antibodies were raised to the catalytic domain (starting at amino acid 63) of murine β4GalT1. These protein-specific antibodies proved suitable to detect murine surface β4GalT1 overexpressed in HeLa cells (Nguyen *et al.*, 1994) and on the anterior cap of bull sperm, where a supportive role in gamete binding seems plausible

(Tengowski *et al.*, 2001). In fact, using an elaborate histochemical reaction for β4GalT activity, staining over the anterior head and the midpiece of mouse sperm cells has been confirmed as appropriate (Benau *et al.*, 1990). Further applications of this method have (to my knowledge) not been published.

What is the function of sperm surface β4GalT1? It was hoped that β4GalT1 knockout mice would provide a clear answer, but one group showed that β4GalT1-null mice remain fertile despite impaired growth and semilethality (Asano *et al.*, 1997), whereas Shur and associates reported impaired acrosomal reaction by knocking out the long form of β4GalT1 (Nixon *et al.*, 2001). Considered together, these data rule out an essential function of surface β4GalT1 in fertilization but confirm an accessory role of this enzyme in binding to ZP3 and inducing the acrosomal reaction. Another function of surface β4GalT1 postulated by Shur and colleagues concerns signaling by activation of heterotrimeric G proteins following clustering (Shi *et al.*, 2001) or tyrosine phosphorylation of focal adhesion kinase in 3T3 fibroblasts (Wassler and Shur, 2000). In a follow-up article, in 3T3 fibroblasts, β4GalT has been localized to the tip of filopodial protrusions using polyclonal antibodies to mouse β4galT expressed in *E. coli*. Interestingly, at this specific site, a cytoskeletal element (Src suppressed C kinase substrate) interacting with the cytoplasmic domain of β4GalT was colocalized (Wassler *et al.*, 2001).

Further evidence for surface expression of β4GalT1 has also been provided by Marchase *et al.* (1988) in *ex vivo* prepared rat parotid gland acinar cell under conditions of growth stimulation. The polyclonal antibody used for surface staining and flow cytometry was obtained against bovine milk β4GalT1 and may not have been monospecific (Humphreys-Beher *et al.*, 1986). However, a 40-fold enrichment of β4GalT1 activity in a plasma membrane fraction supported the immunocytochemical data. Recently, using a mAb, β4GalT1 was localized to the junctional complex of human pancreatic acinar cells (Yamamoto *et al.*, 1999).

Ceramide:β-galactosyltransferase (GT family 1, E.C. 2.4.1.45, cerebroside synthase)

This glycosyltransferase is unique in the sense that its topology belongs to type I transmembrane proteins exposing the N-terminus to the extracytoplasmic compartment (Sprong *et al.*, 1998). Conversely, the C-terminus bearing the KKVK ER-retention signal is oriented to the cytoplasm and explains its predominant localization to the ER and the nuclear envelope. Unfortunately, in this study localization was only investigated in transfected cells. Putatively the same enzyme was investigated in rat brain sections using a polyclonal antiserum to the natural enzyme purified from rat brain (Roussel *et al.*, 1987). In agreement with Sprong *et al.* (1998), little Golgi staining was observed, whereas cytoplasmic (ER?) staining was intense. At the level of electron microscopy using immunoperoxidase, peripheral but no ER staining was observed, raising the question of antibody accessibility. In addition and in accordance with the cell-specific differentiation pattern, intense staining was seen in myelin sheaths, more specifically the inner- and outermost lamellae.

α2,6Sialyltransferase (GT family 29, E.C. 2.4.99.1, ST6Gal, β-galactoside-α2,6-sialyltransferase)

This enzyme was localized to *trans*-Golgi cisternae and the *trans*-Golgi network (TGN) in rat hepatocytes at the ultrastructural level using polyclonal antibody to the natural rat liver enzyme (Roth *et al.*, 1985b). No "ectopic" localization was noted, except perhaps for TGN labeling. Further studies showed colocalization of this enzyme with β4GalT1 by immunofluorescence in the GA in rat liver cells under different conditions. Importantly, in this report it was noted for the first time that the distribution of a glycosyltransferase may depend on the type of specimen investigated. In semithin frozen sections, that is, differentiated cells, ST6Gal appeared as spotlike structures scattered throughout the whole cytoplasm, whereas in primary monolayer cultures, a ringlike perinuclear staining with tubular extensions was observed (Taatjes *et al.*, 1987). In HepG2 cells, the GA is even more restricted to a more compact juxtanuclear staining (Berger *et al.*, 1993). Whether β4GalT1 and ST6Gal occur in different subcellular compartments may thus depend on the cell type studied. In primary cultures of bovine kidney fibroblasts, evidence for different sites was obtained using polyclonal antibodies to the respective native enzymes (Berger and Hesford, 1985): β4GalT1 showed the classical compact juxtanuclear Golgi staining, whereas ST6Gal was distributed also in vesicles scattered around the GA. Because trafficking mechanisms appear to be different for these enzymes (as shown by their different reaction to Golgi disturbing agents), steady-state accumulation at different subcellular sites seems possible (Dinter and Berger, 1998). Significantly, because all the mentioned evidence did not take into account cross-reactive carbohydrate epitopes, Taatjes *et al.* (1988) used protein-epitope purified polyclonal antibodies to ST6Gal and, by immunoelectron microscopy on perfusion-fixed ultrathin sections of the colon, found significant immunogold label over microvilli, basolateral membrane, and apically located cytoplasmic vesicles in addition to Golgi staining. Again, ectopic localization was detected in tissue specimens, that is, in differentiated cells.

α2,3Sialyltransferase (GT family 29, E.C. 2.4.99.6, ST3Gal III, ST3N, Galβ1,3(4)GlcNAc α2,3sialyltransferase)

Unexpected localizations were found in perfusion-fixed tissue sections of rat, whereas in cell lines regular Golgi staining was observed using polyclonal antibodies to a fusion protein consisting of the soluble part of ST3Gal III and β-galactosidase expressed in *E. coli*. In epithelial cells of proximal tubules ST3Gal III is strongly coexpressed with H^+ATPase in a subapical compartment that stains for actin filaments. In addition, ST3Gal III also localized to the GA as shown by similar localization of ST3Gal III on parallel kidney sections (Burger *et al.*, 1998). The function (if any) of ST3Gal III in the subapical compartment remains to be determined.

Lactosylceramide α2,3sialyltransferase (E.C. 2.4.99.9, GM3 synthase, ST3GalV)

This enzyme is involved in biosynthesis of GM3 (NeuAcα3Galβ4GlcCer), which is a Golgi-associated biosynthetic function as shown by fractionation (Lannert *et al.*, 1998). By using polyclonal antibodies to a stretch of 52 aminoacids of the catalytic domain expressed as a fusion protein in *E. coli*,

unexpected localizations were found in tissue sections as well as primary cell cultures (Stern and Tiemeyer, 2001). In cerebellum, staining was found in axons coursing through the granule and Purkinje cell layers, and another antiserum to this enzyme directed against a more aminoterminal region of the enzyme (i.e., closer to the stem region) identified this enzyme in almost complete colocalization with giantin, an authentic Golgi protein (Stern *et al.*, 2000). The subcellular localization of ST3GalV was also investigated in PC12 cells following induction with nerve growth factor, a subset of immunostained protein moved to outgrowing neurites. The function of this ectopically located glycosyltransferase has not been defined.

α1,3Fucosyltransferase 6 (GT family 10, E.C. 2.4.1.152, FucT6)

Recently, FucT6 was shown to be expressed in the GA of HepG2 cells (Borsig *et al.*, 1999) and Weibel-Palade bodies (WPbs) of human umbilical vein endothelial cells (HUVECs) (Schnyder-Candrian *et al.*, 2000) in addition to the GA. Because WPbs are well-characterized storage granules of endothelial cells (Weibel and Palade, 1964) this finding was unexpected. The polyclonal antibody involved was raised against a FucT6-specific peptide to distinguish this enzyme from the closely related FucT3 and FucT5. The fluorescent signal in WPbs was strong and overlapped with expression of von Willebrand factor. These data prompted biochemical identification of the antigen recovered by immunoprecipitation from endothelial cells, followed by sequencing of internal peptides. Because all sequences matched the predicted amino acid sequence and, independently, another polyclonal antiserum to FucT6 was able to identify this enzyme in a purified fraction of WPb, there is strong evidence that WPbs contain FucT6, presumably a different molecular form as indicated by differences in immuno-recognition and absence of enzyme activity. Its function in WPbs, if any, is unknown and remains to be determined.

α1,3/4Fucosyltransferase 3 (GT family 10, E.C. 2.4.1.69, Lewis enzyme, FucT3)

Narimatsu and colleagues developed mAbs to human FucT3 (Kimura *et al.*, 1995), an enzyme known to transfer fucose to GlcNAc substituted by Gal in a β1→3 as well as a β1→4 linkage. As mentioned above, this enzyme is homologous to FucT5 and FucT6, thus cross-reactivities of antibodies are expected. However, the mAb FTA1-16 to FucT3 used by Narimatsu and others appeared monospecific. Immunohistochemical staining of normal and malignant human colon tissue was found in the supranuclear Golgi area as well as in the apical region (Narimatsu *et al.*, 1996).

α1,2Fucosyltransferase 1 (GT family 11, H enzyme, E.C. 2.4.1.69, FucT1)

A recent report provides evidence to show expression of FucT1 on the surface of a subset of prostatic epithelial cells involved in ductular branching in neonatal rats (Marker *et al.*, 2001). The mAb used to show expression of FucT1 on the cell surface of neonatal ventral prostatic cells was obtained by immunizing mice with rat embryonic pancreatic ductal epithelial cell lines. This antibody identified a surface antigen on prostatic cells subsequently identified as FucT1 by expression cloning. Interestingly, growth and branching morphogenesis of neonatal rat prostates appeared to be specifically inhibited in the presence of this mAb.

The exceptions to the rule? Polypeptide-N-acetylgalactosaminyltransferases (GT family 27, E.C. 2.4.1.41, protein N-acetylgalactosaminyltransferase, GalNAc-T) and blood group A/B specifying enzymes (GT family 6, E.C. 2.4.1.40)

The family of human GalNAc-Ts comprises at least nine members. Localization studies have been performed using polyclonal antibodies to the purified natural enzyme in porcine submaxillary glands at the ultrastructural level; the identity of the enzyme investigated with respect to the nine cloned members, however, has not been reported. The enzyme showed unique *cis*-Golgi localization in mucous and serous glandular cells (Roth *et al.*, 1985b). However, using mAbs to three different members of the human GalNAc-T family, immunolabel was found distributed throughout the whole cisternal stack of HeLa cell GAs (Rottger *et al.*, 1998). In addition, the same mAbs have been used to investigate distribution of these GalNAc-Ts in stratified epithelia and squamous cell carcinomas (Mandel *et al.*, 1999). Interestingly, in all instances label was confined to the GA, thus no ectopic localization of any GalNAc-T was observed. Notably though, a strong signal on human sperm was specifically found for GalNac-T3 (Mandel *et al.*, 1999); its topology as an ectoenzyme, however, remains to be defined. In addition, as mentioned previously, no evidence for ectopic localization of blood-group specifiying enzymes were found using mAbs for immunohistochemical stainings of a variety of intestinal tissues (White *et al.*, 1990; Mandel *et al.*, 1990, 1992).

A common denominator for ectopic localization

On compiling localization studies of glycosyltransferases, it became obvious that all glycosyltransferases (except the enzymes mentioned above) that were investigated in tissue specimens or primary cell cultures exhibited some ectopic localizations, provided that all this evidence relies on monospecificity of the antibodies used as suggested by published data. In fact, Taatjes *et al.* (1987) were the first to point out that in primary cultures, ST6Gal appeared in a pattern very different from Golgi staining, for instance, scattered in numerous cytoplasmic vesicles. As summarized in Table I, ectopic localizations were usually found in primary cell cultures, cells obtained *ex vivo*, and cells in tissue sections, that is, in differentiated cells expressing those components necessary for the formation of contact sites as they occur in a tissue. The discrepancy between unique Golgi localization in cell lines and ectopic localizations as described above addresses the following three issues.

Differentiation status of investigated cells

Differentiated cells, such as those investigated in tissue specimens or obtained *ex vivo*, exhibit morphological and biochemical differentiation markers that are lost in derived cell lines or on long-term cultivation, for example, HeLa cells. *In vivo*, the cytoarchitecture of differentiated cells depends on the various contacts with adjacent cells and the extracellular matrix (Drubin and Nelson, 1996). Examples are epithelial

cells that lose their polarity on isolation or secretory cells whose storage vesicles are usually not maintained over prolonged passaging of primary cells (e.g., HUVECs, goblet cells).

Static vs. dynamic Golgi membrane components

It has been known since early work by Thyberg and Moskalewski (1985) that the GA scatters in cells treated with microtubular depolymerizing agents. Since then it has become clear that the GA is linked to various cytoskeletal elements, such as actin and a spectrin/ankyrin network (Kreis *et al.*, 1997), that shape cell morphology. These scaffolding elements are likely to exhibit different dynamics than itinerant proteins as recently inferred from the use Golgi disturbing agents such as Brefeldin A (Seemann *et al.*, 2000) or monensin (Berger *et al.*, 2001). It is conceivable that rearranged cytoskeletal elements maintain scaffolding functions in the sense that they may influence post-Golgi trafficking of itinerant proteins, such as glycosyltransferases.

The cell adhesion/signaling/trafficking connection

Cells embedded in their natural tissue environment form various contacts with adjacent cells and the extracellular matrix. They reorganize their morphology on transformation to a cell line growing on a monolayer, thereby also rearranging their cytoskeleton (Gumbiner, 1996). In fact, cell adhesion receptors interact with signaling pathways that govern cell growth and differentiation. Some of these signaling pathways also have an impact on the organization of cytoskeletal elements and on cdc42, a Golgi-located rho family GTPase involved in regulating a variety of cellular functions, such as cytoskeletal remodeling, membrane trafficking, transcriptional activation, and cell growth control (Van Aelst and D'Souza-Schorey, 1997). The role of cdc42 seems particularly relevant because it appears to interact with γ-COP, a subunit of coatomer (Wu *et al.*, 2000). Hence, extracellular cues resulting from specific adhesion sites may influence post-Golgi trafficking routes, which may be reflected by ectopic localization of Golgi enzymes that remain Golgi-associated in the absence of these adhesion-mediated signaling events. There is indirect evidence for the presence of Golgi enzymes in COP I vesicles *in vitro* (Lanoix *et al.*, 1999), but these findings need to be confirmed. The possible links between adhesion and post-Golgi trafficking of glycosyltransferases are somewhat simplistic and speculative as outlined here but may provide some grounds for further investigation. In fact, recent evidence on interaction of the cytoplasmic domain of β4GalT with the src-suppressed C kinase substrate, a modulator of cyto-architecture (Gelman *et al.*, 1998) provides the first example of such a connection (Wassler *et al.*, 2001).

Functions for ectopic glycosyltransferases?

Ectopic localization of Golgi glycosylation enzymes (glycosyltransferases and glycosidases) suggest stoichiometric instead of catalytic functions. These have been proposed in the case of sperm–egg interactions (Shur *et al.*, 1998) but could also be involved in intracellular trafficking in the case of FucT6, which coexpresses with von Willebrand factor in BPbs (Schnyder-Candrian *et al.*, 2000). The most likely function for ectopic glycosyltransferases may be related to their intrinsic carbohydrate binding specificities as suggested by structural and functional studies (Rauvala *et al.*, 1983; Ma and Colley, 1996; Hagen *et al.*, 1999). Alternatively, movement of glycosyltransferases to ectopic sites merely reflect intracellular membrane dynamics.

Abbreviations

ER, endoplasmic reticulum; GA, Golgi apparatus; HUVEC, human umbilical vein endothelial cell; mAb, monoclonal antibody; TGN, *trans*-Golgi network; WPbs, Weibel-Palade bodies.

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