Protein *N*-Glycosylation along the Secretory Pathway: Relationship to Organelle Topography and Function, Protein Quality Control, and Cell Interactions

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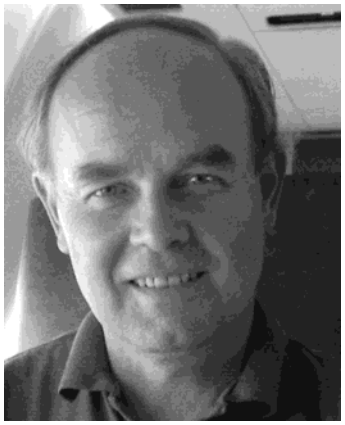
Contents



Jürgen Roth studied medicine and graduated from the Friedrich-Schiller-University Jena in 1969. He is Professor of Cell and Molecular Pathology and the head of the Division of Cell and Molecular Pathology of the Department of Pathology, University of Zurich. Since 1970 his major interest is almost exclusively related to cellular glycoconjugates while working at the Universities of Jena, Geneva, Basle, and Zurich. He has made major contributions in the development of lectin–gold techniques and for immunoelectron microscopy by developing the protein A-gold technique, as well as for low-temperature resin embedding of tissues. He and his group are studying cellular and molecular aspects of protein glycosylation in the ER and the Golgi apparatus and of cell surface glycotope expression during development and in cancer. Current research focuses on aspects of protein quality control under physiological and diseased states.

1. Introduction

Eukaryotic cells contain different highly specialized, membrane-bounded compartments fulfilling specific functions. Among them, the endomembrane system that constitutes the secretory pathway is most prominent and consists of the endoplasmic reticulum (ER), pre-Golgi intermediates, the Golgi apparatus, and different types of post-Golgi apparatus carriers and vesicles. The secretory membrane system accomplishes a multitude of interrelated functions encompassing the translocation and transport of de novo synthesized membrane, secretory, and lysosomal proteins; posttranslational modifications of proteins; the quality control of glycoprotein folding and assembly; and the sorting of glycoproteins to their final cellular destinations, such as the plasma membrane, to name some of the most important ones. Inherent to these functions are highly dynamic processes of membrane and cargo transport between the ER, pre-Golgi intermediates, the Golgi apparatus, and the plasma membrane. This paper will review architectural, topofunctional, and molecular aspects of the biosynthesis of *N*-linked oligosaccharides in the ER and the Golgi apparatus and relate them to protein quality control. Furthermore, functional organization principles of the Golgi apparatus with emphasis on *N*-glycosylation will be discussed, and selected examples of the physiological importance of

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specific oligosaccharide structures on cell surface glycoproteins will be reviewed. Although the biosynthesis and trafficking of lysosomal enzymes represents an import aspect of the secretory pathway, this will not be covered here, and the interested reader is referred to excellent reviews.[1−3]

2. Structure/Function Aspects of the Secretory Pathway

This section will focus on the structure of the secretory pathway and transport carriers as well as current models put forward to explain the mechanism of anterograde and retrograde cargo transport. These aspects are important for the understanding of the relationship of protein glycosylation and protein quality control, which will be discussed later on.

The ER represents not only the entry point into the secretory pathway but also constitutes its largest part and represents a highly dynamic organelle.[4−7] Classically, it is subdivided into three morphologically distinguishable domains: the ribosome-studded rough ER, the ribosome-free smooth ER, and the nuclear envelope (Figure 1). Regarding the latter, only the ribosome-covered outer nuclear membrane is considered to be part of the rough ER. The three ER domains are continuous with each other and extend through most of the cytoplasm to form an intricate network composed of fenestrated cisternae and anastomosing tubules.[8,9] Quantitative variation in the content of the ER may be observed, depending on the cell type and as a consequence of variations of the functional state of a given cell type. Despite membrane continuities between the ER domains, some specific marker proteins have been discovered for each of them.[10−15] In addition to the classical domains, subdomains of the ER either defined by characteristic morphology or by the presence/absence of specific proteins have been identified.[16−20] Over the past decade, intense research has focused on a particular subdomain of the ER, the so-called transitional elements of the rough ER (Figures 1 and 2).[16,21] They are characteristically partly devoid of ribosomes, exhibit buds, are continuous with the rough ER, and appear to be rather static structures. In close spatial and functional relationship to the transitional elements exist clusters of smooth membrane vesicular tubular structures, which have been the subject of intense studies as well (Figures 1 and 2).[22−24] They are referred to as the intermediate (or salvage) compartment, ERGIC-53, vesicular tubular clusters (VTCs), or pre-Golgi intermediates. The

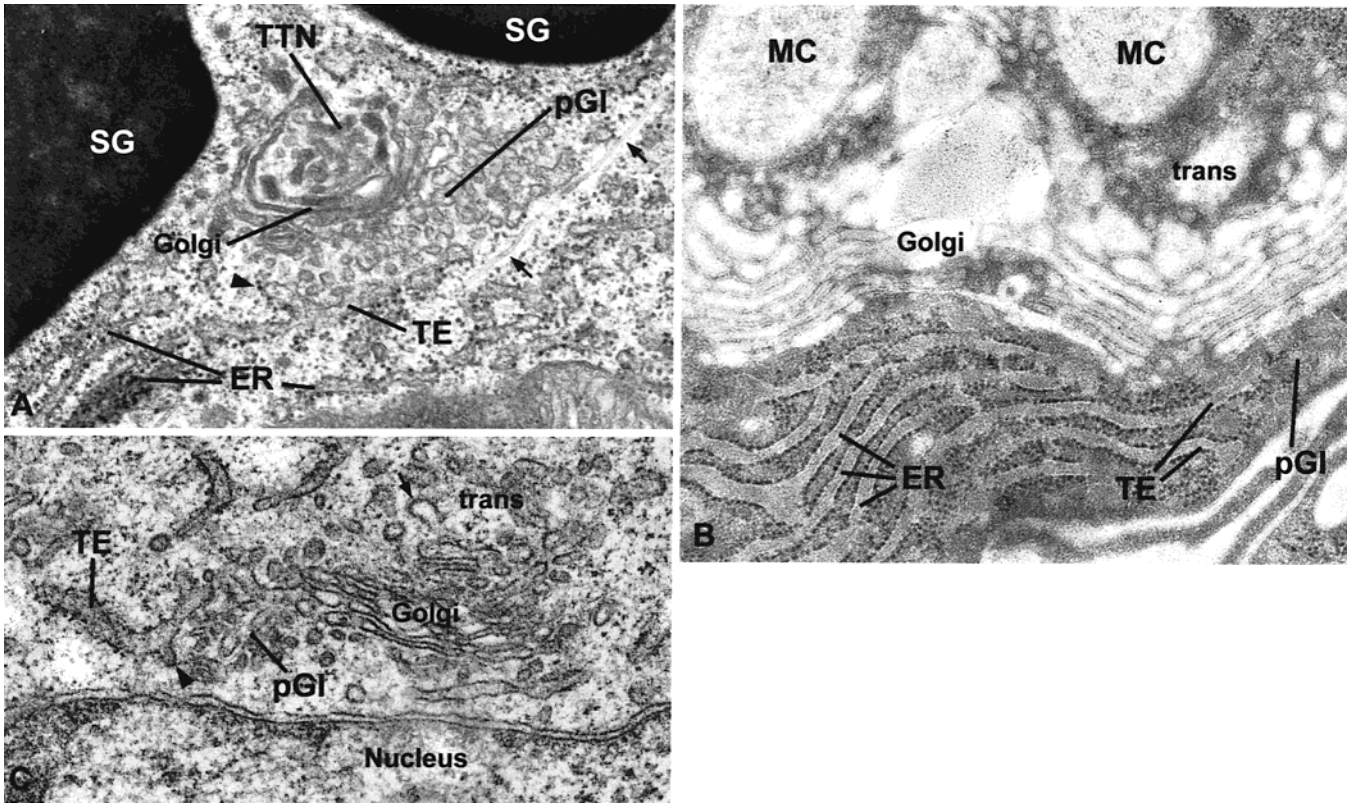

Figure 1. Transmission electron micrographs from ultrathin sections showing examples of highly active secretory cells and of a cell line grown in culture to illustrate the morphological appearance of the secretory pathway. (A) Part of a cell from the salivary gland of the fruit fly *Drosophila melanogaster*; (B) part of a mucus-producing goblet cell from rat colon; (C) part of a Chinese ovary hamster (CHO) cell. ER: cisternae of the rough endoplasmic reticulum covered by ribosomes. TE: transitional element of the rough endoplasmic reticulum partly devoid of ribosomes. Arrowheads in A and C point to membrane buds on TE with a COPII coat. pGI: pre-Golgi intermediates consisting of vesicular tubular smooth membrane structures that are located between TE and the cis face of the Golgi apparatus. TE and pGI exist also in the periphery of cells not in association with the Golgi apparatus. The Golgi apparatus appears as a stack of cisternae with the trans-tubular network (TTN). The arrow in C points to a clathrin-coated element of the TTN. In secretory cells, the trans face of the Golgi apparatus can be unequivocally identified by the presence of secretory granules such as enzyme-containing zymogen granules (SG in A) and mucus droplets (MC in B).

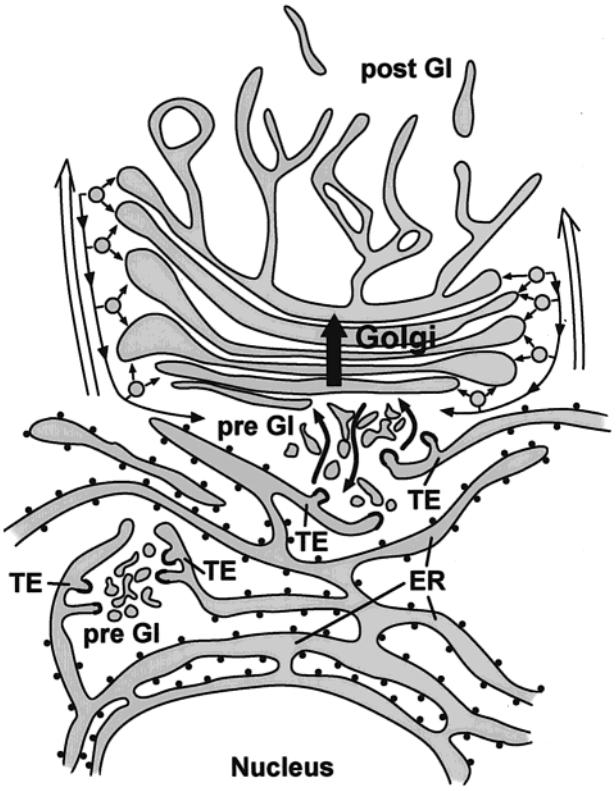

Figure 2. Schematic drawing of the secretory pathway of a cell. ER: rough endoplasmic reticulum cisternae. TE: transitional elements of the rough ER with COPII-coated buds and tubules located either in the periphery of the cell or closely associated with the Golgi apparatus. Pre-GI: pre-Golgi intermediates located between TE and the cis face of the Golgi apparatus or in the periphery of the cell. The bold arrow in the Golgi apparatus indicates the slow cisternal route and the open arrows the fast route by percolating vesicles for anterograde cargo transport. The possibility for retrograde cargo and residential protein transport is also indicated. Post-GI: post-Golgi intermediates.

identification and characterization of these structures has been greatly facilitated by the discovery of marker proteins, p58 (in rat) and p53 (in human) (Figure 3).[25,26] All available evidence indicates that p58 cycles between the ER, pre-Golgi intermediates, and part of the Golgi apparatus,[27] whereas the major recycling route of p53 seems to bypass the Golgi apparatus.[28] The pre-Golgi intermediates represent dynamic structures that can be found either between the ER and the Golgi apparatus or in the periphery of the cell from where they move along microtubules to the Golgi apparatus.[29−32] It is now undisputed that the transitional elements and pre-Golgi intermediates are functionally close partners, and they have been proposed to represent endoplasmic reticulum export complexes.[33−36] The export of cargo from the ER commences at the level of the transitional elements of the ER, and many of the molecular details of this process have been unraveled during the past decade.[37−39] The anterograde traffic of cargo is accomplished by specific sequential interactions of cytosolic coat proteins (COP) with the transitional ER membrane, and COP II coated vesicles are thought to exclusively ferry anterograde cargo.[36,40] Initially, Sar 1p is recruited to the membrane to which Sec23p-Sec24p and finally Sec13p-Sec31p bind, resulting in the formation of a coated bud or tubule into which anterograde cargo is actively sorted, probably through the action of cargo selection proteins, such as the p24 family of proteins.[41−44] COP II-coated buds are thought to give rise to coated vesicles, which rapidly become uncoated and apparently fuse with each other or neighboring vesicular tubular clusters. Thus, transitional elements represent the exit sites of anterograde cargo from the ER that deliver cargo to pre-Golgi intermediates. The current view is that these pre-Golgi intermediates target and fuse with the Golgi apparatus, although many details of this process need to be established.[45,46] It has to be emphasized that pre-Golgi intermediates fulfill a function not only in anterograde but also in retrograde transport that is thought to be mediated by the COP I pathway (Figure 2).

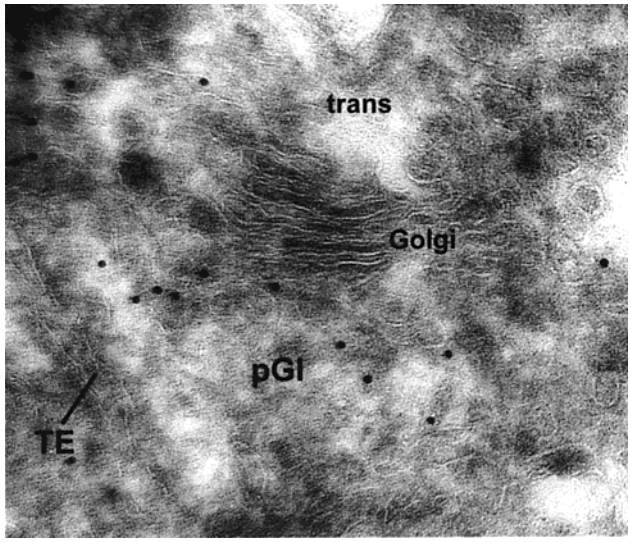

Figure 3. Immunogold labeling for the pre-Golgi intermediate marker protein p58 in an ultrathin cryosection from a cultured hepatocyte. Gold particle labeling is detectable in pre-Golgi intermediate (pGI) at the cis face of the Golgi apparatus. A transitional element (TE) of the rough endoplasmic reticulum is unlabeled.

The boundary between the pre-Golgi intermediates and the Golgi apparatus has remained largely undefined, as one would expect for a highly dynamic and pleomorphic structure. Actually, under the steady-state conditions of immunoelectron microscopic investigations, the marker protein p58/p53 exhibits a dual localization by being detectable in both the pre-Golgi intermediates and in one or two cis Golgi cisternae.[25,26] It must be stressed that some Golgi residential proteins such as a polypeptide: UDP-GalNAc *N*-acetylgalactosaminyltransferase[47] and endomannosidase[48] also exhibit a dual localization by being additionally detectable in pre-Golgi intermediates.

The Golgi apparatus occupies a central place in the secretory pathway in that it is involved, inter alias, in the posttranslational modification and traffic of secretory and membrane glycoproteins as well as lysosomal enzymes.[32,49,50] Its architecture[51,52] is as complex as is its function, and models to explain the mechanism of traffic through the Golgi apparatus remain vividly debated.[49,53−55] The basic structural

frame of the Golgi apparatus as a single organelle consists of a continuous perinuclear ribbon of stacked cisternae that is composed of compact and noncompact regions (Figure 1).[51] The Golgi apparatus is a polar organelle with the cis face representing the entry site for anterograde cargo from the pre-Golgi intermediates and the trans face being the exit site. Both the cis and trans face are characterized by the presence of more or less elaborate and dynamic tubular networks.[56−64] In addition, vesicles can be observed on either face of the Golgi apparatus, COPI- and COPII-coated ones cis and clathrin-coated ones trans, and COPI-coated vesicles at the rims of cisternae. It remains to be emphasized that depending on the cell type not only the number of cisternae making up the stack may be significantly different (as few as three and as many as 20) but also the extensiveness of the cis and trans tubular networks.[51]

To explain the nature of anterograde transport of secretory and membrane proteins across the Golgi apparatus, two alternative models have been proposed: the cisternal progression/maturation model and the stationary cisternae/vesicular transport model.[49,53−55] The cisternal progression/maturation model assumes that Golgi cisternae are formed at the cis face and that entire cisternae mature while moving en bloc up the stack to its trans face. Inherent to this model is that secretory and membrane proteins would move synchronously across the stack. On the other hand, the stationary cisternae/vesicular transport model implies that transport to and across the Golgi apparatus occurs via COPI vesicles pinching off from and fusing with the dilated rims of cisternae. Although both models have received experimental support, neither can account for all principles of Golgi traffic, namely, (i) that membrane and secretory proteins have been shown to move at different rates across the Golgi apparatus and (ii) that certain cargos, for instance scales in algae[65] and procollagen fibers,[66] are simply too large to fit into transport vesicles of a diameter of 60−90 nm. Recently, the fast transport of engineered protein aggregates as large as 400 nm in diameter via megavesicles pinching off the rims of cisternae was reported.[67] However, it was noted that only about 20% of such aggregates were enclosed in megavesicles, and the generality of this phenomenon is doubtful. A recently proposed unifying model is a kind of hybrid of the two models mentioned above and tries to reconcile their pros and cons (Figure 2).[55] Its essence is that COPI vesicles, proposed to percolate up and down the cisternal stack along the dilated rims of the cisternae, would provide the fast track for transport of cargo across the stack, whereas the slow track would be furnished via cisternal progression. In addition, a distinct population of COPI vesicles is postulated to exist for the retrograde transport to the ER and the intra-Golgi transport of Golgi residential proteins.

After traversing the Golgi apparatus, cargo is packed into transport carriers to reach its final destination. Such post-Golgi carriers are represented by small vesicles and large irregularly shaped tubular structures (Figure 2).[68−70] They represent dynamic structures that undergo dramatic changes in shape[71−73] before reaching the plasma membrane.

3. Early Processing Reactions of Asparagine-Linked Oligosaccharides and Protein Quality Control

The biosynthesis of asparagine-linked oligosaccharides proceeds in distinct steps, and key events of this process are summarized in Figure 4. Initially, a lipid-linked oligosaccharide precursor is assembled stepwise, and this process seems to be highly conserved evolutionarily.[74−76] Then, the pre-assembled oligosaccharide, whose structure is $Glc_3Man_9GlcNAc_2$-Dol-P-P, is transferred en bloc to an asparagine in a nascent protein and subjected to a series of processing reactions that result in the removal of all three glucose residues and some of the mannose residues (Figure 5).[77,78] The trimming reactions occurring in the ER have been shown to be conserved from lower to higher eukaryotes. The biological significance of these trimming reactions has remained obscure for a long time, until it became evident that they actually represent key events in the process of protein quality control.[79,80]

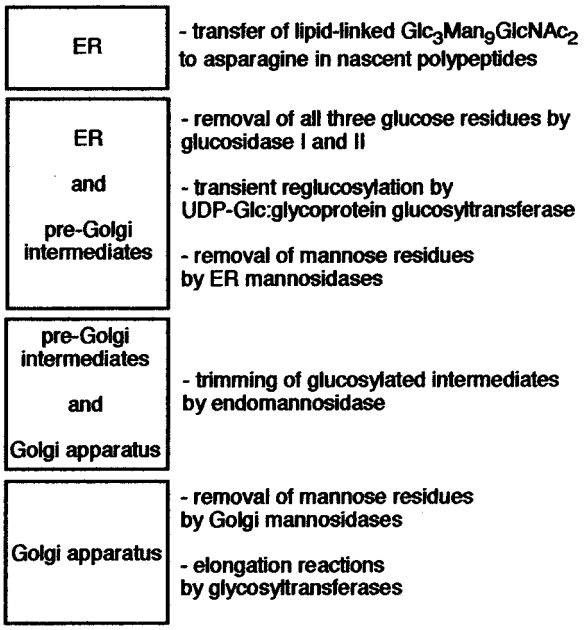

Figure 4. Schematic presentation of key steps of *N*-glycosylation occurring in the endoplasmic reticulum (ER), pre-Golgi intermediates, and the Golgi apparatus.

3.1. The Machinery Proteins

In the following, only those components of protein quality control machinery will be considered whose action is in one or another way related to specific oligosaccharide structures. Details about molecular chaperones involved in this process can be found in many excellent reviews.[81−90]

3.1.1. α-Glucosidase I

Trimming of the asparagine-linked $Glc_3Man_9GlcNAc_2$ oligosaccharide by α- glucosidase I (encoded by CWH41 in yeast) occurs immediately after transfer from the lipid and involves the removal of the

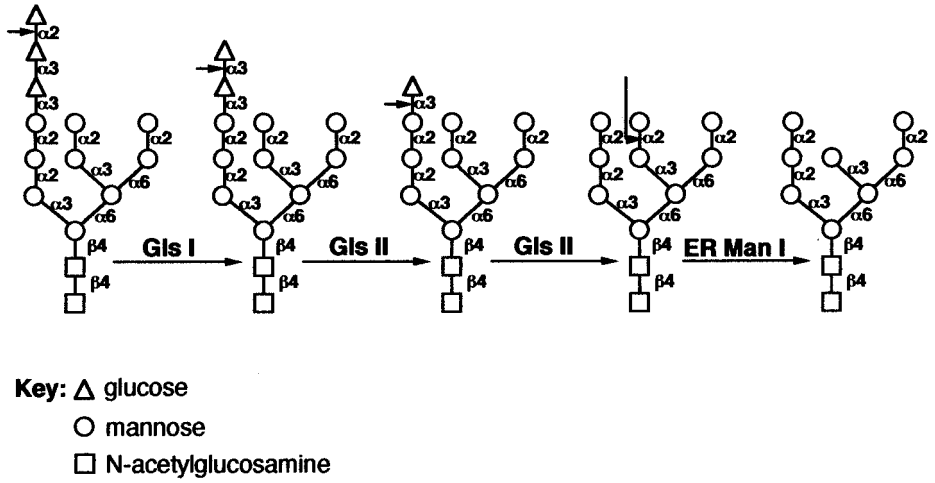

Figure 5. Schematic representation of glucose- and mannose-trimming reactions occurring on asparagine-linked oligosaccharides in the endoplasmic reticulum and pre-Golgi intermediates. Gls I: glucosidase I which removes the outer α1,2-linked glucose. Gls II: glucosidase II which removes the two α1,3-linked glucose residues. ER Man I: ER α1,2-mannosidase I, which removes a single α1,2-linked mannose residue to form the $Man_8GlcNAc_2$ B isomer. The arrows indicate the site where the hydrolysis by the respective glycosidase occurs.

terminal α1,2-linked glucose residue (Figure 5).[91,92] Glucosidase I has a pH optimum of 6.2, which is different from the more acidic one of lysosomal glucosidase, its subunit molecular mass is about 85 kDa, and the native enzyme appears to exist as a tetramer.[93−95] The enzyme, which has no apparent requirement for cations, is inhibited by 1-deoxynojirimycin and its derivatives and by castanospermine.[93,94,96] A glucosidase I cDNA has been isolated from human brain encoding in a single open reading frame a polypeptide of 834 amino acids corresponding to a molecular mass of 92 kDa,[97] and the glucosidase I gene has been mapped to chromosome 2p12-p13.[98] A single *N*-glycosylation site exists at Asn655, which, due to endoglycosidase H sensitivity, appears to bear a high-mannose type oligosaccharide. The primary structure of glucosidase I is consistent with a type II transmembrane protein composed of a 35 amino acid long N-terminal cytosolic domain followed by a 20 amino acid transmembrane domain and a large C-terminal catalytic domain present in the lumen of the ER. In COS 1 cells overexpressing the enzyme, glucosidase I by immunofluorescence microscopy exhibited a distribution indicative of an ER localization.

3.1.2. α-Glucosidase II

Following removal of the terminal α1,2-linked glucose residue, the two inner α1,3-linked glucose residues are sequentially trimmed by α-glucosidase II (encoded by *ROT2* in yeast) (Figure 5).[99,100] The hydrolysis of the first α1,3-linked glucose by glucosidase II occurs very rapidly, whereas the second α1,3-linked glucose is removed more slowly.[92,101] It has been proposed that two different substrate binding sites of glucosidase II are responsible for this kinetic difference.[102] Glucosidase II, like glucosidase I, has a near neutral pH optimum, no apparent requirement for cations, and is inhibited by 1-deoxynojirimycin and its derivatives and by castanospermine. It appears to exist as a tetramer composed of ~110 kDa subunits that carry one high-mannose type oligosaccharide at Asn97.[99,100,103−105] Circumstantial biochemical evidence indicated that glucosidase II is neither a soluble nor a transmembrane but rather a hydrophilic, loosely membrane-associated protein.[100,103] This was supported by the results of cloning the enzyme.[105] A glucosidase II cDNA with an open reading frame of about 2.9 kb was obtained encoding a polypeptide of 944 amino acids. The primary sequence did not contain known ER retention signals of the C-terminal KDEL type nor a double lysine C-terminal or a double arginine N-terminal motif or any hydrophobic region characteristic of transmembrane proteins. The mechanism by which glucosidase II is retained in the ER and pre-Golgi intermediates is not fully understood. It has been proposed that a tightly bound, noncatalytic 80 kDa protein (the so-called β subunit, the α unit being the catalytically active 110 kDa glucosidase II), which contains a HDEL ER retention sequence, is involved.[106,107]

The primary sequence of glucosidase II is highly conserved from yeast to mammals[105,108] with over 90% amino acid identity between the pig and human enzymes and approximately 40% identity between pig and yeast glucosidase II. Glucosidase II seems to exist in two isoforms of 112 and 107 kDa,[104,105] and this could be confirmed by DNA sequencing.[105,109,110,111] Due to alternative splicing, an additional 66 nucleotides coding for a peptide of 22 amino acids are inserted into position 188 of pig liver glucosidase II, and the identical peptide was observed in the amino acid sequence of embryonic, postnatal, and adult rat kidney but was undetectable in insect cells and yeast.[110] The extrapeptide in human[111] and mouse T lymphoma cells[112] showed 95% and 90% identity to the extrapeptide of pig and rat glucosidase II. It has been proposed that this extrapeptide is involved in the interaction of glucosidase II with the β subunit or other proteins in the ER.[113,114] It has also been speculated that the ubiquitous occurrence of the two glucosidase II isoforms may indicate their differential involvement in glucose trimming, but this remains to be elucidated.

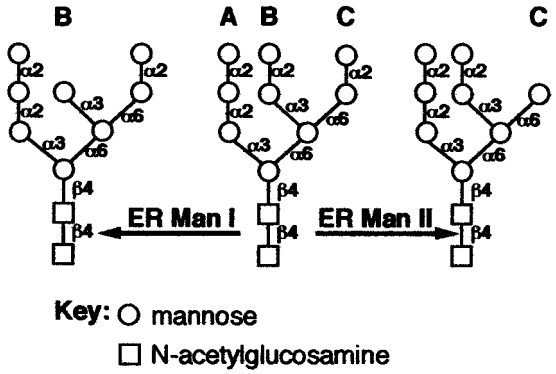

Figure 6. Processing by ER mannosidases. ER Man I: ER α1,2-mannosidase I forms the $Man_8GlcNAc_2$ B isomer. ER Man II: ER α1,2-mannosidase II forms the $Man_8GlcNAc_2$ C isomer.

3.1.3. ER α1,2-mannosidase I

Concurrently with or immediately following glucose trimming, removal of mannose residues can occur by neutral α1,2-mannosidases (Figures 5 and 6).[78,115−117] Although several such ER α1,2-mannosidases have been described on the basis of different sensitivity toward inhibitors and the specific $Man_8GlcNAc_2$ isomers formed, to date only one of these mannosidases, the ER α1,2-mannosidase I (encoded by *MNS1* in yeast),[118,119] has been characterized in greater detail.[120,121] This mannosidase catalyzes the first mannose trimming step by cleaving a single α1,2-mannose residue from $Man_9GlcNAc_2$ to yield a specific $Man_8GlcNAc_2$ isomer (Figures 5 and 6). It is a type II transmembrane protein composed of an N-terminal 47 amino acid long cytoplasmic tail, a single 22 amino acid transmembrane domain, and a large 594 amino acid C-terminal catalytic domain that is ubiquitously expressed in human tissues.[121] The enzyme is inhibited by 1-deoxymannojirimycin and kifunensine but not by swainsonine and requires cations. Thus, because of its similarity in primary sequence, its biochemical properties and catalytic mechanism, ER α1,2-mannosidase I is a class I mannosidase.[116] By immunofluorescence, ER α1,2-mannosidase I exhibited an ER-like pattern in stably transfected NRK cells.

An additional mannosidase, termed ER α1,2-mannosidase II, has been identified in rat liver and several mammalian cell lines and shown to produce a $Man_8GlcNAc_2$ isomer distinct from that of ER α1,2-mannosidase I (Figure 5).[119,122] ER α1,2-mannosidase I and II can be distinguished by their different sensitivity toward kifunensine, with ER α1,2-mannosidase II being kifunensine-resistant and having no specific cation requirements.

3.1.4. ER α-Mannosidase-like Proteins

Very recently, α-mannosidase-like proteins were identified: Mnl1p[123,124] in *Saccharomyces cerevisiae* and EDEM in mouse.[125] However, they all seem not to affect oligosaccharide trimming. Specifically, Mnl1p lacks cysteine residues that are essential for mannosidase activity,[123] EDEM has no mannosidase activity,[125] and deletion of Mnl1p has no effect on oligosaccharide trimming.[124] They all seem to correspond to a mannose-specific ER lectin, postulated to be involved in protein quality control,[101] and their possible role will be discussed below.

3.1.5. UDP-glucose: Glycoprotein Glucosyltransferase

Although trimming reactions seem to prevail on asparagine-linked oligosaccharides of glycoproteins during their stay in the ER, transient reglucosylation of glucose-free high mannose-type oligosaccharides of glycoproteins, which was initially observed in trypanosomatids,[126] seems to occur in a high percentage of oligosaccharides in mammalian cells and tissues as well as fungi, plants, insects, and certain yeast.[127−132] This transient reglucosylation was shown to be due to the activity of an UDP-Glc: glycoprotein glucosyltransferase, a 160 kDa soluble glycoprotein with an absolute requirement for Ca^{2+}.[130,133] Activity for this enzyme was undetectable in *S. cerevisiae*,[134] although a gene (*KRE5*) with homology (20% identity) to glucosyltransferase exists in this yeast.[135] The enzyme exists in *Schizosaccharomyces pompe* but seems not to be essential.[136] Glucosyltransferase transfers a single glucose residue from UDP-Glc to the distal mannose of the A branch (Figure 6) of $Man_9GlcNAc_2$ oligosaccharides in a α1,3-linkage.[131] Reglucosylation may also occur on $Man_8GlcNAc_2$ and $Man_7GlcNAc_2$ oligosaccharides but with lower efficiency.[137]

The glucosyltransferase cDNA from rat liver encodes in a single open reading frame a protein of 1527 amino acids, which contains a C-terminal retrieval tetrapeptide (HEEL), characteristic of soluble endoplasmic reticulum proteins.[138] cDNAs encoding glucosyltransferase have been cloned from *Drosophila melanogaster*,[132] *S. pompe*,[136] and *Caenorhabditis elegans*.[139] The C-terminal part of the enzyme from diverse organisms has a 65−70% amino acid identity, which is in harmony with the fact that the catalytic site of the glucosyltransferase is contained in a 37 kDa C-terminal portion of the protein, with the residues D1334, D1336, Q1429, and N1433 being necessary for its catalytic activity. It became quickly clear that a non-native protein conformation was required in order that the enzyme could reglucosylate a glycoprotein.[137,140−144] It should be stressed that the molecular details of this interaction remain to be fully elucidated. Apparently, an efficient glucosyltransferase acceptor substrate consists of a protein recognition element and part of the oligosaccharide. The former is assumed to be represented by clusters of exposed charged amino acids that must be closely related to the oligosaccharide. It is assumed that the innermost GlcNAc residue of the oligosaccharide must be accessible for the glucosyltransferase, although the glucose is transferred to the terminal mannose residue.

3.1.6. Endo-α-mannosidase

An enzyme that acts preferentially but not exclusively on $Glc_1Man_9GlcNAc_2$ oligosaccharides, like glucosidase II, but that releases the Glcα1,3Man disaccharide (Figure 7) was discovered in Golgi fractions from rat liver.[145] This endo-α-mannosidase is still the only known endoglycosidase among all

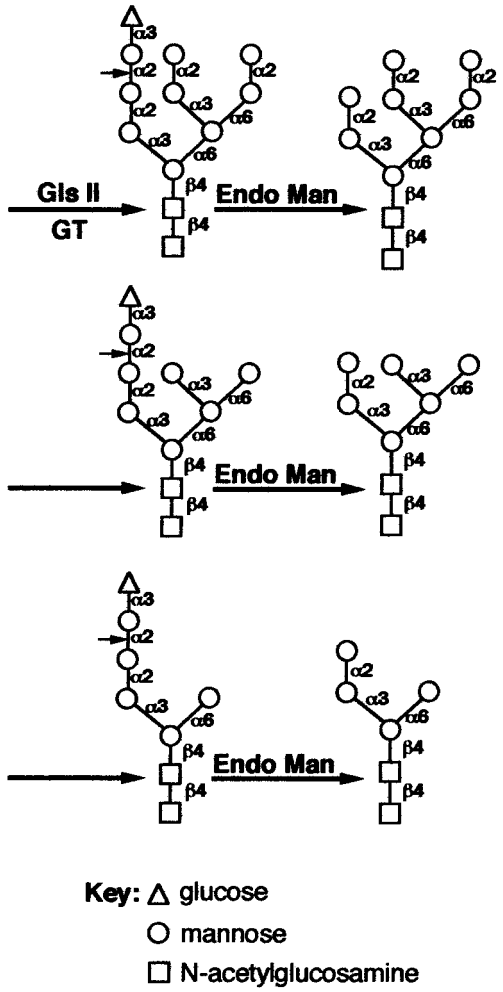

Figure 7. Processing of trimming intermediates by endo-α1,2-mannosidase. Different possible substrates for endomannosidase are indicated. They may be the products of processing by glucosidase II (Gls II) or reglucosylation by glucosyltransferase (GT), as shown in the upper panel, or of processing by mannosidases (middle and lower panel). The arrows indicate the site of hydrolysis by endomannosidase.

trimming glycosidases and provides an alternate, glucosidase II-independent pathway for the formation of complex-type oligosaccharides.[146,147] Detailed analyses of its substrate specificity have shown that, in addition to the monoglucosylated oligosaccharide, di- and triglucosylated oligosaccharides are also trimmed and that monoglucosylated oligosaccharides with truncated mannose chains ($Glc_1Man_{7-5}GlcNAc$) are preferred substrates (Figure 7).[148] The latter distinguishes endomannosidase from glucosidase II, which acts very poorly on such substrates. Endomannosidase has a neutral pH optimum, has no specific cation requirements, and is not sensitive to inhibitors of glucosidase I and II or other neutral trimming mannosidases.[145,149] A specific inhibitor, Glcα1,3(1-deoxy)mannojirimycin, has been developed.[149] The molecular mass of 56 kDa estimated for endomannosidase affinity-purified from rat liver Golgi membranes[150] was basically confirmed through cloning its cDNA, which in a single open reading frame encodes a polypeptide of 457 amino acids, corresponding to a molecular mass of 52 kDa. Its amino acid sequence exhibited no sequence homology with any other protein, and no consensus sequence for *N*-glycosylation was found.[151] Endomannosidase mRNA could be detected in many rat tissues.[151] This was confirmed by in situ immunocytochemistry, although endothelial cells of blood vessels were the sole cell type unreactive with the specific antibody.[152] Enzymatic activity for endomannosidase has been detected in many cell lines but not CHO cells.[48,149] A phylogenetic survey has demonstrated that endomannosidase, in contrast to other processing glycosidases, occurred late during evolution, since it is primarily confined to the phylum *Chordata*.[153]

3.1.7. Calnexin and Calreticulin

Calnexin[154−158] and calreticulin[159−161] are lectins present in the ER, the former being a transmembrane protein and the latter a soluble protein. The luminal domain of calnexin and calreticulin exhibit high homology and contain the high affinity binding site for calcium. Calnexin is a type I transmembrane protein of 65 kDa that is nonglycosylated. Its 89 amino acid cytosolic tail contains a C-terminal RK-PRRE sequence that represents an ER localization signal.[162] Calreticulin is a soluble protein of 46 kDa and contains a KDEL sequence for ER retention as well as a highly negatively charged region for low-affinity Ca^{2+} binding at the C-terminus.[163] Although both proteins perform certain functions characteristic of chaperones, such as transient interaction with folding intermediates and prevention of aggregate formation and of premature protein degradation, their mode of action appears to be distinct from classical chaperones and more similar to that of lectins. Both specifically associate with monoglucosylated high-mannose type oligosaccharides ($Glc_1Man_{9-5}GlcNAc_2$), and protein−protein interactions do not seem to be an essential feature of this interaction.[161,164−171] Although the oligosaccharide specificity of calnexin and calreticulin is identical[169,172,173] and despite that both have been shown to interact with soluble and membrane proteins, a preferential interaction with some glycoproteins has been demonstrated.[162,165,166,174−176] From these observations it became clear that calnexin and calreticulin might interact with the same glycoprotein but that the kinetics of interaction may be distinct, namely, simultaneous or successive binding or differential dissociation. These differences in glycoprotein selection are apparently related to differences in topology, with calnexin having its lectin binding site closely confined to the ER membrane[177] and calreticulin being a soluble protein, and this notion has received experimental support.[161] Recent in vitro studies on calreticulin have provided strong evidence for conformational changes occurring as a consequence of Ca^{2+}-, Zn^{2+}-, and Mg^{2+}-ATP binding.[178] The role of these conformational changes in vivo on calreticulin's lectin function remains to be elucidated.

3.2.Topo-Functional Aspects

The ER provides a unique folding and assembly environment for newly synthesized secretory and membrane proteins[88,179] and at the same time exerts a stringent quality control which ensures that only

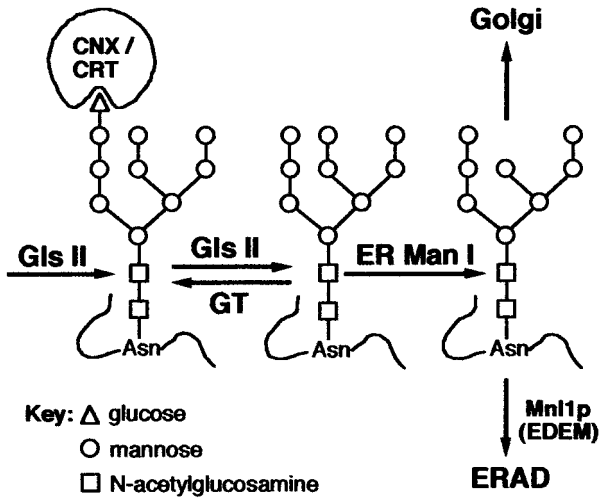

Figure 8. Schematic presentation of the protein quality control mechanism. Calnexin or calreticulin will bind to monoglucosylated $Man_9GlcNAc_2$ oligosaccharides resulting from trimming by glucosidase II (Gls II). Such complexes will be dissociated by glucosidase II mediated glucose removal. If the glycoprotein exhibits a non-native conformation, interaction with glucosyltransferase (GT) and reglucosylation will occur and a new round of calnexin/calreticulin−glucosidase II interactions will commence. Finally, mannose removal by ER mannosidase I (ER Man I) will take place to yield the $Man_8GlcNAc_2$ B isomer. Correctly folded glycoproteins will be exported to the Golgi apparatus. Non-native conformations will be recognized by mannosidase-like lectins (Mnl1p, EDEM) specifically reacting with the $Man_8GlcNAc_2$ B isomer to initiate the ER associated degradation (ERAD).

correctly folded or assembled glycoproteins become exported.[79,80,180,181] Posttranslationally, folding or assembly of glycoproteins may be impeded either due to translational errors or to structural and chemical damage caused by various types of cellular stress and reactive oxygen species, respectively.[182−184] Such misfolded glycoproteins may become rescued by the action of various chaperones[81−90] or, if this mechanism fails, become destroyed by the cytosolic proteasome[181,185−189] or form intracellular protein aggregates.[190−197] Failure of chaperone-mediated rescue of misfolded proteins occurs in a whole spectrum of congenital human diseases characterized by the synthesis of faulty proteins due to a simple point mutation.[198−201] Retention and degradation or formation of protein aggregates represents the common pathogenetic axiom of seemingly unrelated human diseases, which can be classified as a single group of protein folding/retention diseases (Table 1).

3.2.1. The Glucosidase II–Calnexin/ Calreticulin–Glucosyltransferase Cycle

Protein quality control can be seen as a prime example of the intricate relationship between co- and posttranslational protein *N*-glycosylation, protein folding, cargo traffic, and disease.[202,203] In the following, the current view of this process will be discussed with particular emphasis on ER trimming and reglucosylation reactions as well as ER lectins involved either in chaperoning or degradation of glycoproteins (Figure 8).

Different well-defined steps of protein quality control can be distinguished that occur in a stepwise, interdependent manner. An initial step involves trimming by glucosidase I and II, which results in the formation of monoglucosylated high-mannose type oligosaccharides on proteins, a very rapidly occurring event in vivo (see also section 3.1.2). This provides the substrate for binding to and interaction with two ER lectins, calnexin and calreticulin (see also section 3.1.7) (Figure 8), which also have chaperone-like properties, since they are involved in protein folding.[157,180,204−206] Depending on the position of the oligosaccharide in the protein, namely, within the first 50 amino acids of the N-terminus, calnexin/calreticulin binding may occur cotranslationally.[176] The duration of this interaction may vary depending on the folding state of the protein. Some molecular aspects of this lectin/oligosaccharide interaction have been revealed recently.[171,207−209] Thus, it seems that the glycoprotein can form a transient ternary complex with calnexin/calreticulin and glucosidase II and that the substrate interaction between the lectins and glucosidase II occurs at topologically distinct sites of the monoglucosylated oligosaccharide. The interaction of calreticulin with its substrate proceeds as a single-step bimolecular association and dissociation reaction. Recently, the structure of the calreticulin P-domain was revealed by NMR to adopt a bent hairpin-type of fold in solution. It has been proposed that this domain may directly interact with the folding catalyst ERp57 rather than be involved in the binding of the oligosaccharide. ERp57 is a thiol oxidoreductase that establishes transient disulfide bonds with calnexin/calreticulin bound substrates and is as such involved in protein folding.[210−212] Apparently, it is the ERp57 in the calnexin/calreticulin complex with misfolded glycoproteins that modulates glycoprotein folding. Dissociation of calnexin/calreticulin−substrate complexes is mediated by deglucosylation through glucosidase II (Figure 8).[167,213] Depending on the folding state of the released glycoprotein, it may undergo further trimming by ER mannosidase I, followed by export from the ER in case a native conformation was achieved, or it may be retained in the ER for a further round of binding to calnexin/calreticulin if still misfolded. In the latter event, glucosyltransferase (see also section 3.1.5) functions as a folding sensor and will reglucosylate only incompletely folded glycoproteins, providing the substrate for another calnexin/clareticulin interaction (Figure 8).[141,143,144,214,215] Thus, the opposing functions of glucosidase II and glucosyltransferase provide the basis for the on-and-off cycle. It is clear that these interactions cannot persist indefinitely and it has been proposed that ER mannosidase I functions as a timer for the entry of a not correctly folded glycoprotein in the degradative pathway (Figure 8).[101,216−225] At this point, it should be emphasized that these aspects of the protein quality control appear to be conserved between yeast and mammalian cells and have been observed for various types of glycoproteins. So far, there is no evidence that ER mannosidase I discriminates between native and non-native protein configurations. Therefore, the specific $Man_8GlcNAc_2$ B isomer (Figure 7) produced by ER mannosidase I exists on transport-competent native and transport-

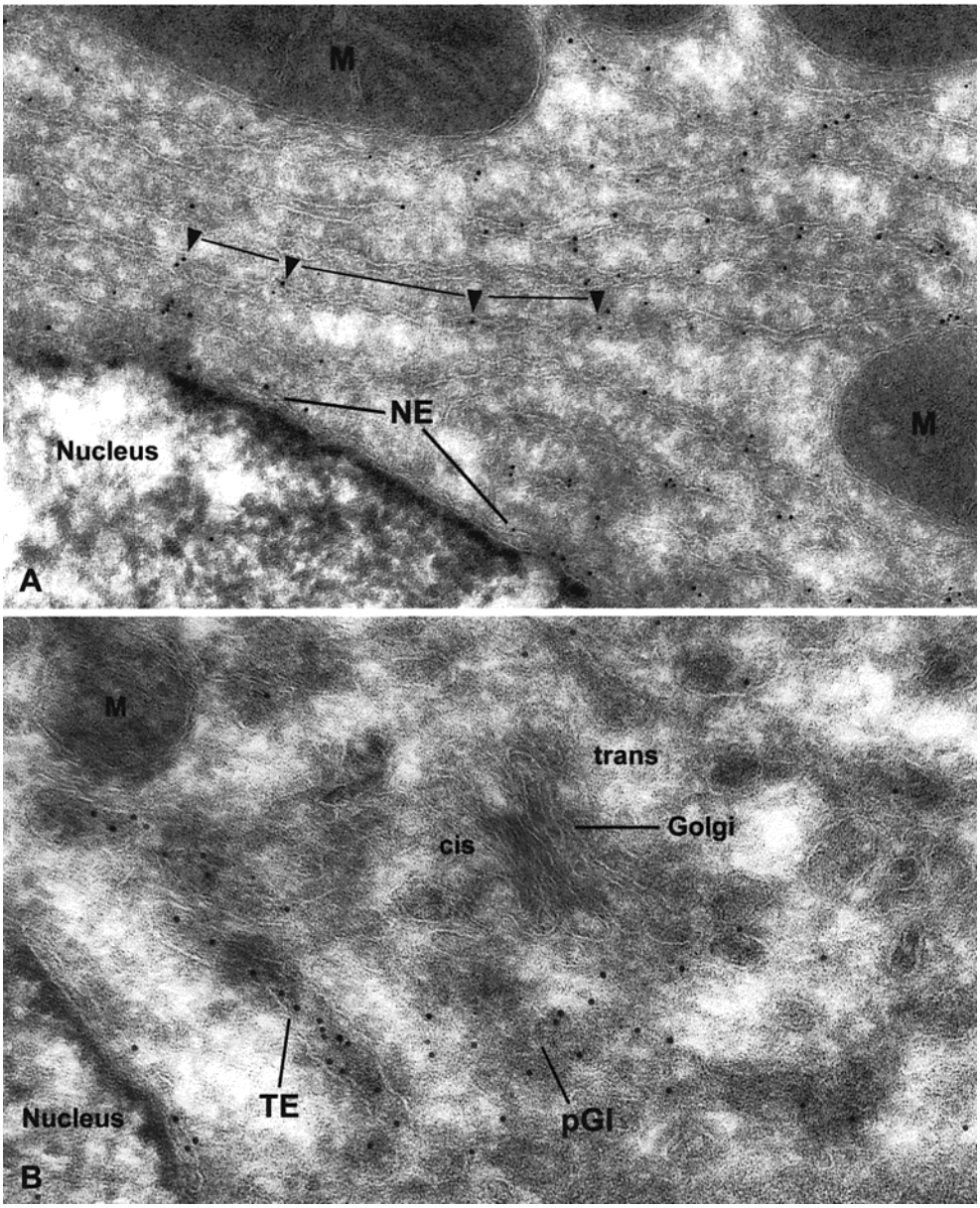

Figure 9. Immunogold localization of glucosyltransferase in the endoplasmic reticulum and pre-Golgi intermediates. (A) Rat liver. Gold particle labeling in a cisterna of the rough endoplasmic reticulum is indicated by arrowheads and unlabeled cisternal regions by the intervening lines. NE: labeling of the nuclear envelope. M: mitochondria. (B) Clone 9 rat hepatocyte cell line. Immunogold labeling for glucosyltransferase in nuclear envelope, transitional element of ER (TE) and pre-Golgi intermediate (pGI). The cisternal stack of the Golgi apparatus is unlabeled.

incompetent glycoproteins. Although a role of the specific $Man_8GlcNAc_2$ B isomer in routing glycoproteins to degradation had been demonstrated experimentally and in vivo and a specific lectin-like molecule recognizing and targeting misfolded glycoproteins to the degradation pathway had been postulated,[101] some evidence for a molecule fulfilling such a function was obtained only recently.[123−125] In yeast, the deletion of the *MNL1* gene encoding a mannosidase-like protein (Mnl1p) caused retardation of misfolded carboxypeptidase Y and other mutant glycoproteins but not of the unglycosylated form of the α-mating prehormone nor the nonglycosylated mutant Sec61-2p. Likewise, overexpression of the corresponding mouse gene (*Edem*) in HEK 293 cells resulted in acceleration of degradation of a mutant α1-antitrypsin. The mode of action of these noncatalytic, mannosidase-like proteins, in particular the way they distinguish the $Man_8GlcNAc_2$ isomer B bearing native from non-native glycoproteins remains elusive at present. Likewise, any possible interaction of these mannosidase-like proteins with other components of ERAD[189,226−235] that direct misfolded glycoproteins for retrotranslocation remains to be elucidated.

Some complementary results have been obtained by the immunoelectron microscopic localization of glucosidase II,[48,236] calreticulin,[48] and glucosyltransferase.[237] As one would expect, all are detectable in the rough ER, but a substantial fraction was also detected in the smooth ER, whose function in detoxification but not in quality control is well-established. Both glucosidase II and glucosyltransferase (Figure 9) were not homogeneously distributed in the rough ER with variously sized labeled and unlabeled cisternal regions alternating. Although this pattern was

observed for both enzymes, it rarely overlapped when double immunogold labeling was applied. This microdomain-like distribution pattern was interpreted as a meshwork of multiple checkpoints[237] engaged in controlling protein quality as originally proposed by Tatu and Helenius.[238] As it was already observed earlier for glucosidase II,[236] glucosyltransferase was also detectable in pre-Golgi intermediates (Figure 9) at labeling intensities 2-fold that of the rough ER.[237] The presence of glucosidase II and glucosyltransferase and of calreticulin in pre-Golgi intermediates indicates that this part of the secretory pathway in addition to the ER is involved in protein quality control. Additional support for an involvement of pre-Golgi intermediates in protein quality comes form electron microscopic studies showing accumulation of misfolded glycoproteins in what may be structurally altered, i.e., expanded, pre-Golgi intermediates.[239−241]

From the above it is evident that a specific oligosaccharidic structure is involved in directing glycoproteins for export out of the ER or for ERAD by the proteasome. This represents an important but certainly not the sole pathway for protein quality control. Since glucosyltransferase is not detectable in *S. cerevisiae* additional mechanisms must exist. It has been demonstrated that ERAD of misfolded carboxypeptidase in *S. cerevisiae* is glycosylation-dependent, since the nonglycosylated misfolded enzyme is retained but not degraded.[217] It has not been excluded that this may be related to the formation of Russel bodies. Furthermore, glycosylation-independent pathways for ERAD seem to exist,[101,217,242,243] although they remain to be elucidated. However, an important question remains to be investigated that relates to the degradation of naturally existing nonglycosylated proteins. That such a mechanism must exist has been indirectly demonstrated by studies on misfolded proinsulin[244] and may also apply to misfolded aquaporin 2 retained and degraded in the ER.[245]

3.2.2. Glucose Trimming beyond the Endoplasmic Reticulum

Traditionally, the ER has been considered as the only subcellular site of glucose trimming, although this additionally occurs in the pre-Golgi intermediates based on the immunoelectron microscopic localization of glucosidase II.[48,236] As described above (section 3.1.6), endomannosidase acts preferentially on monoglucosylated oligosaccharides, like glucosidase II. However, in strong contrast to glucosidase II, endomannosidase activity was found to be highly enriched in Golgi membrane fractions as compared to ER membrane fractions.[145] More recently, immunoelectron microscopy has precisely determined the subcellular distribution of endomannosidase.[48] By quantitative immunogold labeling, 84% of the immunolabeling was found in cis and medial Golgi apparatus cisternae, but none was detectable in the trans Golgi apparatus and the ER. Remarkably, a substantial fraction (15%) of the immunolabeling for endomannosidase was detectable in p58 positive pre-Golgi intermediates. Thus, endomannosidase and glucosidase II exhibit a mutually exclusive subcellular distribution. However, the preferential Golgi apparatus localization of endomannosidase demonstrated that glucose trimming also occurs in the Golgi apparatus in addition to ER and pre-Golgi intermediates. The question arises about the functional significance of endomannosidase-mediated deglucosylation that remains hypothetical at present. One possibility would be that glycoproteins bearing glucosylated oligosaccharides that have escaped the ER will become deglucosylated to ensure that processing to mature oligosaccharides in the Golgi apparatus can continue. When the relative glycosidase activity of glucosidase II and endomannosidase for various oligosaccharide substrates were compared, both enzymes exhibited the same relative activity for the $Glc_1Man_9GlcNAc$ substrate.[246] However, and in contrast to glucosidase II, the in vitro relative substrate activity of endomannosidase increased with progressive excision of mannose residues to be 2-fold higher for $Glc_1Man_5GlcNAc$ as for the $Glc_1Man_9GlcNAc$ substrate. How this differential substrate activity is related to the situation in vivo and which function it could have, if any, remains to be established. It is unknown if endomannosidase will trim oligosaccharides of correctly folded or misfolded glycoproteins or will be nondiscriminatory. It is of interest that endomannosidase was found to form complexes with calreticulin.[172] Subsequently, both endomannosidase and calreticulin were detected in the Golgi apparatus by immunoelectron microscopy.[48] However, only about 6% of the calreticulin immunolabeling was confined to the Golgi apparatus. This observation was interpreted to indicate that endomannosidase is involved in the dissociation of calreticulin−substrate complexes that have escaped the ER,[172] and this would permit the Golgi-associated oligosaccharide processing to take place.

4. Glycosylation in the Golgi Apparatus and Cell Surface Expression of Specific Glycotopes

With regard to protein glycosylation, the Golgi apparatus represents the organelle in which the synthesis of *O*-linked oligosaccharides commences[47,247,248] and trimming as well as elongation of *N*-linked oligosaccharides takes place.[78,249,250] The resulting *N*-linked oligosaccharides may exhibit enormous diversity, ranging from relatively simple biantennary to complicated tetraantennary structures (Figure 10). The functions of particular oligosaccharides present on proteins are quite diverse and encompass the modulation of the activity of cell surface receptors and hormones, aspects of the activation and function of the immune system, cell−cell and cell−substratum interactions of normal and tumor cells, as well as trafficking of lysosomes, to name only a few, and these functions may vary, depending on the cell type and on the degree of cellular differentiation.[3,251−263] Most of the involved glycosidases and glycosyltransferases have been characterized in great detail, as have been the pathways leading to the synthesis of the diverse oligosaccharides.[250,264−267]

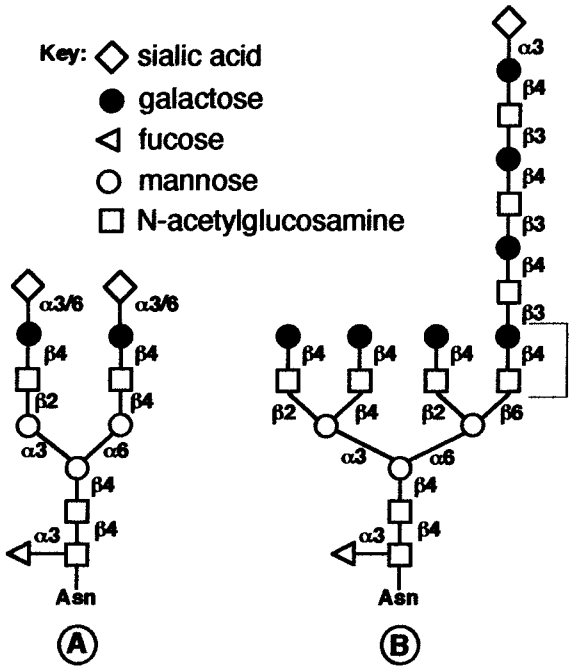

Figure 10. Trimming and elongation reactions in the Golgi apparatus result in complex-type asparagine-linked oligosaccharides. They may appear as simple biantennary oligosaccharide (A) or as complicated tetraantennary oligosaccharides (B). The latter contains the so-called β1,6-branch, which can be found in increased amounts in tumor cell lines and some malignant tumors. The α2,3-linked sialic acid of a tetraantennary oligosaccharide of the neural cell adhesion molecule may become elongated by a homopolymer of α2,8-linked sialic acid.

4.1. Topographical Aspects of Golgi-Associated *N*-Glycosylation: Compartmentation without Boundaries

Trimming reactions in the Golgi apparatus proceed through the action of endomannosidase (see above) and the Golgi mannosidases IA, IB, and II.[78,116] Related and depending on trimming, elongation by the families of *N*-acetylglucosaminyl-, fucosyl-, galactosyl-, and sialyltransferases takes place.[250,265,266,268−271] Studies on the distribution of these enzymes have provided a thorough picture of the relationship between the organization and the function of the Golgi apparatus. Although various classical histochemical stains provided information about subdivision in the Golgi apparatus, this could not be related to specific functions of this organelle. Since studies on the biosynthesis of *N*-linked oligosaccharides had shown that glycosidases and glycosyltransferases often act sequentially, the existence of glycosylation-related Golgi subcompartments was predicted. Refined subcellular fractionation techniques in conjunction with specific glycosyltransferase assays, as well as novel immunoelectron microscopic techniques in conjunction with specific antibodies, provided the kind of resolution required for such analysis. The first localization of a glycosyltransferase by immunoelectron microscopy showed that galactosyltransferase (UDP-Gal: β1,4GlcNAc galactosyltransferase) was restricted to two or three trans Golgi cisternae[272] (Figure 11), and this observation could be extended to other cell types[273,274] and

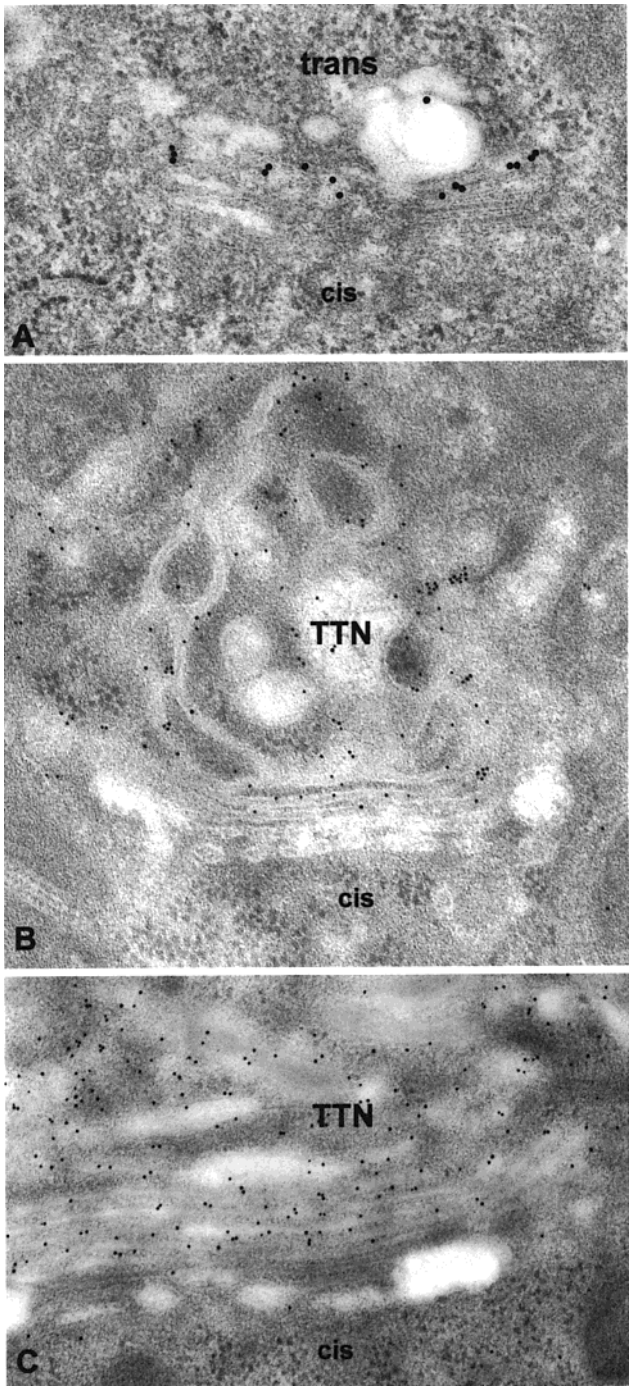

Figure 11. Immunogold localization of glycosyltransferases in the Golgi apparatus. (A) Immunolabeling for galactosyltransferase is confined to trans Golgi apparatus cisternae in a HeLa cell. (B) Sialyltransferase is detectable in trans cisternae and throughout the trans-tubular network of liver hepatocytes. (C) In contrast, sialyltransferase is distributed across the cisternal stack with the exception of a cis cisterna in absorptive enterocytes of rat colon.

matched by the detection of galactose residues with the *Ricinus communis* lectin.[275] Another late-acting glycosyltransferase, sialyltransferase (CMP-NANA: Galβ1,4GlcNAc: α2,6-sialyltransferase), was detectable in trans cisternae and throughout the trans-tubular network of the Golgi apparatus, and this coincided with the occurrence of sialic acid as detected by the *Limax flavus* lectin.[276] Furthermore, an earlier acting glycosyltransferase, *N*-acetylglucosami-

nyltransferase, was detected in medial Golgi cisternae.[277,278] Density gradient centrifugation of cellular membranes revealed galactosyl- and sialyltransferase in a dense fraction, separated from Golgi mannosidase I and II and *N*-acetylglucosaminyltransferase I and II, which were in a less dense membrane fraction.[279−281] Collectively, these data were interpreted to reflect the functional subdivision of the Golgi apparatus in cis, medial, and trans compartments. At that time, due to the limited availability of antibodies, double labeling for sequentially acting glycosyltransferases was not possible and their spatial relationship could not be directly studied by immunoelectron microscopy. However, with the cloning of glycosyltransferase genes and molecular tagging techniques available, two glycosyltransferases, β1,4-galactosyltransferase and *N*-acetylglucosaminyltransferase I, were shown to exhibit broad and partially overlapping distributions in shared trans Golgi cisternae,[282] which from a functional point of view made sense. The concept of distinct, nonetheless overlapping, distributions of glycosidases and glycosyltransferase could be extended to additional enzymes.[283] A detailed analysis of Golgi mannosidase I and II demonstrated their broad distribution in medial and trans Golgi cisternae.[284] As already mentioned above, endomannosidase also exhibited a broad cis/medial distribution in the Golgi apparatus.[48] Another level of specialization relates to observations of cell type-specific differential distribution of glycosyltransferases. Although α2,6-sialyltransferase was present in trans cisternae and the transtubular network in hepatocytes (Figure 11) and intestinal goblet cells, in absorptive enterocytes both the sialyltransferase and sialic acid residues were distributed in an increasing gradient from cis to trans Golgi cisternae[285] (Figure 11), and this could be confirmed for other cell types.[286] Cell-type-related differential distribution patterns were also observed for Golgi mannosidase I and II.[284] Altogether, these data provided evidence for the high degree of topographical specialization of glycosylation reactions in the Golgi apparatus consisting of groups of cisternae with overlapping distributions of enzymes. A third level of specialization is related to tissue specific expression patterns of Golgi mannosidases and glycosyltransferases. Although some of these enzymes show widespread tissue distributions as evidence for housekeeping function, others exhibit highly tissue specific expression patterns that emerge due to the action of specific promoter regions or the presence of multiple promoters.[268−270,287−296] Taking into account all this heterogeneity and specialization, starting at the molecular level and extending up to complex tissues, the observed diversity of asparagine-linked oligosaccharide structures on glycoproteins is not surprising. The following section will focus on functional implications as a consequence of the presence of particular oligosaccharides of cell surface glycoproteins.

4.2. Expression of Cell Surface Glycotopes during Development and in Disease

Numerous studies have shown that particular oligosaccharide structures may exhibit not only specific but also temporary expression patterns that are related to embryonic and fetal development and differentiation of cells and tissues to specific adult tissues and organs and to the malignant transformation of cells.[253,297−312] Despite a great deal of information about carcinoma-associated glycosylation changes, their importance for invasive and metastatic growth and as predictive prognostic markers could be revealed only in few instances, and such examples will be discussed below. It has also become clear that care must be taken in generalizing such observations, since they may apply only to specific types of malignant human tumors. Furthermore, attempts to use this information for therapy are often hampered by the fact that the underlying mechanism is not sufficiently understood. The family of congenital disorders of glycosylation will not be discussed here.[313]

4.2.1. β1,6-Branched Oligosaccharides, Tumor Progression, and Metastasis

A long known, commonly observed change following malignant transformation of cells consists of the formation of large complex-type asparagine-linked oligosaccharides.[314,315] Detailed analysis demonstrated this to be due to the increased synthesis of tri- and tetraantennary oligosaccharides,[316,317] specifically of *N*-acetylglucosamine β1,6-mannose branches (Figure 10). The enzymatic basis for the increased synthesis of the so-called β1,6-branches was shown to be increased levels of *N*-acetylglucosaminyltransferase V[316,318−320]. In their seminal paper, Dennis and co-workers[320] demonstrated that enhanced levels of β1,6-branched oligosaccharides due to elevated levels of *N*-acetylglucosaminyltransferase V were directly associated with the metastatic potential of rodent tumor cells, and this observation could be confirmed in subsequent studies.[321−331] When these observations from rodent and human cell lines and transgenic mice were extended to human tumors, clinically important conclusions could be drawn. In human breast and colon carcinoma, increased amounts of β1,6-branched oligosaccharides were histochemically detectable,[332,333] and in esophageal carcinomas, such structures were increased in regions of the carcinoma invading the surrounding normal tissue.[334] In a single study, histochemically detectable levels of β1,6-branched oligosaccharides were correlated to various pathohistological and clinical parameters of colorectal carcinoma.[335] Although β1,6-branched oligosaccharides were undetectable in normal colonic mucosa, colorectal carcinomas were positive (Figure 12). Importantly, this investigation established the value of β1,6-branched oligosaccharides as an independent prognostic indicator for tumor recurrence and patient survival and its association with the presence of lymph node metastasis. These findings were confirmed in an immunohistochemical study applying an antibody against *N*-acetylglucosaminyltransferase V.[336] However, it seems that these findings do not generally apply to human carcinomas, and this may be related to the fact that almost all epithelia in normal adult organs are histochemically positive for β1,6-branched oligosaccharides (Figure 12).[337] For example, all epithelia along the gastrointestinal tract were positive, with the exception of those of the

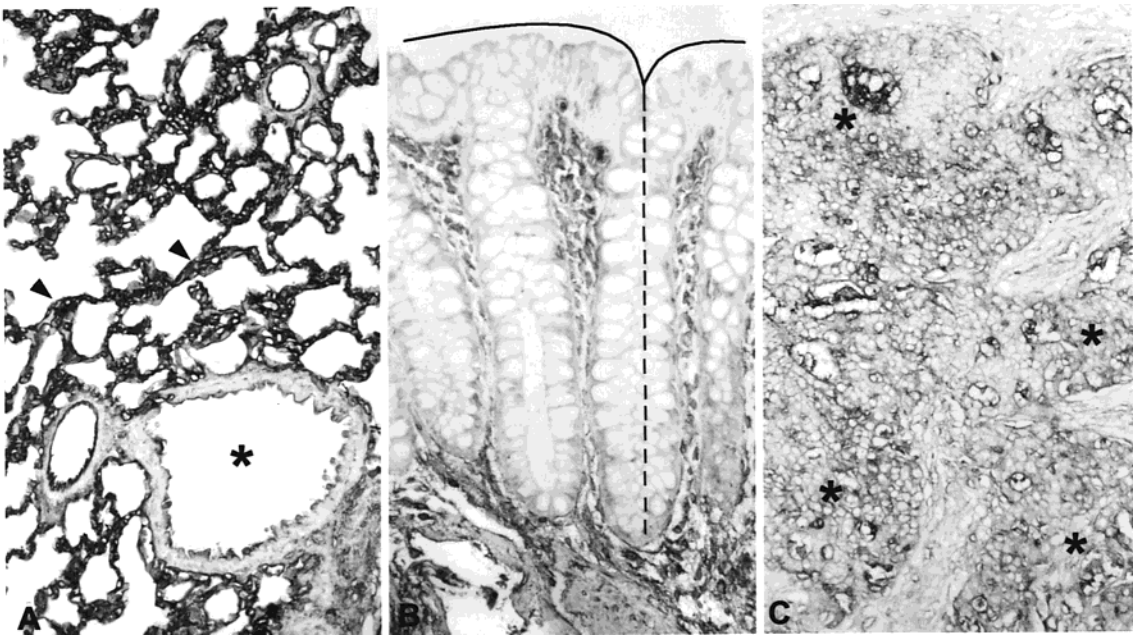

Figure 12. Histochemical demonstration of β1,6-branched oligosaccharides with the leukoagglutinating *Phaseolus vulgaris* lectin. (A) Intense lectin labeling is observed in lung tissue. Arrowheads point to positive alveolar septs, and the asterisks mark a positive small bronchus. (B) The epithelium of the normal human colon is unstained, which is in contrast to positively stained connective tissue elements. The differentiated surface epithelium is marked by the plain line and the crypt epithelium composed of immature and differentiating cells by the dashed line. (C) A colon carcinoma exhibits intense lectin staining (asterisks).

colonic mucosa. Furthermore, epithelia of nonlactating breast were unreactive, but studies in human breast carcinoma with regard to the prognostic value of β1,6-branched oligosaccharides produced contradictory results.[333,338] Studies performed on insulinomas[339] and melanomas[340] also failed to establish a correlation with malignancy and metastasis.

4.2.2. Sialic Acids and Colorectal Carcinoma

Sialic acids occur widely in nature as constituents of oligosaccharide side chains of glycoproteins and glycolipids and are involved in various biological phenomena.[341−343] The importance of sialic acid in general in determining the metastatic potential of human colon carcinoma cells is a long-known fact.[344−347] Studies on some specific sialoglycoconjugates have validated the concept of their carcinoma-associated expression. One is the Neu5Acα2,6GalNAc disaccharide, which represents the sialyl-Tn antigen.[348−353] Its prognostic importance in colorectal carcinomas could be demonstrated by immunohistochemistry using a monoclonal antibody. It should be mentioned that the sialyl Tn antigen exists in normal colonic epithelium, but with the sialic acid being *O*-acetylated[354−357] and thus not recognized by the monoclonal antibody TKH2, which is commonly used for the immunohistochemical detection of sialyl Tn antigen. Another reproducible finding in human colon carcinoma tissues consists of an enhanced activity of CMP-NeuAc: Galβ1,4GlcNAc: α2,6-sialyltransferase.[358−360] Since the sialyltransferase activity measurements are typically performed on tissue pieces, the contribution of the various cell types remains unclear. More detailed information could be obtained by the use of lectins reactive with sialic acids in specific linkage. It was observed that normal human colonic epithelium was strongly positive for α2,3-linked sialic acid[361,362] but unreactive for α2,6-linked sialic acid,[361] and this fact is illustrated in Figure 13. Subsequently it was shown that α2,6-linked sialic acid was detectable in human colon carcinoma (Figure 13).[361,363] The histochemical detection of α2,6-linked sialic acid may be useful for early diagnosis of colon cancer, since it can be found in severe dysplasia, which represents a noninvasive carcinoma in situ. In a multivariate regression study, the independent prognostic value of α2,6-linked sialic acid in predicting overall patient survival was established.[364] Currently, the basis for the importance of α2,6-linked sialic acid in colorectal carcinoma remains unclear, and the possible involvement of I-type lectins, so-called Siglecs, in cell−cell interactions related to tumor spread should be interesting to study.[365,366]

4.2.3. Polysialic Acid of the Neural Cell Adhesion Molecule

Although sialic acids are ubiquitous, this does not apply to a specific polymer formed by α2,8-linked sialic acid, which can be found as part of tri- and tetraantennary oligosaccharides.[367,368] Polymers of α2,8-linked *N*-acetylneuraminic acid are present on oligosaccharides of the neural cell adhesion molecule (NCAM)[367,369] and the sodium channel α-subunit in brain.[370] This unique structure has been shown to modulate cell−cell and cell−substratum interactions[305,371−373] and be involved in various aspects of brain development and neuronal functions in the adult.[374−384] Quite unexpected, polysialylated NCAM was detected in embryonic kidney[308,385] and also shown to be re-expressed in the Wilms tumor, an embryonic-type, highly malignant kidney tumor[386−388]

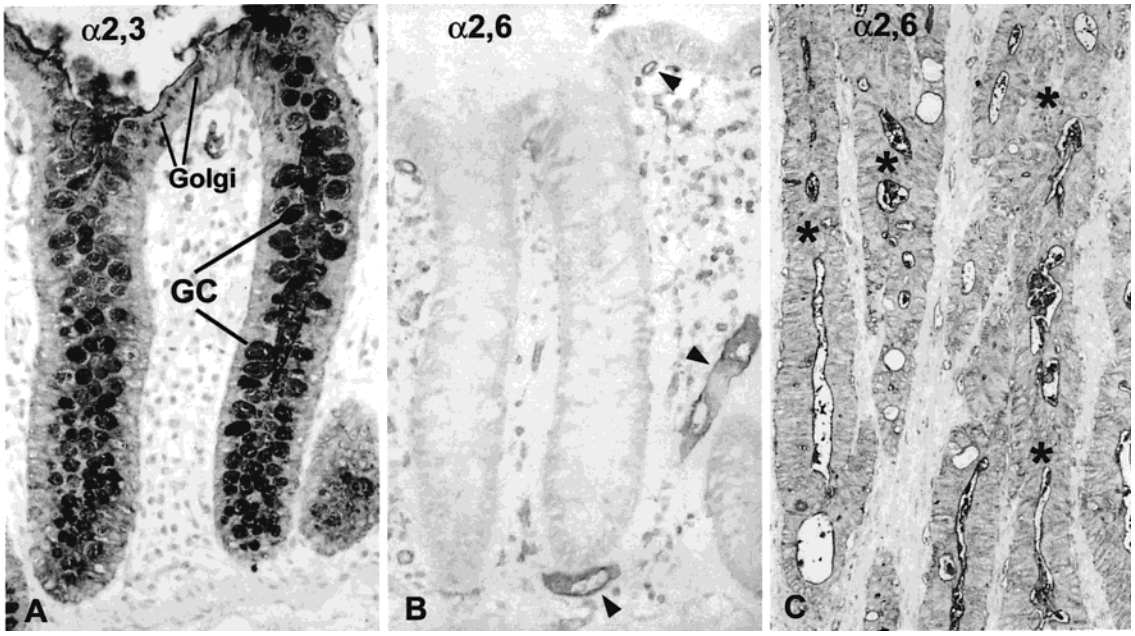

Figure 13. Demonstration of α2,3- and α2,6-linked sialic acid residues in human colon by lectin histochemistry. (A) Both the surface and crypt epithelium is positive for α2,3-linked sialic, as detected by the *Maackia amurensis* lectin. The positive staining of the Golgi apparatus of absorptive enterocytes can be clearly recognized. GC: positive goblet cell mucus. (B) In contrast, no staining for α2,6-linked sialic by the *Sambucus nigra* lectin I is detectable in surface and crypt epithelium, although endothelia of capillaries are positive (arrowheads). (C) A colon carcinoma exhibits intense staining for α2,6-linked sialic, as detected by the *Sambucus nigra* lectin I (asterisks).

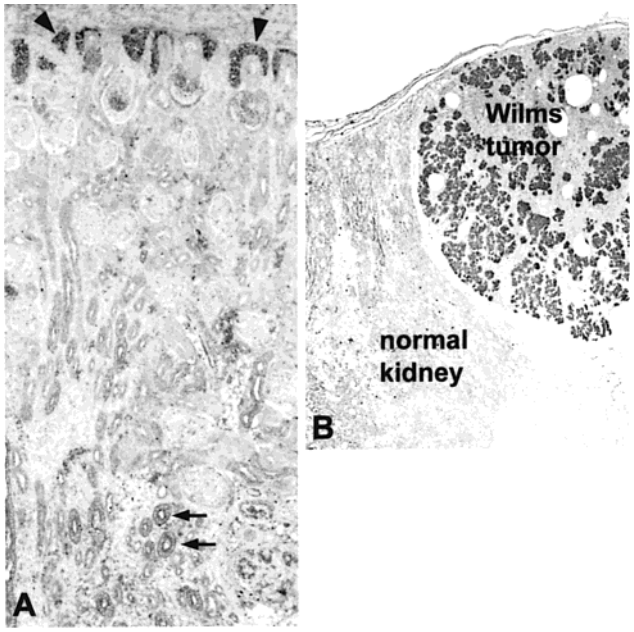

Figure 14. Immunohistochemical demonstration with a monoclonal antibody of polysialic acid of the neural cell adhesion molecule in human kidney. (A) Immature nephrons derived from the metanephrogenic mesenchyme (arrowheads) and immature collecting ducts derived from the ureter bud anlage (arrows) are positive. (B). Although normal adult kidney exhibits no immunostaining for polysialic acid, the Wilms tumor is strongly positive.

(Figure 14). This was of particular interest, because the presence of polysialic acid was known to modulate the adhesive properties of NCAM,[389−392] with the kinetics of adhesion being inversely correlated to the chain length of polysialic acid. The underlying mechanism of this effect was proposed to be the large excluded volume of this highly hydrated polyglycan.[393,394] We speculated that in malignant tumors, the presence of polysialylated NCAM would be of importance for the invasive and metastatic growth properties of tumor cells,[386,387] and this was confirmed by in vitro adhesion and invasion tests and in vivo experiments of metastasis formation.[395] An analysis of various types of malignant kidney tumors demonstrated that polysialylated NCAM was specific for Wilms tumor and therefore of importance for histopathological differential diagnosis.[396] This study also provided clear-cut evidence that a broad spectrum of human carcinomas did not express polysialic acid. However, it was not surprising that various malignant neuroendocrine tumors such as neuroblastoma, pheochromocytoma, small cell lung carcinoma, and medullary thyroid carcinoma were positive for polysialic acid.[397−403] But even in neuroendocrine tumors, this finding was not without exceptions. In the thyroid gland, medullary but no other type of carcinoma expressed polysialic acid, in the adrenal gland, pheochromocytoma but not adrenocortical carcinoma, and in lung small cell carcinoma but not so-called carcinoid. In benign and malignant insulinoma, polysialic acid was undetectable, despite the presence of NCAM.[339]

Clinical studies subsequently revealed the importance of monitoring polysialic acid for neuroblastoma, since its serum levels were dramatically elevated in children with advanced stages and fatal courses of disease, whereas children with differentiated tumor types and limited disease had low or normal levels. Furthermore, serum concentrations correlated with the polysialylated NCAM content of the tumors, and they decreased during successful therapy.[404] Similar observations were reported for rhabdomyosarcoma, another malignant childhood tumor.[405] In nonsmall

lung carcinoma, levels of polysialic acid expression correlated with tumor stage as well as the presence of lymph node and distant metastasis.[406] High expression levels of polysialic acid were correlated with a low 5-year survival rate, and multivariate analysis demonstrated polysialic acid to be an independent factor to predict poor prognosis in nonsmall cell lung carcinoma.[407] Collectively, these results suggested that polysialic acid represents a useful clinical marker for some malignant tumors.

5. Conclusions

Research in protein glycosylation has dramatically changed from a descriptive and structurally oriented discipline to a biological and application-oriented one. This is well-illustrated by the progress made in understanding the trimming reactions occurring on oligosaccharides in the ER in terms of their importance for protein quality control and of cargo traffic between the ER and the Golgi apparatus in health and disease. Despite this success, many aspects at the molecular level remain to be elucidated in order to fully understand this process and to apply this information for corrective intervention in human disease due to protein misfolding. Although oligosaccharide-mediated aspects of protein quality control have become more clear, the possible involvement of pre-Golgi intermediates and of the Golgi apparatus deserves attention. Likewise, the mechanism of degradation of misfolded but nonglycosylated proteins remains to be explored. Studies on trimming and elongation reactions on oligosaccharides during their stay in the Golgi apparatus have greatly contributed to our knowledge about spatial division of labor in a highly dynamic organelle of enormous structural complexity, and this is of general interest for cell and molecular biologists. A most exciting field has been and continues to be the elucidation in functional terms of the role of specific oligosaccharides on glycoproteins of the cell surface, since this is the interface that plays a fundamental role in cell−cell and cell−substratum interaction not only during ontogenesis and in the adult but also in various diseased states including cancer. It has become obvious that glycosylation changes following malignant transformation of cells found in experimental settings can also be detected in malignant human tumors. However, it is important to realize that even most commonly observed changes, such as increased synthesis of β1,6-branched oligosaccharides, are not generally occurring in the different types of malignant human tumors, and this may be related to tissue- and cell-type specific glycosylation patterns.

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