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Review

Role of *N*-linked polymannose oligosaccharides in targeting glycoproteins for endoplasmic reticulum-associated degradation

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Abstract. Misfolded or incompletely assembled multisubunit glycoproteins undergo endoplasmic reticulum-associated degradation (ERAD) regulated in large measure by their *N*-linked polymannose oligosaccharides. In this quality control system lectin interaction with $Glc_3Man_9GlcNAc_2$ glycans after trimming with endoplasmic reticulum (ER) α-glucosidases and α-mannosidases sorts out persistently unfolded glycoproteins for *N*-deglycosylation and proteolytic degradation. Monoglucosylated ($Glc_1Man_9GlcNAc_2$) glycoproteins take part in the calnexin/calreticulin glucosylation-deglucosylation cycle, while the $Man_8GlcNAc_2$ isomer B product of ER mannosidase I interacts with EDEM. Proteasomal degradation requires retrotranslocation into the cytosol through a Sec61 channel and deglycosylation by peptide:*N*-glycosidase (PNGase); in alternate models both PNGase and proteasomes may be either free in the cytosol or ER membrane-imbedded/attached. Numerous proteins appear to undergo nonproteasomal degradation in which deglycosylation and proteolysis take place in the ER lumen. The released free oligosaccharides (OS) are transported to the cytosol as OS-$GlcNAc_2$ along with similar components produced by the hydrolytic action of the oligosaccharyltransferase, where they together with OS from the proteasomal pathway are trimmed to $Man_5GlcNAc_1$ by the action of cytosolic endo-β-*N*-acetylglucosaminidase and α-mannosidase before entering the lysosomes. Some misfolded glycoproteins can recycle between the ER, intermediate and Golgi compartments, where they are further processed before ERAD. Moreover, properly folded glycoproteins with mannose-trimmed glycans can be deglucosylated in the Golgi by endomannosidase, thereby releasing calreticulin and permitting formation of complex OS. A number of regulatory controls have been described, including the glucosidase-glucosyltransferase shuttle, which controls the level of $Glc_3Man_9GlcNAc_2$-P-P-Dol, and the unfolded protein response, which enhances synthesis of components of the quality control system.

Key words. Protein quality control; free polymannose oligosaccharides; *N*-glycanase; endomannosidase; protein folding; ER mannosidases; lectin chaperones; protein retrotranslocation.

Introduction

It has become evident in recent years that the highly sophisticated processing that *N*-linked oligosaccharides of proteins undergo shortly after their cotranslational attachment as $Glc_3Man_9GlcNAc_2$ units is intimately involved in the molecular inspection known as quality control [1–5]. This latter process selects misfolded or incompletely assembled multisubunit proteins for endoplasmic reticulum-associated degradation (ERAD) and is believed to be a characteristic of all eukaryotic cells [6–10]. The early retention and subsequent destruction of abnormal proteins is a major protective mechanism of cells, as it prevents the passage of defective molecules

through the secretory channels or their insertion into membranes. Enhanced proteolytic degradation has moreover been found to serve in the regulation of metabolic pathways which are prone to wide physiological fluctuations [11–13] as well as in a number of pathological states [14], including cystic fibrosis [15], α_1-antitrypsin deficiency [16] and congenital goiter [17], in which mutated proteins are produced; furthermore certain viruses evade cellular immune defenses by promoting degradation of host components [18].

Studies from a number of investigators have indicated that the processing intermediates of *N*-linked oligosaccharides produced by the α-glucosidases and α-mannosidases of the endoplasmic reticulum (ER) and pre- or cis-Golgi compartments [1–5, 19–22] provide specific retention or recycling signals. Thus, for the first time a biological rationale has been given to the now well delineated processing that the newly attached glucosylated polymannose oligosaccharides undergo on their way to becoming mature *N*-linked units, which is a strikingly different approach from the simple sequential step-by-step sugar attachment which prevails in the biosynthesis of *O*-linked oligosaccharides [23]. In particular it is now evident that the glucosyl cap of the newly formed branched polymannose glycan is essential not only for the initial *N*-glycosylation but also for quality control [3].

Since the first report that quality control of proteins is brought about by proteolysis in the ER [6], it has become clear that in many well-defined cases this is accomplished by the ubiquitin-proteasome system after retrotranslocation of the protein destined for degradation through a Sec61 channel to the cytosolic side of the ER membrane [8–10, 24–26]; however, an increasing number of instances have also been reported in which ERAD occurs without involving the proteasomes [12, 27–33.]. At some stage of ERAD, deglycosylation through the action of a peptide:*N*-glycanase (PNGase) occurs with the release of free oligosaccharides terminating at their reducing end in a di-*N*-acetylchitobiose moiety (OS-GlcNAc_2) [34, 35]. Proteins subject to proteasome action are believed to undergo oligosaccharide excision prior to peptide degradation [36, 37]. Indeed, the release of substantial amounts of such glucosylated and unglucosylated polymannose oligosaccharides was observed in vivo and in cell-free systems before the concept of ERAD was formulated [34]. Subsequent studies indicated that these oligosaccharides, which are released shortly after cotranslational *N*-glycosylation, are in fact to a large extent the anticipated by-products of ERAD [35], and their disposal, constituting a major metabolic route of the cytosol, has been described [35, 38].

While the subject of protein quality control presents a very large field of active research, it is the purpose of this review to focus on the continually expanding role which the *N*-linked oligosaccharides play in ERAD, with particular emphasis on how they serve to target aberrant proteins for retention and degradation through their interaction with lectin-like proteins. The subcellular sites of oligosaccharide release will be evaluated and put in context with other aspects of the ERAD machinery. The major disposal challenge which the release of oligosaccharides imposes on the metabolic machinery of the cell will be delineated, and the role of the unfolded protein response (UPR) on genes encoding glycan-binding and processing proteins involved in the quality control system will be indicated.

N-glycosylation of proteins and early processing steps of Asn-linked oligosaccharides

An understanding of the modifications which the $Glc_3Man_9GlcNAc_2$ glycans undergo shortly after their cotranslational attachment by the oligosaccharyltransferase [39, 40] to asparagine residues in the Asn-X-Ser(Thr) consensus sequence on polypeptide chains [23] is important in evaluating the role which these *N*-linked intermediates play in ERAD (fig. 1). The pertinent glycosidases which initiate the processing of the triglucosyl polymannose oligosaccharides have now been studied in some detail and are believed to be situated in the ER and the pre-Golgi intermediate compartments (IC); since it has been reported that ER-Golgi cycling can take place before ERAD, the actions of Golgi trimming enzymes may also be relevant. Various glycosidase inhibitors have been effectively employed in exploring the involvement of processing intermediates in the ERAD system (fig. 1).

Glucosidase I, an integral membrane enzyme with a luminally oriented catalytic domain, rapidly acts to specifically remove the terminal α1-2-linked residue of the triglucosyl sequence, which in almost all eukaryotes is essential for the effective action of the oligosaccharyltransferase. The release of the remaining glucose components is effected by glucosidase II, which in cleaving the Glc remove1-3Glc and Glc remove1-3Man linkages shows a dual specificity that has been attributed to two distinct active sites [21]. This glycosidase is a soluble luminal enzyme which is primarily located in the ER. Glucosidase II acts optimally on oligosaccharides with intact mannose branches but manifests substantially reduced activity on substrates with trimmed mannose branches [41]. Both glucosidases can be effectively inhibited by castanospermine (CST) so that in the presence of this agent the triglucosyl cap remains intact even if trimming of the unglucosylated B and C branches has occurred. Glucose removal can also be achieved by endomannosidase, a processing enzyme that uniquely brings about an internal cleavage between the glucose-substituted mannose and the remainder of branch A [42, 43] (fig. 1). While this enzyme acts optimally on monoglucosylated

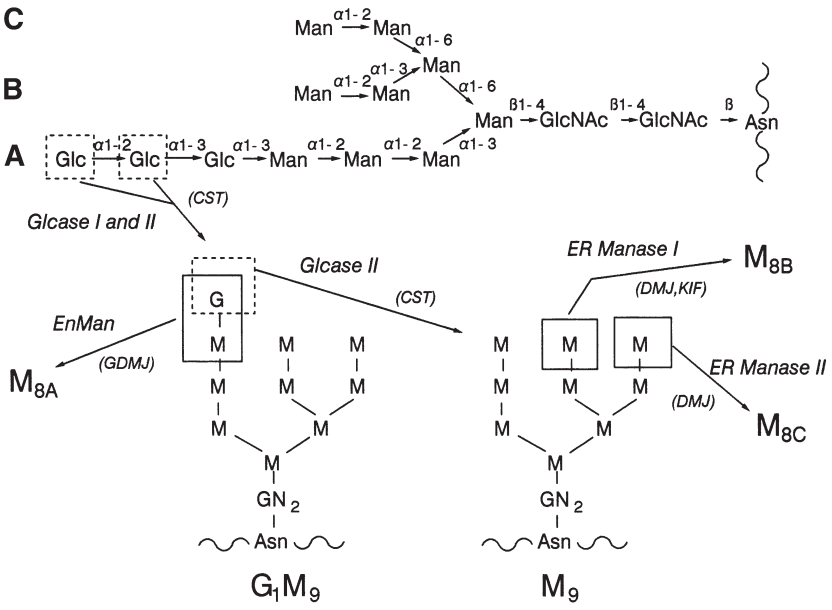

Figure 1. Diagrammatic representation of the early processing of the triglucosylated polymannose oligosaccharides subsequent to their *N*-glycosidic attachment to asparagine residues of proteins. The action of glucosidases (*Glcase*), mannosidases (*ER Manase*) and endomannosidase (*EnMan*) are shown leading to the formation of $Glc_1Man_9GlcNAc_2$ (G_1M_9), $Man_9GlcNAc_2$ (M_9) and the three distinct isomers of $Man_8GlcNAc_2$ (M_{8A}, M_{8B} and M_{8C}). Inhibitors that block these enzymatic steps are shown in parentheses. The three branches of the oligosaccharide are designated by the letters *A*, *B* and *C*, and, accordingly, the $Man_8GlcNAc_2$ isomers resulting from the removal of the terminal mannose residue from these chains are identified by the appropriate subscript.

oligosaccharides, with the release of Glc remove1-3Man it can also act on the di- and triglucosylated polymannose species with the release of di- and triglucosylated mannose, respectively [43]. In pronounced contrast to glucosidase II, the endomannosidase acts most favorably on oligosaccharides with truncated mannose chains down to $Glc_1Man_5GlcNAc$ [43] (table 1). Although the endomannosidase has been found to be predominantly a Golgi enzyme [42], immunoelectron microscopic examination has localized this endoglycosidase to the IC in addition to the cis/medial Golgi cisternae [44]. In the pre-Golgi site it appears to be spatially situated apart from glucosidase II. In contrast to the wide phylogenetic distribution of the glucosidases in eukaryotic cells, the endomannosidase is primarily found among members of the phylum *Chordata* [45]. Endomannosidase offers a route of deglucosylation alternative to glucosidases I and II [46], which insures that the biologically important complex oligosaccharides of higher organisms can be formed. This enzyme is not inhibited by CST or mannosidase inhibitors [43], although Glcα1-3-deoxymannojirimycin (GDMJ) and related Glcα1-3 derivatives block its activity [47].

Mannosidase trimming in mammalian cells is initiated in the ER by two distinct enzymes, ER α1-2 mannosidases I and II [48, 49]. The primary action of the former enzyme is to remove the terminal sugar of the middle branch to generate the $Man_8GlcNAc_2$ isomer B, while mannosidase II, which is related to the cytosolic enzyme, preferentially releases the terminal mannose of the α1-6-linked branch to yield $Man_8GlcNAc_2$ isomer C (fig. 1). Although both enzymes can in vitro trim other mannose residues to yield more extensively processed products, their major action in vivo appears to be limited to the removal of a single specific residue. The two enzymes have many distinguishing properties [49], but most relevant is

Table 1 Specificities of several components of the quality control system toward *N*-linked oligosaccharides*.

Oligosaccharide	Relative activity			
	Glucosidase II	Glucosyltransferase	Endomannosidase	Calreticulin
$Glc_1Man_9GlcNAc$	100	100	100	100
$Glc_1Man_8GlcNAc$	21	50	120	81
$Glc_1Man_7GlcNAc$	9	15	150	64
$Glc_1Man_5GlcNAc$	3	–	220	62

*Data are taken from studies on rat liver, as indicated in the text.

the fact that ER mannosidase II is resistant to kifunensine (KIF) inhibition; both enzymes are, however, inhibited by 1-deoxymannojirimycin (DMJ) (fig. 1). A third α1-2 mannosidase that may be involved in early processing is Man_9 mannosidase, which can trim Man_9 to Man_6 and eventually Man_5 [50]. This enzyme appears to be present in the ER and Golgi, perhaps due to cycling between these compartments; its activity is inhibited by both KIF and DMJ. At least two α-mannosidases situated in the Golgi apparatus have the potential to cleave all four α1-2-linked mannose residues to yield $Man_5GlcNAc_2$, and they are inhibited by DMJ as well as KIF [19]. Subsequently, attachment of a GlcNAc residue to the A branch makes possible the cleavage of the α1-3 and α1-6 mannose residues by Golgi mannosidase II to yield a core unit with three mannose residues [19]. The action of this latter mannosidase does not appear to be pertinent to ERAD.

In contrast to mammalian cells, yeast have only one ER α-mannosidase. This enzyme is similar to the ER mannosidase I of higher eukaryotes and acts specifically to remove one mannose residue from the B chain [51]; further trimming of the *N*-linked polymannose glycans does not occur in yeast before the mature carbohydrate unit is built up by multiple additions of single mannose residues.

Interaction of glucosylated polymannose processing intermediates with lectin chaperones

The first suggestion that the glucose residues on the nascent *N*-linked glycans play a role in the quality control system came from experiments which indicated that blockage of glucose excision by inhibitors so as to retain the triglucosyl cap intact resulted in an accelerated degradation of glycoproteins [52–54]. These observations were clarified by the finding that the chaperones calnexin and calreticulin acting as lectins specifically bind *N*-linked monoglucosylated oligosaccharides in a large array of glycoproteins [55]. Binding studies with calnexin [56] as well as calreticulin [57] indicated that this interaction did not occur with di- and triglucosylated polymannose oligosaccharides, and it was further shown that even after extensive trimming of the 6′-pentamannosyl portion (branches B and C), the chaperone binding activity was retained [57] (table 1). However, excision of the α1-6 mannose residue from $Glc_1Man_5GlcNAc_2$ resulted a complete loss of reactivity [57]. The two chaperones have been observed in a variety of eukaryotic cells, although in *Saccharomyces cerevisiae* a gene that expresses a homologue of these two proteins is believed to be present [58]. While calnexin is located exclusively as an integral ER membrane protein, calreticulin is a soluble chaperone [55], which in addition to the ER has also been observed in the IC and the Golgi apparatus [44, 57]. The two lectins appear to interact in vivo with a different set of glycoproteins [55]. This may be attributed to the possibility that oligosaccharides located in the proximity of the ER membrane would more readily bind to the membrane-bound calnexin, while more distant luminally oriented glycans would preferentially bind to the soluble calreticulin.

While the importance of the ER in promoting the folding and oligomerization of newly synthesized proteins through the action of a number of chaperones and the establishment of an oxidizing environment had been well established [59], it became apparent from the unique affinity of calnexin and calreticulin for monoglucosylated oligosaccharides [55–57] and the discovery of the UDP-Glc:glycoprotein glucosyltransferase [60] that *N*-linked glycans can play a major role in those events which underlie the quality control of proteins. Since the glucosyltransferase can reglucosylate $Man_9GlcNAc_2$ glycans only when linked to misfolded proteins, it provides the key component of a sensing apparatus that has the capacity to retain aberrant molecules and ultimately direct them to degradation. A cyclic model has been formulated [1] in which this enzyme acts in concert with glucosidase II and calreticulin/calnexin (fig. 2). The terminal mannose of the A branch of $Man_9GlcNAc_2$ oligosaccharides on the incompletely folded protein is sequentially glucosylated and deglucosylated, permitting it alternatively to bind to and dissociate from the lectin chaperones until the proper conformation has been obtained. It is only when that stage has been reached that reglucosylation will no longer occur that the now native protein can proceed in its migration into the secretory channels and thereby escape a degradative fate. Because it is known that the glycoprotein glucosyltransferase [61], like glucosidase II, acts poorly on trimmed monoglucosylated *N*-linked glycans (table 1), the cyclic model would not be effective if excision of mannose residues from branches B and/or C through the action of the ER mannosidases I and II had occurred. Indeed, in view of recent evidence, to be discussed below, that cycling between ER and Golgi takes place as part of the quality control of certain proteins, the action of mannosidases in the latter compartment or the IC may also trim the monoglucosylated glycans. To circumvent this difficulty, it has been hypothesized [3, 57] that processed monoglucosyl-containing glycoproteins still attached to the soluble calreticulin, which can effectively bind oligosaccharides down to $Glc_1Man_5GlcNAc_2$ [57], would be dissociated in post-ER locations by endomannosidase, which like calreticulin interacts well with such trimmed glycans (table 1). The finding that endomannosidase and calreticulin both occur in the Golgi in similar amounts [57, 62] and have been colocalized in the IC [44] is consistent with such a proposal.

While the absence of the glycoprotein glucosyltransferase in *S. cerevisiae* [63, 64] would suggest that the

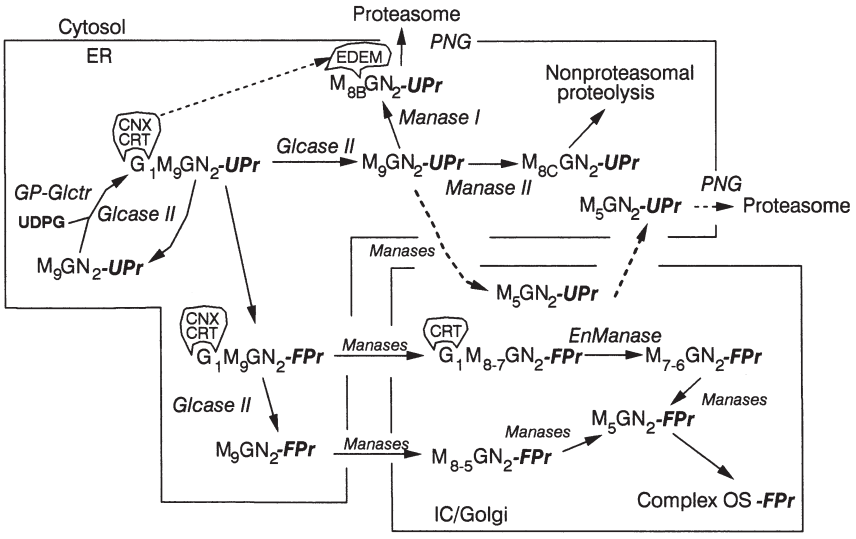

Figure 2. Schematic representation of the role of *N*-linked oligosaccharides in regulating protein folding or degradation during quality control. The monoglucosylated ($G_1M_9GN_2$) glycan linked to an unfolded protein (*UPr*) interacts with calnexin (CNX) or calreticulin (CRT), and this complex participates in a cycle involving glucose attachment and release catalyzed by glycoprotein glucosyltransferase (*GP-Glctr*) and glucosidase II (*Glcase II*), which promotes formation of a folded protein (*FPr*) containing an M_9GN_2 oligosaccharide. This glycoprotein then undergoes trimming by ER, IC and Golgi mannosidases (*Manases*) and ultimately is converted to a protein containing the mature complex oligosaccharides (Complex OS-*FPr*) by Golgi enzymes. If the $G_1M_9GN_2$ glycan still attached to luminal CRT is trimmed by ER mannosidases to truncated monoglucosylated glycans which are resistant to glucosidase II action, an alternate deglucosylation is brought about by Golgi endomannosidase (*EnManase*) which then permits formation of complex carbohydrate units. If folding of the glycoprotein does not occur or is extended over a long time period, a terminal mannose can be released through the action of ER mannosidase I (*Manase I*) or mannosidase II (*Manase II*), leading to formation of $M_{8B}GN_2$ or $M_{8C}GN_2$, respectively. The former ($M_{8B}GN_2$-*UPr*) then interacts with the EDEM lectin followed by PNGase (*PNG*)-catalyzed deglycosylation and proteolysis in proteasomes, while the latter ($M_{8C}GN_2$-*UPr*) undergoes nonproteasomal proteolysis. It is believed that the membrane-associated CNX attached to the unfolded protein can interact with EDEM, as indicated by the dashed arrow. Recycling of unfolded glycoproteins between the ER and post-ER compartments can lead to degradation of proteins with extensively trimmed glycans, as indicated by the dashed arrows. Abbreviations for the monosaccharides are Glc (G), Man (M) and GlcNAc (GN).

deglucosylation-glucosylation cycle may not exist in this organism, it has been reported that the glucosidase II in this yeast strain may take on a chaperone function by itself and discriminate between folded and unfolded proteins [64].

Involvement of a $Man_8GlcNAc_2$-specific lectin in ER quality control of glycoproteins

Evidence that trimming of mannose residues is involved in facilitating ERAD was first obtained by the observation that degradation of yeast prepro-α-factor expressed in mammalian cells treated with the mannosidase inhibitor DMJ was markedly inhibited, while in contrast, as already indicated, inhibition of ER glucosidases considerably enhanced its destruction [65]. Subsequently mannosidase inhibitors have been found to arrest glycoprotein degradation in a number of mammalian cells and yeast [30, 66–70]. The studies employing inhibition by KIF in mammalian cells, which contain two distinct ER mannose-trimming enzymes, provided more precise information than those which used exclusively DMJ, as the former agent is known to block the action of ER mannosidase I but not ER mannosidase II [48, 49]. Elegant studies carried out with the misfolded mutants of human α_1-antitrypsin and yeast carboxypeptidase Y (CPY*) in the laboratories of Sifers [4] and Aebi [71], respectively, implicated the $Man_8GlcNAc_2$ isomer B, which in mammalian cells is formed by ER mannosidase I action, as the signal that is involved in this mannosidase-promoted degradation (fig. 2). Recent studies in mammalian cells have shown that ERAD is enhanced by overexpression of this enzyme [69]. In an attempt to place these findings into a conceptual framework of ERAD control, it has been proposed that the action of the ER mannosidase I acts as a 'timer' of ER glycoprotein residence [4]; in this model the slow in vivo action of the ER mannosidase I would select proteins which spend an inordinate amount of time in the ER, presumably due to incomplete folding. The above-mentioned studies led to the postulation of a $Man_8GlcNAc_2$ isomer B-specific lectin that could in turn transmit the message to the remainder of the ERAD apparatus (fig. 2). Indeed, a recent study identified in the mouse [72] and *S. cerevisiae* [73, 74] such a protein that is a mannosidase I homologue but does not have glycosidase activity. Overexpression of this receptor, which has been called EDEM and Mnl1/Htm1 in mouse and yeast,

respectively, has been shown to accelerate the degradation of misfolded proteins [75, 76], while the absence of the receptor in yeast was found to stabilize such proteins [73]. In contrast to the monoglucosyl polymannose-specific chaperones, the lectin directed toward the Man_{8B} oligosaccharide has not yet been found to have the ability to recognize misfolded proteins and thereby distinguish them from molecules with a native configuration that also contain such *N*-linked glycans. However, it has been reported that EDEM reacts with the transmembrane region of calnexin, and it has been postulated that the mannose-specific lectin accepts substrates from this monoglucosyl polymannose-specific lectin [75, 76] (fig. 2); overexpression of EDEM resulted in an enhanced release of misfolded proteins from the calnexin cycle with subsequent degradation [75, 76]. Binding of EDEM to calreticulin was not observed, and this has been attributed to the fact that this chaperone, contrary to calnexin, does not contain a transmembrane region [75]. If there is a transfer of misfolded glycoproteins from calnexin to EDEM, one might anticipate that the latter lectin would bind $Glc_1Man_8GlcNAc_2$; even though this has not been directly tested, it is known that ER mannosidase I can act on $Glc_1Man_9GlcNAc_2$ in mammalian cells [48] and yeast [71], and it has been reported that proteins from the last-mentioned cells containing the monoglucosylated product ($Glc_1Man_8GlcNAc_2$) undergo quite rapid degradation, in marked contrast to those which have the *N*-linked diglucosylated isomer [71]. At this juncture it is not known whether the cycle involving calnexin acts in tandem with EDEM/Mnl1(Htm1) in designating proteins for ERAD or whether they represent alternate routes. In favor of the latter concept may be the finding that *S. cerevisiae,* while possessing a Man_{8B} detection system [73, 74], lacks the glycoprotein glucosyltransferase [63, 64]. Studies with a mutant thyroglobulin may also be relevant to the state of glucosylation, as it was shown that the extent of degradation of this protein in the presence of KIF was not affected by the further addition of CST [67].

Involvement of the Golgi apparatus and other post-ER compartments in quality control

Before discussing the *N*-deglycosylation and proteolysis which are the ultimate events of the quality control system, the results of a number of recent studies will be presented which suggest that movement of misfolded and unassembled proteins between the ER and the Golgi as well as IC compartments takes place prior to ERAD [77–83]. The KDEL sequence has been shown to mediate the retrieval mechanism operative in ERAD [84], and the mannose-specific ERGIC-53 lectin [85] has been implicated in the transport of glycoproteins to the IC.

If recycling of glycoproteins into post-ER compartments does take place, it would have the potential of exposing the *N*-glycans of misfolded proteins to an array of glycosidases which can carry out processing reactions well beyond those which can occur in the ER (fig. 2). Recent reports have shown that proteins destined for degradation can be trimmed down to $Man_{5\text{-}7}GlcNAc_2$-*N*-linked glycans [69, 70, 86], and moreover it was observed that in a misfolded ribophorin I variant, $Man_5GlcNAc_2$ glycans can be converted to $Man_{3\text{-}4}GlcNAc_2$ prior to proteasomal degradation [87]. Studies from the laboratory of Lederkremer have identified a 'quality control' compartment, distinct from the IC and Golgi, which functions as a gateway to proteasomal degradation [88]. In any case it is evident that misfolded proteins trafficking through Golgi and early post-ER compartments could be cleaved by Golgi mannosidases Ia and Ib as well as by the M_9 mannosidase with its broad specificity [19, 50, 89] (fig. 2). The movement of the misfolded glycoproteins into the IC and Golgi compartments would delegate their deglucosylation and dissociation from calreticulin to the action of endomannosidase [3] for the reasons already discussed (fig. 2). Examination of the ts045 G protein of vesicular stomatitis virus at nonpermissive temperature, at which it has been shown to recycle between the ER and cis-Golgi compartments [77], indicated that it can be deglucosylated by endomannosidase although remaining endo H sensitive [32]. Since endomannosidase cleaves the linkage between the glucosylated mannose residue and the remainder of branch A (fig. 1), reglucosylation cannot take place. In any event, even though calreticulin binds monoglucosylated mannose-trimmed glycans effectively [57], the UDP-glucose:glycoprotein glucosyltransferase [61] and glucosidase II [41] do not act well on these truncated polymannose oligosaccharides (table 1). Identification of extensively trimmed oligosaccharides on ERAD-destined proteins has been limited to mammalian systems, as in yeast only the terminal mannose of the B-chain is excised before the mature *N*-linked polymannose glycans are formed [51]. Although ERAD involving signalling by oligosaccharides has been investigated for a large array of glycoproteins, including the mutants of α_1-antitrypsin, yeast carboxypeptidase Y, and thyroglobulin, as well as yeast prepro-α-factor and unassembled immunoglobulin subunits and major histocompatibility complex (MHC) class I heavy chains, as indicated in the review of Cabral et al. [4], the quality control of nonglycosylated proteins must be mediated by nonlectin chaperones through protein-protein interactions. Indeed, it has been postulated that even in the case of glycoproteins, the lectin binding may be followed by polypeptide interactions [90].

It is apparent from the information presented so far that *N*-linked polymannose oligosaccharides play a major role in directing the quality control system, and accordingly there are a large number of proteins in the form of en-

zymes and lectins, distributed among several subcellular compartments, which interact directly with these glycans, as summarized in table 2. The former are involved in the transfer, trimming, release and disposal of these carbohydrate units, while the latter play a role in targeting proteins destined for degradation. Moreover, several glycosidase inhibitors are currently available which have proved to facilitate the exploration of the oligosaccharide interconversions (table 2).

Proteolytic degradation in quality control

Clearly, the ultimate step in quality control is the proteolysis of the misfolded or unassembled peptide chain which has been recognized by a glycan signal as indicated above. Through the extensive work of a number of investigators with in vitro and in vivo systems it has been shown that the ubiquitin-proteasome system is responsible for the degradation of an assortment of biologically important proteins [8–10, 24–26]. From these studies a model has been formulated, to a large extent through the work of the Ploegh laboratory on MHC class I molecules in human cytomegalovirus-infected cells [24, 91], in which the proteins destined for degradation are retrotranslocated via the channel provided by the Sec61 complex. While the pore size of the translocon has been reported to be 40–60 Å [92], which would be large enough to accommodate the *N*-linked glycans of glycoproteins, a recent study found the channel opening to be only 5–8 Å [93], which would preclude the passage of an *N*-glycosylated protein and would suggest that deglycosylation takes place at the luminal face of the ER membrane. However, no pore measurements have been made on the channel involved in retrotranslocation, which may not be identical to the translocon, as discussed in a subsequent section. In any case, *N*-deglycosylation would have to take place prior to entry of the protein into the lumen of the 20S proteasomes, which is unlikely to be able to accommodate bulky carbohydrate units [37]. Two distinct models (fig. 3) have been formulated for the locale and mechanism of this critical step in the proteasome system, as will be discussed in a subsequent section.

Although the role of proteasomes subsequent to retrotranslocation has so far received the most detailed attention, there is a growing body of evidence that an alternate route of proteolytic degradation exists, and that this occurs in the ER compartment [12, 27–33]. Studies on the PI Z variant of α_1-antitrypsin in murine hepatoma cells [30] and more recently on mutant yeast carboxypeptidase Y (CPY*) in CHO cells [33] have shown that a substantial portion of these misfolded proteins is degraded by a nonproteasomal pathway. ER membrane bound cysteine/serine proteases have been implicated in at least some of the degradation of human thyroperoxidase [31], HMG-CoA reductase [29] and an integral membrane protease in the ER has been noted to proteolyze stearoyl-coenzyme A desaturase [12]. Furthermore, quality control of human P-glycoprotein has been found to be carried out by a luminal ER protease [28]. The nonproteasomal degradation of ER mannosidase I has most recently been reported, although the proteolytic mechanism for this has not yet been identified [69]. It is not yet evident in what manner these nonproteasomal proteases distinguish between native and misfolded molecules which may coexist inside the ER; the finding that KIF blocked the nonpro-

Table 2. Components of the quality control system which interact directly with *N*-linked oligosaccharides*.

Enzyme/lectin	Substrate/ligand	Product	Subcellular location	Inhibitor
Oligotrans/hydrol	$G_3M_9GN_2$-PPDol + protein/H_2O	$G_3M_9GN_2$-Pr/$G_3M_9GN_2$	ER	–
Glucosidase I	$G_3M_9GN_2$-Pr	$G_2M_9GN_2$-Pr	ER	CST
Glucosidase II	$G_{2\text{-}1}M_9GN_2$-Pr	M_9GN_2-Pr	ER, IC	CST
Glucosyltrans	UDPG + M_9GN_2-Pr	$G_1M_9GN_2$-Pr	ER	–
Calnexin	$G_1M_{9\text{-}5}GN_2$-Pr	CNX- $G_1M_{9\text{-}5}GN_2$-Pr	ER	–
Calreticulin	$G_1M_{9\text{-}5}GN_2$-Pr	CRT-$G_1M_{9\text{-}5}GN_2$-Pr-	ER, IC, Gol	–
ER manase I	M_9GN_2-Pr	$M_{8B}GN_2$-Pr	ER	DMJ, KIF
ER manase II	M_9GN_2-Pr	$M_{8C}GN_2$-Pr	ER	DMJ
M_9-manase	M_9GN_2-Pr	$M_{8\text{-}5}GN_2$-Pr	ER, IC, Gol	DMJ, KIF
Endomanase	$G_1M_{9\text{-}5}GN_2$-Pr	$M_{8\text{-}4}GN_2$-Pr	ER, IC, Gol	GDMJ
EDEM	$M_{8B}GN_2$-Pr	EDEM-$M_{8B}GN_2$-Pr	ER	–
ERGIC-53	ER-$M_{8\text{-}7}GN_2$-Pr	IC-$M_{8\text{-}7}GN_2$-Pr	ER, IC	–
Gol manase Ia,b	M_9GN_2-Pr	$M_{8\text{-}5}GN_2$-Pr	Golgi	DMJ, KIF
PNGase	OS-GN_2-Pr	OS-GN_2 + Pr	ER, Cyt	–
EndoGNase	OS-GN_2	OS-GN_1	Cyt	–
Cyt manase	$M_{9\text{-}6}GN_1$	M_5GN_1	Cyt	DMJ

* References are given in text; abbreviations are G, glucose; M, mannose; GN, GlcNAc; Oligotrans, $G_3M_9GN_2$-transferase; OS, polymannose oligosaccharide; PPDol, dolichylpyrophosphoryl; Pr, protein; Gol, Golgi; Cyt, cytosol; trans, transferase; hydrol, hydrolase; CNX, calnexin; CRT, calreticulin; EndoGNase, endo-β-*N*-acetylglucosaminidase. M_{8B} and M_{8C} refer to the B and C isomers of $Man_8GlcNAc_2$, respectively. Other abbreviations are defined in the text.

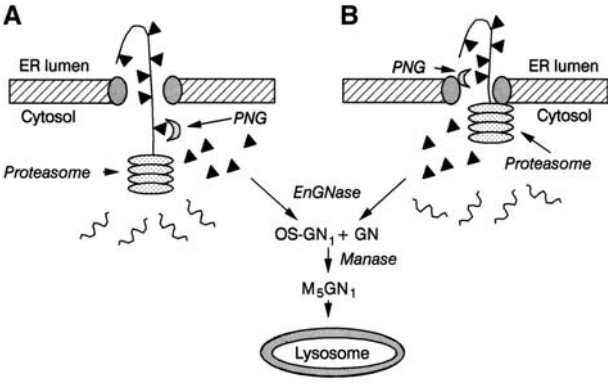

Figure 3. Comparison of two proposals for the retrotranslocation, *N*-deglycosylation and proteasomal degradation of misfolded glycoproteins during ERAD. In both models (*A* and *B*) the unfolded polypeptide with its *N*-linked OS-GlcNAc$_2$ glycans (*solid triangles*) is being translocated from the ER lumen to cytosol through a Sec61 channel. In model *A* both the PNGase (*PNG*) and the proteasome are free in the cytosol, and accordingly act on the retrotranslocated protein in a sequential manner in that compartment. In contrast, model *B* indicates that the PNGase is situated in the ER membrane and releases the oligosaccharides in a co-retrotranslocational fashion, while the proteasomes are positioned on the cytosolic side of the membrane. In both models the oligosaccharides are converted from OS-GlcNAc$_2$ to OS-GlcNAc$_1$ (OS-GN$_1$) by the action of the cytosolic endo-β-*N*-acetylglucosaminidase (*EnGNase*) and are subsequently trimmed down to Man$_5$GlcNAc$_1$ (M$_5$GN$_1$) by the mannosidase of the cytosol (*Manase*), after which it can be transported into the lysosomes for further degradation. OS refers to polymannose oligosaccharides which can have undergone varying degrees of processing by ER glucosidases and mannosidases (Glc$_{3-0}$Man$_{9-7}$GlcNAc$_2$) before being released by the PNGase; the presence of glucose residues, however, prevents the cytosolic mannosidase-trimmed oligosaccharide (Glc$_{3-1}$Man$_5$GlcNAc$_1$) from entering the lysosomal compartment, as shown in a subsequent figure. Due to variation in the quality control of different glycoproteins and cell types, models *A* and *B* are not mutually exclusive. The role of ubiquitin ligases and chaperones is not shown in these schemes.

teasomal pathway in CPY*-transfected CHO cells suggests that Man$_8$ isomer B may play a signaling role [33]. In contrast, the nonproteasomal system involved in such degradation in hepatoma cells was observed to be favored by ER mannosidase II action, which produces Man$_8$ isomer C, and moreover was observed to be sensitive to tyrosine-phosphatase inhibition [30].

N-deglycosylation of glycoproteins during quality control: action and location of *N*-glycanase

The observation that substantial amounts of free polymannose oligosaccharides were produced by mammalian in vitro and in vivo systems suggested that they were the products of the necessary deglycosylation stage of the quality control system [34]. Further work indicated that indeed the oligosaccharides which appeared shortly after *N*-glycosylation and terminated in a di-*N*-acetylchitobiose sequence (OS-GlcNAc$_2$) [35] were the product of deglycosylation brought about by a peptide:*N*-glycanase, which like the familiar enzyme from bacterial sources [94] cleaves the β-aspartylglucosamine glycopeptide bond. This important enzyme has been found in both the ER [95] and microsomes [96] as well as in the cytosol [97]. The PNGase from the latter compartment, in which most of the recoverable activity of the cell is located, has been purified and cloned from *S. cerevisiae* [98] by Lennarz and his collaborators, who furthermore identified its gene in higher eukaryotes [99]. While the ER and purified cytosolic PNGase were assayed with glycopeptides, a recent report indicated that yeast enzyme or its human homologue when transfected into mammalian cells can act on intact glycoproteins [37]. This PNGase was found to act preferentially on polymannose oligosaccharides such as would be expected to be present on glycoproteins undergoing ERAD [37].

The reports that PNGase has been observed in both the cytosol and the ER can be explained by the presence of alternate ERAD routes (fig. 3, models A and B). It would certainly appear likely that those previously listed glycoproteins which undergo nonproteasomal degradation inside the ER [12, 27–33, 66] would require an *N*-glycanase situated in this compartment, and indeed, the *N*-glycanase observed in this subcellular compartment, was found to have its catalytic site in a luminal orientation [95]. Moreover, studies in HepG2 cells infected with the ts045 mutant of the vesicular stomatitis virus indicated that degradation of its G protein in the presence of CST resulted in the exclusive release of the characteristic vesicular OS-GlcNAc$_2$ oligosaccharides which were then trapped in the ER. These components were shown to originate from this virus envelope glycoprotein [32]. Since *N*-glycanase action on ER-situated proteins would need to distinguish between glycoproteins with aberrant and native configurations, a signal to identify the former would appear to be required; such a determinant could be situated in the unfolded polypeptide chain such as has been found for the UDP-Glc:glycoprotein glucosyltransferase. Indeed it has recently been reported that the cytosolic *N*-glycanase can distinguish between native and misfolded glycoproteins [37], although in this case in which the protein is believed to have already been separated from those with normal configurations by retrotranslocation, it would not appear to be necessary.

The *N*-deglycosylation site of glycoproteins which undergo proteasomal degradation and accordingly need to be retrotranslocated from the ER to the cytosol by way of the Sec61 channel presents a more difficult interpretive puzzle and has led to disparate although not mutually exclusive models (fig. 3, A and B) in which the *N*-glycanase is either present in the cytosol and acts following the translocation [24] or is situated in the membrane and cleaves the glycopeptide bond in a co-retrotranslocational manner [36], analogous to the function of the oligosac-

charyltransferase in the import of proteins through the translocon of the ER membrane. The former proposal (model A) is based on the findings of studies which noted that when proteasomal degradation of various glycoproteins is inhibited, the deglycosylated protein intermediates appear in the cytosol [24, 91, 100, 101]. However, these investigations did not demonstrate in which location the oligosaccharide excision actually took place, and the presence of the proteins in various stages of deglycosylation was noted in the microsomal fraction.

An increasing number of reports do suggest that the *N*-glycanase is located in the ER membrane itself (model B) since in the presence of proteasome inhibitors the glycosylated protein or deglycosylated intermediates are either exclusively or predominantly associated with the ER rather than the cytosol [67, 70, 88, 102–106]. These studies included such diverse glycoproteins as immunoglobulin (Ig) subunits [106], MHC class I heavy chains [88], a ribophorin I variant [70], *cog* thyroglobulin mutant [67], cystic fibrosis transmembrane regulator [104] and T-cell receptor (TCR) α-subunits [105]. In fact, in the studies of Wiertz et al., [24] only the deglycosylated protein was noted to be associated with the Sec61 complex of the ER membrane, indicating that this channel must be accessible to the action of the *N*-glycanase. The studies from Ivessa's laboratory on truncated ribophorin I have raised the possibility that the cleavage of the *N*-glycans may actually take place in the lumen of the ER prior to retrotranslocation [70, 102]. Moreover, the site-specific *N*-deglycosylation of diglycosylated ovalbumin can be attributed to an ER situated *N*-glycanase [96], and a particulate PNGase is also believed to convert concanavalin A to an active lectin by cleaving the *N*-glycan of the newly synthesized protein [107, 108].

Direct examination of the polymannose oligosaccharides in mouse T-lymphoma cells in which accelerated degradation of the TCR α-subunit takes place indicated that there was a reduction in their release into the cytosol in the presence of proteasome inhibitors [36]. These findings suggest that *N*-deglycosylation and proteasome-mediated degradation of proteins are coupled events as further discussed below.

Since the molecular properties of the cytosolic PNGase have been defined in much greater detail than those of the enzyme present in the ER, it is not possible at this time to understand how the two are related to each other. It is conceivable that the cytosolic enzyme subsequent to its synthesis is translocated into the ER compartment, as has been found to be the case for the ER mannosidase II [49] as well a number of other proteins (see [49, 109] for other examples). In the case of the ER mannosidase II, its cytosolic form [110], like the soluble PNGase [98], does not contain a terminal hydrophobic signal sequence. A pertinent analogy can be made with the yeast vacuolar α-mannosidase which is similar in a number of ways to the mammalian cytosolic/ER II mannosidase and like the latter does not contain a signal sequence or membrane-spanning domain [111]; furthermore, the yeast enzyme like the ER mannosidase II appears to be imported into its compartment by a route distinct from the secretory pathway and involves a proteolytic cleavage [112].

Concerted function and subcellular location of ER-cytosol quality control system

In preparation for degradation by proteasomes, misfolded or improperly oligomerized proteins need to be de-*N*-glycosylated and frequently to be polyubiquinated in addition to passing through the ER membrane via a channel provided by the Sec61 complex. Although the sequence and location of these events may vary for different proteins and in different cells, a scheme (fig. 3, model B) in which all the necessary components are closely attached to or an integral part of the ER membrane [36] would appear to merit consideration as an alternative to the proposal of Wiertz et al. [24, 91] (model A), particularly in view of numerous reports indicating that the steps in quality control are closely coupled [9, 106, 113, 114]. In the former scheme (model B), the relevant proteasomes would be attached to the cytosolic side of the ER membrane, as reported by Rivett and associates [115, 116] in mammalian cells and by Enenkel et al. [117] in yeast. In the integrated model (fig. 3B) the PNGase would be associated with the translocon for the reasons discussed in the previous section. Ubiquitination, which has also been reported to occur in the ER [118], may take place on the cytosolic side of the membrane in the vicinity of the proteasomes [119], and this event appears to take place in concert with deglycosylation and proteasomal degradation. The action of the ubiquitin ligases and proteasomes is believed to be the driving force of protein movement through the Sec61channel [102, 118–120]. These interactions of the degradative machinery in the ER membrane may be reflected by the report that Fbs2, a member of the E3 ubiquitin ligase family, recognizes polymannose glycans which have the internal di-*N*-acetylchitobiose sequence ($Man_{7\text{-}9}GlcNAc_2$) [121]. Recently, Park et al., [122] reported that PNGase can interact with a component of the ubiquitin complex, although co-immunoprecipitation of the *N*-glycanase with other proteins has not been observed by other investigators [37]. While this interaction, if it indeed occurs physiologically, was tested with soluble PNGase, it may reflect a similar potential of the ER-localized enzyme.

Despite the abundant findings delineated above that *N*-glycans plays an important role in directing aberrant proteins for degradation by the ubiquitin-proteasome system, the ultimate signal by which these molecules are channeled into the ER translocon has not yet been identi-

fied. While the concept that a Sec61 complex is involved in the retrotranslocation of proteins destined for proteasomal degradation has been well established through the work of a number of laboratories [24-26], it has not been clearly determined whether this translocon is identical in all respects to the channel through which newly synthesized proteins pass from the cytosol into the ER lumen. It is difficult to visualize how the identical translocon can carry out such quite distinct operations with quite different signals [123]. Studies of mutants have indicated that deletion of certain components of the heteromeric Sec61 complex can affect one process without altering the other [124]. Although it has been assumed that the Sec61 channel operative in retrotranslocation is situated in the ER, it has been shown in mammalian cells by immunofluorescence microscopy and subcellular fractionation that its core components are located in both the ER and IC [125]. While these findings may simply indicate that the translocon cycles between these two subcellular sites but is primarily a resident of the ER, it may also reflect the possibility that it performs a specific function in the post-ER compartment. If the latter situation pertains, it may have important bearing on the subcellular site of retrotranslocation, as several studies have indicated that quality control occurs in an intermediate compartment [69, 70, 86, 88] and might suggest that misfolded or incorrectly assembled proteins may reach the cytosol in other than ER locations.

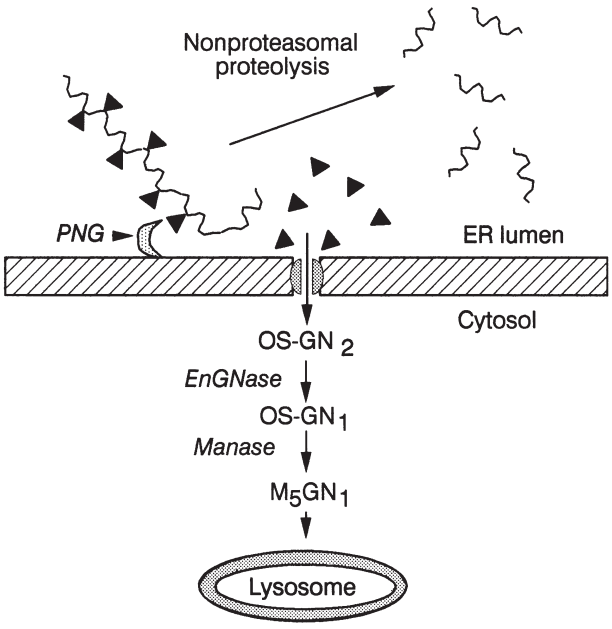

Figure 4. Diagrammatic representation of nonproteasomal ERAD. The unfolded protein with its attached *N*-linked oligosaccharides (*solid triangles*) is being degraded by an ER membrane PNGase and luminal proteases, such as described in the text, with the formation of peptides and the release of free oligosaccharides (OS-GN_2). The latter, after glucose removal (see fig. 5), are then believed to be translocated into the cytosol by a transporter where they are degraded by endo-β-*N*-acetylglucosaminidase (*EnGNase*) and mannosidase (*Manase*) to yield $Man_5GlcNAc_1$ (M_5GN_1), which is taken up by the lysosome.

Nature, origin and disposal of oligosaccharides released during quality control

It has been known for some time now that mammalian cells release substantial amounts of free polymannose oligosaccharides into intracellular compartments and that these occur predominantly as neutral components which terminate with either a di-*N*-acetylchitobiose sequence (OS-$GlcNAc_2$) or a single *N*-acetylglucosamine residue (OS-$GlcNAc_1$) at the reducing end [34]. Further studies with intact cells and in vitro systems [32, 34–36, 126] indicated that these glycans originate to a large extent from the *N*-deglycosylation of glycoproteins (figs 3, 4) although, as will be discussed in a subsequent section, a portion of the OS-$GlcNAc_2$ species originate from dolichyl pyrophosphate-linked glycans. A study with HepG2 cells revealed that the OS-$GlcNAc_2$ components occurred exclusively as large glycans, $Glc_3Man_9GlcNAc_2$ to $Man_8GlcNAc_2$, while the cytosolic species were present as OS-$GlcNAc_1$ and ranged over a broader size spectrum ($Glc_1Man_9GlcNAc_1$ to $Man_5GlcNAc_1$), with a predominance of $Man_9GlcNAc_1$ and $Man_8GlcNAc_1$ glycans [35]. Pulse-chase experiments in these cells demonstrated that the OS-$GlcNAc_2$ species inside the vesicles were expeditiously chased into the cytosol where they appeared as OS-$GlcNAc_1$ (figs 3, 4). The cytosolic OS-$GlcNAc_1$ glycans underwent demannosylation down to the $Man_5GlcNAc_1$ stage, after which they disappeared from the cytosol and entered into another vesicular compartment that was believed to be the lysosomes [35] for further degradation (figs 3, 4). The identity of this compartment was directly demonstrated in a subsequent study [127]. The free polymannose oligosaccharides are entirely metabolized within the cell and do not enter the secretory channel or appear in the media of cultured cells [35] except under abnormal conditions such as glucosidase blockade, as discussed below. Oligosaccharides released by *N*-glycanase as OS-$GlcNAc_2$ within the lumen of the ER are transported into the cytosol where they would mix with the free oligosaccharides derived from the action of PNGase located in the ER membrane or cytosol. This entire pool of oligosaccharides would undergo rapid conversion from the OS-$GlcNAc_2$ product of *N*-glycanase to OS-$GlcNAc_1$ through cleavage of their di-*N*-acetylchitobiose sequence by the endo-β-*N*-acetylglucosaminidase, which has been shown to be present in cytosol [128]. The excision of mannose residues down to $Man_5GlcNAc$ is believed to be carried out by the KIF-resistant cytosolic α-mannosidase [49]. The characterization of the Man_5

GlcNAc cytosolic limit product as Manα1-2Manα1-2Manα1-3(Manα1-6)Manβ1-4GlcNAc indicated that it was distinct from the more extensively branched *N*-linked $Man_5GlcNAc$ formed by Golgi enzymes [35]. While demannosylation of $Man_9GlcNAc$ to the $Man_5GlcNAc$ can occur in the cytosol, entry into this compartment of glycan species already partially processed on the glycoprotein by ER, IC and Golgi mannosidases can also occur, as already discussed. Characterization of the cytosolic $Man_8GlcNAc_1$ component indicated that it occurred as about an equimolar mixture of isomer B and C in contrast to the $Man_8GlcNAc_2$ constituent that was present exclusively as the B-isomer. Since the cytosolic mannosidase would yield only isomer C [49, 89], the presence of the B-isomer [129] would suggest a contribution from partially processed glycoproteins and, as has already been stated, the *N*-linked $Man_{8B}GlcNAc_2$ in particular is involved as a signal for ERAD [4, 71].

It would appear that the cytosol does not contain an α-glucosidase, and accordingly any glucosylated polymannose glycans which do enter into this subcellular compartment (see next section) will not have the A-branch trimmed by the cytosolic mannosidase. It was observed that pulse-chase experiments conducted in HepG2 cells under normal conditions resulted in the accumulation in the cytosol of $Glc_1Man_5GlcNAc_1$ as well as the characteristic $Man_5GlcNAc_1$ glycan [35]. This phenomenon became more evident in CST-treated cells in which there was a pronounced accumulation of the triglucosylated oligosaccharide ($Glc_3Man_5GlcNAc_1$) as the cytosolic mannosidase end product [35], since apparently the presence of glucose residues prevented its uptake into the lysosomes. The transporter responsible for the transfer of oligosaccharides from cytosol to lysosomes was found to act optimally on glucose-free partially demannosylated polymannose glycans, including the characteristic $Man_5GlcNAc_1$ [130] (fig. 5).

Recently it was shown that free polymannose oligosaccharides are also produced in *Saccharomyces cerevisiae*, and that the $Man_8GlcNAc_2$ isomer B is the predominant species initially generated, as might be anticipated if it is a product of protein quality control [131]. During an extended chase smaller degraded polymannose oligosaccharides appeared in wild-type cells, but this time-related degradation did not occur in a vacuolar α-mannosidase-deficient mutant, suggesting that this second yeast α-mannosidase was involved in the ultimate catabolism of the released polymannose oligosaccharides. Since the peptide sequence of yeast vacuolar mannosidase [111, 112] has little in common with that of mammalian lysosomal mannosidase but shows pronounced homology to the α-mannosidase of mammalian cells [110], which is involved in the degradation of cytosolic glycans originating in the ER, it appears likely that the yeast vacuolar enzyme may carry out the trimming of the mannose chains which are released during ERAD. A further analogy between the yeast vacuolar and mammalian cytosolic mannosidase can be found in the observations, already mentioned, that both enzymes while synthesized in the cytosol do not have signal sequences and appear to be translocated into the vacuoles and ER, respectively, by an SRP-independent targeting route which moreover involves a proteolytic cleavage [49, 112].

Release of oligosaccharides from oligosaccharide lipids

It has been shown that free intracellular polymannose oligosaccharides, in addition to their genesis from *N*-deglycosylation during protein quality control, can originate from lipid-linked oligosaccharides and contribute to the oligosaccharide pool in the ER lumen [132] (fig. 5). It would appear that the release of $Glc_3Man_9GlcNAc_2$ by cleavage of its linkage to Dol-P-P through a hydrolytic action of the ER oligosaccharyltransferase serves to control the size of the lipid-oligosaccharide pool [132], which is substantial [133, 134] and is in its triglucosylated form responsible for the *N*-glycosylation of proteins in almost all eukaryotic species [3]. The hydrolytic action of oligosaccharyltransferase was demonstrated in thyroid microsomes [34] and ER vesicles as well as intact cells [132]; in the latter case the OS-$GlcNAc_2$ glycans were observed to be released, albeit at a reduced rate, when protein synthesis and its attendant *N*-glycosylation were inhibited by puromycin [132]. Further studies in thyroid with the use of CST indicated that there was a preferential turnover of the glucose residues of the dolichol-linked $Glc_3Man_9GlcNAc_2$, and these findings led to the postulation of a glucosidase-glucosyltransferase shuttle that operates through a selective removal and readdition of glucose residues by the action of the ER glucosidases and glucosyltransferases [132] (fig. 5). The observation that there is an accumulation of deglucosylated oligosaccharide lipids in cells exposed to an energy-deficient state [133] can be attributed to inhibition of the glucosylation aspect of this shuttle. The state of glucosylation of free and lipid-linked oligosaccharides as well as of *N*-linked glycans can readily be assessed with the use of recombinant endomannosidase, which by cleaving the linkage between the tri- di- or monoglucosylated mannose residue of chain A and the rest of the polymannose glycan releases the characteristic cleavage products of lipid-linked or free oligosaccharides and brings about changes in the electrophoretic mobility of glycoproteins [135].

In addition to the neutral oligosaccharides it has been reported that a specific pyrophosphatase located on the cytosolic face of the ER releases small nonglucosylated OS-$GlcNAc_2$-P, in particular $Man_5GlcNAc_2$-P, from the

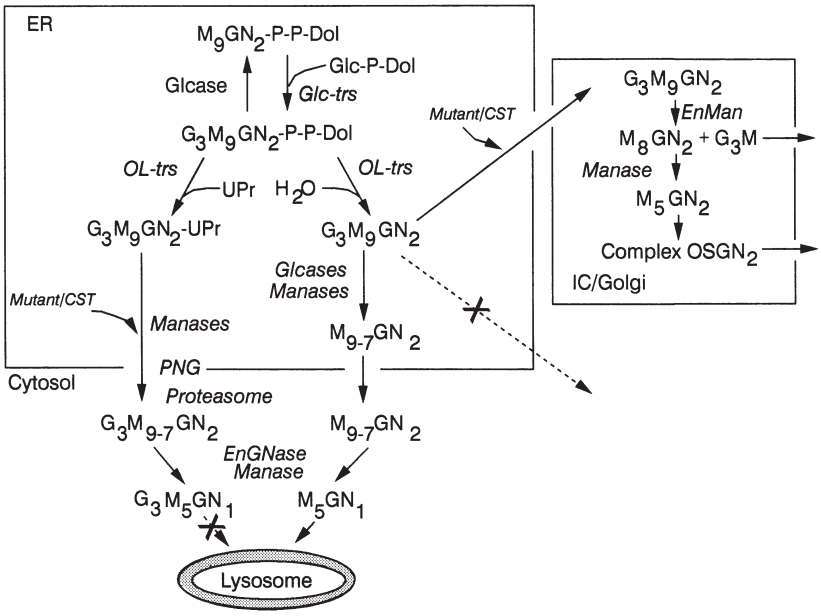

Figure 5. Schematic portrayal of the glucosyltransferase-glucosidase shuttle and hydrolytic action of oligosaccharyltransferase involved in regulating the level of the dolichylpyrophosphate (Dol-P-P)-linked $Glc_3Man_9GlcNAc_2$ ($G_3M_9GN_2$) *N*-glycosylation precursor. The addition and removal of glucose residues by the ER-situated glucosyltransferases (*Glc-trs*) and glucosidases (*Glcase*), respectively, are shown. Moreover, the transfer and hydrolytic activities of the oligosaccharyltransferase (*OL-trs*) are depicted, leading to an *N*-glycosylated unfolded protein (*UPr*) or the release of the free triglucosylated polymannose glycan ($G_3M_9GN_2$). The latter product cannot exit into the cytosol (indicated by an X) unless it is deglucosylated by the ER glucosidases. Subsequently, with or without trimming of the mannose residues by the ER mannosidases (*Manases*), the free glycan can be translocated into the cytosol by an oligosaccharide transporter where it, along with the OS-GN_2 from the proteasomal and nonproteasomal degradation pathways (figs 3, 4), is trimmed to the $Man_5GlcNAc_1$ (M_5GN_1) species by the action of the cytoplasmic endo-β-*N*-acetylglucosaminidase (*EnGNase*) and mannosidase before entering the lysosomes. In the glucosidase I-deficient mutant or in the presence of CST (*Mutant/CST*) the $G_3M_9GN_2$ can enter the Golgi compartment where through the action of endomannosidase (*EnMan*) it can be converted to $Man_8GlcNAc_2$ (*M_8GN_2*). The latter can be converted to M_5GN_2, which in turn can be processed by Golgi enzymes into sialylated complex oligosaccharides which are then secreted by the cell along with the Glc_3Man (G_3M) product of endomannosidase action. In the presence of CST or a glucosidase-deficient mutant, the *N*-glycosylated unfolded protein ($G_3M_9GN_2$-UPr) can, however, be deglycosylated by PNGase (*PNG*) and exit through the Sec61 channel to undergo proteasomal degradation (see fig. 3). The $G_3M_5GN_1$ glycan which is ultimately formed is, however, precluded from entering the lysosomes due to its glucosylated state (indicated by an X).

dolichol phosphate attachment; these are subsequently degraded to OS-$GlcNAc_1$ in the cytosolic compartment [136].

Transport of polymannose oligosaccharides and glycopeptides from ER to cytosol and the effect of glucosidase blockade on their translocation

It is apparent from the information already presented that polymannose glycans in the OS-$GlcNAc_2$ form are released into the lumen of the ER through the hydrolysis by the oligosaccharyltransferase of oligosaccharide lipids as well as by the action of PNGase on a number of glycoproteins which undergo nonproteasomal degradation (figs 4, 5). Since such oligosaccharides do not enter the secretory channels under normal conditions, they must be translocated through the ER membrane into the cytosol where they can undergo further degradation by the endo-β-*N*-acetylglucosaminidase and α-mannosidase situated in that compartment (figs 4, 5). The requirements for this translocation have been studied by Moore et al. [137] and shown to be ATP as well as Ca^{++} dependent; moreover, it was shown that while the polymannose glycans ($Man_{9-8}GlcNAc_2$) were readily transported through the ER membrane, this did not occur when these oligosaccharides were in their triglucosylated form ($Glc_3Man_{9-8}GlcNAc_2$) (fig. 5). The inability of glucosylated oligosaccharides to move from the ER lumen to the cytosol appears to account for the finding that in CST-treated or glucosidase I-deficient cells free triglucosylated polymannose glycans were secreted into the medium [138]; furthermore, it was reported that sialylated complex oligosaccharides also appeared in the extracellular space (fig. 5). The presence of the latter components was attributed to their passage through the secretory channels where they would be deglucosylated by the Golgi-situated endomannosidase, which would permit further processing by the mannosidases and glycosyltransferases that are known to generate complex carbohydrate units. The appearance of the characteristic endo-

mannosidase cleavage product, Glc_3Man, in the medium supported this contention, particularly since this component was no longer evident in the presence of brefeldin, which inhibits the secretory pathway by fusing the Golgi cisternae with the ER. An analogous situation appears to exist in a human congenital disorder of glycosylation caused by glucosidase I deficiency [139]; in this disease a pronounced accumulation of the Glc_3Man occurs in the urine. The occurrence of this tetrasaccharide reflects the operation of the alternate pathway of deglucosylation provided by endomannosidase [46, 140] without which processing of *N*-linked oligosaccharides would be totally precluded in this glucosidase-deficient disorder.

Since studies on a number of proteins that have already been cited have indicated that their proteolysis by the quality control system occurs within the ER compartment rather than by the proteasomal pathway, it is possible that glycopeptides could be generated which would have to be translocated to the cytosol through the ER membrane for ultimate degradation. Indeed, a number of investigations have focused on glycopeptide export into the cytosol [141–145], and it has become evident that a transport mechanism distinct from that employed for oligosaccharide translocation is involved. Although ATP is required for both transporters, their cation requirements differ, and the glycopeptide unlike the oligosaccharide transporter required cytosolic factors. Most prominently it was observed that fully glucosylated glycopeptides were translocated through the ER membrane [142, 143] in contrast to the oligosaccharides that did not pass through the ER membrane in the glucosylated state [137]. Studies with yeast mutants have further defined the glycopeptide transporter; these investigations indicated that although the Sec61 channel involved in glycoprotein translocation also mediates glycopeptide transport, deletion of various components of the Sec61 complex affected the transport of these two types of molecules differently [144, 145].

In view of the finding that glucosylated free oligosaccharides cannot exit the ER into the cytosol [137], the observation that glucosylated polymannose oligosaccharides can appear in the cytosol during glucosidase blockade [35, 138] suggests that their export from the ER can occur while they are still attached to peptide alternatively to their entering the secretory channel as free glycans.

Unfolded protein response as a regulator of glycoprotein quality control

The activation of UPR by the accumulation of aberrant proteins in the ER is a characteristic of all eukaryotic cells which have so far been examined [10, 146, 147]. It represents an ER-to-nucleus signalling pathway which upregulates a large number of genes involved in the various aspects of protein folding and the ERAD system so that the cellular damage by the aggregation of abnormal proteins induced by various stress situations, such as heat shock, inhibition of *N*-glycosylation, disruption of calcium homeostasis and reduction of disulfide bonds, can be avoided. Although an extensive literature dealing with all aspects of UPR, employing yeast and mammalian cells, has emerged over several decades, the present review will only comment on those studies which have dealt with aspects which relate directly to the role of *N*-linked glycans. The investigations of Lehrman's laboratory have shown that the incomplete or inhibited formation of the dolichol-linked $Glc_3Man_9GlcNAc_2$ glycans, induced by glucose deprivation or tunicamycin, as well as interference by CST with their processing subsequent to *N*-glycosylation, brings about glycoprotein misfolding and induces the UPR response which counteracts these stress situations [5, 148]. Furthermore it was shown that cells from humans with type I congenital disorders of glycosylation that have defects in oligosaccharide-lipid synthesis underwent a chronic ER stress in which synthesis of the dolichol-linked saccharides was continuously activated [149]. The UPR response has also been shown to bring about enhanced synthesis of various lectin-like proteins including the mannose specific ERGIC-53 cargo receptor [150], the Man_8-specific EDEM [72] as well as the $Glc_1Man_{9-5}GlcNAc_2$-binding calnexin [151]. Furthermore, activation of the UDP-Glc:glycoprotein glucosyltransferase [152] and the glycan-binding ubiquitin ligase E3 [153] have been shown to be activated by the UPR, indicating that these components play a major role in the quality control system.

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