

A novel lectin in the secretory pathway

An elegant mechanism for glycoprotein elimination

How important can sugars be? Sure, glycoproteins need their glycans during synthesis for proper folding: the sugar chain increases solubility and prevents aggregation, and it mediates interactions with various proteins. On the other hand, so many different glycans exist on glycoproteins that it is difficult to imagine that each would have a specific function. Still, now and again, a new protein is identified that interacts with a particular carbohydrate chain, and that turns out to be crucial for a particular

stage in the biosynthesis and maturation of glycoproteins. Will there be many, or do we have only a few? Whichever the answer, glycobiology is celebrating a certain revival.

The first step of N-linked glycosylation is highly conserved amongst eukaryotic organisms: the $\text{Glc}_3\text{Man}_9\text{GlcNAc}_2$ glycan chain is co-translationally attached to the Asn residue in the consensus motif Asn-X-Ser/Thr (Kornfeld and Kornfeld, 1985). Immediately after attachment, monosaccharides are removed one by one (Figure 1), only to prepare the glycan for the later addition of other monosaccharides. This apparently futile sequence of events is a consequence of the necessary signaling to glycan-specific lectins with various gate-keeper functions in the secretory pathway (Helenius and Aebi, 2001).

Lectins are defined as non-enzymatic, sugar-binding proteins that lack enzymatic activity towards carbohydrates. Up to now, only four lectins had been identified early in the secretory pathway, each with specific glycan recognition, and each with a specific function. Calnexin and calreticulin act as molecular chaperones in the endoplasmic reticulum (ER; Ellgaard *et al.*,

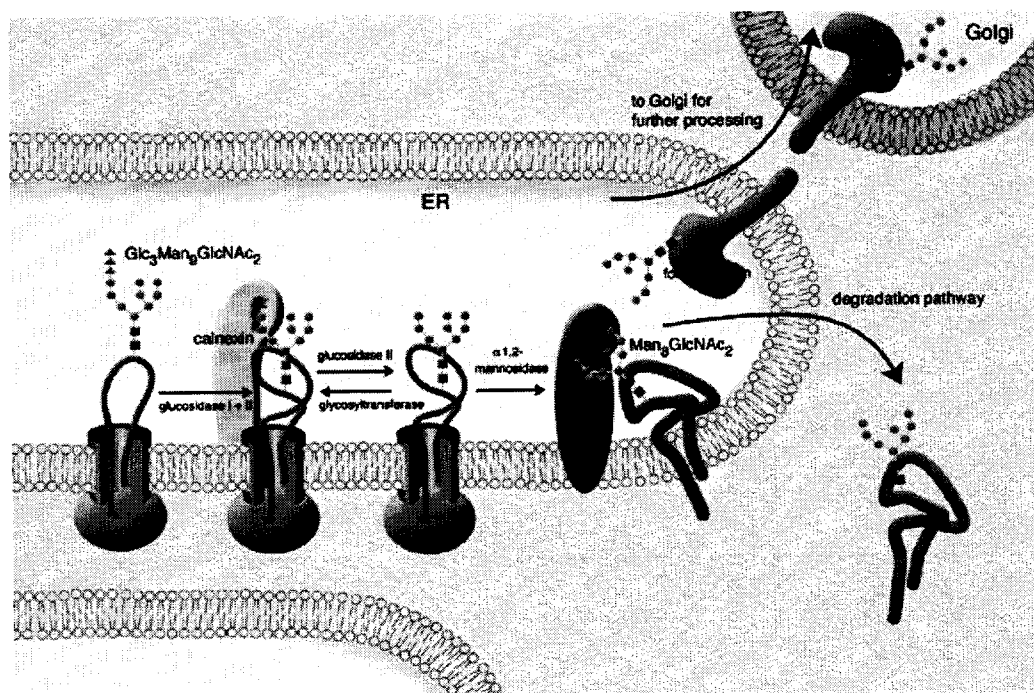


Fig. 1. Modifications of the N-linked glycan on proteins. Glycans are attached as the high mannose $\text{Glc}_3\text{Man}_9\text{GlcNAc}_2$ chain. Immediately after attachment to the protein, the first glucose residue (triangle) is removed by glucosidase I, the second and third by glucosidase II. The third glucose can be reattached by a glucosyltransferase that recognizes only misfolded and unfolded proteins (Parodi, 2000; Helenius and Aebi, 2001). Calnexin and its soluble family member calreticulin (not depicted) associate with the monoglucose chain to retain immature glycoproteins in the ER, to allow folding to proceed, and to prevent release into the secretory pathway (Helenius *et al.*, 1997; Parodi, 2000; Helenius and Aebi, 2001). The ER $\alpha 1,2$ -mannosidase removes a single mannose, resulting in $\text{Glc}_0\text{Man}_8\text{GlcNAc}_2$, before transport of the properly folded protein to the Golgi complex where further processing occurs. The Man_8 glycan, containing a yet unknown number of glucose residues, is recognized by Htm1p/EDEM and targeted for degradation.

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2000; Parodi, 2000), ERGIC-53 functions as a cargo receptor for transport from the ER to the intermediate compartment between the ER and the Golgi complex (ERGIC; Hauri *et al.*, 2000), and VIP36 may function as a cargo receptor within the *cis*-Golgi (Fiedler *et al.*, 1994). The presence of a fifth lectin, which targets proteins with an attached Glc₀₋₃Man₈GlcNAc₂ for degradation, was suggested by several reports (Su *et al.*, 1993; Liu *et al.*, 1997; Jakob *et al.*, 1998; Fagioli and Sitia, 2001). In 1998, Jakob *et al.* published a detailed study in which they showed that, in *Saccharomyces cerevisiae*, specifically the Man₈GlcNAc₂ intermediate allows rapid degradation of the protein to which it is attached (mutant carboxypeptidase Y in this case). This and the other reports prompted the speculation that an ER-localized lectin recognizing Man₈GlcNAc₂ glycans should be responsible for targeting misfolded and unfolded proteins to the proteasome for degradation.

And indeed, three papers now report the identification of α 1,2-mannosidase-like proteins in *S. cerevisiae* and in mouse cells. These two proteins are homologous to one another and lack enzymatic mannosidase activity, suggesting that they are mannoside-binding lectins (Hosokawa *et al.*, 2001; Jakob *et al.*, 2001; Nakatsukasa *et al.*, 2001). A thorough biochemical study from the group of Nagata (Hosokawa *et al.*, 2001) shows that the mouse protein EDEM has no mannosidase activity, it localizes to the ER, and it is upregulated upon ER stress. Overexpression of this protein resulted in increased degradation of misfolded α 1-antitrypsin, but not of a non-glycosylated protein. As EDEM co-immunoprecipitates with misfolded protein, its role in degradation is more than suggestive. The presence of residues crucial for mannoside binding completed the picture of EDEM as a mannoside-binding lectin important for ER-associated degradation.

The group of Aebi (Jakob *et al.*, 2001) reached similar conclusions about the Htm1 protein by different means. Jakob *et al.* identified the yeast Htm1 protein via a search for mannosidase homologs. They then used mostly genetic strategies to demonstrate that deletion of the protein from a yeast cell stabilizes glycoproteins but not a non-glycosylated protein. The results of the *htm1* deletion were similar to the consequences of gene deletions that prevented the formation of a Man₈ intermediate via a defect in either biosynthesis or trimming. The authors therefore suggest that Htm1p acts through Man₈.

The third report, by the group of Endo (Nakatsukasa *et al.*, 2001), describes the identification of the yeast Mnl1 protein, which is identical to Htm1p. The experiments were similar to those of Hosokawa *et al.*, but were performed in *S. cerevisiae*.

Newly synthesized proteins may reside in the ER for a long time, and folding intermediates must be distinguished from misfolded proteins and removed. Both may be associated with calnexin and calreticulin, which retain them in the ER, but at some point the decision for degradation must be made for proteins that will never reach a properly folded conformation. A specific signal for degradation is needed at this stage. Glycan trimming and the subsequent recognition of particular glycans have been suggested to act as a timer for degradation (Su *et al.*, 1993; Helenius *et al.*, 1997). α 1,2-mannosidase, which removes one mannose from Man₉, apparently acts slowly, leaving sufficient time for folding. If a protein folds extremely slowly or into the wrong conformation, it ultimately reaches the Man₈ state that targets it for degradation. The Man₈-related literature indicates degradation periods of hours, compatible with the timer-hypothesis. We cannot, however, assume this mechanism

to be simply time-dependent. The normal folding rates of proteins are highly variable, and the α 1,2-mannosidase may remove mannose groups on different glycans at rates that differ depending on factors such as accessibility.

Ignored in many papers dealing with ER-associated degradation is the substantial amount of breakdown of nascent chains, already during protein synthesis. Hosokawa and colleagues show that ³⁵S metabolic glycoprotein labeling of newly synthesized proteins (including EDEM) is strongly increased when protein degradation is inhibited (Hosokawa *et al.*, 2001), suggesting a co-translational effect of the inhibitors. Mannosidase inhibition, which prevents removal of the ninth mannose residue, and hence the formation of the Man₈ glycan, also increased labeling, indicating an at least partially co-translational degradation mediated by mannoside trimming. Proteasome inhibitors only mildly inhibited degradation induced by overexpression of EDEM. Thus, an additional, alternative degradation pathway is likely to exist. Contrasting with the timer hypothesis, which requires slow mannosidase activity, co-translational degradation mediated by the Man₈ lectin must be rapid, taking place within the minutes it takes to synthesize a protein of average size. Since EDEM contains five N-linked glycans, it may be subject to the same mannoside-mediated control as the proteins it regulates.

The argument for Man₈-lectin triggering protein degradation is very convincing, and Htm1p/EDEM seems to play a role in this process. On the other hand, Man₈ cannot be necessary or sufficient. Alternative pathways of glycan trimming may exist in different cell types and species (Herscovics, 1999), and many glycoproteins that move successfully to the Golgi from the ER will have had a Man₈ intermediate. Perhaps the enzyme that checks the conformational quality of proteins earlier in the ER, the UDP-glucose:glycoprotein glucosyltransferase (Parodi, 2000), attaches a glucose to the Man₈ glycan as well, and provides the specific signal for degradation. This would require Htm1p/EDEM to recognize only Glc₁Man₈ glycans and not any other Man₈ glycans. An alternative scenario would predict that Htm1p/EDEM is not purely a lectin, but recognizes misfolded protein stretches as well.

Now that one lectin with a role in targeting proteins for degradation has been identified, it is tempting to speculate that more will follow. Candidates are lectins recognizing the Man₉ or Man₅ intermediate, alternative Man₈ forms, and perhaps even every other common intermediate in the trimming pathway. The papers on Htm1p/Mnl1p and EDEM signify only the next step in the long, hard search for the diverse roles of sugars in cell biology.

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