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., 2001).

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 \( \text{Glc}_2\text{Man}_8\text{GlcNAc}_2 \), before transport of the properly folded protein to the Golgi complex where further processing occurs. The \( \text{Man}_n \) glycan, containing a yet unknown number of glucose residues, is recognized by Htm1p/EDEM and targeted for degradation.
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published a detailed study in which they showed that, in
Jakob either biosynthesis or trimming. The authors therefore suggest
into the wrong conformation, it ultimately reaches the Man 8
sufficient time for folding. If a protein folds extremely slowly or
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, Saccha-
romyes cerevisiae, specifically the Man,GlCNAC, 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
Man,GlCNAC, glycans should be responsible for targeting
misfolded and unfolded proteins to the proteasome for degradation.

And indeed, three papers now report the identification of α1,2-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
mannose-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 α1-antitrypsin,
but not of a non-glycosylated protein. As EDEM co-immuno-
precipitates with misfolded protein, its role in degradation is more
than suggestive. The presence of residues crucial for mannose
binding completed the picture of EDEM as a mannose-binding
lectin important for ER-associated degradation.

The group of Aebl (Jakob et al., 2001) reached similar conclusions about the Htm1 protein by different methods. 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 the Man9 intermediate via a defect in
either biosynthesis or trimming. The authors therefore suggest
that Htm1 acts through Man9.

The third report, by the group of Endo (Nakatsukasa et al.,
2001), describes the identification of the yeast Mn1 protein,
which is identical to Htm1. 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). α1,2-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 Man9 state that targets it for degradation. The Man9-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 α1,2-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
cotranslational 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 cotranslational degradation
mediated by mannose 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, cotranslational 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 mannose-mediated control as the proteins it regulates.

The argument for Man9-lectin triggering protein degradation is
very convincing, and Htm1p/EDEM seems to play a role in this
process. On the other hand, Man9 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 Man9 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 Man9 glycan as well, and
provides the specific signal for degradation. This would require
Htm1p/EDEM to recognize only Glc-Man9, glycans and not any
other Man9 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
Man9 intermediate, alternative Man9 forms, and perhaps even
every other common intermediate in the trimming pathway. The
papers on Htm1p/Mn1p 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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