Minireview

# What can yeast tell us about *N*-linked glycosylation in the Golgi apparatus?

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**Abstract The *N*-glycans found on eukaryotic glycoproteins occur in a vast range of different structures. A universal *N*-glycan core is attached to proteins during synthesis in the endoplasmic reticulum, and then diversity is generated as the proteins pass through the Golgi apparatus. Many of the Golgi-localised glycosyltransferases have now been identified in both yeast and mammalian cells, but it is still unclear how these enzymes are integrated into the Golgi and the rest of the cell so as to ensure efficient and specific processing of passing substrates. This review discusses the potential of the yeast system to address these issues.** 

*Key words:* Glycosylation; Golgi apparatus; Yeast

## 1. Introduction

The synthesis of *N*-glycans starts in the endoplasmic reticulum (ER) where a core structure is assembled on a lipid donor, and then transferred to nascent proteins during their translocation into the ER lumen [1]. This core structure is the same in all eukaryotes so far examined, and after initial trimming of glucoses (a process linked to protein folding) the assembled glycoproteins are collected into transport vesicles and delivered to the Golgi apparatus. It is in the Golgi that the diversity of *N*-glycan structures is generated by a series of glycosidases and glycosyltransferases acting in a manner that varies depending on the glycoprotein, tissue and species. This raises questions as to how cells generate this diverse range of structures, and what functions the glycans serve.

The budding yeast *Saccharomyces cerevisiae* has proven to be a valuable model system for the study of many aspects of eukaryotic biology, including the trafficking of proteins through the secretory pathway. Indeed many of the enzymes that synthesise the *N*-linked core structure were identified by yeast genetics based on the *alg* (*a*sparagine-*l*inked *g*lycosylation) mutants in which this process is defective [2]. The conservation of this process during eukaryotic evolution is so high that it has allowed human genetic diseases in *N*-glycan core synthesis to be analysed by comparing the structure of the glycan made in the patients with those of yeast *alg* mutants, and hence identify the gene which is likely to be defective in the patient [3]. However although the core structures made in the ER of humans and yeast are very similar, the final *N*-glycan structures generated by processing in the Golgi could hardly be more different. Whilst mammalian *N*-glycans are found in a very wide range of structures containing diverse sugars such as fucose, sialic acid and galactose, the *N*-glycans of yeast occur in only two basic forms, both elaborated with just one sugar type, mannose (Fig. 1). Indeed the Golgi glycosyltransferases are arguably the only part of the secretory pathway that is not conserved between mammals and yeast! However, despite the more than superficial differences between the two systems, there are also underlying similarities which suggest that yeast could prove informative in answering basic questions about how cells generate diversity in *N*-linked glycans, and what function this serves. In this review I will summarise what is currently known about *N*-glycan processing in the yeast Golgi, what issues are still outstanding, and what aspects of this system may be relevant to mammalian cells. The large amount of work from many labs that is briefly covered by this summary is reviewed in greater detail elsewhere [4,5].

## 2. Glycan processing in the yeast Golgi

The two different *N*-glycan structures of yeast are found on distinct sets of proteins. Many of the structural proteins destined for incorporation into the cell wall, and some of the enzymes of the periplasm, receive a large 'mannan' structure that consists of a backbone of about 50 mannoses with short side branches (Fig. 1). In contrast the proteins of the internal organelles of the cell generally have a much smaller 'core-type' structure with only a few mannoses being added in the Golgi. The mannan structure is not essential for viability, and its synthesis was investigated by Ballou and co-workers who initially screened for *mnn* (*mann*an defective) mutations in which mannan was absent or altered [6,7]. By then examining the partial structures found in the different *mnn* mutants they were able to order the steps in mannan synthesis. Some additional mutants with altered mannan were found by subsequent screens based on the fact that defects in mannan alter the surface properties and chemical sensitivities of yeast (*ktr*, *ldb*, *ngd*, *och*, *vrg* mutants [8–12]). Some of the genes corresponding to the *mnn* and other mutants were cloned by complementation, and further enzymes involved were identified by

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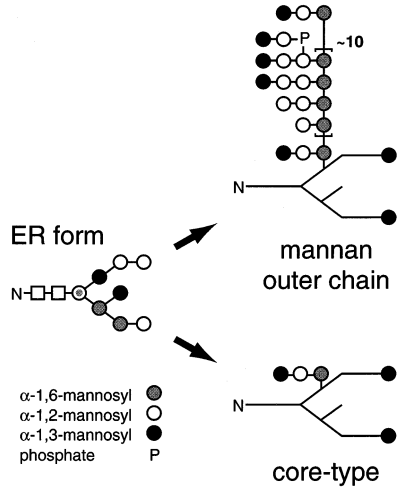


Fig. 1. The two *N*-glycan structures generated in the yeast Golgi. The *N*-glycan core is attached in the ER, and three glucoses and a mannose are removed before arrival in the Golgi. The residues are as indicated along with the two *N*-acetylglucosamines (squares), and the β-1,4-linked mannose in the stem of the core structure. The side branches in the mannan backbone are variable, and the four shown are to indicate the various structures predicted, not their actual ratio. The order of the different side chains on the backbone is unknown. Phosphomannose can also be present on the core as well as in the mannan outer chain.

homology, or by being found in a complex with a known protein [13–16].

Combining the in vitro activities of the individual enzymes with the mannan structure found in cells that lack them, suggests a model that can account for the complete pathway of mannan synthesis [5,17] (Fig. 2). Upon arrival in the Golgi all *N*-glycans receive a single α-1,6-mannose from the Och1p transferase. On a subset of proteins this mannose is then extended by the sequential action of two enzyme complexes (mannan polymerase (M-Pol) I and M-Pol II) to form the long mannan backbone. The branches are then made by the sequential action of Mnn2p, Mnn5p and Mnn1p. All of the proteins or complexes involved have mannosyltransferase activity in vitro. On some of the branches a phosphomannose is attached, an addition that apparently requires both Mnn4p and Mnn6p [18,19], and although their individual roles are unclear, Mnn4p has homology to a known phospho-ligand transferase [20]. In contrast, the 'core-type' *N*-glycans receive just one α-1,2-linked mannose after the action of Och1p, followed by α-1,3-linked mannoses from Mnn1p.

## 3. Relevance to higher eukaryotes

The pathway of mannosyltransferases in the yeast Golgi is clearly distinct from the pathway of mannosidases and glycosyltransferases that acts in mammalian cells. In fact the only sugar found in mammalian *N*-glycans that is never added in the Golgi is mannose, and the yeast mannosyltransferases share little homology with mammalian glycosyltransferases beyond the DxD catalytic site motif conserved in many families of nucleotide-sugar using glycosyltransferases [21]. However beyond the basic enzymology there are further questions of how Golgi enzymes receive their substrates, how they are located within the Golgi, and how they serve the cell by efficiently modifying the right glycoproteins. These questions about the integration of a seemingly simple pathway of glycosyltransferases into the rest of the cell apply to all eukaryotes, and so may reflect underlying mechanisms and components which are better conserved in evolution. I shall consider here what is known in the yeast system about these more general issues.

### *3.1. Substrate specificity*

The mechanisms by which different glycoproteins receive distinct modifications as they pass through the Golgi are generally very poorly understood. Presumably some feature of the peptide part of the glycoprotein is recognised by something in the Golgi. In the case of yeast the main bifurcation point in processing occurs when the mannan backbone is initiated on just a subset of proteins by M-Pol I, after Och1p has attached the first α-1,6-mannose to all *N*-glycans. It has been suggested

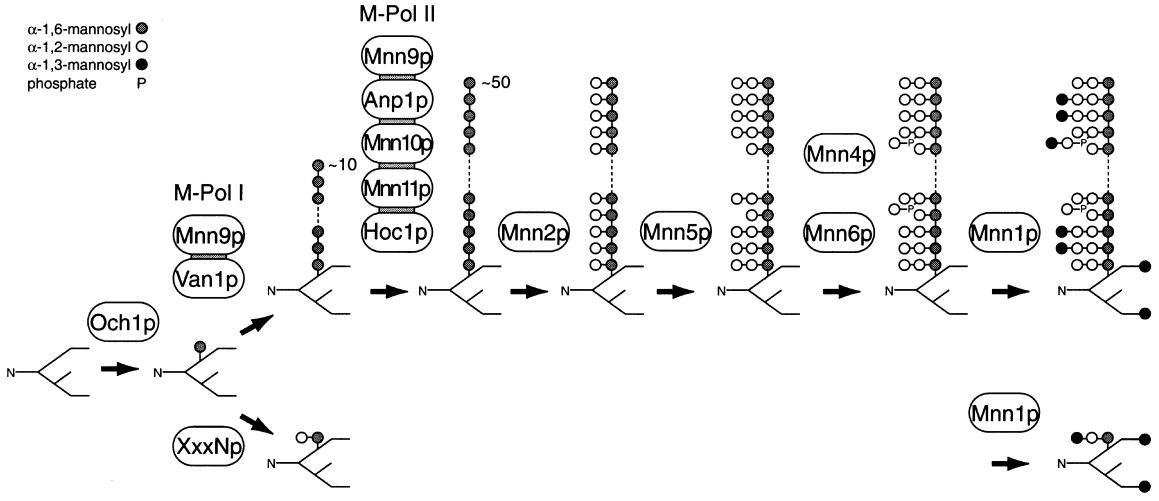


Fig. 2. Pathways of *N*-glycan modification in the yeast Golgi. The enzymes that are believed to be responsible for each step in the synthetic pathway are indicated. The α-1,2-mannosyltransferase responsible for the first addition in the pathway to the core-type structure is currently unknown (XxxNp).

that the addition of an α-1,2-linked mannose to the non-mannan proteins acts as a capping structure which prevents action of M-Pol I [22]. In vitro M-Pol I has mannosyltransferase activity on even very simple carbohydrate substrates [14,17], which would be consistent with it not contributing to specificity in vivo. However in cells lacking the Mnn9p component of M-Pol I, and hence lacking mannan, the α-1,2-mannose cap is found on all glycoproteins, suggesting that its addition is a default that occurs after the initial selective action of M-Pol I. Moreover, the ability to modify simple acceptors in the absence of an attached protein is a feature of many mammalian Golgi glycosyltransferases that show glycoprotein specificity, and it may be that in vivo efficient recognition of the intact *N*-glycan when attached to the rest of the protein is sensitive to adjacent surface features of the substrate proteins. This is the mechanism that appears to apply in the selective addition of GlcNAc-phosphate to mammalian lysosomal hydrolases [23]. However, a hint that the selection of substrates for mannan addition may be more complex comes from the observation that when the cell wall enzyme invertase is expressed as a fusion to part of the vacuolar protein CPY, it passes through the Golgi on the way to the vacuole, but no longer receives mannan addition [24].

In considering how an enzyme could recognise a broad range of substrates with widely divergent sequences, it is perhaps of interest to note that the transport of proteins from ER to Golgi also seems to involve recognition of a broad range of protein substrates, in this case by cargo receptors proposed to collect proteins into COPII vesicles [25,26]. These cargo receptors have mostly proven elusive, but it is tempting to speculate that they could present proteins to the early Golgi enzymes, and so specificity need not lie in how the enzyme recognises substrate, but rather the cargo receptor/substrate complex.

### 3.2. Spatial organisation

It is generally assumed that newly made glycoproteins move through the compartments of the Golgi from *cis* to *trans*, and hence the order in which they are exposed to the Golgi enzymes will be dictated by the distribution of the enzymes between the cisternae. Although this distribution could be important for ensuring that the right modifications are added in the right order, in reality the specificity generally lies within the enzymes themselves, and they cannot modify substrates until they have been modified by earlier enzymes in the pathway. A similar situation seems to apply in mammalian cells, although there are some cases where enzymes compete for the same acceptor and for these location in the stack could play a role in determining specificity. Nonetheless, it is clearly important for the cell to ensure that either all enzymes are in all cisternae, or instead that the enzymes are at least broadly arranged within the Golgi in the order in which they act. It appears that both mammals and yeast adopt the latter approach – presumably the saving in enzyme synthesis outweighs the costs of maintaining sorting mechanisms to restrict enzymes to a subset of cisternae. In yeast it is possible to use temperature sensitive secretion mutants to trap proteins as they move through the Golgi compartments [27], and in addition the compartments are relatively easy to distinguish by immunofluorescence as they are not arranged in a stack but rather scattered throughout the cytosol (the reason for this is unknown, and even amongst budding yeasts it occurs in only a subset of species [28,29]). The exact number of compartments is unclear as many markers show partially overlapping distributions, and indeed if the cisternae are maturing then in reality the only defined compartments are the *cis* (arrival) and the *trans* (exit), with a continuum in between. Nonetheless the early acting enzymes in mannan synthesis are clearly localised differently to the later ones such as Mnn1p [15,30].

The mechanisms by which proteins are distributed to different parts of the Golgi still remains unclear. All of the Golgi glycosyltransferases so far found in yeast and mammals are membrane proteins with a single transmembrane domain (TMD) near their N-termini. This type II orientation is relatively uncommon in membrane proteins from other compartments and its universality amongst Golgi glycosyltransferases is an enduring enigma. Many transferases in yeast and mammals are homodimers, and in a few cases form multienzyme complexes, such as M-Pol II, a common feature of biosynthetic pathways [14,31–33].

The TMDs of yeast Golgi enzymes are substantially shorter than those of plasma membrane proteins [34], a feature shown to be important for Golgi retention in mammalian cells [35,36]. However, although the TMDs of some yeast Golgi enzymes have been found to be involved in retention, other regions also appear to play a role, consistent with multiple mechanisms acting to specify not only Golgi retention but also location to specific cisternae [30,37–39]. Since much of the machinery of membrane traffic is conserved between mammals and yeast, as is the lipid remodeling in the Golgi proposed to cause an increase in bilayer thickness, it seems likely that they will use similar mechanisms to arrange their glycosyltransferases between compartments. Indeed several mutations in genes involved in Golgi membrane trafficking also show defects in mannan synthesis, although whether these reflect altered enzyme locations rather than spatial disorganisation of Golgi membranes remains to be established [40,41].

### 3.3. Metabolic integration

Golgi glycosyltransferases act within the Golgi lumen and yet require the divalent cation $Mn^{2+}$, and nucleotide-sugars which are made in the cytosol. Yeast genes encoding transporters for these compounds have been isolated, some in the screens for mutants with defects in mannan synthesis. These include Vrg4p, a GDP-mannose/GMP antiporter [42], and Pmr1p, a $Ca^{2+}/Mn^{2+}$ ATPase [43]. The GDP released by transferase action is converted to GMP by the nucleoside phosphatases Gda1p and Ynd1p [44,45], but the phosphate exporter has yet to be identified. Homologues to all these proteins exist in mammals although the sugars and nucleosides vary [46,47]. The possibility that there may be some association of these components to allow substrate channeling between cytosol and active site has yet to be fully explored, but alternatively it may be that the substrates simply accumulate to high levels in the Golgi lumen to ensure efficient and rapid glycan synthesis.

### 3.4. Function of different N-glycans

Mannan forms the outer most layer of the yeast cell wall, covering the glucan and chitin polymers underneath, and thus provides a relatively featureless outer coat that shields the rest of the wall and the plasma membrane from digestive enzymes released by hostile neighbours. Cells which lack mannan have elevated levels of chitin [48], and become dependent on intact

stress-signaling and mitotic check point pathways for survival [49,50]. There are suggestions that some mannan could be covalently linked to β-1,6-glucan, and so contribute directly to cell wall structure [51]. Mannan is also used by yeast to recognise each other in processes such as mating and flocculation [52]. Mannan structure varies greatly between different yeast species with different linkages used, and in some cases additional sugars incorporated. This indicates a strong evolutionary pressure to diversify for reasons of survival, analogous to the idea that some of the diversity of mammalian glycans reflects a pressure to evade microorganisms and viruses. The core-type glycan structures attached to internal proteins have no known function beyond those generally associated with *N*-glycan attachment in folding and quality control in the ER [1]. However it is possible that further functions may emerge. In mammalian cells glycan structures have been proposed to have various roles in protein sorting [25]. There are yeast homologues of ERGIC53, a putative lectin-like receptor for ER to Golgi transport, although so far no phenotypes have been found to be associated with their deletion [53]. Moreover it is not understood how yeast cell wall proteins are specifically targeted to the cell exterior. Secretion of invertase was originally reported to be slowed in *mnn9* strains, an observation which suggests that mannan may possibly contribute a secretion signal [22]. In addition the phosphomannose residues may have roles beyond providing negative charge to mannan where it is thought to affect the surface binding properties of the yeast. Phosphomannose is also attached to internal proteins like CPY, and this appears to be mediated by a distinct transferase as CPY, but not invertase, can acquire the modification in the ER [54].

## 4. Conclusion

Golgi modification of *N*-glycans in yeast may at first appear irrelevant to studies of mammalian cells. However the history of yeast biology has shown many times that such apparent differences can prove deceptive. Indeed it was long argued that yeast had no Golgi since no cisternal stack could be seen in electron micrographs. Thus it seems not unreasonable to suppose that behind the facade of the evolutionarily harassed glycosyltransferases, many of the underlying mechanisms by which they are integrated into the secretory and metabolic pathways of the cell, and so perform their tasks accurately and efficiently, are likely to be well conserved, and hence amenable to study in the tractable and increasingly well understood yeast system.

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