Letting go of O-glycans

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Haddas Saad, Chaitanya Patel, and Gerardo Z. Lederkremer

From the School of Molecular Cell Biology and Biotechnology, George Wise Faculty of Life Sciences, Tel Aviv University, Tel Aviv 69978, Israel

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The generation of free N-glycans, or unconjugated oligosaccharides derived from N-linked glycoproteins, is well understood, but whether a similar fate awaits O-linked glycoprotein carbohydrates was unknown. Hirayama et al. now reveal, by using only mannose as an energy source, the generation of free O-glycans in Saccharomyces cerevisiae, in the lumen of a secretory compartment, possibly the vacuole. These findings uncover the presence of a possible regulated degradation pathway for O-mannosylated glycoproteins.

Glycosylation is the most common co- and post-translational protein modification in eukaryotes and also in many prokaryotes. O- and N-glycosylation are involved in various biological processes, including immune responses; protein quality control, targeting, and degradation; signal transduction; cell interactions and differentiation; and other crucial processes (1). N-Linked glycosylation is performed in the ER,2 where it is tied to quality control events in protein folding; progressive trimming of the N-glycans before their removal serves as a code for glycoprotein folding status. Proteins that fail to fold properly are targeted to endoplasmic reticulum–associated degradation (ERAD) (2) (3). In this process, the misfolded glycoproteins are retrotranslocated from the ER to the cytosol, where, before post-translational degradation, they are exposed to PNGase, a glycosidase that releases the N-glycans from asparagine residues, producing free N-glycans (FNGs) (Fig. 1). FNGs can also be generated in the lumen of the ER from dolichol-linked oligosaccharides by oligosaccharyltransferase. Whereas much is clearly known about N-glycan release and degradation, the same cannot be said for O-glycans. Free oligosaccharides generated from O-linked sugar chains (FOGs) have been detected in extracellular medium, but nothing was known about their generation or even their existence inside cells. Hirayama et al. (4) now report on the characterization of FOGs and propose a mechanism for their formation and disposal in yeast, suggesting a regulation in their generation. This opens the possibility for similar pathways of release of O-glycans in higher eukaryotes.

There are several types of O-glycosylation of serine and threonine residues in mammalian cells. Whereas the most common O-linked sugar chains in mammals are linked to the protein moiety through GalNAc residues, in yeast, the protein-conjugated sugar is mannose. O-Mannosylation begins with the first mannose being added in the ER, both in yeast and in higher eukaryotes. Other sugars are added after transfer of the glycoprotein to the Golgi (5); in Saccharomyces cerevisiae, up to a total of five mannose residues are added. O-Mannosylation has a major role in cell wall integrity in yeast (6, 7). In addition, O-mannosylation appears to terminate folding attempts of misfolded glycoproteins in the yeast ER, targeting them to ERAD (8).

It had been assumed that O-glycans are removed before degradation of glycoproteins bearing them. However, FOGs could never be detected intracellularly, presumably because of their fast disposal. Previous studies from the Suzuki group on the FNGs produced by PNGase (PNG1) had pointed to a cytosolic/vacuolar α-mannosidase Ams1 as likely responsible for FNG degradation. Only FNGs and no FOGs were detected in those studies (9). In an effort to learn more about Ams1 activation, the authors now surprisingly find that novel types of free oligosaccharides are formed upon using mannose (YPMan medium) as the sole carbon source for yeast (4). Treatment with jack bean α-mannosidase, along with extensive structural and linkage analyses, clearly indicated that these oligosaccharides are structurally identical to the O-mannosyl glycans that are covalently attached to yeast glycoproteins. Mutations in enzymes responsible for transfer of mannose residues to glycoproteins in the ER and the Golgi led to a reduction in the generation of the FOGs, suggesting that they are probably generated from protein-linked O-glycans by the action of a putative endo-α-mannosidase. Experiments with trafficking mutants suggested that FOGs appear to be generated in the lumen of a post-Golgi compartment, possibly vacuoles; production of FOGs was reduced when ER-Golgi trafficking of glycoproteins was disrupted. Similarly, FOGs were depleted when an α-mannosidase with a vacuole-targeting signal capable of degrading FOGs was exogenously expressed, but not when Golgi-to-vacuolar transport was simultaneously interrupted.

The authors then looked in more detail at the impact of the medium used. As expected, in YPMan medium, there was an increase in the levels of the nucleotide-sugar GDP-Man; however, this translated only into an increase in FOGs and not in FNGs or in general protein N- and O-glycosylation. Therefore, the most likely scenario is that excess O-glycan synthesis is compensated for by increased glycoprotein degradation or removal of the O-glycans. The addition of a small amount of glucose to the YPMan medium strongly reduced FOG detection, suggesting that FOG generation might require repression

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1 To whom correspondence should be addressed. E-mail: gerardol@taux.tau.ac.il.
2 The abbreviations used are: ER, endoplasmic reticulum; ERAD, ER-associated degradation; FNG, free N-glycan; FOG, free O-glycan; PNGase, peptide N-glycosidase; PKC, protein kinase C.
of genes related to glucose catabolism. Indeed, there was a drastic increase in FOG generation upon deletion of Cyc8, a component of one of the general transcription complexes involved in glucose-mediated repression of genes implicated in processing other carbon sources. Interestingly, these cells also exhibited very slow growth in the YPMan medium. The authors found that the cells showed activation of the cell wall integrity pathway (PKC pathway), which could not be further activated by a cell wall stressor, leading to lethal cell wall defects. The sensor of cell wall integrity, Wsc1, is known to be highly O-mannosylated, suggesting that demannosylation associated with the increased FOGs could destabilize this protein. In agreement with this idea, the authors observed a substantial reduction in Wsc1 at the post-transcriptional level in Cyc8 knockout cells, providing a possible explanation for dysregulation of the PKC pathway.

The discovery of the FOGs raises many questions that should be addressed in future research. What is the mechanism by which Cyc8 controls the generation of FOGs? Does this pathway exist constitutively at a low level, and not only in the absence of glucose and presence of mannose? Does the pathway function when O-mannosylated proteins are degraded? Perhaps the FOGs are normally formed but are quickly degraded; thus, they can only be detected when mannose is present in excess, which causes activation of glycoprotein O-mannosylation and then de-O-mannosylation. The O-glycan detachment could be faster than the subsequent degradation to mannose, which would ultimately serve as an energy source. What is the putative endo-O-α-mannosidase, and what is its subcellular location? Are FOGs degraded by a vacuolar α-mannosidase, like the FNGs? Are the deglycosylated proteins degraded in the vacuole, or are they secreted to any extent from the cell (Fig. 1)? Could FOGs function as signaling molecules? Is the degradation regulated, similarly to what occurs in ERAD, or is it constitutive? Is there a quality control component (e.g., identification of defective O-mannosylated proteins)? And finally, does the pathway exist in mammalian cells? In humans, a lack of O-mannosylation causes congenital muscular dystrophies that are associated with brain abnormalities (10). Would an up-regulated demannosylation pathway lead to a similar functional impairment? It will be sweet to find answers to these and many more questions.

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