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Review

Plant glyco-biotechnology

Jennifer Schoberer, Richard Strasser*

Department of Applied Genetics and Cell Biology, University of Natural Resources and Life Sciences, Vienna, Austria

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ABSTRACT

Glycosylation is an important protein modification in all eukaryotes. Whereas the early asparagine-linked glycosylation (*N*-glycosylation) and *N*-glycan processing steps in the endoplasmic reticulum are conserved between mammals and plants, the maturation of complex *N*-glycans in the Golgi apparatus differs considerably. Due to a restricted number of Golgi-resident *N*-glycan processing enzymes and the absence of nucleotide sugars such as CMP-*N*-acetylneuraminic acid, plants produce only a limited repertoire of different *N*-glycan structures. Moreover, mammalian mucin-type *O*-glycosylation of serine or threonine residues has not been described in plants and the required machinery is not encoded in their genome which enables *de novo* build-up of the pathway. As a consequence, plants are very well-suited for the production of homogenous *N*- and *O*-glycans and are increasingly used for the production of recombinant glycoproteins with custom-made glycans that may result in the generation of biopharmaceuticals with improved therapeutic potential.

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1. Introduction (objective)

In recent years, plants have gained increasing attention as manufacturing hosts for recombinant proteins, particularly for glycoprotein production including different human immunoglobulin subtypes or enzymes for treatment of lysosomal storage diseases. While mammalian expression systems like Chinese hamster ovary (CHO) cells are still widely used by industry, plants provide an interesting alternative to established hosts. One major advantage of plants is their comparable simple*N*-glycan processing pathway and the complete absence of mammalian-type *O*-glycosylation. While mammalian cells produce quite heterogeneous glycans on recombinant proteins and need extensive genome editing to make glycans more uniform [1], the same result can be achieved in plants by few engineering steps [2]. Importantly, plant cells and whole plants like the tobacco related species *Nicotiana benthamiana* tolerate the introduction of novel*N*- and *O*-glycan processing steps quite well.

* Corresponding author at: Department of Applied Genetics and Cell Biology, University of Natural Resources and Life Sciences, Muthgasse 18, 1190 Vienna, Austria.
E-mail address: richard.strasser@boku.ac.at (R. Strasser).

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Here, in this review we compare important aspects of the plant and mammalian glycosylation pathways, discuss current developments in glyco-engineering of plants and focus on the underlying cell biology whose understanding is a prerequisite for further improvements and future applications.

2. *N*- and *O*-glycosylation pathways in plants

2.1. N-*Glycosylation*

A large number of proteins that enter the secretory pathway are glycosylated by the attachment of a preassembled oligosaccharide ($Glc_3Man_9GlcNAc_2$) precursor in the lumen of the endoplasmic reticulum (ER) (Fig. 1). The oligosaccharyltransferase (OST) complex scans the nascent proteins for the presence of the *N*-glycosylation consensus sequence Asn-X-Ser/Thr (X ≠ proline) and transfers the oligosaccharide to asparagine resulting in the formation of *N*-glycosylated proteins [3]. However, not all sites carrying the consensus sequence are modified and efficient *N*-glycosylation depends on additional factors such as the local protein conformation, the position of the site within the protein and the presence or absence of distinct subunits of the multimeric OST complex [4]. While the OST complex is quite well characterized in budding yeast and mammals, we still have an incomplete picture in plants. Nonetheless, it is well established that deficiency of both catalytic subunits of the OST complex is gametophytic lethal in *Arabidopsis thaliana* [5]. Despite some recent advances in *Arabidopsis* and other plants, the exact number of glycoproteins and their *N*-glycosylation site occupancy has not been determined [6–8]. Moreover, for most of the identified plant glycoproteins the composition of the *N*-glycan structures is unknown. Thus, for many plant glycoproteins the particular role of *N*-glycans is largely unexplored. Interestingly, among the large class of membrane-localized receptor kinases there are many examples of heavily *N*-glycosylated proteins like the EF-Tu receptor EFR [9], the flagellin receptor FLS2 [9] or BRASSINOSTEROID INSENSITIVE 1 (BRI1) [10]. All 17 members of the *Catharanthus roseus* receptor-like kinase subfamily in *Arabidopsis* carry several conserved*N*-glycosylation sites in their extracellular domain [11] and glycosylation plays an important role for the function of FERONIA and its homologs [12]. Modifications of the*N*-glycan processing pathway indirectly affect also the *endo*-β1,4-glucanase KORRIGAN1 involved in cellulose biosynthesis [13,14].

Upon transfer of the preassembled oligosaccharide, the*N*-glycan is subjected to several processing steps which take place in the lumen of the ER. Glucosidases I and II trim off the terminal and penultimate glucose residues. The arising monoglucosylated glycan can be recognized by the lectins calnexin (CNX) or calreticulin (CRT) that are part of the glycan-dependent ER quality control and promote folding [15]. Glycoproteins with a native conformation are released from the CNX/CRT cycle and allowed to enter the Golgi apparatus and other compartments of the secretory pathway. Incompletely folded or aberrant glycoproteins, on the other hand, are recognized by the folding sensor UDP-glucose:glycoprotein glucosyltransferase (UGGT) or sent for degradation via a glycan-dependent ER-associated degradation (ERAD) pathway. In the current view of UGGT function, UGGT binds to exposed hydrophobic sequence patches and catalyses the transient re-glucosylation of the glycan enabling another interaction of the incompletely folded glycoproteins with CNX/CRT. In contrast to mammals, UGGT-deficiency does not affect the viability of *Arabidopsis* [10]. However, UGGT is required for the biogenesis of plant pathogen receptors and *uggt* mutants display shorter roots and a delay in growth of aerial parts [16–18]. While the knockout of all three *Arabidopsis* CRTs or the two CNX forms is well tolerated in plants [19,20], the absence

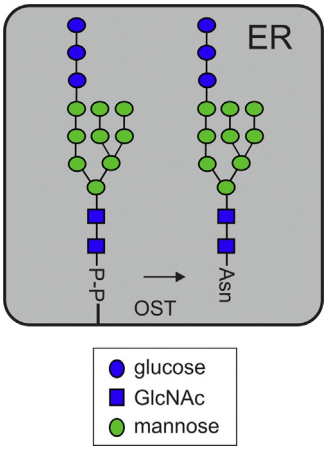

Fig. 1. Schematic illustration of the preassembled oligosaccharide precursor.

of all CNX/CRTs is lethal suggesting that a single round of CNX/CRT interaction with a monoglucosylated glycoprotein is essential for plants.

As the ER is a biosynthetic organelle that lacks a machinery for protein degradation, non-native proteins have to be removed from the organelle by a regulated process to prevent their aggregation or transport of defective proteins to other compartments of the secretory pathway. The ERAD pathway is commonly used to clear these aberrant proteins. ERAD in mammals and yeast is a multistep pathway involving recognition of the substrate in the ER lumen, transport to a membrane-embedded E3 ligase complex, translocation across the membrane from the ER into the cytosol, polyubiquitination and subsequent degradation by the 26S proteasome [21]. In *Arabidopsis*, several ERAD factors involved in the early steps of glycoprotein degradation have been identified. This glycan-dependent ERAD pathway requires recognition of a bipartite signal composed of an aberrant protein conformation and at least one attached oligomannosidic*N*-glycan [22,23]. In yeast, a complex of the α-mannosidase HTM1 and the oxidoreductase PDI1 preferentially processes non-native protein conformations and thus appears crucial for the first committed step of glycan-dependent ERAD [24,25]. Within this complex PDI1 might direct the HTM1 activity to distinct misfolded glycoproteins. MNS4 and MNS5, the *Arabidopsis* HTM1 homologs, remove a single terminal mannose residue from the oligomannosidic*N*-glycans of ERAD substrates but are not required for processing of properly folded secretory proteins [26,27]. How MNS4/MNS5 distinguish between native and non-native glycoproteins is currently unknown. Upon MNS4/MNS5-catalysed glycan trimming, a specific α1,6-linked mannose is exposed enabling interaction with the luminal lectin-like protein OS9 and the SEL1L-HRD1 ERAD complex [27–31]. Genetic and biochemical evidence further indicates that the interaction of an aberrant glycoprotein with the OS9-SEL1L-HRD1 complex leads to the disposal of misfolded glycoproteins by a degradation route that is still ill-defined in plants.

As soon as a glycoprotein destined for secretion passes all quality control steps in the ER, it is allowed to enter other compartments of the endomembrane system. For the vast majority of glycoproteins, the major trafficking route will send them through the Golgi where they encounter different glycosidases and glycosyltransferases that mediate the formation of complex*N*-glycans [3]. In the first step, three additional mannose residues are cleaved off by Golgi α-mannosidase I thereby generating $Man_5GlcNAc_2$ that is used by*N*-acetylglucosaminyltransferase I (GnTI) to initiate complex*N*-glycan formation (Fig. 2). Further processing to $GlcNAc_2Man_3XylFucGlcNAc_2$ containing glycans is carried out by Golgi α-mannosidase II,*N*-acetylglucosaminyltransferase II,

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