



Review article

Function of *N*-glycosylation in plantsYukihiro Nagashima^a, Antje von Schaewen^b, Hisashi Koiwa^{a,*}^a Department of Horticultural Sciences, Texas A&M University, College Station, TX, 77843, USA^b Molekulare Physiologie der Pflanzen, Institut für Biologie & Biotechnologie der Pflanzen, Westfälische Wilhelms-Universität Münster, Schlossplatz 7, 48149, Münster, Germany

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ABSTRACT

Protein *N*-glycosylation is one of the major post-translational modifications in eukaryotic cells. In lower unicellular eukaryotes, the known functions of *N*-glycans are predominantly in protein folding and quality control within the lumen of the endoplasmic reticulum (ER). In multicellular organisms, complex *N*-glycans are important for developmental programs and immune responses. However, little is known about the functions of complex *N*-glycans in plants. Formed in the Golgi apparatus, plant complex *N*-glycans have structures distinct from their animal counterparts due to a set of glycosyltransferases unique to plants. Severe basal underglycosylation in the ER lumen induces misfolding of newly synthesized proteins, which elicits the unfolded protein response (UPR) and ER protein quality control (ERQC) pathways. The former promotes higher capacity of proper protein folding and the latter degradation of misfolded proteins to clear the ER. Although our knowledge on plant complex *N*-glycan functions is limited, genetic studies revealed the importance of complex *N*-glycans in cellulose biosynthesis and growth under stress.

1. Introduction

N-glycosylation is a major post-translational modification in eukaryotes and involves a large number of enzymes that are necessary for the production of lipid-linked and nucleotide-conjugated sugar monomers, enzymes for *core* oligosaccharide synthesis, their transfer to asparagine residues within nascent polypeptides, and further maturation of the oligosaccharides [1–4]. The biosynthetic pathway of the sugar monomers overlaps with the biosynthesis of ascorbate as well as with those used for cell wall polysaccharide precursors, including mannose, fucose, xylose and galactose [5]. The *N*-glycosylation reaction in the endoplasmic reticulum (ER) is an essential process for cell viability, because the attached *core* *N*-glycans are part of the protein-folding machinery operating on secreted glycoproteins and membrane proteins in the ER [1,2]. Fig. 1 summarizes enzymes and *N*-glycan structures in the plant *N*-glycosylation pathway. Structural modifications of these *N*-glycans mark the protein folding status in making decisions whether or not to allow exit from the protein folding cycle mediated by ER chaperones [1]. Upon successful folding of their polypeptide backbones, glycoproteins are exported from the ER to the Golgi apparatus, where the *N*-glycan moieties undergo further maturation to form characteristic complex-type *N*-glycans. Alternatively, glycoproteins may exit the ER via an alternative route to vacuoles retaining high-mannose *N*-glycan structures without Golgi modifications. In comparison to human

complex *N*-glycans, plant complex *N*-glycans uniquely contain *core* α 1,3-fucose (Fuc) and β 1,2-xylose (Xyl) modifications, and may contain terminal Lewis-a epitopes (β 1,3-galactose (Gal) and α 1,4-Fuc linked to terminal *N*-acetylglucosamine, GlcNAc) (Fig. 2). Human complex *N*-glycans are often sialylated and may contain different epitopes, like Lewis x, *N*-acetylglucosamine (LacNAc), and *N,N*-di-*N*-acetylglucosamine (LacDiNAc). While glycan epitopes of human complex *N*-glycans are often targets of lectins important for cell-cell communication, the biological significance of plant *N*-glycans beyond protein folding, in particular modifications in the Golgi apparatus and post-Golgi compartments, had long been neglected. However, studies in the last decade started to uncover specific roles of *N*-glycans in various biological processes. This review will highlight the current view of *N*-glycosylation biology in plants. For consistency, the nomenclature of genes follows that of *Arabidopsis thaliana* unless indicated otherwise.

2. *N*-glycosylation in the ER

2.1. Oligosaccharyltransferase

Protein *N*-glycosylation in the strict sense starts with *en-bloc* transfer of pre-assembled *core* oligosaccharide structures (glucose₃mannose₉*N*-acetylglucosamine₂ (Glc₃Man₉GlcNAc₂)) to nascent polypeptide chains emerging from the translocation channel of the ER [6] (Fig. 1). The

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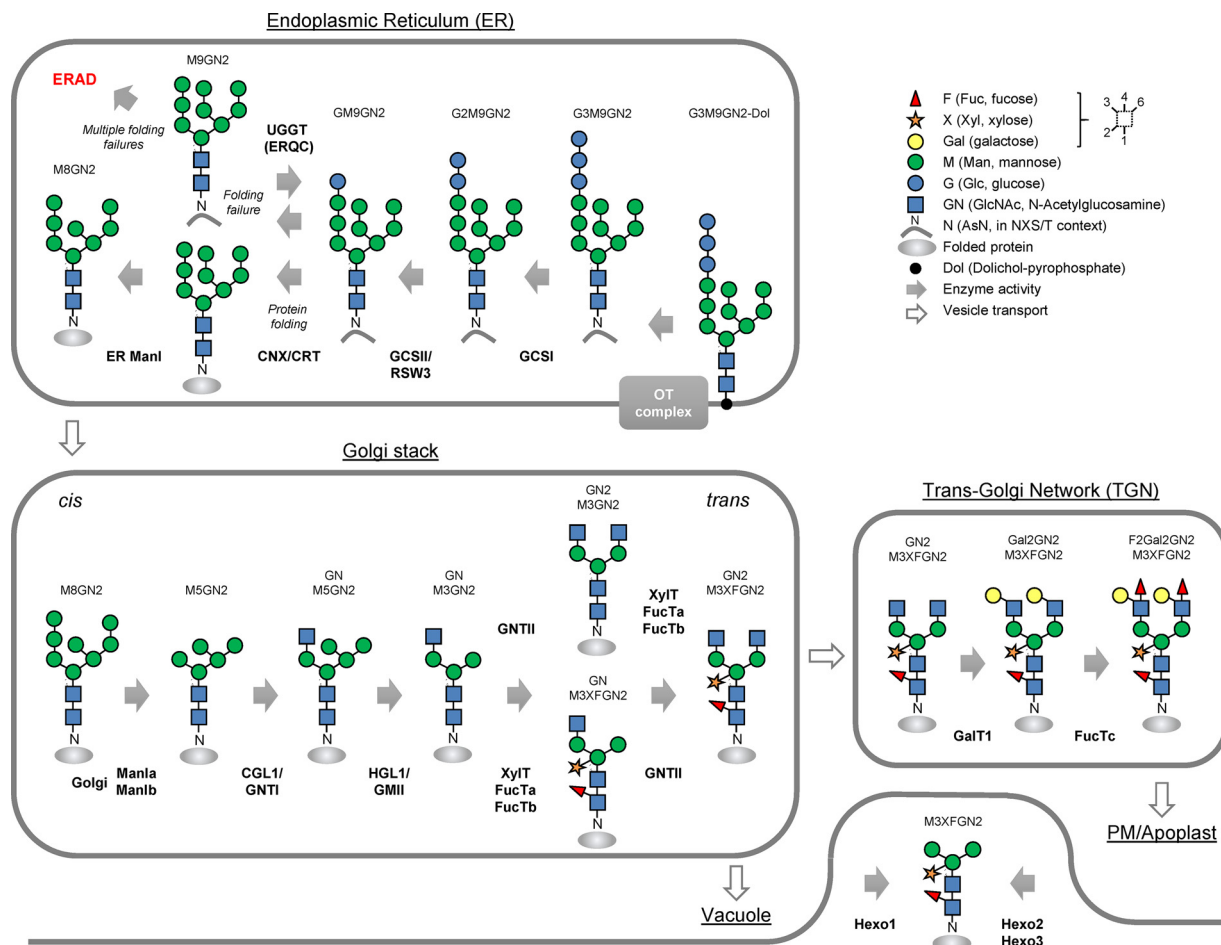


Fig. 1. Formation of complex *N*-glycans in plants. Structures of the lipid-linked core-oligosaccharide substrate of the membrane-bound Oligosaccharyltransferase (OT complex) and asparagine-linked oligosaccharides at each step of processing are shown. The lipid-linked oligosaccharide is transferred to side chain amino group of asparagine in the *N*-glycosylation sequon (NXS/T). Resulting *N*-linked core oligosaccharides undergo folding-coupled *N*-glycan processing in the ER. Successfully folded proteins will be processed by ER-mannosidase I and exported to the Golgi apparatus. In the Golgi apparatus, *N*-glycans are modified by a series of glycosidases and glycosyltransferases, while glycoproteins travel through the Golgi stack from the *cis* to the *trans* side and through the *trans*-Golgi network (TGN) to form mature complex *N*-glycans. Some *N*-glycans of secreted glycoproteins are further modified by TGN-localized glycosyltransferases to form Lewis-a epitopes at their termini. At the plasma membrane (PM) and in the Apoplast or vacuole, hexosaminidases produce highly immunogenic complex *N*-glycans (exposed core xylose and fucose). ERAD, ER-associated degradation; RSW, radially swollen (root tips of mutant plants). For further explanations, see text.

acceptor site is the side-chain amino group of asparagine residues in the sequon "Asn-X-Ser/Thr" (NXS/T). In the sequon, X represents any amino acid except proline, and the third amino acid - on rare occasions - may also be a cysteine [7]. This reaction is mediated by a conserved oligosaccharyltransferase (OT) complex in eukaryotes including plants. The subunit composition of OT complexes varies slightly among organisms. The OT from the yeast *Saccharomyces cerevisiae* contains eight subunits encoded by nine genes (OST1 [OLIGOSACCHARYLTRANSFERASE 1], OST2, OST3/6, OST4, OST5, WHEAT GERM AGGLUTININ-BINDING PROTEIN 1 [WBP1], SUPPRESSOR OF WBP1 [SWP1] and STAUROSPORINE AND TEMPERATURE SENSITIVE 3 [STT3]), of which seven are shared with animal and plant OT complexes [8,9]. Three additional subunits DC2, keratinocyte-associated protein 2 (KCP2), selenoprotein T (SelT), and OT-interacting Selenoprotein K (SelK) have been reported for the human OT [10–14]. Our recent study established that Arabidopsis OT complexes contain seven conserved subunits encoded by twelve genes (Table 1, Fig. 3), which are conserved among yeast and animal OT complexes [15]. A three-dimensional structure model of the Arabidopsis OT based on electron microscopy of purified OT complexes revealed 1:1 association with a ribosome. The overall structure was similar to that of the *in vitro*-reconstituted OT-ribosome supercomplex from yeast [16]. In addition, the Arabidopsis genome encodes homologs of human DC2 (At2g19340/At4g29870) and KCP2 (At1g77350), SelT

(At3g47300/At5g58640) and SelK (At4g08230), but no yeast OST5 subunit homologs, suggesting that plant OT complexes are closer to the two human ones than the single yeast OT (Table 1, Fig. 3). Extensive duplication of Arabidopsis OT subunit genes likely originated from ancient whole genome duplication events, which made functional diversification between isoforms possible. Indeed, the catalytic OT subunit (STT3) is encoded by two genes in the human as well as in the Arabidopsis genome, and human OT complexes with different STT3 isoforms display different catalytic properties [17]. Genetic analysis using Arabidopsis mutants showed that cells depleted for any of the conserved OT subunits produce *N*-glycosylation defects and often result in lethality [18–20,15]. Functions of some individual subunits have been characterized in detail in other systems [21,22]. STT3 is the catalytic subunit that binds the lipid-linked core-oligosaccharide substrate [17], OST3/6 contains a thioredoxin domain that prevents aggregation of the nascent polypeptide chain during *N*-glycosylation reactions [23], and OST1 may bind to the polypeptide substrate [24].

2.2. ER quality control (ERQC)

Once core oligosaccharides are transferred to Asn (N) residues in the polypeptide chain, terminal glucose residues are removed from the core oligosaccharides by glucosidases I and II (GCS1 and GCS2) in the ER

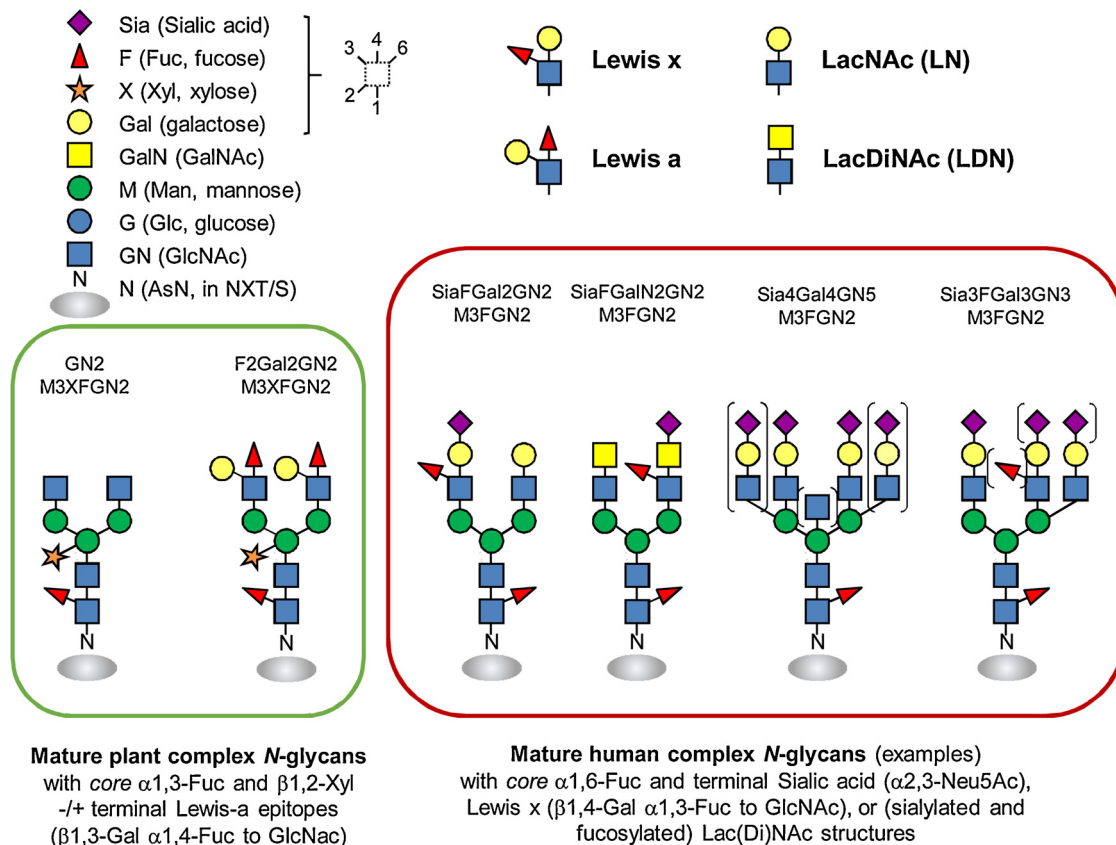


Fig. 2. Comparison of plant and human complex *N*-glycan structures. Mature complex *N*-glycans of plants typically contain core α1,3-Fuc and β1,2-Xyl residues, whereas those of humans contain core α1,6-fucose. Some plant complex *N*-glycans carry terminal Lewis-a epitopes (β1,3-Gal and α1,4-Fuc linked to GlcNAc). In contrast, antennae of human complex *N*-glycans often contain sialylated Lewis-x epitope(s) or sialylated Gal (also with Fuc).

lumen (Table 1, Fig. 1). Such trimmed *N*-glycan structures are recognized by the lectin chaperones calnexin (CNX) and calreticulin (CRT), which are associated with peptide disulfide isomerase (PDI)/ERp57, at least in animal cells [47]. If protein folding cannot be completed, unfolded proteins are recognized and re-glucosylated by UDP-glucose:glycoprotein glucosyltransferase (UGGT), an ER-resident protein-folding sensor [48] (Fig. 1). As a result, the glycoprotein re-associates with CNX/CRT for an additional folding cycle. Successfully folded glycoproteins are exported to the Golgi apparatus, whereas proteins that fail to adopt their native conformation after multiple rounds of folding attempts are degraded by an ER-associated degradation pathway [49,50]. Trimming of terminal mannoses from *N*-glycans by class I α-mannosidases MNS4 and MNS5 (homologs of mammalian ER Degradation-Enhancing α-Mannosidase-like, EDEM) in the ER marks misfolded glycoproteins for degradation [31]. The resulting Man₇GlcNAc₂ Nglycans with a terminal α1,6-linked mannose are recognized by the lectin domain of OS9 [51,52] and directed to the E3 ligase complex HRD1/SEL1L for retrograde transport coupled with cytosolic ubiquitination and subsequent degradation by the 26S-proteasome pathway [53,54].

2.3. Unfolded protein response (UPR)

Underglycosylation results in failure to fold properly and accumulation of protein aggregates in the ER lumen. For eukaryotic cells, such states pose a severe stress (ER stress), and they try to restore normal ER function by inducing a set of genes important for promoting protein folding and eliminating abnormal proteins. A classic example of such a protein is luminal IgG Binding protein BiP (BiP1, BiP2, and BiP3 in Arabidopsis), an ER isoform of the heat shock protein 70 (Hsp70) class. BiP, together with co-chaperone ERdj isoforms [55], promotes folding

of (thermally) unfolded proteins in the ER. This cellular response is known as the ER stress response or unfolded protein response (UPR). In Arabidopsis, UPR genes are mainly induced by two key transcription factors, bZIP28 and bZIP60 (Fig. 4). Under unstressed conditions, Transcription factor bZIP28 is an integral membrane protein of the ER. However, upon ER stress, bZIP28 migrates from the ER to the Golgi apparatus, where the protein is cleaved by Golgi-localized site-2 protease (S2P) [56,57]. Thus released cytosolic domain (with transcriptional activity) moves into the nucleus and initiates transcription of its target genes. On the other hand, bZIP60 is activated by unusual mRNA splicing involving INOSITOL-REQUIRING ENZYME 1 (IRE1), which is an ER-localized type-I transmembrane sensor/transducer protein. Also Arabidopsis IRE1 homologs (IRE1a/IRE1b) likely sense unfolded proteins through their *N*-terminal domains in the ER lumen. Based on research in other species [58], BiP binds to the IRE1 N-terminal domain in the resting state, which prevents activation of IRE1. Upon increase of unfolded proteins in the ER lumen, BiP is released from IRE1, allowing IRE1 to dimerize for activation. The RNase domain of IRE1 is activated through autophosphorylation of its kinase domain in the cytosol [59–61], and removes a 23-nucleotide intron from the *bZIP60* mRNA by cleaving at two specific points on the two stem-loop forming regions. The digested fragments are ligated by tRNA ligase [62]. Unspliced *bZIP60* (*bZIP60u*) codes for 295 amino acids, including a transmembrane domain (TMD), whereas spliced *bZIP60* (*bZIP60s*) codes for 258 amino acids, lacking the TMD. Whereas bZIP60u resides in the ER membrane due to the TMD, bZIP60s can translocate to the nucleus and induce its target genes. TMD removal creates a new nuclear localization signal (NLS) in addition to the already existing NLS [63,58,64]. Besides unconventional splicing of *bZIP60*, IRE1 cleaves mRNAs at the ER membrane in order to reduce protein loading into the ER, termed regulated IRE1-dependent decay (RIDD) [65]. Moreover, IRE1b- but

Table 1
Arabidopsis genes involved in N-glycosylation and N-glycan maturation pathways.

Name	Locus	Human ortholog	Description	Reference
N-glycosylation				
OT subunits				
OST1a	At2g01720	RPN1	Ribophorin I	[15]
OST1b	At1g76400			
OST3/6	At1g61790	N33	Thioredoxin-like	[25]
OST3/6-like	At1g11560			
OST4a	At4g02502	OST4		[25,15]
OST4b	At3g12587			
STT3a	At5g19690	STT3B	Catalytic subunit	[18,15]
STT3b	At1g34130	STT3A		
DGL1	At5g66680	DDOST		[20,15]
HAP6	At4g21150	RPN2	Ribophorin II	[19,15]
DAD1	At1g32210	DAD1		[26,15]
DAD2	At2g35520			
DC2a	At2g19340	DC2		N/A
DC2b	At4g29870			
KCP2	At1g77350	KCP2	Keratinocyte-associated protein 2	N/A
SELTa	At3g47300	SELT	Thioredoxin reductase-like	N/A
SELTb	At5g58640			
SELK	At4g08230	SELK		N/A
N-glycan maturation				
ER				
GCS1/KNOPF/HAP2	At1g67490	MOGS	α -glucosidase I	[27,28,19]
GCS2/RSW3	At5g63840	GANAB	α -glucosidase II	[29]
GCS2 β /PSL4	At5g56360	GLU2B		
MNS3	At1g30000	MAN1B1	ER α -mannosidase I	[30]
MNS4	At5g43710	EDEM1	EDEM-like α -mannosidase	[31]
MNS5	At1g27520	EDEM2		
		EDEM3		
Golgi apparatus				
MAN1a/MNS2	At1g51590	MAN1A1	Golgi α -mannosidase I	[30,32]
MAN1b/MNS1	At3g21160	MAN1A2		
CGL1/GNTI	At4g38240	MGAT1	N-acetylglucosaminyltransferase I	[33–35]
HGL1/GMII	At5g14950	MAN2A1	Golgi α -mannosidase II	[36]
GNTII	At2g05320	MGAT2	N-acetylglucosaminyltransferase II	[37]
FUCTa/FUT11	At3g19280	–	core α -1,3-fucosyltransferase	[38]
FUCTb/FUT12	At1g49710			
XYLT	At5g55500	–	α -1,2-xylosyltransferase	[38,39]
GALT1	At1g26810	–	β -1,3-galactosyltransferase	[40]
FUCTc/FUT13	At1g71990	–	α -1,4-fucosyltransferase	[41–43]
Apoplast/Vacuole				
HEX01	At3g55260		β -hexosaminidase	[44–46]
HEX02	At1g65590			
HEX03	At1g05590			

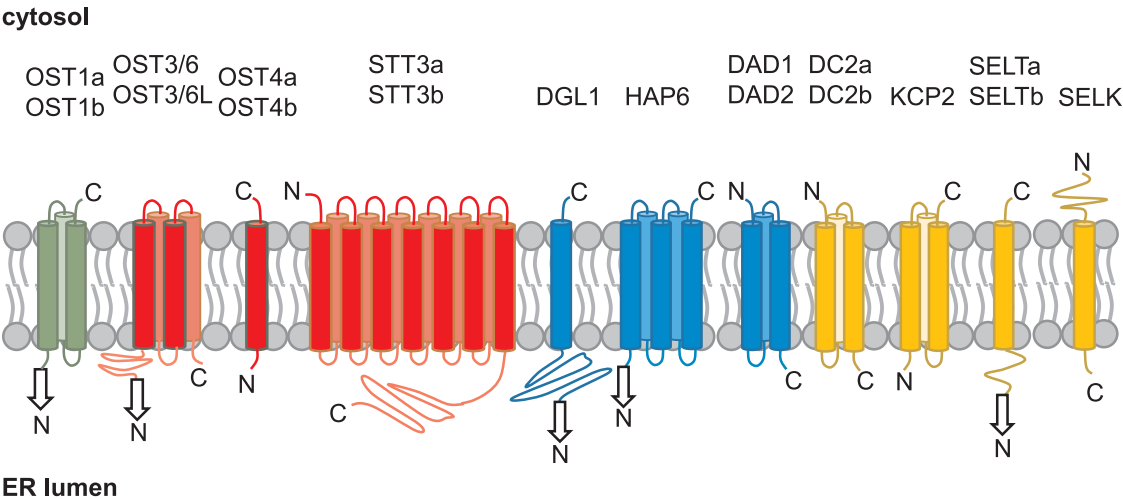


Fig. 3. Subunits of the plant OT complex. A model for *Arabidopsis* OT subunit composition and membrane topology is shown. The color code for the subunits (green, red and blue) designates subcomplexes proposed for eukaryotic OTs. Topologies of previously uncharacterized subunits (DC2s, KCP2, SELTs and SELK) were predicted based on amino acid sequence similarities and topology of the human homologs and are shown in orange. Open box arrows represent predicted signal peptides.

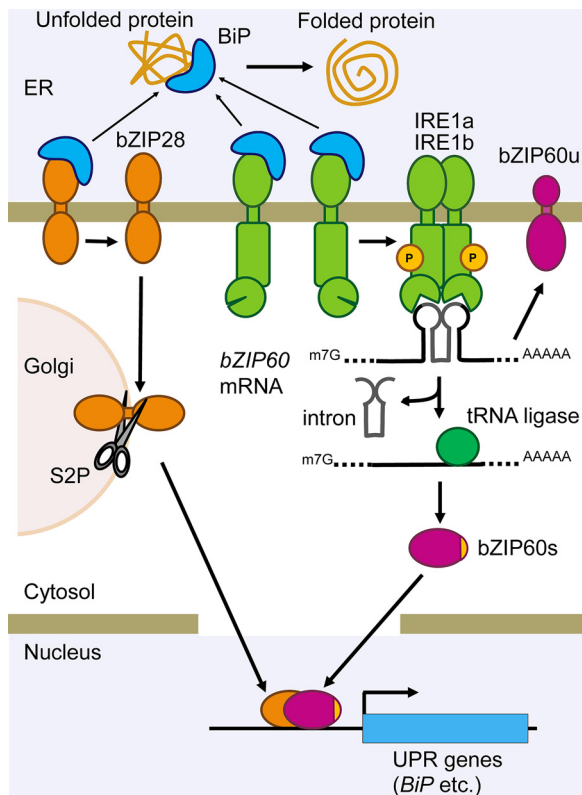


Fig. 4. Unfolded protein response signaling in plants. When cells undergo stress that promotes accumulation of unfolded proteins in the ER, BiP proteins are released from luminal domains of integral membrane proteins bZIP28 and IRE1a/b. Chaperone activity of free BiP promotes folding of ER proteins. Simultaneously liberated bZIP28 is exported to the Golgi apparatus and processed by site-2 protease (S2P). The clipped cytoplasmic transcriptional activator domain is then transported into the nucleus. On the other hand, BiP release activates IRE1a/b proteins by dimerization that catalyze, together with tRNA ligase, cytoplasmic splicing of *bZIP60* mRNA. Before cytoplasmic splicing, *bZIP60* mRNA encodes a transcriptionally inactive integral membrane protein bZIP60u. The cytoplasmic splicing causes a frameshift in bZIP60 mRNA. Upon splicing, *bZIP60s* encodes a soluble, nuclear targeted transcription factor that can dimerize with the S2P-released bZIP28, or homodimerize and activate various UPR genes.

not IRE1a-dependent autophagy has been reported to occur under ER stress [66].

3. N-glycan maturation in the Golgi apparatus and post-Golgi compartments

Upon exit from the ER, glycosidases and glycosyltransferases in the Golgi apparatus convert oligomannosidic *N*-glycan intermediates to mature complex *N*-glycans [4] (Table 1, Fig. 1). Complex *N*-glycans of plants contain *core* α 1,3-Fuc and β 1,2-Xyl residues that are absent in mammalian *N*-glycans [67,68] (Fig. 2). Also notably, maturation of complex *N*-glycans is not uniform among plants and different tissues analyzed [69,40]. In the *cis*-Golgi, oligomannosidic *N*-glycans undergo sequential processing by Golgi α -mannosidase I (MAN1a/MAN1b) [30,32] and β 1,2-*N*-acetylglucosaminyltransferase I (GNTI). The latter is encoded by *CGL1* (*complex glycan-less 1*) in Arabidopsis [70]. Then a group of enzymes comprising Golgi α -mannosidase II (ManII, encoded by *HGL1* for *hybrid glycan 1*) [36], GNTII [37], β 1,2-xylosyltransferase (XylT) [71], and α 1,3-fucosyltransferase (FucTa/FucTb) [38] work in parallel to produce complex *N*-glycan structures in the *medial*- and *trans*-Golgi cisternae. Some *N*-glycans of secreted glycoproteins are further modified to Lewis-a epitopes [72] by the action of β 1,3-

galactosyltransferase (GalT1) [40] and α 1,4-fucosyltransferase (FucTc) [73]. At their final destinations, *N*-glycan structures are trimmed by hexosaminidases (Hexo) in the apoplast/cell wall and vacuole with time, cleaving at terminal GlcNAc residues, which results in paucimannosidic and highly immunogenic Man₃XylFucGlcNAc₂ structures (Fig. 1, M3XFGN2) [45,46]. In Arabidopsis, about three quarters (73.9%) of all *N*-linked glycans are converted to various complex-types. The rest are oligomannosidic *N*-glycans of different sizes [38].

Together with the biosynthesis of cell wall polysaccharide precursors in the Golgi apparatus, these reactions produce a large amount of GDP/UDP released from sugar-nucleotide substrates. These dinucleotide byproducts are processed by Golgi apyrases (APY1/APY2) [74], and the liberated orthophosphate (Pi) is exported to the cytosol for recycling, likely by PHT4;6 [75]. The remaining UMP/GMP moieties are exported in exchange for import of activated (GDP/UDP) sugar monomers into the Golgi/ER by sugar-nucleotide transporters [76,77]. Mutant *phT4;6* and transgenic plants simultaneously silenced for *APY1* and *APY2* expression exhibited phenotypes similar to *N*-glycosylation mutants (originally found for cellulose biosynthesis mutants), suggesting that nucleotide recycling is an integral part of the *N*-glycosylation pathway in plants.

Historically, biosynthetic enzymes of plant-specific complex-*N*-glycans have also been studied by the biotechnology sector, due to a concern for their potential antigenicity to elicit unintended immune reactions when presented as part of plant-produced clinical proteins, such as antibodies. Although it is still controversial, whether plant complex *N*-glycans are involved in eliciting allergic reactions in patients [78–80], most therapeutic protein production studies in plants use host plant modification to avoid plant specific *N*-glycan structures, like *core* β 1,2-Xyl and α 1,3-Fuc [81,82].

4. Target-specific functions of N-glycosylation

4.1. Receptor protein biogenesis/function

Studies have demonstrated that biogenesis and function of several receptor-like kinases (RLKs) are particularly prone to perturbations by compromised ER *N*-glycosylation/quality control pathways. The first reported example for such an observation was the Arabidopsis EF-TU receptor (EFR), a pattern recognition receptor (PRR) involved in the perception of bacterial Elongation factor Tu (EF-Tu) fragments [83–86]. EFR was specifically impaired by loss-of-function, not only of STT3a (OT catalytic subunit), but also of calreticulin3 (CRT3), UDP-glucose:glycoprotein glycosyltransferase (UGGT), and two ER chaperone complex components (STROMA DERIVED FACTOR 2 [SDF2] and ER DnaJ-like3b [ERdj3b]). This suggested that protein folding during EFR biogenesis, rather than the lack of any particular *N*-glycan was important for EFR function [83–86]. However, mutation at a single conserved *N*-glycosylation site (N143Q) in the EFR ectodomain was sufficient to inactivate the receptor for the ability to bind its ligand [86]. In related defense signaling pathways, underglycosylation in *stt3a* mutant plants inhibited plant cell death in *bir1* and *bak1 serk4* mutants [87]. In the latter, *stt3a* likely prevented cell death by interfering with biogenesis/function of another group of cell death-inducing receptor kinases (Cysteine-Rich Kinases CRK4 and CRK5). Of note, biogenesis of another BAK1-interacting receptor kinase, BRASSINOSTEROID INSENSITIVE 1 (BRI1), is also under control of the ERQC pathway. Here, *bri1-5* and *bri1-9* mutant alleles produced aberrant BRI1 proteins that are retained in the ER prior to being sent for degradation; yet when ERQC was compromised, some aberrant receptor proteins escaped degradation [88,89]. Interestingly, mutations in different ERQC components affected *bri1-5* and *bri1-9* differently, suggesting several folding status checkpoints that call for different sets of ERQC components [89]. By contrast, another PRR family receptor kinase, FLAGELLIN SENSITIVE 2 (FLS2), was not affected in *stt3a* or by ERQC pathway mutations, indicating that folding of some receptor kinase proteins is more robust

[83–86].

N-glycosylation is also important for gametophyte recognition. Mutations in a uridine diphosphate (UDP)-glycosyltransferase superfamily protein (TURAN, TUN) and a dolichol kinase (EVAN, EVN), both involved in early *core N*-glycan assembly processes, resulted in inhibition of pollen tube (PT) rupture and sperm release [90]. Less severely affected, *A. thaliana* harboring the *ost3/6* mutation can accept own pollen but no longer pollen from the related species *A. lyrata*, due to failure to induce PT rupture and sperm release [91]. Central to this process is another receptor-like kinase, FERONIA, and the pollen-specific FER homologs ANXUR1 (ANX1) and ANX2 [92–94]. In addition to their common role during PT reception by the synergids, both genes have distinct functions in the pollen: EVN is essential for pollen development, whereas TUN is required for biogenesis and stabilization of ANX1/ANX2 during PT growth, and prevention of premature PT rupture [90]. However, complex *N*-glycans do not seem to play a major role during these phases, since we did not observe deviations from the expected 1:1 transmission ratios upon reciprocal crosses between *CGL1/cgl1* and *cgl1* (K. Fischer and A. von Schaewen, unpublished observations for *cgl1-2* and *cgl1-3* [32]).

A unique type of receptor whose function is dependent on *N*-glycosylation is vacuolar sorting receptor VSR1 [95]. There are three *N*-glycosylation sites in VSR1, and loss of two or more *N*-glycans resulted in secretion of vacuolar proteins from the cells due to the reduction of binding affinity of underglycosylated VSR1 to vacuolar sorting signal peptides. The role of *N*-glycans in VSR1 function is not clear. Underglycosylation of VSR1 did not affect folding or subcellular localization of VSR1, and requirement for specific *N*-glycan modification has not been analyzed. Interestingly, VSR1 is required for osmotic stress tolerance and ABA biosynthesis [96], which may partially contribute to the salt sensitivity of *N*-glycosylation pathway mutants [18,35].

4.2. Cell wall biosynthesis and salt stress tolerance

4.2.1. KORRIGAN1 as a target of complex *N*-glycan function

In human and mouse cells, defects in complex *N*-glycan maturation cause congenital disorders of glycosylation (CDG) [97–100]. In human CDG, as well as in analogous perturbation mutants of *Drosophila melanogaster* (fruit fly) and *Caenorhabditis elegans* (nematode), development of the central nervous system and the immune systems are often compromised or altered [98,101]. In Arabidopsis plants, the first functional target of complex *N*-glycan modification was identified by a knowledge-based approach: KORRIGAN1 (KOR1) is a type II transmembrane glycoprotein with beta-1,4-endoglucanase activity [102]. Arabidopsis *stt3a* and *cgl1* mutations exhibited strong genetic enhancer activities when they were combined with *radially swollen 2-1* (*rsw2-1*), a temperature-sensitive mutant allele of KOR1 [103,104]. Presently, *rsw2-1* is the sole mechanistic target that has been used to test the function of individual complex *N*-glycans in the Arabidopsis model. The receptor proteins discussed above apparently function properly in complex glycan mutants, suggesting their roles are not dependent on *N*-glycan maturation in the Golgi apparatus, as long as *core N*-glycans are attached to the receptors during their biosynthesis in the ER [86].

Similar to other *RSW* genes, KOR1 is required for normal cellulose biosynthesis. However, the exact function of KOR1 is still not clear. KOR1 localizes to the plasma membrane (PM), trans-Golgi network (TGN), and tonoplast [105,106], and interacts with cellulose synthase complexes (CSCs) [107,108] (Fig. 5). KOR1 has been suggested to function in cleavage of the sterol-linked primer molecule (sitosterol-glucoside) during the cellulose chain elongation [109], however, recent studies showed that KOR1 facilitates cellulose biosynthesis by increasing the amount of non-crystalline cellulose [110] and proposed that KOR1 prevents the formation of cellulose aggregates [111]. Interestingly, a recent report showed that a biochemically inactive allele of KOR1 (*jiaoyao1*) partially retained *in vivo* function [112]. Together, these reports indicate that KOR1 might function in more than one way

during cellulose biosynthesis.

Upon biosynthesis in the ER, KOR1 undergoes *N*-glycosylation and further modification of its *N*-glycans in the Golgi apparatus (Figs. 2 and 5). Eight *N*-glycosylation sites are present in KOR1 amino acid sequences [106] (Fig. 6A). Disruption of some *N*-glycosylation sites affected the cellular distribution and its enzymatic activity [113,106]. The importance of complex *N*-glycans for KOR1 function was inferred from genetic interaction between *cgl1* and *rsw2-1*. However, even a non-glycosylated variant of KOR1 still required CGL1 (GNTI in the *cis*-Golgi) for *in vivo* function. This suggested that complex *N*-glycans also affect KOR1 *in trans*, likely via interaction with other glycoproteins [106]. Genetic identification of additional factors that depend on complex *N*-glycans has been difficult, because it requires the comparison of mutant phenotypes, between a wild-type background and a mutant complex *N*-glycan background, such as *cgl1*. Also, the comparison requires partial loss-of-function mutations in a gene-of-interest to test protein function with or without complex *N*-glycans, as opposed to widely available insertion mutants that abrogate protein production. An inhibitor of mannosidase I (kifunensine) can phenocopy complex *N*-glycan mutant phenotypes [30]: several viable point mutations affecting cellulose biosynthesis, like *rsw2-1* (*kor1*) or *cobra-1*, were hypersensitive to kifunensine, suggesting that complex *N*-glycan-dependent phenotypes may be screened by using a chemical hypersensitivity assay.

4.2.2. The intracellular transport of the cellulose biosynthesis machinery is a target of salt stress

CSC, which is assembled in the Golgi apparatus [114], and KOR1 are actively shuttled between the plasma membrane (PM) and the TGN (Fig. 5). Functionally compromised KOR1 tended to accumulate in tonoplast membranes, suggesting that the cellular transport of KOR1 is closely linked to its physiological function [106]. Movement of KOR1 and CSC to and from the PM, as well as at the PM, is guided by microtubules (MT) [115,116]. MT structure and organization (cortical MTs, below the PM) determines the directional deposition of cellulose microfibrils. Recent studies identified multiple factors that connect the CSC to microtubules, and importantly, mutations in these factors phenocopy *rsw* mutants.

The distribution of KOR1, CSC, and the microtubule network undergoes a dynamic reorganization during salt stress [117]. Upon exposure to salt, plant cells induce depolymerization of their cortical MTs, CSCs at the PM are internalized and accumulated below the PM in so-called *small CesA-containing compartments* (SmaCCs) [116] or *microtubule-associated CesA compartments* (MASCs) [115]. Stress-induced MT depolymerization is likely triggered by tubulin kinase activity of PROPYLAMIDE-HYPERSENSITIVE 1 (PHS1) [118]. High salt treatment induces a transient increase of phosphorylated α -tubulin, which cannot be incorporated into microtubules. Re-organization of CSCs and MTs is facilitated by CC1/CC2 (COMPANION OF CSC 1 and 2) - with two potential *N*-glycosylation sites in their ectodomains, and CELLULOSE SYNTHASE INTERACTING PROTEIN 1 (CSI1/POM2) - a cytosolic protein that forms a bridge between CSC and MTs [119,117]. Inhibition of MT depolymerization (by paclitaxel) decreased salt tolerance, but promoting depolymerization (by oryzalin) improved salt tolerance [120]. Interestingly, *sos1* (*salt overly sensitive 1*) and *hkt1* (*H⁺/K⁺ transporter 1*) mutations that alter the cellular Na⁺ status also affect the direction of microtubule orientation [121–124]. Currently, no information is available for a possible function of complex *N*-glycans in these factors.

4.2.3. A unique role for complex *N*-glycans in monocot species

Hints about specific functions of complex *N*-glycans could be found in non-Arabidopsis models. To date, mutants affected in the *N*-glycosylation pathway have been reported for rice (monocot) [125], *Physcomitrella patens* (moss) [126], and *Chlamydomonas reinhardtii* (alga) [127], whereas *GNTI*-antisense/RNAi plants have been produced for tobacco, potato, and tomato [128,82,80,129]. The rice mutant for the

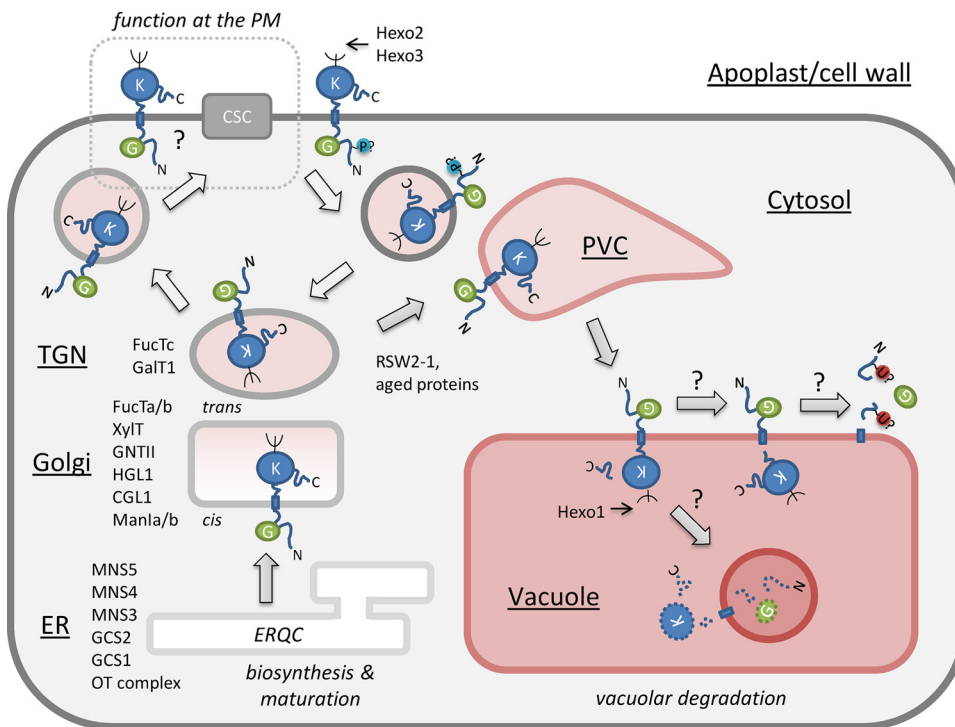


Fig. 5. Biogenesis and transport of KOR1 protein in a plant cell. KOR1 is synthesized on the rough ER, becomes N-glycosylated in the ER lumen, and undergoes trimming of terminal oligosaccharide residues to monitor its folding status by ER quality control (ERQC) mechanisms. Upon exit from the ER and transport to the Golgi apparatus, N-glycans (depicted as fork-like structure) are processed by Golgi glycosidases and glycosyltransferases to form complex N-glycans. KOR1 (K) is transported to the *trans*-Golgi network (TGN) for further maturation of its N-glycans, and shuttled between the TGN and the plasmamembrane (PM). Functional association of KOR1 (K) with cellulose synthase complexes (CSC) probably occurs at the PM. When KOR1 is compromised, either by mutation (RSW2-1) or aging, the protein is removed from the TGN and directed to tonoplast membranes via the prevacuolar compartment (PVC). In the apoplast/cell wall or vacuole, hexosaminidase isoforms (Hexo) cleave at the terminal GlcNAc, exposing the immunogenic xylose and fucose residues of plant complex N-glycans. Transport routes are indicated by grey arrows; progressive luminal acidification by increasing red color. G, GFP; N/C, N-/C-terminal; ~P, phosphorylation; ~U, ubiquitination; ?, unclear process/modification. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article).

minus; ~P, phosphorylation; ~U, ubiquitination; ?, unclear process/modification. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article).

DGL1 (DEFECTIVE GLYCOSYLATION 1) homolog produced similar phenotypes as *Arabidopsis dgl1*, indicating that the function of OT complexes is similar between monocots and dicots [125]. On the other hand, mutations in rice homologs for *CGL1* (*GNT1*) [130] and *core FUCT* [131] showed various defects. Rice *cgl1* was defective in cell wall biosynthesis and cytokinin responses, and unable to complete a life cycle due to failure in the formation of tiller buds. The effect of the rice *fuct* mutation was much milder, yet showed abnormal tiller angle, internode and panicle lengths, besides poor grain filling, which resembles phenotypes caused by defects in polar auxin transport [131]. These findings suggest that complex N-glycan modification in the Golgi apparatus affects tissue-specific functions of phytohormone levels and/or transport. Based on these premises, the monocot-specific phenotypes may reflect a specific interaction with phytohormones, giving rise to the unique structure of this plant clade.

5. Perspectives

In the twenty-five years since the first discovery of *Arabidopsis cgl1* mutant plants lacking complex N-glycans [33], the connection between plant core N-glycosylation/complex N-glycan modifications for salt stress responses, cellulose biosynthesis and microtubule association has been well established. In addition, the importance of ER protein quality control (ERQC) in the biogenesis of several receptor-like kinases, and a connection to stress signaling in plant UPR pathways has been revealed. However, compared to other areas in plant biology, our understanding of the role of plant (complex) N-glycan biology is still scarce. The shortage of knowledge is partially due to the limitation of identified complex N-glycan-related phenotypes and mutant collections, lack of simple methods to detect complex N-glycans in vivo, limited methods to change the structure of N-glycans at specific sites, and limited information for individual protein dynamics within the secretory system. Deep phenotyping of existing complex N-glycan mutants as well as developing a screening system for complex N-glycan-dependent mutants are essential to fill this gap. To date, efficient strategies to conduct in vivo screenings of protein functions affected by the presence or

absence of (complex) N-glycans have not been reported. We recently initiated a genetic screen in *Arabidopsis* based on hypersensitivity to an inhibitor of complex N-glycan maturation: plants with hypersensitivity to sub-inhibitory kifunensin concentration were selected, and a large number of plants whose growth is likely dependent on complex N-glycan formation were isolated. Another important issue to address is the relationship between complex N-glycan decoration and the kinetics of protein mobility. Although lack of complex N-glycans may not affect overall distribution of proteins in the secretory system, it may affect the rate of transport (secretion/internalization/recycling/degradation) that could be important for mounting proper protective responses under stress. We are currently monitoring protein dynamics during protein transport and delivery using fluorescent timer proteins [132–134]. In Fig. 6, we demonstrate that newly synthesized KOR1 (green fluorescence at early time points) and aged KOR1 (red fluorescence at later time points) can be distinguished. It should be noted that the green fluorescence at the later time points could originate from a mixture of aged proteins and new proteins synthesized at the later time point. Detailed subcellular localization of distinct KOR1 populations are currently being analyzed, and we expect to visualize transport dynamics of KOR1 under different N-glycosylation conditions. This novel approach, together with related studies [135,43], may visualize transport routes and different dwell time of proteins in various subcellular domains of the secretory pathway. Finally, as described above, we recently established a preparative scale production-purification pipeline for recombinant proteins in *Arabidopsis* host cells. This system enabled isolation of a large protein complex from *Arabidopsis* cells, specifically the OT complex, for which we successfully conducted electron microscopic analysis. This system will be explored further to obtain higher resolution structures similar to those reported for human and yeast OT complexes using cryo-electron microscopy approaches [136–138], as well as for glycoprotein preparation from different *Arabidopsis* hosts for further analyses. We expect that these efforts/tools will shed new light on the biology of plant-specific N-glycosylation.

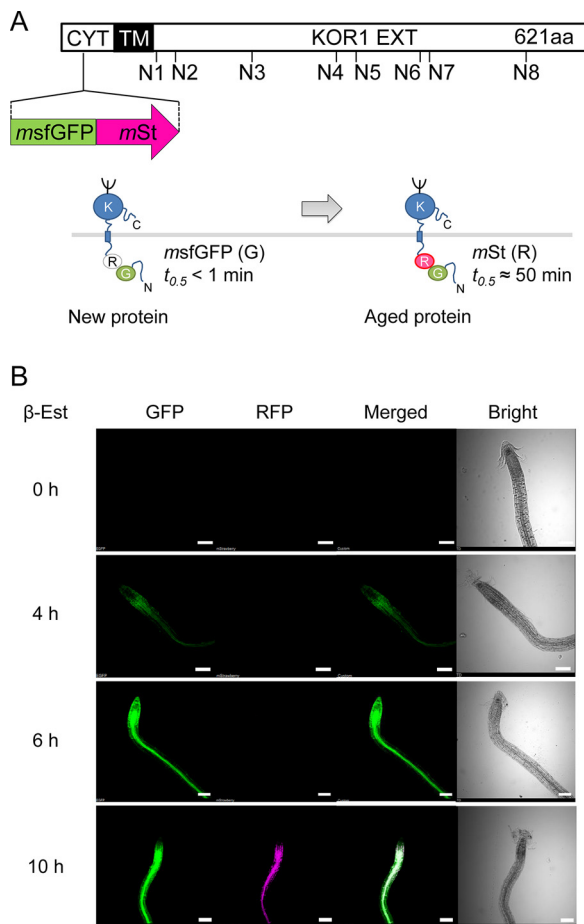


Fig. 6. Expression of tandem-fluorescent timer (tdFT)-labeled KOR1 in Arabidopsis roots. **A)** Structure of tdFT-KOR1. The tdFT part was prepared by fusing monomeric superfolder GFP (*msfGFP*, G) and monomeric Strawberry (*mSt*, R). tdFT was inserted in the cytoplasmic (CYT) region of KOR1. The extracellular domain (EXT) of KOR1 contains eight N-glycosylation sites (N1–N8). TM, transmembrane domain. Halftime ($t_{0.5}$) indicates 50% reporter maturation at 37 °C. **B)** Time course of fluorescent profiles upon expression of inducible tdFT-KOR1. Images were taken at the indicated time points after application of β -estradiol (β -Est) at 22 °C. GFP and RFP channels show total and aged tdFT-KOR1, respectively. Aged KOR1 is detected in both channels and appear white in the merged images. Bright field images are shown for reference. Bars indicate 100 μ m.

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Conflict of interest

None declared.

References

- [1] A. Helenius, M. Aebi, Intracellular functions of N-linked glycans, *Science* 291 (2001) 2364–2369.
- [2] M. Aebi, N-linked protein glycosylation in the ER, *Biochimica et Biophysica Acta (BBA) - Mol. Cell Res.* 1833 (2013) 2430–2437.
- [3] R. Strasser, Biological significance of complex N-glycans in plants and their impact on plant physiology, *Front. Plant Sci.* 5 (2014) 363.
- [4] R. Strasser, Plant protein glycosylation, *Glycobiology* 26 (2016) 926–939.
- [5] N. Smirnov, G.L. Wheeler, Ascorbic acid in plants: biosynthesis and function, *Crit. Rev. Biochem. Mol. Biol.* 35 (2000) 291–314.
- [6] P. Walter, R. Gilmore, M. Muller, G. Blobel, The protein translocation machinery

- of the endoplasmic reticulum, *Philos. Trans. R. Soc. Lond. B Biol. Sci.* 300 (1982) 225–228.
- [7] M.S. Lowenthal, K.S. Davis, T. Formolo, L.E. Kilpatrick, K.W. Phinney, Identification of novel N-glycosylation sites at noncanonical protein consensus motifs, *J. Proteome Res.* 15 (2016) 2087–2101.
- [8] D. Karaoglu, D.J. Kelleher, R. Gilmore, The highly conserved Stt3 protein is a subunit of the yeast oligosaccharyltransferase and forms a subcomplex with Ost3p and Ost4p, *J. Biol. Chem.* 272 (1997) 32513–32520.
- [9] R. Knauer, L. Lehle, The oligosaccharyltransferase complex from yeast, *Biochim. Biophys. Acta* 1426 (1999) 259–273.
- [10] T. Shibatani, L.L. David, A.L. McCormack, K. Frueh, W.R. Skach, Proteomic analysis of mammalian oligosaccharyltransferase reveals multiple subcomplexes that contain Sec61, TRAP, and two potential new subunits, *Biochemistry* 44 (2005) 5982–5992.
- [11] V.A. Shchedrina, et al., Selenoprotein K binds multiprotein complexes and is involved in the regulation of endoplasmic reticulum homeostasis, *J. Biol. Chem.* 286 (2011) 42937–42948.
- [12] C.M. Wilson, A. Magnaudeix, C. Yardin, F. Terro, DC2 and keratinocyte-associated protein 2 (KCP2), subunits of the oligosaccharyltransferase complex, are regulators of the gamma-secretase-directed processing of amyloid precursor protein (APP), *J. Biol. Chem.* 286 (2011) 31080–31091.
- [13] A. Hamieh, et al., Selenoprotein T is a novel OST subunit that regulates UPR signaling and hormone secretion, *EMBO Rep.* 18 (2017) 1935–1946.
- [14] S. Shrima, N.A. Cherepanova, R. Gilmore, DC2 and KCP2 mediate the interaction between the oligosaccharyltransferase and the ER translocon, *J. Cell Biol.* 216 (2017) 3625–3638.
- [15] I.S. Jeong, et al., Purification and characterization of Arabidopsis thaliana oligosaccharyltransferase complexes from the native host: a protein super-expression system for structural studies, *Plant J.* 94 (2018) 131–145.
- [16] Y. Harada, H. Li, H. Li, W.J. Lennarz, Oligosaccharyltransferase directly binds to ribosome at a location near the translocon-binding site, *Proc. Natl. Acad. Sci. U. S. A.* 106 (2009) 6945–6949.
- [17] C. Ruiz-Canada, D.J. Kelleher, R. Gilmore, Cotranslational and posttranslational N-glycosylation of polypeptides by distinct mammalian OST isoforms, *Cell* 136 (2009) 272–283.
- [18] H. Koiwa, et al., The STT3a subunit isoform of the Arabidopsis thaliana oligosaccharyltransferase controls adaptive responses to salt/osmotic stress, *Plant Cell* 15 (2003) 2273–2284.
- [19] M.A. Johnson, et al., Arabidopsis hapless mutations define essential gametophytic functions, *Genetics* 168 (2004) 971–982.
- [20] O. Lerouxel, et al., Mutants in DEFECTIVE GLYCOSYLATION, an Arabidopsis homolog of an oligosaccharyltransferase complex subunit, show protein underglycosylation and defects in cell differentiation and growth, *Plant J.* 42 (2005) 455–468.
- [21] D.J. Kelleher, R. Gilmore, An evolving view of the eukaryotic oligosaccharyltransferase, *Glycobiology* 16 (2006) 47R–62R.
- [22] E. Mohorko, R. Glockshuber, M. Aebi, Oligosaccharyltransferase: the central enzyme of N-linked protein glycosylation, *J. Inher. Metab. Dis.* 34 (2011) 869–878.
- [23] B.L. Schulz, et al., Oxidoreductase activity of oligosaccharyltransferase subunits Ost3p and Ost6p defines site-specific glycosylation efficiency, *Proc. Natl. Acad. Sci. U. S. A.* 106 (2009) 11061–11066.
- [24] Q. Yan, G.D. Prestwich, W.J. Lennarz, The Ost1p subunit of yeast oligosaccharyltransferase recognizes the peptide glycosylation site sequence, -Asn-X-Ser/Thr, *J. Biol. Chem.* 274 (1999) 5021–5025.
- [25] A. Farid, et al., Specialized roles of the conserved subunit OST3/6 of the oligosaccharyltransferase complex in innate immunity and tolerance to abiotic stresses, *Plant Physiol.* 162 (2013) 24–38.
- [26] P. Gallois, et al., An Arabidopsis thaliana cDNA complementing a hamster apoptosis suppressor mutant, *Plant J.* 11 (1997) 1325–1331.
- [27] M. Boisson, et al., Arabidopsis glucosidase I mutants reveal a critical role of N-glycan trimming in seed development, *EMBO J.* 20 (2001) 1010–1019.
- [28] C.S. Gillmor, P. Poindexter, J. Lorieau, M.M. Palic, C. Somerville, Alpha-glucosidase I is required for cellulose biosynthesis and morphogenesis in Arabidopsis, *J. Cell Biol.* 156 (2002) 1003–1013.
- [29] J.E. Burn, et al., The cellulose-deficient Arabidopsis mutant rsw3 is defective in a gene encoding a putative glucosidase II, an enzyme processing N-glycans during ER quality control, *Plant J.* 32 (2002) 949–960.
- [30] E. Liebminger, et al., Class I alpha-mannosidases are required for N-glycan processing and root development in Arabidopsis thaliana, *Plant Cell* 21 (2009) 3850–3867.
- [31] S. Huttner, et al., Arabidopsis class I alpha-mannosidases MNS4 and MNS5 are involved in endoplasmic reticulum-associated degradation of misfolded glycoproteins, *Plant Cell* 26 (2014) 1712–1728.
- [32] H. Kajiura, et al., Two Arabidopsis thaliana golgi alpha-mannosidase I enzymes are responsible for plant N-glycan maturation, *Glycobiology* 20 (2010) 235–247.
- [33] A. von Schaeuwen, A. Sturm, J. O'Neill, M. Chrispeels, Isolation of a mutant Arabidopsis plant that lacks N-acetyl glucosaminyl transferase I and is unable to synthesize Golgi-modified complex N-linked glycans, *Plant Physiol.* 102 (1993) 1109–1118.
- [34] J. Frank, H. Kaulfurst-Soboll, S. Rips, H. Koiwa, A. von Schaeuwen, Comparative analyses of Arabidopsis cgl1 (complex glycan 1) mutants and genetic interaction with stt3a (staurosporin & temperature-sensitive 3a), *Plant Physiol.* 148 (2008) 1354–1367.
- [35] J.S. Kang, et al., Salt tolerance of Arabidopsis thaliana requires maturation of N-glycosylated proteins in the Golgi apparatus, *Proc. Natl. Acad. Sci. U. S. A.* 105 (2008) 5933–5938.

- [36] R. Strasser, et al., Molecular cloning and characterization of *Arabidopsis thaliana* Golgi alpha-mannosidase II, a key enzyme in the formation of complex N-glycans in plants, *Plant J.* 45 (2006) 789–803.
- [37] R. Strasser, et al., Molecular cloning of cDNA encoding N-acetylglucosaminyl-transferase II from *Arabidopsis thaliana*, *Glycoconj. J.* 16 (1999) 787–791.
- [38] R. Strasser, F. Altmann, L. Mach, J. Glossl, H. Steinkellner, Generation of *Arabidopsis thaliana* plants with complex N-glycans lacking beta1,2-linked xylose and core alpha1,3-linked fucose, *FEBS Lett.* 561 (2004) 132–136.
- [39] J.S. Bondili, et al., Molecular cloning and heterologous expression of beta1,2-xylosyltransferase and core alpha1,3-fucosyltransferase from maize, *Phytochemistry* 67 (2006) 2215–2224.
- [40] R. Strasser, et al., A unique {beta}1,3-galactosyltransferase is indispensable for the biosynthesis of N-glycans containing lewis x structures in *Arabidopsis thaliana*, *Plant Cell* 19 (2007) 2278–2292.
- [41] I.B. Wilson, Identification of a cDNA encoding a plant Lewis-type alpha1,4-fucosyltransferase, *Glycoconj. J.* 18 (2001) 439–447.
- [42] I.B. Wilson, et al., Cloning and expression of cDNAs encoding alpha1,3-fucosyltransferase homologues from *Arabidopsis thaliana*, *Biochim. Biophys. Acta* 1527 (2001) 88–96.
- [43] S. Rips, M. Frank, A. Elting, J.N. Offenborn, A. von Schaeuwen, Golgi alpha1,4-fucosyltransferase of *Arabidopsis thaliana* partially localizes at the nuclear envelope, *Traffic* 18 (2017) 646–657.
- [44] M. Gutterrugg, et al., Biosynthesis of truncated N-linked oligosaccharides results from non-orthologous hexosaminidase-mediated mechanisms in nematodes, plants, and insects, *J. Biol. Chem.* 282 (2007) 27825–27840.
- [45] R. Strasser, et al., Enzymatic properties and subcellular localization of *Arabidopsis* beta-N-acetylhexosaminidases, *Plant Physiol.* 145 (2007) 5–16.
- [46] E. Liebminger, et al., Beta-N-acetylhexosaminidases HEXO1 and HEXO3 are responsible for the formation of paucimannosidic N-glycans in *Arabidopsis thaliana*, *J. Biol. Chem.* 286 (2011) 10793–10802.
- [47] J.D. Oliver, F.J. van der Wal, N.J. Bulleid, S. High, Interaction of the thiol-dependent reductase ERp57 with nascent glycoproteins, *Science* 275 (1997) 86–88.
- [48] J.J. Caramelo, A.J. Parodi, Getting in and out from calnexin/calreticulin cycles, *J. Biol. Chem.* 283 (2008) 10221–10225.
- [49] Y.-J. Shin, U. Vavra, C. Veit, R. Strasser, The glycan-dependent ERAD machinery degrades topologically diverse misfolded proteins, *Plant J.* 94 (2018) 246–259.
- [50] S.S. Vembar, J.L. Brodsky, One step at a time: endoplasmic reticulum-associated degradation, *Nat. Rev. Mol. Cell Biol.* 9 (2008) 944–957.
- [51] S. Huttner, C. Veit, J. Schoberer, J. Grass, R. Strasser, Unraveling the function of *Arabidopsis thaliana* OS9 in the endoplasmic reticulum-associated degradation of glycoproteins, *Plant Mol. Biol.* 79 (2012) 21–33.
- [52] W. Su, Y. Liu, Y. Xia, Z. Hong, J. Li, The *Arabidopsis* homolog of the mammalian OS-9 protein plays a key role in the endoplasmic reticulum-associated degradation of misfolded receptor-like kinases, *Mol. Plant* 5 (2012) 929–940.
- [53] W. Su, Y. Liu, Y. Xia, Z. Hong, J. Li, Conserved endoplasmic reticulum-associated degradation system to eliminate mutated receptor-like kinases in *Arabidopsis*, *Proc. Natl. Acad. Sci. U. S. A.* 108 (2011) 870–875.
- [54] Y. Liu, et al., EBS7 is a plant-specific component of a highly conserved endoplasmic reticulum-associated degradation system in *Arabidopsis*, *Proc. Natl. Acad. Sci. U. S. A.* 112 (2015) 12205–12210.
- [55] M. Ohta, F. Takaiwa, Emerging features of ER resident J-proteins in plants, *Plant Signal. Behav.* 9 (2014) e28194.
- [56] J.X. Liu, S.H. Howell, Endoplasmic reticulum protein quality control and its relationship to environmental stress responses in plants, *Plant Cell* 22 (2010) 2930–2942.
- [57] Y. Iwata, et al., Activation of the *Arabidopsis* membrane-bound transcription factor bZIP28 is mediated by site-2 protease, but not site-1 protease, *Plant J.* 91 (2017) 408–415.
- [58] Y. Nagashima, et al., *Arabidopsis* IRE1 catalyses unconventional splicing of bZIP60 mRNA to produce the active transcription factor, *Sci. Rep.* 1 (2011) 29.
- [59] N. Koizumi, et al., Molecular characterization of two *Arabidopsis* Ire1 homologs, endoplasmic reticulum-located transmembrane protein kinases, *Plant Physiol.* 127 (2001) 949–962.
- [60] P. Walter, D. Ron, The unfolded protein response: from stress pathway to homeostatic regulation, *Science* 334 (2011) 1081–1086.
- [61] Y. Deng, R. Srivastava, S.H. Howell, Protein kinase and ribonuclease domains of IRE1 confer stress tolerance, vegetative growth, and reproductive development in *Arabidopsis*, *Proc. Natl. Acad. Sci. U. S. A.* 110 (2013) 19633–19638.
- [62] Y. Nagashima, Y. Iwata, K. Mishiba, N. Koizumi, *Arabidopsis* tRNA ligase completes the cytoplasmic splicing of bZIP60 mRNA in the unfolded protein response, *Biochem. Biophys. Res. Commun.* 470 (2016) 941–946.
- [63] Y. Deng, et al., Heat induces the splicing by IRE1 of a mRNA encoding a transcription factor involved in the unfolded protein response in *Arabidopsis*, *Proc. Natl. Acad. Sci. U. S. A.* 108 (2011) 7247–7252.
- [64] L. Zhang, H. Chen, F. Brandizzi, J. Verchot, A. Wang, The UPR branch IRE1-bZIP60 in plants plays an essential role in viral infection and is complementary to the only UPR pathway in yeast, *PLoS Genet.* 11 (2015) e1005164.
- [65] K. Mishiba, et al., Defects in IRE1 enhance cell death and fail to degrade mRNAs encoding secretory pathway proteins in the *Arabidopsis* unfolded protein response, *Proc. Natl. Acad. Sci. U. S. A.* 110 (2013) 5713–5718.
- [66] Y. Liu, et al., Degradation of the endoplasmic reticulum by autophagy during endoplasmic reticulum stress in *Arabidopsis*, *Plant Cell* 24 (2012) 4635–4651.
- [67] K.D. Johnson, M.J. Chrispeels, Substrate specificities of N-acetylglucosaminyl-, fucosyl-, and xylosyltransferases that modify glycoproteins in the Golgi apparatus of bean cotyledons, *Plant Physiol.* 84 (1987) 1301–1308.
- [68] M. Laurière, C. Laurière, M.J. Chrispeels, K.D. Johnson, A. Strum, Characterization of a xylose-specific antiserum that reacts with the complex asparagine-linked glycans of extracellular and vacuolar glycoproteins, *Plant Physiol.* 90 (1989) 1182–1188.
- [69] H. Steinkellner, R. Strasser, Glycosyltransferases in the plant Golgi, in: D.G. Robinson (Ed.), *Golgi Apparatus and the Plant Secretory Pathway*, Blackwell Publishing, Oxford, 2003, pp. 181–192.
- [70] A. von Schaeuwen, A. Sturm, J. O'Neill, M. Chrispeels, Isolation of a mutant *Arabidopsis* plant that lacks N-acetyl glucosaminyl transferase I and is unable to synthesize Golgi-modified complex N-linked glycans, *Plant Physiol.* 102 (1993) 1109–1118.
- [71] R. Strasser, et al., Molecular cloning and functional expression of beta1, 2-xylosyltransferase cDNA from *Arabidopsis thaliana*, *FEBS Lett.* 472 (2000) 105–108.
- [72] A.C. Fitchette-Laine, et al., N-glycans harboring the Lewis x epitope are expressed at the surface of plant cells, *Plant J.* 12 (1997) 1411–1417.
- [73] R. Leonard, et al., The presence of Lewis x epitopes in *Arabidopsis thaliana* glycoconjugates depends on an active alpha4-fucosyltransferase gene, *Glycobiology* 12 (2002) 299–306.
- [74] T.Y. Chiu, et al., AtAPY1 and AtAPY2 function as Golgi-localized nucleoside diphosphatases in *Arabidopsis thaliana*, *Plant Cell Physiol.* 53 (2012) 1913–1925.
- [75] B. Cubero, et al., The phosphate transporter PHT4;6 is a determinant of salt tolerance that is localized to the Golgi apparatus of *Arabidopsis*, *Mol. Plant* 2 (2009) 535–552.
- [76] L. Norambuena, et al., Transport of UDP-galactose in plants. Identification and functional characterization of AtUTR1, an *Arabidopsis thaliana* UDP-galactose/UDP-glucose transporter, *J. Biol. Chem.* 277 (2002) 32923–32929.
- [77] M.G. Handford, F. Sicilia, F. Brandizzi, J.H. Chung, P. Dupree, *Arabidopsis thaliana* expresses multiple Golgi-localised nucleotide-sugar transporters related to GONST1, *Mol. Genet. Genomics* 272 (2004) 397–410.
- [78] R. Piron, F. Santens, A. De Paepe, A. Depicker, N. Callewaert, Using GlycoDelete to produce proteins lacking plant-specific N-glycan modification in seeds, *Nat. Biotechnol.* 33 (2015) 1135–1137.
- [79] Y. Shaaltiel, Y. Tekoah, Plant specific N-glycans do not have proven adverse effects in humans, *Nat. Biotechnol.* 34 (2016) 706–708.
- [80] H. Kaulfurst-Soboll, M. Mertens, R. Brehler, A. von Schaeuwen, Reduction of cross-reactive carbohydrate determinants in plant foodstuff: elucidation of clinical relevance and implications for allergy diagnosis, *PLoS One* 6 (2011) e17800.
- [81] M. Schahs, et al., Production of a monoclonal antibody in plants with a humanized N-glycosylation pattern, *Plant Biotechnol. J.* 5 (2007) 657–663.
- [82] R. Strasser, et al., Generation of glyco-engineered *Nicotiana benthamiana* for the production of monoclonal antibodies with a homogeneous human-like N-glycan structure, *Plant Biotechnol. J.* 6 (2008) 392–402.
- [83] J. Li, et al., Specific ER quality control components required for biogenesis of the plant innate immune receptor EFR, *Proc. Natl. Acad. Sci. U. S. A.* 106 (2009) 15973–15978.
- [84] V. Nekrasov, et al., Control of the pattern-recognition receptor EFR by an ER protein complex in plant immunity, *EMBO J.* 28 (2009) 3428–3438.
- [85] Y. Saijo, et al., Receptor quality control in the endoplasmic reticulum for plant innate immunity, *EMBO J.* 28 (2009) 3439–3449.
- [86] H. Häweker, et al., Pattern recognition receptors require N-glycosylation to mediate plant immunity, *J. Biol. Chem.* 285 (2010) 4629–4636.
- [87] M.V.V. de Oliveira, et al., Specific control of *Arabidopsis* BAK1/SERK4-regulated cell death by protein glycosylation, *Nat. Plants* 2 (2016) 15218.
- [88] H. Jin, Z. Yan, K.H. Nam, J. Li, Allele-specific suppression of a defective brassinosteroid receptor reveals a physiological role of UGGT in ER quality control, *Mol. Cell* 26 (2007) 821–830.
- [89] Z. Hong, H. Jin, T. Tzfira, J. Li, Multiple mechanism-mediated retention of a defective brassinosteroid receptor in the endoplasmic reticulum of *Arabidopsis*, *Plant Cell* 20 (2008) 3418–3429.
- [90] H. Lindner, et al., TURAN and EVAN mediate pollen tube reception in *Arabidopsis* synergids through protein glycosylation, *PLoS Biol.* 13 (2015) e1002139.
- [91] L.M. Muller, H. Lindner, N.D. Pires, V. Gagliardini, U. Grossniklaus, A subunit of the oligosaccharyltransferase complex is required for interspecific gametophyte recognition in *Arabidopsis*, *Nat. Commun.* 7 (2016) 10826.
- [92] J.M. Escobar-Restrepo, et al., The FERONIA receptor-like kinase mediates male-female interactions during pollen tube reception, *Science* 317 (2007) 656–660.
- [93] A. Boisson-Dernier, et al., Disruption of the pollen-expressed FERONIA homologs ANXUR1 and ANXUR2 triggers pollen tube discharge, *Development* 136 (2009) 3279–3288.
- [94] S. Miyazaki, et al., ANXUR1 and 2, sister genes to FERONIA/SIRENE, are male factors for coordinated fertilization, *Curr. Biol.* 19 (2009) 1327–1331.
- [95] J. Shen, Y. Ding, C. Gao, E. Rojo, L. Jiang, N-linked glycosylation of AtVSR1 is important for vacuolar protein sorting in *Arabidopsis*, *Plant J.* 80 (2014) 977–992.
- [96] Z.-Y. Wang, et al., The *Arabidopsis* vacuolar sorting receptor1 is required for osmotic stress-induced abscisic acid biosynthesis, *Plant Physiol.* 167 (2015) 137–152.
- [97] H.H. Freeze, Human disorders in N-glycosylation and animal models, *Biochim. Biophys. Acta* 1573 (2002) 388–393.
- [98] H.H. Freeze, M. Aebi, Altered glycan structures: the molecular basis of congenital disorders of glycosylation, *Curr. Opin. Struct. Biol.* 15 (2005) 490–498.
- [99] M.E. Losfeld, et al., A new congenital disorder of glycosylation caused by a mutation in SSR4, the signal sequence receptor 4 protein of the TRAP complex, *Hum. Mol. Genet.* 23 (2014) 1602–1605.
- [100] P. Stanley, What have we learned from glycosyltransferase knockouts in mice? *J. Mol. Biol.* 428 (2016) 3166–3182.
- [101] H. Schachter, Mgal1-dependent N-glycans are essential for the normal development of both vertebrate and invertebrate metazoans, *Semin. Cell Dev. Biol.* 21

- (2010) 609–615.
- [102] J.S. Kang, et al., Salt tolerance of *Arabidopsis thaliana* requires maturation of N-glycosylated proteins in the Golgi apparatus, *Proc. Natl. Acad. Sci. U. S. A.* 105 (2008) 5933–5938.
- [103] L.C. Peng, C.H. Hocart, J.W. Redmond, R.E. Williamson, Fractionation of carbohydrates in *Arabidopsis* root cell walls shows that three radial swelling loci are specifically involved in cellulose production, *Planta* 211 (2000) 406–414.
- [104] D.R. Lane, et al., Temperature-sensitive alleles of RSW2 link the KORRIGAN endo-1,4-beta-glucanase to cellulose synthesis and cytokinesis in *Arabidopsis*, *Plant Physiol.* 126 (2001) 278–288.
- [105] S. Robert, et al., An *Arabidopsis* endo-1,4-beta-D-glucanase involved in cellulose synthesis undergoes regulated intracellular cycling, *Plant Cell* 17 (2005) 3378–3389.
- [106] S. Rips, et al., Multiple N-glycans cooperate in the subcellular targeting and functioning of *Arabidopsis* KORRIGAN1, *Plant Cell* 26 (2014) 3792–3808.
- [107] N. Mansoori, et al., KORRIGAN1 interacts specifically with integral components of the cellulose synthase machinery, *PLoS One* 9 (2014) e112387.
- [108] T. Vain, et al., The cellulase KORRIGAN is part of the cellulose synthase complex, *Plant Physiol.* 165 (2014) 1521–1532.
- [109] L. Peng, Y. Kawagoe, P. Hogan, D. Delmer, Sitosterol-beta-glucoside as primer for cellulose synthesis in plants, *Science* 295 (2002) 147–150.
- [110] J. Takahashi, et al., KORRIGAN1 and its aspen homolog PttCel9A1 decrease cellulose crystallinity in *Arabidopsis* stems, *Plant Cell Physiol.* 50 (2009) 1099–1115.
- [111] S.Y. Ding, M.E. Himmel, The maize primary cell wall microfibril: a new model derived from direct visualization, *J. Agric. Food Chem.* 54 (2006) 597–606.
- [112] L. Lei, et al., The *jiaoyao1* mutant is an allele of *korrigan1* that abolishes endoglucanase activity and affects the organization of both cellulose microfibrils and microtubules in *Arabidopsis*, *Plant Cell* 26 (2014) 2601–2616.
- [113] E. Liebinger, J. Grass, F. Altmann, L. Mach, R. Strasser, Characterizing the link between glycosylation state and enzymatic activity of the endo-beta 1,4-glucanase KORRIGAN1 from *Arabidopsis thaliana*, *J. Biol. Chem.* 288 (2013) 22270–22280.
- [114] Y. Zhang, et al., Golgi-localized STELLA proteins regulate the assembly and trafficking of cellulose synthase complexes in *Arabidopsis*, *Nat. Commun.* 7 (2016) 11656.
- [115] E.F. Crowell, et al., Pausing of Golgi bodies on microtubules regulates secretion of cellulose synthase complexes in *Arabidopsis*, *Plant Cell* 21 (2009) 1141–1154.
- [116] R. Gutierrez, J.J. Lindeboom, A.R. Paredez, A.M. Emons, D.W. Ehrhardt, *Arabidopsis* cortical microtubules position cellulose synthase delivery to the plasma membrane and interact with cellulose synthase trafficking compartments, *Nat. Cell Biol.* 11 (2009) 797–806.
- [117] A. Endler, et al., A mechanism for sustained cellulose synthesis during Salt stress, *Cell* 162 (2015) 1353–1364.
- [118] S. Fujita, et al., An atypical tubulin kinase mediates stress-induced microtubule depolymerization in *Arabidopsis*, *Curr. Biol.* 23 (2013) 1969–1978.
- [119] M. Bringmann, et al., POM-POM2/cellulose synthase interacting1 is essential for the functional association of cellulose synthase and microtubules in *Arabidopsis*, *Plant Cell* 24 (2012) 163–177.
- [120] C. Wang, J. Li, M. Yuan, Salt tolerance requires cortical microtubule reorganization in *Arabidopsis*, *Plant Cell Physiol.* 48 (2007) 1534–1547.
- [121] H. Shi, M. Ishitani, J.-K. Zhu, The *Arabidopsis thaliana* salt tolerance gene SOS1 encodes a putative Na⁺/H⁺ antiporter, *Proc. Natl. Acad. Sci. U. S. A.* 97 (2000) 6896–6901.
- [122] A. Rus, et al., AtHKT1 is a salt tolerance determinant that controls Na⁺ entry into plant roots, *Proc. Natl. Acad. Sci. U. S. A.* 98 (2001) 14150–14155.
- [123] A. Rus, et al., AtHKT1 facilitates Na⁺ homeostasis and K⁺ nutrition in planta, *Plant Physiol.* 136 (2004) 2500–2511.
- [124] T. Shoji, et al., Salt stress affects cortical microtubule organization and helical growth in *Arabidopsis*, *Plant Cell Physiol.* 47 (2006) 1158–1168.
- [125] C. Qin, et al., OsDGL1, a homolog of an oligosaccharyltransferase complex subunit, is involved in N-glycosylation and root development in rice, *Plant Cell Physiol.* 54 (2013) 129–137.
- [126] A. Weise, et al., High-level expression of secreted complex glycosylated recombinant human erythropoietin in the *Physcomitrella delta-fuc-t delta-xyl-t* mutant, *Plant Biotechnol. J.* 5 (2007) 389–401.
- [127] S. Schulze, et al., N-glycoproteomic characterization of mannosidase and xylo-transferase mutant strains of *Chlamydomonas*, *Plant Physiol.* 176 (2017) 1952–1964.
- [128] I. Wenderoth, A. von Schaewen, Isolation and characterization of plant N-acetyl glucosaminyltransferase I (GntI) cDNA sequences. Functional analyses in the *Arabidopsis* cgl mutant and in antisense plants, *Plant Physiol.* 123 (2000) 1097–1108.
- [129] J. Limkul, et al., The production of human glucocerebrosidase in glyco-engineered *Nicotiana benthamiana* plants, *Plant Biotechnol. J.* 14 (2016) 1682–1694.
- [130] W.I. Fanata, et al., N-glycan maturation is crucial for cytokinin-mediated development and cellulose synthesis in *Oryza sativa*, *Plant J.* 73 (2013) 966–979.
- [131] R. Harmoko, et al., N-glycan containing a core alpha1,3-fucose residue is required for basipetal auxin transport and gravitropic response in rice (*Oryza sativa*), *N. Phytol.* 212 (2016) 108–122.
- [132] F.V. Subach, et al., Monomeric fluorescent timers that change color from blue to red report on cellular trafficking, *Nat. Chem. Biol.* 5 (2009) 118–126.
- [133] T. Tsuboi, T. Kitaguchi, S. Karasawa, M. Fukuda, A. Miyawaki, Age-dependent preferential dense-core vesicle exocytosis in neuroendocrine cells revealed by newly developed monomeric fluorescent timer protein, *Mol. Biol. Cell* 21 (2010) 87–94.
- [134] A. Khmelinskii, et al., Tandem fluorescent protein timers for in vivo analysis of protein dynamics, *Nat. Biotechnol.* 30 (2012) 708–714.
- [135] J. Schoberer, E. Liebinger, S.W. Botchway, R. Strasser, C. Hawes, Time-resolved fluorescence imaging reveals differential interactions of N-glycan processing enzymes across the Golgi stack in planta, *Plant Physiol.* 161 (2013) 1737–1754.
- [136] S. Pfeffer, et al., Structure of the mammalian oligosaccharyl-transferase complex in the native ER protein translocon, *Nat. Commun.* 5 (2014) 3072.
- [137] L. Bai, T. Wang, G. Zhao, A. Kovach, H. Li, The atomic structure of a eukaryotic oligosaccharyltransferase complex, *Nature* 555 (2018) 328–333.
- [138] R. Wild, et al., Structure of the yeast oligosaccharyltransferase complex gives insight into eukaryotic N-glycosylation, *Science* 359 (2018) 545–550.