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Article Addendum

Role of complex *N*-glycans in plant stress tolerance

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In plant cells, glycans attached to asparagine (N) residues of proteins undergo various modifications in the endoplasmic reticulum and the Golgi apparatus. The *N*-glycan modifications in the Golgi apparatus result in complex *N*-glycans attached to membrane proteins, secreted proteins and vacuolar proteins. Recently, we have investigated the role of complex *N*-glycans in plants using a series of *Arabidopsis thaliana* mutants affected in complex *N*-glycan biosynthesis.[1] Several mutant plants including *complex glycan 1* (*cgl1*) displayed a salt-sensitive phenotype during their root growth, which was associated with radial swelling and loss of apical dominance. Among the proteins whose *N*-glycans are affected by the *cgl1* mutation is a membrane anchored β1,4-endoglucanase, KORRIGAN1/RADIALLY SWOLLEN 2 (KOR1/RSW2) involved in cellulose biosynthesis. The *cgl1* mutation strongly enhanced the phenotype of a temperature sensitive allele of KOR1/RSW2 (*rsw2-1*) even at the permissive temperature. This establishes that plant complex *N*-glycan modification is important for the in vivo function of KOR1/RSW2. Furthermore, *rsw2-1* as well as another cellulose biosynthesis mutant *rsw1-1* exhibited also a salt-sensitive phenotype at the permissive temperature. Based on these findings, we propose that one of the mechanisms that cause salt-induced root growth arrest is dysfunction of cell wall biosynthesis that induces mitotic arrest in the root apical meristem.

In eukaryotic cells, both soluble and membrane proteins that enter the endoplasmic reticulum (ER) system may undergo post-translational modifications called *N*-glycosylation. *N*-glycosylation occurs in two phases, namely, core glycosylation in the ER and glycan maturation in the Golgi apparatus.[2,3] The process and roles of core glycosylation in the ER are well established and ubiquitous for eukaryotes. In the ER, pre-assembled core oligosaccharides ($Glc_3Man_9GlcNac_2$) are transferred to asparagine residues of the Asn-X-Ser/Thr motives in nascent polypeptides by the function of an oligosaccharyltransferase complex (OST). Terminal glucose residues are recognition sites for ER chaperones calnexin and calreticulin, and thus core *N*-glycans in the ER function in correct folding of newly synthesized proteins.[2,3]

Greater diversity exists in the *N*-glycan maturation steps in the Golgi apparatus and conspicuous roles for the resulting complex *N*-glycans.[2,4] In general, mature *N*-glycan structures are classified as oligomannosidic type, hybrid or complex type. Glycoprotein precursors that are exported from the ER carry high-mannose type *N*-glycan intermediates. Numerous enzymes are involved in the conversion of high-mannose type *N*-glycans to mature complex *N*-glycans. The functions of *N*-glycan modifications in the Golgi apparatus are well established in humans, because lack of *N*-glycan maturation results in Type II Congenital Disorders of Glycosylation.[5] In *Drosophila melanogaster*, the Golgi pathway is necessary for development and function of the central nervous system,[6] whereas in *Candida albicans*, it is necessary for cell wall integrity and virulence.[7]

The first *Arabidopsis thaliana* mutant lacking complex *N*-glycans was reported in 1993.[8] Since then, several mutants and transgenic plants altered in *N*-glycan maturation in the Golgi apparatus have been reported.[9-12] Plants with altered *N*-glycan modification pathways that are devoid of potentially immunogenic complex *N*-glycans are used for the production of pharmaceutical proteins[12,13] and could serve as potential food crops with reduced allergenicity. Until recently, however, plant complex *N*-glycans have not been associated with essential biological functions in their host plants due to lack of obvious phenotypes of mutant plants defective in complex *N*-glycan biosynthesis. We recently reported that mutants defective in complex *N*-glycans show enhanced salt sensitivity, establishing that complex *N*-glycans are indispensable for certain biological functions.[1]

Our previous study using an OST subunit mutant *stt3a* indicated that protein glycosylation could affect salt tolerance and root growth of *A. thaliana*.[14] Since OST functions upstream of protein folding processes in the ER, *stt3a* caused an unfolded protein response (UPR), which is a general ER stress response to protein folding defects, as well as accumulation of under-glycosylated proteins. In our recent study, we tried to address whether the salt stress response of the mutant is caused by an activation of UPR, or by a shortage of functional glycoproteins produced by the cells.[1] The *cgl1* mutant is defective in *N*-acetylglucosaminyltransferase in the Golgi apparatus[15] and only able to produce oligomannosidic-type *N*-glycans but not complex-type *N*-glycans.[8] *cgl1* mutants exposed to salt stress

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exhibited root growth arrest and radial swelling similar to *stt3a* mutants, however, unlike *stt3a*, the *cgl1* mutation did not cause UPR as judged by expression of an UPR marker gene, *BiP_{pro}-GUS*. This indicated that salt sensitivity of *cgl1* (and likely also of *stt3a*) is due to lack of mature *N*-glycans essential for functionality of certain glycoprotein(s).

We have determined that a membrane-anchored β1,4-endoglucanase, KORRIGAN1/RADIAL SWELLING2 (KOR1/RSW2), which functions in cellulose biosynthesis, is a target of CGL1 and involved in the salt stress response of *A. thaliana*.[1] A temperature sensitive *rsw2-1* allele[16] showed specific genetic interaction with both *cgl1* and *stt3a* mutations. The corresponding double mutants exhibited spontaneous growth defects at the permissive temperature that were reminiscent of those of *rsw2-1* at the restrictive temperature, of *cgl1* and *stt3a* plants treated with salt, and of the *rsw1-1 rsw2-1* double mutant that combines two cellulose deficiency mutations. This showed that *cgl1* and *stt3a* enhance cellulose deficiency of *rsw2-1*, and in turn indicate that the KOR1/RSW2 protein requires complex *N*-glycans for its function in vivo. Further pyramiding of these mutations resulted in incremental enhancement of growth defects as well as developmental defects of the host plants (Kang et al., (2008), and Fig. 1). Importance of functional cellulose biosynthesis for salt tolerance was further supported by the novel finding of increased salt-sensitivity of *rsw2-1* and *rsw1-1* single mutants.[1]

Our previous and current data have implications that affect our view of protein *N*-glycosylation in plants. First, after all, plant complex *N*-glycans confer important in vivo functions to secreted/secretory glycoproteins, i.e., protect root growth from salt/osmotic stress. In contrast to core oligosaccharides in the ER, which globally affect protein folding, complex *N*-glycans appear to function at the individual protein level. Second, one of the targets of salt/osmotic stress is a component of the cellulose biosynthesis machinery, namely KOR1/RSW2 that requires complex *N*-glycans for its function. KOR1/RSW2 provides a link to how complex *N*-glycans protect plants from salt/osmotic stress. However, the mechanism by which salt stress triggers the growth arrest via KOR1/RSW2 dysfunction is not yet understood. We have previously shown that the root apical meristem of *stt3a* exhibits cell cycle arrest under salt stress, but cell differentiation and lateral root formation continued in the same root tip.[14] This implies that plants, in response to salt stress and compromised cell-wall biosynthesis at the root apical meristem, specifically attenuate cell cycle progression at the old meristem and initiate new meristems. A signal transduction pathway that coordinates cell-wall integrity and cell proliferation is well documented in *Sacchromyces cerevisiae*, where Protein kinase C1 (Pkc1) and a MAP kinase cascade play essential roles.[17] Interestingly, both *S. cerevisiae* Stt3 and Och1 (a mannosyltransferase in the Golgi apparatus) are involved in the cell-wall integrity pathway.[17] In *A. thaliana*, mutations in the receptor kinase THESEUS1 suppressed hypocotyl elongation defects and ectopic lignification in several cellulose deficient mutants.[18] However, since *THE1* is expressed in elongation zones but not in cell division zones of root tips, and *the1* did not suppress the *kor1-1* phenotype,[18] it is unlikely that THE1 is involved in the regulation of the salt stress response at the root apical meristem. This implies that dividing cells and expanding cells employ distinct mechanism to sense cellulose deficiency. Understanding how complex *N*-glycans regulate cell-wall biosynthesis and cell proliferation is an exciting task for the coming years.

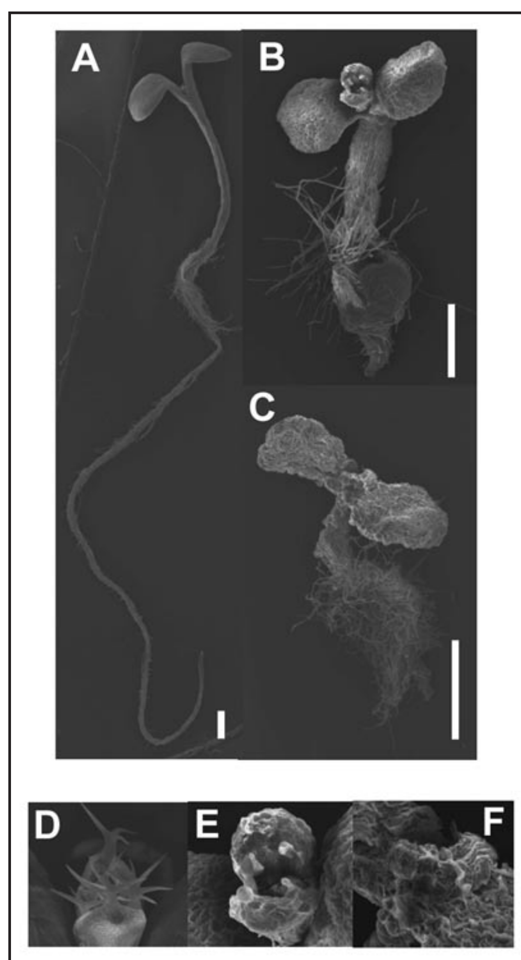

Figure 1. Scanning electron micrograph of one-week-old wild type (A and D), *rsw2-1 stt3a-2 cgl1-T* (B and E) and *rsw2-1 rsw1-1 stt3a-2 cgl1-T* (C and F) seedlings grown at 18°C. Severe growth defects in mutants are obvious. In shoot apical meristem (D–F), aberrant trichome development is seen in *rsw2-1 stt3a-2 cgl1-T* (E). In *rsw2-1 rsw1-1 stt3a-2 cgl1-T* (F), the meristem is transformed into unorganized mass of cells. Bars indicate 0.5 mm.

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