Cellulase in Cellulose Synthase: A Cat among the Pigeons?

Arabidopsis (*Arabidopsis thaliana*) KORRIGAN1 (KOR1) and its close relatives are endo-1,4-β-D-glucanases whose hydrolyzing activity on amorphous cellulose has been demonstrated (Mølhøj et al., 2001; Master et al., 2004; Liebminger et al., 2013). An outstanding characteristic of this group is the existence of a transmembrane domain; thus, these cellulases act on the endomembrane and/or plasma membrane, in contrast to the other cellulases that are secreted into the extracellular space. Similar to Korrigans in the scenes of folklore, KOR1 has hidden behind a mysterious fog for a long time; only paradoxical functions and localizations have been reported thus far.

Mutations in *KOR1* impair cellulose synthesis, resulting in defective cell elongation and dwarfism (Nicol et al., 1998; Zuo et al., 2000; Lane et al., 2001; Sato et al., 2001; Szyjanowicz et al., 2004). Fractionation experiments demonstrated that KOR1 is on the plasma membrane and intracellular organelles (Nicol et al., 1998). However, GFP-tagged KOR1 (GFP-KOR1) expressed under the regulation of the Cauliflower mosaic virus *35S* promoter is not detected on the plasma membrane, although this construct partially complements the growth defect of the *kor1-1* mutant (Robert et al., 2005). The biggest question regarding this cellulase is its function. How does the degrading enzyme cellulase mediate cellulose synthesis? A plausible explanation would be that KOR1 acts in the cellulose synthase complex (CSC) as a regulatory component, possibly by relaxing the tension of cellulose microfibrils, preventing microfibrils from unfavorable cross talk, regulating the length of each glucan chain, or releasing the CSC from the microfibril for its sequestration into the cytoplasm. However, attempts to detect an interaction between the CSC and KOR1 have not been successful (Szyjanowicz et al., 2004; Desprez et al., 2007).

In this issue, Vain et al. (2014) reexamined the effect of the *kor1-1* mutation on CSC dynamics on the plasma membrane and determined that KOR1 is required for the proper movement of the CSC on the plasma membrane. This result may imply an interaction between KOR1 and the CSC on the plasma membrane. These authors then made a significant achievement: they found that GFP-KOR1, driven by its own promoter, was localized to the plasma membrane, where it partly colocalized and comigrated with the CSC in a cortical microtubule-dependent manner. This result suggests a possible interaction between KOR1 and the CSC on the plasma membrane and indicates that the recruitment of KOR1 to the plasma membrane is a strictly regulated process and that the timing and/or the level of its expression is quite critical. GFP-KOR1 was also observed in intracellular punctate organelles, including the Golgi, trans-Golgi network, and late endosomes, in addition to the vacuolar membrane. This punctate localization might represent intermediates in the delivery of KOR1 to the plasma membrane or to the vacuole for degradation. However, it is also possible that the intracellular population of KOR1 could play some role in CSC trafficking, given that the relocalization of the CSC to microtubule-associated compartments in response to treatment with a cellulose synthesis inhibitor was affected by the *kor1-1* mutation.

Finally, the physical interaction between CSC and KOR1 was verified in yeast (*Saccharomyces cerevisiae*) and plant cells by the split-ubiquitin and bimolecular fluorescence complementation methods, respectively. In good agreement with the results of the gel filtration analysis indicating the existence of a high-M_r complex comprising KOR1 and CSC, KOR1 interacted with CESA1, CESA3, and CESA6 in yeast and plant cells. These results indicate that KOR1 is a component of the CSC. The details of this interaction that remain to be defined include the determination of the region of KOR1 necessary for the interaction with CESAs, the stoichiometry between CESA and KOR1 in the CSC, the timing and the place of the interaction and its regulatory mechanisms, the molecular basis of endocytic internalization and recycling, and the function of microtubule-associated compartments. Addressing these subjects could unravel the exact function and regulation of KOR-mediated cellulose synthesis.

Takashi Ueda
Department of Biological Sciences, Graduate School of Science, University of Tokyo, Bunkyo-ku, Tokyo 113–0033, Japan; and Japan Science and Technology Agency, PRESTO, Saitama 332–0012, Japan

LITERATURE CITED

Desprez T, Juraniec M, Crowell EF, Jouy H, Pochylova Z, Parcy F, Höfte H, Gonneau M, Vernhettes S (2007) Organization of cellulose synthase complexes involved in primary cell wall synthesis in Arabidopsis thaliana. Proc Natl Acad Sci USA **104:** 15572–15577

Lane DR, Wiedemeier A, Peng L, Höfte H, Vernhettes S, Desprez T, Hocart CH, Birch RJ, Baskin TI, Burn JE, et al (2001) Temperature-sensitive alleles of *RSW2* link the KORRIGAN endo-1,4-β-glucanase to cellulose synthesis and cytokinesis in Arabidopsis. Plant Physiol **126:** 278–288

Liebminger E, Grass J, Altmann F, Mach L, Strasser R (2013) Characterizing the link between glycosylation state and enzymatic activity of the endo-β1,4-glucanase KORRIGAN1 from Arabidopsis thaliana. J Biol Chem **288:** 22270–22280

Address correspondence to tueda@bs.s.u-tokyo.ac.jp.
www.plantphysiol.org/cgi/doi/10.1104/pp.114.245753

 © 2014 American Society of Plant Biologists. All Rights Reserved.

Master ER, Rudsander UJ, Zhou W, Henriksson H, Divne C, Denman S, Wilson DB, Teeri TT (2004) Recombinant expression and enzymatic characterization of PttCel9A, a KOR homologue from Populus tremula × tremuloides. Biochemistry **43:** 10080–10089

Mølhøj M, Ulvskov P, Dal Degan F (2001) Characterization of a functional soluble form of a *Brassica napus* membrane-anchored endo-1,4-β-glucanase heterologously expressed in *Pichia pastoris*. Plant Physiol **127:** 674–684

Nicol F, His I, Jauneau A, Vernhettes S, Canut H, Höfte H (1998) A plasma membrane-bound putative endo-1,4-beta-D-glucanase is required for normal wall assembly and cell elongation in Arabidopsis. EMBO J **17:** 5563–5576

Robert S, Bichet A, Grandjean O, Kierzkowski D, Satiat-Jeunemaître B, Pelletier S, Hauser MT, Höfte H, Vernhettes S (2005) An *Arabidopsis* endo-1,4-β-D-glucanase involved in cellulose synthesis undergoes regulated intracellular cycling. Plant Cell **17:** 3378–3389

Sato S, Kato T, Kakegawa K, Ishii T, Liu YG, Awano T, Takabe K, Nishiyama Y, Kuga S, Sato S, et al (2001) Role of the putative membrane-bound endo-1,4-beta-glucanase KORRIGAN in cell elongation and cellulose synthesis in Arabidopsis thaliana. Plant Cell Physiol **42:** 251–263

Szyjanowicz PM, McKinnon I, Taylor NG, Gardiner J, Jarvis MC, Turner SR (2004) The irregular xylem 2 mutant is an allele of korrigan that affects the secondary cell wall of Arabidopsis thaliana. Plant J **37:** 730–740

Vain T, Crowell EF, Timpano H, Biot E, Desprez T, Mansoori N, Trindade LM, Pagant S, Robert S, Höfte H, et al (2014) The cellulase KORRIGAN is part of the cellulose synthase complex. Plant Physiol **165:** 1521–1532

Zuo J, Niu QW, Nishizawa N, Wu Y, Kost B, Chua NH (2000) KORRIGAN, an *Arabidopsis* endo-1,4-β-glucanase, localizes to the cell plate by polarized targeting and is essential for cytokinesis. Plant Cell **12:** 1137–1152