Title
Plant cell wall: Never too much acetate

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PLANT CELL WALL

Never too much acetate

Plant cell walls incorporate a variety of acetylated polysaccharides. In addition to enzymes catalysing acetylation (acetyltransferases), plants could produce enzymes to remove acetyl groups (acetylesterases). Previously, pectin acetylesterases were known and now a xylan acetylesterase has been identified — and it has many surprises.

Henrik V. Scheller

Plant cell walls are largely composed of polysaccharides, including cellulose, hemicelluloses and pectins. Hemicelluloses, such as xylans, xyloglucans, mannans and glucomannans, usually contain acetyl groups, which are important for the physicochemical properties of the polysaccharides and their interactions. For example, deficiency in xylan acetylation causes severe dwarfism in Arabidopsis plants1. A large family of acetyltransferases is proposed to be responsible for polysaccharide acetylation2. During developmental processes, polysaccharides in the cell wall are modified or degraded, and therefore acetylesterases might be present for deacetylation of different polysaccharides. There are four families of carbohydrate esterases (CEs) known in plants (www.cazy.org)3: CE6, CE8, CE11 and CE13. CE13 consists of pectin acetylesterases and CE11 is a large family of pectin methylesterases, while CE6 and CE8 are small families that have not been reported to be involved in cell wall metabolism. Now, in a paper published in *Nature Plants*, Zhang *et al.* report the identification of a rice xylan acetylesterase, BRITTLE LEAF SHEATH1 (BS1), which belongs to a new family of carbohydrate esterases4.

The new esterase is important for the structure of xylans and plant vascular development; *bs1* loss-of-function mutants have significantly elevated xylan acetylation and defects in plant growth and development. BS1 belongs to the large family of putative GDSL lipases/esterases, which are characterized by a distinct GSDL sequence motif. In *Arabidopsis* and rice, there are 107 and 112 members of the family, respectively, and very few members have a documented biochemical function; for example, FUCOSYLATED XYLOGLUCAN 1 (FXG1), which has been reported to be an α-fucosidase5. It is surprising that glycosidases and carbohydrate esterases are found in the same protein family, as they have different catalytic properties. Esterases often have low specificity *in vitro* and it may be worth having a second look at the FXG1 protein and other members of the GDSL family with reported glycosidase activity to see if they have acetylesterase activity and what their functions are *in vivo*. The broad specificity of esterases *in vitro* is a major challenge in assigning their functions, but in the case of the Zhang *et al.* study, the xylan acetylesterase activity *in vitro* is consistent with the phenotype of the mutant plants, which provides confidence in the functional assignment.

With a few exceptions (for example, cellulose), all cell wall polysaccharides have backbones with substitutions by other sugars. The backbones or the side-chain sugars are often esterified with acetyl groups, methyl groups or hydroxycinnamate groups. Plants produce a very large number of hydrolases that are involved in the turnover of polysaccharides in the cell wall. In general, these enzymes are secreted to the apoplast where polysaccharide modification or turnover takes place. Most known pectin acetylesterases and methylesterases are secreted to the apoplastic cell wall for further action. In contrast, the new xylan acetylesterase reported by Zhang *et al.* is a membrane-bound protein located in the Golgi apparatus. This is supported by the results of fluorescent confocal microscopy of BS1 protein in rice protoplast, immunoblotting of BS1 in different membrane fractions, and immune-gold electronic microscopy of BS1 in rice

root tip cells. Although BS1 protein is localized in the Golgi, it should be pointed out that the absence of BS1 from the cell wall has not been demonstrated, and it therefore remains a possibility that the protein is secreted but temporarily retained in the Golgi, as has been observed for some pectin methylesterases6.

Polysaccharide-degrading enzymes in the plant cell wall are involved in cell growth and developmental processes, which require modification or breakdown of polysaccharides. They may also be required to remove substitutions that keep the polysaccharide soluble during intracellular transport and prevent polysaccharide incorporation into the cell wall. It is highly possible that polysaccharide hydrolases in the Golgi are playing an editing role, for instance, by correcting 'mistakes' made by biosynthetic enzymes (Fig. 1). It appears that BS1 has such a function in removing too much acetylation of xylose residues to ensure the right amount of xylan acetylation. Furthermore, xylans in *bs1* mutant plants have a substantial amount of acetylated arabinose, which is not usually found in the wild-type plants. Therefore, BS1 is also playing a role in removing this unusual acetylation.

The GDSL family is very large and consists of three subclades — with the BS1 xylan acetylesterase in subclade B. Subclade B includes the aforementioned FXG1 and also EARLY NODULIN 8, which is found in root nodules of legumes. The GDSL family has undergone substantial gene duplication and there is probably a lot of functional redundancy within the protein family. Nevertheless, we could expect that some of the homologues in subclade B, or even in the other subclades, are carbohydrate acetylesterases with specificities for different polysaccharides, such as (gluco)mannans and xyloglucans. Furthermore, xylans are complex polysaccharides with at least two different types of domains, and there are different types of xylans in primary and secondary cell walls. Therefore, multiple xylan acetylesterases with different specificities in plants would be expected.

The work of Zhang *et al.* is important because it identifies a new family of carbohydrate esterases and it suggests that polysaccharide deacetylation may take place in the Golgi prior to deposition of the nascent polymer in the plant cell wall. The paper also raises many interesting questions that can be addressed in future work: are other members of the GDSL family also carbohydrate esterases and what are their specificities? What difference does it make whether deacetylation takes place in the Golgi or *in muro*? Why does BS1 not completely deacetylate xylans? How does excess acetylation of xylans affect their functional properties?

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References

1. Xiong, G., Cheng, K. & Pauly, M. *Mol. Plant* **6,** 1373–1375 (2013).
2. Gille, S. *et al. Plant Cell* **23,** 4041–4053 (2011).
3. Lombard, V., Ramulu, H. G., Drula, E., Coutinho, P. M. & Henrissat, B. *Nucleic Acids Res.* **42,** D490–D495 (2014).
4. Zhang, B. *Nat. Plants* **3,** 17017 (2017).
5. de la Torre, F., Sampedro, J., Zarra, I. & Revilla, G. *Plant Physiol.* **128,** 247–255 (2002).
6. Wolf, S., Rausch, T. & Greiner, S. *Plant J.* **58,** 361–375 (2009).

Competing interests

The author declares no competing financial interests.

Figure 1. Acetylesterases may be needed to trim excess acetylation from nascent polysaccharides. Xylans are cell wall polysaccharides that are made from xylose and usually acetylated, with acetyl groups on some of the backbone xylose residues. However, esterases such as BS1 may be required to trim excess acetylation from the nascent xylan polymers. In the absence of BS1 activity, rice xylans are more heavily acetylated and surprisingly contain

acetylated arabinose residues, which are not usually present. Xylans are very important components of secondary cell walls and essential for proper function and development of plant vasculature. The right amount of xylan acetylation is required; too much or too little acetylation has adverse effects on plant growth and development.