Xylan decoration patterns and the plant secondary cell wall molecular architecture

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Abstract
The molecular architecture of plant secondary cell walls is still not resolved. There are several proposed structures for cellulose fibrils, the main component of plant cell walls, and the conformation of other molecules is even less well known. GUX enzymes, in CAZy family GT8, decorate the xylan backbone with various specific patterns of glucuronic acid. It was recently discovered that dicot xylan has a domain with the side chain decorations distributed on every second unit of the backbone (xylose). If the xylan backbone folds in a similar way to glucan chains in cellulose (2-fold helix), this kind of arrangement may allow the undecorated side of the xylan chain to hydrogen bond with the hydrophilic surface of cellulose microfibrils. Molecular dynamic simulations suggest that such interactions are energetically stable. We discuss the possible role of this xylan decoration pattern in building of the plant cell wall.

Introduction
Plants are not only a source of oxygen, and the primary means of food production. They also supply industrial materials such as wood, cotton, and jute for building construction, and the production of paper, clothing, and rope. Recently, interest has been generated in using plant material as a renewable source of transport fuel. The principal component of plant biomass is secondary cell walls. Despite the importance of this, our knowledge of the cell wall structure is still tentative. Cell wall architecture arises from interactions between major components of the secondary cell wall; namely cellulose, the hemicelluloses and lignin [1]. Knowledge of the chemical composition of cell walls alone is insufficient to understand how the molecular architecture influences biomass properties.

Structure of cellulose in the plant cell wall
The cell wall is composed primarily of cellulose. Cellulose fibrils contain polymers of β-(1, 4) linked glucan organised in a 2-fold screw conformation, with alternating monomers rotated through 180°. It is thought that between 18 and 24 of these polysaccharide chains assemble into fibrils. The polymers are connected within the plane of the crystal by hydrogen bonds. Different surfaces of the fibril exhibit different properties, with hydrophobic surfaces exposing the “top” of sugar ring and hydrophilic surfaces exposing hydroxyl groups of the sugars. A number of studies have addressed the structure of cellulose fibrils and differences in the results and proposed structures may also reflect possible variability across different plants and tissues [2-5].

Xylan decorations are added by specialized groups of enzymes
Hemicellulose structures are more diverse than cellulose, and differ across plants and tissues. In the secondary cell walls of the dicotyledonous plant *Arabidopsis thaliana*, the predominant hemicellulose is xylan, a β-(1, 4) linked xylose polymer, where the monomers may be variably decorated with acetyl and [4-O-Methyl]Glucuronic acid ([Me]GlcA) groups [6, 7].

Acetyl decorations are fairly frequent with around half the xylose residues carrying this moiety. The acetyl functional group (CH_3COO-) is ester linked to

the xylan backbone at either the 2-OH and/or 3-OH position and in extracted xylan it has a tendency to migrate between the two positions, provided one is free [8]. Xylan acetylation is catalyzed by members of the Trichome Birefringence Like (TBL) family, which includes the xylan acetyltransferase TBL29 [9-11]. Acetyl groups occur on xylan with a regular distribution, on every second xylose residue [12, 13]. Analysis of acetylated xylan isolated from Arabidopsis microsomes revealed that an even pattern of decorations is observable before secretion, and that this is similar to the type of pattern seen in the secondary cell wall. It is thus likely that the process of pattern formation is controlled, in an unknown manner, during synthesis in the Golgi apparatus and prior to deposition into the wall [12].

In contrast to acetylation, glucuronic acid (GlcA) decorations exist on only one in eight xylose residues, and are strictly limited to a glycosidic linkage at the 2-OH position. The GlcA residues themselves may or may not become methylated [Me] at the 4-OH position through the actions of one of the three DUF579 GlucuronoXylan Methyltransferases (GXM), [14, 15]. Methylation occurs after the GlcA moieties have been transferred onto the xylan backbone. The proteins responsible for addition of GlcA to the backbone are known as the Glucuronic Acid substitution of Xylan (GUX) enzymes [16, 17]. These belong to the CAZy Glycosyl Transferase (GT) 8 family and catalyse the addition of unmethylated GlcA to the backbone (Figure 1). Five *Arabidopsis* GUX enzymes are currently classified. Of those GUX1, -2 and -4 have been demonstrated to have GlcA transferase activity *in vitro* [18], whereas *in plantae* studies have demonstrated that GUX1 and -2 alone are responsible for addition of the GlcA to the xylan backbone in secondary cell wall [16] and that GUX3 activity is necessary and sufficient for glucuronosylation of primary cell wall xylan [19].

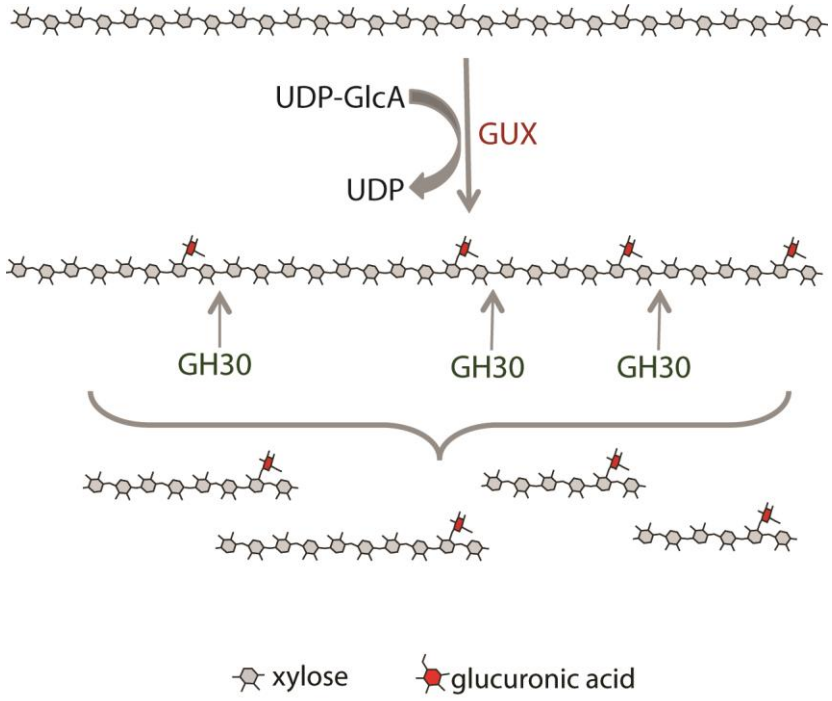

Figure 1. GUX enzymes catalyse addition of GlcA (red) to the xylan backbone (grey) using UDP-GlcA as a substrate. The spacing between the glucuronic acid branches can be analysed using glucuronoxylanase GH30. The length of the oligosaccharide resulting from the digestion corresponds to the interval between GlcA moieties. For simplicity the acetylation of the xylan backbone and methylation of glucuronic acid have been omitted.

Carbohydrate active enzymes – powerful tools to study xylan decorations

Even though [Me]GlcA decorations are on average on every eight xylosyl residue, the actual distribution of residues on an intact xylan backbone is not necessarily regular. It is possible to employ glycoside hydrolases (GH) with specific and known activities to understand how these decorations are positioned relative to each other. Xylanases of the CAZy GH30 family accommodate the [Me]GlcA decoration in a binding pocket at their -2 subsite and hydrolyse the glycosidic bond two residues toward the reducing end of the xylan chain [20-22]. The size of the various oligosaccharides products after hydrolysis thus corresponds to the spacing between [Me]GlcA decorations [23] (Figure 1). This approach was used to determine the spacing of [Me]GlcA decorations on Arabidopsis xylan. It was found that after hydrolysis a wide dispersion of various oligosaccharide lengths was produced, ranging in size from DP 4 to 20. In particular there was a significant enrichment for oligosaccharides with an even number of xylose residues above those with an odd number of residues [17]. In Arabidopsis primary cell walls on the other hand, [Me]GlcA is positioned regularly, on every sixth xylose residue [19].

GUX enzymes produce different patterns of xylan decoration

GUX1 and GUX2 both decorate the secondary cell wall xylan, but the patterns they generate differ [17]. GUX1 is responsible for around 70% of all GlcA decorations. In *gux2* mutant plants, where only GUX1 is active, nearly all [Me]GlcA decorations are spaced with an even number of xylose residues between the [Me]GlcA decorations. Conversely, approximately 30% of GlcA decorations are added by GUX2. In the *gux1* mutant plant [Me]GlcA decorations are found to be more tightly clustered and positioned randomly, primarily every 5, 6, or 7 residues apart and do not display any precedence for an even number of residues between decorations.

Overexpression analysis has demonstrated that GUX2 is unable to decorate more than 30% of the xylan backbone, even in the absence of GUX1. Similarly, overexpression of GUX1 has only limited effect on decoration frequency, resulting in a few more, yet still evenly spaced decorations. It is tempting to postulate that these different domains are present on different xylans, but, as isolated secondary cell wall xylan cannot be separated based on charge or size, this indicates that that GUX1 and GUX2 decorate discrete regions on the same molecule [17]. The even pattern of 6 residue spacing seen on primary cell wall xylan is generated by GUX3 alone [19].

Phylogenetic analysis of Arabidopsis GUX and homologous protein sequences in GT8 from rice, poplar and Physcomitrella (Figure 2) reveals that GUX1 and GUX3 are clustered within a single clade, whereas GUX2 is within a separate branch. This suggests that the sequence and structure of the GUX

proteins may be linked to their activity, because enzymes decorating xylan with an even number of residues between decorations (GUX1 and GUX3) are distinct from those decorating xylan more randomly (GUX2). *In vitro* assays using xylohexaose acceptors support the view that positional specificity is a property of the enzyme [18]. It will be important to understand how GUX enzymes are able to generate these remarkably different patterns.

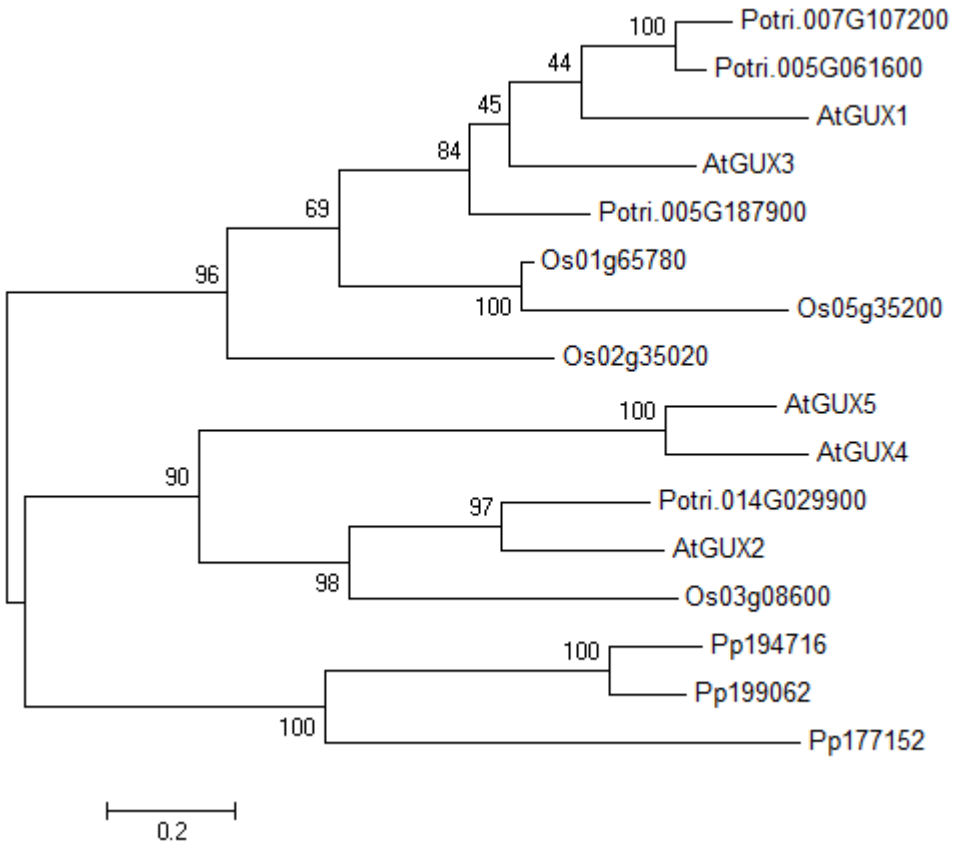

Figure 2: Phylogenetic analysis of *A. thaliana* GUX1 – 5 (AtGUX1 -5) and their putative orthologues from *Populus trichocarpa* (poplar, Potri), *Oryza sativa* (rice, Os) and *Physcomitrella patens* (moss, Pp) performed using protein amino acid sequences. Despite originating from same species as AtGUX1 and AtGUX3, Arabidopsis GUX2 is positioned within a separate clade together with one putative GUX enzyme from *O. sativa* and *P. trichocarpa*. Both AtGUX1 and AtGUX3 are known to generate the cellulose binding compatible pattern of GlcA decoration. AtGUX2 creates an incompatible pattern of GlcA decoration. The split of Arabidopsis GUX1/3 and AtGUX2 into two clades suggests that the amino acid sequence of GUX proteins might be associated with the function as either compatible or incompatible pattern generating enzymes. Both rice and poplar have one putative GUX enzyme in the same clade as AtGUX2 and all other enzymes in the same clade as AtGUX1/3. This observation further supports the hypothesis that the amino acid sequence of GUX enzymes might be related to its function. Glucuronoxylan is present in moss cell walls [24] and the putative *P. patens* GUX enzymes were used as an out-group. Phylogenetic analysis was performed using MEGA6 [25].

Compatible decoration pattern for xylan - cellulose interaction

Although xylose is a pentose and glucose is a hexose, the two polysaccharides are similar in that both are composed of β-(1,4) linked pyranose sugars with identical ring hydroxyl configurations; this may permit a

stable interaction between the two. These polysaccharides are however, not formed in unison, as cellulose is produced directly at the cell membrane whereas xylan is synthesised in the Golgi and secreted via vesicles into the wall [26-29].

When in solution, free xylan adopts a three-fold helical screw conformation [30]. The conformation of xylan in the cell wall is not yet known, but recent solid state NMR suggests it may adopt different conformations in the cell wall [31]. If xylan is folded as a two-fold helical screw it would be similar to the two-fold screw glucan chain conformation in cellulose, and provide a surface for interaction with cellulose [32]. Indeed, molecular dynamic simulations predict evenly decorated xylan would adopt a two-fold helical screw confirmation upon interaction with cellulose [12]. Docking of the two-fold helical screw to the hydrophilic cellulose surface is sterically permissible only in sections of xylan which carry evenly spaced decorations, as this would result in all the decorations being positioned along one side of the xylan chain, whilst the other face would be free of decorations. Such xylan could be referred to as being *compatible* for binding with cellulose. Conversely, xylan that has decorations which are not evenly distributed would obstruct interactions with hydrophilic cellulose surface and could be termed as being *incompatible*. In the secondary cell wall, the different regions of xylan decorated by GUX1 and GUX2 may therefore regulate interactions between xylan and cellulose, either by permitting or preventing direct association of xylan and the hydrophilic surface of cellulose.

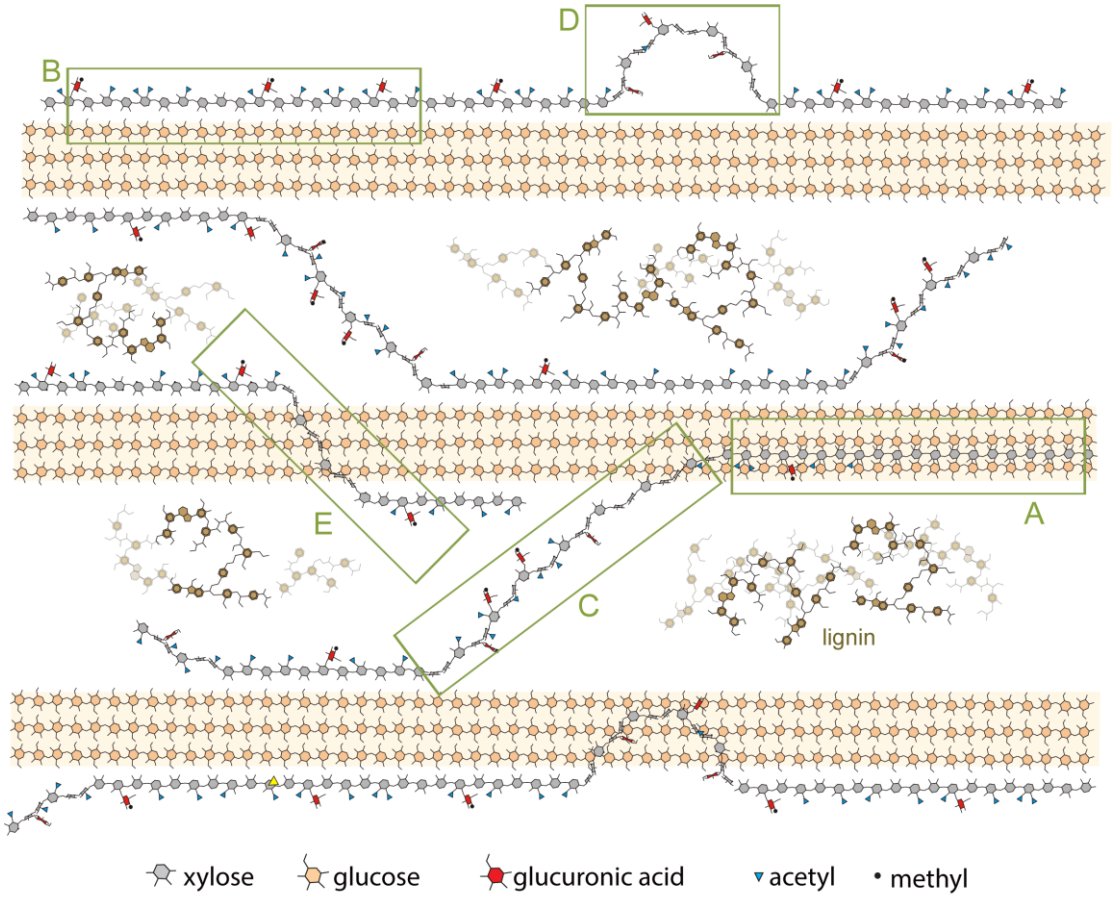

Figure 3: Molecular model indicating possible roles of xylan in maintaining dicot secondary cell wall architecture. Xylan can interact with hydrophobic surface of cellulose (top view, A), and the compatible domain is also able to tightly associate with hydrophilic face of cellulose (B). The incompatible domains may allow tethering of adjacent cellulose fibrils (C), form loops which dissociate from the fibril and extend into the matrix and associate back to the same or different fibril (D), or span the fibrils and dock onto a different hydrophilic groove of the same fibril (E).

The existence of these discrete domains may give insight into the role of xylan *in vivo*. Some possible arrangements of secondary cell wall xylan are shown in Figure 3. Whereas both domains are able to interact with hydrophobic surface of cellulose, the compatible domain is also able to associate tightly with the hydrophilic face of cellulose. The sheathing of the hydrophilic surface of cellulose with xylan chains may provide a hydrophobic surface for interaction of the fibrils with lignin, or protect the cellulose fibril from aggregation with other adjacent fibrils. The incompatible domain may form loops that dissociate from the microfibril and extend into the matrix and associate back to the same or different fibril. An incompatible domain may thus form a tether between two compatible domains, cross-linking separate cellulose microfibrils and stabilising the extracellular matrix; thereby maintaining the architecture of the secondary cell wall.

Conclusions

While the molecular architecture of the secondary plant cell wall remains unresolved, it is known that xylan can bind to cellulose. Recent findings

regarding the unexpected patterning of the substitutions on xylan allow building and *in silica* testing of a new and molecularly detailed model of xylan – cellulose interactions. The model predicts that the compatible domain of xylan, which has decorations precisely spaced, solely on alternating residues of the backbone, would be able to bind to the hydrophilic surface of the cellulose. All xylan domains, regardless of their decoration patterns, can bind in a less defined manner on the hydrophobic surfaces of cellulose. In this manner, the cellulose fibril could be covered by xylan on all its surfaces. It will be important to test these speculative proposals, as they have profound implications for our understanding of the basis of biomass properties such as strength and recalcitrance to enzymatic attack.

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