Mini Review

The Glycosyltransferases involved in Synthesis of Plant Cell Wall Polysaccharides: Present and Future

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Abstract

Glycosyltransferases are enzymes which transfer an activated sugar to an acceptor substrate such as polysaccharides, peptide, lipid or various small molecules. In the past 10-15 years, substantial progress has been made in the identification and cloning of genes that encode polysaccharide synthesizing glycosyltransferases. However, majority of these enzymes remain structurally and mechanistically uncharacterized. This short review will focus on the questions in biochemistry of polysaccharide synthesizing glycosyltransferases to be answered in coming years.

INTRODUCTION

Plant cell walls have been proposed to be a source of renewable energy in the form of lignocellulosic liquid biofuels. In the past 10-15 years, significant progress has been made in understanding of cell wall polysaccharide biosynthesis, particularly, in identifying and characterizing the numerous genes involved in this complex process. With respect to the challenges of revealing the genes required, molecular biology, reverse-genetics, and genomics has provided many powerful tools and significantly advanced our understanding of plant cell wall formation. A significant body of recent reviews describes the advances and the current state in understanding of plant polysaccharide biosynthesis [1-5]. However, the progress in biochemical characterization of the gene products, glycosyltransferases, is being much slower and currently falls behind the successful genetic studies. In part, this is due to the low solubility of these enzymes and the lack of suitable enzyme assays. For example, the small stereo-chemical differences between sugar moieties and the multiple ways these moieties can be linked to each other, which were used by nature to achieve the wide diversity of oligo- and polysaccharide present in different types of plant cell walls, limits selection of suitable substrates and complicates characterization of products. Despite current limitations, the structural characterization and mechanisms of catalysis of plant glycosyltransferases will certainly be a subject of intensive research in the coming years due their essential function in plant cell wall biosynthesis. We present here the brief overview on the long-standing unanswered questions and directions we believe the field of cell wall polysaccharide synthesizing glycosyltransferases is headed.

Synthesis of the branched and heterogeneous polysaccharide structures requires action of multiple specific glycosyltransferases and synthases which transfer a donor sugar substrate to oligosaccharide acceptor. The donor substrates are typically activated sugars such as nucleotide sugars (UDP or GDP-bound) or, more rarely, phosphorylated sugars [6]. The structures solved for GTs from other organisms, the majority of which are involved in glycosylation of small lipophilic molecules, showed that the catalytic domains of most GTs have two types of fold, GT-A and GT-B. However, the existence of the different type, GT-C was also proposed [6]. GT-A contains two $\beta/\alpha/\beta$ Rossmann-like folds tightly associated forming a continuous β-sheet and while GT-B also has two $\beta/\alpha/\beta$ Rossmann-like folds, they are not tightly intertwined but face each other with the active site residing between them. GT-A folds are metal dependent, which is coordinated by the well documented DxD motif [7], while GT-B are metal independent. In addition to these two structural folds, GTs is further characterized whether the chemical bond formed is an inversion or retention of stereochemistry with respect to the donor substrate. The most common donor substrate is nucleotide sugars where the sugar is linked via alpha bond. If a GT catalyzes the formation of the glycosidic bond attaching the sugar to the acceptor molecule via beta bond, the stereochemistry is inverted, while if the new glycosidic bond formed is alpha the stereochemistry is retained. The catalytic mechanism of inverting glycosyltransferases has been demonstrated to be a direct displacement S_N2-like reaction in which an active site residue acts as a general base to deprotonate the acceptor which performs a nucleophilic attack on the donor anomeric

carbon [6]. The mechanism of retaining glycosyltransferases has yet to be elucidated but numerous possibilities have been proposed such as a double displacement mechanism or a front-side single displacement (S_Ni) mechansism [8-10]. The plant cell wall biosynthetic enzymes are found in at least three of these classifications.

Glycosylsynthases

Cellulose Synthase (Ces) and Cellulose Synthase-Like (CSL) are integral, membrane proteins with multiple transmembrane domains (TD) which span the Golgi membrane (or plasma membrane in the case of Ces) multiple times and belong to CAZy family GT2 [11]. The first solved structure for a protein from GT2 family was the structure of the catalytic domain of polysaccharide synthesizing protein, SpsA, from B. subtilis [12], which demonstrated that catalytic domains of GT2 proteins adopt GT-A fold. The 3D structure of SpsA allowed prediction of the active site amino acids important for substrate binding and catalysis [13] and served as a prototype for the organization of other family GT2 synthases. More recently, the structure for *R. sphaeroides* Ces domains BcsA and BcsB demonstrated that TDs form a pore through which the synthesized glucan chain is translocated across the plasma membrane [14]. Another study resulted in a 3D computational model of the predicted cytosolic domain of cotton CESA (GhCESA1) [15], which showed good structural agreement between BcsA and GhCESA1. In both structures, the catalytic residues within GT-A fold included the matching motifs DDG, DCD and TED. The DDG and DCD motifs coordinate UDP and divalent cation and the D of the TED motif, most likely, acts as the catalytic base [14, 16]. The motif QRGRW in BcsA is positioned near the plasma membrane and was shown to interact with cellulose acceptor substrate, whereas in GhCESA1 a QVLRW motif was proposed to have similar function and similar positioning near plasma membrane [16]. These solved structures dismissed the long standing speculation about two active sites possibly present within the same peptide [17,18] to explain cellulose synthesis, presenting convincing model of

how it is done by a single active site in concert with the pore for glucan translocation [14].

Although the β-glycan synthases in the gene family GT2 synthesize the polysaccharides with different linkages (1,3-β, 1,4-β or mixed 1,3-β;1,4-β), they have related sequences and conservative motifs implicated in substrate binding and catalysis. Therefore, it was proposed that they most likely have similar folding patterns and mechanisms of catalysis [19]. For example, the topologies of AtCSLC4 involved in synthesis of xyloglucan backbone [20] and BdCSLF6 involved in synthesis of mixed (1,3;1,4)-β-glucan [21] suggest that these two synthases translocate the corresponding glucan chain into Golgi lumen (Figure 1). However, AtCSLC4 has only six predicted TDs, so it is unclear whether six TDs are able to form wide enough pore to accommodate the glucan chain similar to BcsA. On the other hand, although BdCSLF6 has eight TDs, it was shown to synthesize mixed (1,3;1,4)-β-glucan which has a structure with a kink formed by 1,3-β-linkage. So, how can non-linear glucan chain with two different linkages be formed and accommodated inside the presumably tightly organized pore? The structural characterization of additional family GT2 members which have different number of predicted TDs and synthesize different products would aid in answering those questions.

Glycosyltransferases

In addition to the synthases described above numerous other glycosyltransferases (GT) are involved in cell wall polysaccharide biosynthesis, which are type II membrane proteins residing in Golgi. They possess the short N-terminus domain localized in cytosol, one TD, flexible stem region and a catalytic domain localized in Golgi lumen [22], though the presence of GTs without predicted TD was also reported [23]. To date, none of the plant GTs involved in cell wall biosynthesis has been structurally characterized. The lack of structural data is a significant gap in our current knowledge about glycosyltransferases, which currently precludes further progress in understanding polysaccharide biosynthesis. In addition, determination of substrate binding or
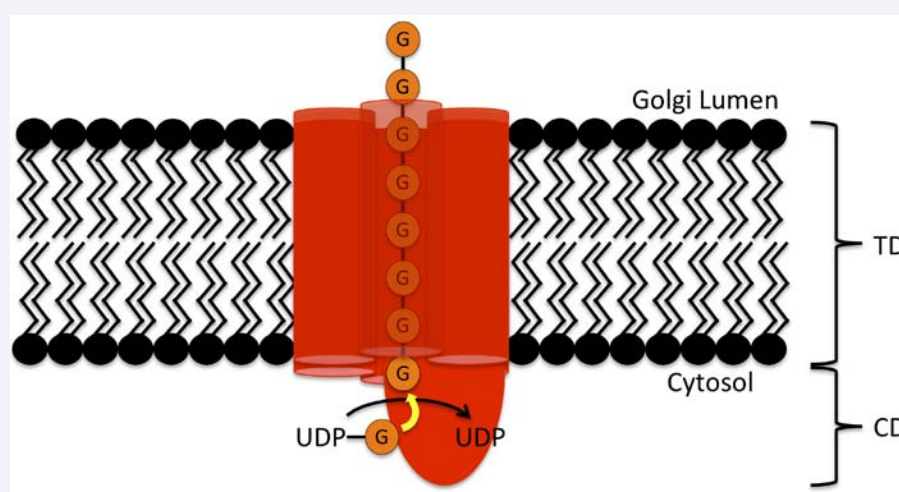

Figure 1 Proposed mode of action of CSLC4. Six predicted transmembrane domains (TD) and one catalytic domain (CD) residing in the cytosol are shown. The catalytic domain transfers glucose from UDP-glucose to a growing glucan chain which is channeled through the protein-formed pore across the membrane into the Golgi lumen.

catalysis of each enzyme is extremely difficult due to the lack of structural similarity and diversity in chemical bond formed, thus one cannot use a single well defined enzyme as a model for another. For example, according to classification system based on amino acid sequence [24,6] two glycosyltransferases with diverse specificity, Xyloglucan Xylosyltransferase (XXT) involved in xyloglucan biosynthesis [25] and Galacturonosyltransferase 1 (GAUT1) involved in homogalacturonan biosynthesis [26] are retaining GTs with the GT-A fold. On the other hand, two xylosyltransferases, which are involved in xylan biosynthesis, are classified differently according to their sequences: Irregular Xylem 14 (IRX14) [27] is an inverting enzyme with the GT-A fold, whereas Irregular Xylem 10-Like (IRX10L), renamed to XYS1 [28], is an inverting enzyme with the GT-B fold. To date, none of plant cell wall synthesizing GTs is predicted to be a retaining enzyme with the GT-B fold [6]. Despite this difficulty, there are few well characterized enzymes from other organisms, which belong to the same gene families as plant cell wall biosynthetic enzymes and in some cases, like described above for Ces proteins, serve as prototypes for initial biochemical analysis.

In addition to the limitations imparted by the lack of structural data, it is also difficult to deduce any information in regards to acceptor substrate binding due to its complexity and diversity. This diversity of acceptor substrates requires the screening of various synthetic acceptor substrates, which are not readily available, for each specific glycosyltransferase. Cavalier and Keegstra [25] investigated the position and processivity of XXT1 and XXT2 catalysis of the xyloglucan backbone. It was found that both XXT1 and XXT2 are capable of xylosylation of cellohexaose at two positions forming α-1,6 bonds and all of the acceptor substrate was first xylosylated at the fourth glucose from the reducing end and then once all acceptor was monoxylosylated, catalysis proceeds at the third xylose from the reducing end (Figure 2A). These results indicate that both UDP and the xylosylated acceptor are released and the xylosylated acceptor rebinds along with a fresh UDP-Xylose for another round of catalysis (Figure 2B). In addition, a oligoglucan with degree of polymerization (DP) of six was found to be the most suitable as the acceptor substrate, and enzyme activity decreased with DP was decreased. Increasing the DP further (e.g., celloheptaose) results in low solubility of the molecule and thus could not be analyzed, yet it cannot be ruled out that longer acceptor substrates may further increase XXT2 activity. In contrast to this, IRX10L/XYS1 was found to have an optimal DP of four and activity decreased with less or higher DP [28]. This is intriguing because XXT catalyze addition of sugar in the middle of the chain, while IRX10L/XYS1 glycosylated at the non-reducing end. Yet it is difficult to compare these two enzymes because XXTs are retaining enzymes with predicted GT-A fold, and IRX10/XYS1 is inverting enzyme with predicted GT-B fold GT.

There are also putative non-catalytic GTs involved in plant cell wall biosynthesis including GAUT7, IRX9, and IRX14 [26,29]. XXT5 has also been proposed to be non-catalytic due to lack of *in vitro* activity, yet no evidence has confirmed this [30]. The function of these enzymes has been proposed to anchor other catalytic GTs to the golgi membrane which lack TDs or to channel the donor substrate to the active enzyme. For example, GAUT1 TD is post-translationally proteolytically cleaved *in vivo* yet is retained in the Golgi via physical interactions with GAUT7 [26], and IRX14 was proposed to channel UDP-Xylose to IRX10 [29].
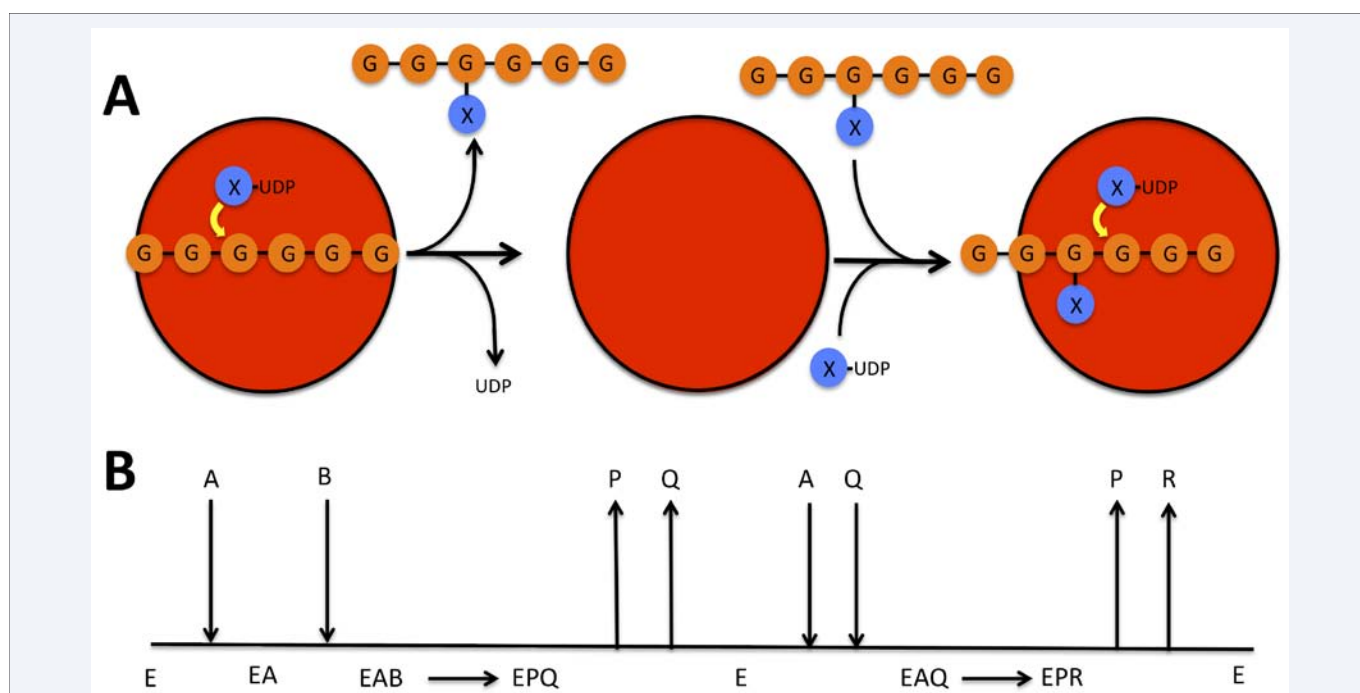

Figure 2 Proposed mode of action of XXT1 and XXT2. A) Positions of xylosylation and the main product formed, GGXGGG, which is then released and rebound for the second xylosylation which primarily forms GGXXGG. B) Kinetic mechanism of XXTs showing that both products are released before the second xylosylation occurs. Based on previous reports for other retaining GT-A fold glycosyltransferases, it is plausible that UDP-Xylose binds first followed by acceptor binding yet no data has been reported to confirm this. E: Enzyme; A: UDP-Xylose; B: Cellohexaose; P: UDP; Q: Monoxylosylatedcellohexaose; R: Bixylosylatedcellohexaose.

However, the hypothesis about IRX14 functioning has yet to be experimentally confirmed.

CONCLUSION

Genetic studies have significantly advanced the field of plant cell wall biology, and currently, most of GT gene families, members of which are predicted to be involved in cell wall polysaccharide biosynthesis, possess at least one functionally characterized plant GTs. However, a simple gene family assignment is not sufficient for the accurate prediction of GT's substrate specificities and mechanism of catalysis and there are a number of outstanding questions outlined in this brief overview. In order to engineer a plant cell wall for a specific purpose we must first understand the enzymes involved. This requires knowledge of not only the phenotypical changes of transgenic knock-out plants, but also the structure and mechanism of the specific enzyme. Characterization of these enzymes *in vitro* will aid in targeting specific enzymes for engineering of a bioenergy dedicated plant, or to engineer an enzymes with new specific functions to obtain the desired characteristics within the plant molecular framework. Although donor substrate binding can be predicted based on previous work on other glycosyltransferases in the same GT family, modes of acceptor binding are is still yet to be characterized due to the complexity and diversity of these substrates. This work will be aided by recently emerged new expression systems for recombinant protein production [28,31] and advanced technologies in structural biology, enzymology, and computational simulations. Future advances in the field of polysaccharide synthesizing glycosyltransferases will aid in the biotechnological production of specific recombinant glycosyltransferases or plants with modified or even novel cell wall components which are engineered for specific applications such as biofuels [32], biomaterials [33], or pharmaceuticals [34].

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