



Golgi-mediated synthesis and secretion of matrix polysaccharides of the primary cell wall of higher plants

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The Golgi apparatus of eukaryotic cells is known for its central role in the processing, sorting, and transport of proteins to intra- and extra-cellular compartments. In plants, it has the additional task of assembling and exporting the non-cellulosic polysaccharides of the cell wall matrix including pectin and hemicelluloses, which are important for plant development and protection. In this review, we focus on the biosynthesis of complex polysaccharides of the primary cell wall of eudicotyledonous plants. We present and discuss the compartmental organization of the Golgi stacks with regards to complex polysaccharide assembly and secretion using immuno-electron microscopy and specific antibodies recognizing various sugar epitopes. We also discuss the significance of the recently identified Golgi-localized glycosyltransferases responsible for the biosynthesis of xyloglucan (XyG) and pectin.

Keywords: cell wall, immunocytochemistry, glycosyltransferases, Golgi, polysaccharides, plants

INTRODUCTION

One of the most important functional properties of the plant Golgi apparatus is its ability to synthesize complex matrix polysaccharides of the cell wall. Unlike cellulose which is synthesized at the plasma membrane and glycoproteins whose protein backbones are generated in the endoplasmic reticulum, the cell wall matrix polysaccharides (pectin and hemicelluloses) are assembled exclusively in the Golgi cisternae and transported to the cell surface within Golgi-derived vesicles (Driouich et al., 1993; Lerouxel et al., 2006).

The synthesis of cell wall matrix polysaccharides occurs through the concerted action of hundreds of glycosyltransferases. These enzymes catalyze the transfer of a sugar residue from an activated nucleotide–sugar onto a specific acceptor. The activity of these enzymes depends, in turn upon nucleotide–sugar synthesizing/interconverting enzymes in the cytosol, and also on the nucleotide–sugar transporters necessary for sugar transport into the lumen of Golgi stacks and subsequent polymerization.

Because cell wall matrix polysaccharides exhibit a high structural complexity, their biosynthesis must be adequately organized and a certain degree of spatial organization/coordination must prevail within Golgi compartments, not only between glycosyltransferases themselves, but also between glycosyltransferases nucleotide–sugar transporters and nucleotide–sugar interconverting enzymes (Seifert, 2004; Reiter, 2008).

Cell wall matrix polysaccharides are known to confer important functions to the cell wall in relation with many aspects of

plant life including cell growth, morphogenesis and responses to abiotic, and biotic stresses. Plant cell walls are also an important source of raw materials for textiles, pulping and, potentially, for renewable biofuels or food production for humans and animals (Cosgrove, 2005; Pauly and Keegstra, 2010).

THE PRIMARY CELL WALL IS COMPOSED OF DIVERSE COMPLEX CARBOHYDRATES

Plants invest a large proportion of their genes (~10%) in the biosynthesis and remodeling of the cell wall (Arabidopsis Genome Initiative, 2000; International Rice Genome Sequencing Project, 2005; Tuskan et al., 2006).

Cell walls of flowering plants are highly diverse and heterogeneous (Popper et al., 2011). They are composed of a variety of complex carbohydrates whose compositional and structural properties are controlled spatially and temporally as well as at the tissue and cell levels (Roberts, 1990; see also Burton et al., 2010).

The primary wall of eudicotyledonous plants comprises cellulose microfibrils and a xyloglucan network embedded within a matrix of non-cellulosic polysaccharides and proteins (i.e., glycoproteins and proteoglycans). Four major types of non-cellulosic polysaccharides are found in the primary walls of plant cells (in taxa outside the gramineae), namely the neutral hemicellulosic polysaccharide xyloglucan (XyG) and three main pectic polysaccharides, homogalacturonan (HG), rhamnogalacturonan I and

rhamnogalacturonan II (RG-I and RG-II; Carpita and Gibeaut, 1993; Mohnen, 2008; Scheller and Ulvskov, 2010).

XyG consists of a β -D-(1-4)-glucan backbone to which are attached side chains containing xylosyl, galactosyl-xylosyl, or fucosyl-galactosyl-xylosyl residues. In eudicotyledonous plants, XyG is the principal polysaccharide that cross-links the cellulose microfibrils. It is able to bind cellulose tightly because its β -D-(1-4)-glucan cellulose-like backbone can form numerous hydrogen bonds with the microfibrils. A single XyG molecule can therefore interconnect separate cellulose microfibrils. This XyG-cellulose network forms a major load-bearing structure that contributes to the structural integrity of the wall and the control of cell expansion (Cosgrove, 1999, 2005; Scheller and Ulvskov, 2010).

The pectic matrix is structurally complex and heterogeneous. HG domains consist of α -D-(1-4)-galacturonic acid (GalA) residues, which can be methyl-esterified, acetylated, and/or substituted with xylose to form xylogalacturonan (Willats et al., 2001; Vincken et al., 2003). De-esterified blocks of HG can be cross-linked by calcium producing a gel matrix which is believed to be essential for cell adhesion (Jarvis, 1984). RG-I domains contain repeats of the disaccharide [-4α -D-GalA-(1-2)- α -L-Rha-(1-)] and the rhamnosyl residues can possess oligosaccharide side chains consisting predominantly of β -D-(1-4)-galactosyl- and/or α -L-(1-5)-arabinosyl-linked residues (McNeil et al., 1982). Side chains of RG-I can also contain α -L-fucosyl, β -D-glucuronosyl, and 4-O-methyl β -D-glucuronosyl residues and vary in length depending on the plant source (O'Neill et al., 1990). These chains are believed to decrease the ability of pectic molecules to cross-link and form a stable gel network, and are thereby able to influence the mechanical properties of the cell wall (Hwang and Kokini, 1991). In addition, the structure and tissue distribution of arabinan- or galactan-rich side chains of RG-I have been shown to be regulated during cell growth and development of many species (for review see Willats et al., 2001; Scheller et al., 2007; Harholt et al., 2010).

RG-II is the most structurally complex pectic polysaccharide discovered so far in plants and is of a relatively low molecular mass (5–10 KDa; Ridley et al., 2001). It occurs in the cell walls of all higher plants as a dimer (dRG-II-B) that is cross-linked by borate di-esters (Matoh et al., 1993; Ishii and Matsunaga, 1996; Kobayashi et al., 1996; O'Neill et al., 1996). The backbone of RG-II is composed of a HG-like structure containing at least eight α -D-(1-4)-GalA-linked residues to which four structurally different oligosaccharide chains, denoted A, B, C, and D, are attached. The C and D side chains are attached to C-3 of the GalA residues of the backbone whereas A and B are attached to C-2 of GalA residues (O'Neill et al., 2004). The C chain corresponds to a disaccharide that contains rhamnose and 2-keto-3-deoxy-D-manno-octulosonic acid (Kdo), whereas the D chain is a disaccharide of 2-keto-3-deoxy-D-lyxo-heptulosaric acid (Dha) and arabinose (O'Neill et al., 2004). The A and B oligosaccharide chains are both composed of eight to ten monosaccharides and are attached by a β -D-apiose residue to O-2 of the backbone. A D-galactosyl residue (D-Gal) occurs on the B chain. RG-II plays an important role in the regulation of porosity and mechanical properties of primary cell walls (Ishii et al., 2001). This in turn has an important impact on growth and development as has been shown from studies using

various *Arabidopsis* mutants (O'Neill et al., 2001; Reuhs et al., 2004; Voxeur et al., 2011).

As for hemicellulosic polysaccharides, it is worth to note that glucurono(arabino)xylan (GAX) does exist in the primary cell walls of eudicotyledonous although at very limited amounts. It is however mostly present in the secondary walls of eudicotyledonous as well as in both the primary and secondary walls of grasses (see also Vogel, 2008 for a difference in polysaccharide composition of the cell walls between grasses and eudicots). GAX of eudicotyledonous primary cell wall is composed of a linear β -D-(1-4)-xylose backbone substituted with both neutral and acidic side chains. The acidic side chains are terminated with glucuronosyl or 4-O-methyl glucuronosyl residues, whereas the neutral side chains are composed of arabinosyl and/or xylosyl residues (Darvill et al., 1980; Zablackis et al., 1995; Vogel, 2008). GAX is also known to be synthesized within Golgi stacks and significant advances have recently been made in understanding its biosynthesis (Faik, 2010). However, this is beyond the focus of this review and only major polysaccharides of the primary cell walls of eudicotyledonous are considered here below.

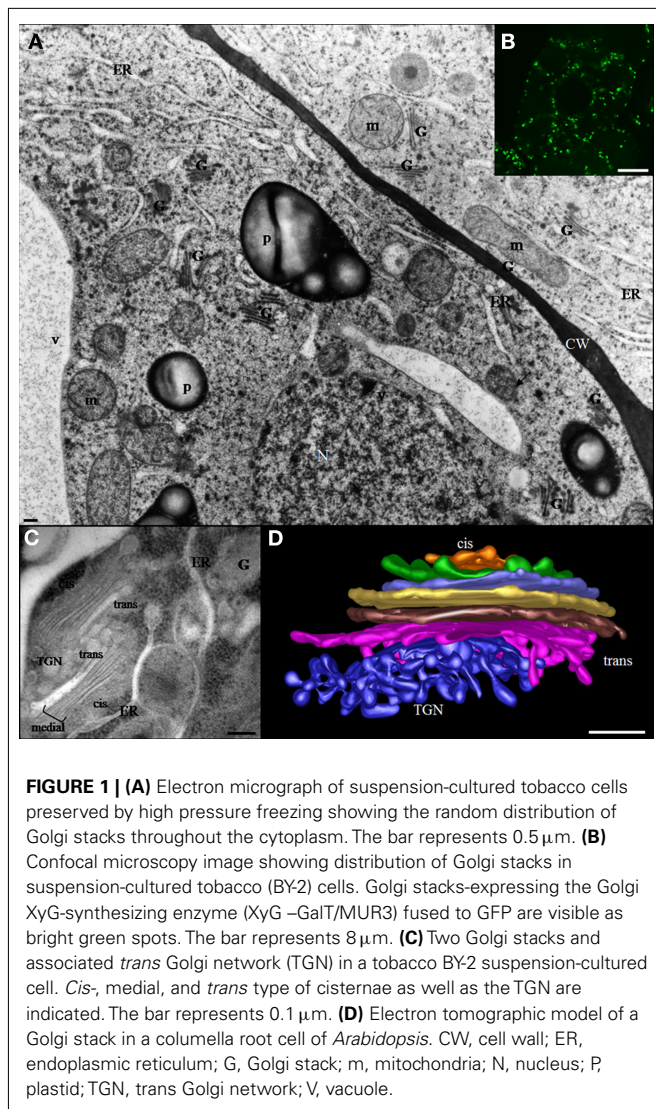
THE ROLE OF THE GOLGI APPARATUS IN COMPLEX POLYSACCHARIDE BIOSYNTHESIS

THE GOLGI IMPLICATION IN THE CONSTRUCTION OF THE CELL WALL

In higher plants, the Golgi apparatus plays a fundamental role in “the birth” of the cell wall. During cytokinesis, a new cell wall is formed and starts to assemble with the transport of Golgi-derived secretory vesicles to the center of a dividing cell. Fusion of these vesicles gives rise to a thin membrane-bound structure, the cell plate, which undergoes an elaborate process of maturation leading to a fully functional cell wall (Staehelin and Hepler, 1996; Cutler and Ehrhardt, 2002; Segui-Simarro et al., 2004). Also to perform the plant cell growth, new polysaccharides are delivered to growing cell wall by fusion of Golgi vesicles with the plasma membrane (Cosgrove, 2005).

The Golgi apparatus of plant cells is a dynamic and organized organelle consisting of a large number of small independent Golgi stacks that are randomly dispersed throughout the cytoplasm. At the confocal microscopy level, individual green fluorescent protein (GFP)-tagged Golgi stacks (around 1 μ m in diameter) appear as round disks, small rings, or short lines depending on their orientation (Nebenführ et al., 1999; Ritzenthaler et al., 2002). At the level of transmission electron microscopy in high pressure frozen/freeze substituted cells, each stack appears to consist of three types of cisternae, designated *cis*, *medial*, and *trans* that are defined based on their position within a stack and their unique morphological features (Staehelin et al., 1990; Staehelin and Kang, 2008). This morphological polarity reflects different functional properties of Golgi compartments (Figure 1; Staehelin et al., 1990; Driouch and Staehelin, 1997). The number of stacks per cell, as well as the number of cisternae within an individual stack, varies with the cell type, the developmental stage of the cell and the plant species (Staehelin et al., 1990; Zhang and Staehelin, 1992).

The *trans* Golgi network (TGN) is a branched tubulo-vesicular structure that is frequently located close to *trans* cisternae. However, the TGN can be found remote from the Golgi stack located



throughout the cytosol as an independent compartment. Two types of TGN compartments have been described and referred to as an early and a late TGN (see Staehelin and Kang, 2008). The plant TGN plays a major role in sorting of proteins and it represents a meeting point of secretory, endocytosis, and membrane recycling pathways. Recent studies have shown that certain TGN types, can serve also as early endosomes and thus have been termed TGN-Early endosomes (Dettmer et al., 2006; Richter et al., 2009; Viotti et al., 2010).

In contrast to the Golgi complex in mammalian cells that has a fixed location near the centrosomes, Golgi units in plants appear to move actively throughout the cytoplasm (Boevink et al., 1998; Nebenführ et al., 1999). GFP-fusions have allowed the study of Golgi dynamics *in vivo* and have shown that each Golgi unit can move at a slow or high speed (up to 5 $\mu\text{m}/\text{s}$) without losing structural integrity (Boevink et al., 1998; Nebenführ et al., 1999; Brandizzi et al., 2002). In addition, cytoskeletal depolymerization studies have indicated that the movement of Golgi stacks depends on actin filaments rather than on microtubules (Nebenführ et al.,

1999). Indeed, it is now established that the movement of Golgi stacks in plant cells occurs along actin filaments driven by myosin motors (Staehelin and Kang, 2008). In the context of this review, it is worth noting that actin filaments interact with Golgi stacks *via* an actin-binding protein, KATAMARI 1/MURUS3 – that is also known as a glycosyltransferase required for cell wall biosynthesis (see below; Tamura et al., 2005). KATAMARI 1 has been shown to be involved in maintaining the organization and dynamics of Golgi membranes.

As in animal cells (Rabouille et al., 1995), the plant Golgi apparatus functions in the processing and modification of N-linked glycoproteins (Pagny et al., 2003; Saint Jore Dupas et al., 2006; Schoberer and Strasser, 2011); but the bulk of the biosynthetic activity of this organelle is devoted to the assembly of different subtypes of complex, non-cellulosic polysaccharides of the cell wall such as pectin and hemicelluloses.

The first studies implicating plant Golgi stacks in cell wall biogenesis date back to the 60 and 70 and involved cytochemical staining as well as autoradiographic experiments with radiolabeled sugars (Pickett-Heaps, 1966, 1968; Harris and Northcote, 1971; Dauwalder and Whaley, 1974). These investigations have shown that Golgi cisternae and Golgi-derived vesicles are rich in carbohydrates and that a similar carbohydrate content is found in the cell plate, the cell wall and in Golgi-enriched fractions. Additionally, biochemical evidence for the role of the Golgi apparatus in the assembly of cell wall polysaccharides was obtained from fractionation experiments in which several glycosyltransferase activities (e.g., xylosyltransferase, arabinosyltransferase, fucosyltransferase) were detected in Golgi membranes (Gardiner and Chrispeels, 1975; Green and Northcote, 1978; Ray, 1980). Further biochemical investigations, reported in the eighties and nineties, allowed the identification and partial characterization of Golgi-associated enzymes specifically involved in the synthesis of XyG and pectic polysaccharides (Camirand et al., 1987; Brummell et al., 1990; Gibeaut and Carpita, 1994).

More recently, a proteomic method called LOPIT, for Localization of Organelle Proteins by Isotope Tagging, has been developed in order to determine the subcellular localization of membrane proteins in organelles of the secretory pathway such as Golgi stacks (Dunkley et al., 2004, 2006). This approach has allowed the identification of the α 1,6-xylosyltransferase (AtXT1) involved in XyG biosynthesis, in the Golgi apparatus (Dunkley et al., 2004).

In connection with XyG biosynthesis, it is remarkable to note that the structure of XyG present in isolated Golgi membranes has been investigated using oligosaccharide mass profiling (OLIMP) method (Obel et al., 2009). This study has revealed subtle differences in the structure of the polymer with XXG and GXXG fragments being more abundant in Golgi-enriched fraction as compared to the cell wall. However, due to the inability of sub-fractionating plant Golgi stacks into *cis*, medial, and *trans* cisternae, it is not possible to associate a XyG oligosaccharide to a specific sub-compartment of the Golgi apparatus. For similar reasons, it is still not possible neither to determine how the enzymes are spatially organized and how complex polysaccharides are assembled within Golgi sub-compartment using biochemical methods.

SPATIAL ORGANIZATION OF THE COMPLEX POLYSACCHARIDE ASSEMBLY PATHWAY IN GOLGI STACKS: INSIGHTS FROM IMMUNO-ELECTRON MICROSCOPY

Progress toward understanding the compartmentalization of matrix cell wall polysaccharide biosynthesis has come from immuno-electron microscopical analyses with antibodies directed against specific sugar epitopes. In most cases, these immunolabeling studies have been performed on a variety of cells prepared by high pressure freezing, a cryofixation technique that is recognized as providing excellent preservation of Golgi stacks thereby allowing different cisternal subtypes to be easily distinguished (Staehelin et al., 1990; Zhang and Staehelin, 1992; Driouich et al., 1993; Follet-Gueye et al., 2012). More recently, a combination of cryofixation/freeze substitution with Tokuyasu cryosectioning have been used to immunolocalize polysaccharide epitopes in Golgi stacks of *Arabidopsis* (Stierhof and El Kasmi, 2010). This technology is quite promising and should be able to provide interesting information on the localization of epitopes within plant Golgi cisternae.

Quantitative immunolabeling experiments using antibodies recognizing either the XyG backbone (anti-XG antibodies: Moore et al., 1986; Lynch and Staehelin, 1992) or an α -L-Fucp-(1-2)- β -D-Galp epitope of XyG side chains in sycamore cultured cells have shown that the epitopes localize to *trans* cisternae and the TGN (Zhang and Staehelin, 1992). These data suggested that the synthesis of XyG occurs exclusively in late compartments of the Golgi and that no precursor forms of XyG are made in *cis* and medial cisternae. The use of these antibodies on clover and *Arabidopsis* root tip cells have also suggested that the synthesis of XyG takes place in *trans* Golgi cisternae and the TGN (Moore et al., 1991; Driouich et al., 1994). Nevertheless, it could be argued that the sugar epitopes recognized by both antibodies are not accessible until they reach the *trans* and TGN compartments, or that the antibodies do not bind XyG precursor forms in *cis* and medial cisternae. More recently, Viotti et al. (2010) have also shown that fucosylated epitopes of XyG occur preferentially in *trans* cisternae and the TGN using thawed ultrathin cryosections of high pressure frozen/freeze substituted and rehydrated *Arabidopsis* root tip cells. Therefore, it is necessary to localize XyG-synthesizing enzymes within Golgi cisternae to determine whether XyG synthesis is exclusively limited to *trans* and TGN cisternae. Although no antibody against any XyG-synthesizing enzyme is currently available, one possible approach of addressing this issue is to produce transgenic plants expressing GFP-tagged glycosyltransferases, followed by localization with anti-GFP antibodies. Such a strategy has been successfully used to study the compartmentation of enzymes involved in the processing of N-linked glycoproteins, including a β -1,2-xylosyltransferase responsible for the addition of β -1,2 xylose residues and an α -1,2-mannosidase responsible for the removal of α -1,2 mannose residues in tobacco suspension-cultured cells (Follet-Gueye et al., 2003; Pagny et al., 2003; Saint Jore Dupas et al., 2006). The same strategy has been recently applied to enzymes involved in the biosynthesis of XyG side chains including α -1,6-xylosyltransferase (AtXT1), β -1,2-galactosyltransferase (AtMUR3), and α -1,2-fucosyltransferase (AtFUT1; Chevalier et al., 2010). Immunogold localization of these GFP-tagged glycosyltransferases has demonstrated that AtXT1-GFP is mainly located in the *cis* and medial cisternae,

AtMUR3-GFP is predominantly associated with medial cisternae and AtFUT1-GFP mostly detected over *trans* cisternae suggesting that initiation of XyG side chains occurs in early Golgi compartments in tobacco suspension-cultured (BY-2) cells.

Another interesting approach to study sub-Golgi localization of glycosyltransferases is through development of *in silico* predictors based on the transmembrane domain of these enzymes. Such an approach employing a support vector machine (SVM) algorithm has been recently used to predict the localization of several human and mouse glycosyltransferases and glycohydrolases within Golgi sub-compartments (van Dijk et al., 2008). This is an interesting methodology to apply for the prediction of sub-Golgi localization of the known plant glycosyltransferases involved in cell wall biosynthesis. Using this approach AtRGXT1 and AtRGXT2 -two α -1,3-xylosyltransferases involved in the synthesis of the pectic polysaccharide RG-II- have been predicted to be confined to the TGN (P. Ulaskov, personal communication).

As for XyG synthesis, similar immunocytochemical studies using antibodies raised against pectin epitopes (including JIM7, anti-PGA/RG-I, and CCRCM2) has allowed a partial characterization of the assembly pathway of homogalacturonan (HG) and RG-I within Golgi cisternae (Zhang and Staehelin, 1992). The polyclonal anti-PGA/RG-I antibodies (recognizing un-esterified HG) were shown to label mostly *cis* and medial cisternae in suspension-cultured sycamore cells as well as in clover root cortical cells (Moore et al., 1991; Zhang and Staehelin, 1992). In contrast, JIM7 (specific for methyl-esterified HG) labeling was mostly confined to medial and *trans* cisternae (Figure 2). Consistent with this data is the localization of the putative pectin methyltransferase, QUASIMODO2 (Mouille et al., 2007) fused to GFP which was found mainly in medial and *trans* cisternae of Golgi stacks in *Arabidopsis* root tip cells (Chevalier et al., unpublished; Figure 2). In addition, the mAb CCRCM-2 which is believed, but not proven, to bind RG-I side chains was found to label *trans* cisternae in sycamore cultured cells (Zhang and Staehelin, 1992). These data suggest that HG is synthesized in its un-esterified form in *cis* and medial Golgi cisternae and, that (i) the methylesterification occurs in both medial and *trans* compartments, and (ii) that side chains of RG-I are added in *trans* cisternae. However, as discussed above for XyG labeling, the absence of labeling in *trans* cisternae and the TGN by anti-PGA/RG-I antibodies, might be due to the inaccessibility of the recognized epitopes in these compartments (because of the methylesterification for instance). This idea is supported by the fact that the same epitopes are localized predominantly in *trans* Golgi cisternae and the TGN in the epidermal cells of clover. Thus, it is not surprising that the compartmentation of cell wall matrix polysaccharides within Golgi cisternae varies in a cell type specific manner. The distribution of XyG and HG in Golgi membranes has also been investigated immunocytochemically in root hair cells of *Vicia villosa* preserved by high pressure freezing (Sherrier and VandenBosch, 1994). Although no quantitative analyses were performed, methyl-esterified HG epitopes recognized by JIM7 were detected within medial and *trans* cisternae, whereas the fucose-containing epitope of XyG (recognized by CCRCM1) was found over *trans* Golgi cisternae. These observations are consistent with those made in sycamore suspension-cultured cells and clover root cortical cells using the same antibodies (Moore et al., 1991; Zhang

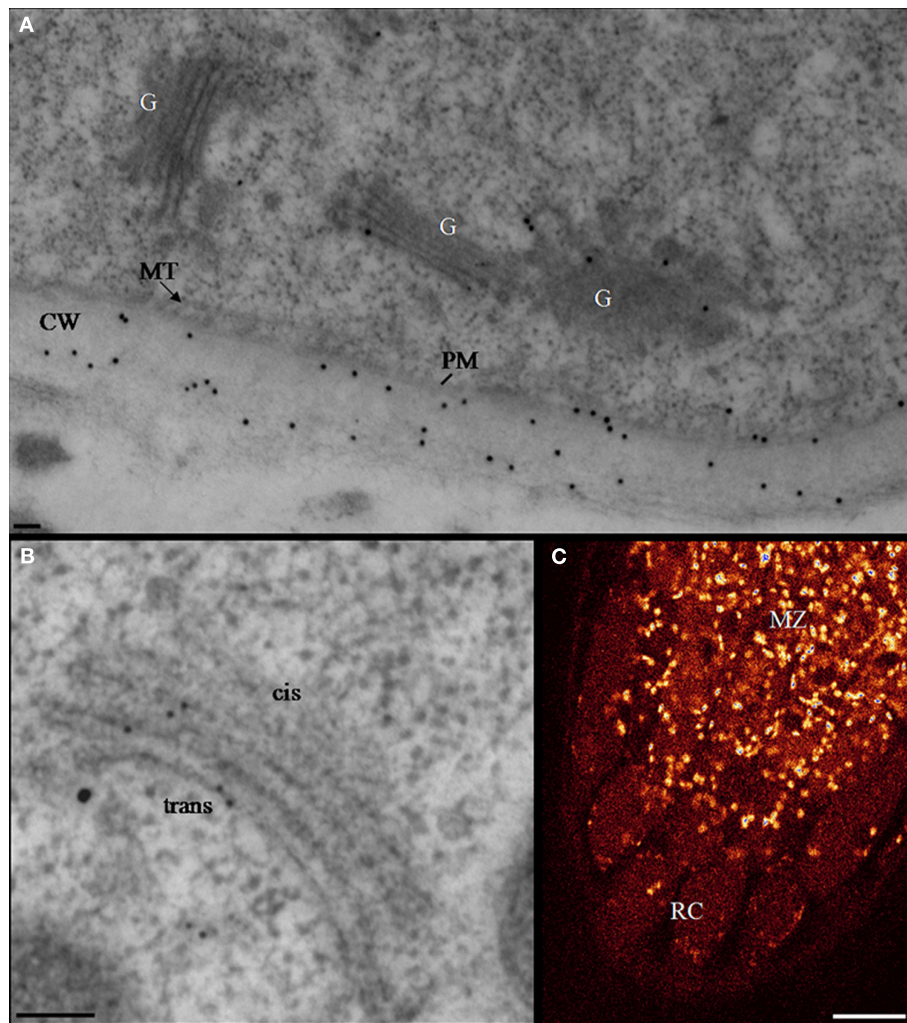


FIGURE 2 | Homogalacturonan localization in transgenic *Arabidopsis* root tips expressing the QUA2-GFP fusion protein.

(A,B) Electron micrographs illustrating typical double-immunogold labeling of Golgi stacks (G) in *Arabidopsis* root cells with the mAbs JIM7 (20 nm gold particles) or with anti-GFP (10 nm gold particles). HG epitopes and the QUA2-GFP are predominantly associated with the

trans cisternae and the TGN. The cell wall (CW) is labeled with JIM 7. Bar represents 100 nm. (C) A confocal laser fluorescence image of *Arabidopsis* root tip expressing the fusion protein QUA2-GFP. Bright spots represent GFP-stained Golgi units. Scale Bar: 16 μm (Chevalier et al., unpublished). CW, cell wall; G, Golgi stack; MT, microtubule; PM, plasma membrane; MZ, meristematic zone; RC, root cap.

and Staehelin, 1992). However, the *V. villosa* study did not address the issue of RG-I side chain distribution within Golgi stacks using the mAb CCRCM2. Therefore, the use of the more recently produced monoclonal antibodies LM5 and LM6, recognizing β -1,4-D-galactan and α -1,5-D-arabinan epitopes, respectively (Jones et al., 1997; Willats et al., 1998) should prove very useful for extending the “current map” of the pectin assembly pathway within the Golgi cisternae of sycamore cultured cells and *V. villosa* root hairs. Both antibodies have been widely used to study the distribution of galactan and arabinan epitopes within the cell walls, but relatively very little is known concerning their localization within the endomembrane system. In flax root cells, LM5-containing epitopes have been shown to be present mostly in *trans* cisternae and the TGN (Vicré et al., 1998). Similarly, epitopes recognized by LM5 and LM6 have been quantitatively localized to TGN in

tobacco (BY-2) cultures (Bernard et al., unpublished). Therefore, it appears that galactan- and arabinan-containing side chains of RG-I are assembled in the TGN. Whether the enzymes responsible for the addition of these residues are confined to the same Golgi sub-compartments remains to be determined by future studies. One of the genes involved in the synthesis of RG-I side chains, namely *ARAD1* (encoding a putative α -1,5-D-arabinosyltransferase) has been recently identified and cloned (see below). The generation of specific antibodies against this glycosyltransferase, or the generation of a GFP-tagged protein should help us to understand more about its specific localization within Golgi compartments. To extend our understanding of RG-I synthesis within Golgi stacks, the use of the new monoclonal antibodies specifically raised against the RG-I backbone (Ralet et al., 2010) or the LM13 recognizing longer oligoarabinosides than LM6, should be very helpful.

In contrast to RG-I, information on the localization and assembly of RG-II within the endomembrane system is missing. RG-II has a complex structure consisting of a HG-like backbone and four side chains that contain specific and unusual sugars including Kdo and apiose (see above). It would certainly be interesting to find out whether the backbone is assembled in the same compartments as the side chains and whether different side chains are assembled in the same or distinct compartments. The elucidation of RG-II assembly within sub-compartments of Golgi stacks requires the generation of antibodies specific for the sugar epitopes of the backbone and for the epitopes associated with different side chains, as well as the associated immuno-electron microscopy studies.

It is generally accepted that the transport of Golgi products, including glycoproteins and complex polysaccharides, to the cell surface occurs by bulk flow (Hadlington and Denecke, 2000). To date, no specific signals responsible for targeting and transport of such products to the cell wall have been found associated with any protein or polysaccharide. It has been shown that Golgi-derived secretory vesicles mediating such a transport vary in size and are capable of carrying mixed classes of complex polysaccharides (Sherrier and VandenBosch, 1994) or both polysaccharides and glycoproteins (see Driouich et al., 1994). Nevertheless, using secretory carrier membrane protein 2 (SCAMP2) from *Nicotiana tabacum*, a mobile secretory vesicle cluster (SVC) generated from the TGN has been recently identified in *Arabidopsis* tissues and tobacco suspension-cultured BY-2 cells (Toyooka et al., 2009). These clusters formed by budding of clathrin coated vesicles from the late TGN, are highly labeled with antibodies specific for either complex glycan or homogalacturonan epitopes (JIM7), suggesting that SVC are involved in mass transport from Golgi stacks to the cell surface. It is also possible that the processing of Golgi products such as cell wall polymers may continue to occur within the vesicles during their transport to the cell surface (Zhang et al., 1996).

GLYCOSYLTRANSFERASES AND SUGAR-CONVERTING ENZYMES INVOLVED IN THE ASSEMBLY OF COMPLEX POLYSACCHARIDES

The Golgi-mediated assembly of complex polysaccharides requires the action of a set of Golgi glycosyltransferases, in addition to nucleotide-sugar transporters and nucleotide-sugar interconversion enzymes (Keegstra and Raikhel, 2001; Seifert, 2004; Reiter, 2008; Reyes and Orellana, 2008). It has been postulated that these partners could interact physically to form complexes within Golgi membranes that would coordinate sugar supply and polymer synthesis (Seifert, 2004; Nguema-Ona et al., 2006).

XYLOGLUCAN BIOSYNTHESIS: GLYCOSYLTRANSFERASES AND OTHER PROTEINS

XyG biosynthesis has long been an interesting, but a challenging, area of investigation. Biosynthesis of the XyG core is expected to require two different catalytic activities, a glucan synthase activity for the backbone and a xylosyltransferase activity adding xylosyl substitutions. Interestingly, Ray (1980) suggested a “cooperative action of β -glucan synthase and UDP-xylose xylosyltransferase in Golgi membranes for the synthesis of a XyG-like polysaccharide.” In that study, a UDP-xylose xylosyltransferase activity was measured in Golgi membranes isolated from pea, and the

incorporation of xylose was shown to be stimulated by the addition of UDP-glucose. Furthermore, the stimulating effect of UDP-glucose on xylosyltransferase activity was shown to occur only in a pH range where β -glucan synthase is active, suggesting that UDP-glucose stimulates UDP-xylose incorporation by promoting β -glucan synthase activity. Here, the β -glucan synthase produces the required β -1,4-glucan substrate molecule necessary for XyG xylosyltransferase activity. At about the same time, Hayashi and Matsuda (1981a,b) performed a detailed characterization of XyG synthase activity in soybean suspension-cultured cells and demonstrated that XyG synthesis requires the cooperation of XyG β -1,4-glucan synthase and the XyG xylosyltransferase. The authors not only demonstrated that the incorporation of one sugar (xylose or glucose) depended on the presence of the other, but also that xylose was not transferred to a preformed β -1,4-glucan. This observation strongly supports the existence of a multienzyme complex responsible for XyG biosynthesis where glucan synthase and xylosyltransferase activities cooperate tightly (Hayashi, 1989). Since then, considerable efforts have been devoted to the characterization of XyG biosynthesis at the molecular level using functional genomics and the model plant *Arabidopsis thaliana* (Lerouxel et al., 2006; Scheller and Ulvskov, 2010).

We have currently gained a better picture of XyG biosynthesis by identifying and characterizing some of the genes involved (Table 1), although without much (if any) understanding of how these enzymes could potentially cooperate to achieve the biosynthesis. For example, the XyG fucosyltransferase AtFUT1 (CAZY GT37, Cantarel et al., 2009) was the first type II glycosyltransferase characterized at the biochemical level (Perrin et al., 1999), and for a long time the only member of this gene family containing nine putative glycosyltransferases to be characterized, as analyses of three other members failed to demonstrate any XyG fucosylation activity (Sarria et al., 2001). Wu et al. (2010) have recently demonstrated the involvement of AtFUT4 and AtFUT6 genes in arabinogalactan proteins fucosylation.

Later, the identification and characterization of the *mur2* mutant of *Arabidopsis* provided the unequivocal proof that the *AtFUT1* gene encodes the unique α -1,2-fucosyltransferase activity responsible for XyG fucosylation in *Arabidopsis* plants (Vanzin et al., 2002). Likewise, one XyG β -1,2-galactosyltransferase (AtMUR3; CAZY GT47) activity was successfully characterized using both mutant analysis (*mur3*) and heterologous expression of the enzyme (Madson et al., 2003). Nevertheless, as the galactose residue of the XyG molecule can be found in two different positions, it seems that at least one XyG galactosyltransferase remains to be identified and characterized. The galactosyl-residues of XyG are known to be O-acetylated and only one XyG-O-acetyltransferase (AXY4) has been identified so far (Gille et al., 2011) and found to be localized within Golgi stacks.

An important contribution to understanding XyG biosynthesis was also made by the characterization of two α -1,6-xylosyltransferase activities required for XyG xylosylation (CAZY GT34). First, one xylosyltransferase activity (named AtXT1) was identified based on sequence homology with a previously identified α -1,6-galactosyltransferase from fenugreek (Edwards et al., 1999), and characterized as an α -1,6-xylosyltransferase using heterologous expression in *Pichia pastoris* (Faik et al., 2002). Cavalier

Table 1 | List and characteristics of the glycosyltransferase and nucleotide–sugar interconversion enzymes.

Gene				Enzyme characteristics				Arabidopsis mutant				Notes	
Designation	Code	CAZy	Activity	Localization	Designation	Phenotypes	Category	Reference					
AtSLC4	At3g28180	GT2	β -1,4-Glucan synthase	Golgi	None	None		Cocuron et al. (2007)					
AtXT1	At3g62720	GT34	α -1,6-Xylosyltransferase	Golgi	<i>xxt1</i>	10% Reduction in XyG	Xyloglucan	Faik et al. (2002), Cavalier and Keegstra (2006), Cavalier et al. (2008)					
AtXT2	At4g02500	GT34	α -1,6-Xylosyltransferase	Golgi	<i>xxt1</i>	20% Reduction in XyG	Xyloglucan	Cavalier and Keegstra (2006), Cavalier et al. (2008)					
AtMUR3	At2g20370	GT47	β -1,2-Galactosyltransferase	Golgi	<i>mur3</i>	Hypocotyls wall strength is 50% reduced, xyloglucan lacks fucogalactosyl sidechain		Madson et al. (2003), Pena et al. (2004)					
AtFUT1	At2g03220	GT37	α -1,2-Fucosyltransferase	Golgi	<i>Mur2</i>	Hypocotyls wall strength is slightly reduced, xyloglucan lacks fucose substitution		Perrin et al. (1999), Vanzin et al. (2002)					
AXY4	At1g70230	GT	O-Acetyltransferase	Golgi	<i>axy4</i>	Cell wall XyG extract is non-acetylated		Pena et al. (2004), Gille et al. (2011)					
AtFUT4	At2g15390	GT37	α -1,2-Fucosyltransferase	Golgi	None	None	Arabinogalactan proteins						
AtFUT6	At1g4080	GT37	α -1,2-Fucosyltransferase putative	Golgi	None	None							
AtQUA1	At3g25140	GT8	α -1,4-Galacturonosyltransferase	Predicted	<i>qua1</i>	Dwarf, reduced cell adhesion, 25% reduction in cell wall GalA content		Bouton et al. (2002)					
AtGAUT1	At3g61130	GT8	α -1,4-Galacturonosyltransferase	Golgi	None	None		Sterling et al. (2006), Dunkley et al. (2004)					
AtRGX1	At4g01770	GT77	α -1,3-Xylosyltransferase	Golgi	<i>rgx1</i>	RG-II from mutant (but not from WT) is an acceptor for α -1,3-xylosyltransferase activity		Egelund et al. (2006)					
AtRGX2	At4g01750	GT77	α -1,3-Xylosyltransferase	Golgi	<i>rgx2</i>	RG-II from mutant (but not from WT) is an acceptor for α -1,3-xylosyltransferase activity		Egelund et al. (2006)					
AtRGX4	At4g01220	GT77	Xylosyltransferase	Golgi	<i>mgp4</i>	Defective for root and pollen tube growth		Fangel et al. (2011), Liu et al. (2011)					
AtARAD1	At2g35100	GT47	α -1,5-Arabinosyltransferase	Predicted	<i>arad1</i>	Cell wall composition altered, decrease in RG-I arabinose content	Pectins	Harholt et al. (2006)					
AtXGD1	At5g33290	GT47	β -1,3-Xylosyltransferase	Golgi	<i>xgd1</i>	<i>xgd1</i> mutant lacks detectable XGA in pectin enriched fraction		Jensen et al. (2008)					

(Continued)

Table 1 | Continued

Enzyme characteristics				Arabidopsis mutant			Notes
Gene	CAZy	Activity	Localization	Designation	Phenotypes	category	Reference
<i>NpGUT1</i>	GT47	Putative β -glucuronidase	Predicted Golgi	<i>nolac-H18</i>	Callus harbored a cell-cell adhesion defect, and a reduced RG-II dimerisation ability		Iwai et al. (2002)
<i>AtEPC1</i>	GT64	Unknown	Predicted Golgi	<i>epc1</i>	Reduced growth habit, defects in vascular formation and reduced cell-cell adhesion in hypocotyls		Singh et al. (2005)
<i>AtQUA2</i>	–	Putative methyltransferase	Golgi	<i>qua2/tds2</i>	Dwarf, reduced cell adhesion, 50% reduction in HG content		Mouille et al. (2007), Krupková et al. (2007)
<i>AtQUA3</i>	At4g00740	Putative methyltransferase	Golgi	<i>qua3</i>	No detectable phenotype		Miao et al. (2011)
<i>AtCGR3</i>	At5g65810	Putative methyltransferase	Golgi	<i>cgr3</i>	Decrease HG methylesterification		Held et al. (2011)
<i>AtUGE4</i>	Atlg64440	UDP-glucose epimerase	Cytosolic and Golgi associated	<i>rhd1 (uge4); reb1</i>	Reduced root elongation rate, bulging of trichoblast cells, xyloglucan, and arabinogalactan galactosylation defects in roots		Andème-Onzighi et al. (2002), Barber et al. (2006), Nguema-Ona et al. (2011)
<i>AtRGP1</i>	GT75	Putative UDP-arabinopyranose mutase	Cytosolic and Golgi associated	<i>rgp1</i>	Decrease in L-Ara, <i>rgp1</i> <i>rgp2</i> double mutant is lethal (pollen development altered)	Interconversion enzymes	Rautengarten et al. (2011)
<i>AtRGP2</i>	GT75	Putative UDP-arabinopyranose mutase	Cytosolic and Golgi associated	<i>rgp2</i>	Decrease in L-Ara <i>hpRGP1/2</i> cell wall is deficient in L-Ara		

arad1, arabinose deficient 1; *At*, *Arabidopsis thaliana*; CSLC4, cellulose synthase-like C4; *cgr3*, cotton golgi-related 3; *epc1*, ectopically parting cells1; *FUT*, fucosyltransferase; *hpRGP1/2*, hairpin construct targeting RGP1 and RGP2 genes; *mgp4*, male gametophyte defective 4; *nolac-H18*, non-organogenic callus with loosely attached cells-H18; *Np*, *Nicotiana plumbaginifolia*; *qua1*, *quasimodo1*; *qua2*, *quasimodo2*; *reb1*, root epidermal bulging1; *rgp1*, reversibly glycosylated protein1; *rgp2*, reversibly glycosylated protein2; *rhd1*, root hair deficient1; *rgxt1*, rhamnogalacturonane II xylosyltransferase1; *rgxt2*, rhamnogalacturonane II xylosyltransferase2; *xgd1*, xylogalacturonan deficient1; *xxt1*, xyloglucan xylosyltransferase1; *xxt2*, xyloglucan xylosyltransferase2.

and Keegstra (2006) extended this work to the characterization of a second xylosyltransferase activity (named AtXT2), encoded by a gene closely related to AtXT1, that promotes an identical reaction to the one catalyzed by AtXT1. Interestingly, the authors also demonstrated that both AtXT1 and AtXT2 are able to catalyze multiple addition of xylosyl residues onto contiguous glucosyl residues of a celohexaose acceptor *in vitro* (even though the β -linkage introduce a 180° rotation from one glucosyl residue to the other), but non-xylosylated celohexaose was the preferred acceptor. The observation that both AtXT1 and AtXT2 xylosyltransferase activities were able to perform multiple xylosylation might indicate a reduced substrate specificity of these enzymes as compared to the high specificity of the XyG fucosyl- or galactosyl-transferases. Nevertheless, these results raise the intriguing possibility that AtXT1 and AtXT2 would be fully redundant *in planta* and that both would be able to perform multiple xylosylation. Such a hypothesis is consistent with the characterization of an *Arabidopsis* double mutant for AtXT1 and AtXT2 genes (named *xxt1 xxt2*; xyloglucan xylosyltransferase 1 and 2), which is lacking detectable amounts of XyG *in planta* (Cavalier et al., 2008).

Another enzyme involved in XyG biosynthesis is glucan synthase. While XyG glucan synthase activity has long been studied biochemically (discussed above), efforts to purify and ultimately characterize this enzyme have not been successful. A gene from the Cellulose Synthase-Like C family (*AtCSLC4*; CAZY GT2) was shown to encode a Golgi-localized β -1,4-glucan synthase activity providing a strong candidate for the, as yet, unidentified XyG β -glucan synthase (Cocuron et al., 2007). The candidate gene was identified using a transcriptional profiling strategy taking advantage of nasturtium's capacity to perform rapidly XyG biosynthesis during seed development (Desveaux et al., 1998). This observation supports the hypothesis that the β -1,4-glucan synthase activity identified was involved in XyG biosynthesis. Interestingly, this study also indicates that *AtCSLC4* is co-regulated with *AtXT1* at the transcriptional level and that some degree of interaction occurs at the protein level could possibly alter the length of the β -1,4-glucan synthesized by the *AtCSLC4* protein (Cocuron et al., 2007). The results of this study are consistent with earlier reports showing that *in vitro* synthesis of XyG was shown to involve a cooperative action between the glucan synthase and the xylosyltransferase activities (Hayashi, 1989). Recently, the topology of *AtCSLC4* was examined using protease protection experiments (Davis et al., 2010). *AtCSLC4* was predicted to have six transmembrane domains and the active site facing the cytosol. These data suggest that *AtCSLC4* protein would delimitate a pore, the glucan chain being synthesized at the cytosolic side of Golgi cisternae, translocated into the lumen and further substituted by an α -1,6-xylosyltransferase to prevent β -1,4-glucan chain aggregation. A physical cooperation between *AtCSLC4* and *AtXT1* or *AtXT2* could then be envisioned to rationalize this process. Recently, Chevalier et al. (2010) have shown that *AtXT1*-GFP is mainly associated with *cis* and medial Golgi cisternae in transformed tobacco suspension-cultured (BY-2) cells. A similar approach to localize the *AtCSLC4* would be highly instructive. Another approach would be the design of a tagged version of either *AtXT1* or *AtXT2*, taking advantage of the characterization of *xxt1/xxt2* double mutant (Cavalier et al., 2008) and further use the tagged version of the xylosyltransferases to pull down interacting

candidate proteins. Then the interaction could be examined using bimolecular fluorescence complementation (BiFC) approach as previously performed for cellulose biosynthesis (Desprez et al., 2007).

XyG biosynthesis does not only depend upon the cooperation between glycosyltransferase activities, but might also require close interaction between glycosyltransferases and nucleotide-sugar interconversion enzymes. The study on the *reb1/rhd1* mutant of *Arabidopsis*, deficient in one of the five UDP-glucose 4-epimerase isoforms (UGE4) involved in the synthesis of UDP-D-galactose, provided indirect evidence for a possible interaction between UGE4 and a XyG- β -1,2-galactosyltransferase (Nguema-Ona et al., 2006). The authors showed that the galactosylation of XyG, unlike that of pectins (RG-I and RG-II), was absent in specific cells of the mutant and that UGE4 and XyG- β -1,2-galactosyltransferase are co-expressed at the transcriptional level in the root. Thus, it was postulated that the two enzymes might be associated in specific protein complexes involved in the galactosylation of XyG within Golgi membranes. Such an association could be required for an efficient galactosylation of XyG, where UGE4 would channel UDP-Gal to XyG-galactosyl-transferases. The existence of such a hypothetical association was supported by the demonstration that UGE4 was not only present in the cytoplasm but also found associated with Golgi membranes (Barber et al., 2006).

Although it is not the focus of this review, it is of interest to note that some glycosylhydrolases involved in XyG modification have been identified and characterized (Sampedro et al., 2001, 2012; Günl and Pauly, 2011; Günl et al., 2011). These are known to be transported via Golgi-derived vesicles to the apoplastic compartment where they act on the polymers. However, whether these enzymes are transported in the same Golgi vesicles and whether they are able to act on polymers within the vesicles during transport remain to be elucidated.

PECTIN BIOSYNTHESIS: TOWARD IDENTIFICATION OF THE GLYCOSYLTRANSFERASES INVOLVED

Because there is a considerable diversity of monosaccharide units and glycosidic linkages making up pectic polysaccharides, it has been proposed that a minimum of 67 glycosyltransferases would be needed for pectin biosynthesis (Mohnen, 2008). In addition, the complexity of pectic polysaccharides has made the identification and characterization of such glycosyltransferases difficult and, consequently, only a handful have been assigned precise biochemical functions (Egelund et al., 2006; Sterling et al., 2006; Jensen et al., 2008) or suggested to have certain functions (Iwai et al., 2001, 2002; Bouton et al., 2002; Harholt et al., 2006, 2010; Egelund et al., 2007; Fangel et al., 2011; Held et al., 2011; Liu et al., 2011). An additional degree of complexity in pectin synthesis is related to the control of the homogalacturonan methylesterification that requires specific methyltransferase activities, but also specific esterases to adjust methyl-esterification level during plant development (Wolf et al., 2009).

As for XyG biosynthesis, many biochemical studies have been devoted to the characterization of the enzymes involved in HG biosynthesis (Doong and Mohnen, 1998; Scheller et al., 1999). These studies showed that α -1,4-galacturonosyltransferase (GalAT) and HG methyltransferase activities are located in the

lumen of isolated Golgi membranes (Goubet and Mohnen, 1999; Sterling et al., 2001). A HG-GalAT activity was partially purified from solubilized membrane proteins isolated from *Arabidopsis* suspension-cultured cells and trypsin-digested peptides sequencing led to the identification of a candidate gene, named *AtGAUT1* for GALactUronosylTransferase1 (Sterling et al., 2006). Genome analysis revealed that *AtGAUT1* belongs to a large gene family comprising 25 members in *Arabidopsis* that is present in CAZy GT8 along with galactinol synthases and bacterial α -galactosyltransferase (Yin et al., 2010). Among the 25 homolog genes to *AtGAUT1* in the *Arabidopsis* genome, two distinct groups have been described for the GAUT genes and the GAUT-related genes (GATL), which comprise 15 and 10 genes, respectively (Sterling et al., 2006). Heterologous expression of *AtGAUT1* in human embryonic kidney cells, showed that *AtGAUT1* cDNA encodes a galacturonosyltransferase activity able to transfer radiolabeled GalA onto α -1,4-oligogalacturonide acceptors, whereas the heterologous expression of *AtGAUT7* cDNA (36% amino-acid sequence identity with *AtGAUT1*) carried out in parallel did not yield any transferase activity. More recently, Atmodjo et al. (2011) have demonstrated that the association of *AtGAUT1* with *AtGAUT7* is necessary to retain GAUT1 galacturonosyltransferase activity in Golgi membranes. In that study the authors have proposed that two GAUT1 proteins would remain associated to each other (and thus retain) *via* one GAUT7 protein anchored in the Golgi membrane. However, whether the *AtGAUT1* activity is directly responsible for the biosynthesis of the backbone of HG or that of RG-II polysaccharide in *Arabidopsis* has not been determined. Characterization of cell wall composition of *Arabidopsis* mutants altered for 13 out of the 15 GAUT genes and 3 of the 10 GATL genes has failed to clearly establish which polysaccharide is affected (Caffal et al., 2009; Kong et al., 2011).

The *Arabidopsis* mutant *quasimodo1* (*qua1*) is characterized by a reduced cell adhesion phenotype combined with a 25% decrease in cell wall galacturonic acid content, supporting the hypothesis that the *AtQUA1* gene encodes a putative glycosyltransferase activity involved in pectin biosynthesis (Bouton et al., 2002; Durand et al., 2009). *AtQUA1* (also named *AtGAUT8*) belongs to CAZy GT 8 family and shows 77% similarity to *AtGAUT1* which has a characterized HG-galacturonosyltransferase activity (Sterling et al., 2006). In addition, α -1,4-galacturonosyltransferase activity was shown to be significantly reduced in *qua1* providing further support for *AtQUA1* involvement in pectin biosynthesis (Orfila et al., 2005). Another *Arabidopsis* mutant *quasimodo2* (*qua2*), having a 50% decrease in HG content, has been described (Mouille et al., 2007). Interestingly, *AtQUA2* does not show any similarity with other glycosyltransferases, but it is a Golgi-localized protein (Figure 2) that contains a putative S-adenosyl methionine dependent methyltransferase domain, and appears strongly co-regulated with *AtQUA1*. This study supports the hypothesis that *AtQUA2* is a pectin methyltransferase required for HG biosynthesis and that both enzymes work interdependently. The observation that an alteration in a putative methyltransferase can impair HG synthesis led the authors to suggest the existence of a protein complex containing galacturonosyltransferase and methyltransferase where the latter enzyme would be essential for the functioning of the protein complex (Mouille et al., 2007). Whereas *Arabidopsis* genome

contains 29 putative genes encoding for pectin methyltransferases (Krupková et al., 2007), in addition to the *AtQUA2*, a novel putative S-adenosyl methionine dependent HG methyltransferase, showing a high amino-acid similarity with *AtQUA2*, the *AtQUA3* has been recently characterized (Miao et al., 2011). *AtQUA3* is a Golgi-localized type II membrane protein which is highly expressed and abundant in *Arabidopsis* suspension-cultured cells. More recently, it has been shown that *AtQUA3* is co-expressed with *AtGAUT1* and *AtGAUT7*; supporting the idea that HG methylation is closely associated with HG synthesis (Atmodjo et al., 2011). In addition, Held et al. (2011) have identified an *Arabidopsis* ortholog of a cotton protein containing a domain similar to SAM methyltransferase domain named cotton Golgi-related 3 (CGR3) involved in the methylesterification of HG. Finally, a third *Arabidopsis* mutant – *ectopically parting cells 1* (*epc1*) – was characterized on the basis of cell adhesion defects, however cell wall analyses did not support the hypothesis that pectin biosynthesis was specifically altered in *epc1* (Singh et al., 2005).

The HG backbone in *Arabidopsis* can also be decorated with β -1,3-xylose residues thus forming a xylogalacturonan domain (Zandleven et al., 2007). The characterization of the *xylogalacturonan deficient1* (*xgd1*) *Arabidopsis* mutant along with the heterologous expression of XGD1 protein in *Nicotiana benthamiana* led to the demonstration that the *XGD1* gene is involved in XGA biosynthesis (Jensen et al., 2008).

Four other genes involved in pectin biosynthesis, encoding a small glycosyltransferase family with four members named RG-II xylosyltransferase (RGXT1–4; CAZy GT77), have been described (Egelund et al., 2006, 2008; Fangel et al., 2011; Liu et al., 2011). The authors convincingly demonstrated that *AtRGXT1*, *AtRGXT2*, *AtRGXT3*, and *AtRGXT4* encode α -1,3-xylosyltransferase activities that are involved in the synthesis of the pectic polysaccharide RG-II. Whereas no mutant has been found for *RGXT3* (Egelund et al., 2008), *Arabidopsis* mutants have been described for *RGXT1*, *RGXT2* (Egelund et al., 2006) and *RGXT4* (Liu et al., 2011). Among these, only *rgxt4* mutant, also named male gametophyte defective 4 (*mgd4*), presents visible phenotypes in both RG-II structure and pollen tube growth (Liu et al., 2011). The peculiar role of *RGXT4* could be explained by its ubiquitous expression in vegetative and reproductive organs whereas *RGXT1* and *RGXT2* expression is restricted to the vegetative organs. Other genes have also been proposed to function in pectin biosynthesis although their involvement has not been firmly established. Mutant lines altered in pectin biosynthesis have been screened based on cell adhesion defects – a process that involves pectin (Iwai et al., 2001, 2002; Bouton et al., 2002; Mouille et al., 2007). A gene named *NpGUT1*, for *Nicotiana glauca* glucuronosyltransferase 1, has been identified using *nolac-H18*, a tobacco callus mutant line that exhibits a “loosely attached cells” phenotype (Iwai et al., 2002). It was proposed that *NpGUT1* encodes a putative glucuronosyltransferase involved in RG-II biosynthesis based on the analysis of *nolac-H18* mutant cell walls. However, recent studies have shown that homologs of *Arabidopsis NpGUT1* are related to the biosynthesis of the hemicellulosic polysaccharide glucuronoxylan rather than to that of RG-II (Wu et al., 2009).

As compared to HG and RG-II biosynthesis, relatively little is known about the glycosyltransferases involved in RG-I

biosynthesis and only one glycosyltransferase has been characterized so far. A reverse genetics approach with putative glycosyltransferases from the CAZy GT47 family led to the identification of the *arabinan deficient 1* (*arad1*) mutant of *Arabidopsis* showing a reduced arabinose content in the cell wall (Harholt et al., 2006). Characterization of the *arad1* cell wall demonstrated that the *ARAD1* gene probably encodes an arabinan α -1,5-arabinosyltransferase activity important for RG-I biosynthesis. Another Golgi glycosyltransferase of GT47 family sharing a high homology with *ARAD1*, named *ARAD2*, has been recently characterized (Harholt et al., 2012). Using biochemical and microscopical approaches, the study has revealed that *ARAD1* and *ARAD2* form homo and heterodimers when they are co-expressed in *N. benthamiana* plants (Harholt et al., 2012). It is interesting to note that although arabinofuranose is normally incorporated in RG-I during its biosynthesis *in planta*, it is the arabinopyranose that is actually incorporated during *in vitro* assays, using detergent-solubilized membranes. To explain this discrepancy between *in planta* and *in vitro* observations, it has been hypothesized that a mutase activity responsible for the conversion is lost upon solubilization (Nunan and Scheller, 2003). A UDP-arabinose mutase (UAM) that converts UDP-arabinopyranose into UDP-arabinofuranose was characterized in rice (Konishi et al., 2007) and found to share more than 80% identity with the reversibly glycosylated proteins (RGPs) of *Arabidopsis* that have long been known to be cytosolic proteins capable of associating with Golgi membranes (Dhugga et al., 1991, 1997; Delgado et al., 1998). Based on the fact that RGPs can be reversibly glycosylated by glucose, xylose, and galactose residues, it has been postulated that they could be involved in XyG or xylan biosynthesis. Among the five isoforms of RGPs (RGP1–5) identified in the *Arabidopsis* genome, the mutase activity has been recently shown for AtRGP1, AtRGP2, and AtRGP3 (Rautengarten et al., 2011). This UAM activity of RGPs suggests their potential role in the biosynthesis of arabinofuranosyl-containing polymers such pectin or arabinogalactan proteins. A double mutant *rgp1/rgp2* was shown to harbor pollen development defects, thereby leading the authors to hypothesize that RGPs might be involved in cell wall polysaccharide biosynthesis (Drakakaki et al., 2006). Moreover, Rautengarten et al. (2011) analyzed the phenotype of homozygous T-DNA insertion mutants (*rgp1-1* and *rgp1-2*), and described severe developmental phenotypes associated with subsequent reduction ($\geq 69\%$) in cell wall arabinose content.

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CONCLUDING REMARKS

The knowledge of XyG and pectin biosynthesis has progressed significantly over the past 10 years with respect to the identification of the enzymes involved in their biosynthesis, using functional genomics. The challenge now is, to determine how these players (and partners) cooperate, in a timely and probably spatially resolved manner, in order to achieve the coordinated and efficient synthesis of these polymers.

In plants, the only approach that has so far provided evidence for the compartmental organization of the Golgi with regards to complex polysaccharide biosynthesis is immunogold microscopy using antibodies raised against specific sugars of different polysaccharides (Follet-Gueye et al., 2012). Now we need to move forward with studies addressing how the glycosyltransferases are distributed within plant Golgi stacks and to determine whether distinct enzymes are preferentially compartmentalized within distinct Golgi cisternae, or if they are always localized in all Golgi sub-compartments and in all cell types. Another interesting issue relates to whether glycohydrolases are able to act on polysaccharides within Golgi-derived vesicles before secretion. Finally, in light of the recent identification of glycosyltransferase complexes involved in HG and arabinan synthesis (Atmodjo et al., 2011; Harholt et al., 2012), as well determination of their membrane topologies (Søgaard et al., 2012), it is of special interest to understand location and interactions of glycosyltransferases with partners including nucleotide–sugar interconversion enzymes and sugar transporters within Golgi membranes. Clearly, a better understanding of such interactions, their dynamics along with membrane topologies of the enzymes and their distribution within Golgi cisternae will provide new mechanistic insights into the biosynthesis and secretion of cell wall polysaccharides in flowering plants.

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