

Evans Review:

Plant cell walls: the skeleton of the plant world

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Abstract. Plants are our major source of renewable biomass. Since cell walls represent some 50% of this biomass, they are major targets for biotechnology. Major drivers are their potential as a renewable source of energy as transport fuels (biofuels), functional foods to improve human health and as a source of raw materials to generate building blocks for industrial processes (biobased industries). To achieve sustainable development, we must optimise plant production and utilisation and this will require a complete understanding of wall structure and function at the molecular/biochemical level. This overview summarises the current state of knowledge in relation to the synthesis and assembly of the wall polysaccharides (i.e. the genes and gene families encoding the polysaccharide synthases and glycosyltransferases (GlyTs)), the predominant macromolecular components. We also touch on an exciting emerging role of the cell wall–plasma membrane–cytoskeleton continuum as a signal perception and transduction pathway allowing plant growth regulation in response to endogenous and exogenous cues.

Additional keywords: cell wall–plasma membrane–cytoskeleton continuum, glycosyltransferase, polysaccharide structure and biosynthesis, synthase.

Plant cell walls

A distinguishing feature of plant cells is the presence of a polysaccharide-rich wall. The wall encloses each cell while at the same time allowing the transfer of solutes and signalling molecules between cells via specific structures such as plasmodesmata (symplastic transport), or pores within the gel-like matrix of the wall itself (apoplastic movement). Similar to the skeleton of animals, the plant wall is a key determinant of overall plant form, growth and development. In contrast to having a specialised skeletal system as occurs in animals, the shape and strength of plants rely on the properties of the wall. The wall also plays a significant role in plant defence and responses to environmental stresses. Plant walls are important not simply because they are integral to plant growth and development but also because they determine the quality of plant-based products. The texture, nutritional and processing properties of plant-based foods for human and animal consumption is heavily influenced by the characteristics of the wall. Fibre for textiles, pulp and paper manufacture, and timber products and increasingly for fuel and composite manufacture is largely composed of walls, or derived from them. Due to their relevance to these industries, the chemical structures of the constituent polymers (polysaccharides, (glyco) proteins, polyphenolics) and the biochemical processes involved in the synthesis, maturation and turnover of the wall have been the subjects of research for many years. This research is now intensifying with the prospect of using plant ligno-cellulosic biomass as a viable and sustainable alternative to fossil fuels

in the production of transportation fuels. Advances are likely to occur through molecular biological and functional genomics approaches, including public availability of genome sequences for various species including *Arabidopsis*, *Populus*, *Vitis* (grapevine), *Oryza* (rice), *Brachypodium* and *Sorghum*, coupled with extensive, associated genomics resources. To date, a combination of these and traditional biochemical technologies has facilitated the identification of genes and enzymes involved in wall biosynthesis and is likely to continue to generate progress in the years to come. This review will cover current work that is being undertaken to elucidate the genes and gene families encoding polysaccharide synthases and glycosyltransferases (designated GlyTs) required for the synthesis of wall polysaccharides. The processes involved in polysaccharide modification by non-glycosyl constituents (e.g. methylation, acetylation and phenolic acids) and wall modification by wall-residing proteins and enzymes, although immensely important, are outside the scope of this review. We will also briefly overview an exciting emerging role of the wall as an external sensor of the environment and the proposed mechanisms by which the external signals are sensed/perceived and then transduced across the plasma membrane (PM) to the cytoskeleton/cytoplasm where signalling pathways are activated. Many important aspects of wall biology, including their dynamic re-modelling during growth and development as well as in response to abiotic and biotic stresses are omitted but are critical to understanding the roles of walls in these processes.

We recommend some excellent reviews covering these topics (Baskin 2005; Cosgrove 2005; Obroucheva 2008; Szymanski and Cosgrove 2009).

Cell wall structure

Primary plant walls are composed predominantly of a complex array of polysaccharides (~90%) and some protein (~10%). In all cell-types, rigid cellulose microfibrils are embedded in a gel-like matrix of non-cellulosic polysaccharides and pectins. These polysaccharides are intimately associated with one another, both non-covalently and covalently, and often with proteins and lignins. Walls are complex, diverse and dynamic, changing throughout the processes of cell division, growth and differentiation. The types of polysaccharides present in the wall vary depending on the plant species, cell type and location, developmental stage and history of responses to biotic and abiotic stresses (Carpita *et al.* 2001b; Trethewey and Harris 2002). Growing cells are surrounded by thin (100 nm or less), highly hydrated (~60% of wet weight) primary walls that are sufficiently flexible to yield to the hydrostatic forces exerted by the protoplast that drives growth. Primary walls of dicots, gymnosperms and non-commelinoid monocots (commonly referred to as Type I walls) consist of a cellulosic network embedded in a matrix of complex polysaccharides, of which xyloglucans (XyGs) and pectic polysaccharides are most abundant (Fig. 1a; Fincher and Stone 1986; Bacic *et al.* 1988; Carpita and Gibeau 1993; McCann and Roberts 1994). Primary walls of the Poales and related commelinoid monocots (commonly referred to as Type II walls) are organised in essentially the same way except that glucuronoarabinoxylans (GAXs) and (1,3;1,4)- β -D-glucans predominate in the matrix phase of these species, and levels of pectic polysaccharides and XyGs are relatively low (Bacic *et al.* 1988; Carpita and Gibeau 1993; McCann and Roberts 1994; Smith and Harris 1999). The concept of Type I/II walls has been extremely useful to guide wall researchers. However, it is now becoming somewhat redundant as surveys across the plant kingdom, particularly driven by the availability of high-throughput antibody profiling techniques of wall polymers (Moller *et al.* 2007), suggests a continuum of wall structures rather than clearly delineated wall types (Harris *et al.* 1997; Harris 2005).

Cells that have ceased enlarging and are required to withstand large compressive forces mature by depositing secondary walls (up to several microns thick) that increase the strength of the cell and reduce flexibility (Fig. 1b). During secondary wall development cellulose, and matrix phase polysaccharides with a lower degree of backbone substitution, such as heteroxylans and heteromannans, are deposited in a highly ordered pattern. Together with the deposition of lignin this results in a dehydration of the wall compartment, which becomes increasingly hydrophobic in nature. In some cell types, lignin is also deposited throughout the wall during secondary development. Hydrophobic lignins overlie and encrust the cellulose microfibrils and matrix phase polysaccharides, and can also be covalently complexed with wall polysaccharides. To date much effort has centred on manipulating the composition and levels of lignins. This work is extensive and important to understanding wall structure and biosynthesis (Boerjan *et al.* 2003; Boudet *et al.*

2003; Vanholme *et al.* 2008), but lignin biology will not be addressed here.

The lignin–polysaccharide associations (together with the dehydration process described above) are factors contributing to the ‘recalcitrance’ of lignified walls to enzymatic digestion and must be broken as a preliminary to the enzymatic digestion, or ‘deconstruction’ phase in ‘cellulosic’ bioethanol production and in ruminant digestion. The more thoroughly this can be achieved, the higher the yields of fermentable sugars. Given the surge in interest in plant biomass for bioethanol production it is important to recognise that the degree of ‘recalcitrance’ is not only influenced by the chemistry of the cell walls but also by the anatomical structures of the cells and tissues that are the primary feedstock. Although these factors are only now being considered for biofuels research, the exhaustive research findings from fibre digestion and utilisation of forage crops (particularly grasses and legumes) by ruminant animals provides some useful guidance. A summary of the chemical composition of the walls of the plant groups that are the major sources of feedstocks is in Table 1 and summarised in an excellent review by Harris and Stone (2008). The extent and type of lignification is a major factor affecting degradability and, in general, non-lignified walls are easily degraded whereas lignified walls are much less degradable (Table 2; Buxton and Redfearn 1997; Wilson and Hatfield 1997). Other physical and chemical structures, such as the presence of waxes/cuticles and a high proportion of cells with a high surface area : volume ratio (e.g. thick-walled parenchyma and sclerenchyma) all contribute to a lower degradability (Table 2). Plant anatomy at the cellular level also affects degradability and different cell types and their proportions influences outcomes (e.g. Chesson 1993; Buxton and Redfearn 1997; Wilson and Hatfield 1997). Pretreatment of feedstocks that cause either physical disruption of the tissue/cells (e.g. high pressure/temperature), chemical modification (e.g. alkali causes both non-covalent (swelling and dissociation of polysaccharide chains) and covalent (ester saponification) modification) are essential in mitigating the influence of these anatomical and chemical characteristics of different feedstocks on enzymic saccharification.

The basic structures of plant wall polysaccharides are depicted schematically in Fig. 2. Cellulose microfibrils are composed of bundles of ~36 linear (1,4)- β -glucan chains (Fig. 2a) held tightly together by hydrogen bonding (Somerville 2006). The width, degree of polymerisation and crystallinity of the cellulose microfibrils are highly variable and depend on the source and age of the tissue (Bacic *et al.* 1988). The molecular backbone of XyGs (Fig. 2b) is similar in structure to cellulose, but the xylosyl (Xylp) side chains and their extensions prevent hydrogen bonding along the length of the substituted chain yielding a more flexible and soluble macromolecule. The side chains of XyGs are quite diverse and vary in frequency and structure depending on the tissue and species (Fry *et al.* 1993; Peña *et al.* 2008). The (1,3;1,4)- β -glucans (Fig. 2c) are also similar in structure to cellulose, except that single (1,3)- β -D-Glcp residues are located between blocks of multiple (1,4)- β -D-Glcp residues, introducing ‘kinks’ into the polysaccharide chain. Adjacent (1,4)- β -D-oligoglucosyl residues (cellodextrin units) usually consist of cellotriosyl and cellotetraosyl units (~90% of the molecule) arranged at random with longer cellodextrin

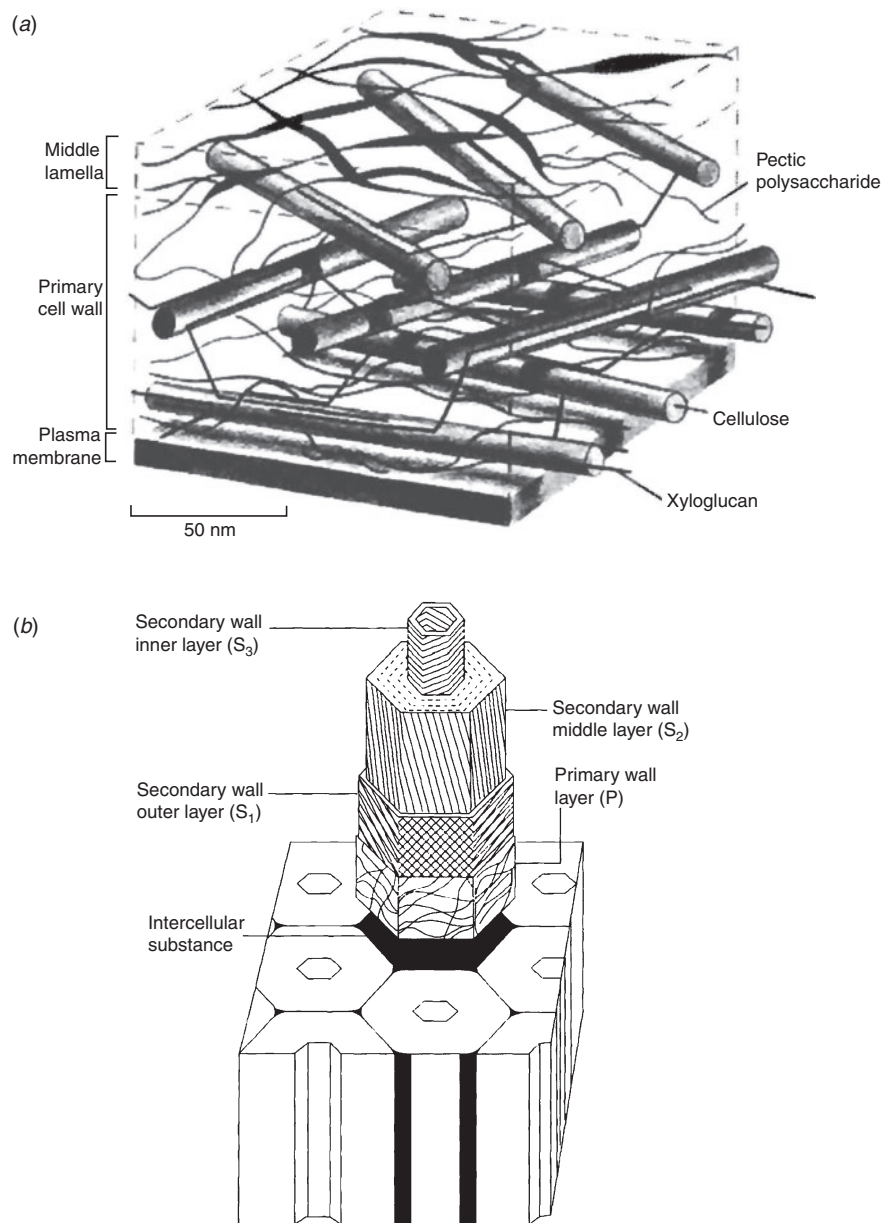


Fig. 1. Structure and organisation of plant cell walls. (a) Simplified schematic, drawn-to-scale representation of the spatial arrangement of polymers in a pectin-rich primary cell wall. Reproduced from McCann and Roberts (1991), with permission. (b) Schematic representation of the cellulose microfibril orientation in the primary (P) and secondary (S₁, S₂, S₃) wall layers of a xylem fibre cell or a tracheid. Reproduced from Wardrop and Bland (1959), with permission.

units (DP 5–20) comprising the remainder of the polysaccharide (Fincher 2009a).

Heteroxylans (Fig. 2d) are composed of a backbone of (1,4)- β -linked Xylp of which ~10% are substituted at C(O)3 and C(O)2 with α -L-arabinofuranosyl (Araf) residues, α -D-glucuronic acid (Glc pA; and 4-O-methyl Glc pA) or a combination of both to give arabinoxylans (AXs), (methyl)glucuronoxylan (GXs) and glucuronarabinoxylans (GAXs), respectively. GXs are the most abundant non-cellulosic polysaccharides in dicot secondary walls whereas GAXs are minor components of the secondary

walls of soft woods. In comparison, AXs are the predominant xylans in grass cell walls, with GAXs being less abundant (York and O'Neill 2008). The backbone Xylp residues can also be acetylated at the C(O)3 and C(O)2 positions or sometimes both (York and O'Neill 2008). The Ara sidechains of the grass wall heteroxylans can be esterified with ferulic and/or *p*-coumaric acid, enabling crosslinking between polysaccharide molecules and between polysaccharides and lignin (Iiyama *et al.* 1993). GXs from birch, spruce and *Arabidopsis*, but not grasses, have been shown to contain a distinct glycosyl sequence

Table 1. Compositions of the cell walls of vegetative parts of 'cellulosic' ethanol feedstocks (modified from Harris and Stone 2008)

Wall type	Plant group			
	Angiosperms	Monocotyledons	Gymnosperms	
	Eudicotyledons e.g. hardwoods [e.g. willows (<i>Salix</i> spp. <i>Eucalyptus</i> spp.)] and legumes	Non-commelinids e.g. sisal (<i>Agave sisalana</i>)	Comelinids e.g. grasses and cereals (Poaceae), palms (Arecales)	e.g. coniferous gymnosperms e.g. softwoods [e.g. spruce (<i>Picea abies</i>)]
Primary walls				
Fibrillar phase	Cellulose	Cellulose	Cellulose	Cellulose
Matrix phase ^A	Pectic polysaccharides	Pectic polysaccharides	Glucuronarabinoxylans ^B (GAXs)	Pectic polysaccharides ^C
	Xyloglucans	Xyloglucans	Pectic polysaccharides	Xyloglucans
	Heteroxylans	Heteroxylans	Xyloglucans	Heteroxylans
	(Galacto)glucmannans (minor)	(Galacto)glucmannans (minor)	[(1,3;1,4)- β -glucans in some Poales families, including Poaceae]	(Galacto)glucmannans (minor)
Phenolics	Ester-linked ferulic and small proportions of <i>p</i> -coumaric acid in 'core' Carophyllales. Known to be linked to RG I side chains in Amaranthaceae		Ferulic, small proportions of <i>p</i> -coumaric, and sometimes sinapic acids, ester-linked to GAXs	Ester-linked ferulic and <i>p</i> -coumaric acids in all families
Secondary walls				
Fibrillar phase	Cellulose	Cellulose	Cellulose	Cellulose
Matrix phase ^A	4-O-methyl-glucuronoxylans	4-O-methyl-glucuronoxylans	Glucuronarabinoxylans (GAXs)	(Galacto)glucmannans
	Glucmannans	Glucmannans	(Galacto)glucmannans (minor)	4-O-methyl-glucuronarabinoxylans
Lignins	Syringyl and guaiacyl; small proportions of <i>p</i> -hydroxy-phenyl units. Acylated with <i>p</i> -hydroxybenzoic acid in Salicaceae and acetic acid in <i>Hibiscus cannibinus</i>		Syringyl and guaiacyl; small proportions of <i>p</i> -hydroxy-phenyl units. Acylated with <i>p</i> -coumaric acid in Poaceae and Musaceae, and with <i>p</i> -hydroxybenzoic acid in Arecales.	Usually mostly guaiacyl units; also <i>p</i> -hydroxyphenyl units in compression wood

^AIn order of relative abundance.

^BPrimary walls of palms (Arecales) are an exception; their matrix polysaccharides are pectic polysaccharides > xyloglucans > glucuronarabinoxylans.

^CDetailed data on matrix polymers are available only for conifers (Coniferales) and *Ginkgo biloba* (Ginkgoales).

Table 2. Relative tissue type proportions for cross-sections of warm- and cool-season perennial grasses (modified from Buxton and Redfearn 1997)

Species ^A	Leaf blade tissue cell types			Stem tissue cell types	
	Bundle sheath (wall moderately thick and weakly lignified)	Mesophyll (thin wall, no lignin)	Sclerenchyma (thick, lignified wall)	Parenchyma (variable thickness and degree of lignification depending on maturity)	Sclerenchyma (thick, lignified wall)
% cross-sectional area					
Warm-season, C ₄					
Bahia grass	11.1	52.4	—	—	—
Indian grass	17.3–19.7	70.0–71.6	—	—	—
Switchgrass	28.9	35.7	2.9	65.2	—
Cool-season, C ₃					
Smooth brome grass ^B	9.6–11.5	60.9–63.5	4.6–4.8	50.9–59.9	20.7–21.6
Kentucky bluegrass	4.1	64.7	6.6	—	—
Tall fescue ('Ky 31')	5.4	60.6	4.9	—	—

^AScientific names of grasses in order of listing are *Paspalum notatum* Flugge, *Sorghastrum nutans* (L.) Nash, *Panicum virgatum*, *Bromus inermis*, *Poa pratensis* L. and *Festuca arundinacea*.

^BRange is based on high and low *in vitro* dry matter digestibility.

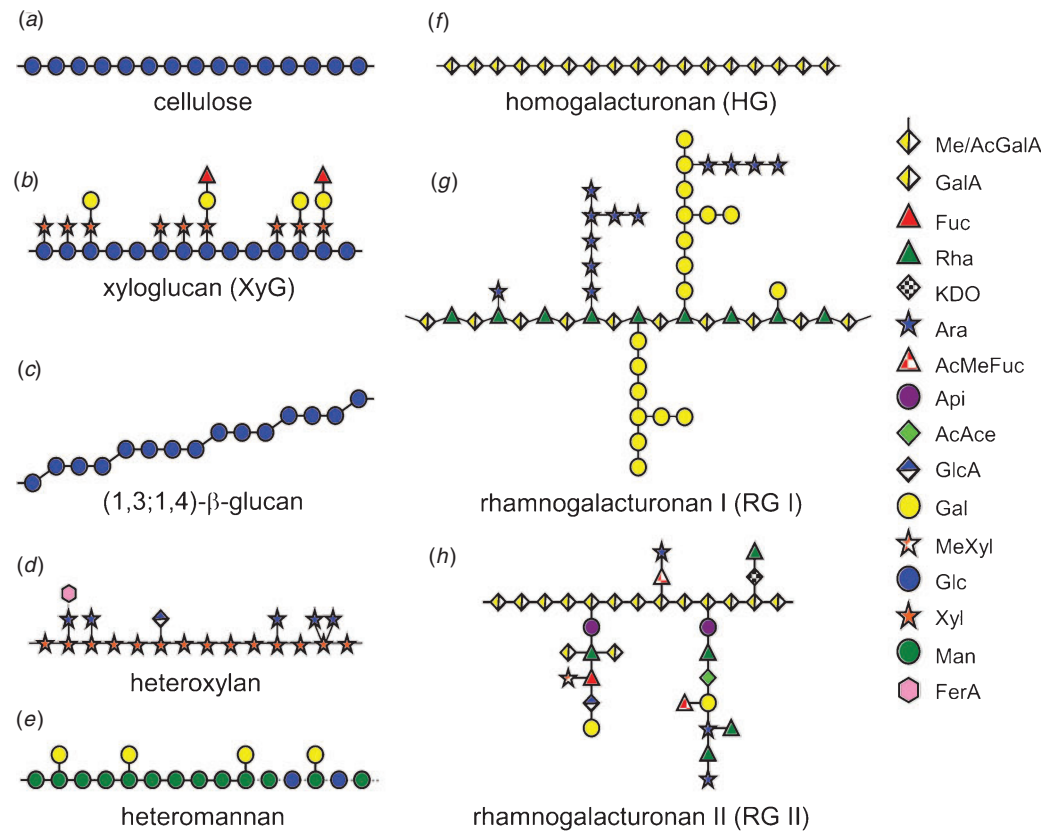


Fig. 2. Schematic representation of the polysaccharide structures found in plant cell walls. Cellulose, xyloglucan (XyG) and (1,3;1,4)-β-glucan have a backbone based on glucose (Glc_p), heteroxylan on xylose (Xyl_p), heteromannan on mannose (Man_p), homogalacturonon (HG) and rhamnogalacturonan II (RG I) on galacturonic acid (Gal_pA), and RGI on an alternating disaccharide of Gal_pA and rhamnose (Rhap).

4)-β-D-Xylp-(1,4)-β-D-Xylp-(1,3)-α-L-Rhap-(1,2)-α-D-GalpA-(1,4)-D-Xylp at the reducing end (Peña *et al.* 2007; York and O'Neill 2008). Heteroxylans comprise approximately one-third of the biomass of wood and as the current microorganisms (yeast) are unable to ferment pentoses into alcohol, their conversion to a fermentable sugar or the discovery of microorganisms that can efficiently utilise heteroxylans as a carbon source is now a high priority to make ligno-cellulosics an effective and sustainable form of alternate transport fuels (Somerville 2006).

The heteromannans (Fig. 2e) consist of the (1,4)- β -mannopyranosyl (Manp)-containing family of wall polysaccharides including mannans, galactomannans, glucomannans and galactoglucomannans. Galactomannans are polymers consisting of a backbone of (1,4)- β -Manp that can be substituted at C(O)6 with β -D-Galp. In glucomannans, the (1,4)- β -Manp backbone is interrupted by (1,4)- β -GlcP residues at irregular intervals. Glucomannans can be acetylated at C(O)2 or C(O)3 of the Manp residues and also substituted with α -D-Galp attached through (1,6)-linkages (galactoglucomannan) (Matheson 1990).

Pectins comprise some of the most complex families of polysaccharides and include the homogalacturonans (Fig. 2f) (HG; homopolymers of (1,4)- α -GalpA); xylogalacturonan (XG; HG substituted at C(O)3 with one or two (1,4)- β -D-Xylp residues); rhamnogalacturonan I (Fig. 2g) (RGI; backbone of [α -D-GalA-(1,2)- α -L-Rha-(1-4)]_n with Rha residues substituted at C(O)2 or C(O)4 with linear or branched polymers of (1,4)- β -D-galactan, (1,5)- α -arabinan, type I arabino-4-galactans, type II (arabino-3,6-galactans or smaller oligosaccharides containing α -L-Araf, β -D-Galp, or α -L-Fucp); and rhamnogalacturonan II (Fig. 2h) (RGI; short HG backbone substituted with complex side chains containing combinations of Rhap, GalpA, GlcP, Api, Fucp, Galp, MeXylp, Kdo, Dha and Ace) (Bacic 2006; Mohnen 2008). Structural complexity is increased by the addition of acetyl and methyl groups to GalpA, Fucp, Xylp and Ace residues, which impact on the physical characteristics and molecular interactions within the wall and in industrial applications. Chemical evidence suggests that these pectic polysaccharides are covalently linked to one another, and to other wall polysaccharides (Mohnen 2008) although the physical arrangement of these different molecules within the wall is unknown.

Wall (glyco)proteins, which comprise less than 10% of the dry weight of the primary wall, are critical to the structural and functional elements of both the wall and the PM–cytoskeleton continuum (see below). In addition to serving a structural role, wall proteins contribute to wall assembly and remodelling during growth and development, and in stress responses. Such proteins include enzymes such as hydrolases, proteases, glycosidases, peroxidases, esterases, as well as expansins, wall-associated kinases (WAKs), hydroxyproline-rich glycoproteins (HRGPs) that include extensins, arabinogalactan-proteins (AGPs) and proline-rich proteins (PRPs) and the glycine-rich proteins (GRPs). Bioinformatic analysis of genomic sequences has revealed that most of these wall proteins are encoded by multigene families. A detailed discussion of the wall proteins is beyond the scope of this overview and readers are referred to several recent comprehensive reviews (Rose 2003).

Since the AGPs are implicated in sensing at the cell surface a brief description of their structure is provided. AGPs are highly glycosylated proteoglycans, usually containing 1–10% protein (Johnson *et al.* 2003). AGP protein backbones are typically rich in Hyp/Pro, Ala, Ser and Thr. The Hyp residues are usually substituted by type II arabino-3,6-galactans (AGs; 5–25 kDa), although short arabinosides are found (Nothnagel 1997). Most AGPs are anchored, sometimes transiently, to the plasma membrane by glycosylphosphatidylinositol (GPI) anchors (Youl *et al.* 1998). AGPs are secreted from plant cells in which they are either anchored to the plasma membrane or are soluble in the

extracellular matrix or cell wall. In *Arabidopsis thaliana* (L.), at least 47 genes are predicted to encode AGP protein backbones, and most of these are predicted to be GPI anchored (Johnson *et al.* 2003). These have been divided into several subclasses on the basis of their biased amino acid composition and their modular structure, and include 13 classical AGPs, 12 AG-peptides (mature protein backbones of 10–17 amino acids), three basic AGPs that include a short Lys-rich region and 21 FLAs (fasciclin-like AGPs) that have, in addition to predicted AGP-like glycosylated regions, putative cell adhesion domains known as fasciclin domains (Johnson *et al.* 2003; Schultz *et al.* 2004). The recent identification of a *salt overly sensitive5* (*sos5*) mutant with a missense mutation in the fasciclin domain of *AtFLA4* (Shi *et al.* 2003) indicates that these domains are important for FLA function.

Biosynthesis of wall polysaccharides

Polysaccharide synthesis can be broken down into four distinct stages: (1) production of activated nucleotide-sugar donors, (2) initiation of polymer synthesis, (3) polymer elongation, and (4) termination of synthesis (Delmer and Stone 1988). Both the initiation and termination of synthesis are poorly understood whereas there has been significant progress on the production of nucleotide sugar donors and the polymer elongation processes. The key enzymes in wall biogenesis are the polysaccharide synthases and glycosyltransferases (GlyTs) that catalyse formation of the bonds between adjacent monosaccharides from activated nucleotide-sugar donors and are the focus of this review. The specificity of these enzymes determines the sequence of sugar residues within a polysaccharide, and the branching pattern. Figure 3 shows where different polysaccharides are synthesised in an idealised plant cell.

The polysaccharides, cellulose and the (1,3)- β -D-glucan callose, are synthesised by polysaccharide synthases directly at the PM (Kudlicka and Brown 1997; Delmer 1999). The rosette, the enzyme complex responsible for cellulose synthesis, has a diameter of ~25 nm and comprises six identical, spherical subunits that aggregate together in a hexagonal arrangement, forming a central pore through which the glucan chains are extruded (Fig. 3). Rosettes have been visualised in transit from the Golgi apparatus (GA) to their destination in the PM using freeze fracture, transmission electron microscopy, and more recently immunofluorescence techniques (Haigler and Brown 1986; Gardiner *et al.* 2003; Somerville 2006; Wightman *et al.* 2009). In contrast, all other non-cellulosic and pectic polysaccharides are synthesised within the endoplasmic reticulum (ER) and GA (Fincher and Stone 1981; Gibeaut and Carpita 1994) using both polysaccharide synthases and GlyTs. The assembled polysaccharides are then transported to the cell surface via Golgi-derived secretory vesicles and incorporated into the wall. Recent progress in understanding the assembly of non-cellulosic polysaccharides suggests that this process may occur in both the Golgi compartment and within the apoplast (see below).

Polysaccharide synthases/glycosyltransferases

Sequence similarity, the presence of certain motifs, hydrophobic cluster analysis (HCA) and catalytic specificity, have been used to categorise polysaccharide synthases and GlyTs into more than 90 families within the overall 'glycosyltransferase' (GT) class of

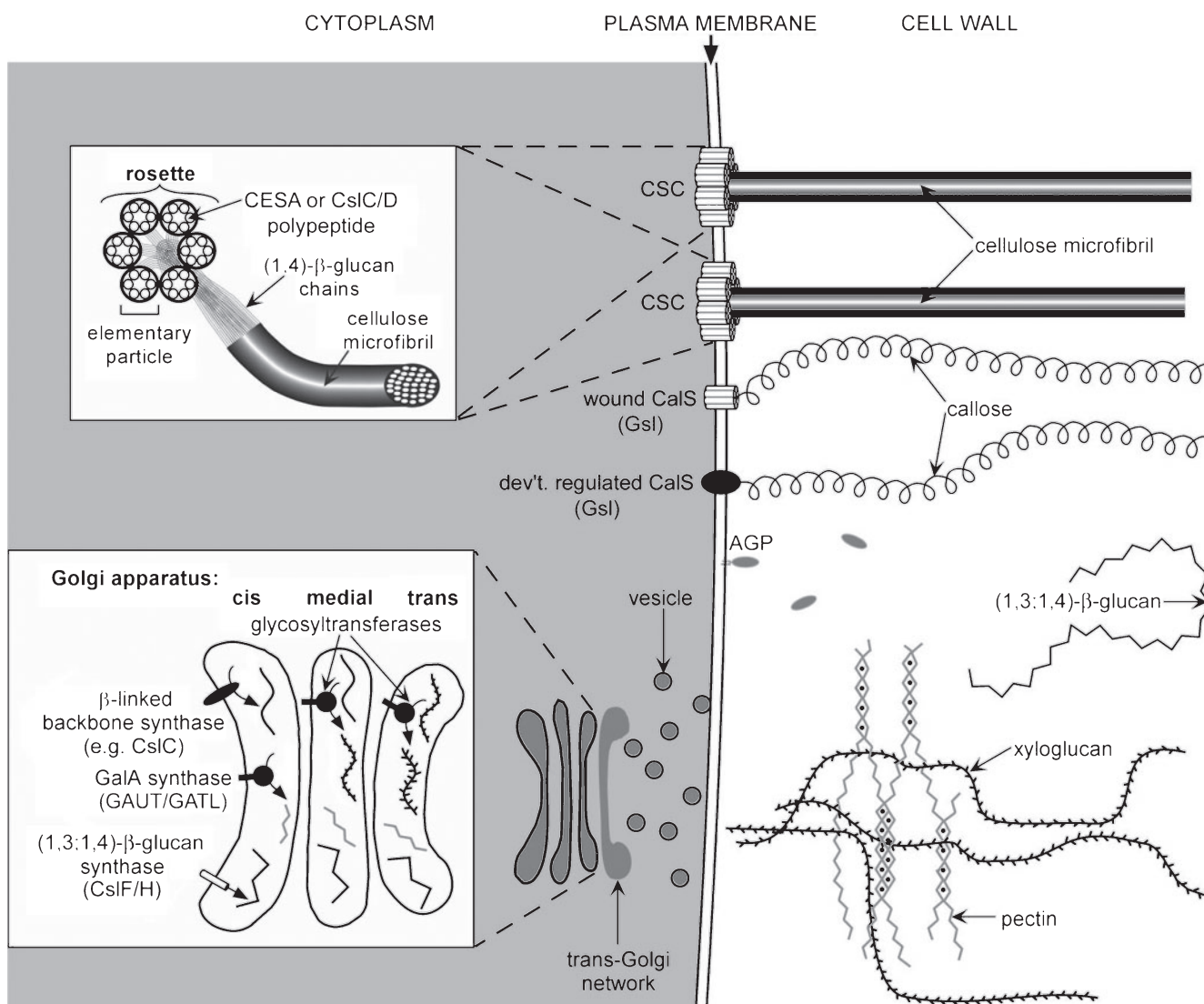


Fig. 3. Gene families involved in plant wall polysaccharide synthesis. This figure shows an idealised primary wall and the proposed arrangement and location of some of the major enzymes involved in polysaccharide biosynthesis. Chains of (1,4)- β -glucan (cellulose) are produced by cellulose synthase enzyme complexes (CSC), or rosettes, at the plasma membrane (PM) that associate with each other to form microfibrils (see Fig. 1a). Members of the Cesa family and possibly the CslC and CslD family are implicated in cellulose synthesis and may form part of the CSC. Callose, a (1,3)- β -glucan, is also synthesised at the PM by a callose-synthesising (CalS/Gsl) complex. Both these complexes use UDP-Glc on the cytoplasmic side of the PM and extrude their products into the apoplast. Glycoproteins, such as AGPs, as well as all other non-cellulosic and pectic polysaccharides are synthesised within the Golgi. Csls, GAUT/GATLs and glycosyltransferases (GlyTs) have all been suggested as being backbone synthases for these polysaccharides. Depending on the location of the catalytic site, the NDP-sugars are either utilised on the cytoplasmic side of the Golgi membrane (straight arrow) or within the Golgi lumen (curved arrow). The sugar sidechains found on branched polysaccharides are most likely added by Golgi-localised GlyTs. The completed polysaccharides are transported to the PM in vesicles for secretion into the wall. Within the wall, further modification of the polysaccharides can occur either during or after deposition. Modified from Doblin *et al.* (2003), with permission.

carbohydrate modifying enzymes collated in the Carbohydrate Active enZymes (CAZy) database (Cantarel *et al.* 2009). Whilst the human genome estimated to contain ~200 GT genes (Scheible and Pauly 2004), in *Arabidopsis* and rice (*Oryza sativa* L.), currently 455 and 565 genes encode CAZy-classified GTs, respectively (July 22, 2009). The sequences of both species are grouped into the same 41 families. This large number of GTs is thought to contribute to the structural and tissue-specific complexity of plant polysaccharides and glycoconjugates

(Coutinho *et al.* 2003). The function/s and biochemical activities of most of the *Arabidopsis* and other plant GTs have not yet been characterised.

GTs are broadly divided into two enzyme classes, the type III polysaccharide synthases and the type II GlyTs. Type III polysaccharide synthases are integral membrane proteins with multiple predicted trans-membrane domains (TMDs) that catalyse the transfer of glycosyl residues from a sugar nucleotide donor to construct the main polymeric backbone of wall

polysaccharides in a processive manner. These enzymes are involved in the biosynthesis of homopolysaccharides such as cellulose, callose, (1,3;1,4)- β -D-glucan and the backbones of most of the other matrix phase heteropolysaccharides, including XyGs and heteromannans, but seemingly excluding the pectins and heteroxylans.

The multigene families of the cellulose synthase (*CesA*) and the nine cellulose synthase-like (*Csl*) groups (*CslA*-H and J) (Fig. 4), and the glucan synthase-like (*Gsl*) genes (Brownfield *et al.* 2009) encode members of the type III polysaccharide synthases. The *CesA* and the related *Csl* enzymes are members of Family GT2 (Table 3) and encode large, integral membrane proteins, (~500–1200 amino acid residues) that contain between three to six predicted TMDs towards the COOH terminus and one to two towards the NH₂ terminus. They share a common D, D, D, QxxRW motif found in all processive GT2 family members, including the chitin and hyaluronan synthases, that is believed to be involved in substrate binding and catalytic activity of the enzyme (Richmond and Somerville 2000). The *Gsl* proteins belonging to Family GT48 (Table 3) are involved in callose biosynthesis, have 13–19 predicted TMDs and molecular masses of greater than 200 kDa, but lack the D, D, D, QxxRW motif (Brownfield *et al.* 2009).

The type II class of GlyTs, transfer single glycosyl residues from the donor to a polysaccharide backbone, although the recently identified (1,4)- α -D-galacturonosyltransferases (GAUTs) that participate in HG synthesis and the putative CAZy GT43 xylan synthases, IRX9 and IRX14, are exceptions (Table 3). They are anchored to the membrane via a single TMD that spans the membrane, have a short cytosolic NH₂ terminus, an extended hydrophilic stem region and a globular catalytic domain towards the COOH terminus of the protein that resides in the Golgi lumen (Keegstra and Raikhel 2001). Examples include the α -D-GalTs that add single α -D-Galp substituents to the (1,4)- β -D-mannan backbone of galactomannans (Edwards *et al.* 1999, 2004) and the α -D-XylTs that add single α -D-Xylp residues to the (1,4)- β -D-glucan backbone of XyGs (Faik *et al.* 2002; Cavalier and Keegstra 2006; Cavalier *et al.* 2008).

Recent developments in understanding wall biosynthesis

Increasingly, genetic manipulation and conventional breeding techniques are being applied in attempts to modify the quality and quantity of individual cell wall components for broader commercial applications. The best candidates for this manipulation are the genes, enzymes and biochemical pathways involved in wall polysaccharide biosynthesis. It has been estimated that well over 2000 different gene products are involved in making and maintaining the wall (Carpita *et al.* 2001a). Until recently, little was known about the polysaccharide synthases and GlyTs involved in wall biosynthesis, mostly due to the difficulty in obtaining active enzymes, particularly the polysaccharide synthases, by traditional biochemical methods. There has now been significant progress in describing some of the genes that are involved in the biosynthesis of cellulose and matrix phase polysaccharides and these genes, and the evidence for their functionality, are summarised in Table 3 and discussed below. A cautionary note, however, is that plants demonstrate incredible plasticity and hence disruption of genes in one pathway results in

pleiotropic effects making interpretation of some gene disruption experiments difficult.

Cellulose

Before 1996, attempts to isolate and purify the cellulose synthase enzyme complex (CSC) were largely unsuccessful as the activity of the enzyme is rapidly lost with cell rupture and detergent solubilisation and the predominant product synthesised from UDP-Glc is callose; this considerably hampered progress towards identifying the catalytic protein(s). A highly significant breakthrough occurred through the use of a bioinformatic approach when Delmer and colleagues made a cDNA library of cotton fibre transcripts at a time of maximal cellulose synthesis and randomly sequenced a small subset of the resulting clones (Pear *et al.* 1996). Two genes, later renamed *GhCesA1* and 2, were identified that shared low but significant sequence similarity with the catalytic subunits of the bacterial cellulose synthases. Unequivocal proof of their involvement in cellulose synthesis came with the identification and characterisation of cellulose-deficient *Arabidopsis* mutants, most notably the *root swelling* (*rsw*) (Baskin *et al.* 1992; Arioli *et al.* 1998) and *irregular xylem* (*irx*) (Turner and Somerville 1997; Taylor *et al.* 1999) mutants, with lesions in *CesA* genes (Desprez *et al.* 2007).

It is now clear that there are multiple *CesA* genes in the genome of each plant species and that there is functional specialisation (and in some cases partial redundancy) between isoforms. Mutant analyses in *Arabidopsis* (but since verified in other species) have shown that a functional CSC has at least three different, non-redundant *CesA* proteins: *CesA*s 1, 3 and 6 or 6-like (*CesA*s 2, 5 and 9 are partially redundant to 6) and *CesA*s 4, 7 and 8 are required for primary and secondary wall cellulose synthesis, respectively (Taylor *et al.* 1999, 2000, 2003; Gardiner *et al.* 2003; Desprez *et al.* 2007; Persson *et al.* 2007b). The exact biochemical activity of each *CesA* isoform, although speculated upon (Read and Bacic 2002), has not yet been shown experimentally apart from one instance in which yeast-expressed *GhCesA1* was able to convert sitosterol- β -glucoside to sitosterol cellodextrins, thus confirming their ability to synthesise (1,4)- β -glucan linkages (Peng *et al.* 2002). The *CesA* isoforms interact directly and when all three are present, they form higher order oligomeric structures involving homodimers (Kurek *et al.* 2002; Gardiner *et al.* 2003; Atanassov *et al.* 2009). Through the use of tagged *CesA* proteins, it may now be possible to purify CSCs from detergent-soluble extracts and establish whether any other proteins are permanent components of the complex. CSCs are also being isolated in lipid rafts with a view to identifying their components and understanding their functions of the complex partners (V. Bulone, pers. comm.). While the *in vitro* cellulose synthase assay conditions have been significantly improved (Lai-Kee-Him *et al.* 2002; Pelosi *et al.* 2003; Colombani *et al.* 2004), synthesis rates are still quite low compared with those observed *in vivo* (~10%) and assay conditions need to be empirically defined and optimised for each plant system.

Several studies have implicated other proteins in the CSC. These include the membrane-bound KORRIGAN-type cellulases, as well as KOBITO (KOB/ELD1/ABI8), a protein of unknown function, the glycosylphosphatidyl inositol (GPI)-

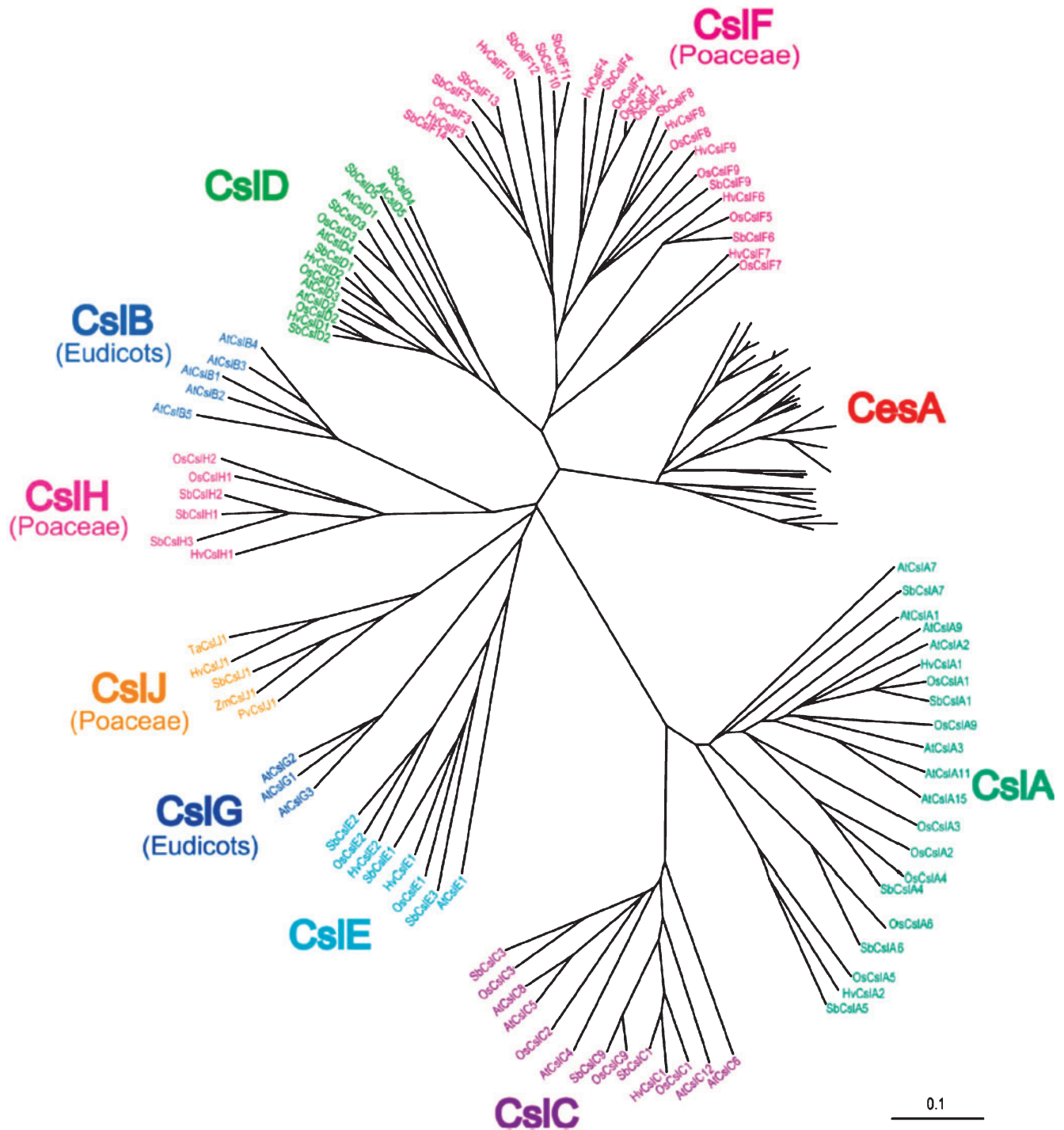


Fig. 4. Phylogenetic tree of the proteins encoded by the cellulose synthase (CesA) and cellulose synthase-like (Csl) gene families in higher plants. Of the nine *Csl* families, the *CslF* and *CslH* genes are found only in cereals (Poaceae), while the *CslB* and *CslG* families appear to be found only in eudicots (Hazen *et al.* 2002). The *CslJ* group is found in certain cereals, including barley, wheat, sorghum, and maize, but not in rice or *Brachypodium*. Reproduced from Fincher (2009b), with permission.

anchored protein COBRA and the chitinase-like CTL1/POM1/ELP1/HOT2 (Robert *et al.* 2004; Somerville 2006), and enzymes involved in N-linked glycan maturation, for example *knopf*, and *rsw3*, glycosidase I and II mutants, respectively. It is not always clear how or why these mutations impact on cellulose assembly.

More recently, correlation analyses of microarray data have been used to search for other genes that may be involved in cellulose synthesis, regulation and/or deposition (Brown *et al.* 2005; Persson *et al.* 2005). Associations between the CSCs and the cytoskeleton are also critical to the orientation of wall cellulose

Table 3. Polysaccharide synthases and glycosyltransferases (GlyTs) involved in plant cell wall polysaccharide biosynthesis

Polysaccharide	Enzyme	CAZy GT family	Gene name	Biochemical activity [demonstrated; expressed or purified enzyme]	Evidence of function [enzyme purification (EP), mutant analysis (MA), heterologous expression (HE), <i>in vitro</i> enzyme assay (EA), subcellular location (SL), transcript correlation (TC)]	References
Cellulose	(1,4)- β -D-glucan synthase	2	<i>CesA</i>	Yes	EA, MA, SL (PM)	Taylor <i>et al.</i> (1999), Peng <i>et al.</i> (2002, 2007), Gardiner <i>et al.</i> (2003), Desprez <i>et al.</i> (2007), Persson <i>et al.</i> (2007b)
		2	<i>HvCslC2</i>	No	TC, SL (PM)	Dwivany <i>et al.</i> (2009)
		2	<i>AtCslDI</i> , 4, 5; <i>NaCslDI</i>	No	TC, SL (ER, Golgi, PM)	Doblin <i>et al.</i> (2001), Manfield <i>et al.</i> (2004), Bernal <i>et al.</i> (2007, 2008), Natera <i>et al.</i> (2008),
Callose	(1,3)- β -D-glucan synthase	48	<i>CFLI</i> ; <i>HvGSLI</i> ; <i>NaGSLI</i>	Yes	EP, EA, SL (ER, Golgi, PM)	Shin and Brown (1998), Cui <i>et al.</i> (2001), Li <i>et al.</i> (2003), Brownfield <i>et al.</i> (2007)
		48	<i>AtGSLI</i> , 2, 5	No	MA, SL (PM)	Vogel and Somerville (2000), Jacobs <i>et al.</i> (2003), Nishimura <i>et al.</i> (2003), Dong <i>et al.</i> (2005), Enns <i>et al.</i> (2005), Nishikawa <i>et al.</i> (2005), Dunkley <i>et al.</i> (2006), Töller <i>et al.</i> (2008)
(1,3;1,4)- β -D-glucan	(1,3;1,4)- β -D-glucan synthase	2	<i>OsCslF2</i> , 4, 9	No	HE	Burton <i>et al.</i> (2006)
Heteromannan	(1,4)- β -D-(gluco)mannan synthase	2 2	<i>HvCslHI</i> <i>CiManS</i>	No Yes	HE, SL (Golgi) HE	Doblin <i>et al.</i> (2009) Dhugga <i>et al.</i> (2004)
		2	<i>AtCslAI</i> , 2, 7, 9; <i>OsCslAI</i> ; <i>PtCslAI</i> , 3 <i>PtaCslAI</i> ; <i>PpCslAI</i> , 2 <i>TjGalT</i>	Yes	HE	Liepmann <i>et al.</i> (2005, 2007), Suzuki <i>et al.</i> (2006)
	α -(1,6)-galactosyltransferase (GalT)	34		Yes	EP, HE	Edwards <i>et al.</i> (1999)
Xyloglucan	(1,4)- β -D-glucan synthase α -(1,6)-xylosyltransferase (XylT)	2 34	<i>TmCslC</i> ; <i>AtCslC4</i> <i>PxXT1</i>	Yes Yes	HE, EA, TC, SL (Golgi) EA	Cocuron <i>et al.</i> (2007) Faik <i>et al.</i> (2002)
		34	<i>AtXXT1</i> , 2	Yes	MA, HE, EA	Faik <i>et al.</i> (2002), Cavalier and Keegstra (2006)
		34	<i>AtXXT5</i>	No	MA, SL (Golgi)	Dunkley <i>et al.</i> (2006), Zabinina <i>et al.</i> (2008)
	β -(1,2)-galactosyltransferase (GalT)	47	<i>MUR3/KAM1</i>	Yes	MA, EA, SL (Golgi)	Madson <i>et al.</i> (2003), Tamura <i>et al.</i> (2005)
	α -(1,2)-fucosyltransferase (FucT)	37	<i>PxFUT1</i>	Yes	EP, EA	Perrin <i>et al.</i> (1999), Faik <i>et al.</i> (2000)

Heteroxylan	Backbone (1,4)- β -D-xylan synthase	37	<i>AtFUT1/MUR2</i>	Yes	EP, M, HE, EA	Perrin <i>et al.</i> (1999), Sarria <i>et al.</i> (2001), Vanzin <i>et al.</i> (2002)
		43	<i>IRX9</i>	Yes	TC, MA, EA, SL (Golgi)	Brown <i>et al.</i> (2005, 2007), Lee <i>et al.</i> (2007a), Peña <i>et al.</i> (2007), Persson <i>et al.</i> (2007a)
		43	<i>IRX14</i>	Yes	TC, MA, EA	Brown <i>et al.</i> (2007), Lee <i>et al.</i> (2007a), Peña <i>et al.</i> (2007), Persson <i>et al.</i> (2007a)
		47	<i>IRX10/AtGUT2</i>	Yes	TC, MA, EA	Brown <i>et al.</i> (2005), Mitchell <i>et al.</i> (2007), Brown <i>et al.</i> (2009), Wu <i>et al.</i> (2009)
		47 2	<i>IRX10-LIKE/AtGUT1</i> <i>AtCslD5</i>	Yes No	TC, MA, EA MA, SL (Golgi)	Brown <i>et al.</i> (2009), Wu <i>et al.</i> (2009) Bernal <i>et al.</i> (2007)
	Reducing-end oligosaccharide xylan glycosyltransferase (GlyT)	47	<i>IRX7/FR48</i>	No	TC, MA, EA, SL (Golgi)	Brown <i>et al.</i> (2005, 2007), Zhong <i>et al.</i> (2005), Lee <i>et al.</i> (2007a), Peña <i>et al.</i> (2007), Persson <i>et al.</i> (2007a)
		8	<i>IRX8/GAUT12</i>	No	TC, MA, EA, SL (Golgi)	Brown <i>et al.</i> (2005, 2007), Lee <i>et al.</i> (2007a), Peña <i>et al.</i> (2007), Persson <i>et al.</i> (2007a)
		8	<i>PARVUS/GLZ1/GATL1</i>	No	TC, MA, EA, SL (ER)	Lao <i>et al.</i> (2003), Shao <i>et al.</i> (2004), Brown <i>et al.</i> (2007), Lee <i>et al.</i> (2007b)
		8	<i>GAUT1</i>	Yes	EP, HE, EA	Sterling <i>et al.</i> (2006), Mohnen (2008)
		8	<i>GAUT7</i>	No	EP	Sterling <i>et al.</i> (2006), Mohnen (2008)
Pectin	HG α -(1,4)-galacturonosyltransferase (GalAT)	8	<i>QUASIMODO1/GAUT8</i>	No	MA, TC, SL (Golgi)	Bouton <i>et al.</i> (2002), Leboeuf <i>et al.</i> (2005), Orfila <i>et al.</i> (2005), Dunkley <i>et al.</i> (2006), Mouille <i>et al.</i> (2007)
		47	<i>XGD1</i>	Yes	MA, HE, EA, SL (Golgi)	Jensen <i>et al.</i> (2008)
		47	<i>AR4D1</i>	No	MA	Harholt <i>et al.</i> (2006)
		47	<i>NpGUT1</i>	No	MA	Iwai <i>et al.</i> (2002, 2006)
		77	<i>RGX71, RGXT2</i>	Yes	MA, HE, EA, SL (Golgi)	Egelund <i>et al.</i> (2006)

and are further discussed in the cell wall–PM–cytoskeleton continuum section.

The cellulose synthase-like D (CslD) family has also been linked with cellulose biosynthesis, although the evidence for their involvement is currently inconclusive. The *CslD* genes are the most similar in sequence to the CesAs in the regions surrounding the D, D, D, QxxRW motif and the cysteine-rich domain near the NH₂ terminus shown to be important for CesA isoform interaction (Kurek *et al.* 2002; Atanassov *et al.* 2009). In *Arabidopsis*, *Nicotiana* spp. and rice, CslD expression is associated with tip-growing cells (root hairs, pollen tubes, xylem fibres) and in moss, protonema cells (Doblin *et al.* 2001; Favery *et al.* 2001; Wang *et al.* 2001; Becker *et al.* 2003; Honys and Twell 2003; Bernal *et al.* 2007, 2008; Kim *et al.* 2007; Roberts and Bushoven 2007), providing correlative evidence for this proposal. CslD proteins have been located in the ER (Favery *et al.* 2001; Bernal *et al.* 2007), GA (Dunkley *et al.* 2006; Bernal *et al.* 2008; Zeng and Keegstra 2008) and PM (Natera *et al.* 2008), making deductions of function from these data impossible. Analysis of mutants has not allowed the function of CslDs to be elucidated unambiguously, although it has confirmed that these genes play an important role in tip-growing cells. Apart from the proposed function in cellulose microfibril synthesis, the CslDs have also been suggested to be involved in both the synthesis of non-crystalline cellulose (Manfield *et al.* 2004) and, surprisingly, in xylan synthesis (Bernal *et al.* 2007). Clearly, additional avenues to link the CslDs to a function need to be explored to clarify the exact role of this gene family.

In a recent study of the *CslC* gene family in barley (*Hordeum vulgare* L.), indirect evidence that also included immunocytochemical location (PM) was provided that is consistent with some family members (*HvCslC2*) being involved in the synthesis of cellulose (Dwivany *et al.* 2009). Unlike *HvCslC3* and *HvCslC1*, the expression profiles of *HvCslC2* and *HvCslC4* were not found to be highly correlated with those of putative XylTs or XET/XTHs, enzymes involved in XyG assembly or re-modelling, suggesting that these genes are not involved in XyG synthesis (Dwivany *et al.* 2009) (see 'XyG' section). Corroborative evidence of these divergent functions is that the *HvCslC2* sequence has a PM location and, is in a clade separate to *AtCslC4* (Dwivany *et al.* 2009), the *CslC* protein with (1,4)- β -glucan activity and a Golgi location (Cocuron *et al.* 2007). From a biochemical perspective, the proposed functional diversification within the *CslC* family is far from implausible as XyG backbone synthases and cellulose synthases both make a (1,4)- β -glucan chain.

Heteromannan

Two different types of enzymes involved in the biosynthesis of mannan polysaccharides have been identified to date: a (1,4)- β -(gluco)mannan synthase (GlcManS) that polymerises the polysaccharide backbone, and a (1,6)- α -galactomannan galactosyltransferase (GMGT) that adds Gal residues to the backbone. The fenugreek (*Trigonella foenum-graecum* L.) seed endosperm GMGT is one of only three type II GTs that have been biochemically purified (Edwards *et al.* 1999) enabling the gene (*TfGalT*) to be cloned from NH₂ terminal peptide sequence (Table 3). *TfGalT* is a member of Family GT34 containing both galactomannan (1,6)- α -GalTs and XyG (1,6)-

α -XylTs. Bioinformatic analyses have shown that *Arabidopsis* may contain two putative galactomannan (1,6)- α -GalTs (Faik *et al.* 2002) and at least one in barley (Doblin *et al.* 2009). However, these genes and their encoded enzymes have not yet been functionally characterised, either *in vitro* or *in vivo*.

The first gene isolated that encoded a mannan synthase was *CtManS*, a *CslA* from guar (*Cyamopsis tetragonoloba* (L.)) endosperm (Dhugga *et al.* 2004). Several other *CslA* genes have since been tested for mannan (and other) synthase activities. In *Drosophila* Schneider 2 (S2) cells, recombinant *AtCslA9*, the *Arabidopsis* *CslA* with highest sequence similarity to the guar enzyme, and *AtCslA2* both displayed ManS and glucomannan synthase (GlcManS) activities *in vitro* when assayed with GDP-Man, or both GDP-Man and GDP-Glc, respectively (Liepman *et al.* 2005). These observations indicate that multiple members of the *Arabidopsis* *CslA* gene family encode β -(gluco)mannan synthases and that a single *CslA* gene is capable of all three ManS, GlcS, and GlcManS activities (Liepman *et al.* 2005). Heterologous expression of *CslA* family members from diverse plant species has yielded proteins with ManS and/or GlcManS activity, at least *in vitro* (Suzuki *et al.* 2006; Liepman *et al.* 2007). As yet, none of the rice, maize and barley *CslA* genes in the cereal-specific clade of the *CslA* family have been shown experimentally to encode ManS (van Erp and Walton 2009).

Xyloglucan (XyG)

Genes encoding the (1,4)- β -glucan synthases involved in synthesising the XyG backbone as well as the (1,6)- α -Xylp-, (1,2)- β -Galp- and (1,2)- α -Fucp transferases that participate in XyG sidechain addition have been identified. These enzymes have been classified into GT families 2, 34, 47 and 37, respectively (Table 3). There are multiple members within each family with only some biochemically characterised with respect to substrate specificity (Fig. 5).

The GT Family 2 gene *AtCslC4* is proposed to encode a XyG glucan backbone synthase. (Cocuron *et al.* 2007) used transcription profiling of developing nasturtium (*Tropaeolum majus* (L.)) seeds in which XyG is the primary seed storage polysaccharide to identify *TmCslC*, a gene with highest sequence similarity to *Arabidopsis* *AtCslC4*. When either *TmCslC* or *AtCslC4* was expressed in *Pichia* cells, short chains of (1,4)- β -glucan accumulated and, upon co-expression of *AtCslC4* with *AtXXT1* (a XylT), a longer (1,4)- β -glucan was synthesised (Cocuron *et al.* 2007). Strong correlative evidence of *AtXXT1* involvement in XyG biosynthesis, as well as the fact that *AtCslC4* and *AtXXT1* are coordinately expressed and co-localise to the GA (Dunkley *et al.* 2006; Cocuron *et al.* 2007; Zabolina *et al.* 2008), provide support that both *AtCslC4* (and *TmCslC*) are involved in XyG backbone synthesis and appear to interact either directly or indirectly with their respective XylTs. Whether all members of the *CslC* clade in dicots/gymnosperms participate in XyG synthesis needs to be confirmed, particularly in light of the recent study by Dwivany *et al.* (2009) proposing a role for *HvCslC2* in cellulose synthesis (see 'Cellulose' section).

The addition of the side chain residues (monosaccharides and oligosaccharides) to the (1,4)- β -D-glucan backbone to form XyG by the GlyTs (XylTs/GalTs/FucTs) has been intensively studied using a combination of heterologous expression of the proteins

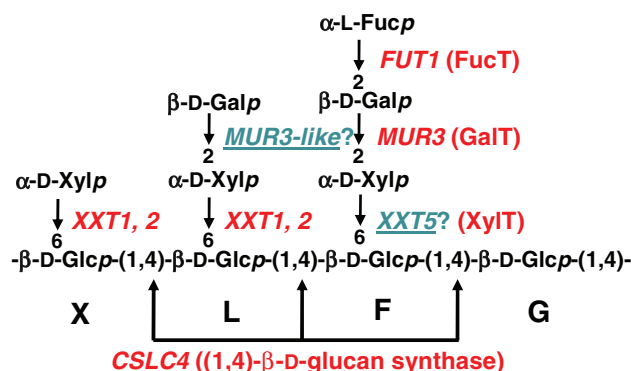


Fig. 5. The polysaccharide synthases and GlyTs involved in xyloglucan biosynthesis in *Arabidopsis*. XyG structures are typically described using a single letter nomenclature to represent the substitution pattern of each backbone Glcp residue (Fry *et al.* 1993). The letters G and X denote an unbranched Glcp residue and the $\alpha\text{-D-Xylp-(1,6)-}\beta\text{-D-Glcp}$ motif, respectively. In *Arabidopsis*, the Xylp residues may be substituted with a $\beta\text{-D-Galp}$ (L sidechain) and the Gal residue of sidechain L is often substituted at O-2 with an $\alpha\text{-L-Fucp}$ residue to form the F sidechain (Peña *et al.* 2007). In XyG extracted from wild-type Col-0 leaves, XXXG (45%) is the major oligosaccharide detected after digestion with a *Trichoderma viride* endo- $\beta\text{-D-glucanase}$ that specifically cleaves the XyG backbone at the unsubstituted Glc residues. Other digestion products include XXFG (24%), XLFG (16%), XXLG (8%), XLXG (3%) and XLLG (4%) (Madson *et al.* 2003). The genes (enzymes in parentheses) known to be involved in the assembly of the XyG backbone and side chains are indicated in italics, those surmised to be involved are underlined.

and biochemical characterisation of their substrate specificity together with mutant analysis. A detailed description is beyond the scope of this review but the salient features and relevant references are summarised in Table 3 and Fig. 5. These elegant studies demonstrate the exquisite substrate specificity of GTs as well as their plasticity under abnormal conditions and reinforce the need to perform biochemical studies on each of the members of multigene families.

(1,3;1,4)- $\beta\text{-D-glucan}$

Like the cellulose synthases, attempts to isolate the components of the (1,3;1,4)- $\beta\text{-D-glucan}$ synthase by biochemical means have been ineffective. More recently, a comparative genomics approach has, however, been successfully applied to uncover putative polysaccharide synthase genes. The identification of a quantitative trait locus contributing to (1,3;1,4)- $\beta\text{-D-glucan}$ content in ungerminated barley grain (Han *et al.* 1995) allowed the syntenic region in the rice genome to be located, leading to the discovery of a cluster of six rice *OsCslF* genes (Burton *et al.* 2006). Given the earlier finding that the *CslF* family is one of three cereal-specific *Csl* families (the others are the *CslHs* and *CslJs*; Hazen *et al.* 2002; Fincher 2009b) and the fact that (1,3;1,4)- $\beta\text{-D-glucan}$ is a polysaccharide unique to the walls of cereals, the *CslF* genes became prime candidates for encoding (1,3;1,4)- $\beta\text{-D-glucan}$ synthases (Burton *et al.* 2006). This possibility was tested by 'gain-of-function' experiments in transgenic *Arabidopsis* plants, a species that contains no (1,3;1,4)- $\beta\text{-D-glucan}$ in its walls nor has any *CslF* genes. Heterologous expression of rice *OsCslF2*, 4 or 9 in

Arabidopsis resulted in the appearance of immunologically detectable levels of (1,3;1,4)- $\beta\text{-D-glucan}$ in leaf epidermal walls, and therefore the *CslF* family probably encodes (1,3;1,4)- $\beta\text{-D-glucan}$ synthases (Burton *et al.* 2006). This conclusion is consistent with the temporal and spatial expression patterns of the seven *CslF* genes in barley (Burton *et al.* 2008).

Similar experiments in which barley *CslH1* was expressed in transgenic *Arabidopsis* plants and the plants analysed immunocytochemically and biochemically to confirm polysaccharide structures have shown that the *CslH* proteins are also capable of generating (1,3;1,4)- $\beta\text{-D-glucan}$ in leaf walls (Doblin *et al.* 2009), suggesting that there are two types of (1,3;1,4)- $\beta\text{-D-glucan}$ synthases in cereals. The transcript level of the single barley *CslH* gene is very low and is restricted to cells that have either mature primary walls (e.g. starchy endosperm) or secondary wall thickenings (e.g. leaf interfascicular sclerenchymal fibre and xylem cells) and hence, it has been suggested that its main role is in (1,3;1,4)- $\beta\text{-D-glucan}$ synthesis during secondary wall synthesis (Doblin *et al.* 2009). That two *Csl* families appear to be involved in (1,3;1,4)- $\beta\text{-D-glucan}$ synthesis is curious. Why have two similar protein families, not just one, evolved to provide a (1,3;1,4)- $\beta\text{-D-glucan}$ synthesis function within the restricted Poalean plant lineage? The answer may lie in the different functional roles of (1,3;1,4)- $\beta\text{-D-glucan}$ in primary v. secondary walls, as a mobilisable, storage or structural polysaccharide, respectively (Burton and Fincher 2009). This hypothesis and others remain to be tested.

The *CslF* and *CslH* gain-of-function experiments in *Arabidopsis* suggest that both genes encode synthases with the same biochemical activity and produce a type of (1,3;1,4)- $\beta\text{-D-glucan}$ that is similar to barley (Doblin *et al.* 2009). Whilst the simplest explanation is that both *CslF* and *CslH* proteins synthesise both the (1,3)- β - and (1,4)- β -linkages, the cumulative evidence is most consistent with the interpretation that both types of *Csl* protein produce only the (1,4)- β -linkage. First, all other *Csl* proteins characterised to date make only (1,4)- β -linkages (see previous sections). Second, the levels of (1,3;1,4)- $\beta\text{-D-glucan}$ deposited in *Arabidopsis* leaf walls were very low (<0.02% w/w) in comparison to barley (0.4% w/w). The restricted pattern of deposition in only certain leaf cell walls despite transgene expression being driven by the constitutive CaMV35S promoter implies that additional protein/s (or other factors) are likely to be involved in (1,3;1,4)- $\beta\text{-D-glucan}$ synthesis (Burton *et al.* 2006; Doblin *et al.* 2009). A second GlyT to make the (1,3)- β -linkage would explain some of the idiosyncrasies of (1,3;1,4)- $\beta\text{-D-glucan}$ structure such as the random arrangement of the cellotriosyl and cellotetraosyl units within (1,3;1,4)- $\beta\text{-D-glucan}$ chains (Staudte *et al.* 1983) and the observation that in cells actively making (1,3;1,4)- $\beta\text{-D-glucan}$, this polysaccharide, unlike AX and heteromannans, is never observed by immunoEM within the GA (Wilson *et al.* 2006), the proposed location of (1,3;1,4)- $\beta\text{-D-glucan}$ synthesis (Henry *et al.* 1983; Gibeau and Carpita 1993). Given the Golgi location of the *HvCslH1* protein, a novel mechanism has been proposed for (1,3;1,4)- $\beta\text{-D-glucan}$ assembly in which cellodextrins are synthesised by *CslF* or *CslH* proteins in the Golgi and joined together by (1,3)- β -linkages by another GlcT at the PM (Burton and Fincher 2009; Doblin *et al.* 2009; Fincher 2009a, 2009b). The *Gsl* proteins, the

XyG endotransglycosylases (XETs) or another as yet unidentified GlcT might be able to catalyse such a reaction (Doblin *et al.* 2009; Fincher 2009b). Co-expression analyses have begun to be used to identify other genes that might be involved in the (1,3;1,4)- β -D-glucan synthesis and assembly process (Schreiber *et al.* 2008; Burton and Fincher 2009). The next step will be to test the function of these candidate genes through various means, including heterologous expression in combination with the CslF and/or H proteins.

Pectin

Homogalacturonan (HG)

Based on structure, a total of ~67 GlyTs, methyltransferases and acetyltransferases are predicted to be required for pectin synthesis (Ridley *et al.* 2001). Assays for some of these activities have been described (Ridley *et al.* 2001; Mohnen 2008) but to date, there is conclusive experimental evidence supporting a role in pectin biosynthesis for only a few genes (Scheller *et al.* 2007; Mohnen 2008). Only one pectin biosynthetic enzyme has been biochemically isolated. The type II HG (1,4)- α -GalpAT, termed GAUT1, has been partially purified from solubilised membrane extracts of *Arabidopsis* suspension-cultured cells (Sterling *et al.* 2006). GAUT1 antibodies immuno-adsorbed HG (1,4)- α -GalpAT activity from these preparations and when expressed in mammalian HEK293 cells, GAUT1 transfers GalA from UDP- α -D-GalpA onto the non-reducing end of HG polysaccharide and oligosaccharide acceptors with a preference for DP > 9 (Sterling *et al.* 2006). GAUT1 was one of two genes, together with GAUT7, identified by peptide sequencing of the partially purified HG (1,4)- α -GalpAT. Both genes belong to a family of 25 genes in *Arabidopsis* that consist of 15 GAUT and 10 GAUT1-like (GATL) genes clustered in CAZy Family GT8 (Lao *et al.* 2003; Sterling *et al.* 2006). To date, mutants in three GAUT1-related family genes have been studied and the observed phenotypes suggest that the encoded proteins, in addition to GAUT1, are involved in the synthesis of wall polysaccharides and may have GalAT activity. The *Arabidopsis* QUASIMODO1 (QUA1), IRX8 and PARVUS/GLZ1 proteins are encoded by the GAUT8, GAUT12 and GATL1 genes, respectively. Interestingly, the mutant phenotypes of all three genes include effects on both HG and xylan content and/or synthase activity, thus preventing their definitive functional identification (Shao *et al.* 2004; Orfila *et al.* 2005; Brown *et al.* 2007; Lee *et al.* 2007b; Peña *et al.* 2007; Persson *et al.* 2007a). While pectins and heteroxylans are generally thought of as separate polymers, structural analyses have suggested that they may be covalently linked (Nakamura *et al.* 2002). Hence, it has been proposed that a lack of an enzymatic activity may affect both polymers through the absence of a specific linkage (Persson *et al.* 2007a; Mohnen 2008). Other explanations that have been proposed to account for the dual polysaccharide effects in the mutants are that HG and xylan synthesis are associated through either a common biosynthetic complex/es or regulatory mechanism, altered polysaccharide deposition or impaired scaffolding between the xylan polymer and specific pectic polymers in the secondary wall (Persson *et al.* 2007a; Mohnen 2008).

A XylT that adds Xylp residues onto the HGA backbone has recently been identified by mutant analysis of the *Arabidopsis*

CAZy GT47 members (Jensen *et al.* 2008). In the *xgd1-1* mutant, less terminal Xylp residues were found in the pectic fraction compared with wild type, indicating a reduction in xylose-substituted HG (XGA). Transformation of the *xgd1-1* mutant with the wild-type gene complemented the XGA-deficient phenotype and when XGD1 was transiently expressed via *Agrobacterium* in leaves of *Nicotiana benthamiana* (Domin.), the protein catalysed the transfer of Xylp from UDP-Xylp onto oligogalacturonides and endogenous acceptors (Jensen *et al.* 2008). The reaction products were hydrolysable with an XGA-specific hydrolase, confirming that XGD1 is an XGA XylT. Additional biochemical analysis of the XGD1 protein is, however, necessary to determine the exact linkage formed by the enzyme (Jensen *et al.* 2008).

Rhamnogalacturonan I (RG I)

The ARAD1 protein, also a CAZy Family GT47 member (Fig. 6), appears to be involved in the biosynthesis of arabinan side-chains of RG I. Walls of ARAD1 mutants contain significant decreases in leaf and stem Ara (75% and 46%, respectively, of wild-type levels) (Harholt *et al.* 2006). Immunohistochemical and linkage analysis indicated a specific decrease in the level but not structure of arabinan side chains of RGI with no change in other pectic domains or wall glycoproteins, indicating that it is likely that the decreased arabinan content is a direct result of the mutation. Transformation of mutant plants with ARAD1 driven by the 35S promoter led to full restoration of the Ara-deficient phenotype, suggesting that ARAD1 is an arabinan (1,5)- α -arabinosyltransferase. A demonstration of this activity by the ARAD1 protein is required as proof of its function, however.

Rhamnogalacturonan (RG II)

Two homologous plant-specific *Arabidopsis* genes, RGXT1 and RGXT2, encode novel type II, Golgi-localised XylTs and are likely to participate in the formation of the A chain in RG II (Egelund *et al.* 2006). These sequences, originally identified in a bioinformatic search aimed at discovering unclassified cell wall GlyTs, seeded the new CAZy GT family 77, of which there are 18 and 15 members in *Arabidopsis* and rice, respectively. Truncated, soluble forms of the corresponding proteins expressed in insect cells displayed XylT activity, transferring D-Xyl from UDP- α -D-Xyl to L-Fuc in a (1,3)- α -linkage, demonstrating the proteins to be (1,3)- α -D-XylTs. As this particular linkage is only known to exist in RG II, it was surmised that RGXT1 and RGXT2 transfer Xylp to the Fucp residue in side chain A of RG II (Egelund *et al.* 2006). In phylogenetic analyses, two other sequences form a cluster with RGXT1 and 2, sharing 68–75% identity with these proteins, hence, they too may be Fuc-specific XylTs. However, the function of the remaining *Arabidopsis* sequences cannot be inferred with any accuracy as they only share low (<19%) identity with the RGXTs.

Another gene that most likely participates in the synthesis of RG II is GUT1 from *Nicotiana plumbaginifolia* (Viv.), yet another member of CAZy Family GT47 (Fig. 6). A T-DNA insertion mutant of NpGUT1, *nolac-H18* (non-organogenic callus with loosely attached cells), has only 14% GlcpA content of normal callus cell walls (Iwai *et al.* 2002). The presence of undetectable levels of GlcpA and a 50% decrease

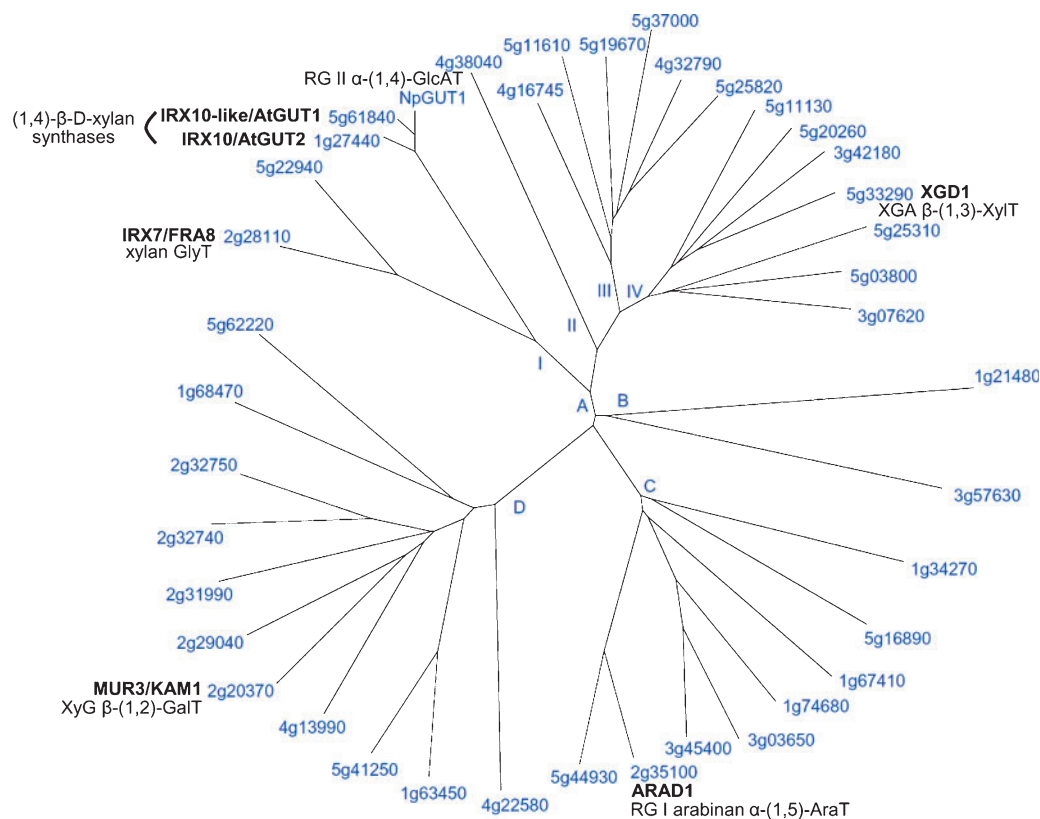


Fig. 6. Phylogenetic tree of the *Arabidopsis* CAZy GT47 family. An unrooted distance phylogenetic tree generated from a CLUSTALW alignment of the amino acid sequences in the putative conserved catalytic domain pfam03016 of the 39 *Arabidopsis* GT47 proteins. The *Arabidopsis* GlyTs are tentatively divided into four major groups A–D, and group A is further separated into four subgroups I–IV. Tobacco NpGUT1 (group AI) is included in the tree to show its relationship to the *Arabidopsis* orthologues. Reprinted from Trends in Plant Science, Vol 8, R Zhong and Z-H Ye, Unraveling the functions of glycosyltransferase family 47 in plants, 565–568, 2003, with permission from Elsevier.

in Gal in RG II isolated from the mutant together with an ~82% reduction in RG II dimer formation *in vitro* suggest that the NpGUT1 protein is an RG II (1,4)- β -GlcAT that transfers GlcA onto the L-Fucp in RG-II side chain A (Iwai *et al.* 2002), the same Fuc acceptor substrate for RGXT1 and 2. This proposed function for NpGUT1 has not yet been substantiated by an *in vitro* enzyme assay. This is particularly important in this case, as the two *Arabidopsis* proteins with highest sequence similarity to NpGUT1, IRX10 and IRX-LIKE with 86% and 92% amino acid identity, respectively, have recently been implicated in xylan chain elongation (Brown *et al.* 2009; Wu *et al.* 2009). If the biochemical activities corroborate these conclusions, then a close sequence comparison of these proteins may yield information regarding what sequence motifs direct donor and/or acceptor specificity and potentially the control of single v. multiple sugar additions.

Heteroxylan

GX biosynthesis is likely to involve multiple GTs responsible for the formation of the backbone, the reducing-end sequence (see ‘Cell wall structure’ section), at least for dicots and gymnosperms, as well as enzymes that add side chains and modify them. No single gene has been unambiguously

described as encoding a xylan synthase, although there is mounting evidence implicating several GlyTs in having such a function, as well as others that appear to be involved in the synthesis of the reducing-end primer sequence. To date in *Arabidopsis*, four genes (*IRX9* and *IRX14*, *IRX10* and *IRX10*-like) and three genes (*IRX7/FRA8*, *IRX8/GAUT12*, *PARVUS/GLZ1/GATLI*) have been hypothesised to be involved in xylan backbone synthesis and synthesis of the reducing-end sequence, respectively (Table 3).

The *IRX* and *PARVUS* genes and their homologues in poplar and rice were all initially identified by correlative expression analyses using EST or microarray data aimed at identifying genes that function in secondary wall polysaccharide synthesis, including xylan (Aspeborg *et al.* 2005; Brown *et al.* 2005; Persson *et al.* 2005; Geisler-Lee *et al.* 2006; Mitchell *et al.* 2007). Biochemical analysis of the corresponding mutants has shown that all have a secondary wall phenotype and exhibit a deficiency in xylan, with two classes of enzymes involved in xylan biosynthesis being identified. NMR and MALDI–TOF MS analysis of xylan extracted from *irx7/fra8*, *irx8* and *parvus* has shown an absence of the reducing-end oligosaccharide sequence, yet xylan synthase activity in these mutants is similar to wild-type (Brown *et al.* 2007; Lee *et al.* 2007a, 2007b; Peña *et al.* 2007). This suggests an important role for the reducing-end sequence in

xylan biosynthesis, and has led to the prediction that IRX7/FRA8, IRX8 and PARVUS catalyse the linkages found within this reducing-end oligosaccharide (Brown *et al.* 2007; Lee *et al.* 2007b; Peña *et al.* 2007). In contrast, the reducing-end sequence is present in *irx9*, *irx14* and the *irx10 irx10-like* double mutant (a mild or no phenotype is observed in the single mutants, respectively, suggesting considerable functional redundancy between these genes) but xylan synthase activity (measured by the ability to transfer [¹⁴C]Xyl from UDP-[¹⁴C]Xyl onto (1,4)- β -xylooligosaccharides) is greatly reduced (Brown *et al.* 2007; Lee *et al.* 2007b; Peña *et al.* 2007). A decreased xylan chain length has also been observed in *irx9* (Peña *et al.* 2007) and the *irx10 irx10-like* double mutant (Brown *et al.* 2009). These data imply that IRX9, IRX14, IRX10 and IRX10-LIKE are required for xylan chain elongation.

IRX9 and IRX14 belong to CAZy Family GT43 and IRX10 and IRX10-LIKE are yet further members of GT47 (Fig. 6). The findings that expression of *IRX10* and to a lesser extent *IRX10-LIKE* is predominantly in cells undergoing secondary wall thickening, that there is no loss of cell adhesion and the majority of RG II is dimerised in the *irx10 irx10-like* double mutant and there appears to be no difference compared with wild-type in either the A or B sidechain of RG II argues against these genes having a function similar to NpGUT1 (Brown *et al.* 2009; Wu *et al.* 2009). However, the recent complementation of the *irx10 irx10-like* mutant with a chimeric IRX10-NpGUT1 protein in which the NH₂-terminal 71 amino acids of IRX10 were fused to the NH₂-terminus of NpGUT1 (Wu *et al.* 2009) calls into question the presumed role of NpGUT1 in RG II biosynthesis. Further work is required to resolve this discrepancy. Whether the individual IRX9/14/10/10-LIKE proteins have the same mechanism of action and whether they are associated in a biosynthetic complex in a similar way to the CesA proteins is unclear (Peña *et al.* 2007; Brown *et al.* 2009). IRX7/FRA8 is also member of the GT47 family (Fig. 6); hence has been proposed to catalyse either the formation of one of several β -linkages of Xylp, for example, Xylp to Rha using UDP- α -Xylp, or the addition of α -linked Rhap to GalpA using UDP- β -Rhap (Peña *et al.* 2007).

Since IRX8 and PARVUS are both members of the GAUT1-related family they have both been hypothesised to catalyse the addition of the α -D-GalA residue to the O-4 of the reducing Xyl residue of the reducing end oligosaccharide, possibly acting redundantly (Peña *et al.* 2007). However, YFP-tagged PARVUS protein is found to predominantly localise to the ER (Lee *et al.* 2007b), a subcellular location distinct from FRA8, IRX8 and IRX9, which have been shown to be Golgi-located (Zhong *et al.* 2005; Peña *et al.* 2007). This difference is consistent with transmembrane predictions indicating that whereas IRX7/FRA8, IRX8 and IRX9 are type II membrane proteins, PARVUS contains a hydrophobic signal peptide sequence but no transmembrane helices (Lee *et al.* 2007b). The ER localisation of PARVUS suggests that it catalyses an earlier step that is different from IRX8 in GX biosynthesis (Lee *et al.* 2007b). It has been hypothesised that the reducing-end sequence might function as either a primer or as a chain terminator for GX biosynthesis (Peña *et al.* 2007; York and O'Neill 2008). The finding that PARVUS is located in the ER and is required for the synthesis of the oligosaccharide sequence at the reducing end of GX favours

the reducing end sequence having a primer function (Lee *et al.* 2007b).

Despite the plausible roles for IRX7/8/9/10/10-LIKE/14 and PARVUS in GAX biosynthesis, experimental proof of their precise enzymatic function is lacking. Attempts to demonstrate the function(s) of FRA8 and IRX9 by heterologous expression in *Pichia* cells have been unsuccessful as no activity was detected (Zhong *et al.* 2005; Peña *et al.* 2007). Hence, further work is required to demonstrate their biochemical activity.

Nucleotide sugar interconversion

Apart from cellulose and callose, the synthesis of the non-cellulosic polysaccharides and pectins occurs within the Golgi apparatus. The active site of the participating polysaccharide synthases (Golgi lumen or cytosol) and GlyTs (Golgi lumen only) may face either the cytosol or the Golgi lumen depending on their membrane topology (Doblin *et al.* 2003). In the latter case, the enzymes are separated from their substrates and cofactors in the cytosol and hence their availability to the synthesis machinery relies on transporters resident in Golgi cisternal membranes as well as the appropriate nucleotide sugar interconversion enzymes (NSIEs). Substantial progress has been made in identifying and characterising the genes and enzymes utilised in this process. These advancements have recently been reviewed (Seifert 2004; Reiter 2008; Reyes and Orellana 2008) and will not be discussed in detail here. Analyses of various mutants with defects in NSIEs have confirmed the essential role for these proteins in wall biosynthesis. It has been hypothesised that transcriptional and post-translational control of NSIEs regulates wall biosynthesis in response to developmental, metabolic and other environmental stimuli (Seifert 2004). Apart from characterising additional NSIEs and transporters, future work will focus upon elucidating the role of NSIEs in regulating wall synthesis, addressing issues such as feedback inhibition and redox sensing, the significance of differing kinetic properties and subcellular location between isoforms and their ability to form multimeric complexes (Seifert 2004; Reiter 2008). Another area of research will be to further understand the role of NSIEs in growth, development and defence responses. Mutants of *Arabidopsis* *UXE1/MUR4/HSR8*, for example, have reduced L-Ara levels due to impaired enzymatic function but also display altered sugar response phenotypes affecting cell division and expansion (Burget and Reiter 1999; Burget *et al.* 2003; Li *et al.* 2007). Such studies will aid in determining the signalling mechanisms linking wall changes to cellular responses (see the wall-PM-cytoskeleton continuum section below).

The cell wall-plasma membrane-cytoskeleton continuum

The cell wall-PM-cytoskeleton continuum is vital for the perception of signals from the external environment and for coordinated cell growth and expansion during plant development. To date, several (glyco)proteins that have been implicated in regulating/sensing the external environment of the cell (Humphrey *et al.* 2007). These include the AGPs (Du *et al.* 1996; Nothnagel 1997; Schultz *et al.* 1998; Majewska-Sawka and Nothnagel 2000; Seifert and Roberts 2007), cellulose synthases (Somerville 2006; Hématy *et al.* 2007; Hématy and Höfte 2008) and more recently PM receptor kinases including the

WAKs (Kohorn 2000; Riese *et al.* 2003), the Ser/Thr CrRLK1 L (*Catharanthus roseus* receptor-like-kinase-1-like) subfamily (Hématy *et al.* 2007) and a clade of the leucine-rich repeat (LRR) RLK family (Xu *et al.* 2008). These are summarised schematically in Fig. 7 and briefly discussed below.

AGPs are situated at the PM wall interface and have long been thought to play a central role as a mediator of these processes. Mounting evidence indicates that AGPs have a specific function during root formation, the promotion of somatic embryogenesis, pollen tube guidance, alternation between sporophytic and gametophytic transitions in the ovules, resistance to root-dependent transformation by *Agrobacterium*, hormone signalling and xylem differentiation/vascular function in *Zinnia* and *Arabidopsis* (Seifert and Roberts 2007). Physical

connections between the wall and the PM were originally inferred from microscopic observations of the presence of AGPs as a fuzzy 'glycocalyx' occupying the PM-wall interface (Roberts 1990) and then as discrete connections (Hechtian strands), particularly in plasmolysed cells upon salt stress (Carpita and Gibeaut 1993; Gens *et al.* 2000; Lampion *et al.* 2006; Pickard 2008). Since AGPs were known to be highly water soluble proteoglycans it was unclear how they might fulfill this role. The discovery that most AGPs have a GPI membrane anchor provided the evidence for their mechanism of association with the PM (Youl *et al.* 1998; Oxley and Bacic 1999). Since GPI anchors do not traverse the entire PM, GPI anchored proteins have been implicated in mediating cell-cell interactions in mammalian systems by interacting with other proteins (Du *et al.* 1996; Plun-

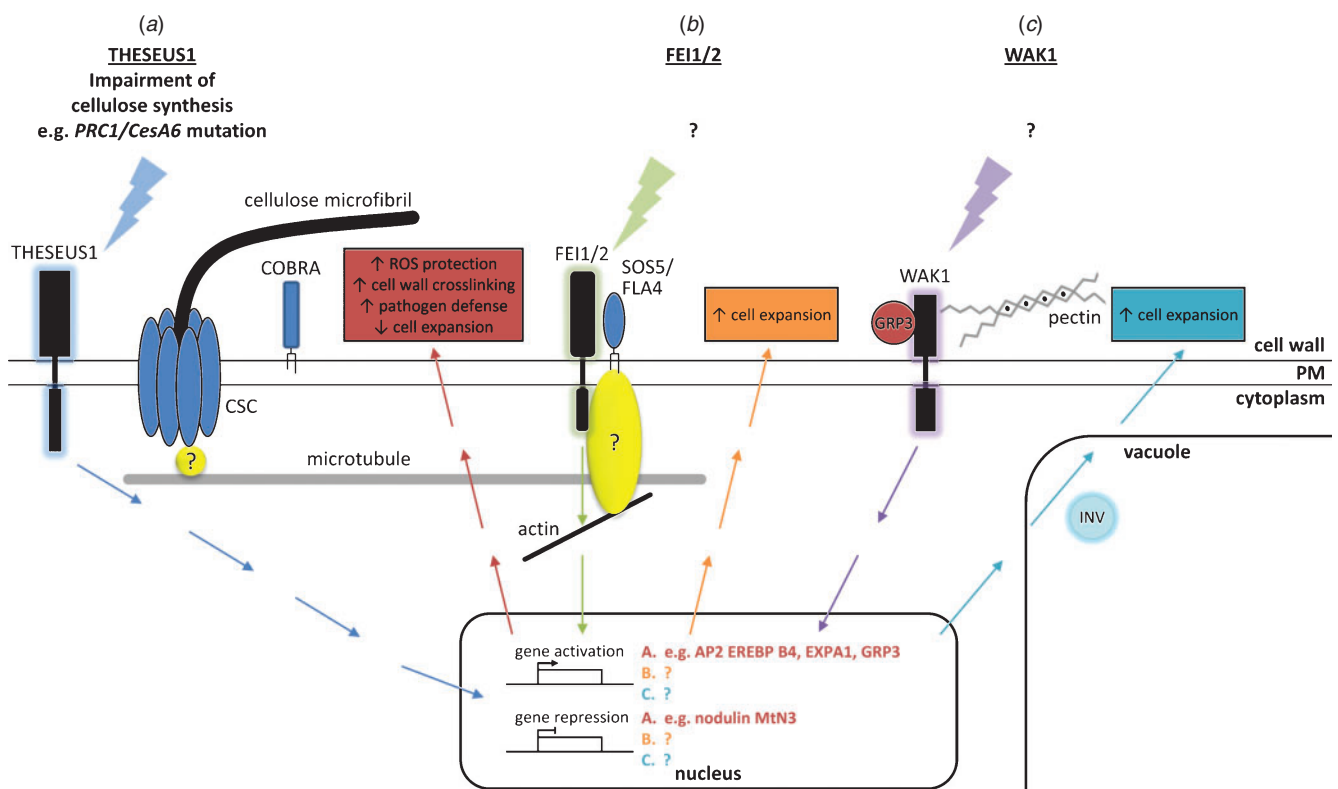


Fig. 7. A schematic representation of the relationship between polysaccharide synthases, PM-bound proteins and RLKs within the cell wall-PM-cytoskeleton continuum. (a) Impairment of cellulose synthesis, for example, via genetic mutation of *CesA6* (Hématy *et al.* 2007), causes an inhibition of cell elongation. THESEUS1, a PM-bound RLK, is activated in this inhibition response and potentially transduces the signal into the cytoplasm, eventually leading to transcriptional changes within the nucleus. Microarray analysis has revealed several genes differentially regulated by THE1 (Hématy *et al.* 2007). Among the genes that are positively regulated are two putative transcription factors *AP2-EREBP B4* and *WRKY45*, genes encoding proteins involved in protection against reactive oxygen species (ROS), pathogen defence proteins and cell wall-related proteins such as expansin (*AtEXPA1*) and glycine-rich proteins *GRP3* and *GRP3S* that have been shown to interact with *WAK1* (Park *et al.* 2001). Hence, THE1 and WAK signalling pathways (C) are connected through these GRPs. A molecule that links the CSC (or other wall protein/s) to the cytoskeleton to control cellulose microfibril orientation has not yet been identified (yellow circle). (b) LRR-RLKs FEI1 and 2 also regulate cell expansion but act in a separate pathway from *CesA6* or *COBRA*, with the GPI-anchored *SOS5/FLA4* AGP potentially acting as a ligand for the FEIs (Xu *et al.* 2008). AGPs are thought to play a role in allowing cell elongation to occur and maintaining cell wall integrity (Humphrey *et al.* 2007; Seifert and Roberts 2007). Interaction of *SOS5/FLA4* with the cytoskeleton has been proposed to be mediated by either a direct interaction with transmembrane protein(s) (e.g. WAKs, CesAs, RLKs, etc.) or an indirect interaction involving lipid rafts (Sardar *et al.* 2006) (yellow oval). (c) PM-localised WAKs have also been implicated in cell expansion (Lally *et al.* 2001; Wagner and Kohorn 2001). The signal/s that induce WAKs have not yet been identified, nor their cellular response. WAKs may sense wall expansion by their attachment to pectin and signal via influencing the activity of vacuolar invertase, thus controlling turgor during cell expansion (Kohorn *et al.* 2006). There is some evidence to suggest *AtGRP3* could be a ligand for the *WAK1* receptor (Park *et al.* 2001). There may also be an association between AGPs and WAKs, based on the co-localisation of AGP-epitopes and *WAK1* in immunofluorescence studies (Gens *et al.* 2000). Signal inputs, arrows pointing towards the nucleus; signal outputs, arrows pointing towards wall.

Favreau *et al.* 2001; Johnson *et al.* 2003). A sub-class of AGPs, the fasciclin-like AGPs (FLAs) are likely candidates as they have, in addition to predicted AGP-like glycosylated regions in their protein backbones, putative cell adhesion domains known as fasciclin domains (Johnson *et al.* 2003; Schultz *et al.* 2004). In other eukaryotes (Elkins *et al.* 1990; Kawamoto *et al.* 1998) and *Volvox* (Huber and Sumper 1994), fasciclin domain-containing proteins are involved in cell adhesion. Interestingly, SOS5/FLA4, a GPI-anchored fasciclin-type AGP, has been proposed as a ligand of the LRR-RLK family members FEI1 and FEI2 (Xu *et al.* 2008). *fei1 fei2* double mutants have defects in cell expansion primarily in roots, are cellulose-deficient and hypersensitive to isoxaben in the presence of sucrose or salt (Xu *et al.* 2008). Genetic analyses indicate that FEI1 and FEI2 act within the same pathway as SOS5/FLA4 but in a pathway independent of COBRA or PROCUSTE1/CESA6 to regulate wall expansion (Fig. 7). Classical AGPs that are GPI-anchored have been implicated in controlling cell shape via a connection, as yet undefined, with the cytoskeleton, due to their involvement in orienting cortical microtubules and influencing F-actin organisation (Andème-Onzighi *et al.* 2002; Sardar *et al.* 2006).

Wall associated kinases (WAKs) are a family of transmembrane proteins that, unlike AGPs, possess the necessary structure to provide a physical link between the wall and the PM and to be directly involved in subcellular signalling (Kohorn 2000; Riese *et al.* 2003; Humphrey *et al.* 2007). They are transmembrane proteins that have a conserved cytoplasmic Ser/Thr protein kinase domain as well as an extracellular wall domain that differs between family members (Fig. 7). An *Arabidopsis* GRP, AtGRP-3 is predicted to be a wall protein that interacts with WAKs, specifically binding to WAK1 both *in vitro* and *in vivo* (Park *et al.* 2001). Both AtGRP-3 and WAKs have conserved Cys residues, and it is possible these are responsible for the interaction between AtGRP-3, WAK1 and other proteins (Park *et al.* 2001). WAKs are also bound to the wall in part by a covalent association with pectin (Anderson *et al.* 2001). The ability of WAKs to bind both GRPs and pectins in their phosphorylated form is proposed to relate to their role in regulating cell expansion (Fig. 7).

The CrRLK1L family includes THESEUS1 (THE1), which appears to be involved in sensing the integrity of the wall through the perturbation of the CSC (Hématy *et al.* 2007; Hématy and Höfte 2008), FERONIA (FER) required for growth cessation of compatible pollen tubes (Escobar-Restrepo *et al.* 2007) and AmRLK involved in the control of the polar conical outgrowth of epidermal cells in *Antirrhinum* petals (Hématy and Höfte 2008). The potential mechanism/s by which these novel Ser/Thr receptor kinases are involved in growth regulation was recently summarised by Hématy and Höfte (2008) and will not be discussed further here as no ligands for these receptors are yet identified.

Evidence accumulated over many decades, including the observations of co-alignment of cortical microtubules and cellulose microfibrils and a loss of directed cell expansion and CSC mobility upon microtubule disruption (Gardiner *et al.* 2003; Somerville *et al.* 2004; Paredez *et al.* 2006; DeBolt *et al.* 2007; Paredez *et al.* 2008) confirm a link between the cortical cytoskeleton and the wall through the cellulose synthase rosette complexes in the PM and the central role this plays in cell growth and expansion. The link between the cytoskeleton and cellulose synthesis is further demonstrated by the fact that *CesA* mutants

have disrupted cytoskeletal organisation and cytoskeleton mutants having disrupted cellulose deposition (Burk and Ye 2002; Chu *et al.* 2007; Paredez *et al.* 2008). However, this relationship is not completely mutually dependent. For example, cellulose microfibril deposition is normal in roots of the temperature-sensitive *microtubule organisation1* (*mor1-1*) mutant that causes cortical microtubule disassembly (Sugimoto *et al.* 2003; Wasteneys 2004), supporting the notion that cellulose microfibrils can self-align in the absence of normal microtubule organisation. By analogy, it is likely that another polysaccharide synthase also located at the PM, callose synthase, could have a similar role as it assembles callose in a similar directed manner and is rapidly activated upon physical or biotic stresses although a link between the cytoskeleton and the synthase complex is yet to be demonstrated.

Future directions

The focus of this review has been on plant wall biosynthesis and, in particular, the synthesis of wall polysaccharides as well as the emerging realisation of the importance of the apoplast, plasma membrane, cytoskeleton in sensing/perceiving the external environment leading to cytoplasmic signalling cascades that regulate plant growth processes. Polysaccharide synthesis and wall assembly occurs through a complex and intricate series of steps that often begin in an intracellular compartment and end in the wall itself, after the polysaccharides have been deposited. The great structural diversity of wall polysaccharides is due to the large number of possible constituent sugars and non-sugar components, the variety of ways these can be linked together, and the many ways in which linear polymers can be branched or modified. Regulation of these steps is central to cell development, because the polysaccharide composition of the wall changes during cell division, elongation and differentiation. At a larger scale, for the plant to assemble its mature organs and tissues in an orderly manner, the stages of wall synthesis in the range of different cell types must be integrated through time and space and must also respond to internal and external developmental cues; how this coordinate regulation of wall synthesis is achieved is unknown and remains a major future research question in plant biology.

The wall is now recognised to be a metabolically active 'organelle' that also plays a critical role in sensing and responding to external signals during growth and development as well as in response to abiotic/biotic stresses. Identifying the (glyco)proteins located at the PM involved in the interaction between the wall, PM and cytoskeleton and understanding their mechanism(s) of signal transduction will be important in devising strategies to optimise growth for plant production.

In the post-genomic era, the greatest challenge is to demonstrate the enzymatic activity/specificity of each GT gene product implicated in wall biosynthesis. GT mutants have proven useful in some instances, providing a phenotype from which a function could be deduced, however, in many cases, an association with a specific biochemical activity has not been forthcoming. This means that additional approaches and tools – bioinformatic, biochemical, genetic, molecular biological, proteomic and others – need to be used in future studies to gather evidence of function. One approach is heterologous expression of GTs within organisms that provide a 'null'

background and where a robust enzyme assay and unequivocal product characterisation can be provided. In other cases, the road to determining a definitive activity for a GT will be more difficult. The use (and availability) of appropriate acceptors and donors in biochemical reactions to test enzymatic activity will be vital in these instances. Importantly, care must be taken in the extrapolation of data obtained from 'proof-of-function' experiments to other GT family members, and in assuming that an enzymatic activity shown *in vitro* is the same as its function *in vivo*; for example, the complexity of the GlyTs in CAZy Family GT47 described above. Continued perseverance of elucidating the suite of genes and enzymes that participate in wall polysaccharide and (glyco)protein biosynthesis is critical, given the rich rewards in terms of the benefits to human society in using plant products for food, fibre, feed and fuel.

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