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Unique aspects of the grass cell wall

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Grasses are amongst the most important crops worldwide, and the composition of their cell walls is critical for uses as food, feed, and energy crops. Grass cell walls differ dramatically from dicot cell walls in terms of the major structural polysaccharides present, how those polysaccharides are linked together, and the abundance and importance of pectins, proteins and phenolic compounds. Recent advances, spurred by the availability of genomic resources for several plant species, include the characterization of cellulose synthase like (Csl) gene families that are unique to the grasses and the demonstration that members of one of those gene families, CslF, are responsible for making the mixed linkage glucans that are unique to the order Poales.

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Introduction

Grasses provide the majority of calories consumed by humans either directly through the consumption of grains or indirectly through animals fed a diet of grains and forage. Grass cell walls are a major source of dietary fiber that provides numerous health benefits beyond simply providing calories [1,2]. Furthermore, grass cell walls are poised to become a significant source of renewable energy because the sugars locked in the polysaccharides of the cell wall can be converted into liquid fuel (e.g. ethanol, butanol) and the entire cell wall can be burned to produce heat or electricity [3•,4•,5].

Significant compositional differences between grass and dicot cell walls have been known for some time (Table 1) [6]. Whereas the overall architectures of grass and dicot cell walls are similar in that they both consist of a network of cellulose fibers surrounded by a matrix of non-cellulosic polysaccharides, they differ considerably in the types and relative abundance of non-cellulosic polysaccharides, cross-linking of the polysaccharides, and abundance of proteins and phenolic compounds. Primary cell walls of flowering plants can be divided into two broad categories [6,7]. Type I cell walls, found in dicots, noncommelinoid monocots (e.g. aroids, alismatids, and lilioids), and gymnosperms, consist of cellulose fibers encased in a network of xyloglucan (XyG), pectin and structural proteins. Type II cell walls, found only in the commelinoid monocots (e.g. grasses, sedges, rushes, and gingers), are composed of cellulose fibers encased in glucuronoarabinoxylans (GAX), high levels of hydroxycinnamates, and very low levels of pectin and structural proteins. In addition, the cell walls of grasses (family Poaceae) and some related families in the order Poales contain significant quantities of mixed linkage glucans (MLG) [8].

Historically, studying the enzymes required for the biosynthesis of cell wall polysaccharides has been extremely challenging for several reasons: these enzymes are integral membrane proteins, activity assays are difficult, and the enzymes quickly lose activity or require unknown cofactors [9••,10•,11]. Studying the role of specific genes has been further complicated by the inherent plasticity of the cell wall and genetic redundancy that interfere with the identification of mutations clearly associated with one cell wall component. Fortunately, the development of powerful genomic tools has provided the toehold necessary to begin solving the mysteries of cell wall biosynthesis. This review will give a brief overview of the compositional difference between dicot and grass cell walls and survey recent advances in our knowledge of the genes responsible for the biosynthesis of the unique grass cell wall.

Polysaccharide component

A milestone in our understanding of the biosynthesis of cell wall polysaccharides was the identification of cellulose synthase A (CesA) as the catalytic subunit of the cellulose synthase complex (reviewed in [12•]). The CesA gene family is present in all seed plants examined to date in addition to the moss *Physcomitrella patens* [13]. Thus, cellulose biosynthesis is probably very similar in both grasses and dicots and will not be considered further here. However, since the β1–4 linkages of cellulose are similar to the linkages found in the backbones of the hemicelluloses (XyG, GAX, (gluco)mannan, and MLG) it has been postulated that cellulose synthase like (Csl) genes might be responsible for the biosynthesis of glycan backbones in the Golgi [14]. Csl genes have been divided into eight families, CslA thru CslH [14,15]. Even at this global level there are obvious differences between grasses and dicots. The CslF and CslH families are unique to the grasses whereas CslB and CslG are unique to the dicots. The remaining families are represented in

Table 1

Approximate composition[a] (% dry weight) of typical dicot and grass primary and secondary cell walls

	Primary wall		Secondary wall	
	Grass	Dicot	Grass	Dicot
Cellulose	20–30[b,c]	15–30[c,d,e]	35–45[c,f]	45–50[c]
Hemicelluloses				
Xylans	20–40[d]	5[c]	40–50[c,g]	20–30[c,g]
MLG	10–30[d]	Absent	Minor	Absent
XyG	1–5[c,d,g]	20–25[g]	Minor	Minor
Mannans and glucomannans	Minor	5–10[d]	Minor	3–5[g]
Pectins	5[c]	20–35[d]	0.1[c]	0.1[c]
Structural proteins	1[d]	10[d,e]	Minor	Minor
Phenolics				
Ferulic acid and ρ-coumaric acid	1–5[c,d]	Minor (except order Caryophyllales)	0.5–1.5[c]	Minor (except order Caryophyllales)
Lignin	Minor	Minor	20[c]	7–10[c]
Silica			5–15[c]	Variable

[a] Numbers in this table were taken from several sources to provide rough approximations of generalized cell wall composition from typical dicots and grasses. Some of the numbers are averages or ranges based on multiple sources.
[b] [32].
[c] [37].
[d] [64].
[e] [65].
[f] [36].
[g] [31].

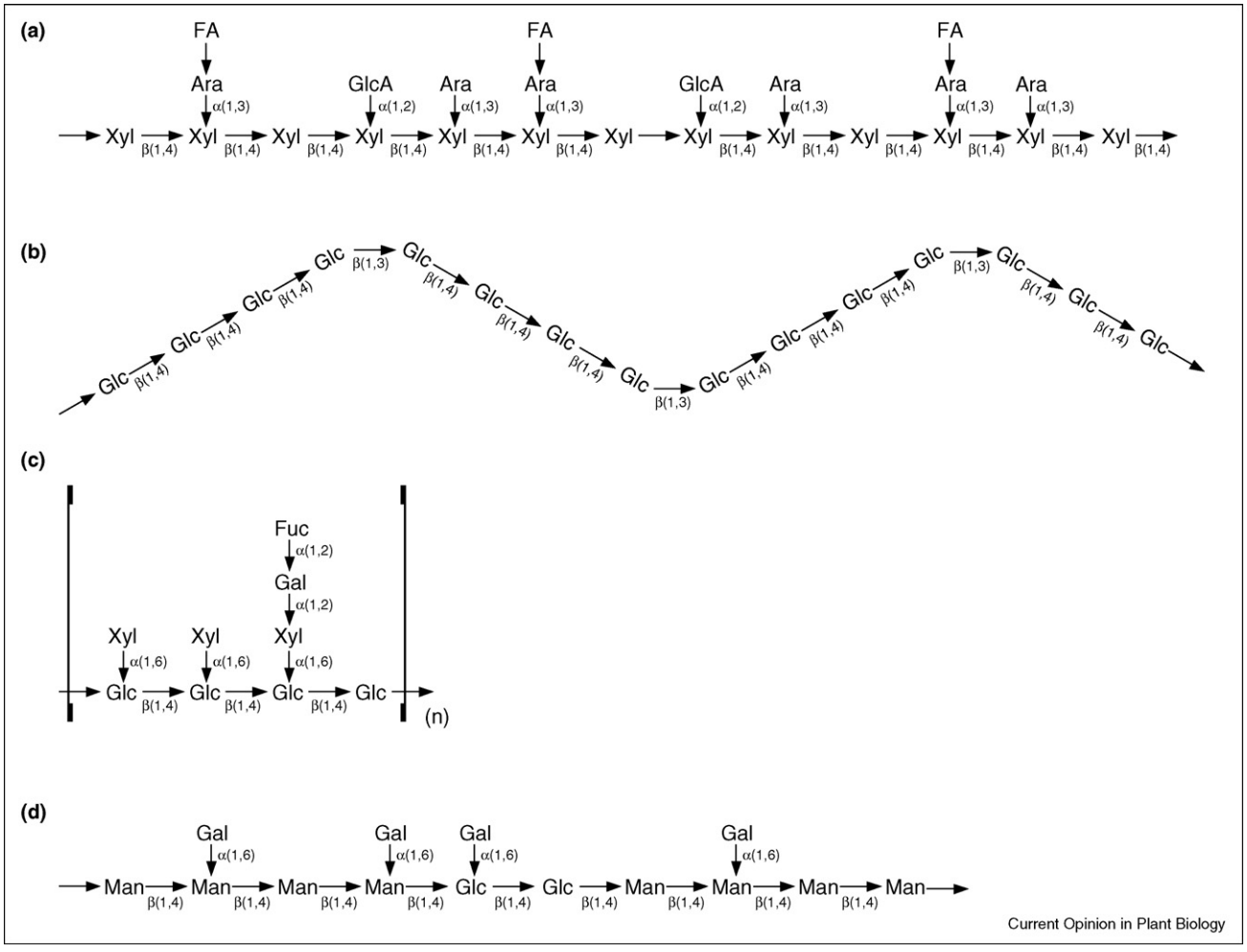
both grasses and dicots. Thus, it seems logical that CslA, CslC, CslD, and CslE might synthesize glycans that occur in both dicots and grasses whereas the other Csls might synthesize lineage-specific glycans.

A CslA family member from guar (a dicot) was shown to synthesize mannan and glucomannan when expressed in transgenic soybean cells [16]. Further examination of the CslA family showed that members from both dicots and grasses synthesize mannan and glucomannan [17] (Gluco)mannans are minor components of both dicot and grass primary cell walls and are the major hemicellulose of gymnosperm wood [17]. Another development in support of the Csl hypothesis was the demonstration that a CslC family member from nasturtium can synthesize the XyG backbone when expressed in yeast [18••]. A number of glycosyl transferases that add side chains to XyG have also been identified, making XyG biosynthesis the best understood of any hemicellulose [10•,19]. However, since XyG and (gluco)mannan occur in dicots and are only minor components of grass cell walls, these developments do not increase our understanding of what makes grass cell walls unique.

MLGs (also known as β-glucans) are unbranched homopolymers of glucose that are unusual in that they contain both β1,3- and β1,4-linkages (Figure 1). MLGs are unique to the cell walls of grasses (family Poaceae) and a few related families from the order Poales [20]. MLGs have been observed in the cell walls of many vegetative cell types using immunogold labeling [21,22] and are found in high concentrations in the endosperm of some grains where they act as storage carbohydrates [23,24]. The concentration of MLG in vegetative cells is highly correlated with cell growth and peaks at the same time as cell expansion suggesting that MLG plays a role in cell expansion [23,25]. A direct role for MLG in cell expansion has yet to be demonstrated, and it is unclear if MLG degradation is necessary or if a slippage mechanism, perhaps mediated by an expansion-like activity, is involved [25,26,27•]. From a practical standpoint, MLG has been shown to be beneficial in the treatment or prevention of several human health conditions (high cholesterol, cardiovascular disease, obesity, and non-insulin dependent diabetes) but to be problematic for brewers and antinutritive for monogastric animals [28,29].

Perhaps the greatest advance in understanding the unique biology of grass cell walls was the demonstration that members of the grass-specific CslF gene family are involved in the synthesis of MLG [30••]. Burton *et al.* mapped a barley quantitative trait locus for MLG content to the corresponding region of the rice genome and found six CslF genes in the region. When these genes were expressed in Arabidopsis, which lacks endogenous CslF genes and MLG, low levels of MLG were detected using anti-MLG antibodies. This remarkable finding indicates that although dicot cells lack CslF, they contain any additional machinery (nucleotide sugar donors, primers and co-factors) required for MLG synthesis. However, the dicot environment is clearly not optimal for MLG synthesis because, despite the presence of large amounts

Figure 1



Hemicellulose structures. **(a)** GAX consists of a β(1,4) linked xylose (Xyl) backbone substituted with arabinose (Ara) (mainly attached at the O-3 position) and less frequently with glucuronic acid (GlcA) (mainly attached at the O-2 position) in a non-repeating fashion. GAX is highly substituted in the Golgi and then some of the Ara and GlcA units are removed in the cell wall. Ferulic acid (FA) is attached to the Ara side chains through various linkages. **(b)** MLG consists of an unbranched polymer of glucose (Glc) in which variable length stretches of β(1,4) linked Glc units are interrupted with single β(1,3) linked Glc units. The β(1,3) linkages cause the polymer to bend. **(c)** XyG is composed of a β(1,4) linked Glc backbone substituted in a repeating pattern of four Glc units. The nature of the side chains varies depending on the species. One typical repeat unit containing Xyl, Gal, and fucose (Fuc) is shown. **(c)** Glucomannans consist of a β(1,4) linked backbone containing both mannose (Man) and Glc. The polymer is variably substituted with galactose (Gal) ranging from not substituted at all to highly substituted. Mannans are similar except they do not contain Glc.

of *CslF* transcript, only minute amounts of MLG were produced.

GAX, the major hemicellulose in grass cell walls, is composed of a β1,4-linked xylose backbone with single arabinose and glucuronic acid side chains primarily attached at the O-3 and O-2 positions, respectively (Figure 1) (for a review of hemicellulose structures see [31], for a general review of xylan biosynthesis, readers are directed to the article by York and O'Neill in this issue). Some grains accumulate large quantities of xylans that contain little glucuronic acid and the polymer is called arabinoxylan. Although xylans are also present in low amounts in dicot primary cell walls, these xylans contain primarily glucuronic acid and methyl glucuronic acid side-chains attached at the O-2 position of the xylosyl units. GAX in grass primary cell walls takes the place of XyG in dicot primary cell walls and cross-links cellulose microfibrils as discussed below. The enzymes that synthesize the xylan backbone remain to be identified, and some evidence suggests that these genes may not belong to the Csl family. Expression of genes from CslA, CslC, CslD, CslE, and CslH families in insect cells did not produce xylans suggesting other roles for these genes [9••]. It is conceivable that insect cells lack co-factors, substrates, or oligosaccharide primers necessary for xylan synthesis. However, when engineered to express

CslA, insect cells do support (gluco)mannan synthesis. A bioinformatic approach to identify candidates for xylan synthase genes from public EST data identified candidates from several glycosyltransferase families including GT43, GT47, and GT61 [32]. Two Arabidopsis glycosyltransferases, IRX8 and FRA8, are strong candidates for genes that add side chains to xylans in dicots [33,34]. Mutations in these genes result in dramatically reduced xylan levels. This suggests that side chains may be added at the time of xylan backbone synthesis and that this addition may be necessary for xylan elongation. However, the grass glycosyltransferases that add arabinose and glucuronic acid are still unknown.

Hydroxycinnamates and protein

An unusual feature of grass primary and secondary cell walls is the presence of significant quantities of the hydroxycinnamates ferulic acid (up to 4%) and ρ-coumaric acid (up to 3%) [35,36]. These hydroxycinnamates exist as unbound acids or ester- and ether-linked to the arabinosyl units of GAX (Figure 1) and to various positions in lignin [37–39]. The ferulate residues can dimerize through ester and ether linkages and cross link adjacent GAX molecules [40]. In addition to dimers, more complex linkages have been observed [39]. Thus, ferulic acid seems to function like the structural proteins that cross-link XyG in dicot cell walls (for a review of cross linking and cell wall structure readers are directed to the article by McCann and Roberts, this issue). The decoration of GAX by hydroxycinnamates contributes to the indigestibility of this cell wall fraction in grasses and makes grass cell walls harder to saccharify for ethanol production. In addition, ferulic acid is inhibitory to the yeast used to ferment the sugars derived from cell walls into ethanol [43]. Ferulate and ρ-coumarate are also present in significant quantities in the primary cell walls in one dicot order, the Caryophyllales, which includes crops like beet and spinach [41]. However, these dicot hydroxycinnamates are linked to arabinosyl and galactosyl units in pectin rather than GAX. Ferulic acid has also been found in significant quantities in the primary cell walls of all gymnosperms examined to date [42].

Cell wall proteins (CWP) range from structural proteins that are strongly or covalently attached to the polysaccharides all the way to loosely attached or soluble proteins. Structural CWP are much less abundant in the grasses than in dicots (Table 1). Recently, water-soluble and loosely ionically bound, CWP from maize were surveyed using a proteomic approach [44] and, perhaps not surprisingly, the majority of the CWP identified had previously been found in dicots. However, a significant proportion (18%) was unique to maize. One of these was an endo-1,3;1,4-β-D-glucanase, which makes sense because the tissue sampled was rapidly elongating roots. Although it is too early to gauge the importance of the protein differences observed in the water-soluble and loosely ionically bound CWP, this data may provide clues to processes like cell expansion.

Secondary cell walls and lignin

The majority of cell wall research in both dicots and grasses has focused on the primary cell wall. However, since the secondary cell walls of grasses comprise at least 50% of the cell wall mass in both leaves and stems, it cannot be ignored [45,46]. Secondary cell walls are deposited inside of the primary cell walls and are prominent features of xylem, fibers and sclerenchyma. The typical grass secondary cell wall is largely composed of cellulose, GAX, and lignin (Table 1). The GAX found in secondary cell walls has fewer side-chains than the GAX of primary cell walls. This results in a stronger GAX-cellulose interaction. Dicot secondary cell walls are also composed of cellulose, xylans, and lignin. However, dicot xylans differ from grass GAX as described above.

Lignin comprises a substantial portion (~20%) of the grass secondary cell wall and essentially fills the pores between the polysaccharides. Grass lignin is similar to dicot lignin in that it is primarily composed of guaiacyl (~35–49%) and syringyl (~40–61%) units. However, grass lignin also contains a small but significant percentage (~4–15%) of ρ-hydroxyphenyl units that are only found in trace levels in dicot lignin [40]. At this time, the biosynthesis and assembly of the monolignols appears to be similar in dicots and grasses [47,48]. Unlike dicots, grass lignin contains substantial amounts of ferulic acid and ρ-coumaric acid [45,46]. Ferulic acid residues attached to GAX may serve as nucleation sites for lignin formation [49–51].

The *brown-midrib* (*bm*) mutations in maize, sorghum, and millet have been known to decrease lignin content and affect lignin composition for many years. In maize, four *bm* loci have been identified, *bm1-bm4*, and readers are directed to a recent review [52] for a more detailed description. Despite having been identified over 40 years ago, only two maize *bm* loci have been associated with genes. Not surprisingly, both *bm* loci affect genes involved in the biosynthesis of lignin monomers: *bm3* by mutations in caffeic acid *O*-methyl transferase and *bm1* is associated with reduced cinnamyl alcohol dehydrogenase activity, possibly because of a mutation in a regulatory gene or region. Recently, expression profiling was used to identify genes differentially expressed among *bm1-bm4*. Using a small macroarray of 144 cell wall-related genes, Guillaumie *et al.* identified 69 genes that were differentially expressed in at least one of the four *bm* mutants [53]. A combination of microarray (containing 9841 genes) and suppression subtractive hybridization analysis was used by Shi *et al.* [54] to identify a large number (>1000) of differentially expressed genes. The large number of genes differentially expressed in the *bm* mutants highlights the interconnected nature of cell wall metabolism.

Few genes that control secondary cell wall biosynthesis have been identified. The cobra gene and several related

cobra-like genes have been shown to be critical for normal secondary cell wall development in Arabidopsis. It has been hypothesized that the glycosylphosphatidylinositol anchored COBRA protein guides the cellulose synthase complex along microtubules to maintain proper cellulose microfibril orientation [55]. The cobra-like genes, brittle culm 1 (bc1) and brittle stalk 2 (bk2), from rice and maize respectively, have been shown to be required for normal secondary wall development [56,57]. When these genes are mutated the stalks become brittle because of greatly reduced secondary wall development. Cellulose content is decreased and lignin makes up a greater portion of the cell wall material, suggesting that these genes are required for normal cellulose deposition in the secondary cell wall. Interestingly, *bc1* and *bk2* mutant plants appear normal until they are mechanically challenged when the compromise in stem strength becomes obvious. This indicates that bc1 and bk2 are only required for secondary cell wall growth. A phylogenic analysis of the cobra like gene family (12 members in Arabidopsis, 11 in rice, and 9 in maize (based on available maize sequence)) revealed one grass-specific clade suggesting that genes in this clade may perform a function unique to the grasses [58].

Conclusions

Over the past several years, much progress has been made in identifying the genes responsible for the biosynthesis of the plant cell wall. However, given that only a handful of the >1000 genes postulated to be involved in synthesizing and remodeling the cell wall have been studied in detail, much work remains. Recent progress stems in large part from the development of powerful genomic tools for the model plant *Arabidopsis thaliana*. Progress in understanding the unique aspects of grass cell walls has lagged that seen for dicot cell walls due in part to the relatively small number of research groups focused on the plant cell wall. This lack of attention to plant cell walls in general and grass cell walls in particular is not in sync with the enormous importance of grasses as food, feed and, increasingly, fuel. However, the last factor in this equation, fuel, promises to change the pace of grass cell wall research. There is considerable interest in developing perennial grasses (e.g. switchgrass and *Miscanthus*) as a source of secure, renewable energy. A glimpse of the new emphasis being placed on understanding grass cell walls can be seen in the commitment by the U.S. Departments of Energy (DOE) and Agriculture (USDA) to develop the small, rapid-cycling grass *Brachypodium distachyon* into a truly tractable grass model system to accelerate the development of grasses as biomass crops [59•]. While the detailed composition of Brachypodium cell walls has not yet been published, preliminary measurements indicate the that lignin and polysaccharide components are typical for a grass (J. Ralph personal communication). The DOE Joint Genome Institute (http://www.jgi.doe.gov) is currently sequencing the *Brachypodium* genome and it is anticipated that sequencing will be completed in 2008. The complete genome sequence when coupled with other *Brachypodium* resources (e.g. facile transformation [60,61], physical maps, BAC libraries [62], EST sequences [63], linkage maps, and insertional mutants) will convert this small, easily grown grass into a powerful model system for grass cell wall research. The increasing resources directed toward developing lignocellulosic biomass as a fuel source along with the current and emerging genomic resources for several species promises to usher in a golden age of cell wall research.

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References and recommended reading

Papers of particular interest, published within the period of the review, have been highlighted as:

- • of special interest
- •• of outstanding interest

1. Spiller GE (Ed): *CRC Handbook of Dietary Fiber in Human Nutrition*, edn 3. CRC Press; 2001.

2. Harris PJ, Smith BG: **Plant cell walls and cell-wall polysaccharides: structures, properties and uses in food products**. *Int J Food Sci Technol* 2006, **41**:129-143.

3. • Ragauskas AJ, Williams CK, Davison BH, Britovsek G, Cairney J, Eckert CA, Frederick WJ Jr, Hallett JP, Leak DJ, Liotta CL *et al.*: **The path forward for biofuels and biomaterials**. *Science* 2006, **311**:484-489.

An overview of the biorefinery paradigm and the steps required to reach this goal.

4. • Perlack RD, Wright LL, Turhollow AF, Graham RL, Stokes BJ, Erbach DC: *Biomass as a feedstock for a bioenergy and bioproducts industry: the technical feasibility of a billion-ton annual supply*. (U.S. Department of Energy and U.S. Department of Agriculture, April 2005; available at feedstockreview.ornl.gov/pdf/billion_ton_vision.pdf).

An overview of available biomass resources under different assumptions about future biomass production. They conclude that 1.3 billion tons of biomass could be used to supply over 30% of the transportation fuel needs of the United States. This study will be of interest to researchers interested in utilizing cell walls as a biofuel feedstock.

5. Service RF: **Biofuel researchers prepare to reap a new harvest**. *Science* 2007, **315**:1488-1491.

6. Carpita NC, Gibeaut DM: **Structural models of primary cell walls in flowering plants: consistency of molecular structure with the physical properties of the walls during growth**. *Plant J* 1993, **3**:1-30.

7. Carpita NC: **Structure and biogenesis of the cell walls of grasses**. *Annu Rev Plant Physiol Plant Mol Biol* 1996, **47**:445-476.

8. Smith BG, Harris PJ: **The polysaccharide composition of Poales cell walls: Poaceae cell walls are not unique**. *Biochem Syst Ecol* 1999, **27**:33-53.

9. •• Liepman AH, Wilkerson CG, Keegstra K: **Expression of cellulose synthase-like (Csl) genes in insect cells reveals that CslA family members encode mannan synthases**. *Proc Natl Acad Sci U S A* 2005, **102**:2221-2226.

The authors expressed genes from five Csl families in insect cells. Only CslA genes produced a polysaccharide. This work highlights the difficulties of assigning function to Csl genes.

10. • Lerouxel O, Cavalier DM, Liepman AH, Keegstra K: **Biosynthesis of plant cell wall polysaccharides—a complex process**. *Curr Opin Plant Biol* 2006, **9**:621-630.

An excellent overview of the biosynthesis of plant cell wall polysaccharides.

11. Richmond TA, Somerville CR: **Integrative approaches to determining Csl function**. *Plant Mol Biol* 2001, **47**:131-143.

12. • Joshi CP, Mansfield SD: **The cellulose paradox—simple molecule, complex biosynthesis**. *Curr Opin Plant Biol* 2007, **10**:220-226.
Provides an overview of cellulose biosynthesis and the many genes required to make a seemingly simple polymer.

13. Roberts AW, Bushoven JT: **The cellulose synthase (CESA) gene superfamily of the moss Physcomitrella patens**. *Plant Mol Biol* 2007, **63**:207-219.

14. Richmond TA, Somerville CR: **The cellulose synthase superfamily**. *Plant Physiol* 2000, **124**:495-498.

15. Hazen SP, Scott-Craig JS, Walton JD: **Cellulose synthase-like genes of rice**. *Plant Physiol* 2002, **128**:336-340.

16. Dhugga KS, Barreiro R, Whitten B, Stecca K, Hazebroek J, Randhawa GS, Dolan M, Kinney AJ, Tomes D, Nichols S *et al.*: **Guar seed β-mannan synthase is a member of the cellulose synthase super gene family**. *Science* 2004, **303**:363-366.

17. Liepman AH, Nairn CJ, Willats WGT, Sørensen I, Roberts AW, Keegstra K: **Functional genomic analysis supports conservation of function among cellulose synthase-like a gene family members and suggests diverse roles of mannans in plants**. *Plant Physiol* 2007, **143**:1881-1893.

18. •• Cocuron J-C, Lerouxel O, Drakakaki G, Alonso AP, Liepman AH, Keegstra K, Raikhel N, Wilkerson CG: **A gene from the cellulose synthase-like C family encodes a β-1,4 glucan synthase**. *Proc Natl Acad Sci U S A* 2007, **104**:8550-8555.
By sequencing ESTs from maturing nasturtium seeds the authors identified a single CslC gene as a candidate for the gene responsible for the synthesis of the β-1,4 glucan backbone of XyG. Yeast cells expressing this CslC gene produced β-1,4 glucan and the Arabidopsis ortholog was coordinately regulated with other genes involved in XyG synthesis providing evidence that CslC synthesized the XyG backbone.

19. Scheible W-R, Pauly M: **Glycosyltransferases and cell wall biosynthesis: novel players and insights**. *Curr Opin Plant Biol* 2004, **7**:285-295.

20. Trethewey JAK, Campbell LM, Harris PJ: **(1 → 3), (1 → 4)-β-D-glucans in the cell walls of the Poales (sensu lato): An immunogold labeling study using a monoclonal antibody**. *Am J Bot* 2005, **92**:1660-1674.

21. Carpita NC, Defernez M, Findlay K, Wells B, Shoue DA, Catchpole G, Wilson RH, McCann MC: **Cell wall architecture of the elongating maize coleoptile**. *Plant Physiol* 2001, **127**:551-565.

22. Trethewey JAK, Harris PJ: **Location of (1 → 3)- and (1 → 3), (1 → 4)-β-D-glucans in vegetative cell walls of barley (*Hordeum vulgare*) using immunogold labelling**. *New Phytol* 2002, **154**:347-358.

23. Buckeridge MS, Rayon C, Urbanowicz B, Tiné MAS, Carpita NC: **Mixed linkage (1 → 3), (1 → 4)-β-D-glucans of grasses**. *Cereal Chem* 2004, **81**:115-127.

24. Wilson SM, Burton RA, Doblin MS, Stone BA, Newbigin EJ, Fincher GB, Bacic A: **Temporal and spatial appearance of wall polysaccharides during cellularization of barley (*Hordeum vulgare*) endosperm**. *Planta* 2006, **224**:655-667.

25. Kim J-B, Olek AT, Carpita NC: **Cell wall and membrane-associated exo-β-D-glucanases from developing maize seedlings**. *Plant Physiol* 2000, **123**:471-485.

26. Whitney SEC, Gidley MJ, McQueen-Mason SJ: **Probing expansin action using cellulose/hemicellulose composites**. *Plant J* 2000, **22**:327-334.

27. • Cosgrove DJ: **Growth of the plant cell wall**. *Nat Rev Mol Cell Biol* 2005, **6**:850-861.
An overview of plant cell growth that highlights the role of expansin in allowing the cell wall to expand.

28. Brennan CS, Cleary LJ: **The potential use of cereal (1 → 3,1 → 4)-β-D-glucans as functional food ingredients**. *J Cereal Sci* 2005, **42**:1-13.

29. Queenan KM, Stewart ML, Smith KN, Thomas W, Fulcher RG, Slavin JL: **Concentrated oat β-glucan, a fermentable fiber, lowers serum cholesterol in hypercholesterolemic adults in a randomized controlled trial**. *Nutr J* 2007, **6**:1-8.

30. •• Burton RA, Wilson SM, Hrmova M, Harvey AJ, Shirley NJ, Medhurst A, Stone BA, Newbigin EJ, Bacic A, Fincher GB: **Cellulose synthase-like CslF genes mediate the synthesis of cell wall (1,3;1,4)-β-D-glucans**. *Science* 2006, **311**:1940-1942.
An elegant study that used the synteny between barley and rice to identify six rice CslF genes at the region syntenic to a barley quantitative trait locus for MLG content. Transgenic Arabidopsis plant expressing the rice CslF genes accumulated MLG in their cell walls. This is the first identification of a gene involved in the synthesis of the unique MLG polymer.

31. Ebringerová A, Ebringerová Z, Heinze T: **Hemicellulose**. *Adv Polym Sci* 2005, **186**:1-67.

32. Mitchell RAC, Dupree P, Shewry PR: **A novel bioinformatics approach identifies candidate genes for the synthesis and feruloylation of arabinoxylan**. *Plant Physiol* 2007, **144**:43-53.

33. Pena MJ, Zhong R, Zhou G-K, Richardson EA, O'Neill MA, Darvill AG, York WS, Yeb Z-H: **Arabidopsis irregular xylem 8 and irregular xylem 9: Implications for the complexity of glucuronoxylan biosynthesis**. *Plant Cell* 2007, **19**:549-563.

34. Persson S, Caffall KH, Freshour G, Hilley MT, Bauer S, Poindexter P, Hahn MG, Mohnen D, Somerville C: **The Arabidopsis irregular xylem 8 mutant is deficient in glucuronoxylan and homogalacturonan, which are essential for secondary cell wall integrity**. *Plant Cell* 2007, **19**:237-255.

35. Saulnier L, Crépeau M-J, Lahaye M, Thibault J-F, Garcia-Conesa MT, Kroon PA, Williamson G: **Isolation and structural determination of two 5,5′-diferuloyl oligosaccharides indicate that maize heteroxylans are covalently cross-linked by oxidatively coupled ferulates**. *Carbohydr Res* 1999, **320**:82-92.

36. Hatfield RD, Wilson JR, Mertens DR: **Composition of cell walls isolated from cell types of grain sorghum stems**. *J Sci Food Agric* 1999, **79**:891-899.

37. Ishii T: **Structure and functions of feruloylated polysaccharides**. *Plant Sci* 1997, **127**:111-127.

38. Lam TBT, Kadoya K, Iiyama K: **Bonding of hydroxycinnamic acids to lignin: ferulic and ρ-coumaric acids are predominantly linked at the benzyl position of lignin, not the β-position, in grass cell walls**. *Phytochemistry* 2001, **57**:987-992.

39. Ralph J, Bunzel M, Marita JM, Hatfield RD, Lu F, Kim H, Schatz PF, Grabber JH, Steinhart H: **Peroxidase-dependent cross-linking reactions of *p*-hydroxycinnamates in plant cell walls**. *Phytochem Rev* 2004, **3**:79-96.

40. Grabber JH, Ralph J, Lapierre C, Barrière Y: **Genetic and molecular basis of grass cell-wall degradability. I. Lignin–cell wall matrix interactions**. *CR Biol* 2004:455-465.

41. Levigne SV, Ralet M-CJ, Quéméner BC, Pollet BN-L, Lapierre C, Thibault J-FJ: **Isolation from sugar beet cell walls of arabinan oligosaccharides esterified by two ferulic acid monomers**. *Plant Physiol* 2004, **134**:1173-1180.

42. Carnachan SM, Harris PJ: **Ferulic acid is bound to the primary cell walls of all gymnosperm families**. *Biochem Syst Ecol* 2000, **28**:865-879.

43. Persson P, Andersson J, Gorton L, Larsson S, Nilvebrant N-O, Jönsson LJ: **Effect of different forms of alkali treatment on specific fermentation inhibitors and on the fermentability of lignocellulose hydrolysates for production of fuel ethanol**. *J Agric Food Chem* 2002, **50**:5318-5325.

44. Zhu J, Chen S, Alvarez S, Asirvatham VS, Schachtman DP, Wu Y, Sharp RE: **Cell wall proteome in the maize primary root elongation zone, I. Extraction and identification of water-soluble and lightly ionically bound proteins**. *Plant Physiol* 2006, **140**:311-325.

45. MacAdam JW, Grabber JH: **Relationship of growth cessation with the formation of diferulate cross-links and ρ-coumaroylated lignins in tall fescue leaf blades**. *Planta* 2002, **215**:785-793.

46. Jung H-JG: **Maize stem tissues: ferulate deposition in developing internode cell walls**. *Phytochemistry* 2003, **63**:543-549.

47. Boerjan W, Ralph J, Baucher M: **Lignin biosynthesis**. *Annu Rev Plant Biol* 2003, **54**:519-546.

48. Humphreys J, Chapple C: **Rewriting the lignin roadmap**. *Curr Opin Plant Biol* 2002, **5**:224-229.

49. Ralph J, Grabber JH, Hatfield RD: **Lignin-ferulate cross-links in grasses: Active incorporation of ferulate polysaccharide esters into ryegrass lignins**. *Carbohydr Res* 1995, **275**:167-178.

50. Jacquet G, Pollet B, Lapierre C, Mhamdi F, Rolando C: **New ether-linked ferulic acid-coniferyl alcohol dimers identified in grass straws**. *J Agric Food Chem* 1995, **43**:2746-2751.

51. Grabber JH, Ralph J, Hatfield RD: **Model studies of ferulate—coniferyl alcohol cross-product formation in primary maize walls: implications for lignification in grasses**. *J Agric Food Chem* 2002, **50**:6008-6016.

52. Barrière Y, Ralph J, Méchin V, Guillaumie S, Grabber JH, Argillier O, Chabbert B, Lapierre C: **Genetic and molecular basis of grass cell wall biosynthesis and degradability. II. Lessons from brown-midrib mutants**. *Comptes Rendus – Biologies* 2004, **327**:847-860.

53. Guillaumie S, Pichon M, Martinant J-P, Bosio M, Goffner D, Barrière Y: **Differential expression of phenylpropanoid and related genes in brown-midrib bm1, bm2, bm3, and bm4 young near-isogenic maize plants**. *Planta* 2007, **226**:235-250.

54. Shi C, Koch G, Ouzunova M, Wenzel G, Zein I, Lübberstedt T: **Comparison of maize brown-midrib isogenic lines by cellular UV-microspectrophotometry and comparative transcript profiling**. *Plant Mol Biol* 2006, **62**:697-714.

55. Roudier F, Fernandez AG, Fujita M, Himmelspach R, Borner GHH, Schindelman G, Song S, Baskin TI, Dupree P, Wasteneys GO *et al.*: **COBRA, an Arabidopsis extracellular glycosyl-phosphatidyl inositol-anchored protein, specifically controls highly anisotropic expansion through its involvement in cellulose microfibril orientation**. *Plant Cell* 2005, **17**:1749-1763.

56. Li Y, Qian Q, Zhou Y, Yan M, Sun L, Zhang M, Fu Z, Wang Y, Han B, Pang X *et al.*: **BRITTLE CULM1, which encodes a COBRA-like protein, affects the mechanical properties of rice plants**. *Plant Cell* 2003, **15**:2020-2031.

57. Ching A, Dhugga KS, Appenzeller L, Meeley R, Bourett TM, Howard RJ, Rafalski A: **Brittle stalk 2 encodes a putative glycosylphosphatidylinositol-anchored protein that affects mechanical strength of maize tissues by altering the composition and structure of secondary cell walls**. *Planta* 2006, **224**:1174-1184.

58. Brady SM, Song S, Dhugga KS, Rafalski JA, Benfey PN: **Combining expression and comparative evolutionary analysis. The COBRA gene family**. *Plant Physiol* 2007, **143**:172-187.

59. DOE (Ed). *Breaking the Biological Barriers to Cellulosic Ethanol: A Joint Research Agenda*: U.S. Department of Energy, Office of Science and Office of Energy Efficiency; 2006, available online at http://genomicsgtl.energy.gov/biofuels/b2bworkshop.shtml.
• This document is the product of a workshop organized by DOE. It outlines the research necessary to establish a viable biofuel industry in the U.S. Considerable emphasis is placed on the need for expanded cell wall research in both grasses and dicots.

60. Vain P, Worland B, Thole V, McKenzie N, Alves S, Opanowicz M, Fish L, Bevan M, Snape J: **Agrobacterium-mediated transformation of the temperate grass Brachypodium distachyon (genotype Bd21) for T-DNA insertional mutagenesis**. *Plant Biotechnol J* 2008, **6**:236-245.

61. Vogel J, Hill T: **High-efficiency Agrobacterium-mediated transformation of Brachypodium distachyon inbred line Bd21-3**. *Plant Cell Rep* 2008, **27**:471-478.

62. Huo N, Lazo G, Vogel J, You F, Ma Y, Hayden D, Colemann-Derr D, Hill T, Dvorak J, Anderson O, *et al.*: **The nuclear genome of Brachypodium distachyon: analysis of BAC end sequences**. *Funct Integr Genom* 2008, **8**:135-147.

63. Vogel J, Gu Y, Twigg P, Lazo G, Laudencia-Chingcuanco D, Hayden D, Donze T, Vivian L, Stamova B, Coleman-Derr D: **EST sequencing and phylogenetic analysis of the model grass Brachypodium distachyon**. *Theoret Appl Genet* 2006, **113**:186-195.

64. O'Neil MA, York WS: **The composition and structure of plant primary cell walls**. In *The Plant Cell Wall.* Edited by Rose JKC. CRC Press; 2003:1-54.

65. Zablackis E, Jing H, Muller B, Darvill AG, Albersheim P: **Characterization of the cell-wall polysaccharides of Arabidopsis thaliana leaves**. *Plant Physiol* 1995, **107**:1129-1138.