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DARWIN REVIEW

Plant cell walls throughout evolution: towards a molecular understanding of their design principles

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Abstract

Throughout their life, plants typically remain in one location utilizing sunlight for the synthesis of carbohydrates, swhich serve as their sole source of energy as well as building blocks of a protective extracellular matrix, called the cell wall. During the course of evolution, plants have repeatedly adapted to their respective niche, which is reflected in the changes of their body plan and the specific design of cell walls. Cell walls not only changed throughout evolution but also are constantly remodelled and reconstructed during the development of an individual plant, and in response to environmental stress or pathogen attacks. Carbohydrate-rich cell walls display complex designs, which together with the presence of phenolic polymers constitutes a barrier for microbes, fungi, and animals. Throughout evolution microbes have co-evolved strategies for efficient breakdown of cell walls. Our current understanding of cell walls and their evolutionary changes are limited as our knowledge is mainly derived from biochemical and genetic studies, complemented by a few targeted yet very informative imaging studies. Comprehensive plant cell wall models will aid in the re-design of plant cell walls for the purpose of commercially viable lignocellulosic biofuel production as well as for the timber, textile, and paper industries. Such knowledge will also be of great interest in the context of agriculture and to plant biologists in general. It is expected that detailed plant cell wall models will require integrated correlative multimodal, multiscale imaging and modelling approaches, which are currently underway.

Key words: 3D organization, chemical composition, deconstruction, evolution, electron microscopy, plant cell wall, spectroscopy.

Introduction

In the evolution of multicellular organisms on our planet, plants arguably hold an exceptional place, for they changed the face of our planet permanently through the production of oxygen, a by-product of an efficient strategy to harvest the physical energy of the sunlight through photosynthesis. The harvested solar energy is stored chemically in the form of carbohydrate-based polymers, a fact that was not lost on competing organisms that were developing their own strategies to utilize this chemical energy as they struggled for their own survival. And so began a fascinating ‘arms race’ between plant cell strategies for protection of their rich chemical energy stores and microbial and animal strategies for the breach of such protective efforts (Stahl and Bishop, 2000). In addition, plants face changes in climate and compete among each other for the scarce resources they have come to rely on, such as sunlight, water, and nutrients from the soil. Since plants are autotrophs they do not rely on predation for their own survival and reproduction, and hence they did not evolve locomotive mechanisms. Adaptation to survive predation, unfavourable environmental conditions, and competition for resources without the ability to move to another location has hence resulted in specialized plant body plans that require highly specialized tissues with distinct properties (Graham et al., 2000; Falster and Westoby, 2003). Such specialization in function is reflected at the molecular level in the design of the cell walls surrounding plant cells in each tissue. Cell walls are composed of a small number of distinct chemical polymers tightly woven into a meshwork with specific 3D architectural organization, allowing the respective cell walls to perform various mechanical and biochemical

functions (McCann and Roberts, 1991; Carpita and Gibeaut, 1993; Somerville et al., 2004; Humphrey et al., 2007). The mechanical functions include providing tensile strength to the plant body and formation of a physical barrier to harsh biotic and environmental insults. The biochemical functions include reorganization of cell wall components and possibly signal transduction in response to pathogen attack, environmental stresses, and during different developmental stages. These characteristics of cell walls allow the plants to grow to remarkable heights, to avoid predation, to minimize water loss, and to function and reproduce successfully in very diverse habitats.

The chemical complexity and compact organization of cell walls make the plants extremely recalcitrant, and hence require the ingenuity of microbial evolution for efficient plant biomass deconstruction (Pauly and Keegstra, 2008). Members of other kingdoms depend on plants for their sustenance, but these organisms are faced with the barrier of cell walls in plants. Strategies of deconstructing cell walls have co-evolved in these organisms along with the evolution of the plant cell wall (Walton, 1994; Warren, 1996; Chisholm et al., 2006; Jones and Dangl, 2006; Cantu et al., 2008b). In recent years, deconstruction of cell walls has become an important area of research (Ragauskas et al., 2006; McCann and Carpita; 2008; Pauly and Keegstra, 2008; Sticklen, 2008). One challenge is to engineer plants whose cell walls can be efficiently broken down and yet have enough mechanical strength to grow tall and resist microbial attacks. Such re-designed plants would allow large-scale production of economically important products such as lignocellulose-based biofuels, timber, textile, and paper. One of the main bottlenecks for these industries, we would contend, is that detailed knowledge of the spatial organization of each cell wall component is missing. Gaining such detailed knowledge would lead to better understanding of the cell wall design principles and will allow the prediction of cell wall properties and function.

Since the early 1970s, the chemistry of cell walls has been a prominent area of research, as cell walls are composed of a relatively small number of basic building blocks (Fig. 1), and hence could be readily extracted and analysed chemically. Comparative biochemical analyses revealed that all plant cell walls share several common features. All plant cell walls are composed of cellulose microfibrils that form the mechanical framework of the wall, and a matrix phase that forms cross-links among the microfibrils and fills the space between the fibrillar framework (McCann and Roberts, 1991; Carpita and Gibeaut, 1993; O'Neill and York, 2003; Somerville et al., 2004). However, it has also become clear that not all cell walls have exactly the same chemical composition and probably exhibit different designs (Fig. 2). Studies of a widespread group of organisms have revealed the diversity of cell walls with respect to their chemical compositions, and provide an insight into their evolutionary relationships (Popper and Fry, 2003, 2004; Matsunaga et al., 2004; Niklas, 2004; Popper et al., 2004; Harris, 2005; Carafa et al., 2005; Nothnagel and Nothnagel, 2007; van Sandt et al., 2007; Popper, 2008). Moreover, cell walls within the lifetime of any individual plant are constantly remodelled and re-constructed to ensure proper growth and development, successful reproduction, and defence against biotic attacks and severe environmental conditions (McCann and Roberts, 1991; Carpita and Gibeaut, 1993; Cosgrove, 2000, 2003; Rose, 2003; Humphrey et al, 2007; Cantu et al., 2008a, b). While the number of monomers of cell wall components is relatively small and chemically well defined, the process of component assembly and the complexity of their macromolecular organization, reorganization, and its regulation are far from simple. Only in recent times have plant biologists begun to employ genetic analyses, which have identified a number of the genes and gene products that are associated with cell wall synthesis and functioning (Roberts and Roberts, 2004; Somerville et al., 2004; Yong et al., 2005; Geisler-Lee et al., 2006; Persson et al., 2007; McCann and Carpita, 2008). For plants, such as Arabidopsis and poplar, it is estimated that 1000–2500 genes are involved in cell wall synthesis, remodelling, and breakdown (Somerville et al., 2004; Yong et al., 2005; Geisler-Lee et al., 2006). Our current knowledge about cell walls is still incomplete and is mostly restricted to indirect biochemical and genetic studies of a handful of plant species. We believe that a comprehensive comparative analysis, including direct visualization of the 3D macromolecular organization along with biochemical and genetic analyses, of various cell walls from diverse plant types is needed to fill some of the gaps in our knowledge of cell wall design.

In this review, the evolution and diversity of cell walls are first discussed based on the biochemical and genetic information available. Then the different aspects of cell wall deconstruction are discussed. Finally, the various techniques and tools that are currently used to study the cell wall 3D macromolecular organization and composition, and which have a high potential to result in a realistic model of plant cell walls, are reviewed.

Trends in evolution of cell walls

While cell walls are a characteristic feature of all plants, they are not exclusive to plants, with most bacterial and algal cells as well as all fungal cells also being surrounded by extracellular macromolecular barriers. The macromolecular composition, however, is characteristically different among the major evolutionary lineages of the living world (Fig. 3). Most bacteria have a peptidoglycan-rich cell wall, while most archaea are surrounded by an

envelope of proteins or glycoproteins. Another cell wall design, which is based predominantly on polysaccharides, is found in the eukaryotic groups and is distinctly different from both types of prokaryotic cell walls (Kandler, 1994). While an evolutionary connection between the cell walls of these three domains cannot be ruled out, it would seem that

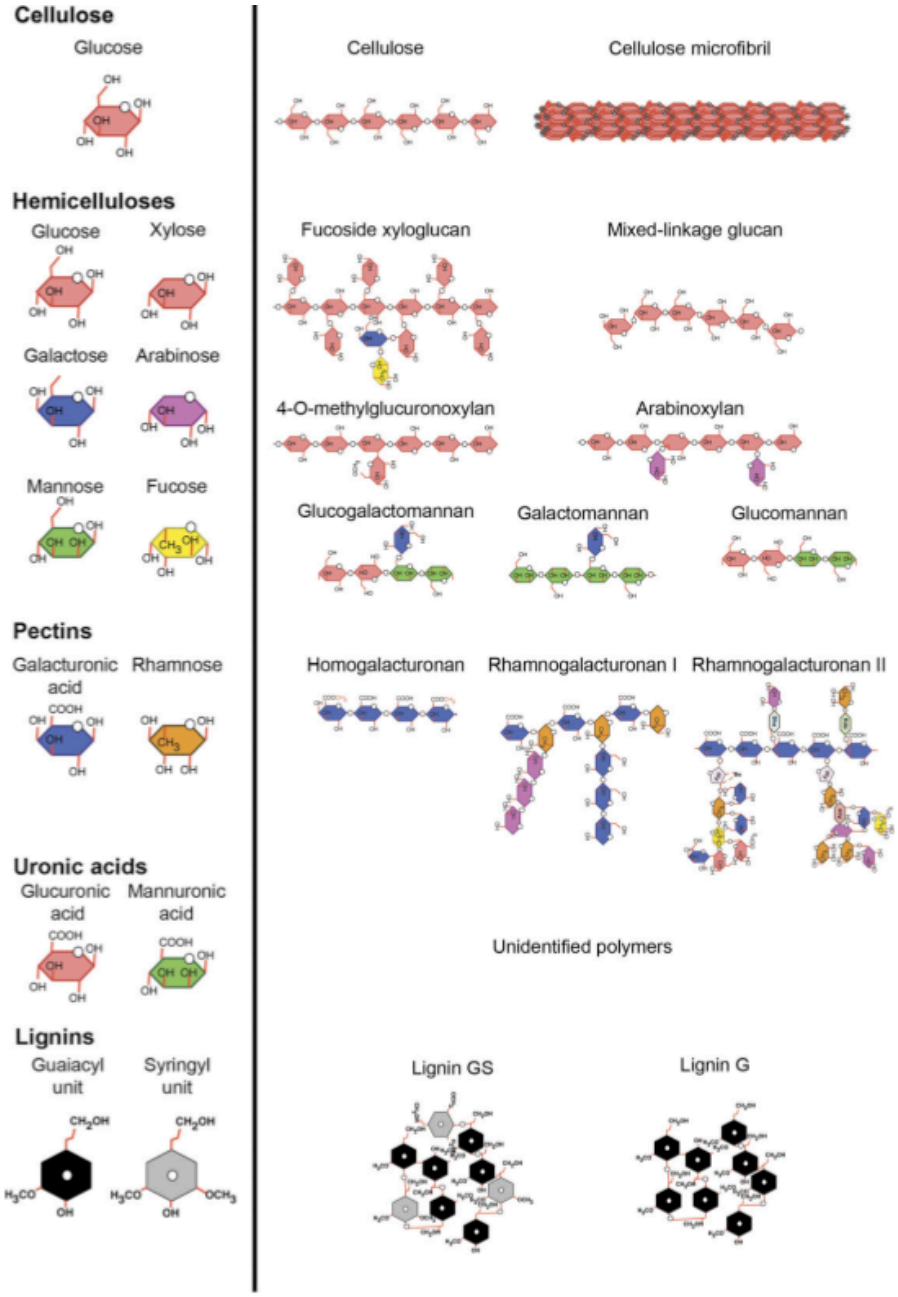

Fig. 1. Chemical structure of the predominant building blocks of plant cell walls. Left panel: monomers. Right panel: subunit of the respective polymers.

nature ‘invented’ the protective properties of cell walls more than once, coming up with vastly different solutions to a common problem. It is widely believed that the cell wall designs in the two prokaryotic domains have evolved independently from a common wall-less ancestor, whereas cell walls in eukaryotes have evolved by lateral gene transfer from previously established cell wall-producing organisms during primary or secondary endosymbiosis (Niklas, 2004).

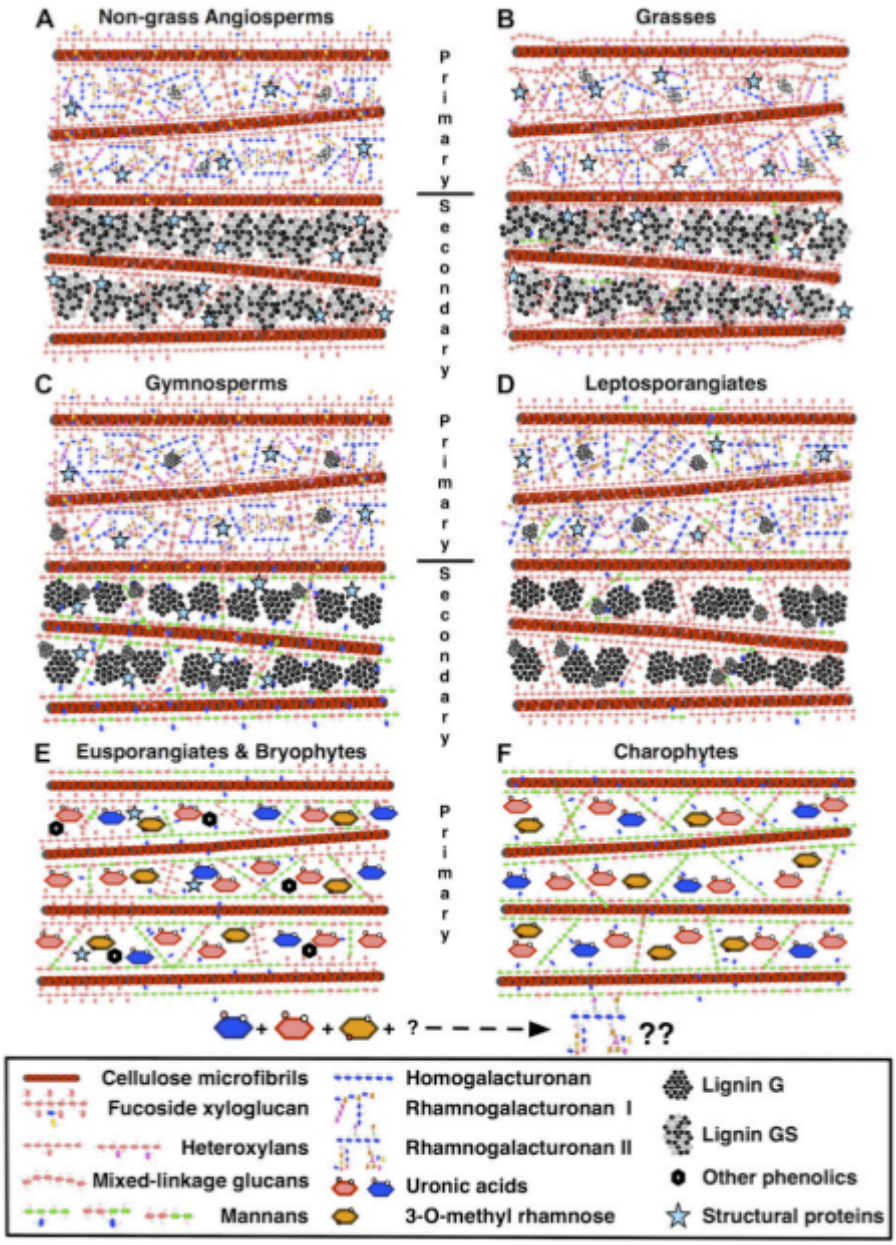

Fig. 2. Simplified 2D representation of general cell wall composition in the different groups of Kingdom Plantae. All groups have cellulose microfibrils. Non-grass angiosperms (A) have high amount of hemicelluloses (fucoside xyloglucan (XG), xylans, some mannans) and structural proteins. The primary walls have high amounts of pectins (homogalacturonans (HG), rhamnogalacturonan (RG) I and II) while the secondary walls have high amounts of lignins with guaicyl (G) and syringyl (S) units. XGs, HGs and RGs are lower in amount in grasses (B), which have higher amount of mixed-linkage glucans instead. Gymnosperms (C) have wall composition similar to non-grass angiosperms except they have higher amount of glucomannans and their lignins are homogeneous consisting primarily of guaicyl units. Leptosporangiates (D) have low amounts of XGs, HGs and RGs, but have high amounts of xylans, mannans, uronic acids, 3-O-methyl rhamnose and lignins. In eusporangiates, bryophytes and charophytes (E, F), the cell walls are not clearly differentiated into primary and secondary walls. Eusporangiates and bryophytes (E), cell walls have compositions similar to leptosporangiate walls except they lack xylans and lignins. Phenolic compounds such as lignans are present instead of lignins. Only mannans, glucuronic acids, mannuronic acids and 3-O-methyl rhamnose have been detected from cell walls of Charophytes (F) until now. Note: Spatial distribution, orientation, size and proportion of polymers in this diagram are not per scale.

While eukaryotes clearly differ in their cell wall design from prokaryotes, a remarkable diversity in cell wall design can also be seen within the eukaryotic kingdoms (Niklas, 2004; Raven et al., 2005). The basic polysaccharide components of plant cell walls are cellulose and hemicellulose, whereas fungal cell walls primarily consist of chitin. The simpler and primitive eukaryotes such as algae, metazoa, oomycetes, etc. have diverse types of cell walls, often having cell wall compositions similar to those of their respective plant or fungal descendants.

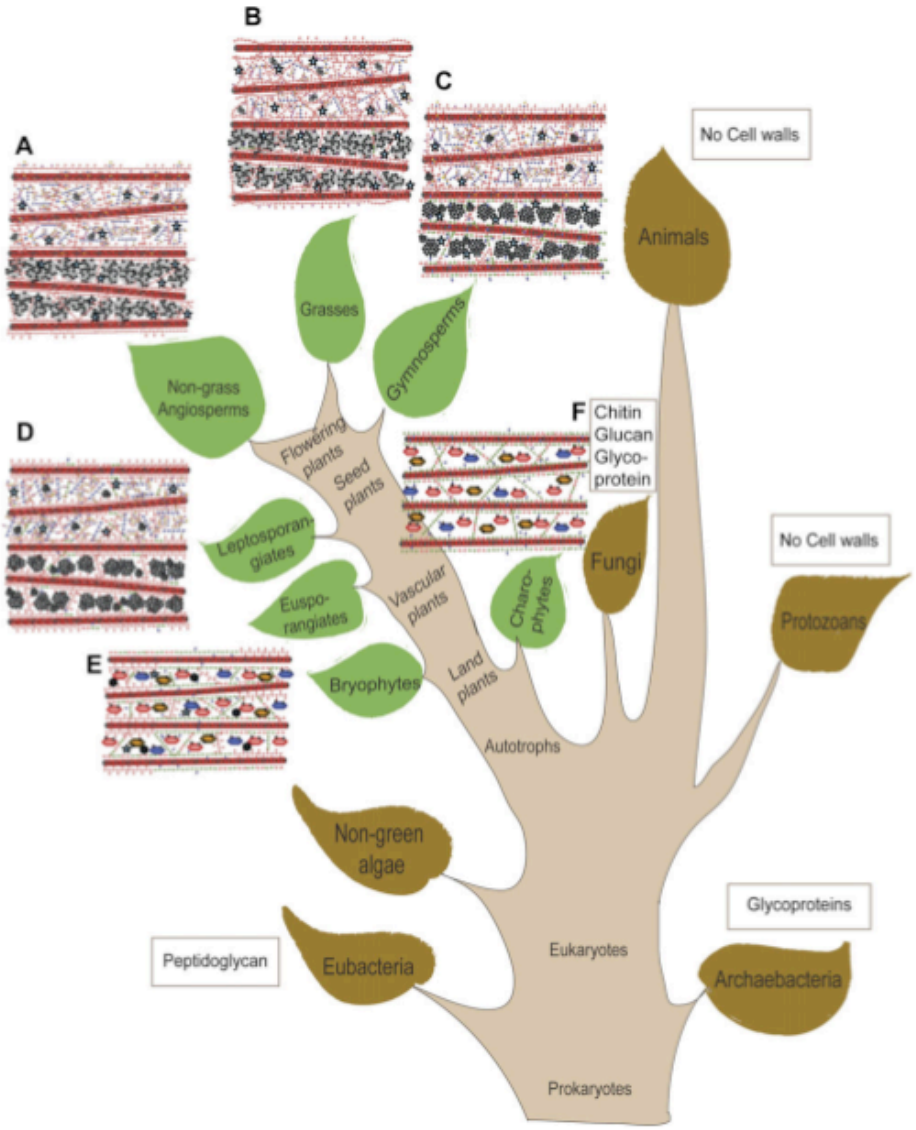

Fig. 3. Diagram showing changes in cell wall composition during the course of evolution.

Protozoans that are probably ancestors of animals lack cell walls. The distinct characteristics of each cell wall design is likely to have played significant roles for the successful survival of these major lineages through the course of evolution.

The earliest green eukaryotes such as euglenoids, which share similarities with both green algae and heterotrophic protozoans, lack cell walls. They have a protein-rich envelope layer called the pellicle and synthesize a storage carbohydrate called b-1–3-glucan. These early autotrophic organisms also have flagella and can swim, and revert to heterotrophism under unfavourable conditions (Becker, 2000; Niklas, 2004; Raven et al., 2005). In contrast, the higher autotrophic multicellular organisms no longer had to search for food, and instead were able to synthesize their own chemical energy source, only by being reliant on sunlight, atmospheric gases, water, and minerals. It is likely that at that point mobility was no longer an evolutionary advantage and therefore was lost. The high concentrations of carbohydrate solutes, which are the result of photosynthesis, cause a rapid uptake of hypotonic solutions (i.e. water) into these cells, resulting in a high osmotic pressure and hence threatening the integrity of cell membranes. Polysaccharide formation reduces the osmotic pressure and provides building blocks for the construction of a rigid cell wall that provides mechanical support, thus preventing rupture of the cells (Raven et al., 2005; Taiz and Zeiger, 2006). Hence, cell wall formation became an effective strategy to deal with even extreme osmotic conditions and was therefore key to survival in all conditions.

While autotrophs are capable of producing their own food, they do require water, sunlight, oxygen, and carbon dioxide, as well as reduced nitrogen, phosphorus, and sulfur from their surrounding. Given their stationary lifestyle and limited resources, plants have continuously faced fierce evolutionary competition among each other to secure a continuous supply of these essential resources (Graham et al., 2000; Falster and Westoby; 2003). An aquatic-to-terrestrial habitat transition, evolution of vascular tissue, increase in height, compartmentalization, and branching seem to have been effective strategies of exploring more resources, in particular sunlight. Such characters, together with the evolution of reproductive strategies, facilitated more widespread dispersal of their offspring and increased chances of survival. From the biochemical and genetic information available at present, it seems that these major events in evolution were accompanied by prominent changes in cell wall characteristics (Niklas, 2004; Harris, 2005; Popper, 2008). Apart from food and reproduction, other major concerns for plants are the defence against microbial, pathogenic, and animal attacks, as well as survival under adverse atmospheric conditions. Since plants are sessile, they cannot escape these unfavourable conditions. Hence, they developed an arsenal of strategies to overcome these challenges, including changes in cell wall ultrastructure (Walton, 1994; Warren, 1996; Chisholm et al., 2006; Jones and Dangl, 2006; Humphrey et al., 2007; Cantu et al., 2008b). Fungi are heterotrophic like animals, but they are stationary like plants. Likewise, they require some mechanical support for survival, reproduction, and defence. Their cell walls are chitin based, which is thought to be weaker than cellulose as the tensile strength of chitin polymers is significantly lower than that of cellulose microfibrils (Muzzarelli, 1977), which probably provides just enough mechanical support for the small fungal plant bodies (Duchesne and Larson, 1989).

Changes in cell wall compositions throughout plant evolution

Aquatic ancestors of land plants

The land plants (embryophyta) are believed to have evolved from green algae. Green algae of the group Charophyta are thought to be the closest ancestor of land plants (Lewis and McCourt, 2004). These algae are multicellular, branched, and have a plant-like appearance, and their cells are surrounded by polysaccharide-based cell walls like plants. However, these cell walls are thin and cannot be distinguished as primary or secondary cell walls. Biochemical analysis showed the presence of cellulose, mannose-containing hemicelluloses, glucuronic acid, man- nuronic acid, and 3-O-methyl rhamnose in Charophytes (Popper and Fry, 2003). Cellulosic cell walls are a common feature of all green algae and all land plants (Niklas, 2004). A single cellulose molecule is a linear, unbranched glucan chain composed of b-(1/4)-linked D-glucose residues. The high tensile strength and chemical stability of cellulose most probably gave it evolutionary advantages over the weaker and less stable wall components such as chitin and glycoproteins found in other kingdoms. Importantly, cellulose-based walls, unlike chitin-based cell walls, do not require high amounts of reduced nitrogen during wall synthesis, making them more favourable in a nitrogen- limited environment (Duchesne and Larson, 1989). Cellulose is synthesized by the cellulose synthase (CeS) enzyme present in the plasma membrane. In less evolved groups of algae, single units of CeS enzymes are arranged in linear arrays, producing individual linear glucan chains, resulting in a weak cell wall. In Charophytes, the CeS enzymes are grouped together to form cellulase synthase complexes (CSCs) that are then arranged in rosettes (Niklas, 2004). Such an arrangement results in the production of thicker closely associated bundles of ;36 glucan chains that form strong microfibrils held together by intra- molecular hydrogen bonding. Cellulose microfibrils have been observed to be ;2.2–3.6 nm in diameter in some angiosperms, depending on the species examined (Kennedy et al., 2007; Xu et al., 2007), which can further coalesce to form thicker macrofibrils in some plants (Ding and Himmel, 2006). The evolution of a stronger microfibrillar network in Charophytes is likely to have helped them to have a bigger, branched body as compared with less evolved groups of algae.

Charophytes were also found to possess high quantities of mannose-containing hemicelluloses, glucuronic acid, mannuronic acid, and some 3-O-methyl rhamnose. These algae lack xyloglucans (XGs), hydroxyproline-rich amino acids, lignins, and cutins, which are common in most land plants (Popper and Fry, 2003). The lack of such essential cell wall components in Charophytes could possibly be responsible for their relatively smaller and weaker plant bodies compared with most land plants. Charophytes are aquatic organisms but can endure dry conditions during prolonged summers. The high amount of uronic acids and 3-O-methyl rhamnose detected in Charophytes might be part of the mucilage that helps them resist desiccation during the dry periods and microbial attacks (Popper and Fry, 2003; Popper et al., 2004). Some structures analogous to the higher plant cuticle have been detected in some Charophytes (Cook and Graham, 1998). Even though XGs have not yet been found in algae, two putative cDNA sequences have been recently identified in some Charophytes that contain all domains crucial for the activity of the XG-modifying enzymes xyloglucan endotransglycosy- lase/hydrolase (XET/XTH) (van

Sandt et al., 2007). These XG-modifying enzymes are usually found in land plants, where they cleave XG chains and transfer the glycosylate residues to other XG chains, thus allowing expansion of the cell wall or modifying cell wall strength (Cosgrove, 2003). Furthermore, XET activity was located at the sites of growth in Chara (a member of Charophyta) and a putative XTH ancestral enzyme was identified in Chara (van Sandt et al., 2007). Structural connections between these XG-modifying enzymes and other enzymes that act on xyloglucan-like polysaccharides have been detected by some researchers. Based on these findings, these researchers suggest that the homologues of XET/XTH found in Charophytes might have a less specific substrate affinity, which might have evolved to be a more specific XG-modifying enzyme in land plants (Strohmeier et al., 2004; Nishitani and Vissenberg, 2006; van Sandt et al., 2007).

Move towards terrestrial habitat

During the course of evolution, a gradual shift from aquatic habitat to terrestrial habitats among both autotrophic and heterotrophic eukaryotes took place. Changes in the atmosphere and geography of the planet, along with an increasing demand and competition for resources in the oceans due to rapidly increasing number of species, presumably forced this gradual shift. Modern bryophytes are believed to resemble the earliest land plants the most, having many similar morphological and physiological features (Mishler et al., 1994). Bryophytes usually grow in moist land, close to the water. Their body plan is more differentiated compared to that of Charophytes. They have root-like rhizoids for anchorage and absorption and a green aerial part called a thallus for photosynthesis, but they do not possess specialized transport systems. Instead, water pressure and simple diffusion appear to be sufficient for water transport in these tiny plants (Raven et al., 2005). The cell walls of bryophytes are thin and can easily absorb and transport water. Cell walls in most bryophytes cannot be clearly distinguished as primary or secondary walls. In addition to cellulose microfibrils, mannose-containing hemi-celluloses are found in high quantities in cell walls of the few bryophytes that have been tested, which may be responsible for the absorbent nature of their cell walls (Popper and Fry, 2003). Bryophytes have high amounts of uronic acids including glucuronic acid, galacturonic acid, and mannuronic acids, as well as 3-O-methyl rhamnose, which are also thought to provide protection against desiccation, similar to strategies used by Charophytes. Popper and Fry (2003) also detected XGs in the cell walls of all bryophyte they tested, which have not yet been detected in algae. XGs are hemicelluloses that are believed to be the major load-bearing structure in cell walls of more evolved plant groups (O'Neill and York, 2003). The occurrence of XGs in bryophytes may therefore be indicative of cell wall strengthening compared with algae. Rhamnogalacturonans (RGs) and hydroxyproline-rich proteins (HRPs) both seem to have originated in bryophytes (Matsunaga et al., 2004; Niklas, 2004). Their presence may also have some role in increased strength of cell walls in bryophytes when compared with algae. None of the bryophytes tested to date possesses lignins, but instead may have other phenolic compounds such as lignan or lignin-like polymers (Ligrone et al., 2008; Popper, 2008). Nevertheless, peroxidase enzymes, which are typically involved in lignin biosynthesis, have been detected in some bryophytes (Duroux and Welinder, 2003), suggesting that the lignin biosynthesis machinery might have already started evolving in these early land plants. Mosses, the most evolved group of bryophytes, can be found in very dry habitats. Unavailability of nearby water probably threatened reproduction during the course of the habitat shift, as the gametes and spores of lower groups of bryophytes and all algae are spread by water. The evolution of spore-bearing reproductive organs (sporangia) most probably helped mosses to overcome this limitation. Moss sporangia have specialized cells that cause a sudden rupture of the sporangia and high-speed liberation of spores. Some higher mosses display thick-walled cells that are connected to each other, which could be viewed as the precursors of vascular tissues (Graham et al., 2000; Raven et al., 2005).

Evolution of vascular plants

With the advent of pteridophytes, there was a shift from the gametophytic (haploid) body plan to a sporophytic (diploid) body plan, increasing the chances of genetic diversification and therefore increasing survival (Raven et al., 2005). The diploid sporophytic plant bodies of pteridophytes are bigger in size than the haploid gametophytic bodies of bryophytes. With the move to drier habitats and an increase in height, parts of the plant body became distanced from the water and nutrients, with other parts of the plant no longer having proper access to atmospheric resources. Hence, water pressure and simple diffusion were no longer sufficient for the efficient transport of water in pteridophytes. This situation probably led to the evolution of the compartmentalized body plan and the evolution of vascular tissues, where water, reduced nitrogen and other nutrients are absorbed from soil by roots or rhizoids, while carbohydrates are being produced in green leaves, thallus, or fronds. The physical

separation of needed resources made an efficient transport system necessary and led to further specializations of the cell walls to meet the demands of the new types of transport cells. Within pteridophytes, there was a shift from the lower eusporagiate (sporangium with multiple cell layered epidermis) club mosses to higher leptosporangiate (sporangium with a single cell layered epidermis) ferns and horsetails (Raven et al., 2005). Sporangium with a single layer of epidermal cells ruptures easily and facilitates a better dispersal of spores, and hence increases the chances of survival for a species. The arrangement of vascular tissue (stele) also changed at the eusporangiates–leptosporangiates transition period towrds a stronger stele arrangement.

A major shift in cell wall characteristics can be noticed during these evolutionary steps among pteridophytes, which possibly resulted from changes in growth and survival requirements (Popper and Fry, 2004). While the cell walls of eusporangiates and lower plant groups cannot be clearly differentiated, there occurs a differentiation of cell walls into primary and secondary layers with the advent of leptosporangiate pteridophytes (Popper and Fry, 2004; Harris, 2005). In leptosporangiates and other more evolved vascular plant groups, the primary wall layer is formed during the growth period of the cells, stabilizing the young plant, while allowing continuous growth and development. The secondary layer is deposited on the primary layer only after the cell has stopped growing, and is responsible for providing increased mechanical strength and protection against microbial attack (Raven et al., 2005). The quantities of some hemicelluloses and pectins are markedly different among the primary and secondary layer of the walls, which may correlate with the respective functions of the different wall layers. In leptosporangiates, galactomannan, glucomannan, mannose, galacturonic acid, and 3-O-methylrhamnose are found in lower quantities in the primary cell walls (Popper and Fry, 2004), but some indirect evidence suggests that these polysaccharides are more concentrated in the secondary cell walls (Harris, 2005). This could mean that these hemicelluloses and pectins are not essential for cell wall growth and probably serve towards other functions such a mechanical support and defence that are associated with secondary cell walls. Another hemicellulose, xylan, has been found in high concentrations in secondary cell walls of many vascular plants (Carafa et al., 2005). Lignin has been detected in some leptosporangiates, most probably residing in their secondary cell walls (Harris, 2005). Emergence of lignocellulosic walls must have played a vital role in shaping the plant body at the next steps of evolution.

Emergence of tall, woody plants

The evolution of plants was accompanied by rapid changes in the abilities of microbes and animals to breach the barrier function of cell walls, including the evolution of enzymes capable of degrading plant cell walls (Walton, 1994; Warren, 1996; Chisholm et al., 2006; Jones and Dangl, 2006; Cantu et al., 2008a). Importantly, the evolution of herbivorous animals also must have endangered the lives of smaller plants. Due to their stationary state, plants could not escape such assaults, and were simply eaten in part or as a whole. Such threat of extinction probably led to the evolution of yet other survival strategies in plants such as the evolution of very tall, woody plant bodies. Gymnosperms represent the early tall, woody plant population. They are also the earliest seed-bearing plants. The evolution of seeds provides complete independence from water as the vehicle for reproduction. Hence, seeds are believed to be the most important reason for successful spreading of gymnosperms into diverse and extreme environmental conditions (Raven et al., 2005). Cell wall compositions of gymnosperm seeds are not available, but the analyses of some seedbearing angiosperms show the presence of high amounts of mannose-containing hemicelluloses in the thick nonlignified walls of seeds (Harris, 2005), while some other angiosperm seeds contain large amounts of XGs and starch as storage polymers (Reid, 1985). Such specialized compositions are possibly suited to provide nourishment to the growing embryo, as these sugars are easier to break down compared with lignins found in other parts of the plant. In the earlier gymnosperms such as Cycads, the leaves and seed-bearing cones are present at the top of an unbranched tree that keeps these vital parts away from soil-borne pathogens and herbivores. Higher gymnosperms, such as conifers, are branched (Raven et al., 2005). Branching of the stem was also beneficial for diversification among higher land plants as branching aids in the competition for light and other atmospheric resources, and helps in widespread dispersal of seeds (Graham, 2000). The diameter of xylem tracheids is wider in gymnosperms, which helps in transport of larger quantities of water required for the tall body (Niklas, 1985). The composition of the different specialized body parts, possibly reflecting respective specialization in function.

Cell walls of gymnosperm contain high quantities of cellulose, fucoside XG, glucomannan, homogalacturonans (HGs), RGI and RGII. The XGs, however, have a higher number of fucose side chains, and RGII has a lower number of methylated side chains compared with the less evolved groups of plants (Popper and Fry, 2004; Harris, 2005). Such changes may play a role in the increase in tensile strength of the cell walls to support the tall up-

right plant body and its advanced water transport systems. The cell wall components of gymnosperms, especially the lignins, can withstand high mechanical pressure exerted by the gravitational pull and the load of the tall plant body, and so act as so-called anti-gravitation materials (Volkmann and Baluska, 2006), providing mechanical strength to the tall trees. In addition, gymnosperms contain a high amount of lignins in the secondary walls of their woody stems, which render the cell walls highly recalcitrant. The phenolic compounds are organized into complex polymers through chemical radical reactions, and therefore the polymers do not display a predictable organization for which appropriate enzyme activity could have evolved. Hence, lignin provides not only mechanical strength but also an advanced level of protection from cell wall-degrading enzymes secreted by pathogens (Iiyama et al., 1994). The emergence of woody cell walls has been extraordinarily successful among plant survival strategies, and has resulted in plants that have a life span of hundreds of years while resisting microbial attack and the threat of herbivore animals.

Rapid diversification of flowering plants

While the increase in height among gymnosperms was clearly beneficial for the survival of the plant under low water environmental conditions in the new land habitats, growth and development of such a body is costly for plants (Falster and Westoby; 2003). A decrease in leaf biomass and a slower reproduction rate is common among gymnosperms, which compensates for the costly body plan (Bond, 1989). Several trade-offs evolved in the most modern group of land plants, i.e. the angiosperms (flowering plants). Early angiosperms (basal angiosperms) have larger, branched plant bodies. Among the modern angiosperms, especially in the group eudicots, smaller herbs, shrubs, and trees are predominant. These plants usually have shorter life cycles compared with gymnosperms and basal angiosperms, but they reproduce faster and more efficiently compared with their predecessors, hence enhancing their chances of survival as a species. The evolution of flowers and fruits clearly helped in such rapid life cycles. Diversification among angiosperms may explain the diverse compositions of cell walls among angiosperms (Harris, 2005). Reproduction via flowers and fruits, however, required help from various animals. These plants developed a remarkable set of strategies that allow for successful reproduction, while at the same time avoiding being eaten up by animals. Variations in different cell types, including cell wall characters, are seen among different parts of the same plant, with the stem, branches, and leaves displaying strong, resistant cell walls for steady growth and development. The general composition of cell walls in dicotyledons and non-grass monocotyledons is almost identical to that found in gymnosperms (Popper and Fry, 2004; Harris, 2005), probably reflecting the fact that all these plant groups have a similar body plan and grow in similar environmental conditions. These plant groups all have cellulose microfibrils, and high amounts of XGs, xylans, and pectins including RGII in their cell walls. While the lignins in the secondary cell walls of gymnosperms, from a chemical point of view, have a fairly homogeneous composition containing mainly guaiacyl units, the lignins in angiosperm secondary cell walls are composed of both guaiacyl and syringyl units (Ros Barcelo et al., 2004; Harris, 2005). Syringyl units are hypothesized to be superior to guaiacyl units in their ability to strengthen cell walls (Li et al., 2001). The heterogeneity of the lignin composition in cell walls possibly gave angiosperms a further adaptive advantage over the homogeneous lignin composition of gymnosperm cell walls as the complex nature of lignin in angiosperms makes mechanical and enzymatic breakdown by fungi or insects more difficult (Hatfield and Vermerris, 2001). Interestingly, cell walls of fruits and flowers do not have secondary walls and contain very low amounts of lignins. Instead, they contain a high level of pectins, which can be modified more easily at different stages of plant development (Rose et al., 2003; Harris, 2005), therefore allowing a fine-tuning of cell wall properties during fruit maturation. Such an arrangement is mutually beneficial for the plants as well as for the animals as the animals help in the reproduction of plants by carrying the seeds around, while obtaining easily digestible, nutritious food. This strategy allows a more efficient mechanism of seed dispersal and thus a higher chance for offspring production. Moreover, the evolution of animals that are attracted to the fruits ensures that the vegetative parts of the plant are not eaten up. In addition to tough lignified secondary walls, specialized defence structures such as trichomes, spines, and thorns are found in angiosperms. The cell walls of some of these plant parts contain silica, which is very effective in keeping herbivores away. Moreover, thick cuticles serve as a physical barrier for pathogens (Raven et al., 2005).

Poaceae (the grasses), on the other hand, possess very different cell walls compared with other angiosperms, including other monocotyledons (Carpita and Gibeaut, 1993; Carpita, 1996; Harris, 2005). They usually do not have woody stems or branches, and their walls have low amounts of XGs. The XGs in grass walls are significantly less branched compared with other angiosperms or gymnosperms, and lack fucoside residues altogether (Harris, 2005). Grasses contain few HGs and RGs but instead they have a higher amount of mixed-linkage glucans (MLGs) and xylans (Carpita and Gibeaut, 1993; Carpita, 1996; Harris, 2005; Fry et al., 2008).

Grass cell walls possess a very different composition and possibly a different 3D organization compared with other angiosperms and lower plants, suggesting that the evolution of grasses was a very successful alternative survival strategy for modern land plants. Unlike tall trees, the grasses typically do not spend as much energy on increasing height and mechanical strength. In stead, they are focused on rapid vegetative growth and reproduction strategies that help them in occupying habitats that are unfavourable for trees. Even though grass cell walls have low amounts of XGs, the number of XTH genes for XG modification found in grasses is close to that of eudicots (Popper, 2008). This supports the idea that while grasses apparently have the genetic capacity to form XG- containing cell walls like eudicots, they probably do not have any use for such cell walls and, hence, most of these genes remain inactive in grasses.

Obviously, evolution is a complex process, with divergence and diversification of organisms through time being predominantly guided by natural selection, although we can merely speculate in hindsight about the reasons for the appearance or disappearance of certain components and designs. It is probably fair to say that nature's design of cell walls is the product of a long evolutionary process, that helped in most effective species propagation, and as such a compromise between the different demands on cell wall function, including flexibility for remodelling. From the viewpoint of human use of plant biomass, cell wall design is not optimized. It seems likely that cell wall properties could be altered within limits to be better adaptated to environmental challenges posed by agriculture. Hence, re-engineering of cell walls may be of enormous value in order for the plants to serve specific functions, such as becoming an ideal feedstock for lignocellulose-based biofuel production.

Remodelling and reconstruction of cell walls

Cell wall properties vary not only among different taxa of the plant kingdom, but also within the same plant and throughout the individual plant's life cycle. Variations in cell wall composition have been detected among different organs, different cell types within one tissue, and even within a single cell (Knox, 2008). For example, the quantity and distribution of certain cell wall components in the triangular cell junctions between three cells differ significantly from the flat portions of the walls where two cells are adjoined. Different layers of the cell wall, namely primary cell wall and secondary cell wall layers (S1, S2, and S3) differ in composition and spatial 3D arrangement of wall components. Such diversity in cell walls clearly suggests that instead of a single model of cell walls, there will be a range of representative cell wall models that need to be determined.

Plants have limited resources that they must use towards their growth, development, storage, defence, and reproduction, hence trade-offs became essential (Herms and Mattson, 1992; Graham et al., 2000; Falster and Westoby, 2003). It is therefore not a surprise that the allocation of resources for these various purposes depends on the status f the various tissues and the stage of life, and is reflected in the variability among cell walls within single individual plants. The growth period requires plenty of resources and high flexibility to accommodate expansion. Hence, young plants have only primary cell walls, which are dynamic in nature. In contrast, secondary cell walls are less flexible and therefore their presence in young plants would probably hinder growth. At maturity, maintaining the physiological water and nutrient transport system and strengthening of the defence system inevitably becomes a higher priority for secondary cell walls. Hence, secondary walls are formed in cells that have stopped growing in the mature parts of the plant. Plant growth can continue in the form of a vulnerable new branch if enough resources become available. Cells of such younger growing parts of the plant usually have only primary cell walls.

So far we have depicted cell walls as being rigid structures that once laid down resist any modification. This picture is not entirely true. In fact, cell walls, in particular the primary cell walls, are highly dynamic in nature and are constantly being remodelled (Knox, 2008; Pauly and Keegstra, 2008). Remodelling of cell walls occurs during cell growth, organ development, fruit ripening, and abscission of plant parts such as leaf, flower parts, and fruits at maturity (Knox, 1992; Roberts et al., 2000, 2002; Rose, 2003; Vicente, 2007). Cell walls are modified by specific enzymes such as cellulases, hemicellulases, pectinases, and peroxidases. Small differences in cell wall ultrastructure and chemistry at different stages of the plant life cycle can have profound effects on the variety of functions that the cell walls perform. Cell wall ultrastructure has also been observed to change in response to different environmental conditions, such as during leaf abscission in winter (Roberts et al., 2002) and in response to sudden flooding at warm temperatures and to hypoxia (Gunawardena et al., 2001; Sarkar et al., 2008). Changes in cell wall composition have been detected in response to chemical exposure (Le Van et al., 1994) and pathogen attack (Vidyasekaran, 2002; Salerno and Gianinazzi, 2004).

Variations in cell wall composition and organization across plant species and within the same plant somewhat complicate the studies of cell walls, requiring statistical sampling and approaches that can detect the changes in composition and 3D architecture. Not too surprisingly, our knowledge about cell walls is still far from

complete. It is anticipated that comprehensive imaging of a variety of cell walls, followed by careful comparisons of their similarities and differences, will allow the existing knowledge gaps to be filled with respect to cell wall design.

Cell wall deconstruction

Deconstruction by pathogens and plant defence strategies

While plants have become increasingly successful in protecting themselves throughout evolution, the microbial world co-evolved with the plants and found novel ways to threaten the integrity of plant cell walls (Walton, 1994; Warren, 1996; Cantu et al., 2008b). For example, pathogenshave evolved an array of cell wall-degrading enzymes including cellulases, hemicellulases, pectinases, and lignin modifying enzymes (LMEs). The different enzymes degrade their respective cell wall components with a wide range of efficiency. While pectin and xylan can be degraded relatively easily, lignins prove to be the most resistant component of
the cell wall (Walton, 1994), probably due to the complex organization of the lignin units. The degradability of the wall depends on the total concentration of lignin monomers in the cell wall, its hydrophobicity, as well as the exact nature of the covalent bonds of the cross-links (Grabber, 2005). Occurrences of LMEs are less common than those of the polysaccharide-degrading enzymes. LMEs have been reported only in certain fungi and in some insects. Due to the aromatic nature of the polymer, lignin degradation requires a lot of energy and hence is not a preferred food source. Even the organisms that can produce LMEs only degrade lignin under unfavourable conditions and at a very slow rate (Ros Barcelo et al., 2004; Grabber, 2005). Lignins are typically found to be most concentrated in cells located close to the dermal layers of a stem. Within individual cells, lignins are usually confined to the secondary walls. Such strategic placement towards the outer portion of plants probably evolved to retain protection while allowing the inner portion of the plants to undergo remodelling and continue their cellular and physiological functions (Grabber, 2005). Cell wall degradation by depolymerizing enzymes results in the formation of so-called active oligosaccharins, which are thought to play important roles in signalling and activate the defence mechanisms of the cell (Darvill et al., 1992; Dumville et al., 1999; Vorwerk et al., 2004).

Deconstruction by herbivores

As the cell wall structure became ever more sophisticated, pathogens and herbivores evolved their own strategies to breach the protective barriers. A number of animals feed exclusively on grass or wood, with the best studied examples being cows and termites, respectively. Animals themselves do not contain the enzymatic make-up for cell wall degradation, but instead entered symbiotic relationships with microbial communities specialized in anaerobic lignocellulose breakdown (Flint et al., 2008). The host may even provide the community with reduced nitrogen and other essential minerals, which are low in the plant biomass. These communities can be of remarkable complexity. Intriguingly, metagenomic sequencing of the termite hind gut revealed ~200 microbial species to participate in this complex community. However, it remains unclear if all ~200 species are needed for lignocellulose degradation or whether a small subset would be sufficient. Only high- resolution spatial mapping of the individual species in the termite gut and the cow rumen communities can reveal their interactions and, together with the knowledge of their respective physiological repertoire, will illustrate their interdependencies. The herbivore animal provides a mechanical and chemical pre-processing of the biomass that allows efficient microbial degradation, and provides a protected stable environment.

Economic importance of cell wall deconstruction

One could argue that human evolution would not have been possible were it not for plants. Throughout the history of human civilization, plants have served as a source of energy (firewood), building material, and medicine. More recently, our civilization has come to recognize that energy production through the burning of fossil plants, such as coal or oil, is no longer sustainable and has led to climate change. Global warming, limited supplies of fossil fuels, and fluctuating fuel prices have resulted in a revived interest in renewable energies, with a new focus on various transportation fuels derived from lignocellulosic plant biomass (Ragauskas et al., 2006; Himmel et al., 2007; Pauly and Keegstra, 2008; Sticklen; 2008). Ethanol as a transportation fuel is currently being produced in modest quantities from the readily extractable sugars of corn and sugar cane, but such efforts are not scalable and sustainable to meet the increasing need for carbon-neutral biofuels. Plant cell walls represent one of the most abundant renewable resources on this planet. Switchgrass and poplar trees have been identified as possible energy crops since they do not require intense farming and grow in marginal lands with low water and/or nutrient

needs. However, due to their recalcitrance and the low yields of fermentable sugars, biomass is currently not a viable alternative for biofuel production. Various physical and chemical pre-treatment steps are currently being explored to allow for subsequent enzymatic cell wall degradation, but such pre-treatment approaches often lead to undesired by-products that are toxic to microbial fermentation. One possible solution is the re-design of plant cell walls, e.g. by replacing the recalcitrant lignins with other phenolic compounds that can be degraded more easily while still supporting tall growth of the plants and resisting unfavourable weather and climate conditions, as well as microbial attack. However, with >1000 gene products estimated to be involved in cell wall formation and remodelling, traditional approaches such as systematic knockout studies are likely to fail. Therefore, it seems crucial to understand better the design principles that guide the many functions of cell walls (Himmel et al., 2007; Pauly and Keegstra, 2008). Both the increase in wall biomass by means of genetic modification, and rendering plants more susceptible to degradation without compromising the life cycle of a plant require a thorough integrative biophysical, developmental, and genetic knowledge of the composition as well as a molecular-resolution 3D structure of the plant cell wall (Somerville et al., 2004).

Armed with such detailed knowledge the large quantities of lignocellulosic waste created through forestry, agricultural activities, and industrial processes, such as breweries, paper-pulp, textile, and timber industries, could be turned into biodegradable biomass (Levine, 1990). With appropriate technology, such biomass could be converted into valuable products such as biofuels, chemical precursors, and cheap energy sources for fermentation, as well as improved animal feeds and human nutrients (Howard et al., 2003; Okafor et al., 2007). Fibres, which are traditionally used in the textile industry, have become of increasing interest in the development of agro-materials for the automobile and building industries. However, there is still a lack of a comprehensive understanding of the link between 3D structures and physicochemical characteristics of the fibres. Likewise, the increasing demands for paper further shows the need to understand the 3D structure of non-woody sources (Khalil et al., 2006)

Moreover, since fungal pathogens, and bacterial and virus infections are known to cause devastating annual crop losses worldwide, detailed knowledge about cell wall lignin and polysaccharide organization might help geneticists engineer less vulnerable cell walls (Dey and Harborne, 1997). While attempts have been made to characterize the modification of the content of the cell wall during ripening of fruits, including the intricate genetically programmed biochemical pathways involved in this process (Giovannoni, 2001), there is still a lack of a detailed understanding about cell wall degradation in the process of fruit softening. Detailed knowledge of the underlying enzymatic and regulatory mechanisms would greatly benefit the storage and transportation of fruits (Tucker and Seymour, 1991) and would have significant commercial impact, as fruit ripening dictates the harvesting time and might allow subsequent handling without damage.

The quest for realistic cell wall models

It may be clear from the previous sections that realistic representative models of cell wall architecture are needed in order to understand and predict the physical properties of the wall polymers, their relationship with the biochemical inventories, and the molecular strategies of the plant cell and/or microbial attackers. Cell walls have been a prominent area of research for a long time, but most of the research has been concentrated on either biochemical analysis or genetic studies.

Biochemical studies have been invaluable to provide detailed information about the chemical nature of the different types of cell wall components, typically by employing organic chemical or enzymatic reactions to break down the complex biopolymers, followed by separation and identification of the breakdown products, e.g. by gas chromatography. While biochemical analysis can yield the composition as well as the stoichiometry of cell wall component monomers and oligomers, analysis of the intact polymers is complicated by polymer insolubility, hence rendering fractionation, purifica tion, and chemical analysis more complicated.

Biochemical analyses have been complemented by genetic analyses, which have identified a number of genes associated with cell wall synthesis and function. Because of the vast numbers of proteins involved in cell wall formation, genetic dissection of the regulation and breakdown pathways is daunting and on its own unlikely to succeed. What is still lacking is a detailed understanding of how the identified gene products utilize the chemical components and arrange them spatially in order to achieve such multifunctional cell walls, in part due to limited knowledge about temporal and spatial patterns of protein and carbohydrate subcellular localization. Nevertheless, a consensus model of plant cell walls has emerged.

Current cell wall model

The current 'general' model of the cell wall features the cellulose being organized into a microfibril scaffold, which confers mechanical strength to the primary cell wall. Cellulose fibres act not unlike steel girders stabilizing a skyscraper's structure (Somerville et al., 2004). Tracks of parallel cellulose microfibrils are laid down in a highly coordinated manner, presumably due to the interaction of the CeS with the underlying cortical microtubule network (Paredez et al., 2006; Lloyd and Chan, 2008). Though the causative relationship between microtubule organization and microfibril orientation is still hotly debated, many researchers hypothesize that a dynamic alteration of the microtubule network is responsible for a change in the directionality of the nascent microfibril scaffold (Somerville, 2006). Recent data suggest that the microfibril's crystalline core is surrounded by a paracrystalline layer of cellulose, followed by hemicellulose, a ramified polymer composed of pentoses and hexoses (Himmel et al., 2007). In secondary cell walls, hemicellulose is thought also to form covalent links with lignin. Lignins are rigid aromatic polymers, whose 3D structure and organization is poorly defined due to the radical chemistry nature of lignin polymer formation. Both the highly organized crystalline structure of cellulose and its tight association with hemicellulose and lignin are physical, steric, and biochemical obstacles for cell wall breakdown.

The current model of cell walls represents an educated guess on the cell wall organization that presumably is not too far off from reality. However, if one wants to redesign cell walls for biofuel or agricultural applications, one requires models that are based on direct experimental data with respect to exact chemical composition and macromolecular 3D structural organization. While invaluable, and clearly the foundation on which future cell wall models will stand, biochemical and genetic analyses alone do not suffice to describe the vast diversity in cell wall organization expected to be found to differ significantly between plant species, within the same plant, organ, tissue, cell, and even neighbouring portions of the same cell wall.

To gain direct experimental insight into cell wall composition and 3D architecture and to deal with the heterogeneity of cell wall designs, biochemical and genetic analyses need to be complemented with advanced microscopic imaging. To date, a small but important number of conventional electron microscopy (EM) and spectroscopy studies on cell walls have helped answer some of the coarse architectural questions, but still a huge knowledge gap persists with respect to the exact molecular organization of cell walls and its regulation (Somerville et al., 2004; Knox, 2008).

Contribution and shortcomings of current imaging approaches

To date, most studies have focused on the dimensions of cellulose microfibrils, whereas little is known about the structural relationship of cellulose, hemicellulose, and lignin within the different layers of the wall, which reflects the difficulties in studying such interaction with current technologies. Atomic force microcopy (AFM) imaging of the maize parenchyma cell wall surface in combination with earlier immuno-transmission electron microscopy (TEM; Kimura et al., 1999) data suggests a 36-chain elementary fibril model (Ding and Himmel, 2006). The authors hypothesize that the elementary fibril is synthesized from rosettes of CesA protein complexes producing 36 b-D-glucans. These 36 glucan chains are thought then to assemble into a crystalline core and a subcrystalline structure of the elementary fibril via hydrogen bonds and vander Waals forces. However, it remains unclear whether and how the composition of the elementary fibrils changes during cell wall growth. TEM and nuclear magnetic resonance (NMR) of some angiosperms have estimated that the cellulose crystallites are 2–5 nm in diameter with disordered surface chains. Since TEM preparations involve solvent extractions that introduce disorder or may remove non-crystalline layers altogether, it is not yet confirmed that these structural disorders indeed exist in native plant cell wall microfibrils, or are a result of the sample preparation process. Ding and Himmel (2006) visualized the parenchyma cell walls in different growth stages, and found that as the cells expand, more cell wall components are deposited on the inner faces in a directional way. Microfibril aggregates (macrofibrils) have been examined by NMR (Hult et al., 2001a, b), scanning probe microscopy (Hanley and Gray 1994, 1999; Fahlen and Salmen 2002, 2003), field emission scanning electron microscopy (FESEM) (Kataoka et al. 1992; Daniel and Duchesne, 1998; Duchesne and Daniel, 1999; Awano et al., 2000; Duchesne et al., 2001; Daniel et al., 2004a, b), and TEM (Fengel, 1970; Bardage et al., 2003; Daniel et al., 2004b). Donaldson (2007) visualized the organization of macrofibrils in different cell wall types comparing normal and reaction wood of radiata pine and poplar as examples of a typical softwood and hardwood respectively using a combination of FESEM and environmental scanning electron microscopy (SEM). They showed the size of macrofibrils to range typically from 10 nm to 60 nm in diameter and to vary between different cell wall types and even slightly between adjacent cells of the same cell wall type. Resin etching was applied in combination with FESEM and allowed macromolecular structures smaller than ~10 nm in diameter to be resolved. Donaldson (2007) also observed that

macrofibrils occur predominantly in a random arrangement, although radial and tangential lamellae may sometimes be seen in individual cells.

Most of the imaging studies have focused on microfibrils, whereas visualization of hemicellulose and lignin remains to be accomplished. Biochemical and genetic studies of lignin mutants (Hu et al., 1999) have been complemented by a few targeted imaging studies, which to date yielded valuable yet limited insight of the role of lignin in the 3D architecture of the cell wall (Fromm et al., 2003).

Towards realistic models of the plant cell wall through integrated imaging

Since there is no single imaging technique—currently being deployed or foreseeable—that on its own can provide both chemical and architectural information at the same time, combining a variety of imaging approaches seems to be the most promising path forward to gain the desired molecular understanding of plant cell wall design. From an un derstanding of design principles, it might be possible to predict and possibly alter cell wall properties. Naively spoken, the hope is that the task is similar to calculating the statics for a skyscraper building and to estimate what forces the various building materials and building designs can withstand. Although undoubtedly, given the diversity and dynamic nature of cell walls and the uncertainties associated with the precise mechanical properties of the cell wall components, calculating the physical properties of cell walls is far more complicated.

Currently, we have substantial amounts of biochemical information with respect to overall cell wall composition, and relative molecular ratios of its constituents, but this information was derived from bulk measurements and therefore represents at best an average, and from different specimens, and tissues at different growth stages. Moreover, biochemical analysis speaks little of the 3D organization of the polymers. What has not yet been accomplished, but is clearly needed, is the integration of biochemical, genetic, and imaging information. Since no single imaging technique can provide all of the desired information, it seems essential to image the same samples by multiple imaging modalities, at multiple levels of scale and resolution, followed by integration of the information obtained by each imaging modality. It is believed that a target correlative multimodal, multiscale in situ imaging study as illustrated in Fig. 4 will help bridge this gap. In order to achieve integration of different imaging modalities, a variety of technical and organizational obstacles need to be overcome, including the development of common sample preparation methods for multimodal imaging, the development of cross-modality- specific labels, correlative widefield 2D and 3D imaging using optical microscopy (Raman, UV, super-resolution) and EM, data integration, and analysis.

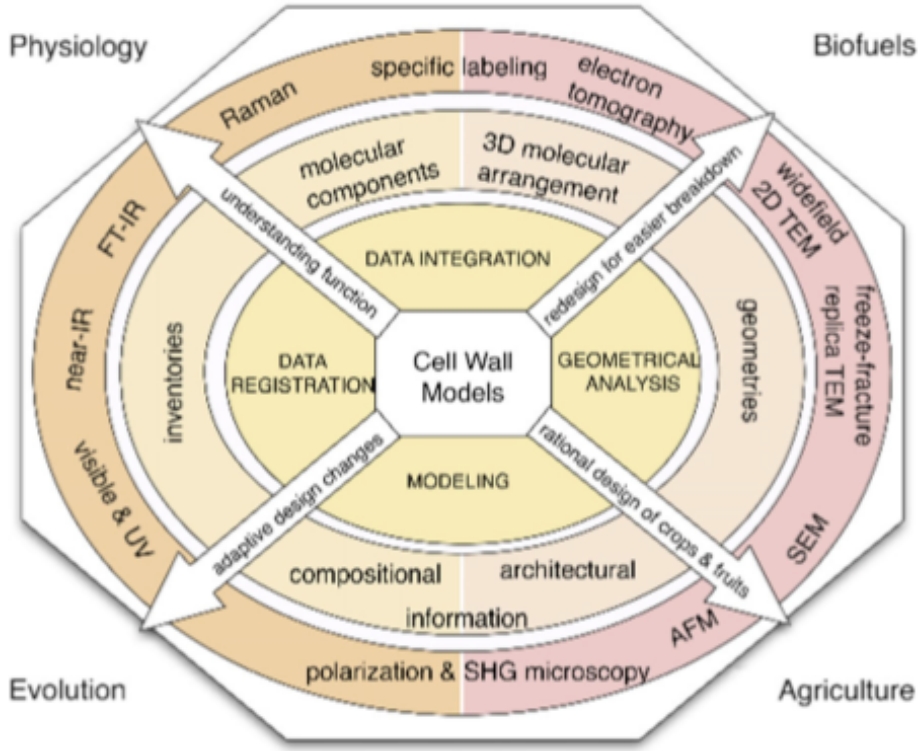

Fig. 4. Summary of the comprehensive, multiscale, multimodal approach that we think is needed to produce a realistic cell wall model and its implications. Integration of the data obtained from different image modalities on the exact same specimen will

provide the exact chemical composition, as well as geometrical constraints that will yield cell wall models, which will reflect the expected diversity of cell walls. TEM, transmission electron microscopy; SEM, scanning electron microscopy; AFM, atomic force microscopy; SHG microscopy, second harmonic generation microscopy; FT-IR, Fourier transform infrared; near IR, near infrared; UV, ultraviolet.

Clearly, both compositional and architectural information is needed from the same specimen. The inventories of molecular components, both carbohydrate polymers and proteins, are needed, but geometries are also needed to determine how the molecular components are arranged in 3D. For precise modelling of cell wall architecture and prediction of cell wall properties, both the parts and the building instructions are needed, including spatial and temporal patterns of precise localization and molecular interactions. Compositional information can be obtained through a variety of optical imaging approaches such as diffraction-limited and super-resolution fluorescence microscopy, visible and UV optical, near infrared (IR), Fourier transform infrared (FT-IR), Raman spectroscopy, and imaging, as well as specific labelling approaches. Architectural information can be obtained though polarization and second harmonic generation (SHG) microscopy, which exploits the effect of preferentially organized macro-molecules (such as the microfibrils) on polarized light, possibly small-angle X-ray scattering analysis, as well as a variety of higher resolution surface scanning and transmission microscopies. AFM detects topological profiles, which with appropriate cantilever tip geometries can yield near-atomic resolution. AFM has been successfully used for the study of Venericardia ventricosa cellulose microfibrils (Hanley et al., 1992; Baker et al., 2000; Lee et al., 2000), pulp fibres (Kirby et al., 1996), cotton fibres (Baker et al., 1997), and plant cell walls (Morris et al., 1997; Vincent et al., 1999). SEM uses backscattering properties of heavy atom-coated surfaces to determine topologies, not unlike AFM, with nanometre resolution, and allows heavy atom elemental analysis. However, unlike AFM, SEM is highly invasive and cannot be done on living samples, and often requires extensive sample preparation. Like AFM, SEM does not yield information about the inside of an object and is strictly a surface technique. Freeze-fracture sample preparation as well as deep-etching electron microscopy can overcome the problem of access to the cell interior and may be of particular interest for cell wall analysis; however, precise location of the fracture lines cannot be predicted, which then leaves TEM as the method of choice for ultrastructural analysis. Alternatively, while being far from a routine imaging tool, dual-beam focused ion beam (FIB)/ SEM has the potential to provide continuous 3D information over a large depth range.

The disadvantage of TEM is that it requires extensive sample preparation, is a destructive technique, and cannot study live specimens, although in-depth analysis of certain time points allows detailed snapshots of a complex biological process to be obtained. Pure ultrastructural analysis ideally is complemented by specific labelling approaches, with conventional immunoaffinity probes being the most commonly deployed. However, due to the shortcomings of affinity probes, including target accessibility and target affinity retention, and the often necessary compromises in ultrastructural preservation, multiple efforts are underway to develop genetically encoded tag-based approaches, which promise higher sensitivity and coverage and which are compatible with exquisite sample preservation. TEM imaging approaches require sophisticated sample preparation, with biological samples being imaged either as whole mounts or as sections, depending on the size of the specimen. Whole mount imaging is typically done in a frozen-hydrated state using rapid liquid ethane plunge-freezing of the sample on a microscope grid, although the less challenging alternative approaches such as negative and positive staining may also be sufficient. Often biological specimens need to be sectioned in order to allow the electron beam to penetrate the sample, and to avoid multiple scattering. Samples are either sectioned in their frozen state or embedded in a hardened plastic resin. In either case, samples are typically ultra rapidly frozen, e.g. by using high-pressure freezing, and then either sectioned at liquid nitrogen temperature or subjected to freeze-substitution, where the cellular water is replaced by an organic solvent in order to allow subsequent plastic resin infiltration, embedding, and polymerization. Freeze-substitution and resin embedding is by far the easiest approach and allows the addition of staining reagents that provide higher contrast in the resulting EM image. Freeze-substitution typically avoids the issues often associated with conventional sample preparation, including aggregation and extraction due to the low temperature at which water-to-organic solvent exchange occurs. Lengthy resin-embedding protocols can also lead to extraction artefacts, and hence microwave- assisted sample preparation protocols in our hands have proven to be very valuable for cell walls, but not for retention of cytoplasmic ultrastructural details. To achieve vitrification of the cellular ice, and therefore the avoidance of damaging ice crystal formation, one usually resorts to a cryo-protectant in addition to the high-pressure conditions present during flash-freezing. The presence of cryo-protectants can disturb tissue osmolarity and lead to unwanted effects. A special case of cryo-sectioning is the Tokuyasu approach where chemically fixed biomass is infiltrated with high levels of sucrose which acts as a cryo-protectant, frozen, and sectioned in a frozen-hydrated state only to be thawed afterwards and subjected to on-section labelling. This approach can result in vastly improved epitope recognition, but suffers from overall lower ultra structural preservation and lower contrast. Samples that

are prepared for TEM imaging can then be subjected to widefield 2D TEM imaging, resulting in large areas of high-resolution high content image montages and/or to electron tomography imaging that allows the visualization of the 3D molecular organization of macromolecular machines in their native cellular context.

There are multiple challenges of multiscale multimodal imaging of a single specimen. The development of sample preparation protocols that are compatible with the different imaging modalities and the development of probes that work across imaging platforms and modalities are two of the major problems. Post-imaging includes overlay of the various imaging information, which requires image data visualization, data registration, geometrical image and data analysis, feature extraction and annotation, as well as data integration and subsequent derivation of models. However, if successful, realistic cell wall models based on qualitative and quantitative information will allow a fundamentally improved understanding of cell wall physiology and function, and will probably allow biologists to reconstruct the adaptive design changes that occurred throughout evolution. Such models will result in a more rational design of cell wall properties of crops and fruits for agriculture and finally allow the re-design of plant cell walls for easier breakdown to enable biofuel production.

A number of the technical components to achieve the ambitious goal of integrated imaging already exist and have been successfully deployed in the past to the study of cell walls. For example, FTIR microspectroscopy has been used to determine the major chemical components present in plant cell walls (Sene et al., 1994; Mouille et al., 2003; McCann et al., 2007). Raman microscopy, due to the shorter wavelength, can achieve a slightly higher resolution at ;500 nm. Surface-enhanced Raman imaging promises to improve the resolution to 30–50 nm; however, the feasibility of this approach for biofuel research remains to be proven but is currently under development. The main advantage of such spectrum-based approaches is that intact cell walls can be analysed and the spatial distribution of their components can be visualized albeit at somewhat low resolution without the need for affinity probes. Several studies identified chemical signatures of different cell wall types, including the localization of cellulose and lignin in Picea mariana, Pinus and Populus (Agarwal, 2006; Gierlinger and Schwanninger, 2006). Another spectroscopic technique that has been successfully applied to the study of cell wall is solid-state cross-polarization magic-angle spinning ^{13}C-NMR spectroscopy. Applied to primary cell walls from a range of angiosperm species, this technique showed that all of the cellulose is in a crystalline state in the form of cellulose I. The calculated cross-sectional dimensions of the cellulose I crystallites from all cell wall sources was found to be in the range of 2–3 nm (Vieter et al., 2002). Both NMR and FTIR spectroscopy have suggested that the cellulose microfibrils contain both crystalline and paracrystalline regions, exhibiting highly disordered structures (Kataoka and Kondo, 1998; Sturcova et al., 2004). However, the relationship between the crystalline cellulose structure and the non-cellulosic poly saccharides remains to be determined (Ding and Himmel, 2006).

Where available, affinity probes can be used for specific labelling of cell wall components which can be visualized by either optical or EM imaging. Traditionally, histochemical stains have been employed to distinguish between different categories of cell wall components such as hemicellulose, pectin, lignin, and glycoproteins. However, in most cases the partial removal of cell wall components was needed to allow these reagents access to their respective target (Krishnamurthy, 1999). By employing antibodies specific for certain cell wall components followed by EM analysis, one can localize the various molecules with high precision within the cell wall. However, such immunohistochemical labelling methods often suffer from the fact that only a small fraction of all epitopes are accessible and recognized by the specific antibody, and optimization of the labelling protocols are tedious and error prone. For some components such as lignin there is no specific antibody, which may be due to the fact that lignin polymerizes through radical formation, which makes the formation of unique epitopes far less likely. A promising set of affinity tools are the carbohydrate-binding modules (CBMs), which are non-catalytic proteins collectively covering a wide range of cell wall polysaccharides. CBMs have been found to have the capacity of distinguishing between in vitro and in vivo forms of the same polysaccharides (McCartney et al., 2006). CBMs nevertheless face challenges similar to antibodies, including accessibility to their substrate and low stoichiometry. All these approaches allow one to probe for the presence of certain candidate components, with some diffraction-limited spatial localization, although recent attempts to use super-resolution microscopy, e.g. PALM imaging, with appropriately modified CBMs appear promising for molecular resolution imaging of the microfibril nextwork (Liphardt et al., unpublished).

For the most part, in order to achieve molecular resolution, TEM is still unsurpassed. Hence, TEM has been the primary study method of in situ cell wall structure for many decades. However, conventional 2D TEM imaging runs into two problems: (i) only small, ideally representative areas of the sample are imaged at high resolution, therefore preventing an adequate statistical analysis; and (ii) the areas imaged constitute a 2D projection of a 3D volume onto the 2D area of a film or CCD camera. Therefore, multiple molecular layers contribute to the final image that for this reason can be difficult to interpret. The recently developed high-resolution wide-field imaging and EM tomography can overcome these two limitations of conventional EM, respectively.

In both approaches, several hundred CCD images are collected and then either assembled into a 2D projection montage or used for a 3D reconstruction of the cellular volume, leading to large amounts of data that require sophisticated computational support for interactive visualization and semi-automated image analysis. EM tomography is of particular interest as it should allow the visualization of the 3D microfibrillar network, as long as the individual microfibrils are at least ~2 nm in diameter and spaced >2–3 nm apart from each other. Any polymer <2 nm in diameter will probably only be visible if it can be stained selectively with a high electron contrast reagent. Moreover, in EM the entire cellular ultrastructure including microtubules, vesicles, mitochondria, chloroplasts, Golgi, and endoplasmic reticulum membrane systems as well as the size, shape, and distribution of large macromolecular machines are readily visible, resulting in high-content data sets that can be examined for a variety of biological questions. EM tomography of resin-embedded samples has been successfully applied to study the 3D organization of the chloroplast thylakoid membranes in lettuce leaves (Shimoni et al., 2005) as well as of the cellulose microfibrils in the S2 layer of the secondary cell wall in Pinus wood tissue (Xu et al., 2007).

Ultrastructure of plant cell walls: sample preparation and TEM imaging

One challenge associated with conventional TEM imaging arises from the fact that electrons can only penetrate specimens thinner than ;1 lm, hence requiring tissue samples to be sliced very thinly, typically some 70–100 nm. Another challenge stems from the fact that the plant tissue needs to be exposed to the high vacuum of the electron microscope, resulting in the immediate evaporation of cellular water and hence the drying out of the plant tissue. One solution to these two problems is to embed the biological specimen in a plastic resin, which is then polymerized into a hard block, which is then suitable for sectioning and resistant in the vacuum of the TEM. This approach typically requires a controlled dehydration of the tissue using organic solvents such as ethanol or acetone because resins are not directly miscible with aqueous solutions and hence would not infiltrate the tissue. In our view, it is this step in the sample preparation that is the most detrimental to ultrastructural preservation as exposure to organic solvents denatures proteins and leads to aggregation artefacts often detectable in conventionally prepared probes. Despite glutaraldehyde fixation steps in conventional protocols, the dehydration and resin infiltration, particularly if carried out at room temperature, lead to aggregation and/or extraction, and therefore to a substantial alteration of the ultrastructure. One can largely overcome such complications by carrying out the dehydration at temperatures well below the freezing point of water (Steinbrecht and Mu¨ ller, 1987; Kellenberger, 1987, 1990; Dahl and Staehlin, 1989; McDo nald, 1994; McDonald and Auer, 2006). This is typically accomplished by the instantaneous vitrification of tissue up to ~200 lm thickness through high-pressure freezing (HPF), followed by the freeze-substitution (FS) protocols. Samples are then infiltrated with resin at either low temperature or room temperature, and the resin is then polymerized either by UV light or thermally, respectively. To enhance the contrast, heavy metal ion solutions can be applied at various steps of the protocol. Osmium tetroxide and uranyl acetate, which are standard EM stains, primarily stain proteins, membranes, and nucleic acids, but to a far lesser degree carbohydrates, resulting in very low contrast.

While every specimen presents its own challenges, and therefore one cannot easily generalize, it has been found that plant cell walls are typically well preserved by either microwave-assisted room temperature sample preparation or the more sophisticated high-pressure freezing and freeze substitution approach, whereas faithful preservation of the cytoplasm depends crucially on the latter. Microwave assisted processing speeds up fixation, dehydration, and infiltration, and therefore avoids lengthy exposure to extracting reagents. High-pressure freezing avoids aggregation artefacts, which otherwise dominate in the cytoplasm, but no systematic difference in microfibril organization between high-pressure frozen, freeze-substituted, and microwave-processed samples was found, except for a possibly higher rate of extraction in microwave-processing (unpublished observation). Upon 3D imaging, microfibrils appear highly organized in both sample preparation scenarios, and individual cross-linked strands of ;3nm in diameter can be readily resolved; hence, we are confident that both approaches do not suffer from molecular aggregation, presumably reflecting the highly cross-linked nature of plant cell walls. Often, internal membranes are not readily visible in HPF/FS samples, but contrast can be improved by the retention of small amounts of water throughout sample preparation or the addition of tannic acid during freeze-substitution. Also, it should be emphasized that contrast in resin-embedded samples arises from the binding of heavy metal atoms to macromolecules. These methods have been optimized over the last five decades for the visualization of nucleic acids, proteins, and membranes, with carbohydrates being somewhat less represented in the standard processing protocol, though certain ‘carbohydrate-specific’ stains are available.

As an alternative to chemical fixation, dehydration, and resin embedding, high-pressure frozen tissue samples can be sectioned in their frozen-hydrated state at 30–100 nm, and then kept at liquid nitrogen temperature for cryo-EM imaging (Al-Amoudi et al., 2003), where the contrast is independent of heavy metal

binding affinity but instead is the result of inherent molecular density. This approach, while having been successfully applied to a number of prokaryotes, yeast, and some mammalian tissue, is technically very challenging, yet promising, and, given the staining behaviour of cell wall polymers in conventional approaches, cryo-sectioning followed by cryo-tomography is of particular interest in the study of the cell wall 3D architecture.

Very recently yet another tool has been added that may allow electron microscopists to obtain ultrathin frozen- hydrated samples: vitrified samples can be subjected to focused ion beam milling, resulting in ultrathin cryo- sections (Marko et al., 2007). Independently of the exact sample preparation protocol, electron microscopic 2D and 3D imaging will probably retain a dominant role for imaging cell walls. An alternative approach to resin embedding or cryo-sectioning is freeze-fracture, where frozen samples are fractured in a vacuum, typically along cellular discontinuities such as membranes or cell walls. Onto the freshly exposed surface one typically evaporates a thin layer of carbon, followed by platinum. Once the organic material is digested away by acid, one is left with a replica of the fracture surface that can be examined by TEM. Freeze-fracture imaging, while rarely performed these days, has revealed the rosettes of the cellulose synthase (Mueller and Brown, 1980), which remains one of the key pieces of evidence for the presumed organization of the cellulose-forming protein complex.

Roadblocks and future outlook

Given all these sophisticated approaches for cell wall characterization, why is it that we do not yet have a model that is grounded in direct ultrastructural evidence? The answer may lie in the complexity and diversity of plant cell walls as well as the small number of investigators and suitable techniques, some of which are very recent or still in the process of being developed. With the renewed interest in lignocellulosic biofuels and the resulting surge in funding, more investigators have become interested in the problem, and hence it is very likely that enormous progress will be made within the next couple of years. However, we think that there is another reason why progress has been slow. Since no single technique can give all the information necessary for a comprehensive cell wall model, it requires the teamwork of a variety of imaging and computer science experts. They must strive to integrate the information from various imaging modalities by subjecting the exact same sample to different imaging approaches, and superimpose and integrate the complementary data sets into a model. Through sample preparation that is compatible with all the different imaging modalities, the details that are needed for a sophisticated model as well as statistics of the homogeneous and therefore representative data will be obtained. For example, we will need to determine the range of cellulose microfibril dimensions and distances, the range of their next neighbour angles, as well as the degree of order and organization of the cell wall components on a local, regional, or global scale. Such integrative multiscale, multimodal imaging combined with sophisticated modelling lies at the heart of our quest for a realistic cell wall model, and we are just beginning now to put the pieces of the puzzle together.

Preliminary widefield TEM imaging of different plant species suggests that there is not a single design that fits all plant cell walls, but instead that we are even likely to find differences within one cell wall, between neighbouring cells, between different tissues, between different plants, and at different times in the life cycle of the plant. Nevertheless, it now seems that we are within reach of having the first model of plant cell walls and its building principles derived from direct experimental data. Science and technology have come a long way since ‘On the origin of species’ appeared in print, but we still marvel at the beautiful designs of plant cell walls that have evolved over the last billion years, and which provide the competitive advantage that will allow the continued survival of the species.

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References

Agarwal UP. 2006. Raman imaging to investigate ultrastructure and composition of plant cell walls: distribution of lignin and cellulose in black spruce wood (Picea mariana). Planta 224, 1141–1153.

Al-Amoudi A, Dubochet J, Gnaegi H, Lu¨ thi W, Studer D. 2003. An oscillating cryo-knife reduces cutting-induced deformation of vitreous ultrathin sections. Journal of Microscopy 212, 26–33.

Awano T, Takabe K, Fujita M, Daniel G. 2000. Deposition of glucuronoxylans on the secondary cell wall of Japanese beech as observed by immuno-scanning electron microscopy. Protoplasma 212, 72–79.

Baker AA, Helbert W, Sugiyama J, Miles MJ. 1997. High-resolution atomic force microscopy of native Valonia cellulose I microcrystals. Journal of Structural Biology 119, 129–138.

Baker AA, Helbert W, Sugiyama J, Miles MJ. 2000. New insight into cellulose structure by atomic force microscopy shows the Ia crystal phase at near-atomic resolution. Biophysical Journal 79, 1139–1145.

Bardage S, Donaldson LA, Tokoh C, Daniel G. 2003. Ultrastructure of the cell wall of unbeaten Norway spruce pulp fibre surfaces: implication for pulp and paper properties. Nordic Pulp and Paper Research Journal 19, 448–452.

Becker B. 2000. The cell surface of flagellates. In: Leadbeater BSC, Taylor JCG, eds. The flagellates: unity, diversity,and evolution. New York: Taylor and Francis, 110–123.

Bond WJ. 1989. The tortoise and the hare: ecology of angiosperm dominance and gymnosperm persistence. Biological Journal of the Linnean Society 36, 227–249.

Cantu D, Vicente AR, Greve LC, Dewey FM, Bennett AB, Labavitch JM, Powell ALT. 2008a. The intersection between cell wall disassembly, ripening, and fruit susceptibility to Botrytis cinerea. Proceedings of the National Academy of Sciences, USA 105, 859–864.

Cantu D, Vicente AR, Labavitch JM, Bennett AB, Powell ALT. 2008b. Strangers in the matrix: plant cell walls and pathogen susceptibility. Trends in Plant Science 13, 610–617.

Carafa A, Duckett JG, Knox JP, Ligrone R. 2005. Distribution of cell-wall xylans in bryophytes and tracheophytes: new insights into basal interrelationships of land plants. New Phytologist 168, 231–240.

Carpita NC. 1996. Structure and biogenesis of the cell walls of grasses. Annual Review of Plant Physiology and Plant Molecular Biology 47, 445–476.

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

Chisholm ST, Coaker G, Day B, Staskawicz BJ. 2006. Host–microbe interactions: shaping the evolution of the plant immune response. Cell 124, 803–814.

Cook ME, Graham LE. 1998. Structural similarities between the surface layers of selected Charophycean algae and bryophytes and the cuticles of vascular plants. International Journal of Plant Science 159, 780–787.

Cosgrove DJ. 2000. Expansive growth of plant cell walls. Plant Physiology and Biochemistry 38, 109–124.

Cosgrove DJ. 2003. Expansion of the plant cell. In: Rose JKC, ed. The plant cell wall. Boca Raton, FL: CRC Press, 237–263.

Dahl R, Staehelin LA. 1989. High-pressure freezing for the preservation of biological structure. Theory and practice. Journal of Electron Microscopy Technique 13, 165–174.

Daniel G, Duchesne I. 1998. Revealing the surface ultrastructure of spruce pulp fibres using field emission-SEM. In: Proceedings of the 7th International Conference on Biotechnology in the Pulp and PaperIndustry. Vancouver, Canada, 81–85.

Daniel G, Duchesne I, Tokoh C, Bardage S. 2004a. The surface and intracellular nanostructure of wood fibres: electron microscope methods and observations. In: Schmitt U, Ander P, Barnett JC, Emmons AMC, Jeromidis G, Saranpaa P, Tschegg S, eds. Proceedings of COST action: wood fibre cell walls: methods to study their formation, structure and properties. ESF, EC, Brussels, 87–104.

Daniel G, Volc J, Niku-Paavola ML. 2004b. Cryo-FE-SEM and TEM immuno-techniques reveal new details for understanding white-rot decay of lignocellulose. Comptes Rendus Biologies 327, 861–871.

Darvill A, Augur C, Bergmann C, et al. 1992. Oligosaccharins—oligosaccharides that regulate growth, development and defense responses in plants. Glycobiology 2, 181–198.

Dey PM, Harborne JB. 1997. Plant biochemistry. London: Academic Press.

Ding SY, Himmel ME. 2006. The maize primary cell wall microfibril: a new model derived from direct visualization. Journal of Agricultural and Food Chemistry 54, 597–606.

Donaldson L. 2007. Cellulose microfibril aggregates and their size variation with cell wall type. Wood Science and Technology 41, 443–460.

Duchesne I, Daniel G. 1999. The ultrastructure of wood fibre surfaces as shown by a variety of microscopical methods—a review. Nordic Pulp and Paper Research Journal 14, 129–139.

Duchesne I, Hult EL, Molin U, Daniel G, Iversen T, Lennholm H. 2001. The effects of hemicellulose on fibril aggregation of Kraft pulp fibres as revealed by FE-SEM and CP/MAS 13C-NMR. Cellulose 8, 103–111.

Duchesne LC, Larson DW. 1989. Cellulose and the evolution of plant life. Science 39, 238.

Dumville JC, Fry SC. 1999. Uronic acid-containing oligosaccharins: their biosynthesis, degradation and signalling roles in non-diseased plant tissues. Plant Physiology and Biochemistry 38, 125–140.
Duroux L, Welinder KG. 2003. The peroxidase gene family in plants: a phylogenetic overview. Journal of Molecular Evolution 57, 397–407.
Fahlen J, Salmen L. 2002. On the lamellar structure of the tracheid cell wall. Plant Biology 4, 339–345.
Fahlen J, Salmen L. 2003. Cross-sectional structure of the secondary cell wall of wood fibres as affected by processing. Journal of Materials Science 38, 119–126.
Falster DS, Westoby M. 2003. Plant height and evolutionary games. Trends in Ecology and Evolution 18, 337–343.
Fengel D. 1970. Ultrastructural behaviour of cell wall polysaccharides. Technical Association of the Pulp and Paper Industry Journal 53, 497–503.
Flint HJ, Bayer EA, Rincon MT, Lamed R, White BA. 2008. Polysaccharide utilization by gut bacteria: potential for new insights from genomic analysis. Nature Reviews Microbiology 6, 121–131.
Fromm J, Rockel B, Lautner S, Windeisen E, Wanner G. 2003. Lignin distribution in wood cell walls determined by TEM and back scattered SEM techniques. Journal of Structural Biology 143, 77–84.
Fry SC, Mohler KE, Nesselrode BHWA, Frankova L. 2008. Mixed-linkage b-glucan:xyloglucan endotransglucosylase, a novel wall-remodelling enzyme from Equisetum (horsetails) and Charophytic algae. The Plant Journal 55, 240–252.
Geisler-Lee J, Geisler M, Coutinho PM, et al. 2006. Poplar carbohydrate-active enzymes. Gene identification and expression analyses. Plant Physiology 140, 946–962.
Gierlinger N, Schwanninger M. 2006. Chemical imaging of poplar wood cell walls by confocal Raman microscopy. Plant Physiology 140, 1246–1254.
Giovannoni J. 2001. Molecular biology of fruit maturation and ripening. Annual Review of Plant Physiology and Plant Molecular Biology 52, 725–749.
Grabber JH. 2005. How do lignin composition, structure, and cross-linking affect degradability? A review of cell wall model studies. Crop Science 45, 820–831.
Graham LE, Cook ME, Busse JS. 2000. The origin of plants: body plan changes contributing to a major evolutionary radiation. Proceedings of the National Academy of Sciences, USA 97, 4535–4540.
Gunawardena AHLAN, Pearce DM, Jackson MB, Hawes CR, Evans DE. 2001. Rapid changes in cell wall pectic polysaccharides are closely associated with early stages of aerenchyma formation, a spatially localized form of programmed cell death in roots of maize (Zea mays L.) promoted by ethylene. Plant, Cell and Environment 24, 1369–1375.
Hanley SJ, Giasson J, Revol JF, Gray DG. 1992. Atomic force microscopy of cellulose microfibrils: comparison with transmission electron microscopy. Polymers 33, 4639–4642.
Hanley SJ, Gray DG. 1994. Atomic force microscope images of black spruce wood sections and pulp fibers. Holzforschung 48, 29–34.
Hanley SJ, Gray DG. 1999. AFM images in air and water of Kraft pulp fibers. Journal of Pulp and Paper Science 25, 196–200.
Harris PJ. 2005. Diversity in plant cell walls. In: Henry RJ, ed. Plant diversity and evolution: genotypic and phenotypic variation in higher plants. Wallingford, UK: CAB International Publishing, 201–227.
Hatfield RD, Vermerris W. 2001. Lignin formation in plants. The dilemma of linkage specificity. Plant Physiology 126, 1351–1357.
Herms DA, Mattson WJ. 1992. The dilemma of plants: to grow or defend. Quarterly Review of Biology 67, 283–335.
Himmel ME, Ding SY, Johnson DK, Adney WS, Nimlos MR, Brady JW, Foust TD. 2007. Biomass recalcitrance: engineering plants and enzymes for biofuels production. Science 315, 804.
Howard RL, Abotsi E, Jansen van Rensburg EL, Howard S. 2003. Lignocellulose biotechnology: issues of bioconversion and enzyme production. African Journal of Biotechnology 2, 602–619.
Hu WJ, Harding SA, Lung J, Popko JL, Ralph J, Stokke DD, Tsai CJ, Chiang VL. 1999. Repression of lignin biosynthesis promotes cellulose accumulation and growth in transgenic trees. Nature Biotechnology 17, 808–812.
Hult EL, Larsson PT, Iversen T. 2001a. Cellulose aggregation—an inherent property of Kraft pulps. Polymer 42, 3309–3314.
Hult EL, Larsson PT, Iversen T. 2001b. A CP/MAS 13C-NMR study of the supermolecular changes in the cellulose and hemicellulose structure during Kraft pulping. Nordic Pulp and Paper Research
Journal 16, 46–52.
Humphrey TV, Bonetta DT, Goring DR. 2007. Sentinels at the wall: cell wall receptors and sensors. New Phytologist 176, 7–21.
Iiyama K, Lam TBT, Stone B. 1994. Covalent cross-links in the cell wall. Plant Physiology 104, 315–320.
Jones JDG, Dangl JL. 2006. The plant immune system. Nature 444, 323–329.
Kandler O. 1994. Cell wall biochemistry and three-domain concept of life. Systematics and Applied Microbiology 16, 501–509.
Kataoka Y, Kondo T. 1998. FT-IR microscopic analysis of changing cellulose crystalline structure during wood cell wall formation. Macromolecules 31, 760–764.
Kataoka Y, Saiki H, Fujita M. 1992. Arrangement and superimposition of cellulose microfibrils in the secondary walls of coniferous tracheids. Mokuzai Gakkaishi 38, 327–335.
Kellenberger E. 1987. The response of biological macromolecules and supramolecular structures. In: Steinbrecht RA, Zierold K, eds. Cryotechniques in biological electron microscopy. Berlin: Springer-Verlag, 149–172.
Kellenberger E. 1990. The potential of cryofixation and freeze substitution: observations and theoretical considerations. Journal of Microscopy 161, 183–203.
Kennedy CJ, Cameron GJ, Sturcova A, Apperley DC, Altaner C, Wess TJ, Jarvis MC. 2007. Microfibril diameter in celery collenchyma cellulose: X-ray scattering and NMR evidence. Cellulose 14, 235–246.
Khalil AHPS, Siti Alwani M, Mohd Omar AK. 2006. Chemical composition anatomy, lignin distribution and cell wall structure of Malaysian plant wastes fibres. Bioresources 1, 220–232.
Kimura S, Laosinchai W, Itoh T, Cui XJ, Linder CR, Brown RM. 1999. Immunogold labeling of rosette terminal cellulose-synthesizing complexes in the vascular plant Vigna angularis. The Plant Cell 11, 2075–2085.
Kirby AR, Gunning AP, Waldron KW, Morris VJ, Ng A. 1996. Visualization of plant cell walls by atomic force microscopy. Biophysical Journal 70, 1138–1143.
Knox JP. 1992. Cell adhesion, cell separation and plant morphogenesis. The Plant Journal 2, 137–141.
Knox JP. 2008. Revealing the structural and functional diversity of plant cell walls. Current Opinion in Plant Biology 11, 308–313.
Krishnamurthy KV. 1999. Methods in cell wall cytochemistry. Boca Raton, FL: CRC Press.
Lee I, Evans BR, Woodward J. 2000. The mechanism of cellulase action on cotton fibers: evidence from atomic force microscopy. Ultramicroscopy 82, 213–221.

Le Van H, Kuraishi S, Sakurai N. 1994. Aluminum-induced rapid root inhibition and changes in cell-wall components of squash seedlings. Plant Physiology 106, 971–976.
Levine JS. 1990. Global biomass burning: atmospheric, climatic, and biospheric implications. EOS, Transactions, American Geophysical Union 71, 37–39.
Lewis LA, McCourt RM. 2004. Green algae and the origin of land plants. American Journal of Botany 91, 1535–1556.
Li L, Cheng XF, Leshkevich J, Umezawa T, Harding SA, Chiang VL. 2001. The last step of syringyl monolignol biosynthesis in angiosperms is regulated by a novel gene encoding sinapyl alcohol dehydrogenase. The Plant Cell 13, 1567–1585.
Ligrone R, Carafa A, Duckett JG, Renzaglia KS, Ruel K. 2008. Immunocytochemical detection of lignin-related epitopes in cell walls in bryophytes and the charalean alga. Nitella. Plant Systematics and Evolution 270, 257–272.
Lloyd C, Chan J. 2008. The parallel lives of microtubules and cellulose microfibrils. Current Opinion in Plant Biology 11, 641–646.
Marko M, Hsieh C, Schalek R, Frank J, Mannella C. 2007. Focused-ion-beam thinning of frozen-hydrated biological specimens for cryo-electron microscopy. Nature Methods 4, 215–217.
Matsunaga T, Ishii T, Matsumoto S, Higuchi M, Darvill A, Albersheim P, O'Neill MA. 2004. Occurrence of the primary cell wall polysaccharide rhamnogalacturonan II in pteridophytes, lycophytes, and bryophytes. Implications for the evolution of vascular plants. Plant Physiology 134, 339–351.
McCann MC, Carpita NC. 2008. Designing the deconstruction of plant cell walls. Current Opinion in Plant Biology 11, 314–320.
McCann MC, Defernez M, Urbanowicz BR, Tewari JC, Langewisch T, Olek A, Wells B, Wilson RH, Carpita NC. 2007. Neural network analyses of infrared spectra for classifying cell wall architectures. Plant Physiology 143, 1314–1326.
McCann MC, Roberts K. 1991. Architecture of the primary cell wall. In: Lloyd CW, ed. The cytoskeletal basis of plant growth and form. Toronto, Canada: Academic Press, 109–129.
McCartney L, Blake AW, Flint J, Bolam DN, Boraston AB, Gilbert HJ, Knox JP. 2006. Differential recognition of plant cell wall s by microbial xylan-specific carbohydrate-binding modules. Proceedings of the National Academy of Sciences, USA 103, 4765–4770.
McDonald KL. 1994. Electron microscopy and EM immunocytochemistry. Methods in Cell Biology 44, 411–444.
McDonald KL, Auer M. 2006. High-pressure freezing, cellular tomography and structural cell biology. Biotechniques 41, 137–143.
Mishler BD, Lewis LA, Buchheim MA, Renzaglia KS, Garbary DJ, Delwiche CF, Zechman FW, Kantz, Chapman RL. 1994. Phylogenetic relationships of the 'green algae' and 'bryophytes'. Annals of the Missouri Botanical Garden 81, 451–483.
Morris VJ, Gunning AP, Kirby AR, Round A, Waldron K, Ng A. 1997. Atomic force microscopy of plant cell walls, plant cell wall polysaccharides and gels. International Journal of Biological Macromolecules 21, 61–66.
Mouille G, Robin S, Lecomte M, Pagant S, Ho¨ fte H. 2003. Classification and identification of Arabidopsis cell wall mutants using Fourier-transform infrared (FT-IR) microspectroscopy. The Plant Journal 35, 393–404.
Mueller SC, Brown RM. 1980. Evidence for an intramembrane component associated with a cellulose microfibril synthesizing complex in higher plants. Journal of Cell Biology 84, 315–326.
Muzzarelli RAA. 1977. Enzymatic synthesis of chitin and chitosan. Occurrence of chitin. In: Muzzarelli RAA, ed. Chitin. New York, NY: Pergamon Press, 87–101.
Niklas KJ. 1985. The evolution of tracheid diameter in early vascular plants and its implications on the hydraulic conductance of the primary xylem strand. Evolution 39, 1110–1122.
Niklas KJ. 2004. The cell walls that bind the tree of life. BioScience 54, 831–841.
Nishitani K, Vissenberg K. 2006. Roles of the XTH protein family in the expanding cell. In: Verbelen J-P, Vissenberg K, eds. The expanding cell. Plant Cell Monographs Series. Berlin: Springer-Verlag, 89–116.
Nothnagel AL, Nothnagel EA. 2007. Primary cell wall structure in the evolution of land plants. Journal of Integrative Plant Biology 49, 1271–1278.
Okafor UA, Okochi VI, Onyegeme-okerenta BM, Nwodo-Chinedu S. 2007. Xylanase production by Aspergillus niger ANL 301 using agro-wastes. African Journal of Biotechnology 6, 1710–1714.
O'Neill MA, York WS. 2003. The composition and structure of primary cell walls. In: Rose JKC, ed. The plant cell wall. Boca Raton, FL: CRC Press, 1–54.
Paredez AR, Somerville CR, Ehrhardt DW. 2006. Visualization of cellulose synthase demonstrates functional association with microtubules. Science 312, 1491–1495.
Pauly M, Keegstra K. 2008. Cell-wall carbohydrates and their modification as a resource for biofuels. The Plant Journal 54, 559–568.
Persson S, Paredez A, Carroll A, Palsdottir H, Doblin M, Poindexter P, Khitrov N, Auer M, Somerville CR. 2007. Genetic evidence for three unique components in primary cell-wall cellulose synthase complexes in Arabidopsis. Proceedings of the National Academy of Sciences, USA 104, 15566–15571.
Popper ZA. 2008. Evolution and diversity of green plant cell walls. Current Opinion in Plant Biology 11, 286–292.
Popper ZA, Fry SC. 2003. Primary cell wall composition of bryophytes and charophytes. Annals of Botany 91, 1–12.
Popper ZA, Fry SC. 2004. Primary cell wall composition of pteridophytes and spermatophytes. New Phytologist 164, 165–174.
Popper ZA, Sadler IH, Fry SC. 2004. 3-O-Methylrhamnose in lower land plant primary cell walls. Biochemical Systematics and Ecology 32, 279–289.
Ragauskas AJ, Williams CK, Davison BH, et al. 2006. The path forward for biofuels and biomaterials. Science 311, 484–489.
Raven PH, Ray FE, Eichhorn SE. 2005. Biology of plants. New York: W.H. Freeman. Reid JSG. 1985. Cell wall storage carbohydrates in seeds: bio-chemistry of the seed 'gums' and 'hemicelluloses'. Plant Physiology and Biochemistry 11, 125–155.
Roberts AW, Roberts E. 2004. Cellulose synthase (CesA) genes in algae and seedless plants. Cellulose 11, 419–435.
Roberts JA, Elliott KA, Gonzalez-Carranza ZH. 2002. Abscission, dehiscence, and other cell separation processes. Annual Review of Plant Biology 53, 131–158.
Roberts JA, Whitelaw CA, Gonzalez-Carranza ZH, McManus MT. 2000. Cell separation processes in plants: models, mechanisms and manipulation. Annals of Botany 86, 223–235.
Ros Barcelo A, Gomez Ros LV, Gabaldom C, Lopez-Serrano M, Pomar F, Carrion JS, Pedren˜ o MA. 2004. Basic peroxidases: the gateway for lignin evolution? Phytochemistry Reviews 3, 61–78.
Rose JKC, Catala´ C, Gonzalez-Carranza ZH, Roberts J. 2003. Cell wall disassembly In: Rose JKC. The plant cell wall. Boca Raton, FL: CRC Press, 264–324.
Salerno M, Gianinazzi S. 2004. Ultrastructural and cell wall modifications during infection of Eucalyptus viminalis roots by a pathogenic Fusarium oxysporum strain. Journal of General Plant Pathology 70, 145–152.

Sarkar P, Niki T, Gladish DK. 2008. Changes in cellular ultrastructure induced by sudden flooding at 25° C in Pisum sativum (Fabaceae) pri mary roots. Ameri can Journal of Botany 95, 1–12.
Sene C, McCann MC, Wilson RH, Grinter R. 1994. Fourier-transform Raman and Fourier-transform infrared spectroscopy (an investigation of five higher plant cell walls and their components). Plant Physiology 106, 1623–1631.
Shimoni E, Rav-Hon O, Ohad I, Brumfeld V, Reich Z. 2005. Three-dimensional organization of higher-plant chloroplast thylakoid membranes revealed by electron tomography. The Plant Cell 17, 2580–2586.
Somerville C. 2006. Cellulose synthesis in higher plants. Annual Review of Cell and Developmental Biology 22, 53–78.
Somerville C, Bauer S, Brininstool G, et al. 2004. Toward a systems approach to understanding plant-cell walls. Science 306, 5705.
Stahl EA, Bishop JG. 2000. Plant–pathogen arms races at the molecular level. Current Opinion in Plant Biology 3, 299–304.
Steinbrecht RA, Mu¨ ller M. 1987. Fr eeze-substi tuti on and freeze-dryi ng. I n: Stei nbrecht RA, Zi erol d K, eds. Cryotechniques in biological electron microscopy. Berlin: Springer-Verlag, 149–172.
Sticklen MB. 2008. Plant genetic engineering for biofuel production:
towards affordable cellulosic ethanol. Genetics 9, 433–443.
Strohmeier M, Hrmova M, Fischer M, Harvey AJ, Fincher GB, Pleiss J. 2004. Molecular modelling of family GH16 glycoside/hydrolases: potential roles for xyloglucan transglucosylases/hydrolases in cell wall modification in the poaceae. Protein Science 13, 3200–3213.
Sturcova A, His I, Apperley DC, Sugiyama J, Jarvis MC. 2004. Structural details of crystalline cellulose from higher plants. Biomacromolecules 5, 1333–1339.
Taiz L, Zeiger E. 2006. Plant physiology, 4th edn. Sunderland, MA: Sinaur Associates Inc. Tucker GA, Seymour GB. 1991. Cell wall degradation during mango fruit ripening. Acta Horticulturae (ISHS) 291, 454–460.
Van Sandt VT, Stieperaere H, Guisez Y, Verbelen JP, Vissenberg K. 2007. XET activity is found near sites of growth and cell elongation in bryophytes and some green algae: new insights into the evolution of primary cell wall elongation. Annals of Botany 99, 39–51.
Vicente AR, Saladie M, Rose JKC, Labavitch JM. 2007. The linkage between cell wall metabolism and fruit softening: looking to the future. Journal of the Science of Food and Agriculture 87, 1435–1448.
Vidhyasekaran P. 2002. Bacterial disease resistance in plants: molecular biology and biotechnological applications. Binghamton, NY: Haworth Press.
ietor RJ, Newman RH, Ha MA, Apperley DC, Jarvis MC. 2002. Conformational features of crystal-surface cellulose from higher plants. The Plant Journal 30, 721–731.
Vincent JFV. 1999. From cellulose to cell. Journal of Experimental Biology 202, 3263–3268.
Volkmann D, Baluska F. 2006. Gravity: one of the driving forces for evolution. Protoplasma 229, 143–148.
Vorwerk S, Somerville S, Somerville C. 2004. The role of plant cell wall polysaccharide composition in disease resistance. Trends in Plant Science 9, 203–209.
Walton JD. 1994. Deconstructing the cell wall. Plant Physiology 104, 1113–1118.
Warren RA. 1996. Microbial hydrolysis of polysaccharides. Annual Review of Microbiology 50, 183–212.
Xu P, Donaldson LA, Gergely ZR, Staehelin LA. 2007. Dual-axis electron tomography: a new approach for investigating the spatial organization of wood cellulose microfibrils. Wood Science and Technology 41, 101–116.
Yong W, O'Malley R, Link B, et al. 2005. Plant cell wall genomics. Planta 221, 747–751.