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This volume is dedicated to the 2004 Anselme Payen Award Winner, Deborah P. Delmer. Dr. Delmer has received international recognition for her contributions to cellulose biosynthesis. She was also elected as a member of the National Academy of Sciences USA in April 2004. Her pioneering research from her challenging heart has stimulated our studies on plant cell wall.

The Anselme Payen Award is named for the distinguished 19th-century French scientist who identified and defined cellulose as the main fibrous component found universally in plant cell walls. The award is recognized internationally as the most significant honor in the science and technology of cellulose.

Takahisa Hayashi

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Preface

My thanks go out to Takahisa Hayashi, the Editor of this book, for organizing such an interesting effort. Taka also asked me to contribute an article, but, as I’ve moved on to a whole new life dealing with issues of agriculture in the developing world, I declined. I did so because of my feeling that the field of plant cell walls now has so many talented individuals, it was definitely time for me to step aside and let you all speak for yourselves. But I did agree to write this preface and reminisce very briefly about my own research with cell walls and with the topic of cellulose synthesis in particular.

I thought that one interesting way to “reminisce” might be to use my accumulated experiences in a different way and instead to ask the question: “If I were young and starting over again to work on plant cell walls and cellulose synthesis, where would I like to focus my efforts?” Obviously, we tend to focus on projects that suit our own particular set of skills, which in my case, are primarily as a biochemist and a Johnny-Come-Lately molecular biologist. But I’ll cheat a little and just imagine I could learn all the new skills I would need in order for me to extend the range of possibilities I see emerging as potential topics for research.

Just to touch briefly on the non-cellulosic part of the wall, I am a bit hesitant to suggest new directions, knowing that others will have a much better perspective than I. But I can say that I think that, in terms of biosynthesis, the powers of both genomics and molecular genetics are beginning to reveal themselves as being huge assets. It is clear that, as for cellulose synthesis for so many years, it has proven exceedingly difficult to demonstrate synthesis of complex non-cellulosic polymers using isolated membrane preparations. One notable exception has been ability to synthesize galactomannans, but when it comes to pectins and other non-cellulosic polysaccharides, the complexity of the systems, the need for suitable primers and/or cooperative interactions among a large number of enzymes, and the low levels of such enzymes in membranes, has made this task exceedingly challenging. Clearly the ability to isolate mutants with altered polysaccharide composition provides one avenue for the...
identification of key genes encoding biosynthetic enzymes. Yet, possible redundancy of genes and/or inability to detect altered phenotypes for whatever reason can still limit this approach. This is where genomics can play an important role, and we are finally beginning to see how searching for important genes based upon their homology with other related glycosyltransferases is beginning to pay off for pathways involved in synthesis of polymers such as the pectins, and xyloglucan. And, when it comes to structure, well, I think it won’t be long before we’ll understand just how all those various structures that we lump as together as pectins really do associate with each other and perhaps also with other non-pectic polysaccharides and/or proteins. One hopes we will also finally get clarification of how expansins and XETs modulate extensibility, and maybe even one day we’ll really understand that great white whale of plant biology—how does auxin really control cell elongation?

Discussion of these possibilities also helps me make one very important point—it is no longer possible just to be one kind of scientist as many of us used to be. Each group now needs to have members who are either skilled themselves in many fields, or find partners who can complement their own skills in order to really make progress. Certainly each group needs to be able to use model systems effectively because they offer ease of manipulation, complete genome sequences, and extensive mutant collections. Yet model systems also have limitations. I think most of us really do hope that our research will one day lead to greater benefits to agriculture. Thus, we hope that, at some point, the results of model systems can to be translated to enhancing our understanding and ultimate improvement of crops such as cotton, trees, and major cereal or legume crops. Model systems also may not be the best for hard biochemistry where large amounts of tissue may be required, and they may lack specialized tissues (like the cotton fiber for cellulose synthesis) where a particular process predominates at one particular stage of development in one distinct and easily-isolated cell type. In short, we need to be flexible and not wedded just to one approach for our entire careers. Certainly my own career took a turn for the better when I was able to make the leap beyond just biochemistry.
Turning to cellulose synthesis, I have to say that I am REALLY pleased to see how many new faces have joined the field and the new insights you have added to our understanding of this process. Through genomics and mutant studies, we now know that there are a number of CesA genes that indeed can be predicted to catalyze glucan chain elongation in some way, and that some of these distinct CESA proteins mysteriously seem to be required to work in combination to synthesize microfibrils. But why this is true still eludes us. It is clear that genomics can only suggest that this is true and may also provide indications of other new partner proteins that may be necessary for the process. But hard work involving good, serious biochemistry will still be important to provide a final confirmation of the structure of the rosette, the function of each CESA within the rosette, and the role of other accessory proteins. Questions also remain as to what distinguishes primary from secondary wall cellulose synthesis. Steryl glycosides seem to function as primers in vitro in membranes of cotton fibers engaged in secondary wall synthesis. Is this some in vitro artefact, is it unique to secondary wall synthesis in vivo, or is it a general phenomenon for all of cellulose synthesis? Is one CESA responsible for elongating primers while others may function in further elongation? What is different about the CESA’s required for primary vs. secondary wall synthesis? Why do rosettes/CESAs appear to have such short half lives? Only time will tell. Why should cellulases like Korrigan be needed? Perhaps to cleave the primers, to edit mistakes in growing microfibrils, or to facilitate chain termination? How do rosettes assemble? What are the domains that associate to form rosettes—e.g., do the zinc finger domains interact in a redox-dependent fashion? (In fact, if there were two topics I’m sure I’d address more seriously if I were to continue would be the general role of redox regulation and the role of protein turnover in the process of cell wall assembly.) Are there scaffolding proteins and/or other accessory proteins embedded within the rosettes? Do microtubules really interact directly with rosettes to guide their movement as suggested by some recent data? The answers to some of these questions are beginning and will continue to be answered with the additional help of the fantastic new tools for imaging that are improving at a very rapid pace. Will we one day really be able to see clearly the rosette moving within the membrane and show with such imaging techniques exactly what
proteins are within the rosette and how its movement is guided? (Simon Turner’s lab seems to be getting close to doing just that!) And what about proteins that may be involved but may not directly interact with the rosette, such as Korrigan or sucrose synthase? And, well, have we really solved the issue of how synthesis of cellulose and callose may or may not be connected? And how did these biosynthetic pathways evolve?

Finally, what may be the practical benefits that could arise from such work? One prediction is that work with enzymes like sucrose synthase may provide new insights into how carbon is partitioned in plant cells. Clearly the cell wall is a major sink for carbon, but, unless you are a ruminant, it’s not exactly the world’s best source of calories. And lignin is even less pleasant than cellulose—clearly of benefit to the plant, but with major impacts on digestibility for both humans and animals, and on the efficiency and safety of processing of plant tissues for production of paper, starch, and ethanol. So understanding how carbon may be partitioned between the digestible and non-digestible polymers may offer opportunities to alter the value of many plant products. As one who invariably seems to choose in the market the toughest old radishes, turnips, rutabagas, and carrots and the stringiest celery and green beans, I’ve also wondered if there may be opportunities to alter cellulose (and lignin) content in such veggies to enhance texture as well as digestibility. With suitable tissue-specific promoters and the right genes, it surely seems quite possible. Finally, I’ve been most gratified to see that some of our research may also prove valuable for the kind of work I’m now doing with the Rockefeller Foundation. Lodging of cereals is a problem throughout the world, not only for maize in the fields of commercial farmers, but also for crops such as rice, millet, sorghum and tef in the developing world. While the green revolution genes have played an important role in helping solve this problem, it now appears there may be opportunities to enhance resistance to lodging by over-expression of CesA genes in specific tissues.

So I leave this fascinating field just when the opportunities seem the greatest. But it makes leaving so much easier to see how many talented young (and older!) scientists have taken up the cause. Taka has asked
you all to speculate as you write the articles for this book. This is surely fitting since I’ve not ever been shy to speculate throughout my career. (How I remember Bruce Stone moaning “Oh Dear! Not another Delmer theory!!!”). But it’s a valuable exercise even when we get it wrong, as it does challenge us and our colleagues to extend our way of thinking. Yet, as valuable as speculation can be, it is no substitute for good hard facts. And that is what’s so gratifying now—you no longer have to do SO MUCH speculating, as the answers to many of the questions posed above are now well within your reach. Enjoy the journey!

Deborah Delmer
Introduction

Plant cell walls are composed of complex carbohydrates, proteins, phenolic compounds, and inorganic ions, all of which play functional roles. Several models for plant cell walls help explain their structure, which consists of primary and secondary walls. The primary wall is the outer wall of a growing plant cell, and elongates and/or expands over the life of the cell. The secondary wall exists inside the primary wall in elongated and expanded cells. Structural analysis has primarily employed microscopy of walls, because “seeing is believing.” Since cellulose microfibrils are skeletal and architectural components, their orientation determines the physical properties of plant cell walls. The fine structure of the wall has been determined by chemical analysis.

Cellulose (1,4-β-glucan) and callose (1,3-β-glucan) are synthesized in the plasma membrane, while other polysaccharides are synthesized in the Golgi. Although cellulose is the most abundant biopolymer on the earth, the details of its biosynthesis have been difficult to elucidate, despite the ongoing work of many scientists for many years. The success story of cellulose biosynthesis, by V. Bulone, serves as an excellent plenary chapter in this book.

Plant cell growth occurs with the loosening of the walls, which may be caused by several enzymatic actions. Many polysaccharides synthesized in the Golgi are exported to the cell wall, where hydrogen bonding occurs between xyloglucan and cellulose, together with chemical cross-linking between polymers. By measuring wall extensibility, tethers between xyloglucan and cellulose have been proven to play important roles. Xyloglucan metabolism is undoubtedly involved in the loosening.

Plant development is related to the morphological changes of cells and tissue, which is caused by structural changes of the walls: the generation of walls from grasses to seeds, induction of secondary wall formation, and cotton fiber development. The key action might be caused by XTHs and proline-rich wall proteins.
A recent genome project has revealed about 248 glycan hydrolases and 214 glycan transferase genes, including 10 cellulose-synthase (CesA) and 30 cellulose-synthase-like (CsI) genes in Arabidopsis thaliana. Genetic analysis of gene knockout mutants in Arabidopsis thaliana has revealed the function of these genes. Proteomics and glycomics also provide new insights into the studies on plant cell walls.

Woody plants make up 80% of total plant biomass, which is the carbon source for living organisms and also the biological sink of CO₂ on the Earth. Cell walls control plant cell growth and define the structure of plants, both in guiding the development of plants and in providing a role in their defense against pathogens. Other benefits of plant cell walls are their uses as food, various materials, and energy for human beings.

Takahisa Hayashi
The primary cell wall is composed of complex carbohydrates and proteins. Many models have been proposed to understand its structure, beginning with the first complete model by Keegstra et al. (1973), which was based on enzymatic and chemical analyses of the walls of growing sycamore cells (Figure 1). Their primary theory was that rhamnogalacturonan, arabinogalactan, xyloglucan, and hydroxyproline-rich proteins are interconnected by covalent bonds, and the connection between cellulose and xyloglucan is hydrogen bonds. The walls of growing plant cells were thought to act as a macromolecular complex. Based on this model, numerous other models were formed using additional findings (Figures 2-5). Figure 2 (Fry 1986) shows the interactions between the molecules in the cell walls, and Figure 3 shows the matrix polysaccharides arranged nearly parallel to the cellulose microfibrils.

The models can be divided into two approximate categories. One type stresses chemistry and the interactions and bonds between molecules, while the other attempts to express an artistic architectural construction of the molecules. Figure 4 (Roberts 1994) illustrates the most popular model of the latter type. It appears in many general biology textbooks; though the drawing is simple, it is a beautiful description of cell wall construction. Since some of the polysaccharides and the cell wall structure of some monocots are different from those of dicots and other monocots, they were drawn separately as Types I and II in Figure 5. Most dicots and noncommelinoid monocots have Type I walls, while commelinoid monocots have Type II walls.

A completely different model has been proposed for secondary walls in woody plants (e.g., Esau 1977; Higuchi 1997). Figure 6 (Liese 1970) shows a gymnosperm tracheid and the alteration of microfibril orientation in the layered secondary wall. Later, the fiber wall of angiosperms was revealed to have the same structure as this model. However, bamboo has cell walls with more layers (Figure 7: Parameswaran and Liese 1981). The secondary wall is known to...
consist of cellulose microfibrils, hemicelluloses, and lignin. A model (Figure 8) has been proposed for their localizations, but no details of the molecular bonding level have been found. How they interact and construct the secondary wall remains unknown.

We can clarify the story by gathering the models together, focusing on each stage of wall material production, and so on. Because of limited space, only two figures for wall formation are shown here. Cellulose microfibrils are produced on the membrane along the cortical microtubules (Figure 9: Gunning and Steer 1996). New cellulose and xyloglucan meet just outside the membrane and immediately bond tightly to each other (Figure 10: Hayashi 1989).

Fig. 1 Tentative structure of sycamore cell walls. This model is not intended to be quantitative, but is instead an effort to present the wall components in approximately correct proportions. The distance between cellulose elementary fibrils is expanded to allow presentation of the interconnecting structure. The circled areas are representative wall fractions released by the degrading enzymes. They are fractions PG-1B and PG-2 released by endopolygalacturonase, fractions C-1 and C-2 released by endoglucanase, and fraction PR-2 released by pronase. The symbols shown at right represent the various components of the cell wall (Keegstra et al. 1973).
Fig. 2 Representative primary structures and possible cross-linking of wall polymers.

This is not a model of the plant cell wall, and no significance is placed on the chain length, orientation, conformation, or spacing of the molecules (Fry 1986).

(*) hydrogen bonds:
1. cellulose-cellulose
2. xyloglucan-cellulose
3. xylan-cellulose

( o ) calcium bridges:
4. homogalacturonan-homogalacturonan

( ± ) other ionic bonds:
5. extensin-pectin
6. extensin-extensin

( : ) coupled phenols:
7. pectin-pectin
8. arabinoxylan-arabinoxylan

( = ) ester bonds:
9. pectin-cellulose

( - ) glycosidic bonds:
10. arabinogalactan-rhamnogalacturonan

( --- ) entanglement:
11. pectin-in-extensin

A, arabinose; F, fucose; G, glucose; L, galactose; R, rhamnose; U, galacturonic acid; U^, galacturonic acid methyl ester; a, amino acid other than tyrosine; y, tyrosine; y:y, isodityrosine; φ, ferulic acid; φ:φ, diferulic acid.

A, arabinose; F, fucose; G, glucose; L, galactose; R, rhamnose; U, galacturonic acid; U^, galacturonic acid methyl ester; a, amino acid other than tyrosine; y, tyrosine; y:y, isodityrosine; φ, ferulic acid; φ:φ, diferulic acid.

Fig. 3 Schematic of suggested polymer organization in pea primary walls, depicted in the plane of the cellulose microfibrils.
The xyloglucan and arabinogalactan layers form a hemicellulosic sheath ("cortex") around each microfibril, the non-crystalline portions of which contain intercalated xyloglucan chains. Interstices between the hemicellulose-coated microfibrils are occupied by pectin. Where microfibrils approach one another more closely, their hemicellulose sheaths may overlap, and some xyloglucan chains may extend from one microfibril to another. The differently labeled polymer types are depicted in the relative portions in which they occur in pea cell walls, according to this study and previous reports. Smooth portions of polyuronide backbones donate homogalacturonan, and side chain-bearing portions are rhamnogalacturonan blocks. The longer blocks represent lengthy runs of substituted galacturonosyl-rhamnose, such as RG-I. Except for extensin, minor components (e.g., mannann and xylan) are not shown (Talbot and Ray 1992).

**Fig. 4** Scale model of a portion of primary cell wall showing the two major polysaccharide networks. The orthogonally arranged layers of cellulose microfibrils are cross-linked into a network by hydrogen-bonded hemicellulose. This network is coextensive with a network of pectin polysaccharides. The cellulose and hemicellulose network provides tensile strength, while the pectin network resists compression. Cellulose, hemicellulose, and pectin are typically present in roughly equal quantities in a primary cell wall. The middle lamella is pectin-rich and cements adjacent cells together (Roberts 1994).
Fig. 5 (A) Architectural model of the Type I cell wall.
The Type I cell wall is a strong but dynamic network of cellulose, tethered by cross-linking xyloglucans and embedded in a gel of matrix pectins, which include simple and complex homogalacturonans (HG) and rhamnogalacturonan I (RG I). To the RG I backbone may be linked α-arabinans, β-galactans, and type I arabinogalactans. The wall is also residence for several structural proteins and hundreds of enzymes. (adapted from Carpita and Gibeaut 1993)

(B) Architectural model of the Type II cell wall.
The microfibrils coated with a dense layer of \((1\rightarrow3),(1\rightarrow4)\)-β-glucan and relatively unsubstituted glucuronoarabinoxylan (GAX) are interlaced primarily by GAX with greater degree of branching by single arabinofuranosyl units. Some highly-substituted GAX remains intercalated in the small amount of pectins that also are found in the primary wall. Unlike the Type I wall, a substantial portion of the non-cellulosic polymers are “wired on” the microfibrils by alkali-resistant phenolic linkages. (adapted from Buckeridge et al. 2004)

Fig. 6 Diagram of a piece of tracheid wall illustrating the layers and their microfibrillar organization.
S₁ to S₃ refers to the secondary wall. S₃ is sometimes interpreted as a tertiary wall layer (Adapted from Liese 1970).
Fig. 7 Polylamellar wall structure of a bamboo culm fiber. 
ML, Middle lamella; P, primary wall; s, secondary wall; l and t denote longitudinal and transverse orientations of microfibrils, respectively (Parameswaran and Liese 1981).

Fig. 8 Localization of and the relationships between cellulose, hemicelluloses, and lignin in the S₂ layer (Ruel et al. 1978).
As soon as the xyloglucan synthesized in Golgi is transported to extracellular sites, cellulose synthase (terminal complex) catalyzes cellulose synthesis at the cell surface, and the association of cellulose with xyloglucan takes place. (Hayashi 1989).

Fig. 9  Schematic diagram showing cellulose synthesis by the membrane synthase complex ("rosette") and its presumed guidance by the underlying microtubules in the cytoplasm (Gunning and Steer 1996).

Fig. 10  Potential model of xyloglucan exocytosis and macromolecular organization.

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References
Introduction
As the son of an artist, I grew up surrounded by drawings, paintings, and sculpture. Perhaps for that reason, making images is central to my work as a scientist. The problem I study, organ morphogenesis, requires analysis of the cell wall. Fertile approaches stretch out in many directions (see the rest of this book) but I have been drawn to architectural problems, revealing how components are integrated into a whole. For structural appreciation, imaging is paramount.

For imaging the cell wall, my laboratory has developed or enhanced several methods. I will describe some of these here, and discuss advantages and limitations, without attempting to review cell wall imaging comprehensively. The first section is on immunocytochemistry at the light-microscope level. The second and third sections are on high-resolution imaging based on field-emission scanning electron microscopy. The first is convenient and allows many samples to be examined and a relatively large area of tissue to be viewed; the second and third are technically more demanding but resolve structure at virtually the level of macromolecules.

Butyl-methyl-methacrylate embedding for immunocytochemistry
Cell wall researchers will be familiar with the use of antibody probes to localize polysaccharide as well as protein epitopes in the cell wall (Knox 1997), but are probably less familiar with the use of butyl-methyl-methacrylate as an embedding matrix. I first encountered this resin while attempting to localize microtubules (Baskin et al. 1992). This methacrylate is easy to section, dry or wet; however, its primary advantage for immunocytochemistry is that following sectioning, the majority of the embedding matrix can be removed with a brief incubation in acetone, in contrast to nearly all other plastic embedments, which can be removed partially if at all only with harsh treatments. By removing the embedment, access to the antigen for the antibody is enlarged. Removability is shared by paraffin and wax but these preserve most samples poorly compared to plastic. At the electron-microscope level, adequate access for antibody to antigen is
provided by various plastics that are sufficiently porous to allow antibodies to penetrate 10 nm or so into the section, thus sampling an appreciable proportion of the volume of an ultra-thin section (60 nm thickness). However, for the semi-thin sections (1 to 2 µm thick) typically used in light microscopy, a 10 nm penetration depth amounts to a negligible proportion of the section volume, and the greater penetration gained by removing the embedment becomes a significant advantage.

Butyl-methyl-methacrylate, like most methacrylates, polymerizes via a free radical-based mechanism. This is useful because it means that polymerization can be catalyzed by ultraviolet light, thus avoiding denaturation caused by high-temperature polymerization. However, early efforts to use this methacrylate mix were frustrated by oxidative damage to the sample that lowered antigenicity, damage presumably mediated by free radicals. I found that adding the free-radical scavenger dithiothreitol to the resin allowed polymerization but blocked the attack on the sample (Baskin et al. 1992). Subsequently, this resin has been used to localize tubulin and other antigens in a variety of samples (e.g., Herman et al. 1994; Stadler et al. 1995; Hoffman et al. 1998; Palmer et al. 2001).

In general, embedding in butyl-methyl-methacrylate is straightforward (Baskin and Wilson, 1997). However, the small size of arabidopsis roots (ca 0.15 mm diameter) makes them easy to lose while changing solutions. To retain the roots, I use a method that is not only convenient but also turns out to be beneficial for sample preservation. Originally, I encased each root in a small droplet of low-gelling-temperature agarose (Baskin et al. 1992), but this is messy and exposes the sample to heat, albeit briefly. Then, I modified a method from cryofixation where samples are mounted on a Formvar film (Baskin et al. 1996). A chemically fixed root tip is placed on a Formvar-coated wire loop, a second Formvar film secures the root tip on the loop. The Formvar films are readily permeated by solvents and small molecules. Between Formvar films, the thin arabidopsis root tip is prevented from bending or twisting. I call this “mechanical fixation” and beyond being convenient, it seems to enhance sample preservation.

Loops are made in advance and coated by casting Formvar rectangles (measuring a little more than the loop diameter on one side and a
little more than twice the loop diameter on the other) and plunging the loop into the water over the rectangle so that the plane of the loop bisects the long axis of the rectangle. The Formvar rectangle wraps around the wire loop and the coated loop is removed at once from the water. Such loops remain stable for months. To secure a sample, the procedure is repeated: After the sample has been fixed and rinsed, a loop (already Formvar coated) is placed horizontally on a drop of water (or buffer) and the sample placed on the Formvar. Excess sample is trimmed if needed, and the loop (with sample) is plunged onto a new Formvar rectangle, thus encasing the sample between Formvar layers, held by the loop. Several loops can be placed in a vial and solutions exchanged without losing the sample. The loop is embedded with the sample, and removed during trimming. I use fine copper wire (36 gauge), which can be trimmed along with the block.

My colleagues and I have recently taken advantage of this methacrylate to characterize cell wall epitopes present in the rhd1/reb1/cst2 mutant of arabidopsis (Andème-Onzighi et al. 2002). We found that selected arabinogalactan-protein epitopes were differentially expressed within the epidermis between root-hair forming cells (trichoblasts) and non-root-hair forming cells (atrichoblasts) and suggested that these proteins may be involved in repressing bulge formation in atrichoblasts (Figure 1).

**Field-emission scanning electron microscopy for cell wall ultrastructure**

The most common approach to cell wall ultrastructure is transmission

**Fig. 1 Transverse sections of arabidopsis roots embedded in butyl-methyl-methacrylate and stained with anti-cell wall antibodies.**

Top panels show JIM5, which recognizes pectin, bottom panels show JIM 14, which recognizes arabinogalactan epitopes. Left hand panels show wild type (Columbia), right hand panels show reb1. Note that JIM 5 stains more or less ubiquitously, as JIM 14 stains the wild type; however, JIM 14 staining in the trichoblasts of reb1 appears specifically decreased. Bars = 25 µm (top), 15 µm (lower right). Figure modified from Andème-Onzighi et al. 2002).