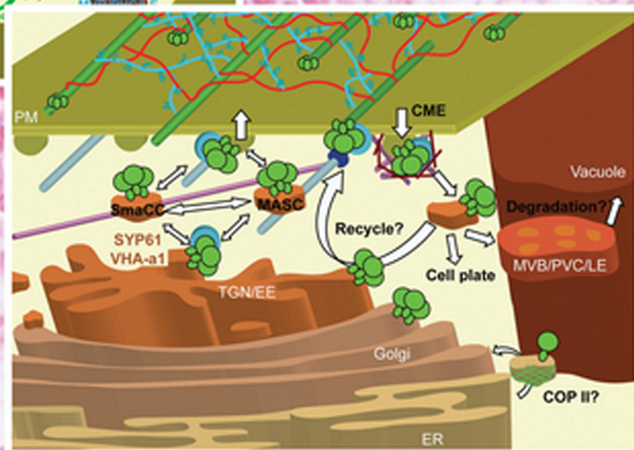
PLANT CELL WALL PATTERNING AND CELL SHAPE

Edited by **Hiroo Fukuda**



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Edited by
HIROO FUKUDA

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Contents

List of Contributors vii
Preface xiii

Section 1 Factors Controlling Plant Cell Wall Patterning 1

1 **The Biosynthesis and Function of Polysaccharide Components of the Plant Cell Wall** 3
Ryusuke Yokoyama, Naoki Shinohara, Rin Asaoka, Hideki Narukawa and Kazuhiko Nishitani

2 **Regulation of Cell Wall Formation by Membrane Traffic** 35
Clara Sánchez-Rodríguez and Staffan Persson

3 **A Blueprint for Cellulose Biosynthesis, Deposition, and Regulation in Plants** 65
Ian S. Wallace and Chris R. Somerville

4 **Cortical Microtubule Array Organization and Plant Cell Morphogenesis** 97
Sidney L. Shaw and Laura Vineyard

5 **Actin Filament Dynamics and their Role in Plant Cell Expansion** 127
Jiejie Li, Ruthie Arieti and Christopher J. Staiger

Section 2 Cellular Mechanisms Underlying Various Cell Shapes 163

6 **The Regulation of Cell Shape Formation by ROP-dependent Auxin Signaling** 165
Shingo Nagawa and Zhenbiao Yang

7 **Xylem Cell Wall Pattern Formation Regulated by Microtubule-associated Proteins and ROP GTPases** 191
Yoshihisa Oda and Hiroo Fukuda

8 **ROP Signaling and the Cytoskeleton in Pollen Tube Growth** 215
Lei Zhu and Ying Fu

9 Phosphoinositide Signaling in Root Hair Tip Growth **239**
Hiroaki Kusano, Rumi Tominaga, Takuji Wada, Mariko Kato and Takashi Aoyama

10 Arabidopsis Trichome Morphogenesis and the Role of Microtubules in Controlling Trichome Branch Formation **269**
M. David Marks

11 Transfer Cells: Novel Cell Types with Unique Wall Ingrowth Architecture Designed for Optimized Nutrient Transport **287**
David W. McCurdy

Section 3 Developmental Regulations of Cell Shape **319**

12 Regulation of Guard Cell Formation by Integration of Transcriptional and Signaling Regulation **321**
Chin-Min Kimmy Ho and Dominique C. Bergmann

13 Transcriptional Regulation of Biosynthesis of Cell Wall Components during Xylem Differentiation **351**
Ruiqin Zhong and Zheng-Hua Ye

14 Phloem Cell Development **379**
Raffael Lichtenberger, Kaori Furuta-Miyashima, Eva Hellmann and Ykä Helariutta

Index **401**

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Preface

Hundreds of thousands of species of plants have evolved to thrive on Earth. One of the reasons for their prosperity must be the acquisition and clever use of cell walls. Plant cell walls dictate the plant life form by preventing cell migration, resulting in immobility of plants. On the other hand, cell walls produce the strength that allows plants to grow as huge trees and enable a unique strategy for the plant body plan by using cells hardened with cell walls as building blocks. Moreover, cell walls define cell shape, cell function, and sometimes cell fate through mechanisms such as asymmetric cell division and intercellular communication. An understanding of plant cell walls is therefore essential for an understanding of plant life.

A number of books about plant cell walls have already been published. Most books focus on the structure and biosynthesis of plant cell walls, providing invaluable knowledge of the biochemical and structural nature of plant cell walls. However, there are few books that provide detailed information about cell wall functions and their underlying mechanisms, despite recent conspicuous progress in these areas. I therefore planned a book entitled *Plant Cell Wall Patterning and Cell Shape*, describing current knowledge of the spatiotemporal regulation of plant cell organization in view of cell walls.

This book is grouped into three sections: (1) *Factors Controlling Plant Cell Wall Patterning*; (2) *Cellular Mechanisms Underlying Various Cell Shapes*; and (3) *Developmental Regulations of Cell Shape*.

Spatiotemporal regulation of cell wall formation appears as cell wall patterns. Section 1 therefore deals with crucial components for cell wall patterning. In Chapters 1 and 2, current knowledge of the biosynthesis of cell wall components, including cellulose microfibrils, is described as a basis for cell wall patterning. Membrane traffic, which is another key component for cell wall patterning, is described in Chapter 3. Mechanisms underlying cell wall patterning involve microtubule and actin filament dynamics beneath the plasma membrane. Chapters 4 and 5 therefore highlight regulation of microtubule and actin filament arrangement, respectively, in cell wall pattern formation.

A conspicuous function of cell walls is the formation of diverse cell shapes. Section 2 therefore describes new insights into cellular mechanisms leading to distinctive cell shapes. In plants, polarization of the plasma membrane leads

to the formation of a locally specialized architecture of cell walls, resulting in various shapes of plant cells with specific functions. Recent progress in this field has revealed that Rho-like GTPases from plants (ROPs) play a crucial role in polarization of the plasma membrane to form distinct plant cell shapes. Chapters 6–8 therefore deal with the role of ROPs in the shape of three different cells such as pavement cells, xylem vessel cells, and pollen tubes. Chapters 9–11 describe current knowledge of the cell shape formation in root hair, trichome, and transfer cells, in which different cellular mechanisms such as lipid signaling and cytoskeleton are discussed.

Section 3 deals with the developmental regulation of specific types of plant cells such as guard cells, xylem cells, and phloem cells. Cell wall pattern and cell shape are both under developmental control. Cell-non-autonomous extracellular signals derived from neighboring cells or environmental cues regulate cell fate. Within the cell, transcriptional cascades finally determine a cell fate and then execute distinct cell-specific wall formation to lead to distinct cell shape. Signaling and transcriptional cascades leading to cell differentiation are discussed in Chapters 12 and 13, respectively. Finally, Chapter 14 describes our current knowledge of inter- and intra-cellular signaling that determines phloem cell differentiation.

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Hiroo Fukuda

Section 1
Factors Controlling Plant Cell Wall Patterning

1 The Biosynthesis and Function of Polysaccharide Components of the Plant Cell Wall

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Introduction

The cell wall of land plants consists of three layers, namely the middle lamella, the primary cell wall, and the secondary cell wall. The middle lamella is directly derived from the cell plate generated during cytokinesis and the primary cell wall is deposited onto the middle lamella during the cell expansion process. The two cell wall layers are generally found in all cell types, whereas the secondary wall is deposited onto the primary cell wall in certain specific cell types after cell expansion has ceased (Albersheim *et al*., 2011; Fig. 1.1).

The three layers differ from each other in terms of their chemical nature and physical properties, and they serve different biological functions. Although both the primary and secondary cell walls directly function as a mechanical housing capable of resisting both turgor pressure from the inside out and compression force from the outside in, only the primary cell wall can extend or deform in response to the force applied and thereby determine the direction and rate of cell expansion (Burgert and Frantzl, 2007; Wasterneys and Collings, 2007; Fig. 1.1). In addition to these mechanical roles, the primary cell wall functions as an information processing system. Typical functions include non-cell-autonomous regulation of cell differentiation via apoplastic signaling (Irving and Gehring, 2012; Wolf *et al*., 2012a), particularly in meristems, defensive responses to pathogens and parasites (Bradley *et al*., 1992; Vorwerk *et al*., 2004), and interactions with symbionts. The dynamics of the primary cell wall therefore play a pivotal role in determining cell shape and function during development and in response to environmental stimuli. Accordingly, in this chapter we will focus on the primary cell wall and the dynamic aspects of its major components, namely cellulose and matrix polysaccharides, in relation to its function.

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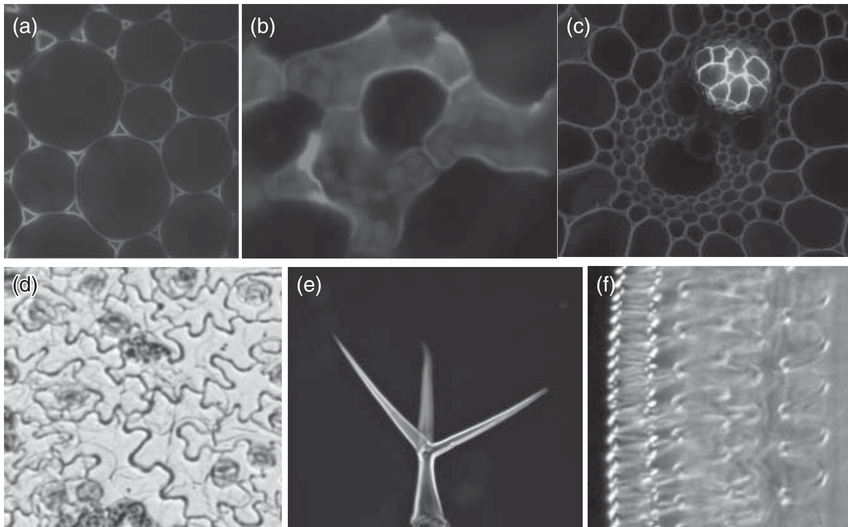

Figure 1.1 Various types of plant cells defined by the cell wall: (a–c) immunofluorescence labeling with monoclonal antibodies against cell wall polysaccharide epitopes; (a) JIM5, specific to homogalacturonan with a low degree of methylesterification; (b, c) CCRC-M1, specific to fucosylated xyloglucan; (d, e) bright field images of unstained specimens; (f) histochemical staining of lignin with phloroglucinol-HCl. A, parenchyma of *Oryza sativa*; B, spongy mesophyll of *Fagus crenata*; C, vascular of *O. sativa*; D, E and F, epidermis, trichome and xylem of *A. thaliana,* respectively.

Overview of the Plant Cell Wall

Plants devote a considerable amount of energy to constructing and maintaining the architecture of the plant cell wall, which is a biphasic composite consisting of crystalline microfibrils and an amorphous gel-like matrix; the former is embedded in the latter, which is intelligent enough to be able to self-organize and regulate cell shape and function during growth and, hence, the morphology of land plants.

For its assembly, remodeling, and disassembly, various types of structural and functional components must be secreted into the cell wall space. These include polysaccharides, structural proteins, enzymes, and small signaling molecules. Examination of the increasing number of currently available genome sequences of land plants tells us that each plant genome contains several thousand cell-wall-related genes which are implicated in biosynthesis, modification, and disassembly of the cell wall, and their regulation with respect to transcription, membrane trafficking, and enzyme actions (Henrissat *et al*., 2001; Coutinho *et al*., 2003; Somerville *et al*., 2004; Yokoyama and Nishitani, 2004; Brown *et al*., 2005). The presence of such a large number

of genes and proteins committed to cell wall dynamics apparently reflects the fact that cell wall type is dependent upon cell type, of which there are estimated to be more than 40 in a land plant. Transcriptomic analysis has demonstrated that different cell types have different expression patterns of cell-wall-related genes (Zhu and Wang, 2000; Demura *et al*., 2002; Birnbaum *et al*., 2003; Imoto *et al*., 2005; Demura and Fukuda, 2007).

In addition to cell-type-specific variations, the chemical and physical nature of the cell wall is also hugely dependent upon the stages of growth and differentiation of the cell. This is rather self-evident as we have seen that the rate and direction of cell growth, and thus the final shape of the cell, is ultimately determined by the nature of the cell wall. Continued reduction in the tensile strength of the cell wall, which is termed 'cell wall loosening', is the direct cause of cell wall expansion followed by cell expansion, the ubiquitous process by which cell expansion is regulated. Accordingly, an anisotropic or localized modification of the primary cell wall within a cell will cause anisotropic cell growth, such as cell elongation in stem cortical cells and polarized cell expansion in leaf trichomes and pavement cells. The chemical and physical nature of the primary cell wall can therefore precisely determine the size and shape of individual cells and play a vital role in determining the morphology of the plant as a whole (Fig. 1.1; Somerville *et al*., 2004; Cosgrove, 2005).

By contrast, the secondary cell wall has a static structure consisting mainly of crystalline cellulose microfibrils impregnated with lignin and suberin, and is responsible for providing mechanical resistance as well as forming a diffusion barrier. In xylem and fiber cells, the secondary cell wall functions to resist compression force as well as tensile force, and it provides the cell with enough strength to support aerial parts of the plant body, or serves as a non-growing cellular pathway for the translocation of water and nutrients (Fig. 1.2; Demura and Fukuda, 2007). On the other hand, the diffusion resistance function of the secondary cell wall is most prominently found in the Casparian strip in the endodermis, in which lignin confers the hydrophobicity necessary for forming a diffusion barrier to the cell wall (Naseer *et al*., 2012). These functions of the secondary wall are not directly related to the determination of cell shape and are therefore not discussed in this chapter.

Components of the Primary Cell Wall

The primary cell wall is composed of cellulose microfibrils, matrix polysaccharides, and structural proteins and can serve as an aqueous microenvironment harboring non-structural soluble components such as enzymes, signaling molecules, and ions (Carpita and Gibeaut, 1993; Cosgrove, 1997). In this section, we first describe the structural features of the cellulose microfibrils and two major matrix polysaccharides – pectin and hemicellulose – before

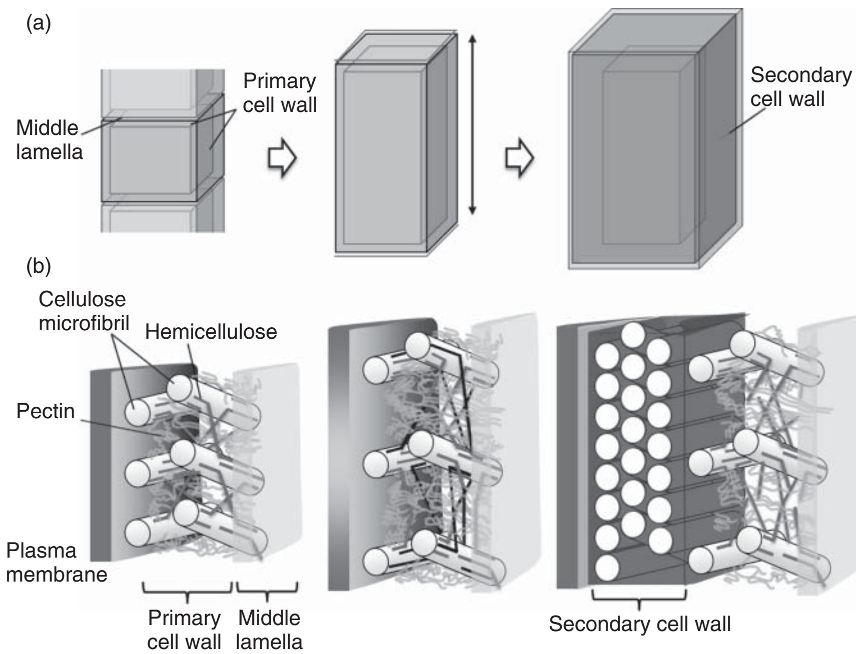

Figure 1.2 Cellulose/hemicellulose and pectin networks in the primary cell wall at successive stages of plant cell growth. (a) Processes of cell elongation and differentiation. (b) Major polymers and their likely arrangement in the cell wall. Newly secreted hemicelluloses (shown in black) and the other polymers (gray) are integrated into the cellulose/hemicellulose network.

describing how they are organized to form the dynamic architecture of the primary cell wall.

Basic Structure and Cellulose Microfibrils

A single microfibril in land plants is circular or square when observed in cross-section. The dimension of the cellulose microfibril in land plants has been estimated by transmission electron microscopy, X-ray scattering (Jakob *et al.*, 1995), and solid-state ^{13}C nuclear magnetic resonance (NMR) (Newman, 1999; Kennedy *et al.*, 2007). The diameters suggested by these analyses range from 2.5 nm to 3.6 nm, which corresponds to 15–32 chains of β-1,4-glucan molecules (Somerville, 2006; Fernandes *et al.*, 2011) if it is assumed that each chain occupies 0.317 nm^2 (Nishiyama *et al.*, 2002).

In cellulose microfibrils, there are two types of domains conforming to a triclinic (termed cellulose I-α) form and a monoclinic (termed cellulose I-β)

form. In land plants, the I-β form predominates. In the crystalline domain, β-1,4-glucan chains are arranged in parallel and undergo self-association via several interactions, which include the formation of intramolecular hydrogen bonds at O3 … O5 and O2 … O5, an intermolecular hydrogen bond at O3 … O3, and hydrophobic intermolecular interactions. This structure renders the cellulose microfibrils insoluble in water, immune to enzymatic attack, and resistant to chemical agents.

Another important characteristic of cellulose is its high tensile strength and elastic modulus. The latter is estimated to be between 124 and 155 GPa for the cellulose I-β form, values that are comparable to that of gray cast iron (Nishino *et al*., 1995). The crystallinity is frequently disrupted by dislocations, resulting in amorphous or *para*-crystalline regions in the microfibril. The cellulose microfibril therefore has a substructure consisting of highly organized crystalline domains linked together by less organized amorphous or para-crystalline regions (O'Sullivan, 1997; Nishiyama *et al*., 2002).

In the primary cell wall, stable crystalline cellulose microfibrils are embedded in amorphous hydrophilic matrix polysaccharides through interaction with the less-organized *para*-crystalline domains. Land plants contain two major classes of matrix polysaccharides: hemicellulose and pectin. The former includes xyloglucans, glucomannans, and arabinoxylans (Scheller and Ulvskov, 2010), and the latter consists of homogalacturonan (HG) and the rhamnogalacturonan (RG) I and II domains (Mohnen, 2008).

Hemicellulosic Polysaccharides

The typical hemicellulosic polysaccharide is a linear polymer composed of a β-D-pyranosyl backbone substituted by short side chains with a single or a few glycosyl residues. Hemicellulosic polysaccharides and cellulose therefore share the structural feature of a β-D-pyranosyl backbone, which allows tight binding of hemicellulosic polysaccharides to the amorphous region of the para-crystalline cellulose microfibril by hydrogen bonding. The hemicellulose/cellulose interaction is of a chemical nature, such that concentrated alkaline solution is required to disrupt the interaction and liberate hemicellulose from the cell wall to aqueous solution (Cosgrove, 1997). Although the side chains on hemicellulosic polysaccharides modulate the efficiency of binding to the microfibrils, the interaction is still strong enough to resist tensile stress derived from turgor pressure.

Since the molecular lengths of certain hemicellulosic polysaccharides such as xyloglucans are longer than the distances between cellulose microfibrils, they can cross-link adjacent microfibrils to tether together and coat the surface of the cellulose microfibrils (Hayashi, 1989; McCann *et al*., 1990), thereby forming a cellulose/hemicellulose network that functions as the major

tension-bearing framework of the primary cell wall. It is worth noting that it is these hemicellulosic cross-links that confer extensibility to the network structure and act as a modulator of mechanical properties of the primary cell wall. Xyloglucans are typical hemicellulosic polysaccharides. These polysaccharides are ubiquitous in land plants and are particularly abundant in dicotyledonous plants (Talbott and Ray, 1992; Popper *et al*., 2011). By contrast, commelinoid monocotyledons, which include cereals such as rice (*Oryza sativa*), have relatively few xyloglucans in most of the tissues, and a relatively large amount of xyloglucans are restricted to certain tissue types such as phloem (Fig. 1.1C; Brennan and Harris, 2011). The predominant glycans in these plant species are glucuronoarabinoxylan and β (1→3),(1→4)-mixed-linkage glucan, which are also hypothesized to cross-link the cellulose microfibrils in these plant species (Vogel, 2008).

In addition to xyloglucans, glucomannans and arabinoxylans may also bind to cellulose microfibrils in the primary cell walls of dicotyledonous and non-graminaceous monocotyledonous plants. The fact that the molecular lengths of these hemicelluloses are significantly shorter than those of xyloglucans, and that their extractability from the cell wall differs from that of xyloglucans, implies that these hemicelluloses may have different interactions with cellulose from those of xyloglucans.

Cellulose/xyloglucan Network

The primary cell wall, which determines cell shape, must be strong enough to withstand the mechanical stresses imposed upon it but flexible enough to allow deformation in response to developmental and environmental cues. Given the load-bearing function of cellulose/xyloglucan networks in the primary cell wall, the rearrangement of these networks is essential during cell growth. Two possible processes have been postulated to be involved in the rearrangement of cellulose/xyloglucan networks. One process envisages a remodeling process in which disruption of hydrogen bonding is followed by immediate reconnection at different positions, allowing remodeling of the cell wall network. The other process postulates remodeling by molecular grafting between xyloglucan cross-links by means of an endotransglycosylation reaction. Both of these processes can be achieved without the loss of cell wall integrity (Fig. 1.2). The protein families expansins (Cosgrove, 2005) and xyloglucan endotransglucosylase/hydrolases (XTHs) (Nishitani and Vissenberg, 2007) are implicated in these processes.

Expansins were originally discovered for their ability to cause acid-induced extension of isolated cell walls (McQueen-Mason *et al*., 1992). Expansins form a family of small cell wall proteins characterized by a certain carbohydrate-binding domain, some of which dissociate interactions between

hemicellulose and cellulose microfibrils (Cosgrove, 2000; Yennawar *et al*., 2006). However, expansins do not exhibit hydrolytic or transglucosylation activity on any matrix polysaccharide examined, despite the fact that their amino acid sequences are similar to the catalytic domain of the family-45 endoglucanases. The expansin EXPB1 has been proposed to facilitate the local movement and stress relaxation of arabinoxylan–cellulose networks within the cell walls of maize by non-covalent rearrangement, but its molecular mechanism remains unclear (Yennawar *et al*., 2006).

In growing cells, wall expansion must be coupled with the synthesis and integration of new wall components to maintain the thickness and mechanical properties of the cell wall. Since expansins cause no significant alterations in the chemical composition of the cellulose/xyloglucan network, their actions alone cannot explain long-term cell expansion in which incorporation of new wall materials is required to compensate for the thinning of wall thickness. Instead, it is likely that expansins are involved in rapid and transient cell expansion processes such as the 'acid growth' process observed during the first phase of auxin-induced cell expansion, which is based on rapid, localized changes in cell wall extensibility without additional polysaccharide synthesis (Rayle and Cleland, 1992).

XTHs form a subgroup in the Glycoside Hydrolase Family 16 (GH16). Several members of the XTH family specifically cleave a β (1→4) glucosidic linkage of an unsubstituted glucosyl residue in a xyloglucan main chain (donor substrate), and reconnect the reducing end generated by cleavage of the donor molecule to the non-reducing end of another xyloglucan molecule (acceptor substrate). This activity is termed xyloglucan endotransglucosylase (XET) activity. Certain XTHs only cleave xyloglucan without reconnecting the split end, an activity termed xyloglucan endohydrolase (XEH) activity (Nishitani, 1997; Nishitani and Vissenberg, 2007).

Both XET and XEH activities enable extension of xyloglucan chains tethering cellulose microfibrils and integrate new xyloglucans into the cellulose/xyloglucan networks (Rose *et al*., 2002). This process provides an effective means of rearranging the cellulose/xyloglucan network and allows sustainable expansion of the cell wall. Although a complete description of the functions of the XTH family of proteins *in muro* is not yet available, the physiological role of XTH is becoming clearer. We will return to this topic in 'Function of Xyloglucan and XTH'.

Pectic Polysaccharides

Hemicellulose serves as a cross-linker between cellulose microfibrils in the primary cell wall, whereas pectin exists in a gel and serves as a space-filling or packing matrix in the primary cell wall.

Pectic polysaccharides are highly hydrophilic and are most soluble in the water of polysaccharides in the primary cell wall. Pectic polysaccharides are easily extracted by hot water, chelating agents, or dilute acidic solutions. These polysaccharides are composed of a complex, heterogeneous group of polysaccharide domains and characteristically contain galacturonic acid and rhamnose, arabinose, and galactose as major sugar components (Ridley *et al*., 2001). The main pectin domains include the homogalacturonan (HG), rhamnogalacturonan I (RG I), and rhamnogalacturonan II (RG II) domains. The HG domain has a relatively simple structure, forming a linear polymer consisting of 1,4-linked α-D-galacturonic acid residues. RG I has a backbone composed of alternating (1→2)α-L-rhamnose-(1→4) α-D-galacturonic acid residues decorated primarily with arabinan and galactan side chains. The RG II domain is a low molecular mass but highly complex carbohydrate domain composed of 11 different glycosyl residues.

Some structural models have been proposed for the pectic matrix in the primary cell wall. One conventional model envisages HG to be a long main chain connected in series to two branching domains, RG I and RG II. In this model, the pectin backbone consists of three connected domains. In a more recent model, however, RG I is postulated to function as a scaffold or the main chain to which long HG domains and RG II domains are covalently attached as side chains (Vincken *et al*., 2003). Pectic polysaccharides are therefore covalently joined *in vivo* (Willats *et al*., 2006).

Pectin Network

The pectic polysaccharides are subject to a number of modifications of conformation and covalent linkage, crucial processes that alter the chemical and physical nature of the matrix in the primary cell wall.

Borate binds to two apiose residues in RG II domains and forms an apiose-borate-apiose diester bridge. Most RG II molecules are spontaneously dimerized through the borate ester bridge upon secretion into the cell wall space (O'Neill *et al*., 2004). Borate-mediated cross-linking contributes to the strengthening of the primary cell wall as well as the control of wall porosity and intercellular adherence (Caffall and Mohnen, 2009).

Another modification of pectic polysaccharides is de-esterification of methylesterified galacturonic acid residues in the HG domain. Nascent HG domains localized in the Golgi are normally fully methylesterified. Methylester groups on the HG domain are removed upon secretion into the cell wall by pectin methylesterases (PMEs) present in the cell wall space. Demethylesterification of pectin is followed by two alternative pectin modification processes.

In one process, free carboxyl groups generated on the HG domain are cross-linked via Ca^{2+} bridges to assemble the pectin into a gel-like network. The resulting HG-Ca^{2+} complex acts as a space-filling hydrophilic filter to prevent aggregation and collapse of the cellulose/hemicellulose network, and is considered to make the network less sensitive to the actions of cell wall enzymes (Cosgrove, 1997) and thereby renders the cell wall more resistant to compression stress. This process is observed in the regulation of cell wall stiffening in basal parts of inflorescence stems (Hongo *et al*., 2012).

In the other process, random demethylesterified HG domains are not fully cross-linked via Ca^{2+} bridges. These domains become more susceptible to hydrolytic degradation and are disassembled. This process is observed during primordial development in the shoot apical meristem (Peaucelle *et al*., 2011a).

In addition to the boron and calcium bridges, pectic polysaccharides are linked to each other by various covalent bonds including ester linkages through phenolic dimers such as diferulic acid (Wallace and Fry, 1994). Furthermore, the pectin networks may be linked to the cellulose/xyloglucan networks and structural proteins via phenolics, such as *p*-coumaryl and feruloyl acids (Caffall and Mohnen, 2009). More recently, pectin was demonstrated to link covalently to arabinoxylan via a rhamnosyl residue in the arabinogalactan (AG) domain of an arabinogalactan protein (AGP). This wall structure, consisting of arabinoxylan, pectin, and AGP, is referred to as Arabinoxylan Pectin Arabinogalactan Protein1 (APAP1; Tan *et al*., 2013). A large macromolecular network, which can interact functionally with the other components, is also thought to be responsible for various physical properties of the primary cell wall (Fig. 1.2).

The precise functions of many of the pectin networks are yet to be determined; however, recent work shows that modifying the pectic polysaccharides is a key process in elucidating functional network formation with respect to plant growth and development. We will return to this topic in ‘Function of Pectin and PME’.

Biosynthesis and Assembly of the Cell Wall

Cellulose is synthesized at the plasma membrane by large complexes called rosette terminal complexes (TCs; Delmer and Amor, 1995), whereas matrix polysaccharides are polymerized exclusively in the Golgi lumen and secreted into the apoplast or cell wall space via a membrane trafficking system. This section focuses on the general mechanisms of the synthesis of the two types of cell wall polysaccharides and how these polysaccharides are assembled into the dynamic architecture of the primary cell wall.

Cellulose Synthesis

A rosette terminal complex consists of six subunits, with each subunit containing six catalytic units of cellulose synthase (CESA) proteins. Each catalytic unit has been proposed to mediate polymerization of one (1→4)-linked β-D-glucan chain using UDP (uridine diphosphate) -glucose as the substrate, which is supplied by a membrane-associated form of sucrose synthase localized in the vicinity of the catalytic units of cellulose synthase (Doblin *et al.*, 2002; Carpita, 2011). According to this model, 36 molecules of (1→4)-linked β-D-glucans are synthesized at the same time on a single rosette. This predicted number of molecules is not however supported by the actual observed numbers, which range from 15 to 32 as discussed in 'Components of the Primary Cell Wall'. Therefore, either two catalytic units are involved in the synthesis of a single glucan chain, or fewer than five of the six catalytic units are actually functional in the rosette. The mechanism underlying cellulose synthesis therefore remains controversial.

Nascent (1→4)-linked β-D-glucan chains are extruded as a self-assembling microfibril, forming a crystalline microfibril. Evidence from genetic experiments indicates that three different *CESA* genes are normally required to produce a functional complex, and that different sets of genes are involved in the formation of the primary and secondary walls. In Arabidopsis, for example, *CESA1*, *CESA3*, and *CESA6* are required for the synthesis of the primary cell wall, whereas *CESA4*, *CESA7*, and *CESA8* are required to form secondary cell walls (Burn *et al.*, 2002; Taylor *et al.*, 2003). Moreover, CESA2 and CESA5 are partially redundant with CESA6 (Desprez *et al.*, 2007).

The master regulatory transcription factors that specifically govern the synthesis of the secondary cell wall have been successfully identified using suspension-culture cell lines, which can be forced to undergo highly synchronized differentiation to tracheary elements (Yamaguchi and Demura, 2010). On the other hand, cellulose synthesis, especially in the primary cell wall, has been proposed to be controlled post-transcriptionally rather than by transcription factors (Somerville *et al.*, 2004).

In addition, a variety of correlative evidence shows that the oriented deposition of cellulose microfibrils seems to be guided by microtubules adjacent to the plasma membrane (Somerville, 2006). A microtubule-associated protein termed CESA interactive protein 1 (CSI1) functions as a bridge between CESA complexes and cortical microtubules. CSI1 plays a crucial role in regulating microtubule-directed cellulose synthesis (Li *et al.*, 2012; Mei *et al.*, 2012).

Chitinase-like (CTL) proteins, including CTL1/POM1 and CTL2, are another class of regulators of cellulose synthesis. These two proteins are secreted to the apoplast and interact with CESA. In *ctl1/ctl2* double mutants the crystalline cellulose content is reduced. This suggests that these two CTLs

affect assembly of the glucan chains, thereby modulating the interactions between xyloglucan and cellulose (Sanchez-Rodriguez *et al*., 2012).

Synthesis of Matrix Polysaccharides

Matrix polysaccharides, including hemicellulosic and pectic polysaccharides, are synthesized in the Golgi and secreted into the wall via an uncharacterized vesicle-mediated trafficking pathway. Given the complexity of the structural features of the matrix polysaccharides in terms of glycosidic linkages and sugar residues, it is obvious that a large number of enzymes are required for their synthesis.

Biosynthesis of xyloglucan (which has a relatively simple structure) is thought to require β (1→4)-glucan synthase, encoded by *CELLULOSE SYNTHASE-LIKE C* (*CslC*), to form the glucan backbone as well as at least three other types of glycosyltransferases – including α-fucosyltransferases, β-galactosyltransferases, and α-xylosyltransferase – to decorate the glucan main chain with side chains (Zabotina, 2012).

For the synthesis of pectic polysaccharides, which are more complex than xyloglucan, at least 67 transferases are thought to be required. These enzymes, which include glycosyl-, methyl-, and acetyltransferases (Mohnen, 2008), are typically encoded by large multigene families classified as glycosyl transferases in the CAZy (carbohydrate-active enzymes) database (Yokoyama and Nishitani, 2004). Most of these synthetic enzymes for matrix polysaccharides are integral membrane proteins and are considered to exist as complexes anchored to the Golgi (Atmodjo *et al*., 2013).

The newly synthesized and retained polysaccharides in the Golgi lumen are secreted as soluble polymers into the cell wall space, where they diffuse within the aqueous extracellular environment to their final destination by an as-yet-unknown mechanism.

Cell Wall Assembly

Upon secretion into the cell wall space, the matrix polysaccharide precursors become associated with the pre-existing cell wall polymers or other newly secreted precursors *in muro*. Some of the polysaccharides are also assembled into larger polysaccharides via poorly understood mechanisms. Thus, the newly synthesized polysaccharides are integrated into the pre-existing framework of the primary cell wall to alter or maintain its chemical and physical nature.

Network formation involves both spontaneous interactions between the polysaccharides and, perhaps, enzymatic cross-linking. Although the precise

molecular processes have not yet been fully identified, there are a few examples in which specific wall enzymes are involved in the assembly of newly synthesized polysaccharides into the pre-existing network. XTH may be the only potential candidate for this function. XTH catalyzes the molecular grafting or disassembly of xyloglucan cross-links within the cellulose/xyloglucan network as well as the integration of newly synthesized xyloglucans into the cellulose/xyloglucan network (Ito and Nishitani, 1999; Rose *et al*., 2002; Eklöf and Brumer, 2010). PME-mediated demethylesterification of the HG domain of pectin is another example, which leads to the formation of Ca^{2+} bridges between the carboxyl groups of HG domains, thereby forming pectin gel (Micheli, 2001). These *in muro* network formations generally require a regulatory system for transporting the enzymes and their substrates to the proper location at the proper time and to perform reactions in a synergistic manner. PMEs are no doubt the key factor involved in the regulatory system for such *in muro* network formation of pectin.

To date, two types of key factors (XTH and PME) have been specifically implicated in the important processes of network formation which directly affects the physical properties of the primary cell wall and defines cell morphology. The functions of these two classes of proteins are discussed in the following sections.

Function of Xyloglucan and XTH

The first indication of the role for xyloglucan in cell wall expansion was obtained when its metabolism was studied using pulse-chase experiments employing ^{14}C-labeled glucose in pea stem tissues. This classical experiment clearly showed that xyloglucan metabolism is enhanced during auxin-induced cell expansion (Labavitch and Ray, 1974a, b). This study was followed by the finding that changes in the molecular weight of cell wall xyloglucans were generally induced by auxin and acidic pH in various land plants, including monocotyledonous plants and gymnosperms (Nishitani, 1997).

These observations provided strong evidence for the hypothesis that hydrolytic cleavage of xyloglucan cross-links between cellulose microfibrils is the key step controlling the mechanical properties of the cell wall. However, it was also observed that cleavage of load-bearing linkages alone cannot account for prolonged cell expansion, in which remodeling of the cell wall is required to integrate new wall components into the pre-existing framework (Nishitani, 1997). To explain this paradox, hypothetical endotransglycosylation, or molecular grafting between cross-linking molecules, was postulated (Albersheim, 1976).

The existence of this hypothetical enzyme was demonstrated when the enzyme capable of mediating molecular grafting between xyloglucans was