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.

Plant Cell Wall Patterning and Cell Shape, First Edition. Edited by Hiroo Fukuda.

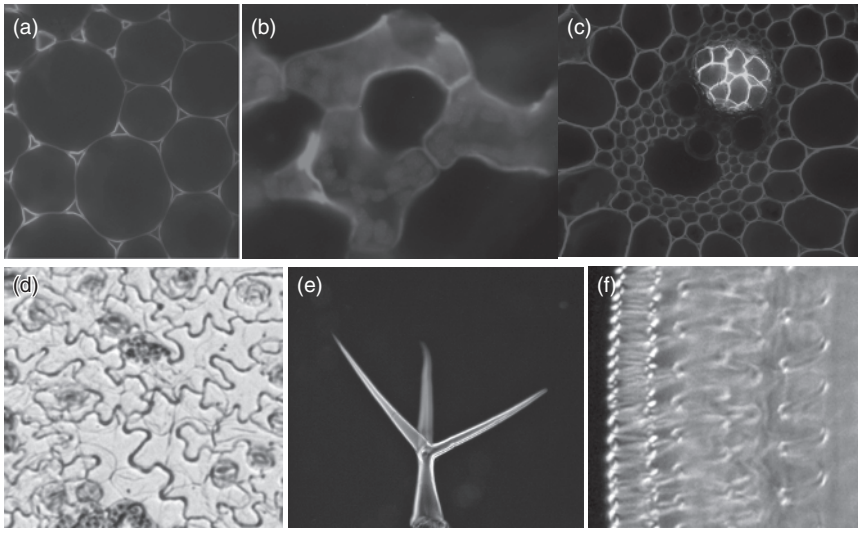

Figure 1.1 Various types of plant cells defined by the cell wall: (a–c) immunofluorescence labeling with monoclonal antibodies against cell wall polysaccharide epitopes; (a) JIM5, specific to homogalacturonan with a low degree of methylesterification; (b, c) CCRC-M1, specific to fucosylated xyloglucan; (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

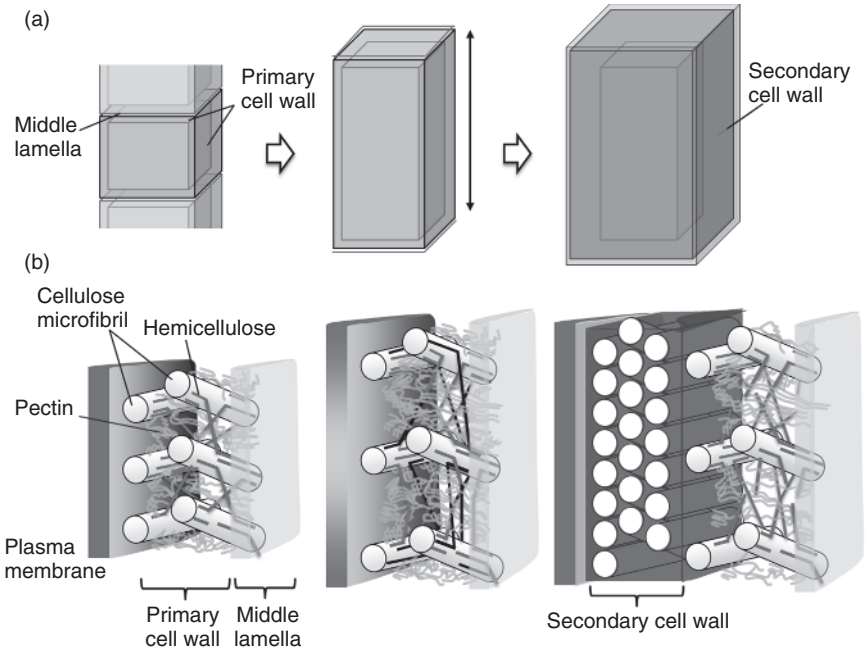

Figure 1.2 Cellulose/hemicellulose and pectin networks in the primary cell wall at successive stages of plant cell growth. (a) Processes of cell elongation and differentiation. (b) Major polymers and their likely arrangement in the cell wall. Newly secreted hemicelluloses (shown in black) and the other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

isolated from the cell wall space of Azuki bean (Fry *et al*., 1992; Nishitani and Tominaga, 1992). This enzyme is currently termed xyloglucan endotransglucosylase/hydrolase (XTH) (Rose *et al*., 2002). In this section, we discuss the roles of XTH family enzymes and their specific substrates, xyloglucans, in determining cell shape in plants.

Xyloglucan Structure and Function

Xyloglucan (Fig. 1.3) is the most abundant cross-linking polymer in land plants (Fry, 1989; Hayashi, 1989; Popper *et al*., 2011), consisting of a β (1→4)-glucan backbone frequently decorated with side chains of α (1→6)-xylosyl residues. The α (1→6)-xylosyl side chains are often further substituted with several different glycosyl residues depending on phylogeny and cell type. In non-commelinoid monocotyledons and non-solanaceous eudicotyledons, xylosyl residues are substituted with β (1→2)-galactosyl residues with or without further substitution with α (1→2)-fucosyl residues; in solanaceous plants, these residues are substituted with β (1→2)-galactosyl or β (1→2)-arabinofuranosyl residues. In Poales (and Commelinales), the β (1→4)-glucan backbone is less frequently substituted by xylosyl residues, and α (1→2)-fucosyl-β (1→2)-galactosyl substitution is only restricted to a specific cell wall type such as that in phloem (Hsieh and Harris, 2009; Brennan and Harris, 2011).

As for other polysaccharides in plant cell walls, hydroxyl groups in xyloglucans are O-acetylated at various positions. In Arabidopsis, for example, the galactosyl moiety of the side chain is intensively O-acetylated. This acetylation affects the hydrolytic degradation properties of xyloglucan side chains. An Arabidopsis mutant with reduced acetylation in xyloglucan (*rwa2*) is more resistant to the pathogen *Botrytis cinerea* than the wild type, but it exhibits no obvious morphological or growth differences (Manabe *et al*., 2011). The role of O-acetylation in the cell wall extension process therefore remains unclear.

As we saw in ‘Components of the Primary Cell Wall’, xyloglucans can non-covalently associate with β (1→4)-glucan chains of the cellulose microfibrils to form the cellulose/xyloglucan network (Pauly *et al*., 1999) which is found in the primary cell walls of flowering plants (Carpita and Gibeaut, 1993). *In vitro* experiments reveal that the side chains of xyloglucans can prevent self-association of xyloglucan molecules and that the terminal fucosyl residue helps to stabilize the planar configuration of the xyloglucan backbone, which is essential for optimal cellulose binding. Arabidopsis mutants lacking a fucosyl (*mur1*) or galactosyl (*mur3*) residue exhibit tissues with slightly reduced mechanical strength, but do not exhibit drastic phenotypic changes in vegetative growth. Moreover, the Arabidopsis double mutant *xxt1/xxt2*, which lacks detectable xyloglucan in its cell wall, exhibits

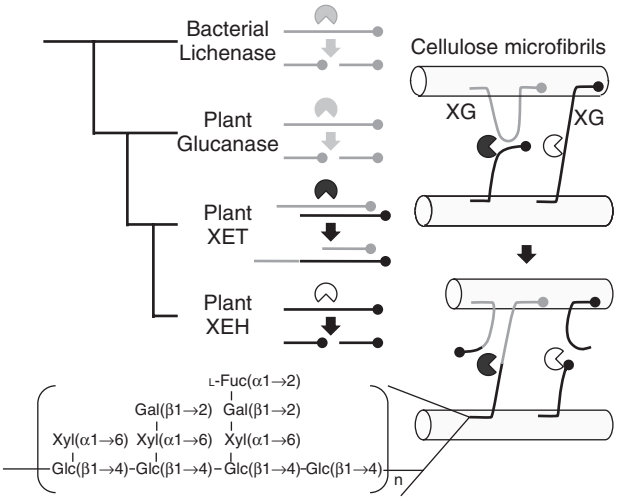

Figure 1.3 Phylogeny of the XTH family of enzymes and their roles in construction, modification, and disassembly of the cellulose/xyloglucan network in the primary cell wall. Black or gray lines indicate xyloglucans (XG), and the circle ends indicate the reducing termini. XET, xyloglucan endotransglucosylase; XEH xyloglucan endohydrolase.

significant changes in the mechanical properties of its tissue, but gross morphological phenotypes are not observed in this mutant (Cavalier *et al*., 2008). These observations suggest that xyloglucans, and hence cellulose/xyloglucan networks, are not essential for basic plant architecture or that they may be compensated for by other components of the cell wall; however, they may be required for modulating the mechanical properties of the cell wall.

XTH Family

A molecular basis for a dynamic aspect of the cellulose/xyloglucan network was first demonstrated in the 1990s, when XET activity was isolated from growing plant tissues (Fry *et al*., 1992; Nishitani and Tominaga, 1992).

The enzymes responsible for such activity (EC 2.4.1.207) are encoded by a multigene family. The genomes of three angiosperm species, Arabidopsis (*Arabidopsis thaliana* (L.) Heynh.), rice (*Oryza sativa* L.), and poplar (*Populus trichocarpa*) contain 33, 29, and 41 members (Yokoyama and Nishitani, 2001; Yokoyama *et al*., 2004; Geisler-Lee *et al*., 2006), respectively, while the basal land plant moss (specifically *Physcomitrella patens*) possesses 32 members (Yokoyama *et al*., 2010). This family of proteins is classified into three

groups – I/II, IIIA, and IIIB – based on amino acid sequence similarity. Biochemical and structural studies have predicted that group I/II XTH exhibits XET activity, whereas group IIIA XTH exclusively exhibits endohydrolase (XEH) activity to xyloglucan molecules (Rose *et al*., 2002; Chanliaud *et al*., 2004; Baumann *et al*., 2007; Eklöf and Brumer, 2010; Kaewthai *et al*., 2013). Accordingly, this family of enzymes was renamed the xyloglucan endotransglucosylase/hydrolase (XTH) family (Rose *et al*., 2002). Each member of this family can mediate either cleavage and reconnection or simple cleavage of xyloglucan molecules. It is worth noting that construction, remodeling, and disassembly of xyloglucan cross-linking in the cellulose/xyloglucan network can be explained by the collaborative actions of XTH family enzymes (Nishitani, 1997).

Phylogenetic analysis indicates that the XTH gene family has diversified during the evolution of land plants (Yokoyama and Nishitani, 2001; Yokoyama *et al*., 2010). In addition, both biochemical and crystallographic data indicate that the functional differences between XET and XEH can be ascribed to differences in the structural features around the acceptor binding site, namely whether a water molecule or the xyloglucan molecule has access to the acceptor substrate site (Baumann *et al*., 2007).

As mentioned in 'Biosynthesis and Assembly of the Cell Wall', the XTH enzymes belong to Glycoside Hydrolase Family 16 (GH16) (Barbeyron *et al*., 1998; Cantarel *et al*., 2009), which comprises a broad range of microbial endoglucanases and endogalactanases, as well as algal β-agarase and κ-carrageenase. Since the closest relatives of XTH in GH16 are bacterial licheninases (EC 3.2.1.73) which specifically hydrolyze β (1→3), β (1→4)-glucans, it has been hypothesized that the XTH genes evolved from these bacterial glucanases (Planas, 2000; Eklöf and Brumer, 2010). This notion was supported by the recent finding of a novel endoglucanase from poplar (*Populus trichocarpa*), which revealed a newly recognized small clade of GH16 genes that represent intermediates between the bacterial licheninases and plant XTHs (Eklöf *et al*., 2013).

Xyloglucan Dynamics as Mediated by XTH

XET activity: Before the discovery of XTH, growth-promoting and growth-inhibitory effects of exogenously applied xyloglucan oligosaccharides were documented, and the 'oligosaccharin' hypothesis was advanced in the late 1980s (McDougall and Fry, 1989; Vargas-Rechia *et al*., 1998). This hypothesis proposes that oligosaccharides derived from cell wall xyloglucans act as signaling molecules (Ryan, 1987).

Currently however, the term 'oligosaccharins' is seldom used for xyloglucan oligosaccharides, which are instead considered to function simply as acceptor substrates for XTH, which mediate incorporation of xyloglucan oligomers into the cellulose/xyloglucan network to facilitate disassembly of xyloglucan cross-links (Takeda *et al*., 2002). This point of view is also supported by the observation that the growth of tobacco suspension-culture cells is accelerated by XTH-mediated incorporation of xyloglucan oligosaccharide into the cell wall (Ito and Nishitani, 1999; Kaida *et al*., 2010).

Since the 1990s, the roles of XTHs in plant growth and differentiation have been extensively investigated (Nishitani and Vissenberg, 2007). These studies show a positive correlation between XET activity and the growth process in specific tissues or organs, such as fruit ripening (Redgwell and Fry, 1993) and root elongation (Ryan, 1987; Pritchard *et al*., 1993; Vissenberg *et al*., 2000). Furthermore, the effects of environmental factors (such as mechanical stimulus, light conditions, anoxia, and developmental signals) on XTH gene expression, as mediated by plant hormones, have been extensively studied (Lee *et al*., 2005; Nishitani and Vissenberg, 2007).

Comprehensive expression analysis of all XTH genes of Arabidopsis reveals that virtually all members of the XTH family have specific expression patterns, many of which overlap. This result indicates that a specific set of XTH family genes are expressed in individual tissues and in response to individual environmental stimuli (Yokoyama and Nishitani, 2001; Lee *et al*., 2005). This ubiquitous nature suggests the functional importance of xyloglucan dynamics throughout the life cycles of land plants.

The overwhelming redundancy in XTH genes has however hampered functional studies employing phenotypic analysis of XTH mutants. Only a few of the 33 Arabidopsis XTH genes have been shown to exhibit morphological phenotypes in their loss-of-function mutants. Nonetheless, some mutants of XTH genes exhibit tissue-specific phenotypes.

For example, *atxth27-1* is a transposon-tagged knockout line of *AtXTH27*. This mutant differentiates morphologically altered tracheary elements, fails to develop tertiary veins in leaves, and exhibits lesion-mimic yellow spots on leaves (Matsui *et al*., 2005), indicating that this gene is involved in the formation of cell walls of tracheary elements. Another Arabidopsis mutant, *atxth28-1* (in which *AtXTH28* is disrupted by a T-DNA insertion), is defective in efficient self-pollination due to its shorter stamens, suggesting that *atxth28* plays a role in the elongation growth of filament cells in the stamen (Kurasawa *et al*., 2009). AtXTH27 and AtXTH28 are similar to each other in terms of both expression profile and amino acid sequence. These proteins belong to the group IIIB subclass of the XTH family and are predicted to exhibit XET activity. However, the phenotype of *atxth28* is not enhanced by *atxth27*, indicating that AtXTH28 is not functionally redundant with AtXTH27 in planta. XTH is involved in a plant's responses to light. In general, plants are highly

sensitive to the quality, quantity, and direction of light, and they usually escape from the shade of other plants by rapidly elongating their shoot. This phenomenon, termed shade avoidance, is triggered under low red/far red light and high green light conditions. These types of light upregulate the expression of *AtXTH9*, *AtXTH15*, *AtXTH16*, *AtXTH17*, *AtXTH19*, and *AtXTH22* (Sasidharan *et al*., 2010). Interestingly, disrupting only one of these genes can retard accelerated shoot growth under such light conditions, indicating the non-redundant action of these genes on shoot growth.

XTH is also involved in plant defenses. Infestation of celery (*Apium graveolens*) by aphids (*Myzus persicae*) causes up-regulation of certain XTH genes, including celery *XTH1* specifically in the phloem, a tissue from which aphids suck plant sap (Divol *et al*., 2007). Arabidopsis *AtXTH33*, which is the closest homolog to celery *XTH1*, is also up-regulated when Arabidopsis is infested by aphids. Since aphids preferentially settle on loss-of-function mutant of *AtXTH33* (*atxth33*) plants compared with wild-type plants, AtXTH33 and its celery counterpart may play a role in protecting plants from aphids. Since over-expression of *AtXTH33* in Arabidopsis does not increase the resistance of plants to aphids, factors other than AtXTH33 are likely to be involved in this defense mechanism.

XTHs are therefore involved in growth responses to environmental stimuli as well as defense to biotic stresses. For other functions of XTH in the hormonal regulation of plant growth and responses to biotic and abiotic environmental stimuli, the reader is referred to previous reviews (Lee *et al*., 2005; Nishitani and Vissenberg, 2007).

XEH Activity: Storage xyloglucan, which occurs widely in plant seeds, was noted as early as the 1830s by Matthias J. Schleiden and was named amyloide because it was stained blue with iodine (Edwards *et al*., 1985). The enzyme, which is capable of specifically hydrolyzing storage xylgolucans, was isolated and characterized from germinated cotyledons of nasturtium (*Tropaeolum majus* L.) and was named xyloglucan-specific endo-(1→4)-β-D-glucanase before the discovery of the XTH family of proteins (Edwards *et al*., 1986). Currently, this hydrolase is classified as a group IIIA XTH.

During germination, storage xyloglucans in cotyledonary cell walls are degraded into monosaccharides by the sequential actions of hydrolytic enzymes. These enzymes include glycosidases acting on xyloglucan side chains as well as XTH members with XEH activity (Dos Santos *et al*., 2004). XEH activity is therefore responsible for supplying carbohydrates used for both respiration and body construction during seed germination.

Interestingly, genes encoding group IIIA XTH are found even in plant species whose cotyledons do not contain storage xyloglucans (Eklöf and Brumer, 2010). Arabidopsis does not contain storage xyloglucans in its seeds, but it does contain two group IIIA XTHs, AtXTH31 and AtXTH32, which

are expressed in various organs other than seeds. This observation suggests that group IIIA XTHs may play other roles than in degradation of storage xyloglucan. In fact, a recent study shows that neither a double mutant of *atxth31* and *atxth32* nor transgenic plants impaired in these genes exhibit prominent phenotypic changes in morphology (Kaewthai *et al*., 2013). These results, together with those obtained using the xyloglucan-less double mutant *xxt1*/*xx2* described in 'Xyloglucan Structure and Function', raise questions about the essential role of xyloglucan in the primary cell wall in determining cell shape.

Another point worth noting is that rice (*Oryza sativa* L.), which contains little xyloglucan in most tissues, still possesses group IIIA XTH with hydrolase activity; the physiological role of this XTH remains elusive (Hara *et al*., 2014).

One possible explanation for these discrepancies is that there is an as-yet-unknown essential factor (or factors) that is in charge of the regulation of the basic properties of the primary cell wall, and xyloglucans serve as modulators of this essential factor. This notion is consistent with the newest structural model of the primary cell wall which has been advanced based on solid-state, high-resolution, carbon-13 cross-polarization/magic angle spinning nuclear magnetic resonance studies. This model envisages that pectin, not xyloglucan, intensively interacts with cellulose microfibrils in the primary cell wall (Peaucelle *et al*., 2012; Wang *et al*., 2012). The possible role of pectin in determining cell shape is discussed in the next section.

Function of Pectin and PME

As for xyloglucans, pectins are broadly conserved among land plants and they act as the major component that fills spaces within the cellulose/xyloglucan network in the primary cell wall. Through the modification of pectin, plants regulate the physical properties of primary cell walls, thereby directly regulating developmental processes including the control of cell shape in plants. There have also been recent breakthroughs in understanding pectin biosynthesis and functionality, and a set of enzymes responsible for pectin biosynthesis has been identified (Mohnen, 2008; Peaucelle *et al*., 2012; Wang *et al*., 2012; Atmodjo *et al*., 2013). In addition, new roles for pectin in plant growth and development are being elucidated (Palin and Geitmann, 2012; Peaucelle *et al*., 2012). Since recent progress in this field has been reviewed by other authors (e.g., Atmodjo *et al*., 2013), this section will focus on PME-mediated modification of pectin and its biological significance in the determination of cell shape in plants.

Basic Mechanisms of PME Action

Pectins as Regulators of the Physical Properties of the Primary Cell Wall: As stated in 'Components of the Primary Cell Wall', pectin consists of three major domains, namely HG (HG), RG I (RG-1), and RG II (RG-II). HG is the most abundant domain, accounting for more than 60% of the pectin in the primary cell wall. HG consists of an unbranched homopolymer of α-D-galacturonic acid residues, while RG I and RG II have complex side chains. Biosynthesis, methylesterification, and the addition of side chains to pectin main chains occur in the Golgi apparatus.

An immunolabeling study using sycamore maple (*Acer pseudoplatanus* L.) suspension-culture cells has revealed that pectins are polymerized at the cis-Golgi, methylesterified at the medial-Golgi, and modified with side chains at the trans-Golgi (Zhang and Staehelin, 1992).

Two galacturonosyltransferases (GalAT), GAUT1 and GAUT7, were recently demonstrated to form a catalytic core of GalAT in Arabidopsis (Atmodjo *et al*., 2011). Interestingly, during the formation of a functional catalytic core, GAUT1 is processed to cleave the N-terminal transmembrane domain and is no longer retained in the Golgi, whereas GAUT7 remains unprocessed. GAUT1 is therefore anchored to the Golgi via association with GAUT7 to form the GAUT1: GAUT7 complex.

As identified in 'Components of the Primary Cell Wall', HG is secreted into the cell wall space in a highly methylesterified state and is then demethylesterified by pectin methylesterases (PMEs). The degree and pattern of methylesterification are critical factors in determining subsequent reactions, i.e., stiffening by cross-linking with Ca^{2+} or degradation by polygalacturonases (PGs). Unraveling the mode of action of PMEs on HG and its regulatory mechanism would therefore constitute an important advance in understanding how polysaccharide modification is controlled in the wall space and how closely it is related to cell wall integrity.

Two Modes of Demethylesterification of Pectin: PME acts on HG in two different manners, i.e., 'random' and 'linear' (Fig. 1.4; Micheli, 2001). In the random mode, PME randomly demethylesterifies galacturonic acid residues in HG and produces discontinuously demethylesterified regions in HG, which is susceptible to degradation by PGs, resulting in cell wall loosening or cell wall disassembly. In the linear mode on the other hand, PME acts on continuous blocks of galacturonic acid residues along HG, giving rise to blocks of demethylesterified residues in HG. This facilitates association among the residues via the highly frequent formation of Ca^{2+} bridges to form a gel structure, which contributes to the stiffening of cell walls. Multiple factors such as pH, cations, and the initial degree of methylesterification (DE) are thought to affect the mode of action and catalytic properties of PME (Catoire *et al*.,

1998; Denes *et al*., 2000; Schmohl *et al*., 2000; Goldberg *et al*., 2001; Kim *et al*., 2005).

Diversification of the PME Family: Pectin is conserved ubiquitously in the Streptophyta, from charophytes to angiosperms (Popper *et al*., 2011). The PME family of enzymes is thought to have arisen along with pectins and undergone diversification. In Arabidopsis, 66 members comprise the PME family. Monocotyledons, especially grasses (whose cell walls contain fewer pectic polysaccharides than dicotyledonous plants), have relatively few PMEs (*c*. 40 members in rice and *Sorghum bicolor* L. Moench). In addition, 15 PME members are found in *Coleochaete orbicularis* (Wang *et al*., 2013). The diversification of PMEs is likely to have contributed to both the functional and morphological diversification of land plants.

PME Inhibitors: The activity of PME is regulated by specific proteinaceous inhibitors termed pectin methylesterase inhibitors (PMEIs). PMEI was first isolated from kiwi fruit (*Actinidia chinensis* Planch) (Balestrieri *et al*., 1990). Kiwi PMEI is a small protein composed of 152 amino acid residues. Its sequence shows significant similarity to the N-terminal region (termed 'PRO region') of plant PMEs and it binds to PMEs, forming a heterodimeric complex.

PMEIs also comprise a multigene family in angiosperms, whereas ferns, mosses, and charophytes have few or no PMEI members (Wang *et al*., 2013). The genome of *A. thaliana* encodes 71 PMEI members. PMEI and PME form a stoichiometric 1:1 complex. Crystallographic analysis of the PME-PMEI complex has revealed that plant PMEI is structurally distinct from bacterial PMEI (Di Matteo *et al*., 2005). This observation suggests that PMEIs specifically inhibit plant endogenous PMEs, unlike other inhibitor proteins such as polygalacturonase inhibitor proteins (PGIPs) which function equally against microbial polygalacturonases. Clustering analysis of PME and PMEI mRNA data in Arabidopsis has revealed that PMEs can be classified into five clusters with respect to their expression sites (seed coat, shoot apex, micropylar endosperm, chalazal endosperm, and pollen), and PMEIs are classified within the same categories as PMEs (Wolf *et al*., 2009a).

The balanced regulation of PME activity via endogenous inhibitors may be beneficial to angiosperms, which may be related to the evolution of floral organs, fruits, and seeds. To date, functional analyses of PMEI reveal that PMEI is involved in various phenomena (which we discuss below), but little is known about the individual functions of PME–PMEI pairs, except for a few such pairs (Peaucelle *et al*., 2008; Rockel *et al*., 2008; Reca *et al*., 2012).

PRO Region of PME: The PME family enzymes belong to Carbohydrate Esterase Family 8 (CE8) and are classified into two groups based on their

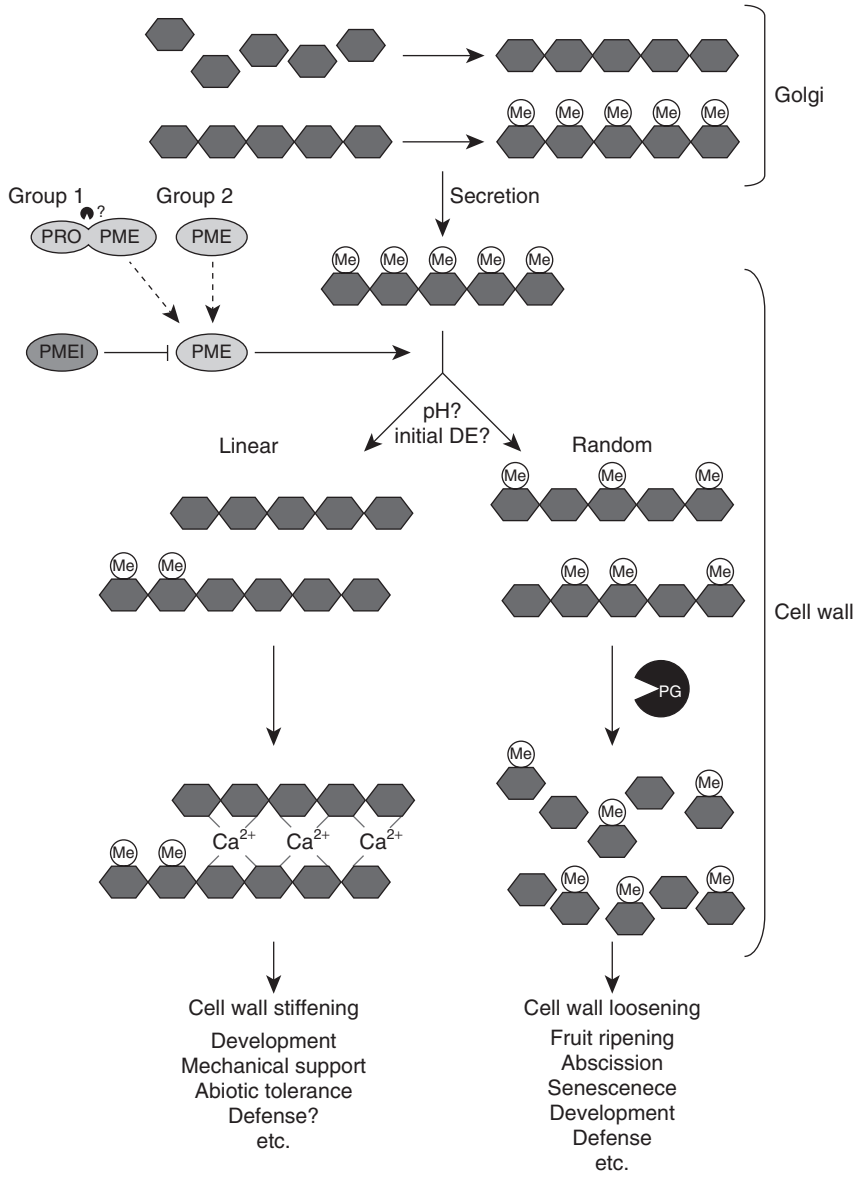

Figure 1.4 Two processes of HG modification. Two types of processes by PME ('linear' and 'random') have different effects on the physical properties of the primary cell wall. Gray hexagons represent galacturonic acid; white circles represent methyl group esterified to galacturonic acid.

primary structures (Pelloux *et al*., 2007). In *A. thaliana*, 47 out of 66 PME members are classified as Group 1 which is characterized by the presence of a PRO region in the N-terminus; the remaining members are classified as Group 2, which lack PRO in the N-terminal region. During the maturation of PMEs, the N-terminal PRO regions are processed by a protease which cleaves the PRO region.

Since PRO regions have structural similarities to PMEI, several possible roles for these regions have been proposed. These regions may serve as: (1) molecular chaperones for PME folding (Shinde and Inouye, 1993); (2) inhibitors of PME; and/or (3) regulators of PME targeting to the cell wall (Micheli, 2001). Experiments using tobacco pollen tubes support the latter two possibilities, namely inhibitory and targeting functions for the PRO region, although the supporting evidence for these roles is rather indirect (Bosch *et al*., 2005). A tobacco transient assay study also indicates that the PRO region is required for the export of PME from the Golgi apparatus to the cell wall (Wolf *et al*., 2009b). Subtilase, a putative proteinase capable of cleaving the PRO region, is also of interest and is currently being investigated. Unveiling the role of PME (as regulated by PMEI and the PRO region) is crucial for understanding the individual roles of pectin modifications in individual cell types at different growth stages.

Other Regulators of Pectin Demethylesterification: The regulatory mechanisms of pectin demethylesterification are likely to vary depending on tissue and organ type as well as developmental events. Recent studies have revealed the emerging roles of indirect regulators of pectin demethylesterification as well as PMEI. Plant hormones may represent one such type of regulator, because plant hormones are master regulators of many developmental events that require PME activity. Recently, PME was shown to act downstream of auxin signaling in phyllotactic events (Peaucelle *et al*., 2011b; Braybrook and Peaucelle, 2013). Another study has also revealed that brassinosteroids have an effect on PME activity in the maintenance of cell wall integrity in growing cells (Wolf *et al*., 2012b). Interestingly, an ubiquitin ligase has emerged as another putative regulator of demethylesterification in seed mucilage pectin (Voiniciuc *et al*., 2013). Several transcription factors that regulate the expression of PMEs in specific cell types were also reported recently (Peaucelle *et al*., 2011b; Phan *et al*., 2011; Negi *et al*., 2013).

Physiological Roles of Pectin Modification

Pectin Modification in Plant Development and Adaptation to Environmental Changes: As mentioned in ‘Components of the Primary Cell Wall’ and at the beginning of this section, the modification of pectins by PMEs and their

regulators plays a crucial role in multiple steps of development by affecting cell wall properties. PME-mediated random demethylesterification, followed by PG-mediated degradation of pectin, is thought to lead to fruit softening in some species such as watermelon, tomato, and peach (Tieman and Handa, 1994; Kagan-Zur *et al*., 1995; Brummell *et al*., 2004; Karakurt and Huber, 2004). PMEI plays an important role in fruit ripening. PMEI purified from ripe fruit is a well-known proteinaceous inhibitor (Balestrieri *et al*., 1990), although the physiological function of this compound has not been fully clarified. PME-mediated enhancement of pectin degradation by PG is also involved in cell adhesion, which contributes to tissue abscission and senescence (Willats *et al*., 2001; Arancibia and Motsenbocker, 2006).

On the other hand, PME and PMEI are involved in multiple steps of plant development because they regulate cell wall stiffening (or suppress stiffening in the case of PMEI). Many studies have suggested the involvement of mutual regulation of PME and PMEI in the growth of hypocotyls, flower stems, roots, and many other organs. Recently, mucilage extrusion in Arabidopsis seed coats was successfully used as a model system to study pectin degradation. Seed mucilage is chiefly composed of unbranched RG-I and small amounts of HG, cellulose, and xyloglucan, and contributes to protecting germinating seeds against drought. Recent work demonstrates that the suppression of pectin demethylesterification by PMEI6 promotes mucilage release, and a subtilase is also involved in this regulatory process (Saez-Aguayo *et al*., 2013).

Another PME member, PME5, plays a role in generating proper shoot phyllotaxis. This PME acts in Arabidopsis shoot meristems and, unexpectedly, increases the elasticity of meristematic cell walls (Peaucelle *et al*., 2008, 2011a, 2011b). By contrast, primary cell wall stiffening mediated by demethylesterification of pectin contributes to the mechanical support to the basal part of the stem (Hongo *et al*., 2012). Pectin modification is therefore important, even in non-growing tissues. These results imply that the mode of action of PME varies depending on its site of action, even in the stem.

Cell wall modification also plays a critical role in the plant's interaction with the environment. PME activity affects a plant's susceptibility to fungi, bacteria, viruses, herbivores, and nematodes (reviewed in Lionetti *et al*., 2012). Highly methylesterified pectin is likely to be most susceptible to degradation by microbial pathogens (Lionetti *et al*., 2007; Volpi *et al*., 2011), whereas the action of fruit-specific PME and the consequent generation of oligogalacturonides by PG reinforce plant defenses against pathogens in strawberries (Osorio *et al*., 2008, 2011). Moreover, the involvement of PME in metal tolerance was recently revealed (Weber *et al*., 2013). Since demethylesterification of HG increases its capacity to interact with apoplastic cations, HG may contribute to the maintenance of metal homeostasis in the cell wall space.

There are other studies showing that polarized or anisotropic demethylesterification occurs within a cell wall through the action of PME, and its inhibition by PMEI is coordinated with the formation of individual cell shape. This phenomenon is well-characterized during pollen tube growth, and the PME–PMEI interaction was shown to be involved in this process. In tobacco (*Nicotiana tabacum* L.) the apical region of the pollen tube is highly methylesterified, and demethylesterification by exogenously applied PME results in thickening of the apical cell wall and inhibition of pollen tube growth (Bosch *et al*., 2005).

Using a transient expression system in tobacco pollen tubes, the growth-promoting activity of Arabidopsis AtPMEI2 and the growth-inhibiting effect of AtPPME1 have been demonstrated. In Arabidopsis, AtPMEI2 accumulates in the apical region of the growing pollen tube, whereas AtPPME1 exhibits a non-polarized localization (Rockel *et al*., 2008).

In addition, PME activity may influence trichome formation and stomatal development although, to date, there is no direct evidence that demethylesterification of pectin is required for polarized growth of the cells (Francis *et al*., 2006; Bischoff *et al*., 2010; Negi *et al*., 2013).

The PME family of enzymes, together with their specific inhibitors in the PMEI family proteins, plays many diverse roles in various developmental processes in almost every tissue. Their individual roles still remain elusive however, and the whole picture of PME/PMEI function has not yet been described. Elucidating these complicated mechanisms is highly important for unveiling the role of the cell wall in determining cell shape, and thus the growth patterns and function of the plant itself.

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