

## *Cell Walls: Structure, Biogenesis, and Expansion*

PLANT CELLS, UNLIKE ANIMAL CELLS, are surrounded by a relatively thin but mechanically strong cell wall. This wall consists of a complex mixture of polysaccharides and other polymers that are secreted by the cell and are assembled into an organized network linked together by both covalent and noncovalent bonds. Plant cell walls also contain structural proteins, enzymes, phenolic polymers, and other materials that modify the wall's physical and chemical characteristics.

The cell walls of prokaryotes, fungi, algae, and plants are distinctive from each other in chemical composition and microscopic structure, yet they all serve two common primary functions: regulating cell volume and determining cell shape. As we will see, however, plant cell walls have acquired additional functions that are not apparent in the walls of other organisms. Because of these diverse functions, the structure and composition of plant cell walls are complex and variable.

In addition to these biological functions, the plant cell wall is important in human economics. As a natural product, the plant cell wall is used commercially in the form of paper, textiles, fibers (cotton, flax, hemp, and others), charcoal, lumber, and other wood products. Another major use of plant cell walls is in the form of extracted polysaccharides that have been modified to make plastics, films, coatings, adhesives, gels, and thickeners in a huge variety of products.

As the most abundant reservoir of organic carbon in nature, the plant cell wall also takes part in the processes of carbon flow through ecosystems. The organic substances that make up humus in the soil and that enhance soil structure and fertility are derived from cell walls. Finally, as an important source of roughage in our diet, the plant cell wall is a significant factor in human health and nutrition.

We begin this chapter with a description of the general structure and composition of cell walls and the mechanisms of the biosynthesis and secretion of cell wall materials. We then turn to the role of the primary cell wall in cell expansion. The mechanisms of tip growth will be contrasted with those of diffuse growth, particularly with respect to the

establishment of cell polarity and the control of the rate of cell expansion. Finally, we will describe the dynamic changes in the cell wall that often accompany cell differentiation, along with the role of cell wall fragments as signaling molecules.

## THE STRUCTURE AND SYNTHESIS OF PLANT CELL WALLS

Without a cell wall, plants would be very different organisms from what we know. Indeed, the plant cell wall is essential for many processes in plant growth, development, maintenance, and reproduction:

- Plant cell walls determine the mechanical strength of plant structures, allowing those structures to grow to great heights.
- Cell walls glue cells together, preventing them from sliding past one another. This constraint on cellular movement contrasts markedly to the situation in animal cells, and it dictates the way in which plants develop (see Chapter 16).
- A tough outer coating enclosing the cell, the cell wall acts as a cellular “exoskeleton” that controls cell shape and allows high turgor pressures to develop.
- Plant morphogenesis depends largely on the control of cell wall properties because the expansive growth of plant cells is limited principally by the ability of the cell wall to expand.
- The cell wall is required for normal water relations of plants because the wall determines the relationship between the cell turgor pressure and cell volume (see Chapter 3).
- The bulk flow of water in the xylem requires a mechanically tough wall that resists collapse by the negative pressure in the xylem.
- The wall acts as a diffusion barrier that limits the size of macromolecules that can reach the plasma membrane from outside, and it is a major structural barrier to pathogen invasion.

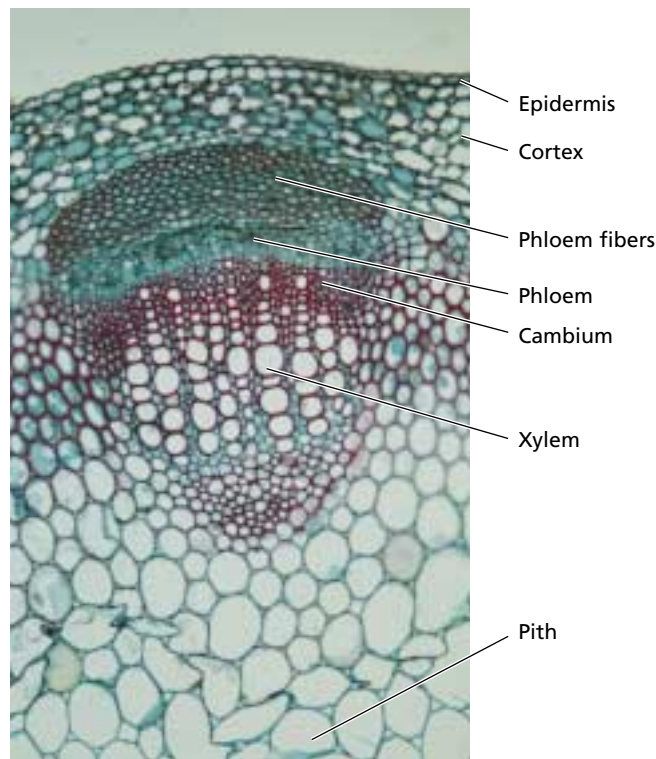
Much of the carbon that is assimilated in photosynthesis is channeled into polysaccharides in the wall. During specific phases of development, these polymers may be hydrolyzed into their constituent sugars, which may be scavenged by the cell and used to make new polymers. This

phenomenon is most notable in many seeds, in which wall polysaccharides of the endosperm or cotyledons function primarily as food reserves. Furthermore, oligosaccharide components of the cell wall may act as important signaling molecules during cell differentiation and during recognition of pathogens and symbionts.

The diversity of functions of the plant cell wall requires a diverse and complex plant cell wall structure. In this section we will begin with a brief description of the morphology and basic architecture of plant cell walls. Then we will discuss the organization, composition, and synthesis of primary and secondary cell walls.

### Plant Cell Walls Have Varied Architecture

Stained sections of plant tissues reveal that the cell wall is not uniform, but varies greatly in appearance and composition in different cell types (Figure 15.1). Cell walls of the cortical parenchyma are generally thin and have few distinguishing features. In contrast, the walls of some specialized cells, such as epidermal cells, collenchyma, phloem fibers, xylem tracheary elements, and other forms of sclerenchyma have thicker, multilayered walls. Often these walls are intricately sculpted and are impregnated with specific substances, such as lignin, cutin, suberin, waxes, silica, or structural proteins.



**FIGURE 15.1** Cross section of a stem of *Trifolium* (clover), showing cells with varying wall morphology. Note the highly thickened walls of the phloem fibers. (Photo © James Solliday/Biological Photo Service.)

The individual sides of a wall surrounding a cell may also vary in thickness, embedded substances, sculpting, and frequency of pitting and plasmodesmata. For example, the outer wall of the epidermis is usually much thicker than the other walls of the cell; moreover, this wall lacks plasmodesmata and is impregnated with cutin and waxes. In guard cells, the side of the wall adjacent to the stomatal pore is much thicker than the walls on the other sides of the cell. Such variations in wall architecture for a single cell reflect the polarity and differentiated functions of the cell and arise from targeted secretion of wall components to the cell surface.

Despite this diversity in cell wall morphology, cell walls commonly are classified into two major types: primary walls and secondary walls. **Primary walls** are formed by growing cells and are usually considered to be relatively unspecialized and similar in molecular architecture in all cell types. Nevertheless, the ultrastructure of primary walls also shows wide variation. Some primary walls, such as those of the onion bulb parenchyma, are very thin (100 nm) and architecturally simple (Figure 15.2). Other primary walls, such as those found in collenchyma or in the epidermis (Figure 15.3), may be much thicker and consist of multiple layers.

**Secondary walls** are the cell walls that form after cell growth (enlargement) has ceased. Secondary walls may become highly specialized in structure and composition, reflecting the differentiated state of the cell. Xylem cells, such as those found in wood, are notable for possessing

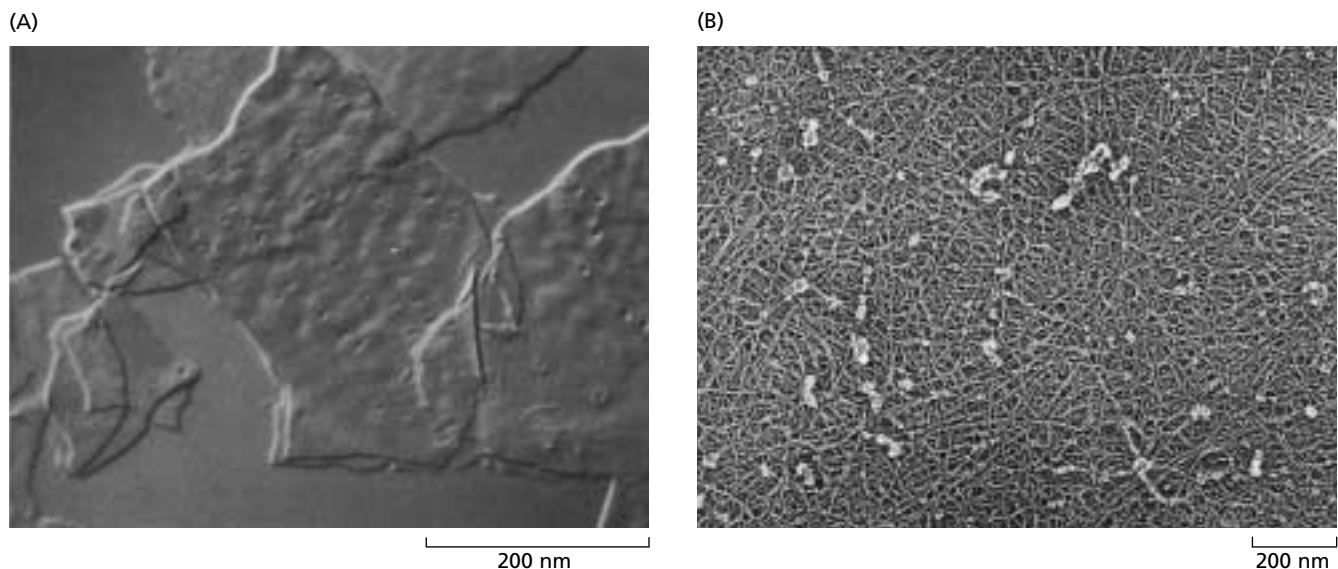
highly thickened secondary walls that are strengthened by **lignin** (see Chapter 13).

A thin layer of material, the **middle lamella** (plural *lamellae*), can usually be seen at the junction where the walls of neighboring cells come into contact. The composition of the middle lamella differs from the rest of the wall in that it is high in pectin and contains different proteins compared with the bulk of the wall. Its origin can be traced to the cell plate that formed during cell division.

As we saw in Chapter 1, the cell wall is usually penetrated by tiny membrane-lined channels, called **plasmodesmata** (singular *plasmodesma*), which connect neighboring cells. Plasmodesmata function in communication between cells, by allowing passive transport of small molecules and active transport of proteins and nucleic acids between the cytoplasm of adjacent cells.

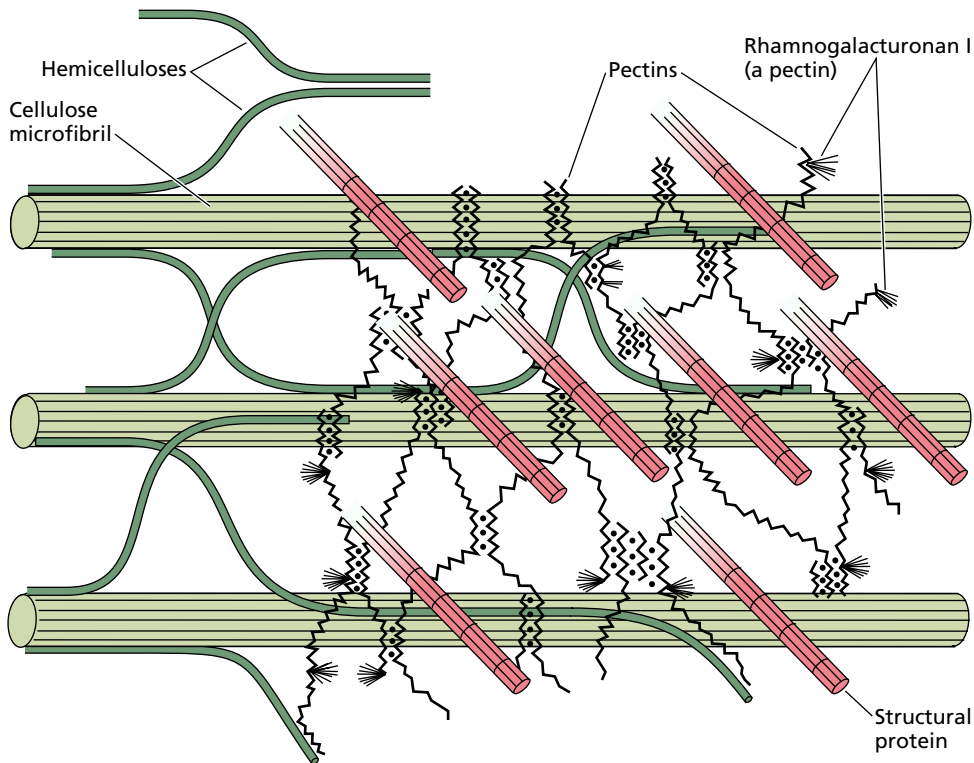
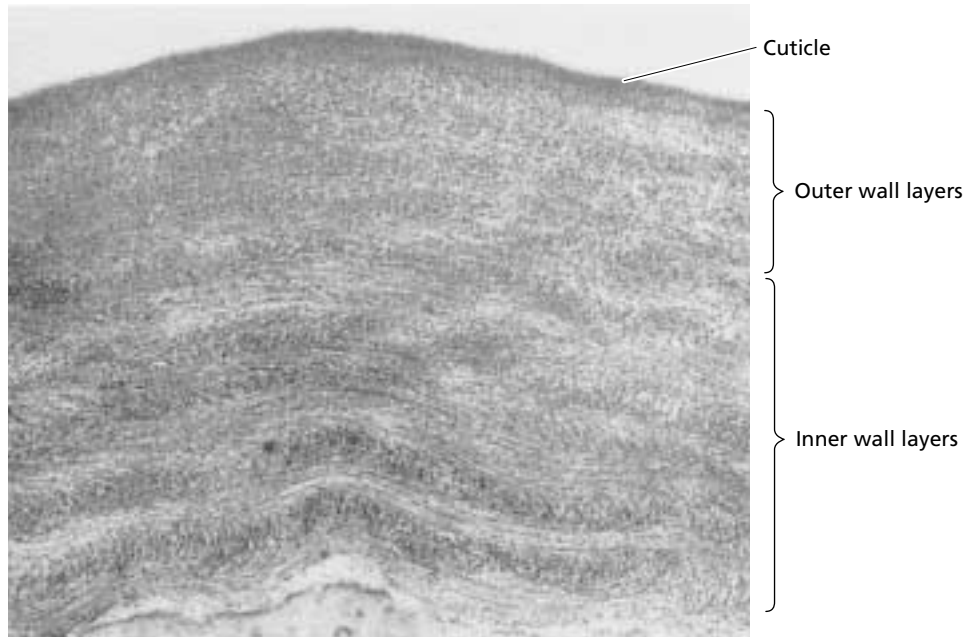
### The Primary Cell Wall Is Composed of Cellulose Microfibrils Embedded in a Polysaccharide Matrix

In primary cell walls, cellulose microfibrils are embedded in a highly hydrated matrix (Figure 15.4). This structure provides both strength and flexibility. In the case of cell walls, the **matrix** (plural *matrices*) consists of two major groups of polysaccharides, usually called hemicelluloses and pectins, plus a small amount of structural protein. The matrix polysaccharides consist of a variety of polymers that may vary according to cell type and plant species (Table 15.1).



**FIGURE 15.2** Primary cell walls from onion parenchyma. (A) This surface view of cell wall fragments was taken through the use of Nomarski optics. Note that the wall looks like a very thin sheet with small surface depressions; these depressions may be pit fields, places where plasmodesmatal connections between cells are concentrated. (B) This surface view of a cell wall was prepared by a freeze-etch replica technique. It shows the fibrillar nature of the cell wall. (From McCann et al. 1990, courtesy of M. McCann.)

**FIGURE 15.3** Electron micrograph of the outer epidermal cell wall from the growing region of a bean hypocotyl. Multiple layers are visible within the wall. The inner layers are thicker and more defined than the outer layers because the outer layers are the older regions of the wall and have been stretched and thinned by cell expansion. (From Roland et al. 1982.)



**FIGURE 15.4** Schematic diagram of the major structural components of the primary cell wall and their likely arrangement. Cellulose microfibrils are coated with hemicelluloses (such as xyloglucan), which may also cross-link the microfibrils to one another. Pectins form an interlocking matrix gel, perhaps interacting with structural proteins. (From Brett and Waldron 1996.)

**TABLE 15.1**  
**Structural components of plant cell walls**

Class	Examples
Cellulose	Microfibrils of (1→4)β-D-glucan
Matrix Polysaccharides	
Pectins	Homogalacturonan Rhamnogalacturonan Arabinan Galactan
Hemicelluloses	Xyloglucan Xylan Glucomannan Arabinoxylan Callose (1→3)β-D-glucan (1→3,1→4)β-D-glucan [grasses only]
Lignin	(see Chapter 13)
Structural proteins	(see Table 15.2)

These polysaccharides are named after the principal sugars they contain. For example, a *glucan* is a polymer made up of glucose, a *xylan* is a polymer made up of xylose, a *galactan* is made from galactose, and so on. *Glycan* is the general term for a polymer made up of sugars. For branched polysaccharides, the backbone of the polysaccharide is usually indicated by the last part of the name.

For example, *xyloglucan* has a glucan backbone (a linear chain of glucose residues) with xylose sugars attached to it in the side chains; *glucuronoarabinoxylan* has a xylan backbone (made up of xylose subunits) with glucuronic acid and arabinose side chains. However, a compound name does not necessarily imply a branched structure. For example, *glucomannan* is the name given to a polymer containing both glucose and mannose in its backbone.

**Cellulose microfibrils** are relatively stiff structures that contribute to the strength and structural bias of the cell wall. The individual glucans that make up the microfibril are closely aligned and bonded to each other to make a highly ordered (**crystalline**) ribbon that excludes water and is relatively inaccessible to enzymatic attack. As a result, cellulose is very strong and very stable and resists degradation.

**Hemicelluloses** are flexible polysaccharides that characteristically bind to the surface of cellulose. They may form tethers that bind cellulose microfibrils together into a cohesive network (see Figure 15.4), or they may act as a slippery coating to prevent direct microfibril–microfibril contact. Another term for these molecules is *cross-linking glucans*, but in this chapter we'll use the more traditional term, *hemicelluloses*. As described later, the term *hemicellulose* includes several different kinds of polysaccharides.

**Pectins** form a hydrated gel phase in which the cellulose–hemicellulose network is embedded. They act as hydrophilic filler, to prevent aggregation and collapse of

the cellulose network. They also determine the porosity of the cell wall to macromolecules. Like hemicelluloses, pectins include several different kinds of polysaccharides.

The precise role of wall **structural proteins** is uncertain, but they may add mechanical strength to the wall and assist in the proper assembly of other wall components.

The primary wall is composed of approximately 25% cellulose, 25% hemicelluloses, and 35% pectins, with perhaps 1 to 8% structural protein, on a dry-weight basis. However, large deviations from these values may be found. For example, the walls of grass coleoptiles consist of 60 to 70% hemicelluloses, 20 to 25% cellulose, and only about 10% pectins. Cereal endosperm walls are mostly (about 85%) hemicelluloses. Secondary walls typically contain much higher cellulose contents.

In this chapter we will present a basic model of the primary wall, but be aware that plant cell walls are more diverse than this model suggests. The composition of matrix polysaccharides and structural proteins in walls varies significantly among different species and cell types (Carpita and McCann 2000). Most notably, in grasses and related species the major matrix polysaccharides differ from those that make up the matrix of most other land plants (Carpita 1996).

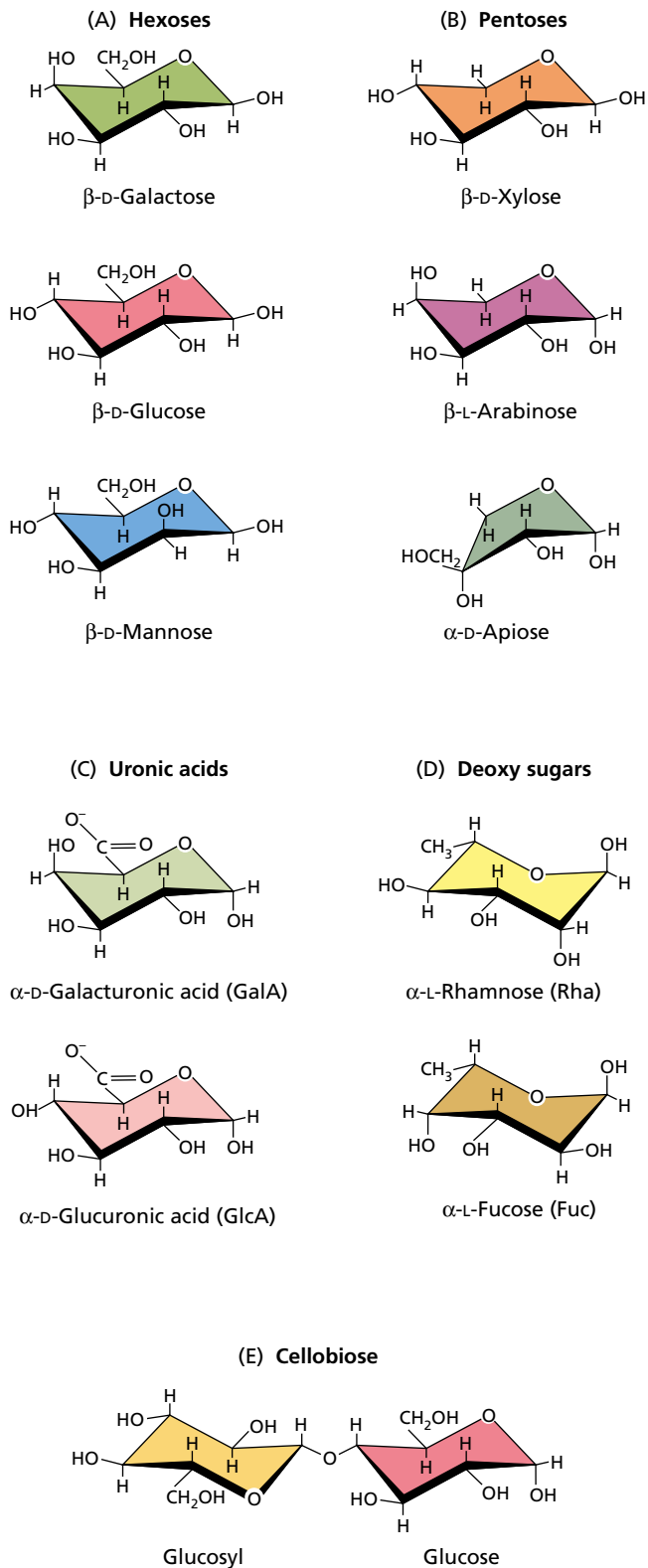
The primary wall also contains much water. This water is located mostly in the matrix, which is perhaps 75 to 80% water. The hydration state of the matrix is an important determinant of the physical properties of the wall; for example, removal of water makes the wall stiffer and less extensible. This stiffening effect of dehydration may play a role in growth inhibition by water deficits. We will examine the structure of each of the major polymers of the cell wall in more detail in the sections that follow.

### Cellulose Microfibrils Are Synthesized at the Plasma Membrane

Cellulose is a tightly packed microfibril of linear chains of (1→4)-linked β-D-glucose (Figure 15.5 and [Web Topic 15.1](#)). Because of the alternating spatial configuration of the glucosidic bonds linking adjacent glucose residues, the repeating unit in cellulose is considered to be cellobiose, a (1→4)-linked β-D-glucose disaccharide.

Cellulose microfibrils are of indeterminate length and vary considerably in width and in degree of order, depending on the source. For instance, cellulose microfibrils in land plants appear under the electron microscope to be 5 to 12 nm wide, whereas those formed by algae may be up to 30 nm wide and more crystalline. This variety in width corresponds to a variation in the number of parallel chains that make up the cross section of a microfibril—estimated to consist of about 20 to 40 individual chains in the thinner microfibrils.

The precise molecular structure of the cellulose microfibril is uncertain. Current models of microfibril organization suggest that it has a substructure consisting of highly crystalline domains linked together by less organized “amor-



**FIGURE 15.5** Conformational structures of sugars commonly found in plant cell walls. (A) Hexoses (six-carbon sugars). (B) Pentoses (five-carbon sugars). (C) Uronic acids (acidic sugars). (D) Deoxy sugars. (E) Cellobiose (showing the (1→4) $\beta$ -D-linkage between two glucose residues in inverted orientation).

phous” regions (Figure 15.6). Within the crystalline domains, adjacent glucans are highly ordered and bonded to each other by noncovalent bonding, such as hydrogen bonds and hydrophobic interactions.

The individual glucan chains of cellulose are composed of 2000 to more than 25,000 glucose residues (Brown et al. 1996). These chains are long enough (about 1 to 5  $\mu\text{m}$  long) to extend through multiple crystalline and amorphous regions within a microfibril. When cellulose is degraded—for example, by fungal cellulases—the amorphous regions are degraded first, releasing small crystallites that are thought to correspond to the crystalline domains of the microfibril.

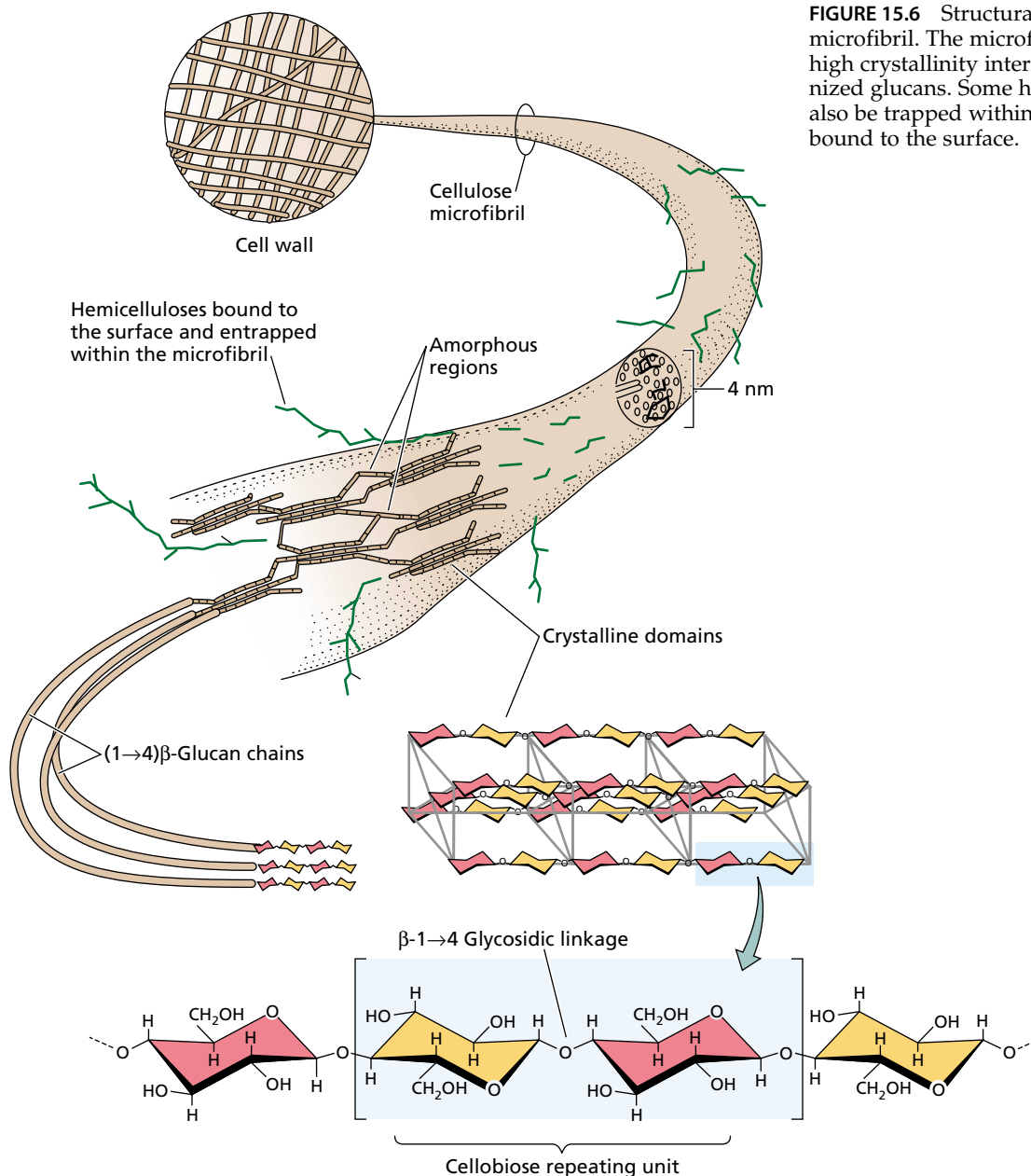
The extensive noncovalent bonding between adjacent glucans within a cellulose microfibril gives this structure remarkable properties. Cellulose has a high tensile strength, equivalent to that of steel. Cellulose is also insoluble, chemically stable, and relatively immune to chemical and enzymatic attack. These properties make cellulose an excellent structural material for building a strong cell wall.

Evidence from electron microscopy indicates that cellulose microfibrils are synthesized by large, ordered protein complexes, called **particle rosettes** or **terminal complexes**, that are embedded in the plasma membrane (Figure 15.7) (Kimura et al. 1999). These structures contain many units of **cellulose synthase**, the enzyme that synthesizes the individual (1→4) $\beta$ -D-glucans that make up the microfibril (see [Web Topic 15.2](#)).

Cellulose synthase, which is located on the cytoplasmic side of the plasma membrane, transfers a glucose residue from a sugar nucleotide donor to the growing glucan chain. Sterol-glucosides (sterols linked to a chain of two or three glucose residues) serve as the primers, or initial acceptors, to start the growth of the glucan chain (Peng et al. 2002). The sterol is clipped from the glucan by an endoglucanase, and the growing glucan chain is then extruded through the membrane to the exterior of the cell, where, together with other glucan chains, it crystallizes into a microfibril and interacts with xyloglucans and other matrix polysaccharides.

The sugar nucleotide donor is probably uridine diphosphate D-glucose (UDP-glucose). Recent evidence suggests that the glucose used for the synthesis of cellulose may be obtained from sucrose (a disaccharide composed of fructose and glucose) (Amor et al. 1995; Salnikov et al. 2001). According to this hypothesis, the enzyme **sucrose synthase** acts as a metabolic channel to transfer glucose taken from sucrose, via UDP-glucose, to the growing cellulose chain (Figure 15.8).

After many years of fruitless searching, the genes for cellulose synthase in higher plants have now been isolated (Pear et al. 1996; Arioli et al. 1998; Holland et al. 2000; Richmond and Somerville 2000). In *Arabidopsis*, the cellulose synthases are part of a large family of proteins whose function may be to synthesize the backbones of many cell wall polysaccharides.



**FIGURE 15.6** Structural model of a cellulose microfibril. The microfibril has regions of high crystallinity intermixed with less organized glucans. Some hemicelluloses may also be trapped within the microfibril and bound to the surface.

The formation of cellulose involves not only the synthesis of the glucan, but also the crystallization of multiple glucan chains into a microfibril. Little is known about the control of this process, except that the direction of microfibril deposition may be guided by microtubules adjacent to the membrane.

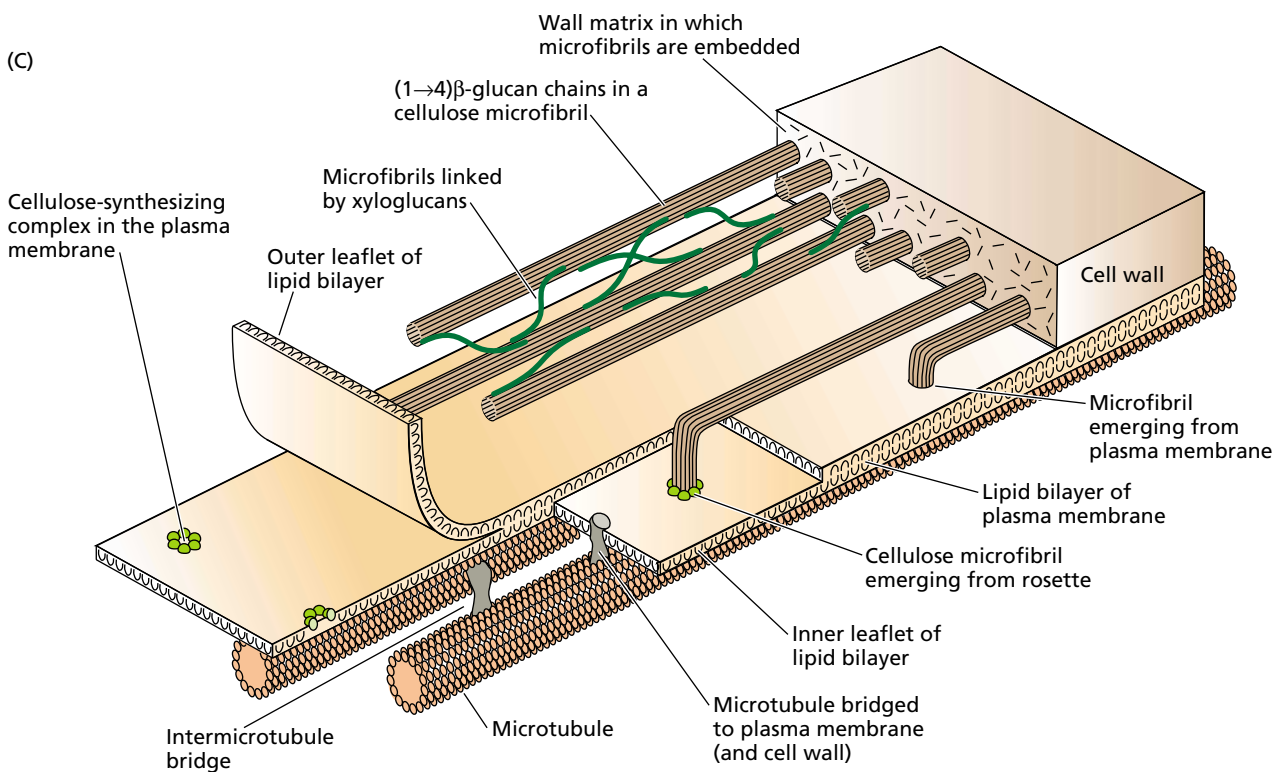
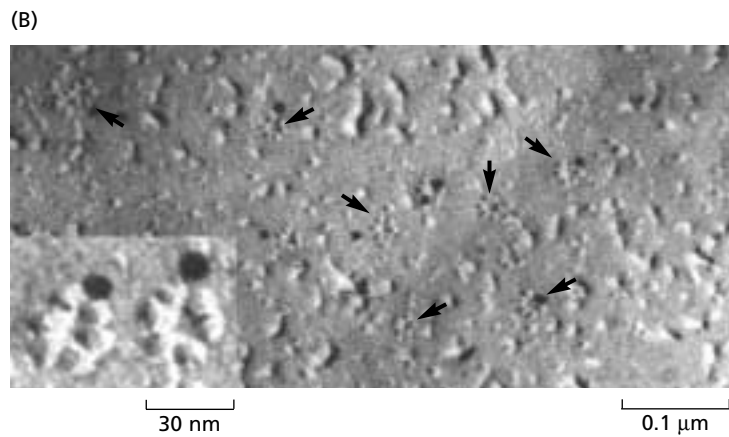
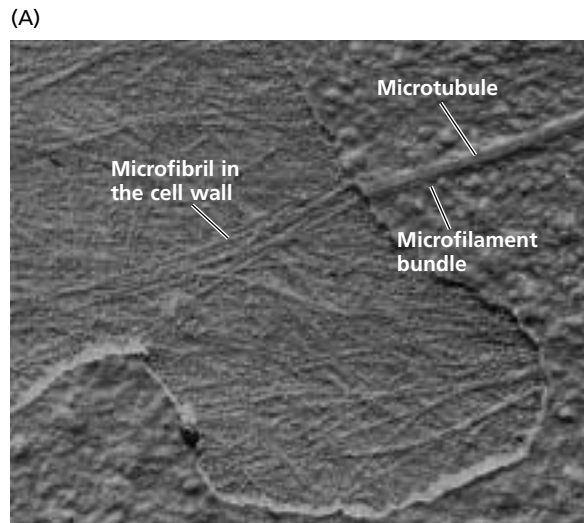
When the cellulose microfibril is synthesized, it is deposited into a milieu (the wall) that contains a high concentration of other polysaccharides that are able to interact with and perhaps modify the growing microfibril. *In vitro* binding studies have shown that hemicelluloses such as xyloglucan and xylan may bind to the surface of cellulose. Some hemicelluloses may also become physically en-

trapped within the microfibril during its formation, thereby reducing the crystallinity and order of the microfibril (Hayashi 1989).

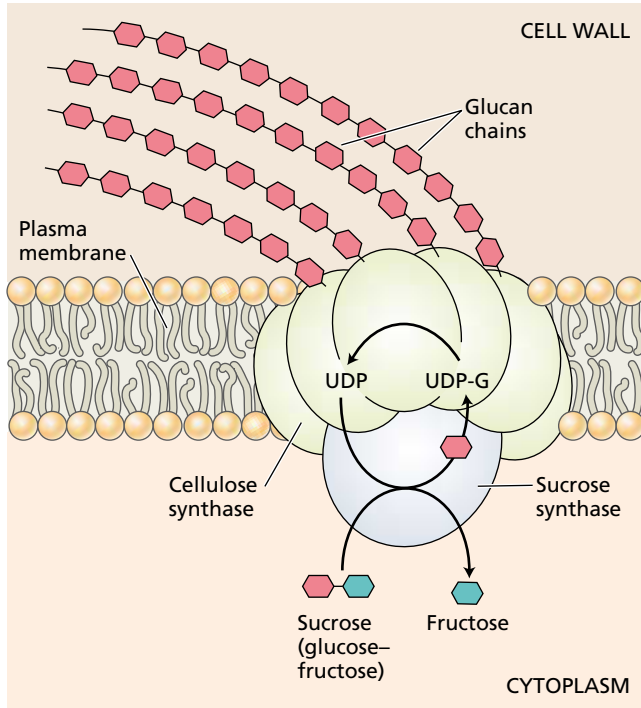
### Matrix Polymers Are Synthesized in the Golgi and Secreted in Vesicles

The matrix is a highly hydrated phase in which the cellulose microfibrils are embedded. The major polysaccharides of the matrix are synthesized by membrane-bound enzymes in the Golgi apparatus and are delivered to the cell wall via exocytosis of tiny vesicles (Figure 15.9 and [Web Topic 15.3](#)). The enzymes responsible for synthesis are *sugar-nucleotide polysaccharide glycosyltransferases*. These

**FIGURE 15.7** Cellulose synthesis by the cell. (A) Electron micrograph showing newly synthesized cellulose microfibrils immediately exterior to the plasma membrane. (B) Freeze-fracture labeled replicas showing reactions with antibodies against cellulose synthase. A field of labeled rosettes (arrows) with seven clearly labeled rosettes and one unlabeled rosette. The inset shows an enlarged view of two selected rosettes (terminal complexes) with immunogold labels. (C) Schematic diagram showing cellulose being synthesized by membrane synthase complex ("rosette") and its presumed guidance by the underlying microtubules in the cytoplasm. (A and C from Gunning and Steer 1996 B from Kimura et al. 1999.)







**FIGURE 15.8** Model of cellulose synthesis by a multisubunit complex containing cellulose synthase. Glucose residues are donated to the growing glucan chains by UDP-glucose (UDP-G). Sucrose synthase may act as a metabolic channel to transfer glucose taken from sucrose to UDP-glucose, or UDP-glucose may be obtained directly from the cytoplasm. (After Delmer and Amor 1995.)

enzymes transfer monosaccharides from sugar nucleotides to the growing end of the polysaccharide chain.

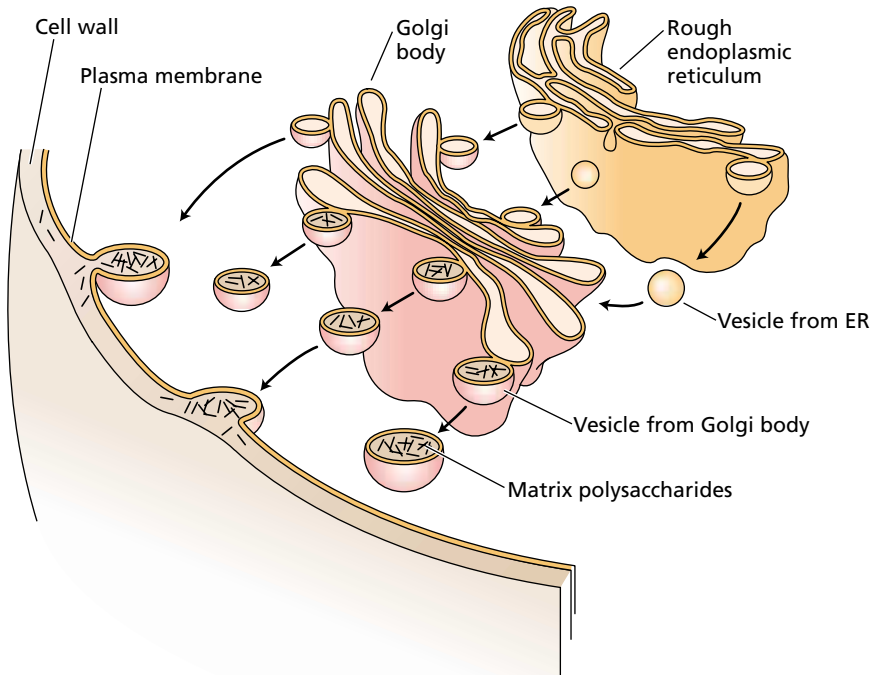
Unlike cellulose, which forms a crystalline microfibril, the matrix polysaccharides are much less ordered and are often described as amorphous. This noncrystalline character is a consequence of the structure of these polysaccharides—their branching and their nonlinear conformation. Nevertheless, spectroscopy studies indicate that there is partial order in the orientation of hemicelluloses and pectins in the cell wall, probably as a result of a physical tendency for these polymers to become aligned along the long axis of cellulose (Séné et al. 1994; Wilson et al. 2000).

**Hemicelluloses Are Matrix Polysaccharides That Bind to Cellulose**

Hemicelluloses are a heterogeneous group of polysaccharides (Figure 15.10) that are bound tightly in the wall. Typically they are solubilized from depectinated walls by the use of a strong alkali (1–4 M NaOH). Several kinds of hemicelluloses are found in plant cell walls, and walls from different tissues and different species vary in their hemicellulose composition.

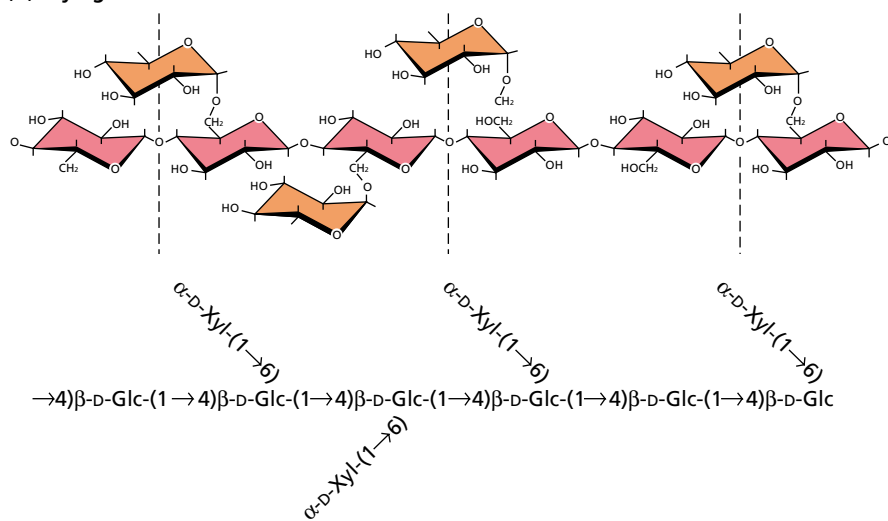
In the primary wall of dicotyledons (the best-studied example), the most abundant hemicellulose is **xyloglucan** (see Figure 15.10A). Like cellulose, this polysaccharide has a backbone of (1→4)-linked β-D-glucose residues. Unlike cellulose, however, xyloglucan has short side chains that contain xylose, galactose, and often, though not always, a terminal fucose.

By interfering with the linear alignment of the glucan backbones with one another, these side chains prevent the

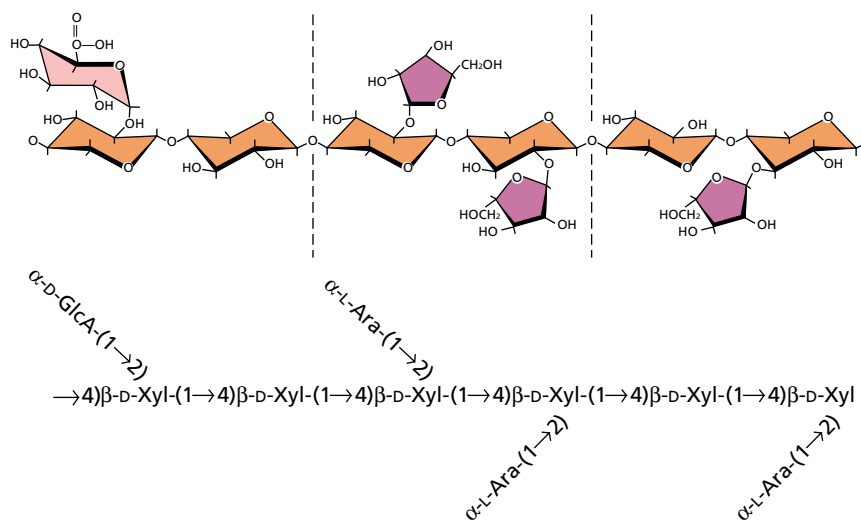


**FIGURE 15.9** Scheme for the synthesis and delivery of matrix polysaccharides to the cell wall. Polysaccharides are synthesized by enzymes in the Golgi apparatus and then secreted to the wall by fusion of membrane vesicles to the plasma membrane.

## (A) Xyloglucan



## (B) Glucuronoarabinoxylan



**FIGURE 15.10** Partial structures of common hemicelluloses. (For details on carbohydrate nomenclature, see [Web Topic 15.1](#).) (A) Xyloglucan has a backbone of  $(1\rightarrow4)$ -linked  $\beta$ -D-glucose (Glc), with  $(1\rightarrow6)$ -linked branches containing  $\beta$ -D-xylose (Xyl). In some cases galactose (Gal) and fucose (Fuc) are added to the xylose side chains. (B) Glucuronoarabinoxylans have a  $(1\rightarrow4)$ -linked backbone of  $\beta$ -D-xylose (Xyl). They may also have side chains containing arabinose (Ara), 4-O-methyl-glucuronic acid (4-O-Me- $\alpha$ -D-GlcA), or other sugars. (From Carpita and McCann 2000.)

assembly of xyloglucan into a crystalline microfibril. Because xyloglucans are longer (about 50–500 nm) than the spacing between cellulose microfibrils (20–40 nm), they have the potential to link several microfibrils together.

Varying with the developmental state and plant species, the hemicellulose fraction of the wall may also contain large amounts of other important polysaccharides—for example, **glucuronoarabinoxylans** (see Figure 15.10B) and **glucomannans**. Secondary walls typically contain less xyloglucan and more xylans and glucomannans, which also bind tightly to cellulose. The cell walls of grasses contain only small amounts of xyloglucan and pectin, which are replaced by glucuronoarabinoxylan and  $(1\rightarrow3,1\rightarrow4)\beta$ -D-glucan.

### Pectins Are Gel-Forming Components of the Matrix

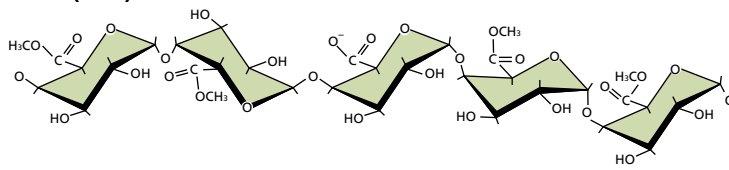
Like the hemicelluloses, pectins constitute a heterogeneous group of polysaccharides (Figure 15.11), characteristically

containing acidic sugars such as galacturonic acid and neutral sugars such as rhamnose, galactose, and arabinose. Pectins are the most soluble of the wall polysaccharides; they can be extracted with hot water or with calcium chelators. In the wall, pectins are very large and complex molecules composed of different kinds of pectic polysaccharides.

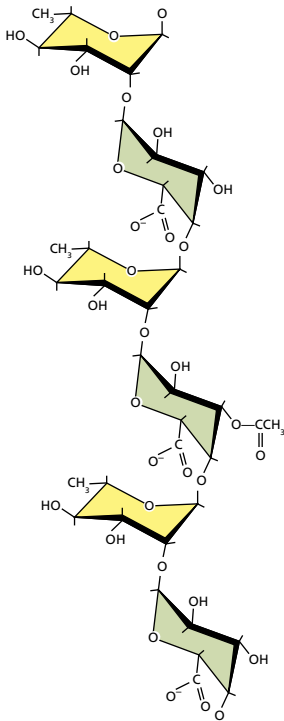
Some pectic polysaccharides have a relatively simple primary structure, such as *homogalacturonan* (see Figure 15.11A). This polysaccharide, also called *polygalacturonic acid*, is a  $(1\rightarrow4)$ -linked polymer of  $\alpha$ -D-glucuronic acid residues. Figure 15.12 shows a triple-fluorescence-labeled section of tobacco stem parenchyma cells showing the distribution of cellulose and pectic homogalacturonan.

One of the most abundant of the pectins is the complex polysaccharide *rhamnogalacturonan I (RG I)*, which has a long backbone and a variety of side chains (see Figure 15.11B). This molecule is very large and is believed to contain highly branched (“hairy”) regions (i.e., with arabinan,

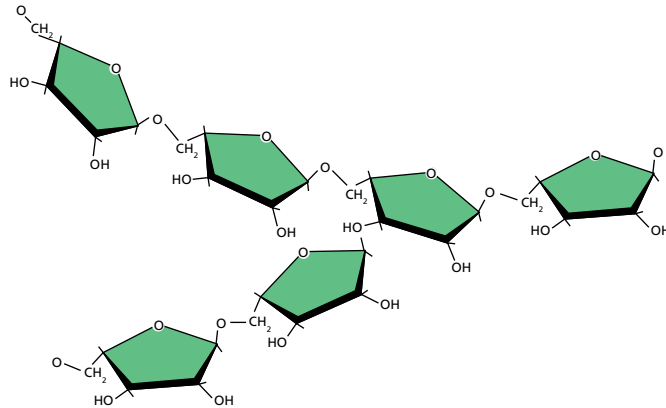
## (A) Homogalacturonan (HGA)



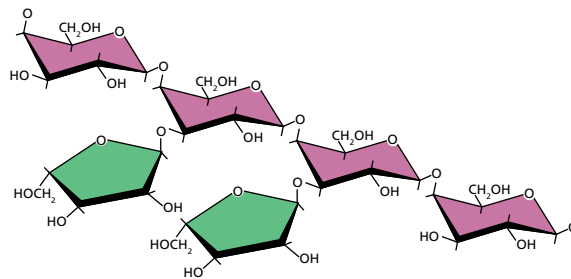
## (B) Rhamnogalacturonan I (RG I)



## (C) 5-Arabinan

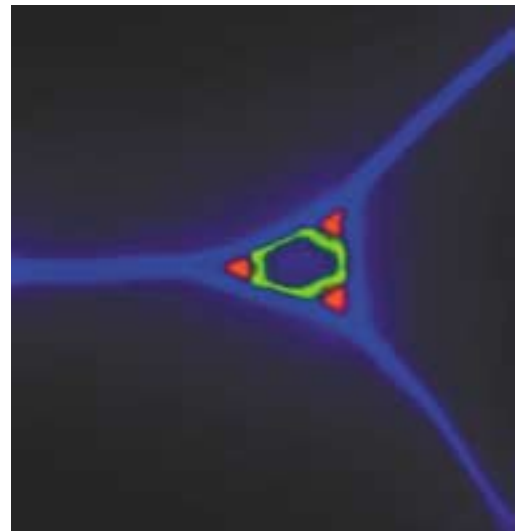


## (D) Type I arabinogalactan



**FIGURE 15.11** Partial structures of the most common pectins. (A) Homogalacturonan, also known as polygalacturonic acid or pectic acid, is made up of (1→4)-linked  $\alpha$ -D-galacturonic acid (GalA) with occasional rhamnosyl residues that put a kink in the chain. The carboxyl residues are often methyl esterified. (B) Rhamnogalacturonan I (RG I) is a very large and heterogeneous pectin, with a backbone of alternating (1→4) $\alpha$ -D-galacturonic acid (GalA) and (1→2) $\alpha$ -D-rhamnose (Rha). Side chains are attached to rhamnose and are composed principally of arabinans (C), galactans, and arabinogalactans (D). These side chains may be short or quite long. The galacturonic acid residues are often methyl esterified. (From Carpita and McCann 2000.)

**FIGURE 15.12** Triple-fluorescence-labeled section of tobacco stem showing the primary cell walls of three adjacent parenchyma cells bordering an intercellular space. The blue color is calcofluor (staining of cellulose), and the red and green colors indicate the binding of two monoclonal antibodies to different epitopes (immunologically distinct regions) of pectic homogalacturonan. (Courtesy of W. Willats.)



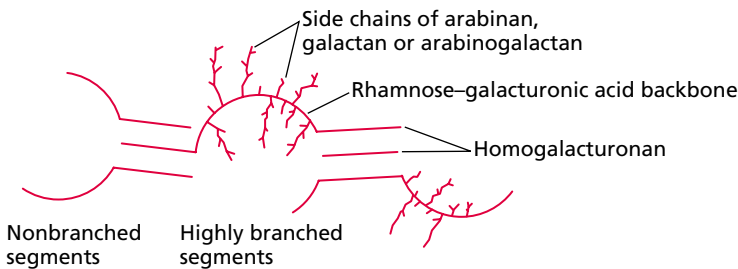
and galactan side chains) interspersed with unbranched (“smooth”) regions of homogalacturonan (Figure 15.13A).

Pectic polysaccharides may be very complex. A striking example is a highly branched pectic polysaccharide called *rhamnogalacturonan II (RG II)* (see Figure 15.13C), which contains at least ten different sugars in a complicated pattern of linkages. Although RG I and RG II have similar names, *they have very different structures*. RG II units may be cross-linked

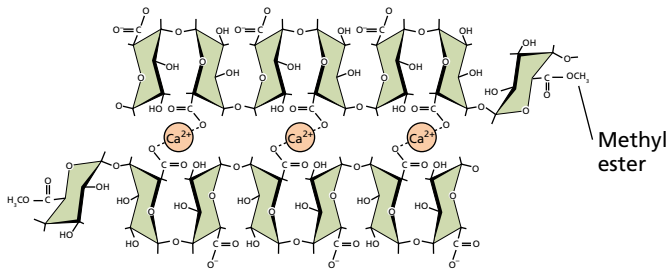
by borate diesters (Ishi et al. 1999) and are important for wall structure. For example, *Arabidopsis* mutants that synthesize an altered RG II that is unable to be cross-linked by borate show substantial growth abnormalities (O’Neill et al. 2001).

Pectins typically form gels—loose networks formed by highly hydrated polymers. Pectins are what make fruit jams and jellies “gel,” or solidify. In pectic gels, the charged carboxyl ( $\text{COO}^-$ ) groups of neighboring pectin chains are linked

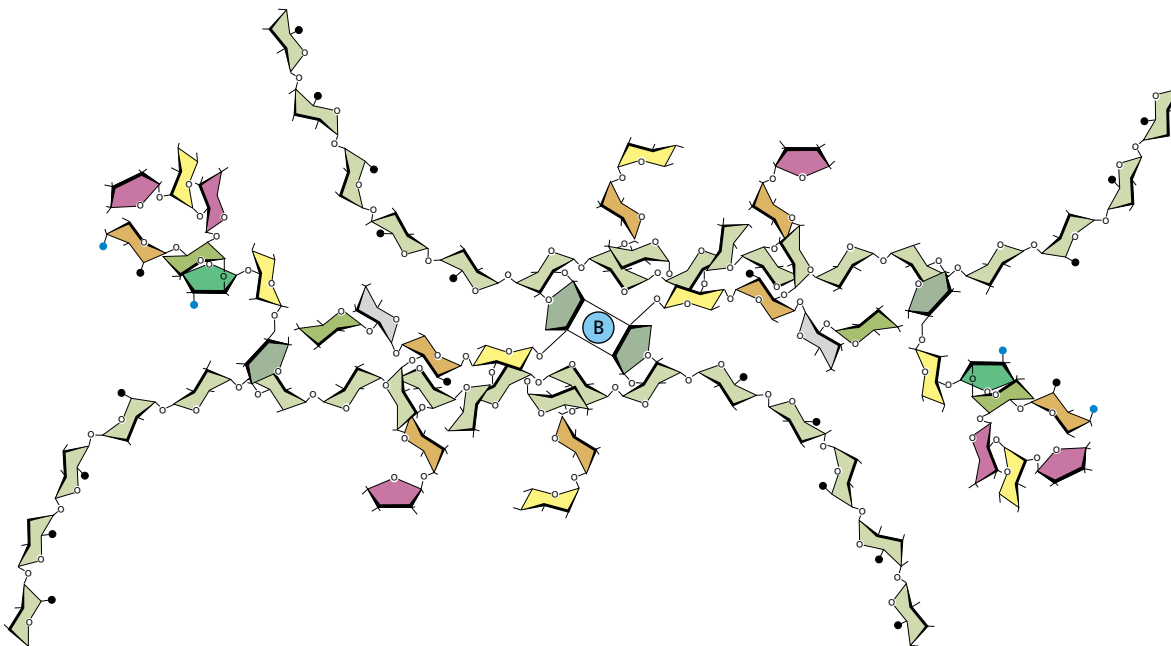
(A) Rhamnogalacturonan I structure



(B) Ionic bonding of pectin network by calcium



(C) Rhamnogalacturonan II (RG II) dimer cross-linked by borate diester bonds



**FIGURE 15.13** Pectin structure. (A) Proposed structure of rhamnogalacturonan I, containing highly branched segments interspersed with nonbranched segments, and a backbone of rhamnose and galacturonic acid. (B) Formation of a pectin network involves ionic bridging of the nonesterified carboxyl groups ( $\text{COO}^-$ ) by calcium ions. When blocked by methyl-esterified groups, the carboxyl groups cannot participate in this type of interchain network formation. Likewise, the presence of side chains on the backbone interferes with network formation. (C) Structure of rhamnogalacturonan II (RG II). (B and C from Carpita and McCann 2000.)

together via  $\text{Ca}^{2+}$ , which forms a tight complex with pectin. A large calcium-bridged network may thus form, as illustrated in Figure 15.13B.

Pectins are subject to modifications that may alter their conformation and linkage in the wall. Many of the acidic residues are esterified with methyl, acetyl, and other unidentified groups during biosynthesis in the Golgi apparatus. Such esterification masks the charges of carboxyl groups and prevents calcium bridging between pectins, thereby reducing the gel-forming character of the pectin.

Once the pectin has been secreted into the wall, the ester groups may be removed by pectin esterases found in the wall, thus unmasking the charges of the carboxyl groups and increasing the ability of the pectin to form a rigid gel. By creating free carboxyl groups, de-esterification also increases the electric-charge density in the wall, which in turn may influence the concentration of ions in the wall and the activities of wall enzymes. In addition to being connected by calcium bridging, pectins may be linked to each other by various covalent bonds, including ester linkages between phenolic residues such as ferulic acid (see Chapter 13).

### Structural Proteins Become Cross-Linked in the Wall

In addition to the major polysaccharides described in the previous section, the cell wall contains several classes of structural proteins. These proteins usually are classified according to their predominant amino acid composition—for example, hydroxyproline-rich glycoprotein (HRGP), glycine-rich protein (GRP), proline-rich protein (PRP), and so on (Table 15.2). Some wall proteins have sequences that are characteristic of more than one class. Many structural proteins of walls have highly repetitive primary structures and sometimes are highly glycosylated (Figure 15.14).

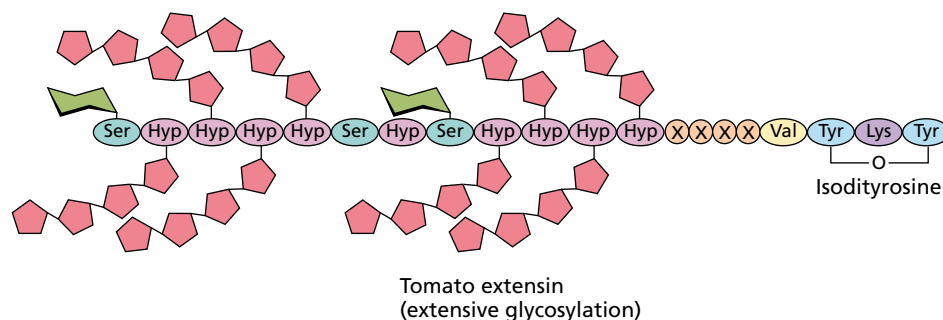
**TABLE 15.2**  
Structural proteins of the cell wall

Class of cell wall proteins	Percentage carbohydrate	Localization typically in:
HRGP (hydroxyproline-rich glycoprotein)	~55	Phloem, cambium, sclereids
PRP (proline-rich protein)	~0–20	Xylem, fibers, cortex
GRP (glycine-rich protein)	0	Xylem

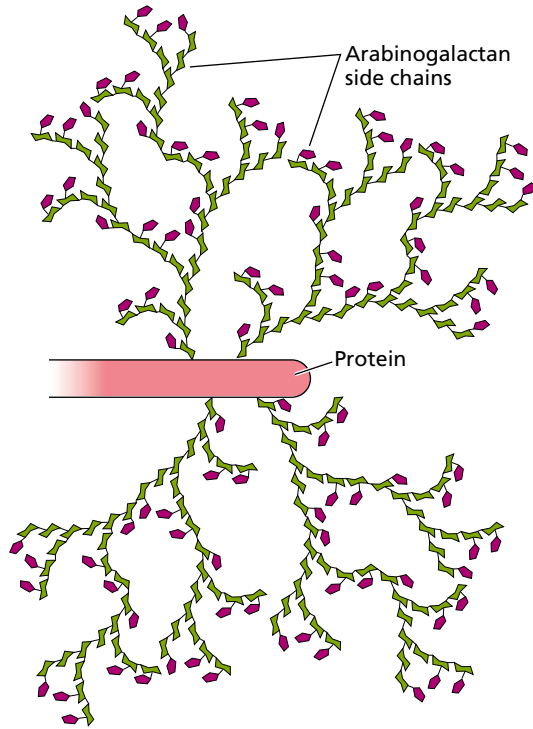
In vitro extraction studies have shown that newly secreted wall structural proteins are relatively soluble, but they become more and more insoluble during cell maturation or in response to wounding. The biochemical nature of the insolubilization process is uncertain, however.

Wall structural proteins vary greatly in their abundance, depending on cell type, maturation, and previous stimulation. Wounding, pathogen attack, and treatment with elicitors (molecules that activate plant defense responses; see Chapter 13) increase expression of the genes that code for many of these proteins. In histological studies, wall structural proteins are often localized to specific cell and tissue types. For example, HRGPs are associated mostly with cambium, phloem parenchyma, and various types of sclerenchyma. GRPs and PRPs are most often localized to xylem vessels and fibers and thus are more characteristic of a differentiated cell wall.

In addition to the structural proteins already listed, cell walls contain **arabinogalactan proteins (AGPs)** which usually amount to less than 1% of the dry mass of the wall. These water-soluble proteins are very heavily glycosylated: More than 90% of the mass of AGPs may be sugar residues—primarily galactose and arabinose (Figure 15.15) (Gaspar et al. 2001). Multiple AGP forms are found in plant tissues, either in the wall or associated with the plasma membrane, and they display tissue- and cell-specific expression patterns.



**FIGURE 15.14** A repeated hydroxyproline-rich motif from a molecule of extensin from tomato, showing extensive glycosylation and the formation of intramolecular isodityrosine bonds. (From Carpita and McCann 2000.)



**FIGURE 15.15** A highly branched arabinogalactan molecule. (From Carpita and McCann 2000.)

AGPs may function in cell adhesion and in cell signaling during cell differentiation. As evidence for the latter idea, treatment of suspension cultures with exogenous AGPs or with agents that specifically bind AGPs is reported to influence cell proliferation and embryogenesis. AGPs are also implicated in the growth, nutrition, and guidance of pollen tubes through stylar tissues, as well as in other developmental processes (Cheung et al. 1996; Gaspar et al. 2001). Finally, AGPs may also function as a kind of polysaccharide chaperone within secretory vesicles to reduce spontaneous association of newly synthesized polysaccharides until they are secreted to the cell wall.

### New Primary Walls Are Assembled during Cytokinesis

Primary walls originate *de novo* during the final stages of cell division, when the newly formed **cell plate** separates the two daughter cells and solidifies into a stable wall that is capable of bearing a physical load from turgor pressure.

The cell plate forms when Golgi vesicles and ER cisternae aggregate in the spindle midzone area of a dividing cell. This aggregation is organized by the **phragmoplast**, a complex assembly of microtubules, membranes, and vesicles that forms during late anaphase or early telophase (see Chapter 1). The membranes of the vesicles fuse with each other, and with the lateral plasma membrane, to become the new plasma membrane separating the daughter cells. The contents of the vesicles are the precursors from which the new middle lamella and the primary wall are assembled.

After a wall forms, it can grow and mature through a process that may be outlined as follows:

Synthesis → secretion → assembly →  
expansion (in growing cells) →  
cross-linking and secondary wall formation

The synthesis and secretion of the major wall polymers were described earlier. Here we will consider the assembly and expansion of the wall.

After their secretion into the extracellular space, the wall polymers must be assembled into a cohesive structure; that is, the individual polymers must attain the physical arrangement and bonding relationships that are characteristic of the wall. Although the details of wall assembly are not understood, the prime candidates for this process are self-assembly and enzyme-mediated assembly.

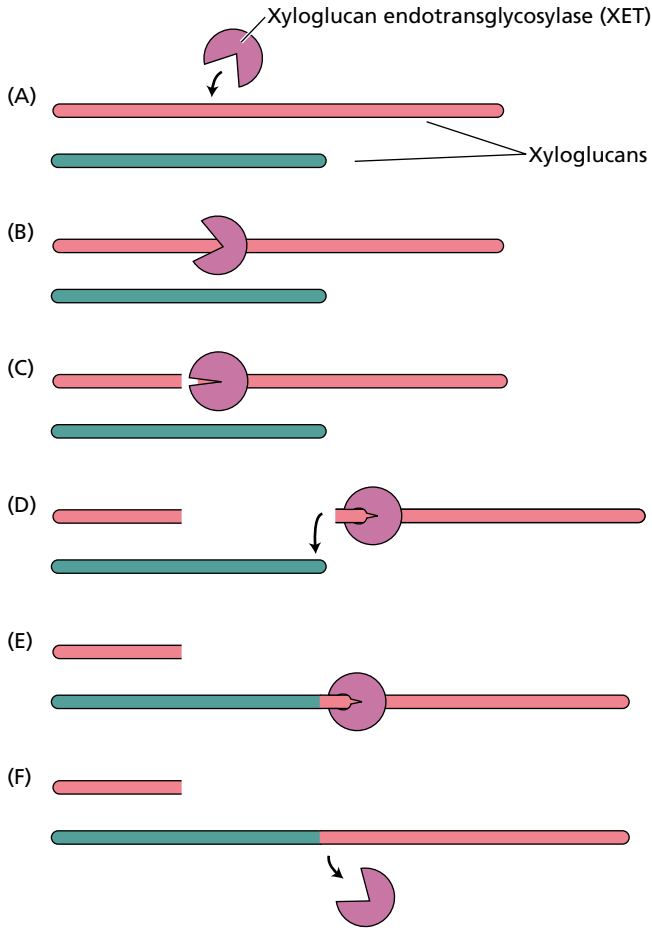
**Self-assembly.** Self-assembly is attractive because it is mechanically simple. Wall polysaccharides possess a marked tendency to aggregate spontaneously into organized structures. For example, isolated cellulose may be dissolved in strong solvents and then extruded to form stable fibers, called rayon.

Similarly, hemicelluloses may be dissolved in strong alkali; when the alkali is removed, these polysaccharides aggregate into concentric, ordered networks that resemble the native wall at the ultrastructural level. This tendency to aggregate can make the separation of hemicellulose into its component polymers technically difficult. In contrast, pectins are more soluble and tend to form dispersed, isotropic networks (gels). These observations indicate that the wall polymers have an inherent ability to aggregate into partly ordered structures.

**Enzyme-mediated assembly.** In addition to self-assembly, wall enzymes may take part in putting the wall together. A prime candidate for enzyme-mediated wall assembly is *xyloglucan endotransglycosylase* (*XET*). This enzyme has the ability to cut the backbone of a xyloglucan and to join one end of the cut xyloglucan with the free end of an acceptor xyloglucan (Figure 15.16). Such a transfer reaction integrates newly synthesized xyloglucans into the wall (Nishitani 1997; Thompson and Fry 2001).

Other wall enzymes that might aid in assembly of the wall include glycosidases, pectin methyl esterases, and various oxidases. Some glycosidases remove the side chains of hemicelluloses. This “debranching” activity increases the tendency of hemicelluloses to adhere to the surface of cellulose microfibrils. Pectin methyl esterases hydrolyze the methyl esters that block the carboxyl groups of pectins. By unblocking the carboxyl groups, these enzymes increase the concentration of acidic groups on the pectins and enhance the ability of pectins to form a  $\text{Ca}^{2+}$ -bridged gel network.

Oxidases such as peroxidase may catalyze cross-linking between phenolic groups (tyrosine, phenylalanine, ferulic



**FIGURE 15.16** Action of xyloglucan endotransglycosylase (XET) to cut and stitch xyloglucan polymers into new configurations. Two xyloglucan chains are shown in (A) with two distinct patterns to emphasize their rearrangement. XET binds to the middle of one xyloglucan (B), cuts it (C), and transfers one end to the end of a second xyloglucan (D, E), resulting in one shorter and one longer xyloglucan (F). (After Smith and Fry 1991.)

acid) in wall proteins, pectins, and other wall polymers. Such phenolic coupling is clearly important for the formation of lignin cross-links, and it may likewise link diverse components of the wall together.

**Secondary Walls Form in Some Cells after Expansion Ceases**

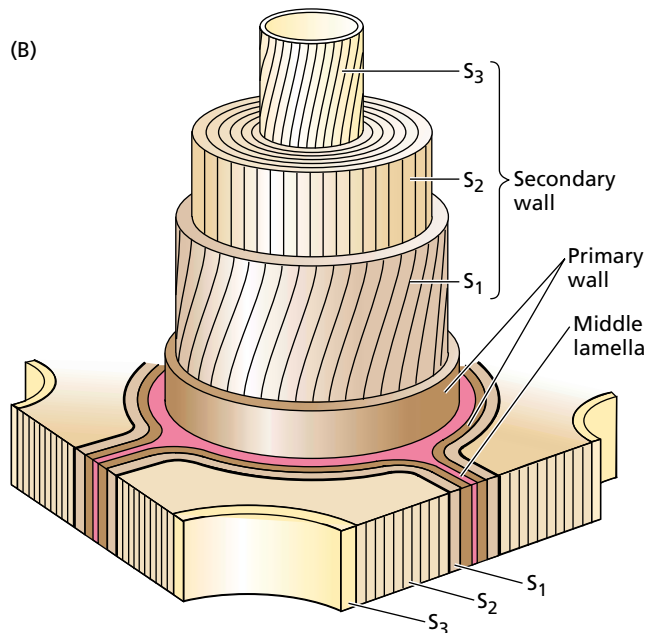
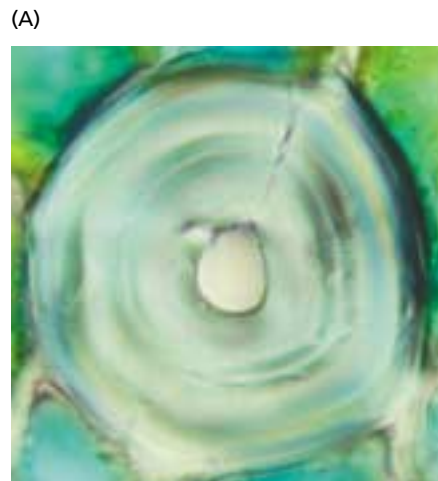
After wall expansion ceases, cells sometimes continue to synthesize a wall, known as a secondary wall. Secondary walls are often quite thick, as in tracheids, fibers, and other cells that serve in mechanical support of the plant (Figure 15.17).

Often such secondary walls are multilayered and differ in structure and composition from the primary wall. For example, the secondary walls in wood contain xylans rather than xyloglucans, as well as a higher proportion of cellulose. The orientation of the cellulose microfibrils may be more neatly aligned parallel to each other in secondary

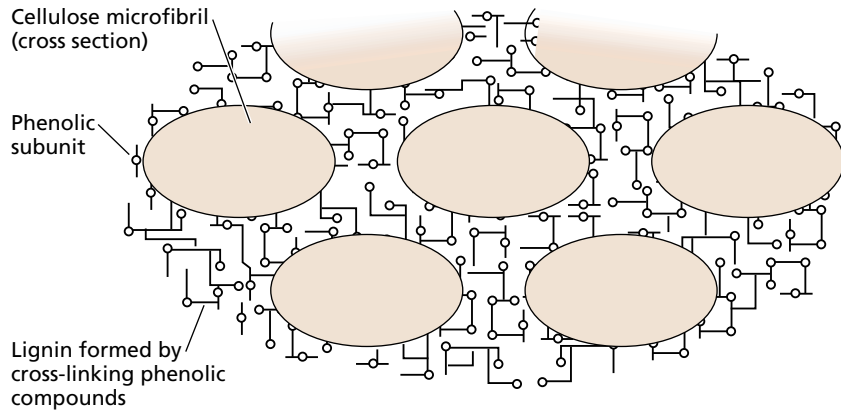
walls than in primary walls. Secondary walls are often (but not always) impregnated with lignin.

Lignin is a phenolic polymer with a complex, irregular pattern of linkages that link the aromatic alcohol subunits together (see Chapter 13). These subunits are synthesized from phenylalanine and are secreted to the wall, where they are oxidized in place by the enzymes peroxidase and laccase. As lignin forms in the wall, it displaces water from the matrix and forms a hydrophobic network that bonds tightly to cellulose and prevents wall enlargement (Figure 15.18).

Lignin adds significant mechanical strength to cell walls and reduces the susceptibility of walls to attack by pathogens.



**FIGURE 15.17** (A) Cross section of a *Podocarpus* sclereid, in which multiple layers in the secondary wall are visible. (B) Diagram of the cell wall organization often found in tracheids and other cells with thick secondary walls. Three distinct layers ( $S_1$ ,  $S_2$ ,  $S_3$ ) are formed interior to the primary wall. (Photo ©David Webb.)



**FIGURE 15.18** Diagram illustrating how the phenolic subunits of lignin infiltrate the space between cellulose microfibrils, where they become cross-linked. (Other components of the matrix are omitted from this diagram.)

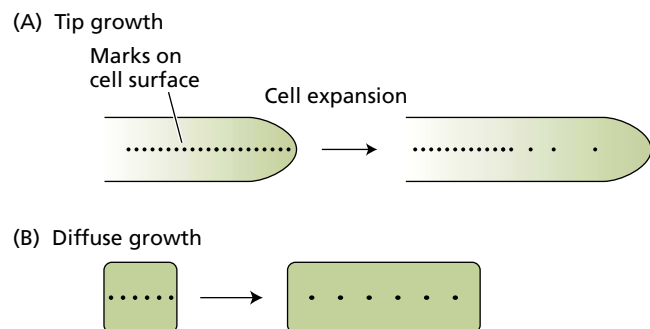
Lignin also reduces the digestibility of plant material by animals. Genetic engineering of lignin content and structure may improve the digestibility and nutritional content of plants used as animal fodder.

## PATTERNS OF CELL EXPANSION

During plant cell enlargement, new wall polymers are continuously synthesized and secreted at the same time that the preexisting wall is expanding. Wall expansion may be highly localized (as in the case of **tip growth**) or evenly distributed over the wall surface (**diffuse growth**) (Figure 15.19). Whereas tip growth is characteristic of root hairs and pollen tubes (see [Web Essay 15.1](#)), most of the other cells in the plant body exhibit diffuse growth. Cells such as fibers, some sclereids, and trichomes grow in a pattern that is intermediate between diffuse growth and tip growth.

Even in cells with diffuse growth, however, different parts of the wall may enlarge at different rates or in different directions. For example, in cortical cells of the stem, the end walls grow much less than side walls. This difference may be due to structural or enzymatic variations in specific walls or variations in the stresses borne by different walls. As a consequence of this uneven pattern of wall expansion, plant cells may assume irregular forms.

**FIGURE 15.19** The cell surface expands differently during tip growth and diffuse growth. (A) Expansion of a tip-growing cell is confined to an apical dome at one end of the cell. If marks are placed on the cell surface and the cell is allowed to continue to grow, only the marks that were initially within the apical dome grow farther apart. Root hairs and pollen tubes are examples of plant cells that exhibit tip growth. (B) If marks are placed on the surface of a diffuse-growing cell, the distance between all the marks increases as the cell grows. Most cells in multicellular plants grow by diffuse growth.



## Microfibril Orientation Determines Growth Directionality of Cells with Diffuse Growth

During growth, the loosened cell wall is extended by physical forces generated from cell turgor pressure. Turgor pressure creates an outward-directed force, equal in all directions. The directionality of growth is determined largely by the structure of the cell wall—in particular, the orientation of cellulose microfibrils.

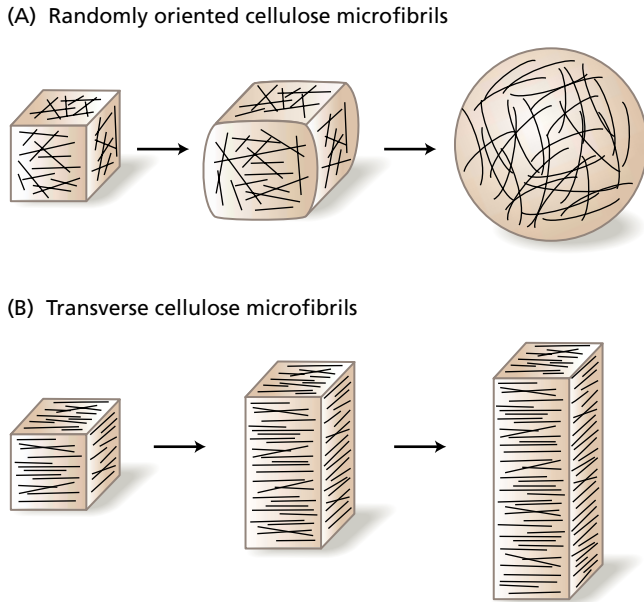
When cells first form in the meristem, they are isodiametric; that is, they have equal diameters in all directions. If the orientation of cellulose microfibrils in the primary cell wall were **isotropic** (randomly arranged), the cell would grow equally in all directions, expanding radially to generate a sphere (Figure 15.20A).

In most plant cell walls, however, the arrangement of cellulose microfibrils is **anisotropic** (nonrandom).

Cellulose microfibrils are synthesized mainly in the lateral walls of cylindrical, enlarging cells such as cortical and vascular cells of stems and roots, or the giant internode cells of the filamentous green alga *Nitella*. Moreover, the cellulose microfibrils are deposited circumferentially (transversely) in these lateral walls, at right angles to the long axis of the cell. The circumferentially arranged cellulose microfibrils have been likened to hoops in a barrel, restricting growth in girth and promoting growth in length (see Figure 15.20B). However, because individual cellulose microfibrils do not actually form closed hoops around the cell, a more accurate analogy would be the glass fibers in fiberglass.

Fiberglass is a *complex composite material*, composed of an amorphous resin matrix reinforced by discontinuous strengthening elements, in this case glass fibers. In complex composites, rod-shaped crystalline elements exert their maximum reinforcement of the matrix in the direction parallel to their orientation, and their minimum reinforcement perpendicular to their orientation. The reinforcement of the wall is greater in the parallel direction because the matrix must physically scrape along the entire length of the fibers for lateral displacement to occur.





**FIGURE 15.20** The orientation of newly deposited cellulose microfibrils determines the direction of cell expansion. (A) If the cell wall is reinforced by randomly oriented cellulose microfibrils, the cell will expand equally in all directions, forming a sphere. (B) When most of the reinforcing cellulose microfibrils have the same orientation, the cell expands at right angles to the microfibril orientation and is constrained in the direction of the reinforcement. Here the microfibril orientation is transverse, so cell expansion is longitudinal.

In contrast, when the material is stretched in the perpendicular direction, the matrix polymers need only slip over the diameters of the fibrous elements, resulting in little or no strengthening of the matrix. Because the glass fibers in fiberglass are randomly arranged, fiberglass is equally strong in all directions; that is, it is mechanically isotropic.

Plant cell walls, like fiberglass, are complex composite materials, composed of an amorphous phase and crystalline elements (Darley et al. 2001). Unlike fiberglass, however, the microfibril strengthening elements of a typical primary cell wall are transversely oriented, rendering the wall structurally and mechanically *anisotropic*. For this reason growing plant cells tend to elongate, and they increase only minimally in girth.

Cell wall deposition continues as cells enlarge. According to the **multinet hypothesis**, each successive wall layer is stretched and thinned during cell expansion, so the microfibrils become passively reoriented in the longitudinal direction—that is, in the direction of growth. Successive layers of microfibrils thus show a gradation in their degree of reorientation across the thickness of the wall, and those in the outer layers are longitudinally oriented as a result of wall stretching (Figure 15.21).

Because of thinning and fragmentation, these outer layers have much less influence on the direction of cell expansion than do the newly deposited inner layers. The inner

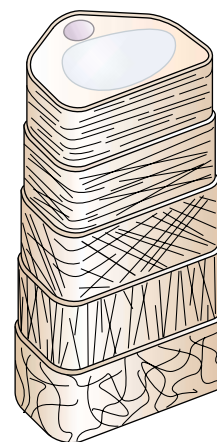
one-fourth of the wall bears nearly all the stress due to turgor pressure and determines the directionality of cell expansion (see [Web Topic 15.4](#)).

### Cortical Microtubules Determine the Orientation of Newly Deposited Microfibrils

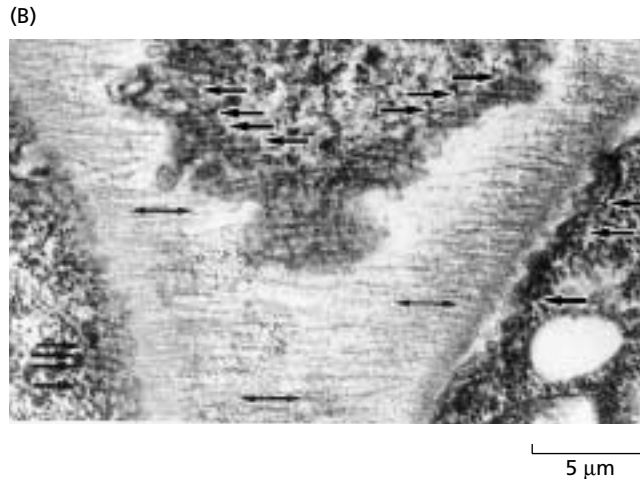
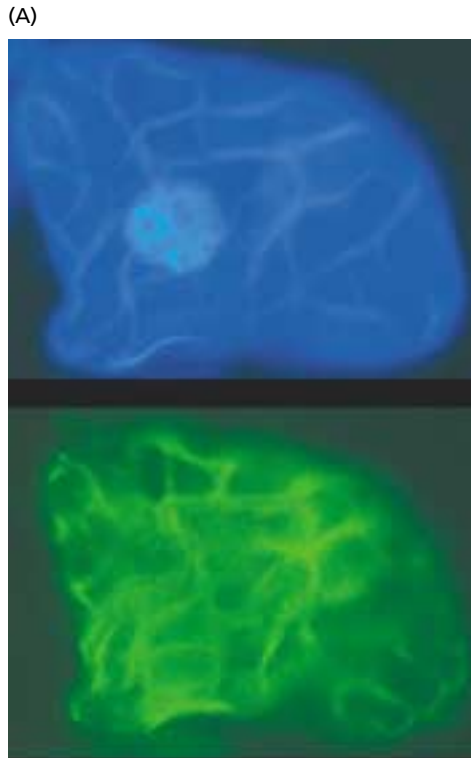
Newly deposited cellulose microfibrils and cytoplasmic microtubules in cell walls usually are coaligned, suggesting that microtubules determine the orientation of cellulose microfibril deposition. The orientation of microtubules in the cortical cytoplasm, the cytoplasm immediately adjacent to the plasma membrane, usually mirrors that of the newly deposited microfibrils in the adjacent cell wall, and both are usually coaligned in the transverse direction, at right angles to the axis of polarity (Figure 15.22). In some cell types, such as tracheids, the microfibrils in the wall alternate between transverse and longitudinal orientations, and in such cases the microtubules are parallel to the microfibrils of the most recently deposited wall layer.

The main evidence for the involvement of microtubules in the deposition of cellulose microfibrils is that the orientation of the microfibrils can be perturbed by genetic mutations and certain drugs that disrupt cytoplasmic microtubules. For example, several drugs bind to tubulin, the subunit protein of microtubules, causing them to depolymerize. When growing roots are treated with a microtubule-depolymerizing drug, such as oryzalin, the region of elongation expands laterally, becoming bulbous and tumorlike (Figure 15.23).

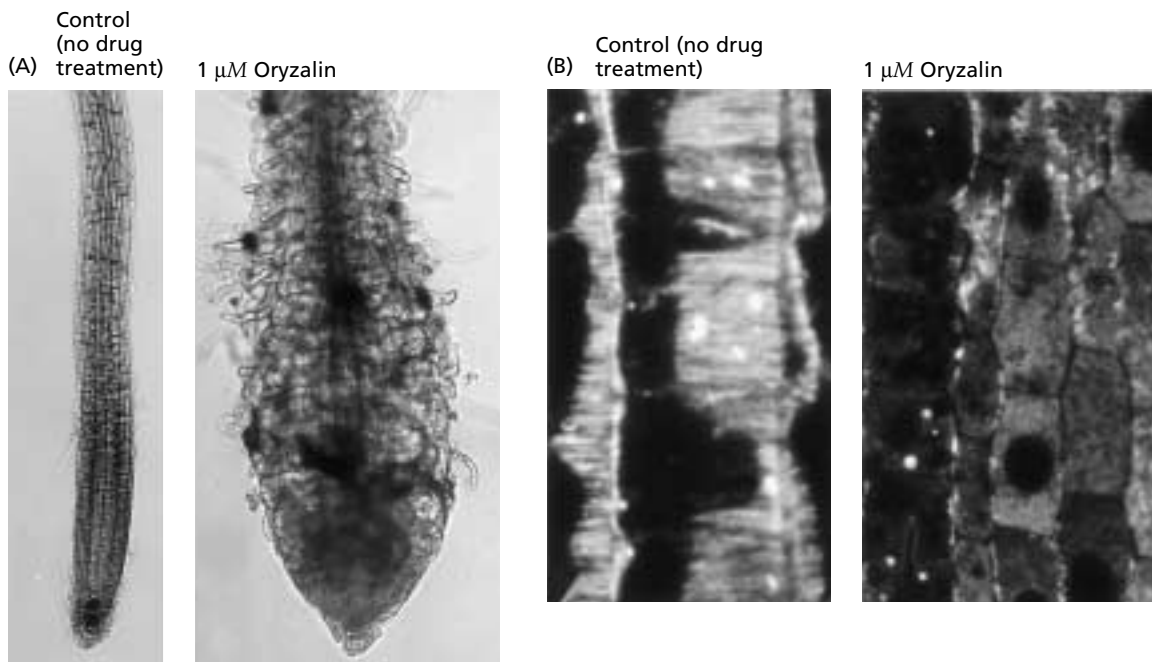
This disrupted growth is due to the isotropic expansion of the cells; that is, they enlarge like a sphere instead of elongating. The drug-induced destruction of microtubules



**FIGURE 15.21** The multinet hypothesis for wall extension. Newly synthesized cellulose microfibrils are continually deposited on the inner surface of the wall in the transverse orientation. As cell elongation proceeds, the older outer wall layers are progressively thinned and weakened, and their cellulose microfibrils are passively rearranged to a longitudinal orientation. The wall mechanical properties are determined by the inner layers.



**FIGURE 15.22** The orientation of microtubules in the cortical cytoplasm mirrors the orientation of newly deposited cellulose microfibrils in the cell wall of cells that are elongating. (A) The arrangement of microtubules can be revealed with fluorescently labeled antibodies to the microtubule protein tubulin. In this differentiating tracheary element from a *Zinnia* cell suspension culture, the pattern of microtubules (green) mirrors the orientation of the cellulose microfibrils in the wall, as shown by calcofluor staining (blue). (B) The alignment of cellulose microfibrils in the cell wall can sometimes be seen in grazing sections prepared for electron microscopy, as in this micrograph of a developing sieve tube element in a root of *Azolla* (a water fern). The longitudinal axis of the root and the sieve tube element runs vertically. Both the wall microfibrils (double-headed arrows) and the cortical microtubules (single-headed arrows) are aligned transversely. (A courtesy of Robert W. Seagull; B courtesy of A. Hardham.)



**FIGURE 15.23** The disruption of cortical microtubules results in a dramatic increase in radial cell expansion and a concomitant decrease in elongation. (A) Root of *Arabidopsis* seedling treated with the microtubule-depolymerizing drug oryzalin ( $1 \mu\text{M}$ ) for 2 days before this photomicrograph was taken. The drug has altered the polarity of growth.

(B) Microtubules were visualized by means of an indirect immunofluorescence technique and an antitubulin antibody. Whereas cortical microtubules in the control are oriented at right angles to the direction of cell elongation, very few microtubules remain in roots treated with  $1 \mu\text{M}$  oryzalin. (From Baskin et al. 1994, courtesy of T. Baskin.)

in the growing cells also disrupts the transverse orientation of cellulose microfibrils in the most recently deposited layers of the wall. Cell wall deposition continues in the absence of microtubules, but the cellulose microfibrils are deposited randomly and the cells expand equally in all directions. Since the antimicrotubule drugs specifically target the microtubules, these results suggest that microtubules act as guides for the orientation of cellulose microfibril deposition.

## THE RATE OF CELL ELONGATION

Plant cells typically expand 10- to 100-fold in volume before reaching maturity. In extreme cases, cells may enlarge more than 10,000-fold in volume (e.g., xylem vessel elements). The cell wall typically undergoes this profound expansion without losing its mechanical integrity and without becoming thinner. Thus, newly synthesized polymers are integrated into the wall without destabilizing it. Exactly how this integration is accomplished is uncertain, although self-assembly and xyloglucan endotransglycosylase (XET) play important roles, as already described.

This integrating process may be particularly critical for rapidly growing root hairs, pollen tubes, and other specialized cells that exhibit tip growth, in which the region of wall deposition and surface expansion is localized to the hemispherical dome at the apex of the tubelike cell, and cell expansion and wall deposition must be closely coordinated.

In rapidly growing cells with tip growth, the wall doubles its surface area and is displaced to the nonexpanding part of the cell within minutes. This is a much greater rate of wall expansion than is typically found in cells with diffuse growth, and tip-growing cells are therefore susceptible to wall thinning and bursting. Although diffuse growth and tip growth appear to be different growth patterns, both types of wall expansion must have analogous, if not identical, processes of polymer integration, stress relaxation, and wall polymer creep.

Many factors influence the rate of cell wall expansion. Cell type and age are important developmental factors. So, too, are hormones such as auxin and gibberellin. Environmental conditions such as light and water availability may likewise modulate cell expansion. These internal and external factors most likely modify cell expansion by loosening the cell wall so that it *yields* (stretches irreversibly). In this context we speak of the *yielding properties* of the cell wall.

In this section we will first examine the biomechanical and biophysical parameters that characterize the yielding properties of the wall. For cells to expand at all, the rigid cell wall must be loosened in some way. The type of wall loosening involved in plant cell expansion is termed *stress relaxation*.

According to the acid growth hypothesis for auxin action (see Chapter 19), one mechanism that causes wall stress relaxation and wall yielding is cell wall acidification, resulting from proton extrusion across the plasma membrane.

Cell wall loosening is enhanced at acidic pH. A little later we will explore the biochemical basis for acid-induced wall loosening and stress relaxation, including the role of a special class of wall-loosening proteins called *expansins*.

As the cell approaches its maximum size, its growth rate diminishes and finally ceases altogether. At the end of this section we will consider the process of cell wall rigidification that leads to the cessation of growth.

## Stress Relaxation of the Cell Wall Drives Water Uptake and Cell Elongation

Because the cell wall is the major mechanical restraint that limits cell expansion, much attention has been given to its physical properties. As a hydrated polymeric material, the plant cell wall has physical properties that are intermediate between those of a solid and those of a liquid. We call these **viscoelastic**, or **rheological** (flow), **properties**. Growing-cell walls are generally less rigid than walls of non-growing cells, and under appropriate conditions they exhibit a long-term irreversible stretching, or **yielding**, that is lacking or nearly lacking in nongrowing walls.

*Stress relaxation* is a crucial concept for understanding how cell walls enlarge (Cosgrove 1997). The term *stress* is used here in the mechanical sense, as force per unit area. Wall stresses arise as an inevitable consequence of cell turgor. The turgor pressure in growing plant cells is typically between 0.3 and 1.0 MPa. Turgor pressure stretches the cell wall and generates a counterbalancing physical stress or tension in the wall. Because of cell geometry (a large pressurized volume contained by a thin wall), this wall tension is equivalent to 10 to 100 MPa of tensile stress—a very large stress indeed.

This simple fact has important consequences for the mechanics of cell enlargement. Whereas animal cells can change shape in response to cytoskeleton-generated forces, such forces are negligible compared with the turgor-generated forces that are resisted by the plant cell wall. To change shape, plant cells must thus control the direction and rate of wall expansion, which they do by depositing cellulose in a biased orientation (which determines the directionality of cell wall expansion) and by selectively loosening the bonding between cell wall polymers. This biochemical loosening enables the wall polymers to slip by each other, thereby increasing the wall surface area. At the same time, such loosening reduces the physical stress in the wall.

Wall stress relaxation is crucial because it allows growing plant cells to reduce their turgor and water potentials, which enables them to absorb water and to expand. Without stress relaxation, wall synthesis would only thicken the wall, not expand it. During secondary-wall deposition in nongrowing cells, for example, stress relaxation does not occur.

## The Rate of Cell Expansion Is Governed By Two Growth Equations

When plant cells enlarge before maturation, the increase in volume is generated mostly by water uptake. This water

ends up mainly in the vacuole, which takes up an ever larger proportion of the cell volume as the cell grows. Here we will describe how growing cells regulate their water uptake and how this uptake is coordinated with wall yielding.

Water uptake by growing cells is a passive process. There are no active water pumps; instead the growing cell is able to lower the water potential inside the cell so that water is taken up spontaneously in response to a water potential difference, without direct energy expenditure.

We define the water potential difference,  $\Delta\Psi_w$  (expressed in megapascals), as the water potential outside the cell minus the water potential inside (see Chapters 3 and 4). The rate of uptake also depends on the surface area of the cell ( $A$ , in square meters) and the permeability of the plasma membrane to water ( $Lp$ , in meters per second per megapascal).

Membrane  $Lp$  is a measure of how readily water crosses the membrane, and it is a function of the physical structure of the membrane and the activity of aquaporins (see Chapter 3). Thus we have the rate of water uptake in volume units:  $\Delta V/\Delta t$ , expressed in cubic meters per second. Assuming that a growing cell is in contact with pure water (with zero water potential), then

$$\begin{aligned} \text{Rate of water uptake} &= A \times Lp (\Delta\Psi_w) \\ &= A \times Lp (\Psi_o - \Psi_i) \end{aligned} \quad (15.1)$$

This equation states that the rate of water uptake depends only on the cell area, membrane permeability to water, cell turgor, and osmotic potential.

Equation 15.1 is valid for both growing and nongrowing cells in pure water. But how can we account for the fact that growing cells can continue to take up water for a long time, whereas nongrowing cells soon cease water uptake?

In a nongrowing cell, water absorption increases cell volume, causing the protoplast to push harder against the cell wall, thereby increasing cell turgor pressure,  $\Psi_p$ . This increase in  $\Psi_p$  would increase cell water potential  $\Psi_w$ , quickly bringing  $\Delta\Psi_w$  to zero. Water uptake would then cease.

In a growing cell,  $\Delta\Psi_w$  is prevented from reaching zero because the cell wall is "loosened": It yields irreversibly to the forces generated by turgor and thereby reduces simultaneously the wall stress and the cell turgor. This process is called **stress relaxation**, and it is the crucial physical difference between growing and nongrowing cells.

Stress relaxation can be understood as follows. In a turgid cell, the cell contents push against the wall, causing the wall to stretch elastically (i.e., reversibly) and giving rise to a counterforce, a wall stress. In a growing cell, biochemical loosening enables the wall to yield inelastically (irreversibly) to the wall stress. Because water is nearly incompressible, only an infinitesimal expansion of the wall is needed to reduce cell turgor pressure and, simultaneously, wall stress. Thus, *stress relaxation is a decrease in wall stress with nearly no change in wall dimensions*.

As a consequence of wall stress relaxation, the cell water potential is reduced and water flows into the cell, causing a measurable extension of the cell wall and increasing cell surface area and volume. Sustained growth of plant cells entails simultaneous stress relaxation of the wall (which tends to reduce turgor pressure) and water absorption (which tends to increase turgor pressure).

Empirical evidence has shown that wall relaxation and expansion depend on turgor pressure. As turgor is reduced, wall relaxation and growth slow down. Growth usually ceases before turgor reaches zero. The turgor value at which growth ceases is called the **yield threshold** (usually represented by the symbol  $Y$ ). This dependence of cell wall expansion on turgor pressure is embodied in the following equation:

$$GR = m(\Psi_p - Y) \quad (15.2)$$

where  $GR$  is the cell growth rate, and  $m$  is the coefficient that relates growth rate to the turgor in excess of the yield threshold. The coefficient  $m$  is usually called **wall extensibility** and is *the slope of the line relating growth rate to turgor pressure*.

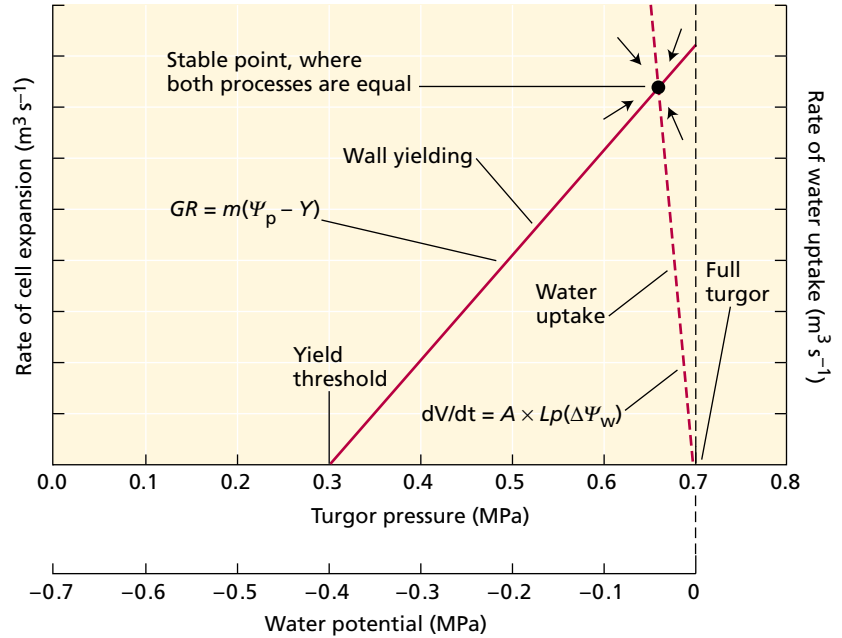
Under conditions of steady-state growth,  $GR$  in Equation 15.2 is the same as the rate of water uptake in Equation 15.1. That is, the increase in the volume of the cell equals the volume of water taken up. The two equations are plotted in Figure 15.24. Note that the two processes of wall expansion and water uptake show opposing reactions to a change in turgor. For example, an increase in turgor increases wall extension but reduces water uptake. Under normal conditions, the turgor is dynamically balanced in a growing cell exactly at the point where the two lines intersect. At this point both equations are satisfied, and water uptake is exactly matched by enlargement of the wall chamber.

This intersection point in Figure 15.24 is the steady-state condition, and any deviations from this point will cause transient imbalances between the processes of water uptake and wall expansion. The result of these imbalances is that turgor will return to the point of intersection, the point of dynamic steady state for the growing cell.

The regulation of cell growth—for example, by hormones or by light—typically is accomplished by regulation of the biochemical processes that regulate wall loosening and stress relaxation. Such changes can be measured as a change in  $m$  or in  $Y$ .

The water uptake that is induced by wall stress relaxation enlarges the cell and tends to restore wall stress and turgor pressure to their equilibrium values, as we have shown. However, if growing cells are physically prevented from taking up water, wall relaxation progressively reduces cell turgor. This situation may be detected, for example, by turgor measurements with a *pressure probe* or by water potential measurements with a *psychrometer* or a *pressure chamber* (see [Web Topic 3.6](#)). Figure 15.25 shows the results of such an experiment.

**FIGURE 15.24** Graphic representation of the two equations that relate water uptake and cell expansion to cell turgor pressure and cell water potential. The values for the rates of cell expansion and water uptake are arbitrary. Steady-state growth is attained only at the point where the two equations intersect. Any imbalance between water uptake and wall expansion will result in changes in cell turgor and bring the cell back to this stable point of intersection between the two processes.

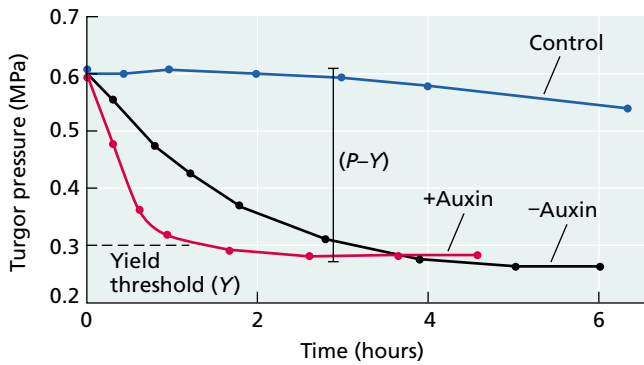


**Acid-Induced Growth Is Mediated by Expansins**

An important characteristic of growing cell walls is that they extend much faster at acidic pH than at neutral pH (Rayle and Cleland 1992). This phenomenon is called **acid growth**. In living cells, acid growth is evident when growing cells are treated with acid buffers or with the drug

fusococcin, which induces acidification of the cell wall solution by activating an H<sup>+</sup>-ATPase in the plasma membrane.

An example of acid-induced growth can be found in the initiation of the root hair, where the local wall pH drops to a value of 4.5 at the time when the epidermal cell begins to bulge outward (Bibikova et al. 1998). Auxin-induced growth is also associated with wall acidification, but it is probably not sufficient to account for the entire growth induction by this hormone (see Chapter 19), and other wall-loosening processes may be involved. Recent work, for example, implicates the production of hydroxyl radicals in wall loosening during auxin-induced growth (Schopfer 2001). Nevertheless, this pH-dependent mechanism of wall extension appears to be an evolutionarily conserved process common to all land plants (Cosgrove 2000) and involved in a variety of growth processes.

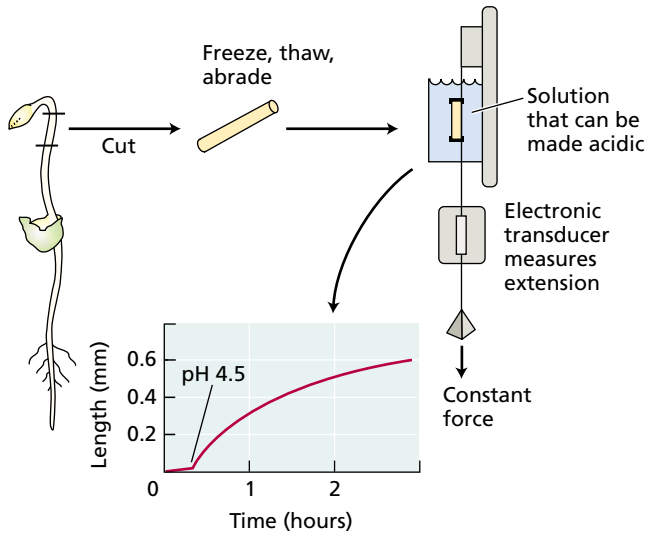


**FIGURE 15.25** Reduction of cell turgor pressure (water potential) by stress relaxation. In this experiment, the excised stem segments from growing pea seedlings were incubated in solution with or without auxin, then blotted dry and sealed in a humid chamber. Cell turgor pressure (*P*) was measured at various time points. The segments treated with auxin rapidly reduced their turgor to the yield threshold (*Y*), as a result of rapid wall relaxation. The segments without auxin showed a slower rate of relaxation. The control segments were treated the same as the group treated with auxin, except that they remained in contact with a drop of water, which prevented wall relaxation. (After Cosgrove 1985.)

Acid growth may also be observed in isolated cell walls, which lack normal cellular, metabolic, and synthetic processes. Such observation requires the use of an extensometer to put the walls under tension and to measure the pH-dependent **wall creep** (Figure 15.26).

The term *creep* refers to a time-dependent irreversible extension, typically the result of slippage of wall polymers relative to one another. When growing walls are incubated in neutral buffer (pH 7) and clamped in an extensometer, the walls extend briefly when tension is applied, but extension soon ceases. When transferred to an acidic buffer (pH 5 or less), the wall begins to extend rapidly, in some instances continuing for many hours.

This acid-induced creep is characteristic of walls from growing cells, but it is not observed in mature (nongrowing) walls. When walls are pretreated with heat, proteases,



**FIGURE 15.26** Acid-induced extension of isolated cell walls, measured in an extensometer. The wall sample from killed cells is clamped and put under tension in an extensometer that measures the length with an electronic transducer attached to a clamp. When the solution surrounding the wall is replaced with an acidic buffer (e.g., pH 4.5), the wall extends irreversibly in a time-dependent fashion (it creeps).

or other agents that denature proteins, they lose their acid growth ability. Such results indicate that acid growth is not due simply to the physical chemistry of the wall (e.g., a weakening of the pectin gel), but is catalyzed by one or more wall proteins.

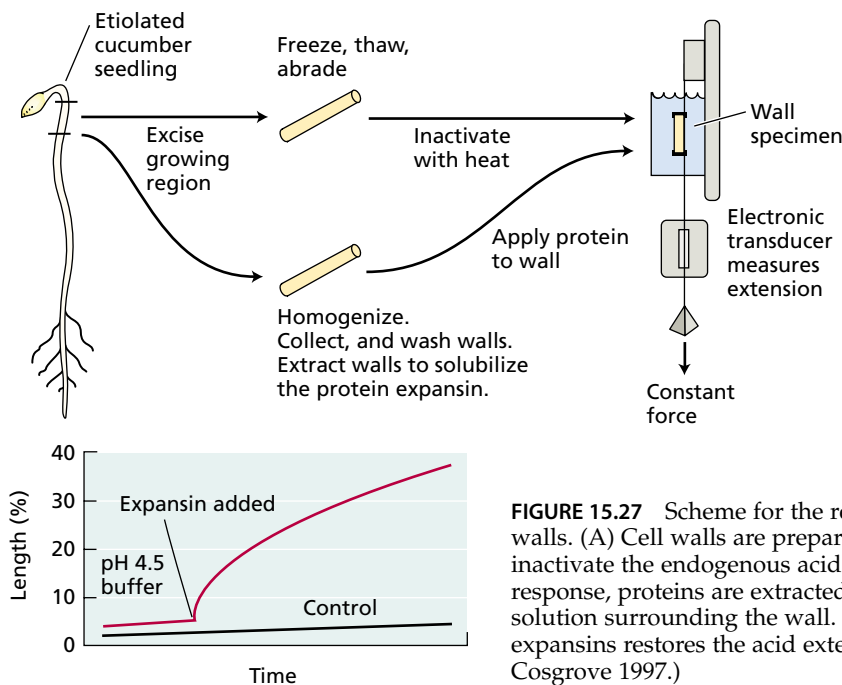
The idea that proteins are required for acid growth was confirmed in reconstitution experiments, in which heat-inactivated walls were restored to nearly full acid growth responsiveness by addition of proteins extracted from growing walls (Figure 15.27). The active components proved to be a group of proteins that were named **expansins** (McQueen-Mason et al. 1992; Li et al. 1993). These proteins catalyze the pH-dependent extension and stress relaxation of cell walls. They are effective in catalytic amounts (about 1 part protein per 5000 parts wall, by dry weight).

The molecular basis for expansin action on wall rheology is still uncertain, but most evidence indicates that expansins cause wall creep by loosening noncovalent adhesion between wall polysaccharides (Cosgrove 2000; Li and Cosgrove 2001). Binding studies suggest that expansins may act at the interface between cellulose and one or more hemicelluloses.

With the completion of the *Arabidopsis* genome, we now know that *Arabidopsis* has a large collection of expansin genes, divided into two families:  $\alpha$ -expansins and  $\beta$ -expansins. The two kinds of expansins act on different polymers of the cell wall (Cosgrove 2000).  $\beta$ -expansins have also been found in grass pollen, where they probably function to aid pollen tube penetration into the stigma and style (Li and Cosgrove 2001).

### Glucanases and Other Hydrolytic Enzymes May Modify the Matrix

Several types of experiments implicate (1→4) $\beta$ -D-glucanases in cell wall loosening, especially during auxin-induced cell elongation (see Chapter 19). For example, matrix glucans



**FIGURE 15.27** Scheme for the reconstitution of extensibility of isolated cell walls. (A) Cell walls are prepared as in Figure 15.21, and briefly heated to inactivate the endogenous acid extension response. To restore this response, proteins are extracted from growing walls and added to the solution surrounding the wall. (B) Addition of proteins containing expansins restores the acid extension properties of the wall. (After Cosgrove 1997.)

such as xyloglucan show enhanced hydrolysis and turnover in excised segments when growth is stimulated by auxin. Interference with this hydrolytic activity by antibodies or lectins reduces growth in excised segments.

Expression of (1→4) $\beta$ -D-glucanases is associated with growing tissues, and application of glucanases to cells in vitro may stimulate growth. Such results support the idea that wall stress relaxation and expansion are the direct result of the activity of glucanases that digest xyloglucan in dicotyledons or (1→3,1→4) $\beta$ -D-glucans in grass cell walls (Hoson 1993).

However, most glucanases and related wall hydrolases do not cause walls to extend in the same way that expansins do. Instead, treatment of walls with glucanases or pectinases may enhance the subsequent extension response to expansins (Cosgrove and Durachko 1994). These results suggest that wall hydrolytic enzymes such as (1→4) $\beta$ -D-glucanases are not the principal catalysts of wall expansion, but they may act indirectly by modulating expansin-mediated polymer creep.

Xyloglucan endotransglycosylase has also been suggested as a potential wall-loosening enzyme. XET helps integrate newly secreted xyloglucan into the existing wall structure, but its function as a wall-loosening agent is still speculative.

### Many Structural Changes Accompany the Cessation of Wall Expansion

The growth cessation that occurs during cell maturation is generally irreversible and is typically accompanied by a reduction in wall extensibility, as measured by various biophysical methods. These physical changes in the wall might come about by (1) a reduction in wall-loosening processes, (2) an increase in wall cross-linking, or (3) an alteration in the composition of the wall, making for a more rigid structure or one less susceptible to wall loosening. There is some evidence for each of these ideas (Cosgrove 1997).

Several modifications of the maturing wall may contribute to wall rigidification:

- Newly secreted matrix polysaccharides may be altered in structure so as to form tighter complexes with cellulose or other wall polymers, or they may be resistant to wall-loosening activities.
- Removal of mixed-link  $\beta$ -D-glucans is also coincident with growth cessation in these walls.
- De-esterification of pectins, leading to more rigid pectin gels, is similarly associated with growth cessation in both grasses and dicotyledons.
- Cross-linking of phenolic groups in the wall (such as tyrosine residues in HRGPs, ferulic acid residues attached to pectins, and lignin) generally coincides with wall maturation and is believed to be mediated by peroxidase, a putative wall rigidification enzyme.

Many structural changes occur in the wall during and after cessation of growth, and it has not yet been possible to identify the significance of individual processes for cessation of wall expansion.

## WALL DEGRADATION AND PLANT DEFENSE

The plant cell wall is not simply an inert and static exoskeleton. In addition to acting as a mechanical restraint, the wall serves as an extracellular matrix that interacts with cell surface proteins, providing positional and developmental information. It contains numerous enzymes and smaller molecules that are biologically active and that can modify the physical properties of the wall, sometimes within seconds. In some cases, wall-derived molecules can also act as signals to inform the cell of environmental conditions, such as the presence of pathogens. This is an important aspect of the defense response of plants (see Chapter 13).

Walls may also be substantially modified long after growth has ceased. For instance, the cell wall may be massively degraded, such as occurs in ripening fruit or in the endosperm of germinating seeds. In cells that make up the abscission zones of leaves and fruits (see Chapter 22), the middle lamella may be selectively degraded, with the result that the cells become unglued and separate. Cells may also separate selectively during the formation of intercellular air spaces, during the emergence of the root from germinating seeds, and during other developmental processes. Plant cells may also modify their walls during pathogen attack as a form of defense.

In the sections that follow we will consider two types of dynamic changes that can occur in mature cell walls: hydrolysis and oxidative cross-linking. We will also discuss how fragments of the cell wall released during pathogen attack, or even during normal cell wall turnover, may act as cellular signals that influence metabolism and development.

### Enzymes Mediate Wall Hydrolysis and Degradation

Hemicelluloses and pectins may be modified and broken down by a variety of enzymes that are found naturally in the cell wall. This process has been studied in greatest detail in ripening fruit, in which softening is thought to be the result of disassembly of the wall (Rose and Bennett 1999). Glucanases and related enzymes may hydrolyze the backbone of hemicelluloses. Xylosidases and related enzymes may remove the side branches from the backbone of xyloglucan. Transglycosylases may cut and join hemicelluloses together. Such enzymatic changes may alter the physical properties of the wall, for example, by changing the viscosity of the matrix or by altering the tendency of the hemicelluloses to stick to cellulose.

Messenger RNAs for expansin are expressed in ripening tomato fruit, suggesting that they play a role in wall disassembly (Rose et al. 1997). Similarly, softening fruits

express high levels of pectin methyl esterase, which hydrolyzes the methyl esters from pectins. This hydrolysis makes the pectin more susceptible to subsequent hydrolysis by pectinases and related enzymes. The presence of these and related enzymes in the cell wall indicates that walls are capable of significant modification during development.

### Oxidative Bursts Accompany Pathogen Attack

When plant cells are wounded or treated with certain low-molecular-weight elicitors (see Chapter 13), they activate a defense response that results in the production of high concentrations of hydrogen peroxide, superoxide radicals, and other active oxygen species in the cell wall. This “oxidative burst” appears to be part of a defense response against invading pathogens (see Chapter 13) (Brisson et al. 1994; Otte and Barz 1996).

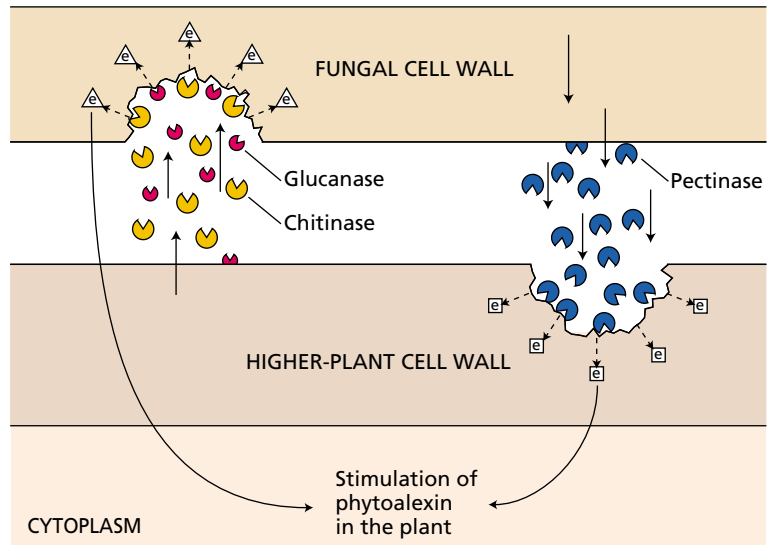
Active oxygen species may directly attack the pathogenic organisms, and they may indirectly deter subsequent invasion by the pathogenic organisms by causing a rapid cross-linking of phenolic components of the cell wall. In tobacco stems, for example, proline-rich structural proteins of the wall become rapidly insolubilized upon wounding or elicitor treatment, and this cross-linking is associated with an oxidative burst and with a mechanical stiffening of the cell walls.

### Wall Fragments Can Act as Signaling Molecules

Degradation of cell walls can result in the production of biologically active fragments 10 to 15 residues long, called **oligosaccharins**, that may be involved in natural developmental responses and in defense responses (see [Web Topic 15.5](#)). Some of the reported physiological and developmental effects of oligosaccharins include stimulation of phytoalexin synthesis, oxidative bursts, ethylene synthesis, membrane depolarization, changes in cytoplasmic calcium, induced synthesis of pathogen-related proteins such as chitinase and glucanase, other systemic and local “wound” signals, and alterations in the growth and morphogenesis of isolated tissue samples (John et al. 1997).

The best-studied examples are oligosaccharide elicitors produced during pathogen invasion (see Chapter 13). For example, the fungus *Phytophthora* secretes an endopolygalacturonase (a type of pectinase) during its attack on plant tissues. As this enzyme degrades the pectin component of the plant cell wall, it produces pectin fragments—**oligogalacturonans**—that elicit multiple defense responses by the plant cell (Figure 15.28). The oligogalacturonans that are 10 to 13 residues long are most active in these responses.

Plant cell walls also contain a  $\beta$ -D-glucanase that attacks the  $\beta$ -D-glucan that is specific to the fungal cell wall. When



**FIGURE 15.28** Scheme for the production of oligosaccharins during fungal invasion of plant cells. Enzymes secreted by the plant, such as chitinase and glucanase, attack the fungal wall, releasing oligosaccharins that elicit the production of defense compounds (phytoalexins) in the plant. Similarly, fungal pectinase releases biologically active oligosaccharins from the plant cell wall. (After Brett and Waldron 1996.)

this enzyme attacks the fungal wall, it releases glucan oligomers with potent elicitor activity. The wall components serve in this case as part of a sensitive system for the detection of pathogen invasion.

Oligosaccharins may also function during the normal control of cell growth and differentiation. For example, a specific nonasaccharide (an oligosaccharide containing nine sugar residues) derived from xyloglucan has been found to inhibit growth promotion by the auxin 2,4-dichlorophenoxyacetic acid (2,4-D). The nonasaccharide acts at an optimal concentration of  $10^{-9}$  M. This xyloglucan oligosaccharin may act as a feedback inhibitor of growth; for example, when auxin-induced breakdown of xyloglucan is maximal, it may prevent excessive weakening of the cell wall. Related xyloglucan oligomers have also been reported to influence organogenesis in tissue cultures and may play a wider role in cell differentiation (Creelman and Mullet 1997).

## SUMMARY

The architecture, mechanics, and function of plants depend crucially on the structure of the cell wall. The wall is secreted and assembled as a complex structure that varies in form and composition as the cell differentiates. Primary cell walls are synthesized in actively growing cells, and secondary cell walls are deposited in certain cells, such as xylem vessel elements and sclerenchyma, after cell expansion ceases.



The basic model of the primary wall is a network of cellulose microfibrils embedded in a matrix of hemicelluloses, pectins, and structural proteins. Cellulose microfibrils are highly ordered arrays of glucan chains synthesized on the membrane by protein complexes called particle rosettes. The genes for cellulose synthase in plants have recently been identified, bringing the realization that a large gene family encodes these and related proteins. The matrix is secreted into the wall via the Golgi apparatus. Hemicelluloses and proteins cross-link microfibrils, and pectins form hydrophilic gels that can become cross-linked by calcium ions. Wall assembly may be mediated by enzymes. For example, xyloglucan endotransglycosylase has the ability to carry out transglycosylation reactions that integrate newly synthesized xyloglucans into the wall.

Secondary walls differ from primary walls in that they contain a higher percentage of cellulose, they have different hemicelluloses, and lignin replaces pectins in the matrix. Secondary walls can also become highly thickened, sculpted, and embedded with specialized structural proteins.

In diffuse-growing cells, growth directionality is determined by wall structure, in particular the orientation of the cellulose microfibrils, which in turn is determined by the orientation of microtubules in the cytoplasm. Upon leaving the meristem, plant cells typically elongate greatly. Cell enlargement is limited by the ability of the cell wall to undergo polymer creep, which in turn is controlled in a complex way by the adhesion of wall polymers to one another and by the influence of pH on wall-loosening proteins such as expansins, glucanases, and other enzymes.

According to the acid growth hypothesis, proton extrusion by the plasma membrane  $H^+$ -ATPase acidifies the wall, activating the protein expansin. Expansins induce stress relaxation of the wall by loosening the bonds holding microfibrils together. The cessation of cell elongation appears to be due to cell wall rigidification caused by an increase in the number of cross-links.

Hydrolytic enzymes may degrade mature cell walls completely or selectively during fruit ripening, seed germination, and the formation of abscission layers. Cell walls can also undergo oxidative cross-linking in response to pathogen attack. In addition, pathogen attack may release cell wall fragments, and certain wall fragments have been shown to be capable of acting as cell signaling agents.

## Web Material

### Web Topics

#### 15.1 Terminology for Polysaccharide Chemistry

A brief review of terms used to describe the structures, bonds, and polymers in polysaccharide chemistry is provided.

#### 15.2 Molecular Model for the Synthesis of Cellulose and Other Wall Polysaccharides That Consist of a Disaccharide Repeat

A model is presented for the polymerization of cellulose units into glucan chains by the enzyme cellulose synthase.

#### 15.3 Matrix Components of the Cell Wall

The secretion of xyloglucan and glycosylated proteins by the Golgi can be demonstrated at the ultrastructural level.

#### 15.4 The Mechanical Properties of Cell Walls: Studies with *Nitella*

Experiments demonstrating that the inner 25% of the cell wall determines the directionality of cell expansion are described.

#### 15.5 Structure of Biologically Active Oligosaccharins

Some cell wall fragments have been demonstrated to have biological activity.

## Web Essay

#### 15.1 Calcium Gradients and Oscillations in Growing Pollen Tube

The role of calcium in regulating pollen tube tip growth is described.

## Chapter References

- Amor, Y., Haigler, C. H., Johnson, S., Wainscott, M., and Delmer, D. P. (1995) A membrane-associated form of sucrose synthase and its potential role in synthesis of cellulose and callose in plants. *Proc. Natl. Acad. Sci. USA* 92: 9353–9357.
- Arioli, T., Peng, L., Betzner, A. S., Burn, J., Wittke, W., Herth, W., Camilleri, C., Hofte, H., Plazinski, J., Birch, R., Cork, A., Glover, J., Redmond, J., Williamson, R. E. (1998) Molecular analysis of cellulose biosynthesis in *Arabidopsis*. *Science* 279: 717–720.
- Baskin, T. I., Wilson, J. E., Cork, A., and Williamson, R. E. (1994) Morphology and microtubule organization in *Arabidopsis* roots exposed to oryzalin or taxol. *Plant Cell Physiol.* 35: 935–942.
- Bibikova, T. N., Jacob, T., Dahse, I., and Gilroy, S. (1998) Localized changes in apoplastic and cytoplasmic pH are associated with root hair development in *Arabidopsis thaliana*. *Development* 125: 2925–2934.
- Brett, C. T., and Waldron, K. W. (1996) *Physiology and Biochemistry of Plant Cell Walls*, 2nd ed. Chapman and Hall, London.
- Brisson, L. F., Tenhaken, R., and Lamb, C. (1994) Function of oxidative cross-linking of cell wall structural proteins in plant disease resistance. *Plant Cell* 6: 1703–1712.
- Brown, R. M., Jr., Saxena, I. M., and Kudlicka, K. (1996) Cellulose biosynthesis in higher plants. *Trends Plant Sci.* 1: 149–155.
- Buchanan, B. B., Gruissem, W., and Jones, R. L., eds. (2000) *Biochemistry, and Molecular Biology of Plants*. Amer. Soc. Plant Physiologists, Rockville, MD.
- Carpita, N. C. (1996). Structure and biogenesis of the cell walls of grasses. *Annu. Rev. Plant Physiol. Plant Mol. Biol.* 47: 455–476.
- Carpita, N. C., and McCann, M. (2000) The cell wall. In *Biochemistry and Molecular Biology of Plants*, B. B. Buchanan, W. Gruissem, and

- R. L. Jones, eds., American Society of Plant Biologists, Rockville, MD, pp. 52–108.
- Cheung, A. Y., Zhan, X. Y., Wang, H., and Wu, H.-M. (1996) Organ-specific and Agamous-regulated expression and glycosylation of a pollen tube growth-promoting protein. *Proc. Natl. Acad. Sci. USA* 93: 3853–3858.
- Cosgrove, D. J. (1985) Cell wall yield properties of growing tissues. Evaluation by in vivo stress relaxation. *Plant Physiol.* 78: 347–356.
- Cosgrove, D. J. (1997) Relaxation in a high-stress environment: The molecular bases of extensible cell walls and cell enlargement. *Plant Cell* 9: 1031–1041.
- Cosgrove, D. J. (2000) Loosening of plant cell walls by expansins. *Nature* 407: 321–326.
- Cosgrove, D. J., and Durachko, D. M. (1994) Autolysis and extension of isolated walls from growing cucumber hypocotyls. *J. Exp. Bot.* 45: 1711–1719.
- Creelman, R. A., and Mullet, J. E. (1997) Oligosaccharins, brassinolides, and jasmonates: Nontraditional regulators of plant growth, development, and gene expression. *Plant Cell* 9: 1211–1223.
- Darley, C. P., Forrester, A. M., and McQueen-Mason, S. J. (2001) The molecular basis of plant cell wall extension. *Plant Mol. Biol.* 47: 179–195.
- Delmer, D. P., and Amor, Y. (1995) Cellulose biosynthesis. *Plant Cell* 7: 987–1000.
- Gaspar, Y., Johnson, K. L., McKenna, J. A., Bacic, A., and Schultz, C. J. (2001) The complex structures of arabinogalactan-proteins and the journey towards understanding function. *Plant Mol. Biol.* 47: 161–176.
- Gunning, B. S., and Steer, M. W. (1996) *Plant Cell Biology: Structure and Function*. Jones and Bartlett Publishers, Boston.
- Hayashi, T. (1989) Xyloglucans in the primary cell wall. *Annu. Rev. Plant Physiol. Plant Mol. Biol.* 40: 139–168.
- Holland, N., Holland, D., Helentjaris, T., Dhugga, K. S., Xoconostle-Cazares, B., and Delmer D. P. (2000) A comparative analysis of the plant cellulose synthase (CesA) gene family. *Plant Physiol.* 123: 1313–1324.
- Hoson, T. (1993) Regulation of polysaccharide breakdown during auxin-induced cell wall loosening. *J. Plant Res.* 103: 369–381.
- Ishii, T., Matsunaga, T., Pellerin, P., O'Neill, M. A., Darvill, A., and Albersheim, P. (1999) The plant cell wall polysaccharide rhamnogalacturonan II self-assembles into a covalently cross-linked dimer. *J. Biol. Chem.* 274: 13098–13104.
- John, M., Röhrig, H., Schmidt, J., Walden, R., and Schell, J. (1997) Cell signalling by oligosaccharides. *Trends Plant Sci.* 2: 111–115.
- Kimura, S., Laosinchai, W., Itoh, T., Cui, X. J., Linder, C. R., and Brown, R. M., Jr. (1999) Immunogold labeling of rosette terminal cellulose-synthesizing complexes in the vascular plant *Vigna angularis*. *Plant Cell* 11: 2075–2085.
- Li, L.-C., and Cosgrove, D. J. (2001) Grass group I pollen allergens (beta-expansins) lack proteinase activity and do not cause wall loosening via proteolysis. *Eur. J. Biochem.* 268: 4217–4226.
- Li, Z.-C., Durachko, D. M., and Cosgrove, D. J. (1993) An oat coleoptile wall protein that induces wall extension in vitro and that is antigenically related to a similar protein from cucumber hypocotyls. *Planta* 191: 349–356.
- McCann, M. C., Wells, B., and Roberts, K. (1990) Direct visualization of cross-links in the primary plant cell wall. *J. Cell Sci.* 96: 323–334.
- McQueen-Mason, S., Durachko, D. M., and Cosgrove, D. J. (1992) Two endogenous proteins that induce cell wall expansion in plants. *Plant Cell* 4: 1425–1433.
- Nishitani, K. (1997) The role of endoxyloglucan transferase in the organization of plant cell walls. *Int. Rev. Cytol.* 173: 157–206.
- O'Neill, M. A., Eberhard, S., Albersheim, P., and Darvill, A. G. (2001) Requirement of borate cross-linking of cell wall rhamnogalacturonan II for *Arabidopsis* growth. *Science* 294: 846–849.
- Otte, O., and Barz, W. (1996) The elicitor-induced oxidative burst in cultured chickpea cells drives the rapid insolubilization of two cell wall structural proteins. *Planta* 200: 238–246.
- Pear, J. R., Kawagoe, Y., Schreckengost, W. E., Delmer, D. P., and Stalker, D. M. (1996) Higher plants contain homologs of the bacterial celA genes encoding the catalytic subunit of cellulose synthase. *Proc. Natl. Acad. Sci. USA* 93: 12637–12642.
- Peng, L., Kawagoe, Y., Hogan, P., and Delmer, D. (2002) Sitosterol- $\beta$ -glucoside as primer for cellulose synthesis in plants. *Science* 295: 147–148.
- Rayle, D. L., and Cleland, R. E. (1992) The acid growth theory of auxin-induced cell elongation is alive and well. *Plant Physiol.* 99: 1271–1274.
- Richmond, T. A., and Somerville, C. R. (2000) The cellulose synthase superfamily. *Plant Physiol.* 124: 495–498.
- Roland, J. C., Reis, D., Mosiniak, M., and Vian, B. (1982) Cell wall texture along the growth gradient of the mung bean hypocotyl: Ordered assembly and dissipative processes. *J. Cell Sci.* 56: 303–318.
- Rose, J. K. C., and Bennett, A. B. (1999) Cooperative disassembly of the cellulose-xyloglucan network of plant cell walls: Parallels between cell expansion and fruit ripening. *Trends Plant Sci.* 4: 176–183.
- Rose, J. K. C., Lee, H. H., and Bennett, A. B. (1997) Expression of a divergent expansin gene is fruit-specific and ripening-regulated. *Proc. Natl. Acad. Sci. USA* 94: 5955–5960.
- Salnikov, V. V., Grimson, M. J., Delmer, D. P., and Haigler, C. H. (2001) Sucrose synthase localizes to cellulose synthesis sites in tracheary elements. *Phytochemistry* 57: 823–833.
- Schopfer, P. (2001) Hydroxyl radical-induced cell-wall loosening in vitro and in vivo: Implications for the control of elongation growth. *Plant J.* 28: 679–688.
- Séné, C. F. B., McCann, M. C., Wilson, R. H., and Grinter, R. (1994) Fourier-transform Raman and Fourier-transform infrared spectroscopy. An investigation of five higher plant cell walls and their components. *Plant Physiol.* 106: 1623–1631.
- Smith, R. C., and Fry, S. C. (1991) Endotransglycosylation of xyloglucans in plant cell suspension cultures. *Biochem. J.* 279: 529–536.
- Thompson, J. E., and Fry, S. C. (2001) Restructuring of wall-bound xyloglucan by transglycosylation in living plant cells. *Plant J.* 26: 23–34.
- Wilson, R. H., Smith, A. C., Kacurakova, M., Saunders, P. K., Wellner, N., and Waldron, K. W. (2000) The mechanical properties and molecular dynamics of plant cell wall polysaccharides studied by Fourier-transform infrared spectroscopy. *Plant Physiol.* 124: 397–405.