Plant Cell Walls

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Introductory article

The cell wall, as the outer coat of a plant cell, controls cell expansion, gives plant cells their characteristic shapes, and provides protection. Cellulose is only one of many polysaccharides and other polymers of the higher plant cell wall; some of these polymers undergo chemical reactions in the walls of living cells.

Article Contents

Introduction

The cell wall is the strong, outermost layer of a plant cell, located external to the plasma membrane. The cell wall often far outlives the protoplast which synthesized it. In cork, for example, the wall serves its particular biological role (physical protection of a tree trunk) for many years after the death of the protoplast. However, the walls of living cells are not inert 'boxes' but complex and dynamic subcellular compartments that play diverse and subtle roles in plant growth, development and defence. The walls range from ~ 0.1 to over 10 μm thick and are composed mainly of polysaccharides; they thus differ fundamentally from cell membranes, which are < 0.01 μm thick and composed of phospholipids and proteins.

This article considers the cell walls of seed plants. The walls of ferns, liverworts and charophycean algae resemble those of seed plants, but have been investigated in less detail. Other algae differ widely and are not discussed here.

General Morphology: Cell Wall Proper, Middle Lamella

Long cellulose microfibrils, lying in the plane of the cell surface, form the wall's scaffolding. Microfibrils are typically 4–10 nm thick and spaced roughly 30 nm apart. Many plant cells are approximately cylindrical, with the neighbouring microfibrils deposited parallel with each other so as to form 'hoops' around the cylinder (**Figure 1**). This arrangement dictates the direction in which the cell can elongate.

New walls are usually formed soon after mitosis at such a location as to divide the mother cell into two (sometimes unequal) daughter cells. The siting of the new wall is directed by a cluster of microtubules, the phragmoplast.

Walls may increase in thickness as new microfibrils are deposited on the inner face. Walls can also expand in area – often unidirectionally (cell elongation, giving roughly cylindrical or rectangular cells); alternatively expansion can be approximately equal in all directions (e.g. in potato tuber parenchyma, giving nearly spherical cells) or irregular (e.g. in spongy mesophyll, giving knobbly cells). In some unidirectionally growing cells, wall expansion occurs uniformly over the area of the side-walls (e.g. in the parenchyma and epidermis of a stem or coleoptile); in others (e.g. fibre cells, pollen tubes and root hairs) it is confined to the tip(s).

The inner face of the cell wall is usually in contact with the plasma membrane, the outer face with a neighbouring wall. Most of the wall's area is not firmly attached to the underlying plasma membrane, as can be seen when the cell

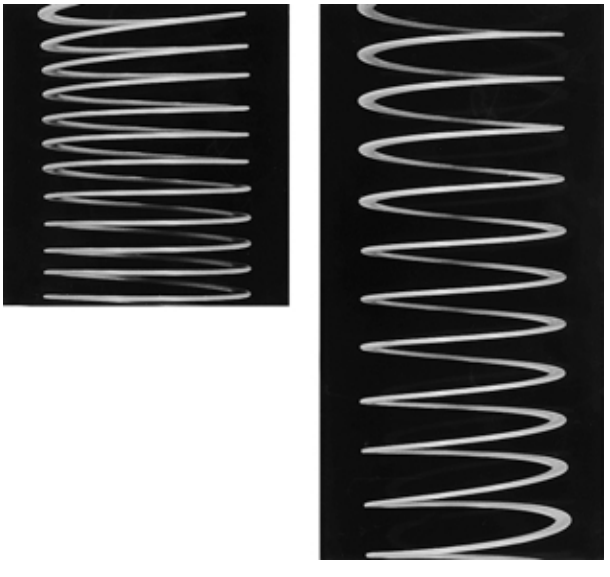


Figure 1 Model showing an arrangement of microfibrils in the side-walls of an approximately cylindrical plant cell. Microfibrils are inextensible, so this hoop arrangement dictates that the cylindrical cell will expand predominantly in one dimension. Many of the cells in an elongating stem or root conform to this model.

is plasmolysed in concentrated sucrose solutions: the membrane of the shrinking protoplast readily withdraws from much of the wall. Adhesion between neighbouring walls is much stronger, and is achieved through a layer of 'glue', the middle lamella, which imparts tissue cohesion. The middle lamella is rich in acidic pectins, which may contribute to its adhesiveness. However, it is often difficult to separate plant cells with pectinases, indicating that the molecular details of the middle lamella await clarification.

Between neighbouring cells there is often an air space. Some tissues (e.g. mesophyll, cortex and especially aerenchyma) have large air spaces; others (e.g. meristems, the stele and collenchyma) usually have no obvious air spaces. The formation of air spaces between a pair of sister cells involves the partial disintegration of middle lamella and primary wall material.

Definitions of Primary and Secondary Cell Walls

Primary cell walls

A wall whose microfibrils were laid down while it was still capable of growing in area is called a primary wall. After deposition, a primary wall may lose its ability to grow: thus, all growing walls are primary, but not all primary walls can grow. Primary walls are often about 0.1 μm thick, but collenchyma primary walls can be 10 μm thick. Sometimes additional components, e.g. lignin, cutin, suberin and extensin (but not new microfibrils), are infiltrated between the microfibrils of a primary wall; that does not alter its status as 'primary'.

Secondary cell walls

Any additional microfibrils deposited after the cell has stopped growing constitute a secondary wall. The secondary wall is deposited on the inner face of the primary wall. Secondary walls are often much thicker than primary walls; secondary walls of fibre cells are often 10–20 μm thick. They differ from primary walls in polysaccharide composition (see below).

Variability in Architecture

Walls differ in thickness, in the orientation of the microfibrils, and in chemical composition. In general, meristematic and parenchymatous cells have thin walls. Collenchyma, sclerenchyma and cork cells have much thicker walls, providing physical strength. In xylem vessel elements there is great diversity, with different walls thickened to different extents and in different patterns – for example with annular, helical, scalariform or pitted secondary wall layers, which resist collapse of the vessels when the sap in them is under suction during transpiration. In the epidermises of stems and leaves, the outer walls (in contact with air) are much thicker than the inner walls; the outer walls function to limit growth, minimize water loss and exclude pathogens. Guard cells are irregularly thickened so that changes in turgor pressure lead to the reversible movements which open and close the stomata. Seeds often have thick walls, some of whose polysaccharides are enzymatically hydrolysed after germination to provide sugars for the growing seedling.

Cells tend to expand in a direction perpendicular to the microfibrils. Treatment of dwarf pea plants with the plant hormone gibberellic acid causes new microfibrils to be deposited mainly perpendicular to the long axis of the stem; the cells then grow in length, leading to a tall, thin stem. Treatment with ethylene causes new microfibrils to be deposited more randomly; the cells then 'balloon', and the stem swells in diameter.

Functions of Cell Walls

Cell shape

The wall governs the cell's shape and size, which remain relatively unchanged if the protoplast is killed (e.g. by freezing, boiling or poisoning) or shrunk (by plasmolysis). Live, isolated protoplasts are always spherical, so the cell's original characteristic shape must have been dictated by the wall.

Cell size

The protoplast of a living cell that is bathed in a hypotonic medium tends to take up water by osmosis. In the absence of a cell wall, this water uptake would swell and eventually burst the protoplast. In an intact cell, however, the hydrostatic (turgor) pressure exerted by the swelling protoplast is opposed by a back-pressure exerted by the wall. Thus, the wall controls cell volume.

Reversible (elastic) cell expansion

When wall material from a mature organ is stretched (e.g. *in vivo* by turgor pressure when a wilted plant is rewatered, or *in vitro* in an extensiometer), some elastic expansion occurs. The new size is reached quickly, as when a rubber band is stretched. In the living plant, the swelling cells press against one another, restoring turgidity to wilted tissues. When the stretching force is removed, however, the wall returns to its original size.

Irreversible (plastic) cell expansion

When wall material from immature tissue is stretched, both elastic and plastic expansion occur, the latter being the increase in wall area that is not reversed when the stretching force is removed. Plastic expansion – the basis of plant growth (defined as irreversible increase in volume) – is slow, occurring over a period of hours, and is at least partly dependent on the activity of wall proteins. A wall that is capable of plastic expansion is described as loose; the opposite is conveniently described as tight (the terms 'rigid' and 'stiff', which mean inflexible, are sometimes misleadingly used: primary walls usually remain flexible, even when they lose the capacity for plastic expansion).

Wall loosening and tightening are the major changes regulating growth in well-watered plants. The expansion of an organ may be largely dictated by the wall properties of one specific tissue – in shoots, the epidermis. Thus, removal of the epidermis from a stem or leaf enables a rapid burst of (mainly elastic) expansion in the underlying tissues.

Permeability

Walls allow free passage of most small, water-soluble molecules and ions such as oxygen, carbon dioxide, nitrate, phosphate, sugars and amino acids into and out of the cell; larger molecules cannot permeate the wall. Two attempts to measure the wall's M_r cut-off gave discrepant estimates (1000–5000 and $\sim 50\,000$).

Cation binding

Walls have a high affinity for certain inorganic cations, and may be a major location of some of these, e.g. Ca^{2+} and Cu^{2+}. The wall may harmlessly sequester heavy metals when taken up by the plant from polluted soils.

Tissue mechanics

The wall, being the outermost layer of the cell, constitutes the first line of defence against mechanical injury and pathogen ingress. Other mechanical roles of walls include lubrication (by mucilaginous root-cap cell walls) of the passage of roots through soil, the control of texture in fruit during ripening, and tissue disintegration enabling leaf abscission. In many of these examples, it is unclear whether the cell wall proper or the middle lamella plays the predominant role.

Source of signalling molecules: oligosaccharins

Several specific oligosaccharides, generated *in vitro* by partial hydrolysis of wall polysaccharides, possess hormone-like or signalling activity, regulating growth, development and metabolism. Such oligosaccharides are termed oligosaccharins. For instance, xyloglucan-derived oligosaccharins can regulate cell expansion, and pectin-derived oligosaccharins can elicit several defence responses such as phytoalexin synthesis. Xyloglucan- and pectin-derived oligosaccharins have been shown to be formed *in vivo*, supporting the view that their production is another role of the plant cell wall.

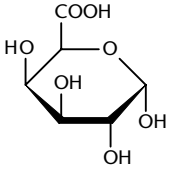
Major Components and their Properties

Primary walls of cultured plant cells are typically $\sim 70\%$ water. The dry matter is typically $\sim 90\%$ polysaccharide and $\sim 10\%$ (glyco)protein. Classically, the wall is fractionated into three polysaccharide classes: pectins, hemicelluloses and cellulose. For details, see **Table 1**.

Polysaccharides

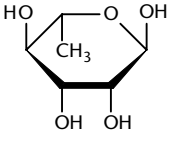
Pectins are polysaccharides which on hydrolysis yield much galacturonate [**I**]; rhamnose [**II**], galactose [**III**] and arabinose [**IV**] are often also present. Pectins are partially extracted from the wall by cold solutions of chelating agents, e.g. EDTA (ethylenediaminetetraacetic acid). More efficiently, they are extracted by hot water (which partially cleaves the polymer backbone) or pectinase (= endo-polygalacturonase, which degrades homogalacturonan, releasing intact rhamnogalacturonans).

Hemicelluloses are diverse polysaccharides with a backbone rich in glucose [**V**], xylose [**VI**] or mannose [**VII**]; many have side-chains containing arabinose, xylose,



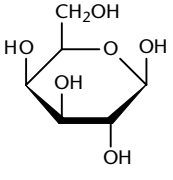
[**I**]

α-D-Galacturonic acid



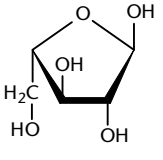
[**II**]

α-L-Rhamnose



[**III**]

β-D-Galactose



[**IV**]

α-L-Arabinose
(furanose form)

Table 1 Polysaccharides and structural proteins of primary cell walls

Polymer	Amount (%)[a]	Q^b	Principal building blocks	Notes
Polysaccharides				
Microfibrillar				
Cellulose	30	0	βGlc	Linear (1→4)-Glc$_n$. Hydrogen-bonded within a microfibril
Matrix				
Pectins				
Homogalacturonan	16	–	αGalA	Linear (1→4)-GalA$_n$. Some of GalA residues methylesterified, some *O*-acetylated. Nonesterified homogalacturonan is hydrolysed by EPG
Rhamnogalacturonan-I	10	–	αGalA, αRha, βGal, αAra*f*, ...	Backbone [-GalA-(1→4)-Rha-(1→2)-]$_n$. ~Half the Rha residues carry Gal/Ara*f*-rich oligomer side-chains. GalA residues *O*-acetylated. Not hydrolysed by EPG
Rhamnogalacturonan-II	4	–	α&βGalA, α&βRha, αGal, αFuc, αAra*p*, βAra*f*, βApi*f*, βGlcA, KDO, βAce*f*A, αXyl, βDHA...	Extraordinarily complex polymer. α-(1→4)-GalA-rich core. Other residues as oligosaccharide side-chains attached via Api and KDO residues. Some Fuc and all Xyl residues as 2-*O*-methyl ethers. Some Api*f* residues esterified with borate. Not hydrolysed by EGP
Apiogalacturonan	±	–	αGalA, Api*f*, ...	Only in certain aquatic angiosperms e.g. *Lemna*
Hemicelluloses				
Xyloglucans	20	0	βGlc, αXyl, βGal, αFuc (±αAra*f*, βXyl)	Backbone (1→4)-Glc$_n$. Frequent repeat units include XXXG and XXFG where X = αXyl-(1→6)-βGlc*, G = βGlc*, F = αFuc-(1→2)-βGal-(1→2)-αXyl-(1→6)-βGlc*; asterisked residues = part of backbone. Some Gal residues *O*-acetylated
Xylans	8	–	βXyl, αAra*f*, α-GlcA, β-D-Gal (± L-Gal, ...)	Backbone (1→4)-Xyl$_n$. Side-chains, linked to C-2 or C-3 of backbone, include Ara*f*, GlcA, Xyl-(1→2)-Ara*f* and longer oligosaccharides. Some Xyl *O*-acetylated. Some Ara*f* residues have ferulate esterified to C-5
Mannans	±	0	βMan, βGlc, αGal	Little studied in primary cell walls. Well known in secondary walls of xylem and in some seeds
Mixed-linkage glucans (MLGs)	±~	0	βGlc	Only in graminaceous monocots. Linear polymer with ~70% (1→4), 30% (1→3)-linkages
Callose	±	0	βGlc	Linear, (1→3)-Glc$_n$
Glucuronomannans	±	–	βGlcA, αMan, Ara*f*, Xyl, Gal	Backbone (-Man-(1→4)-GlcA-(1→2)-)$_n$. Ara*f*, Xyl and Gal residues as side-chains

continued

Table 1 *continued*

Polymer	Amount (%)[a]	Q^b	Principal building blocks	Notes
Proteins				
Extensins	±	+	Hyp, Ser, Lys, Tyr, Val, His; 50–60% sugar [β&αAra*f*, αGal]	Basic polypeptide backbone. Tetrasaccharide (αAra*f*-(1→3)-βAra*f*-(1→2)-βAra*f*-(1→2)-βAra*f*-) *O*-linked to most Hyp residues. Single Gal attached to some Ser residues. Some Tyr coupled to form isodityrosine
Arabinogalactan proteins (AGPs)	±	–	Hyp, Ser, Asp, Thr, Gly; 90–98% sugar [βGal, αAra*f*, GlcA, ...]	Slimes, e.g. gum arabic. Short, acidic polypeptide backbone. Polysaccharide groups (rich in (1→3) & (1→6)-linked Gal) *O*-linked to Hyp. Some AGPs tissue-specific. Some covalently attached to lipids
Proline-rich proteins (PRPs)	±	+	Pro, Hyp, Val, Tyr, Lys	Hyp:Pro ratio ~1:1. May become covalently crosslinked, possibly via Tyr residues. Little or no sugar. Sometimes associated with lignin.
Glycine-rich proteins (GRPs)	±		Gly; also Ser, Ala, ...	Often 60–70% Gly. Not glycosylated. Often associated with lignin

[a]Rough guide to amount of polymer present, as % of dry weight of a typical dicot primary cell wall from a rapidly growing cell culture. ±, not always present; ~, amount varies greatly.

[b]Charge on polymer molecule (at physiological pH): –, negative; +, positive; 0, uncharged.

AceA, L-acerate; Ala, L-alanine, Api, D-apiose; Ara, L-arabinose; Asp, L-aspartate; D, optical isomer; DHA, 3-deoxy-2-D-heptulosarate; DP, degree of polymerization; EPG, endo-polygalacturonase; *f*, furanose ring-form; Fuc, L-fucose; Gal, galactose (D unless otherwise stated); GalA, D-galacturonate; Glc, D-glucose; GlcA, D-glucuronate; Gly, glycine; His, L-histidine; Hyp, L-hydroxyproline; KDO, D-ketodeoxyoctulosonate; L, optical isomer; Lys, L-lysine; Man, D-mannose; *O*-, via oxygen atom; Pro, L-proline, Rha, L-rhamnose; Ser, L-serine; Thr, L-threonine; Tyr, L-tyrosine; Val, L-valine, Xyl, D-xylose. All sugar residues are in the pyranose (*p*) ring-form unless indicated *f*.

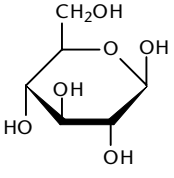
galactose, fucose (= 6-deoxygalactose) or glucuronate [**VIII**]. They are not solubilized from the wall by EDTA but are extracted by aqueous alkali, e.g. 6 mol L^{-1} NaOH at 37°C. Once extracted from the wall, pectins and many hemicelluloses remain soluble even in distilled water.

Cellulose, a rigid, linear polymer of β-(1→4)-linked D-glucose, is the most abundant organic compound on earth. Occurring in paracrystalline bundles (microfibrils), it is insoluble in alkali and is thus a major component of the residue obtained after extraction of hemicelluloses.

Noncarbohydrate components

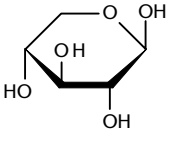
Some pectins and hemicelluloses are decorated with nonsugar groups, e.g. methyl esters [**IX**] and ethers [**X**], acetyl esters [**XI**] and feruloyl esters [**XII**]. These groups increase the hydrophobicity, and may alter the charge and susceptibility to enzymic digestion of the polysaccharides. Ester groups are rapidly removed by cold, dilute alkali.

Primary walls contain several structural proteins (**Table 1**), many of which are glycoproteins. Best studied are the extensins, a group of rod-shaped, strongly basic,



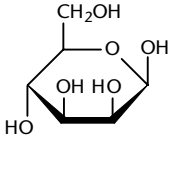
[**V**]

β-D-Glucose



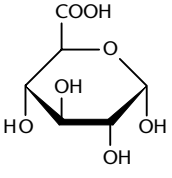
[**VI**]

β-D-Xylose



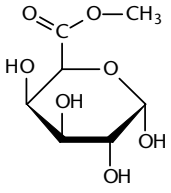
[**VII**]

β-D-Mannose

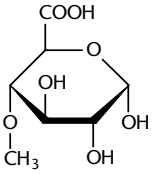


[**VIII**]

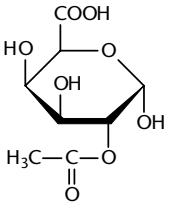
α-D-Glucuronic acid



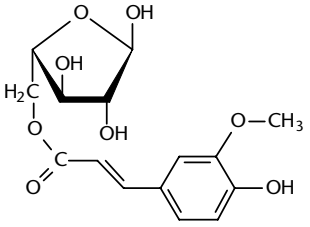
[IX]
Methyl α-D-galacturonate



[X]
4-*O*-Methyl-α-D-glucuronic acid

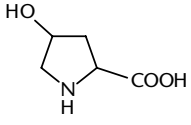


[XI]
2-*O*-Acetyl-α-D galacturonic acid



[XII]
5-*O*-Feruloyl-α-L-arabinose

tightly crosslinked glycoproteins that may serve a defensive role, minimizing wall digestion. Extensins are exceptionally rich in the unusual 'amino' acid, hydroxyproline [**XIII**]. Certain specialized walls are reinforced by additional components that are neither polysaccharide nor protein in nature. These include lignin, cutin, suberin and silica.



[XIII]
L-Hydroxyproline

Chemical differences between different cell walls

There is taxonomic variation in primary wall polysaccharide composition. Grasses (includes cereals) seem to be unique in containing mixed-linkage glucan (MLG); they also contain more xylans (and the xylans carry more ferulate groups) and less pectins and xyloglucans than do dicots and nongraminaceous monocots. Grass xyloglucans contain very little fucose; those of the Solanaceae (potato, tomato, tobacco, etc.) are rich in arabinose. Duckweeds are unusual in having much apiogalacturonan.

Secondary walls of xylem have also been studied in detail. These contain large amounts of cellulose, xylans and mannans, but little or no pectin or xyloglucan.

Macromolecular Organization of Microfibrils

The cell wall is biphasic: microfibrils (the wall's scaffolding) are embedded in and interconnected by, yet also held apart by, the wall matrix. Microfibrils are composed of semicrystalline cellulose and contain little water. Individual cellulose molecules within a microfibril lie in parallel (i.e. all the molecules have their reducing termini pointing towards the same end), attached to each other through numerous hydrogen bonds. At any point along its length a microfibril may contain about 30–50 cellulose molecules. Microfibrils are of indeterminate length – well over 100 μm long, much longer than a cellulose molecule (typical length 1–5 μm). Therefore, there are frequent cellulose chain termini along a microfibril.

Macromolecular Organization of the Wall Matrix

The matrix is less precisely ordered structurally than the microfibrils. It contains essentially all the wall's water, and is sometimes described as 'amorphous'. However, this is an oversimplification and some of the pectins and hemicelluloses of the matrix do adopt preferred orientations.

Most polysaccharides and glycoproteins of the matrix are (after purification) water-soluble, but (while in the wall) firmly bound and therefore water-inextractable. This implies that in the wall the polymer molecules are crosslinked. There is considerable interest in understanding the nature of these crosslinks because they determine the physical properties of the wall. Both covalent and noncovalent crosslinks appear to participate.

Noncovalent crosslinks

Hydrogen bonds

Hydrogen bonds form between suitably orientated groups that undergo fractional ionization, e.g. a hydroxy group and an ether-bonded oxygen.

$$R—O^{\delta-}—H^{\delta+}\cdots{}^{\delta-}O<$$

In vitro, most hemicelluloses (including xyloglucans, MLGs and low-arabinose xylans) readily hydrogen bond to cellulose. An individual hydrogen bond is weak and transient, but the numerous hydrogen bonds between two polysaccharide molecules can add up to a firm linkage. It is

thought that *in vivo* xyloglucan is hydrogen-bonded to microfibril surfaces. Since xyloglucan chains are long (typically 300 nm) compared with the spacing between microfibrils (typically 30 nm), a xyloglucan chain may tether neighbouring microfibrils. This would give xyloglucan a key position in wall architecture.

Full ionic bonds

Full ionic bonds between amino and carboxy groups also exist in the wall matrix.

$$R{-}NH_3^+ \cdots {}^-OOC{-}R'$$

For example, the lysine residues of extensins are attracted to the galacturonate residues of pectins. In addition, Ca^{2+} forms crosslinks between pairs of negatively charged homogalacturonans.

Covalent crosslinks

Phenolic coupling products

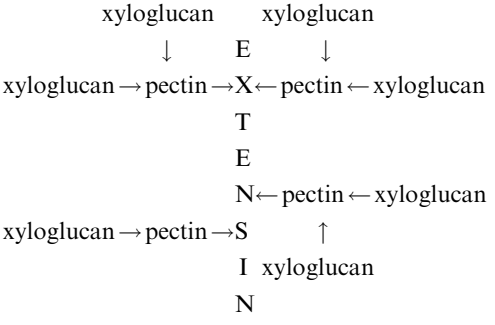
Phenolic groups in the cell wall include tyrosine residues of proteins and feruloyl esters [**XII**] of some polysaccharides. These phenolics may be oxidatively coupled (dimerized) *in vivo*: tyrosine forms isodityrosine [**XIV**], which can itself dimerize to di-isodityrosine; ferulate forms several diferulates e.g. compound [**XV**]. Such coupling may covalently crosslink wall polymers.

Ester and amide crosslinks

Other postulated covalent crosslinks include *O*-galacturonoyl–sugar esters and N^{ε}-galacturonoyl–lysine amides, by which pectins may be bonded to other polysaccharides and to proteins, respectively. Certain sugar residues, especially apiose, form relatively stable borate diesters: pairs of rhamnogalacturonan-II molecules may thus be crosslinked through apiose–borate–apiose diester bridges. This function may explain why boron is an essential element in higher plants.

Glycosidic bonds

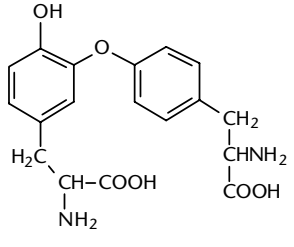
A polysaccharide can, in principle, form a glycosidic bond through its reducing terminus to a nonreducing sugar residue of another polymer. In this way, formerly separate polymer molecules can theoretically join into a linear or tree-like structure. Since no polysaccharide molecule has more than one reducing terminus, true crosslinks (making a net-like structure) through glycosidic bonds are chemically impossible. In 1973, Albersheim and colleagues proposed an influential model in which xyloglucans, pectins and extensin were glycosidically linked (as twigs, branches and trunk, respectively) into a tree-like structure (arrows = glycosidic bonds).



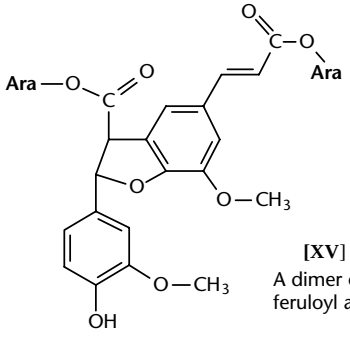
The molecular fragments that supported this model have not been reproducibly detected in wall digests; the model has therefore lost currency. Nevertheless, the possibility that some xyloglucans are glycosidically linked to pectins has not been discounted. In addition, it appears likely that the various types of pectin are glycosidically linked to each other, perhaps in a linear arrangement e.g. …homogalacturonan→rhamnogalacturonan-I→homogalacturonan→rhamnogalacturonan-II… Such an arrangement would explain why endo-polygalacturonase, which hydrolyses homogalacturonan, solubilizes wall-bound rhamnogalacturonans.

Acid Growth Theory

Treatment of excised stem and coleoptile segments with growth-promoting doses of the hormone auxin often results in rapid acidification of the wall. Treatment of tissues with mildly acidic buffers very rapidly promotes



[**XIV**]
L,L-Isodityrosine



[**XV**]
A dimer of feruloyl arabinose

wall loosening even in the absence of auxin (= 'acid growth'), whereas treatment of tissues with neutral buffers tends to suppress wall loosening in the presence of auxin. Therefore, wall acidification has been proposed to mediate auxin-induced wall loosening. The low pH optimum of some wall-loosening proteins, especially expansins (see below), may provide an explanation for the mechanism of acid growth.

Some authors have suggested that auxin-induced acidification is too little and too late to account for auxin-induced growth. However, it is impossible to monitor the precise pH changes that occur within the semisealed compartment (between plasma membrane and cuticle of the epidermis) in which wall loosening is most relevant to the regulation of stem elongation. The acid growth theory therefore has considerable support, at least for the early stages of auxin action.

Mechanism and Regulation of Cell Wall Expansion

The irreversible expansion of the primary wall requires neighbouring microfibrils to move apart from one another. Proposed wall-loosening agents that could enable this movement include hydrolases, transglycosylases, expansins and •OH radicals. Cell expansion is clearly of fundamental significance in plant physiology, and it should not be surprising if there are diverse mechanisms, offering multiple control points.

Since it is thought that microfibrils are tethered by hemicellulose chains, much interest has centred on enzymes that cleave hemicelluloses.

Endo-hydrolases

One such enzyme is cellulase (= endo-β-(1→4)-D-glucanase), an endo-hydrolase that cleaves the backbones of xyloglucan and MLG but has little effect on microfibrillar cellulose. The amount of cellulase in walls often increases in response to auxin, especially at relatively high auxin concentrations that cause cellular swelling rather than elongation. Recent studies have focused on membrane-bound cellulases, which may serve a special role in wall loosening. By digesting xyloglucan tethers, cellulase may loosen the wall and facilitate cell expansion.

Compatible with this view, auxin-induced growth is often accompanied by xyloglucan turnover in dicots and by MLG turnover in grasses. Furthermore, auxin-induced growth can be partially blocked by anti-xyloglucan antibodies in dicots and by anti-MLG antibodies in grasses – these antibodies may act by binding to and protecting the relevant polysaccharides from enzymic hydrolysis.

Exo-hydrolases

Primary walls also contain numerous exo-hydrolases, e.g. β-D-glucosidases, β-D-galactosidases and α-D-galacturonidases. These enzymes remove monosaccharides one at a time from nonreducing termini, so the effect on a large polysaccharide chain may be slight. The biological role of these exo-hydrolases is thus unclear. Inhibition of β-D-glucosidase and β-D-galactosidase by added aldonolactones does not block the growth-promoting action of auxin.

Xyloglucan endotransglycosylase

Recently, attention has focused on xyloglucan endotransglycosylase (XET) as a potential wall-loosening activity. The enzyme cleaves one xyloglucan chain, forming a xyloglucan–enzyme complex, which can persist for some minutes or hours before the xyloglucan moiety is reattached to the nonreducing terminus of a different xyloglucan chain, completing a transglycosylation reaction (**Figure 2**).

By the action of XET, a xyloglucan tether may be cleaved, allowing incremental wall expansion and thus molecular realignment, followed by re-formation of a tether involving a different xyloglucan chain. Since the substrates and products of the transglycosylation reaction do not differ chemically, it is difficult to demonstrate its occurrence *in vivo*; however, by use of ^{13}C(density)/^{3}H(radioactivity) dual-labelling followed by isopycnic centrifugation of the labelled xyloglucans, interpolymeric transglycosylation has been demonstrated in the walls of cultured rose cells.

XET activity often correlates with the rate of cell expansion, suggesting a role of XET in wall loosening.

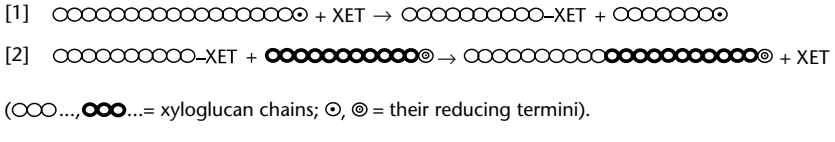


Figure 2 Mechanism of wall loosening caused by the activity of xyloglucan endotransglycosylase (XET).

The *Arabidopsis* genome encodes at least 12 XET-like proteins; unsurprisingly, it has not so far proved possible to generate an XET-less plant in which to test the role of XET. Nevertheless, treatment of pea stem segments with xyloglucan oligosaccharides, which would interfere with tether re-formation, can promote wall loosening, supporting a role for XET in cell expansion.

Expansins

Simultaneously, another novel group of proteins called expansins have been shown to act as wall-loosening agents. The numerous isoforms of expansin fall into two major categories: α-expansins are hydrophobic and firmly wall-bound; β-expansins are hydrophilic glycoproteins, readily extractable in water. Without causing detectable hydrolysis or transglycosylation, exogenous expansin somehow loosens walls whose own proteins have been inactivated, e.g. by boiling. Expansin acts optimally at low pH, and may mediate 'acid growth'. It appears likely that expansin catalyses the cleavage of hydrogen bonds between wall polysaccharide molecules, e.g:

$$\text{cellulose} \cdots \text{hemicellulose} \underset{\rightarrow}{\longleftarrow} \text{cellulose} + \text{hemicellulose}$$

(where $\cdots$ = hydrogen bond). Catalysts cannot alter the equilibrium position, but they increase the rate at which equilibrium is reached. Since the equilibrium in the above 'reaction' may lie far to the left, it is understandable that the effect of expansin can be seen only when some external force (e.g. stretching by turgor or in an extensiometer) moves the two separated molecules out of reach of each other, preventing them rebonding.

Nonenzymatic scission of wall polysaccharides

In addition to protein-mediated wall loosening, nonenzymatic scission of wall polysaccharides may occur. Walls of living cells often contain H_2O_2, and certain wall polymers carry tightly bound copper ions, some of which are probably reduced to Cu^+ by the action of ascorbate or superoxide. H_2O_2 and Cu^+ undergo a Fenton reaction producing the hydroxyl radical (•OH).

$$Cu^+ + H_2O_2 \rightarrow Cu^{2+} + OH^- + \bullet OH$$

•OH is an exceedingly reactive oxidant and will react, within ~ 1 nm of its site of production (i.e. near a wall-bound copper ion), with essentially any organic molecule it hits. *In vitro*, •OH cleaves xyloglucan chains. Current research aims to determine whether •OH acts on polysaccharides *in vivo* and is thus an additional wall-loosening mechanism.

Wall tightening

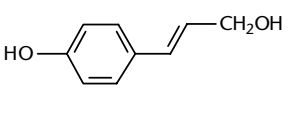
When cells mature, growth ceases, although turgor pressure usually remains high and wall-loosening agents may remain present. It thus appears that the cell can tighten its wall, i.e. render it resistant to further plastic expansion. Tightening presumably involves the formation of more crosslinks, probably including phenolic coupling products, e.g. isodityrosine, diferulate and lignin, by the action of peroxidase. As predicted by this proposal, there are many examples of negative correlations between growth rate and peroxidase activity. Another possible mechanism of wall tightening is the action of pectin methylesterase (PME), which converts neutral, methyl esterified regions of homogalacturonan into polyanionic domains strongly crosslinked by Ca^{2+} bridges.

Lignification

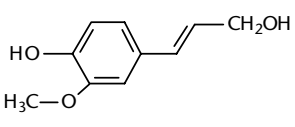
In sclerenchyma cells and xylem vessels, lignin is found initially in the middle lamella and later in both the primary and secondary walls. It often accounts for about 20–35% of the dry weight of wood. Small amounts of lignin have recently been found also in growing tissues (**Figure 3**).

Lignin is an aromatic polymer formed by the oxidative polymerization of one to three monolignols: coniferyl, sinapyl and *p*-coumaryl alcohols [**XVI**–**XVIII**]. Grass lignins contain all three monolignols, dicot lignins the first two, and conifer lignins predominantly the first. The linkages between the monolignols are diverse and the polymerization appears to be largely stochastic rather than in any defined sequence.

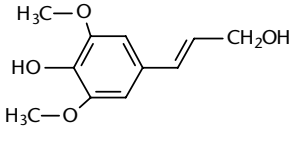
Lignin partially replaces the wall's H_2O. Lignin is highly resistant to enzymic digestion, it waterproofs the side-walls of conducting vessels, and by enabling stress transfer between neighbouring microfibrils it confers on walls



[**XVI**]
p-Coumaryl alcohol



[**XVII**]
Coniferyl alcohol



[**XVIII**]
Sinapyl alcohol

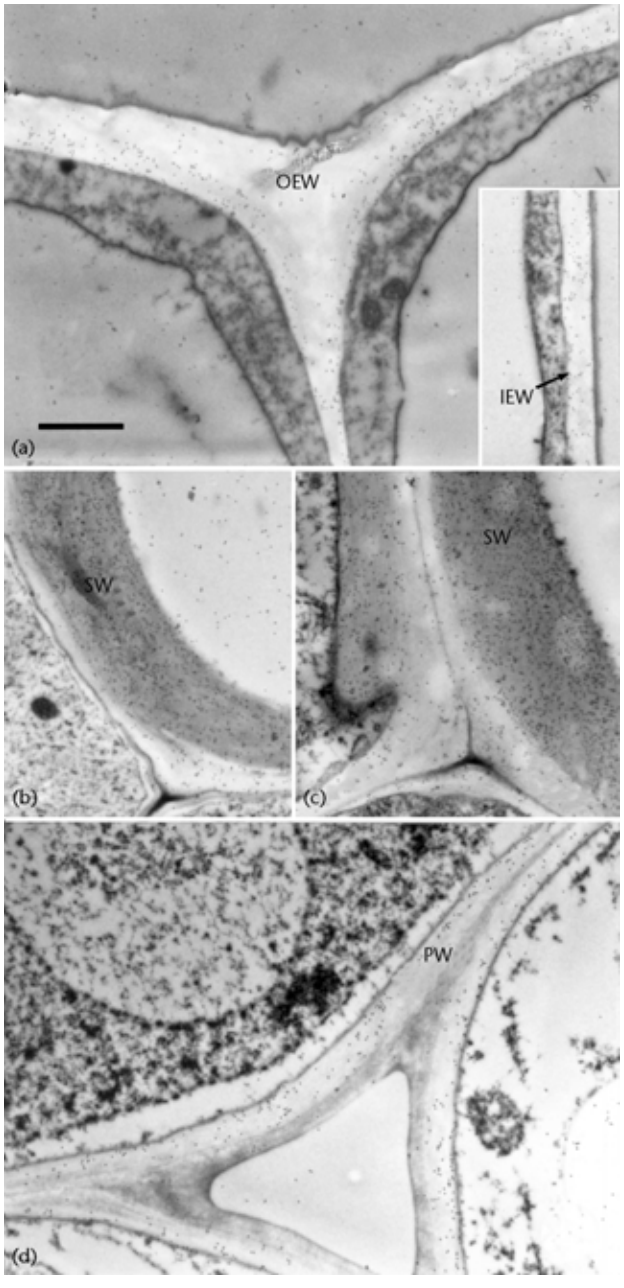


Figure 3 Immunogold labelling of lignin in primary and secondary cell walls. The electron micrographs show cross-sections through various tissues in a maize coleoptile after application of antibodies that recognize lignin. The small dense spots are 10-nm gold particles indicating the location of lignin. (a) Outer epidermis (OEW) and (inset) inner epidermis (IEW); (b, c) xylem cells showing secondary wall layers (SW); (d) parenchyma cells with only primary walls (PW). Although the presence of lignin in xylem walls is well known, its presence in small amounts in the primary walls of expanding cells is a recent discovery. Bar in (a), 1 μm. From Müsel *et al.* (1997) *Planta* **201**: 146–159.

physical resistance to buckling (e.g. under the weight of a tree).

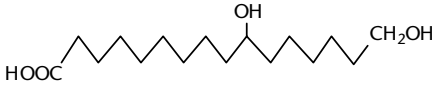
Cuticular Surface Layers

Architecture

The outer epidermal surface of a leaf or stem, whether or not it is growing, generally includes the sequence of layers shown in **Figure 4**. The inner portion of the cuticle (= the cuticular layer of the cell wall) contains cutin and wall polysaccharides. The cuticle proper lacks polysaccharides but contains cutin and soluble cuticular lipids; the latter appear to play the major role in restricting the loss of water vapour from a leaf. The cuticle is covered with low-molecular-weight waxes, which govern the wettability of the leaf surface by mists and sprays.

Cutin and cutan

Cutin contains a high-molecular-weight aliphatic polyester formed by condensation of C_{16} and C_{18} hydroxy-fatty acids e.g. 10,16-dihydroxyhexadecanoate [**XIX**]. The polyester can be degraded by enzymic or alkaline hydrolysis of the ester bonds. Cutin also contains oxidatively polymerized phenolics; it is unclear whether these are covalently linked to the aliphatic polymers. It is also unclear whether existing cutin is restructured *in vivo* to accommodate leaf growth.



[**XIX**]

10,16-Dihydroxy-hexadecanoic acid

Another polymer, 'cutan' has recently been proposed to accompany or even replace cutin in some plants. Cutan

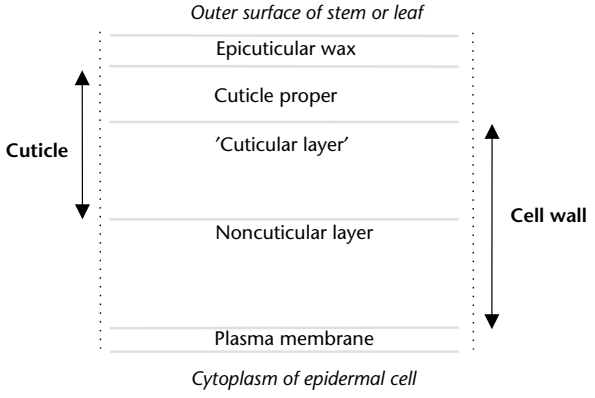


Figure 4 Sequence of layers in the outer epidermal surface of a leaf or stem.

appears to be a polythene-like aliphatic polymer which resists alkaline hydrolysis.

Suberized Cell Walls

Suberin contains a polyester somewhat similar to that of cutin but composed of a different range of fatty acids (especially C_{16} to C_{24} ω-hydroxy-fatty acids **[XX]**, α,ω-dicarboxylates, and simple C_{20} to C_{30} fatty acids) and C_{20} to C_{30} fatty alcohols. Suberin also includes an aromatic polymer, containing *N*-feruloyl-tyramine. Suberin occurs in cork, seed-coats, the Casparian strip of the endodermal cell wall, and at leaf scars after abscission. It can also be induced by wounding (see below), and is thought to confer waterproofness and resistance to microbes.

[**XX**]

An ω-hydroxy-fatty acid

Changes in Response to Environmental Stresses and Pathogens

Most wall components are constitutive; however, others are induced upon wounding. The enhanced synthesis, deposition and/or crosslinking of indigestible wall components in response to wounding or injury may provide effective defence mechanisms.

Injury

Suberin is often induced in newly exposed cells as a wound response, e.g. when a potato tuber is cut open. An extremely rapid response to wounding is the induction of callose (β-(1→3)-D-glucan). Touch stimulation of plants, perhaps mimicking the action of wind, can induce XET expression, and the resulting molecular rearrangement of xyloglucans may confer mechanical strength.

Infection

Infection can also induce the deposition of nonconstitutive wall components. For example, lignin is deposited in wheat leaves challenged by the pathogenic fungus *Puccinia graminis*, and extensin is induced in melons upon attempted infection by *Colletotrichum lindemuthianum*. In addition, structural proteins of the wall can be rapidly crosslinked through di-isodityrosine bridges when cells are exposed to fungal elicitors.

Further Reading

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