GROWTH OF THE PLANT CELL WALL

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Abstract | Plant cells encase themselves within a complex polysaccharide wall, which constitutes the raw material that is used to manufacture textiles, paper, lumber, films, thickeners and other products. The plant cell wall is also the primary source of cellulose, the most abundant and useful biopolymer on the Earth. The cell wall not only strengthens the plant body, but also has key roles in plant growth, cell differentiation, intercellular communication, water movement and defence. Recent discoveries have uncovered how plant cells synthesize wall polysaccharides, assemble them into a strong fibrous network and regulate wall expansion during cell growth.

PROTOPLASM
The contents of living cells, including cytoplasm and nucleus.

CREEP
Slow, time-dependent, irreversible extension, in which the microfibrils and associated matrix polysaccharides slowly slide within the wall, therefore increasing its surface area.

XYLEM
A tissue that comprises a group of specialized cells that are involved in the transport of water and solutes in vascular plants. Mature xylem vessels essentially contain only the cell wall.

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A tree's leaves may be ever so good,
So may its bark, so may its wood

Leaves Compared with Flowers, Robert Frost

Something there is that doesn't love a wall

Mending Wall, Robert Frost

Without cell walls, plants would be pliant piles of PROTOPLASM, more like slime moulds than the stately trees and other greenery that grace our planet. Plants comprise of ~35 cell types, each of which is distinctive in its size, shape, position and wall characteristics (FIG. 1a,b). In growing cells, the wall is typically a thin, flexible layer (0.1–1 μm) that consists primarily of complex polysaccharides and a small amount of structural proteins. The veil-like thinness of the wall can be visualized by light microscopy, whereas electron microscopy reveals its fibrous character at the nanometer scale (FIG. 1c,d). Despite its thinness, the wall forms a strong network that functions like a corset, compressing and giving shape to the protoplast within.

Plant cells grow by expanding their cell walls through a process of controlled polymer CREEP. Because the cells are tightly glued together through their cell walls, cell migration is not possible and plant morphogenesis is mostly a matter of localized cell division and selective cell enlargement. Such enlargement can be impressive: some of the largest plant cells are XYLEM vessels (FIG. 1b), which can increase in volume >30,000-fold from their MERISTEMATIC initials. Similarly, hair cells on the surface of young cotton seeds elongate 1000-fold before maturity. Such growth is accomplished through enlargement of the cell volume owing to water uptake into the VACUOLE and irreversible extension of the pre-existing cell wall. Simultaneously, new polymers are integrated into the wall to prevent it becoming thinner and weaker.

In this article, I focus on recent progress in understanding how the plant cell wall grows, both in terms of the synthesis and integration of new polysaccharides into the wall and the expansion of the polysaccharide network. For many years, the enzymes that synthesize the wall polysaccharides remained elusive. However, many of the genes responsible have been recently identified, and we are now beginning to elucidate the molecular machinery that assembles sugars into the complex polymers that comprise the cell wall. Similarly, for many years the existence of 'WALL LOOSENING' enzymes was proposed, without solid evidence concerning their nature. Now, strong contenders for this process have emerged and provide novel ideas about the biochemical underpinnings of wall expansion. In this review, I discuss only the PRIMARY PLANT CELL WALL and not the SECONDARY CELL WALL, which is deposited after cells cease enlargement and often has a distinctive

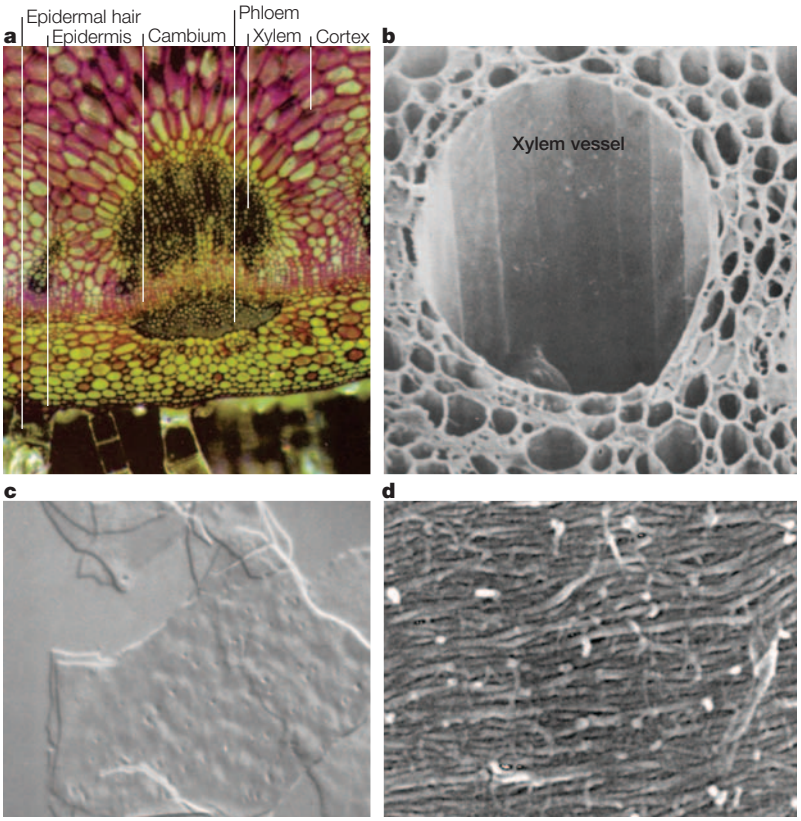


Figure 1 | **Photographic depictions of plant cell walls. a** | A cross-section of the stem of *Zinnia elegans* illustrates the diverse cell sizes, shapes and cell-wall types. Note the tiny meristematic cells in the cambium, versus the huge cells that comprise the epidermal hairs and the cortex. **b** | A scanning electron micrograph of a maize root cross-section[129] illustrates the impressive ability of cells to enlarge. Note the huge xylem vessel. All of the cells in this cross-section originated from tiny meristematic initials. **c** | Micrograph of onion epidermal cell wall[130]. This image, which was obtained by differential interference microscopy of wall fragments, reveals the thinness and delicacy of the primary cell wall. **d** | A scanning electron micrograph of the growing cell wall from cucumber hypocotyls[50]. Note the ordered fibrillar texture, which is the result of the ordered arrangement of cellulose microfibrils, which are coated with a layer of matrix polysaccharide. Part **a** of the figure is reproduced, with permission, from REF.128 © The American Society of Plant Biologists (1995). Part **b** of the figure is reproduced, with permission, from REF.129 © Department of Scientific and Industrial Research, New Zealand (1972). Part **c** of the figure is reproduced, with permission, from REF.130 © The Company of Biologists (1990). Part **d** of the figure is reproduced, with permission, from REF.50 © Blackwell Publishing (2005).

MERISTEM
Region of rapid cell division on a plant; it is where cell initials (or 'stem' cells) are maintained and organogenesis starts. Root meristems, shoot meristems and flower meristems fit this description.

VACUOLE
A membrane-bound cellular compartment, usually filled with a dilute watery solution. Mature plant cells often have very large central vacuoles.

WALL LOOSENING
Modification of the cell wall that enables it to extend in response to the wall stress that is generated by cell turgor.

composition and organization. Not all cells have secondary cell walls, which are made by cells that require great mechanical strength and structural reinforcement.

Building the primary cell wall

The growing cell wall has a fibreglass-like structure[1,2] (FIGS 1d,2), with crystalline CELLULOSE MICROFIBRILS that are embedded in a matrix of complex polysaccharides, which are divided into two classes (BOX 1). PECTINS are wall polysaccharides that are solubilized by aqueous buffers and dilute acidic solutions or calcium chelators. HEMICELLULOSES, on the other hand, require strong alkali for solubilization. Hemicelluloses are cellulose-binding polysaccharides, which together with cellulose form a network that is strong yet resilient. Pectins are perhaps the most complex polysaccharides in the living world and have several functions[3,4]. They form hydrated gels that push microfibrils apart, easing their sideways slippage during cell growth, while also locking them in place when growth ceases. They are important determinants of wall POROSITY and wall thickness and they glue cells together in an adhesive layer called the MIDDLE LAMELLA[5]. Pectins are primary targets of attack by invading microbes and their breakdown products function as potent elicitors of plant-defence responses.

Cellulose and MATRIX POLYSACCHARIDES are made by distinctive pathways (FIG 2; see below). Cellulose is synthesized by large membrane complexes[6–8] which extrude a microfibril from the cell surface, similar to a spider's thread. By contrast, matrix polysaccharides are synthesized in the Golgi apparatus and packaged into tiny vesicles that fuse with the plasma membrane and thereby deliver their cargo to the wall. Matrix polysaccharides then become integrated into the wall network by physical interactions, enzymatic ligations and crosslinking reactions. Unlike cellulose microfibrils, newly secreted matrix polysaccharides can diffuse some distance into the cell wall[9], aided by cell TURGOR PRESSURE, which stretches the cell wall, increases its porosity and provides an energy gradient to drive polymers into the wall[10].

Cellulose synthesis. Plant cellulose synthase (*CESA*) genes were identified in the late 1990s through molecular and genetic studies[11,12]. In the model plant *Arabidopsis thaliana*, the *CESA* family contains ten genes, which are expressed in different tissues and cell types. Growing evidence from genetic experiments and gene-expression analyses indicates that three different *CESA* genes are normally required to make a functional cellulose-synthesizing complex[13–16] and that different sets of genes are involved in the formation of the primary and secondary wall. For example, *CESA1*, *CESA3* and *CESA6* are required for biosynthesis of the primary wall[12,17], whereas *CESA4*, *CESA7* and *CESA8* are required to form secondary walls[18].

CESA proteins are embedded in the plasma membrane in hexameric arrays called particle rosettes[6] (FIG. 3a). The smallest subunits that are visible by electron microscopy are thought to consist of six CESA proteins that are encoded by three genes (FIG. 3b,c). The assembly of CESA subunits into hexamers is not well understood, but is thought to require CESA dimerization, which is mediated by two zinc fingers in the N-terminal region of the CESA proteins[19]. Membrane complexes probably contain other proteins that aid microfibril formation and that link the complexes to nearby microtubules for guidance along the membrane[20].

Each cellulose microfibril is formed from the spontaneous 'bundling' and crystallization of dozens of (1,4)-linked β-D-glucan chains, each made by a CESA protein (FIG. 3c). Microfibrils are 3–5 nm wide and many micrometers in length — long enough to wind around the circumference of a cell many times. It is possible that hemicelluloses, such as xyloglucan, become trapped in the microfibril as it forms, resulting in disordered regions[21].

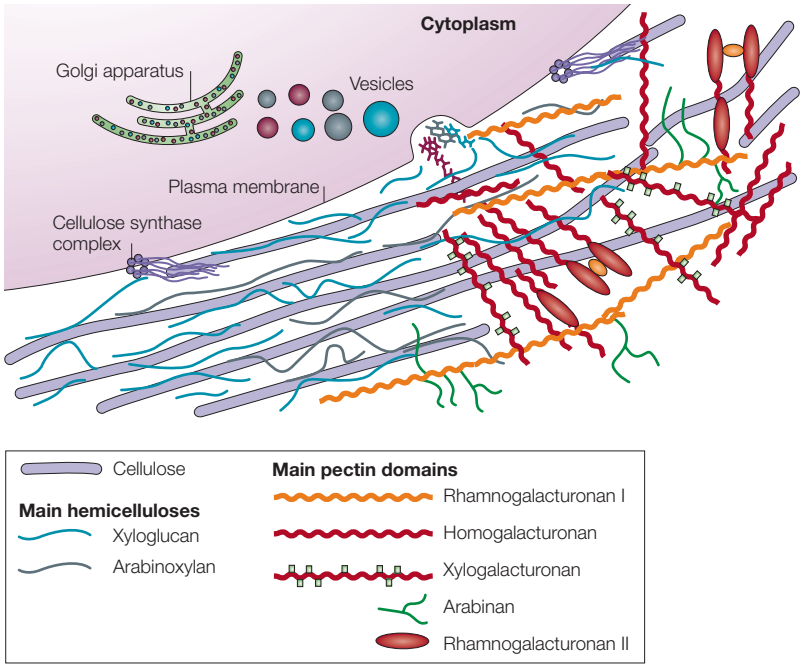


Figure 2 | **Structure of the primary cell wall.** Cellulose microfibrils (purple rods) are synthesized by large hexameric complexes in the plasma membrane, whereas hemicelluloses and pectins, which compose the matrix polysaccharides, are synthesized in the Golgi apparatus and are deposited to the wall surface by vesicles. For clarity, the hemicellulose–cellulose network is shown on the left part of the cell wall without pectins, which are emphasized on the right part of the figure. In most plant species the main hemicellulose is xyloglucan (blue), while hemicelluloses such as arabinoxylans (grey) and mannans (not shown) are found in lesser amounts. The main pectin polysaccharides include rhamnogalacturonan I and homogalacturonan, with smaller amounts of xylogalacturonan, arabinan, arabinogalactan I (not shown) and rhamnogalacturonan II. Pectin domains are believed to be covalently linked together[4] and to bind to xyloglucan by covalent and non-covalent bonds[127,131]. Neutral pectin polysaccharides (green) are also able to bind to cellulose surfaces[121]. See BOX 1 for further information on the structure of these polysaccharides.

If CESA enzymes add glucose residues to an existing glucan, how does the glucan chain initiate? Recent studies indicate that a STEROL GLUCOSIDE might function as the initial acceptor for chain elongation. Sterols are common lipid components of plant cell membranes and sterol-β-glucosides are commonly synthesized in plant plasma membranes, where CESA can use a sterol-glucoside and uridine 5′-diphosphate-glucose to form short sterol-linked glucans[22]. This idea is further supported by studies showing that defects in sterol biosynthesis result in cell-wall gaps and reductions in cellulose content[23].

A membrane-bound endoglucanase (named KORRIGAN, or KOR) is also required for normal cellulose formation[24,25], but its exact function is unclear. *kor* mutants have defects in cytokinesis and cell elongation. Although they make (1,4)-linked β-D-glucan, it is not properly crystallized into a microfibril. It has been postulated that the KOR endoglucanase trims sterol residues from nascent glucan primers or trims out-of-register glucans to aid crystallization of the microfibril[22]. However, the protein is not specifically associated with CESA complexes[26,27] and *kor* mutants have normal amounts of sterol glucoside[28], so its action on microfibril formation might be indirect.

PRIMARY CELL WALL
The flexible extracellular matrix that is deposited while the cell is expanding.

SECONDARY CELL WALL
The flexible extracellular matrix that is deposited while the cell is still expanding is known as the primary cell wall. When expansion ceases, a secondary wall is sometimes laid down inside the primary wall, making it stronger.

CELLULOSE MICROFIBRIL
A tough, inelastic fibre wrapped in layers (lamellae) within the plant cell wall. Composed of (1,4)-linked β-D-glucosyl residues.

Two recent studies[29,30] analysed patterns of gene co-expression to identify a number of genes that are associated with cellulose synthesis of secondary walls. Many of these genes were not identified in previous genetic screens for defective secondary walls, yet analysis of lines bearing mutations in these genes revealed clear cell-wall defects. Further study of these genes should broaden our understanding of the mechanisms that are involved in cellulose synthesis.

Synthesis of matrix polysaccharides. In contrast to cellulose, matrix polysaccharides possess a more diverse set of glycosidic linkages and sugar residues (BOX 1). Identification of the specific genes that encode the relevant glycosyltransferases has proved difficult, but almost 20 of these genes have recently been characterized[16].

The *CESA* family belongs to a larger superfamily of genes called *CELLULOSE SYNTHASE-LIKE* (*CSL*; see Online links box), which includes eight other gene families[31], named *CSLA*, *CSLB* and so on, up to *CSLH*. CSL proteins contain sequence motifs that are characteristic of β-glycosyltransferases, but lack the N-terminal region containing the zinc-finger domains — which is found in CESA — that function in protein dimerization. Because of their similarity to, and divergence from, CESA, CSL proteins are considered good candidates for the synthases that are localized in the Golgi and that form the β-D-GLYCAN backbone of hemicelluloses such as xyloglucan, xylan, mannan and other β-D-glycans in the cell wall.

Recent studies indicate that *CSLA* genes encode β-mannan synthases, the enzymes that are required for the formation of the mannan backbone of certain hemicelluloses. Dhugga *et al.*[32] showed that galactomannan of guar gum is synthesized by a *CSLA* gene product. Galactomannan accumulates as a storage polysaccharide in guar-bean seeds and is used commercially as a food thickener. It has a β-mannan backbone with galactose sidechains. Using a different approach, Liepman *et al.*[33] employed heterologous expression of *A. thaliana CSLA* genes in insect cells to show that these genes encode mannan synthases, and they proposed that CSLA proteins synthesize the mannans and glucomannans of the growing cell wall. Heterologous expression of other *CSL* family members in insect cells (which do not have a cell wall) could help to identify the function of these unique plant proteins.

In addition to the synthases that assemble the backbones of the various matrix polysaccharides, other glycosyltransferases are required to add branches to these glycans. Genes that encode some of these enzymes were recently identified (for a review, see REF. 16). These important advances pave the way for rapid progress in defining the enzymes that synthesize wall polysaccharides.

Forming a network. After the matrix polysaccharides are secreted into the wall, they become associated with newly synthesized cellulose microfibrils, as well

Box 1 | Structure of the main polysaccharides that are present in the growing cell wall

Cellulose. **The primary structure of cellulose is an unbranched (1,4)-linked β-D-glucan. Many parallel glucans snap into register to form a crystalline microfibril that is mechanically strong and highly resistant to enzymatic attack — an almost ideal scaffold material. These long, crystalline ribbons are 3–5 nm wide and, in growing cells, are aligned with each other, giving a structural bias to the cell wall** (FIG. 1d).

Hemicelluloses. **The backbone of hemicelluloses resembles that of cellulose. Hemicelluloses bind to cellulose, but branches and other modifications in their structure prevent them from forming microfibrils by themselves. Xyloglucan and arabinoxylan are two of the most abundant hemicelluloses. Details of their structure vary slightly among plant species. Xyloglucan has a backbone that is similar to that of cellulose, but it is decorated with xylose branches on 3 out of 4 glucose residues. The xylose can also be serially appended with galactose (Gal) and fucose (Fuc) residues. Arabinoxylan consists of a (1,4)-linked β-D-xylan backbone decorated with arabinose branches. Other residues, such as glucuronic acid and ferulic acid esters (FAE), are also attached in arabinoxylans that are particularly abundant in cereal grasses. Mannans are also found in primary cell walls and probably function in the same way as xyloglucan and arabinoxylan.**

Pectins. **This complex and heterogeneous group of polysaccharides consists of distinctive domains, which are believed to be covalently linked together[3,4,119]. Rhamnogalacturan I consists of alternating residues of galacturonic acid and rhamnose, and probably has side branches that contain other pectin domains[4]. Homogalacturonan comprises a linear chain of galacturonic acid residues, whereas xylogalacturonan is modified by the addition of xylose branches. The carboxyl groups of homogalacturonan and xylogalacturonan are often methyl esterified, a modification that 'blocks' the acidic group and reduces their ability to form gels. Rhamnogalacturonan II is a complex pectin domain that contains 11 different sugar residues (for details see** REFS 1,2**) and forms dimers through borate (B) esters. The neutral arabinans and arabinogalactans are also linked to the acidic pectins and it has been proposed that they promote wall flexibility[120] and that they bind to the surface of cellulose[121].**

Pectins and hemicelluloses are modified in many ways. See reviews[1–4,119] for details. The structures of the molecules described above are shown in Supplementary information S1 (figure).

PECTINS
Group of complex polysaccharides that are extracted from the cell wall by hot water, dilute acid or calcium chelators. They include homogalacturonan, rhamnogalacturonans I and II, galactans, arabinans and other polysaccharides.

HEMICELLULOSES
Group of complex polysaccharides, including xyloglucans, xylans and mannans, that are extracted from plant cell walls by use of strong alkali; characteristically they bind tightly to the surface of cellulose and have a backbone made up of (1,4)-β-D-glycans that resembles cellulose.

POROSITY
Property that indicates how readily gases, liquids and other materials can penetrate an object.

MIDDLE LAMELLA
The thin layer that connects two plant cells and is rich in pectin.

MATRIX POLYSACCHARIDES
Complex polysaccharides found in the space between cellulose microfibrils. They are traditionally divided into pectins and hemicelluloses.

TURGOR PRESSURE
Force generated by water pushing outward on the plasma membrane and plant cell wall, that results in plant rigidity. The loss of turgor pressure causes wilting.

STEROL GLUCOSIDE
A molecule consisting of a sterol that is linked to glucose through a glycosidic bond.

β-D-GLYCAN
A polymer built up of sugar residues connected by glycosidic bonds; β-D- identifies the particular stereochemical configuration of the sugar.

ENDOTRANSGLYCOSYLASES
Enzymes that cut a glycan and ligate one of the fragments to the free end of another polymer, usually of the same type.

as with the pre-existing wall polymers, to form a network that is both strong and extensible. Network formation involves spontaneous physico-chemical interactions between the wall polysaccharides and perhaps enzymatic crosslinking (BOXES 2,3). At this level of polymer–polymer interaction, the precise structure of the cell wall has not yet been resolved in detail and remains a key issue for understanding how the cell wall expands.

Xyloglucan is an abundant hemicellulose in primary cell walls, and is believed to crosslink microfibrils, forming either a direct tether between microfibrils[1,21,34] or an indirect link[35] (BOX 2). Breakdown of xyloglucan is increased after treatment with auxin, which is a potent growth hormone[36,37]. The potential crosslinking role of xyloglucan was supported by experiments showing that isolated walls were induced to creep upon digestion with a fungal endoglucanase that digests xyloglucan[38]. Moreover, expression of a fungal xyloglucanase in poplar gave transformants with longer and thicker stems[39]. A physical model of cell-wall growth, which was based on the thermodynamics of hydrogen-bonded networks, was proposed[40]. This model specifically predicts how the abundance and size of xyloglucan 'tethers' should affect wall enlargement. Experiments that attempted to manipulate xyloglucan size in growing cells[41] have confirmed the predictions of this physical model.

Integration of newly secreted matrix polysaccharides into the existing network might also be mediated by enzymes such as ENDOTRANSGLYCOSYLASES, which cut and ligate glycans together[42–44]. One such enzyme, called xyloglucan endotransglucosylase (XET), specifically cuts the xyloglucan backbone and re-forms a glycosidic bond with the free end of another xyloglucan chain (FIG. 4). XET is a member of a large family of plant enzymes called XTH (see Online links box), for xyloglucan endotransglucosylase/hydrolase, because some members have hydrolase activity. XTHs and the genes that encode them have been characterized in detail[42–46]. DOUBLE-LABELLING EXPERIMENTS[47] demonstrate that XET grafts new xyloglucans onto chains that are already part of the wall network. The wall-strengthening action of XET is supported by experiments in which xyloglucans were added to excised pea stems, which consequently became stiffer[41].

Molecular modelling data indicated that some members of the XTH family might target arabinoxylan and (1,3;1,4)-β-D-glucan[48], hemicelluloses that are notably abundant in grass cell walls. However, this hypothesis has yet to be confirmed experimentally. An endotransglycosylase with specificity for mannans has recently been characterized[49]. Endotransglycosylases for xylans and other matrix polysaccharides presumably exist but have not yet been discovered.

In summary, cellulose microfibrils are linked together by non-covalent interactions with matrix polysaccharides, which determine most of the physical properties of the cell wall. The complexity of the cell-wall network allows for many potential sites where loosening and expansion might be initiated, yet physics makes this a tricky business, as discussed below.

The physical dilemma of wall enlargement

The pliant walls of growing cells are under tremendous tension, equivalent to 100–1000 atmospheres

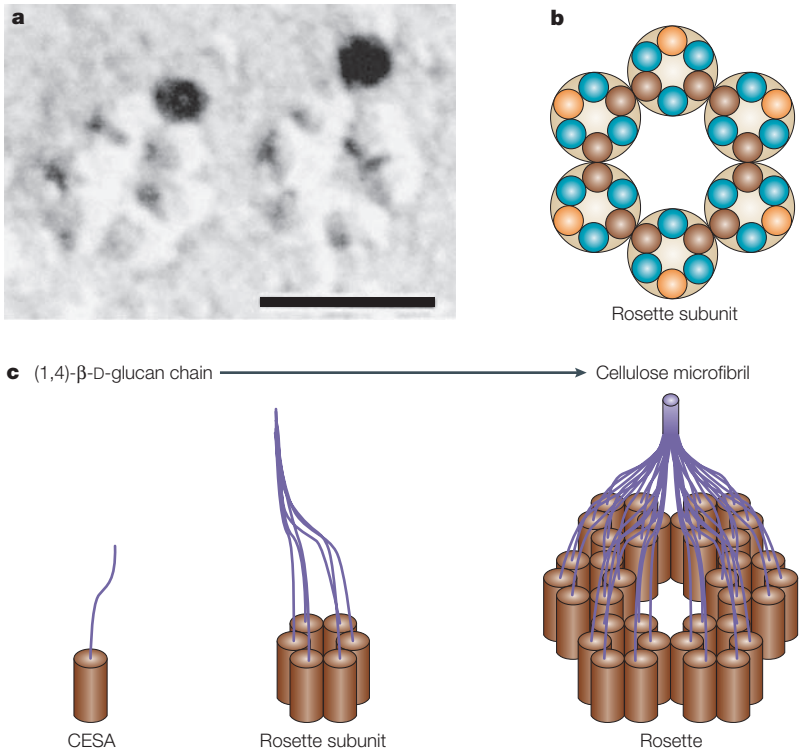


Figure 3 | **The cellulose-synthesizing machinery of the cell. a** | Immunogold labelling shows that CESA is localized to hexameric 'particle rosettes' in the plasma membrane[6]. The black circles represent gold nanoparticles that are attached to antibody against CESA. The smallest subunit in the particle rosette is believed to be made of six CESA proteins. Particle rosettes are sometimes found attached to cellulose microfibrils. Scale bar, 30 nm. **b** | This model of a hexameric particle rosette shows how three different CESA proteins (shown in three different colours: α, orange; β, brown; γ, green) might be organized into rosette subunits and then into a hexameric synthase complex[7]. CESA assembly into rosette subunits is thought to involve oxidatively reversible disulfide bond formation between cysteines in the N-terminal zinc-finger region of CESA[19]. **c** | A model of how CESA complexes synthesize a cellulose microfibril[7]. Each CESA protein can synthesize a single (1,4)-linked β-D-glucan chain. Cellulose is formed as a crystalline ribbon that is composed of many such glucans. In this model, 36 β-D-glucan chains are formed by a particle rosette, which is composed of a hexamer of CESA hexamers. Part **a** of the figure is reproduced, with permission, from REF.6 © American Society of Plant Biologists (1999). Parts **b** and **c** of the figure are modified, with permission, from REF.19 © Oxford University Press (2002).

of tensile stress. Wall stress results from the stretching of wall polymers as they resist cell turgor pressure and provides the mechanical energy that is required to extend the cell wall. But how can this thin material irreversibly extend its surface area without risking a fatal blowout? It does so by a mechanism of carefully controlled polymer creep, in which the matrix yields, allowing the cellulose microfibrils to move apart[50]. Although the mechanical force for this polymer motion comes from wall stress (and therefore turgor pressure), control of the process resides in the selective loosening and shifting of load-bearing linkages between cellulose microfibrils.

In the longer term, wall expansion must be matched by the synthesis and integration of new wall materials to prevent thinning to the point of mechanical instability. However, expansion and synthesis of the wall are separate processes that are only loosely coupled in most cells. Fast growth responses — occuring in a few seconds[51] or even less than a second[52] — are based on rapid changes in wall extensibility without significant alterations in wall composition or structure. By contrast, slower changes, such as the gradual decline in growth that occurs as cells mature and as their walls stiffen, involve substantial changes in wall composition and crosslinking.

'Wall loosening' refers to a molecular modification of the wall network that results in relaxation of wall stress. WALL STRESS RELAXATION might result from scission of a stress-bearing crosslink or from sliding of such a crosslink along a scaffold; in both cases it results in a reduction in wall stress without a substantial change in wall dimensions[53]. Actual enlargement of the wall occurs secondarily, as a consequence of cellular water uptake, in response to the turgor relaxation that inevitably accompanies wall stress relaxation.

Recently, four molecular mechanisms of wall loosening have attracted recent attention and are reviewed below. The candidate wall loosening agents include: expansin, xyloglucan endotransglycolase/hydrolase, endo-(1,4)-β-D-glucanase and HYDROXYL RADICALS. For this discussion, it is useful to distinguish between two classes of wall loosening agents, which I call 'primary' and 'secondary' wall loosening agents[54]. Primary agents catalyse stress relaxation directly — this can be demonstrated by applying the agent to isolated cell walls and measuring either wall stress relaxation or wall extension (creep). By contrast, secondary agents modify the wall without causing stress relaxation and they cause wall extension indirectly, by amplifying the physical effects of primary agents. Both types of loosening agent might be important for the control of wall enlargement by plants.

Expansin

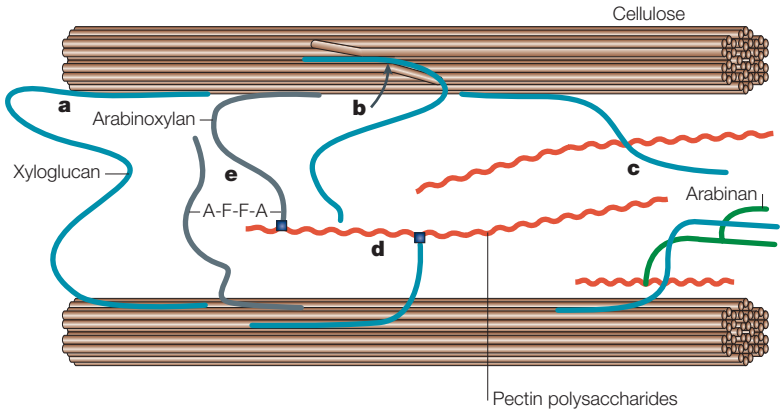
Growing plant cells characteristically exhibit ACID GROWTH[55–57]. Isolated cell walls also exhibit this phenomenon, which results from the action of pH-dependent wall-loosening proteins named EXPANSINS[58]. The pH of the cell wall of growing cells is typically between 4.5 and 6, which is the range in which acidification activates expansin activity. This is biologically important because a number of agents alter cell growth, at least in part, by inducing the cell to alter its wall pH — through control of the plasma membrane proton pump (H^+-ATPase). The plant growth responses that involve changes in cell-wall pH include stem and root tropisms (bending toward or away from a stimulus); hormone-induced growth; light-induced stimulation of leaf expansion and inhibition of stem elongation; responses of shoots, roots and leaves to water deficits and salt stress; initiation and early outgrowth of root hairs; and rapid growth in response to the fungal toxin fusicoccin.

A role for expansins in cell growth. Four lines of evidence support the idea that expansins (see Online links box) regulate cell wall enlargement in growing cells. First, when the acid-growth behaviour of isolated cell walls is eliminated by heat or protease treatment, it

DOUBLE-LABELLING EXPERIMENTS
Experiments in which two different tags (such as the radioisotopes ^3H and ^{14}C) are incorporated in and attached to a molecule. Useful for tracing the origin and fate of a molecule that undergoes complicated processing.

WALL STRESS RELAXATION
Reduction in mechanical stress in the cell-wall network, because of slippage or scission of load-bearing polymers in the cell wall; wall loosening stimulates wall stress relaxation.

Box 2 | **How do hemicelluloses form a network with cellulose microfibrils?**



At least five ideas (see figure, a–e) have been considered and involve direct or indirect linkages between cellulose microfibrils. Hemicelluloses (grey and blue strands) may form a primary network with cellulose, and may also be linked to acidic pectins (red strands). Additionally, neutral pectin polysaccharides, such as arabinans (green strands), are able to bind the cellulose surfaces as well[121].

- **Hemicelluloses might spontaneously bind to the surfaces of cellulose microfibrils and tether adjacent microfibrils together[21,34] (see figure, a).**
- **Xyloglucan might become entrapped during formation of the ordered microfibril[122,123] (see figure, b). The untrapped remainder of the xyloglucan would be free to bind to other cellulose surfaces or to other matrix polymers, thereby anchoring the microfibril firmly to its neighbours. This hypothesis might explain why a significant xyloglucan fraction is released from the microfibril only after treatments that cause microfibril swelling (for example, treatment with a strong alkali). It could also explain why a cellulose-specific endoglucanase stimulates cell enlargement: digestion of the noncrystalline region of a cellulose molecule results in the release of the trapped xyloglucan and the microfibril is freed from its tether[124].**
- **Cellulose microfibrils might be simply coated with xyloglucans (blue strands), which adhere to other matrix polysaccharides, without direct linkage between microfibrils (see figure, c). This idea is based on studies showing that the main polysaccharides can be selectively extracted from the growing wall without evidence for covalent linkage with other polysaccharide species[35].**
- **Xyloglucans (blue strands) might be covalently attached to pectin polysaccharides (red strands), forming a macromolecule that anchors the microfibrils by sticking of xyloglucan to cellulose surfaces (see figure, d). This is a variation of the earliest molecular model of the plant cell wall[125], which was subsequently dismissed owing to lack of evidence of the covalent linkage between xyloglucan and pectin. However, recent studies[126,127] report that a proportion of xyloglucan is covalently attached to acidic residues, possibly homogalacturonan. The nature of the hypothetical xyloglucan–pectin linkage has not been established.**
- **Arabinoxylans (grey strands) might bind cellulose and be crosslinked by ferulic acid esters (A-F-F-A) (see figure, e). This type of phenolic crosslink might also crosslink other hemicelluloses and pectins, particularly in gross cell walls.**

can be almost fully restored by the addition of purified expansin proteins[58]. This indicates that expansins are primary wall loosening agents and that their action alone is sufficient to restore extensibility to cell walls. Second, addition of exogenous expansin to growing cells stimulates their growth[59–61]. These results show that expansin can stimulate cell enlargement under the normal operating conditions of the cell wall and that endogenous expansins are at least partially limiting for cell growth. Third, ectopic expression of expansin genes stimulates plant growth, whereas suppression of expansins by gene silencing decreases plant growth[62–65], indicating that altered expression of expansin genes can modify plant growth. Last, endogenous expansin-gene expression correlates with the onset, increase and cessation of cell growth[66–72]. Expansin genes are expressed at the right time and the right place to exert control of cell growth.

Exploring expansin function. How expansins catalyse wall enlargement remains unclear. They function rapidly — within seconds of expansin addition, isolated walls begin to creep at a fast rate[58]. By contrast, walls that were treated with a fungal endoglucanase, which hydrolyses xyloglucan, required prolonged digestion before the walls began to extend[38]. Whereas endoglucanase digestion physically weakened the cell wall (as measured by plastic and elastic compliances), expansin treatment did not. These compliances are measured in stress/strain experiments and changes indicate modification of cell-wall structure and the degree of crosslinking. On the other hand, expansins promote wall stress relaxation, but endoglucanases have not been found to have this ability. Similarly, expansins and endoglucanases have distinctive mechanical effects on artificial cellulose–xyloglucan composites[73]. It is therefore clear that expansins and endoglucanases modify walls in distinctive ways and with different kinetics.

Sequence analyses[74,75] indicate that expansins consist of two domains: an N-terminal domain (~15 kDa) with distant sequence similarity to the catalytic domain of the family-45 endoglucanases; and a C-terminal domain (~10 kDa) that is related to a family of grass-pollen allergens of unknown function. Despite the

HYDROXYL RADICAL
The most active form of reactive oxygen species, consisting of a free hydroxyl group in which oxygen is missing an electron in its outermost shell. It is a strong oxidant that can steal an electron from — and thereby damage — polysaccharides, proteins, lipids, nucleic acids and other classes of organic molecules.

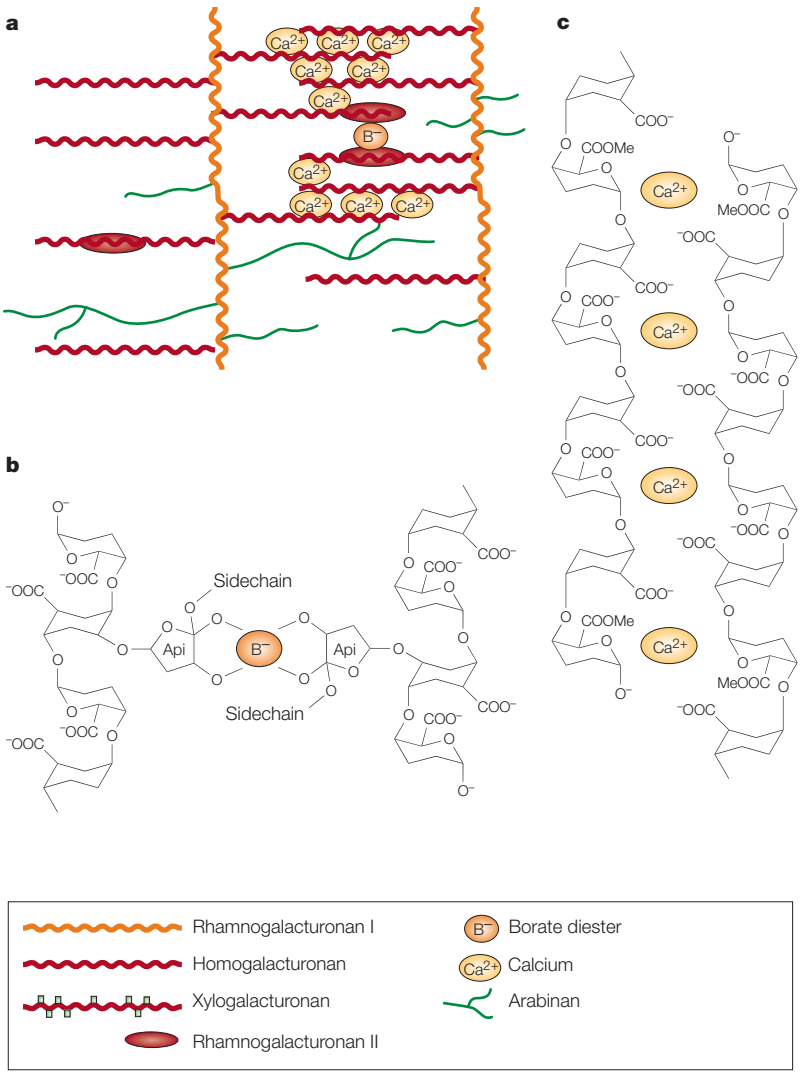
ACID GROWTH
Faster cell elongation under acidic conditions.

EXPANSINS
Wall loosening proteins that induce wall stress relaxation and irreversible wall extension in a pH-dependent manner, but they do not hydrolyze wall polymers.

Box 3 | **Formation of pectin networks by covalent and ionic bonds**

The distinctive pectin domains are believed to be covalently crosslinked to each other (see figure, part **a**), but the nature of the crosslink has not been determined. In addition, two other types of linkage involving boron (part **b**) and calcium (part **c**) are important crosslinking mechanisms.

- Part **a** of the figure shows a model of how the pectin domains may be covalently linked together to form a massively large macromolecular pectin network. This is a simplified version of a recent model by Vincken *et al.*[4], in which rhamnogalacturonan I serves as the backbone and the other pectin domains are attached as branches. Homogalacturonans are ionically crosslinked by calcium (part **c**) whereas boron crosslinked rhamnogalacturonan II through diester linkages (part **b**).
- Rhamnogalacturonan II forms dimers through a borate ester bond (part **b**). This crosslinking is important for normal wall formation as well as for the control of wall porosity and wall thickness.
- Homogalacturonan (also known as polygalacturonic acid) forms stiff gels through Ca^{2+}-mediated crosslinking of its carboxyl groups through ionic and COORDINATE BONDS (part **c**). Growing cells usually synthesize homogalacturonan in which ~75% of the carboxyl groups (COO^-) are methyl esterified (COOMe) — this modification removes the negative charge of the carboxylate ion and blocks its ability to undergo Ca^{2+} crosslinking. Highly esterified homogalacturonans do not form stiff gels and their secretion might help the expanding wall to remain pliant. Carboxyl-based crosslinking sites are unmasked later, as the cells cease growth, owing to the action of pectin methylesterases. These methylesterases, which are secreted by plant cells into their wall space, hydrolyse the methylesters and free the carboxyl group for Ca^{2+} crosslink formation and gel formation.



COORDINATE BOND
Chemical bond involving the sharing of a pair of electrons, each supplied by one atom.

similarity with endoglucanases, no enzymatic activity has been found that accounts for the action of expansin on the wall[76–78]. A report[79] that β-expansin exhibited protease activity when it was recombinantly expressed in yeast was later refuted[78], with the activity apparently being the result of an induced yeast protease[80].

Expansins are believed to disrupt non-covalent binding of wall polysaccharides to one another. This concept has been supported by several studies. First, expansin weakens paper, which is a hydrogen-bonded network of cellulose fibrils, but this weakening action does not involve cellulose hydrolysis[77]. Second, expansin synergistically enhances the hydrolysis of crystalline cellulose by cellulases. Because glucan accessibility is the rate-limiting step in cellulase action, this result could indicate that expansin promotes the release of glucans on the surface of the cellulose microfibril, making them available for enzymatic attack. Third, expansin does not gradually and progressively weaken the cell wall, as would be expected for a hydrolytic enzyme. As soon as expansin enters the wall it stimulates extension, and subsequent removal of expansin restores the wall to an inextensible state[76]. This indicates that expansin does not alter gross wall structure or the degree of crosslinking. Last, 2 M urea, which disrupts hydrogen bonding, mimics some of the physical effects of expansin on the cell wall[77]. On the basis of these and other results, we have proposed that expansin functions to dissociate a polysaccharide complex that links microfibrils together[76,77].

Expansins: growth and beyond? Genomic and phylogenetic analyses show that plant expansins consist of a large superfamily that is divided into four divergent families[81]. It has been demonstrated that two families contain members that have the ability to extend walls (EXPA or α-expansin, and EXPB or β-expansin), whereas the functions of the other two related families (EXLA and EXLB, for expansin-like family A and B) remain to be established. *A. thaliana* has 36 members

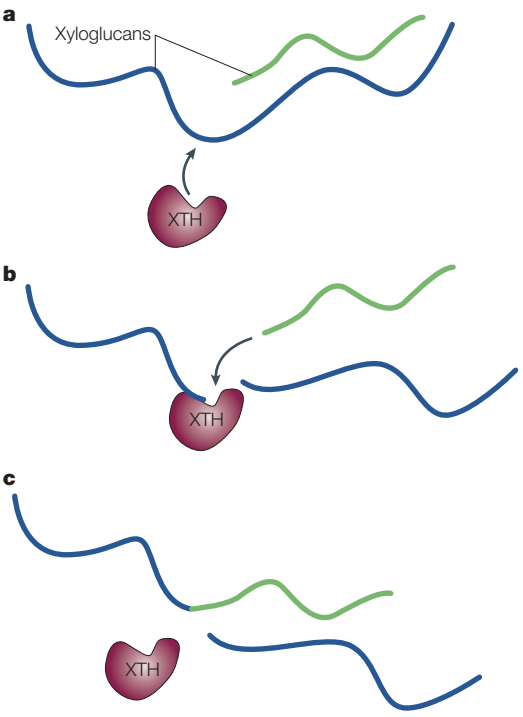


Figure 4 | **The activity of xyloglucan endotransglucosylase/hydrolase (XTH) as an endotransglucosylase.** The enzyme carries out two reactions, first a scission of a glycosidic bond in the xyloglucan backbone, followed by the re-formation of the bond with a second xyloglucan chain. **a** | Two xyloglucan chains are shown (blue and green). XTH binds to one of the chains. **b** | Upon cutting the xyloglucan backbone, one of the strands remains covalently attached to the catalytic site of the enzyme. The free end of a second xyloglucan moves into place for the second reaction. **c** | After ligation, a hybrid xyloglucan is formed, which is indicated as a xyloglucan of two colours and a xyloglucan fragment is released.

of this superfamily, whereas rice has 58 members. The biological function of individual expansin genes is currently being studied. Gene-expression studies have shown that many genes are related to the growth of specific cell types, whereas others promote wall loosening in other situations, such as fruit softening[82,83], ABSCISSION[84] and pollination[85]. A challenge for the future will be to elucidate the specific roles of the genes that belong in this superfamily.

Xyloglucan endotransglucosylase/hydrolase

It has been postulated that XTHs carry out various functions[46]; these include wall loosening[86], wall strengthening[87], integrating new xyloglucans into the wall[47], trimming xyloglucan strands that are not tightly stuck to the surface of cellulose[88], fruit softening[89], and hydrolysing xyloglucans[90,91] particularly during xylem formation[92,93].

Despite assertions in the literature that XTH causes wall loosening, there is little direct evidence in support of XTH as a primary wall-loosening agent – that is, an agent that induces wall stress relaxation and extension by itself. Attempts to measure XTH-induced cell wall creep have yielded only negative results (J. Rose and D.C., unpublished data; REFS 94,95). These data contrast with results obtained using artificial cellulose–xyloglucan composites, in which XTH induced creep in BIAXIAL STRAIN ASSAYS[73]. Such results indicate that XTH can loosen some wall-like networks, but they also point out that results that were obtained with these artificial composites cannot always be extrapolated to the behaviour of real cell walls. When isolated walls of azuki bean HYPOCOTYLS were incubated with XTH, they became more extensible in force/extension assays[96] (this is not the same as creep[53]). This is apparently the only published report showing that XTH reduces the mechanical strength of isolated walls. However, the long incubation times that were used in these experiments (48 hours at 37°C) indicate very weak enzyme activity. Low-level contamination with other enzymes is a potential concern in such situations. Also, the walls were pre-treated with boiling methanol, which — although it kills most enzyme activity — does not reduce expansin activity[56,58]. Therefore, the cell walls that were used in these experiments probably contained active endogenous expansin, as well as exogenous XTH.

However, another study that used living tissues (not isolated walls) demonstrated that xyloglucan metabolism by endogenous XTH is a potential control point for cell elongation[41]. Addition of long xyloglucans, which have the potential to crosslink cellulose microfibrils, reduced growth of pea-stem segments by more than 50%. The opposite response, a growth stimulation and substantial xyloglucan solubilization, was obtained when short xyloglucan oligosaccharides (XGOs) were used. When XGOs are used as acceptors in a transglucosylation reaction by XET, the result is similar to the action of hydrolase — the xyloglucan chain length is reduced.

These experiments — in which xyloglucan is artificially manipulated — confirm the significance of xyloglucans for cell enlargement. However, the millimolar level of XGOs used in these experiments is much higher than that found *in vivo*[97], so the case for XET as a primary wall-loosening enzyme *in vivo* cannot be considered strong on the basis of this result. Because large xyloglucans are continually being deposited at the inner surface of the growing wall, a strengthening function for XET seems more probable.

Seeing XET in action. The activity of XET can be visualized with the use of fluorescently labelled XGOs[98,99]. When roots were labelled in this way, XET activity was greatest in the elongation region[100], as well as at sites of root-hair initiation, where the thick outer epidermal wall begins to bulge out. Studies of XTH gene expression[101] indicate that four XTH genes might account for these XET activities. A recent study[102] noted that XGO fluorescence was associated with cellulose microfibrils in elongating cells, whereas in dividing (non-elongating) cells the fluorescence pattern was diffuse. The authors proposed that some XET isoforms select xyloglucan that is tightly bound to cellulose, whereas others function less specifically. Another possibility is

ABSCISSION
The process by which old parts of a plant break off naturally (for example, leaves).

BIAXIAL STRAIN ASSAYS
Procedures in which a material is stretched not in one direction, but in two directions, as happens with the membrane of an expanding balloon.

HYPOCOTYLS
The stem region of a seedling below the cotyledons (seed leaves).

that newly secreted xyloglucans are particularly accessible to tagging by XET under the conditions of this assay, and they soon become bound to nascent cellulose microfibrils, which are synthesized at high rates in elongating cells. This gives rise to a fibrillar pattern of fluorescence in such cells (see cellulose microfibrils in FIG. 1d).

XTH gene expression is high in regions of active wall formation — that is, in elongation zones and in regions where wall deposition continues after cell enlargement has ceased or where other forms of wall remodelling occur[87,103–105]. But what are the consequences of manipulating XTH levels in plants? Herbers *et al.*[106] suppressed XTH gene expression in tobacco plants using antisense methods; the enzyme activity was reduced as much as 56% and the xyloglucan size increased by 20%, but effects on plant growth were not noted. An XTH mutant with a subtle phenotype in xylem development was recently characterized[93]; the *A. thaliana xth27* mutant had fewer tertiary veins, and where tertiary veins did form, the TRACHEARY ELEMENTS were shorter and misshapen. Although XTH27 is expressed in many cells in the plant, a developmental phenotype was only observed in the tertiary veins of the mutant plants. These results indicate that XTH27 is required for proper morphogenesis of tertiary veins, but the interpretation of this phenotype in terms of wall loosening, xyloglucan removal from secondary walls or other potential biochemical functions of XTH is less clear.

Endo-(1,4)-β-D-glucanase

In addition to XTH, plants also have a family of secreted endo-(1,4)-β-D-glucanases (sometimes called 'cellulases'). These enzymes belong to glycoside hydrolase family 9 (see Carbohydrate-active enzymes in Online links box), of which there are 25 family members in *A. thaliana*. Three are membrane-bound endoglucanases (KOR and its PARALOGUES) that are involved in cellulose formation. The remaining proteins are secreted enzymes, mostly of unknown function.

Although the expression of plant endoglucanase genes has been studied for years in connection with fruit softening, abscission and growth, the enzymes' biochemical properties, substrate specificities and potential function for cell-wall loosening have drawn surprisingly little experimental attention[107,108]. Potential wall substrates include cellulose and xyloglucan. Ohmiya *et al.*[109,110] examined two such enzymes from poplar (PopCel1 and PopCel2) and presented indirect evidence that they digest non-crystalline regions of cellulose. The authors proposed that the enzymes loosen walls by causing the release of xyloglucans trapped in cellulose microfibrils. Overexpression of *PopCel1* in *A. thaliana* resulted in enhanced plant growth and increased plastic extensibility of the cell walls. Conversely, antisense suppression of these enzymes in poplar led to reduced leaf growth, by as much as 32%. Similar results were obtained in *A. thaliana* by silencing a related endoglucanase gene[111].

In summary, the work described in the last two sections indicates that xyloglucan–cellulose interactions are important determinants of the mechanical and growth properties of the cell wall. The role of the XTH family of enzymes in the regulation of cell-wall loosening versus wall strengthening is unclear but the latter role has more support. The role of endo-(1,4)-β-D-glucanases in wall loosening merits greater attention.

Hydroxyl radical (•OH)

•OH is a highly active form of reactive oxygen species, which have important roles in signalling and cell death. Recent studies have advanced the new idea that •OH is harnessed by growing cells to loosen their cell walls and stimulate cell enlargement[112–116]. This hypothesis is based on the observation that •OH can cleave wall polysaccharides (along with almost everything else it touches) by nonenzymatically removing a hydrogen atom from polysaccharides[113]. This idea is supported by results showing that extension of isolated walls could be induced by artificially generated •OH[114]. Moreover, plant cells produced •OH in an auxin-dependent manner and free-radical quenchers suppressed auxin-induced growth[116].

It has been postulated that endogenous •OH might be produced nonenzymically by copper ions that are bound to the cell wall[112] or by wall peroxidases[115] from the superoxide anion and hydrogen peroxide. Hydrogen peroxide is formed by monovalent O_2 reduction by a plasma-membrane NAD(P)H oxidase and takes part in various defence responses, as well as in hormone signalling.

Therefore, various observations implicate the involvement of •OH in auxin-induced cell growth. However, in published studies the total amount of wall extension that was induced by •OH was small (~1% extension) and our attempts to induce more realistic amounts of extension by •OH treatments led to wall breakage rather than extension (L. Zhao and D.C., unpublished data). In comparison, 'acid growth' of walls results in a 40% to 100% extension before the wall is weakened to the point of breakage[56]. Furthermore, it is doubtful that the amount of •OH that is produced by growing cells approaches the high concentration that is required to cause cell-wall extension *in vitro*; such concentrations might be expected to cause widespread damage to living cells.

If •OH does function as a bona fide wall-loosening agent in living cells, its production and release must be carefully controlled, so that its destructive reactivity is targeted specifically to xyloglucan and other load-bearing polysaccharides. How this might be achieved has yet to be determined, as •OH probably reacts with the first suitable molecule it encounters (including proteins, lipids and other biomolecules). Evidence of •OH-induced polysaccharide splitting in growing cell walls might be obtained by finding the predicted products of •OH action in growing cell walls[117]. Evidence for such products has been found in softening fruit[118], but not in growing cells[112].

TRACHEARY ELEMENTS
Specialized cells in the xylem of vascular plants that are responsible for the conductance of water as well as providing mechanical support.

PARALOGUES
Genes or gene families that originated from a common ancestral sequence by a duplication event, not involving speciation.

Conclusions

The growing cell wall in plants is a thin, strong and pliant extracellular layer that is composed of cellulose microfibrils. These microfibrils are embedded in a hydrated matrix that is made of complex polysaccharides and a small amount of structural proteins. Microfibrils are synthesized by membrane complexes that contain cellulose synthases, which are encoded by three *CESA* genes. Matrix polysaccharides are synthesized in the Golgi apparatus and become integrated into the existing wall by enzymes and by spontaneous binding mechanisms. Recent studies have identified the genes that encode the glycosyltransferases that synthesize some of the glycosidic linkages in xyloglucans, mannans and pectins.

Cell-wall enlargement begins with wall stress relaxation, which allows the cells to take up water and physically enlarge. Expansins are a group of nonenzymatic wall proteins that induce wall stress relaxation and extension. They mediate 'acid-induced growth' by disrupting the non-covalent linkages that hold microfibrils in place in the cell wall. Xyloglucan endotransglucosylase cuts and joins xyloglucans. The consequences of this biochemical activity for wall properties are still being investigated. Plant endo-(1,4)-β-D-glucanases might digest the noncrystalline regions of cellulose microfibrils and release trapped xyloglucans, resulting in increased wall extensibility and cell growth. •OH has been proposed as a wall loosening agent that nonenzymatically cuts wall polysaccharides, but questions remain about the physiological significance of this loosening mechanism.

Genetics and genomics have proved to be key tools in the past decade for uncovering the enzymes that assemble sugars into the diverse polysaccharides of the plant cell wall and for characterizing the machinery that underlies the formation and growth of the wall. Enzymes that synthesize and modify the wall are members of moderate to large multigene families, so a challenge for the future will be to dissect the specific functions of all of these genes. We have only recently begun to characterize the transferases that form specific linkages in the cell wall (there are over 400 genes for such transferases in *A. thaliana*[16]), but we can anticipate rapid progress in the coming years. Further progress on more recalcitrant problems, such as understanding the assembly of the macromolecular wall and the molecular bases of crosslinking, will be made by combining these approaches with new biochemical and biophysical methods for assessing wall structure and the changes that underlie its growth. This poses daunting technical challenges, but should reveal key missing pieces in the puzzle of cell-wall growth, and perhaps bring Robert Frost's poetic appreciation of the plant form into new light.

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Competing interests statement

The author declares no competing financial interests.

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