



The molecular basis of plant cell wall extension

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Abstract

In all terrestrial and aquatic plant species the primary cell wall is a dynamic structure, adjusted to fulfil a diversity of functions. However a universal property is its considerable mechanical and tensile strength, whilst being flexible enough to accommodate turgor and allow for cell elongation. The wall is a composite material consisting of a framework of cellulose microfibrils embedded in a matrix of non-cellulosic polysaccharides, interlaced with structural proteins and pectic polymers. The assembly and modification of these polymers within the growing cell wall has, until recently, been poorly understood. Advances in cytological and genetic techniques have thrown light on these processes and have led to the discovery of a number of wall-modifying enzymes which, either directly or indirectly, play a role in the molecular basis of cell wall expansion.

Abbreviations: CDTA, *trans*-1,2-diaminocyclohexane-*N,N,N',N'*-tetraacetic acid; CsExp1, cucumber expansin 1; GAX, glucuronoarabinoxylan; $\cdot\text{OH}$, hydroxyl radical; IAA, indole-3-acetic acid; PME, pectin methyl-esterase; XET, xyloglucan endotransglycosylases

Introduction

Plant cell growth is driven by internal turgor pressure and restricted by the ability of the cell wall to extend under this pressure. The ability of cell walls to extend depends on at least two different, but connected, levels of events. Firstly, extensibility depends on the composition of the wall and how its components are bound to one another. Secondly, wall extension can be controlled by modification of existing wall structures. In this paper we will review the current state of our understanding of the molecular mechanisms that underlie cell wall extension in plants.

Wall synthesis determines the inherent extensibility of the wall material, but this extensibility is fine-tuned by processes within the wall that alter the structure or binding of existing wall polymers. These processes have been described by Cosgrove (1999) as primary and secondary wall-loosening agents. Primary wall loosening agents directly alter the mechanical properties of existing wall material, and secondary loosening processes influence wall extensibility by

altering the composition and structures that primary processes act upon. As both wall synthesis and structure receive extensive review in other parts of this volume the present paper will concentrate solely on aspects of these topics relating directly to wall extension. We will then use this structural background to discuss factors which modulate the extension of existing wall material.

Structural components of the primary cell wall and their assembly

In general, new primary cell wall material must be synthesised in a form that is competent to undergo extension. The primary cell wall is essentially a composite material consisting of a framework of cellulose microfibrils embedded in a matrix of other, mostly polysaccharide, polymers. The matrix polysaccharides can be grouped into two major classes, pectins and cross-linking glycans. Based on the composition of these polysaccharides the primary cell wall can be distinguished into two distinct types: Type I and type II

cell walls (Carpita and Gibeaut, 1993). In Type I cell walls the principal polysaccharide interlinking the cellulose microfibrils is xyloglucan, creating a multi-polymer network embedded in pectic polysaccharides. Most dicotyledonous and some monocotyledonous plants possess Type I cell walls and their properties and assembly constitute the major part of the present review. Type II cell walls are characteristic of those found in grasses (Poaceae) and other related 'commelinoid' species. Although the overall structure of the cellulose-cross linking glycans is similar to Type I cell wall type II cell walls differ in that the major cross-linking polysaccharide is glucuronoarabinoxylan (GAX) not xyloglucan. Another discriminating feature of Type I cell walls is a lower content of pectin, especially in growing regions (Knox *et al.*, 1990). This review will now focus on the role of each cell wall component in cell extension.

The role of cellulose

Alterations in cellulose synthesis can have marked effects on plant growth which may be brought about by alterations in wall extensibility. Studies using cellulose biosynthesis inhibitors on cultured plant cells reveal that the cells can survive in the absence of cellulose (Shedletsky *et al.*, 1990). The walls of these cells are effectively devoid of cellulose, and much of this absence appears to be compensated by increased levels of pectin. It is clear that cell growth is greatly restricted in this condition. In addition, the cells adopt a spherical shape, supporting the hypothesis that the cellulose framework may be essential in determining directionality in plant cell growth. In intact plants, cellulose synthesis inhibitors are used as herbicides, implying that correct cellulose synthesis is essential in normal plant life.

In a similar context, alterations in cellulose structure can have effects on cell growth. Shpigel *et al.* (1998) showed that incubation of tip growing organs (pollen tubes and root hairs) in solutions containing a bacterial cellulose-binding domain would increase their rate of extension. The same protein also inhibited the expansive growth of *Arabidopsis* root cells. In both cases the protein appeared to disrupt the structure of cellulose deposited in the wall leading to the effect on growth.

Mutations that affect the function of cellulose synthase genes in plants have drastic effects on growth and morphology. A temperature sensitive mutation in the *rswl* gene leads to reduced growth and aberrant

cell morphology in *Arabidopsis* (Arioli *et al.*, 1998). Cells of plants grown at high temperature show growth aberrations with many epidermal cells ballooning out, suggesting they can no longer effectively control their expansion. Analysis of the walls of these cells show that there is a general loss of cellulose crystallinity and this probably results in gross changes in extensibility.

The cellulose/cross-linking glycan network

There is general agreement that the cellulose/matrix glycan network serves to hold the primary cell wall together and is the key determinant of wall extensibility. Glycans, such as xyloglucan in dicots and glucuronoarabinoxylan (GAX) in graminaceous monocots, are thought to bind to cellulose microfibrils via extensive hydrogen bonding between the (1→4) β -D-glucan chains in the microfibrils and the glucan or xylan backbone. The matrix glycans are able to bind to more than one microfibril at a time (McCann *et al.*, 1990), which enables them to span between microfibrils and form molecular tethers. An easy assumption to make from this observation is that, by doing this, the tethering matrix glycans would strengthen the wall by endowing interfibril integrity.

Dissociation of cellulose and the tethering polymers from one another requires generally severe extractions such as 4 to 6 M NaOH (Fry, 1988). This suggests that at least a subfraction of the glycans are held to the microfibrils by a more substantial interaction than simple hydrogen bonding to the fibril surface. Indeed, such harsh chemical treatments not only release the matrix glycans but also lead to swelling of the crystalline microfibrils due to partial dissociation of many (1→4) β -D-glucan chains from the crystal structure of the microfibrils themselves. This, in turn, may allow the release of xyloglucan trapped within the structure of the microfibrils.

If cellulose and xyloglucan (or other cross-linking glycans) are mixed together *in vitro* then the amount of glycan that associates with the cellulose is much lower (by as much as a factor of 5) than that found in the wall (Hayashi *et al.*, 1987; Baba *et al.*, 1994). In addition, comparatively mild extractions are sufficient to remove what polymer is bound to the cellulose. Hayashi *et al.* (1987) showed that if crystalline cellulose and xyloglucans were annealed together by heating and cooling, then more stable associations (requiring 4 M NaOH to disrupt) were formed. This suggested that during heating the crystal structure of the cellulose was partly disrupted allowing the xyloglucan to inte-

grate, in part, with the (1→4) β -D-glucans and then to become entrapped in the crystal structure during annealing.

Pectins

The function of pectins in the wall, with regards to extension, is still not clear. However, it is clear that pectins are mainly found in primary cell walls, indicating that they fulfil an important function during wall extension. In this context, it is worth noting that in Type I walls pectic polymers are in much lower amounts than in Type II walls, suggesting that the role is not necessarily dependent on abundance. In solution, pectins exist as highly hydrated gels and concentrations above 2–5% are difficult to obtain, but in the wall pectins are maintained in a highly condensed form, possibly closer to 20%. How the pectins are maintained in this concentrated form in the wall is unclear, but perhaps it is by purely spatial constraints. This high pectin concentration could provide a high hydration potential to the wall, which in turn may help endow the wall with compressive strength. As will be discussed in a later section, there is gathering evidence that pectins may play a role in wall rigidification at the end of growth.

Bacterial cellulose composites: model structures for the cell wall?

Understanding how cellulose and matrix glycans associate in this network may be key to understanding the molecular basis of wall extension. There is a general assumption that the components of the wall are co-secreted in the apoplast and form the structure of the wall by spontaneous assembly. That is to say that the matrix glycans and pectins secreted into the wall spontaneously assemble themselves around the framework provided by cellulose microfibrils synthesised at the outer face of the plasma membrane. Various investigators have tried to manipulate the components of the wall to assemble correctly *in vitro* (Hayashi *et al.*, 1987; Baba *et al.*, 1994). The major failing in this work was simply in obtaining composites that reflect the correct relative amounts of polymers in them. Type I walls typically contain roughly equal quantities of cellulose, xyloglucan and pectin. However, as pectins exist as highly hydrated gels *in vitro*, it is not possible to dissolve them into the concentrations found in the wall. In addition, it has not been possible to devise conditions for cellulose and xyloglucan to associate in the 1:1 ratio that is found in native walls. Part of this

problem is probably related to the substrates used in these types of experiment. For example, commercially available cellulose is generally derived from whole cell walls such as cotton linters, and this is very different in nature (surface area to volume ratio) than dealing with individual cellulose microfibrils as would occur during wall synthesis.

A more effective means of creating wall-like composites has been recently developed. *Acetobacter xylinus* cultures produce strong cohesive mats of cellulose fibrils. Each bacterial cell secretes a single, long cellulose microfibril. In a culture these come together to form a highly entangled mat known as a pellicle. Whitney *et al.* (1998, 1999) showed that when *Acetobacter* cultures are grown in the presence of xyloglucan or glucomannan, they form a pellicle that reflects many of the characteristics of the cellulose/matrix glycan network in plant cell walls. Not only do these composite materials contain a similar proportion of cellulose and cross-linking glycan to that found in cell walls, but chemical extractions, and NMR studies, showed that the two polymer systems associate in a similar manner (Whitney *et al.*, 1998). Microscopic examination of the structure of the composite showed many similarities to structures observed in pectin-depleted wall materials (McCann *et al.*, 1990, 1993). In this system cellulose synthesis occurs in a medium with high concentrations of matrix glycan. It seems likely that associations can form between matrix glycan molecules and glucan chains in the nascent microfibril as it forms. This probably leads to entrapment of a proportion of the glycans into the crystal structure of the fibril. This is probably very similar to what occurs during plant cell wall synthesis.

At the surface of the plasma membrane in plant cells, cellulose synthesis is thought to occur in areas of high local concentrations of glycans released from Golgi secretions, leading to a similar entrapment in microfibrils during synthesis. In support of this idea, Pauly *et al.*, (1999) demonstrated that there are three domains of xyloglucan within a dicot cell wall. One domain is represented by xyloglucan that is easily extractable, readily digested by endo- β -glucanases, and is probably largely held in the wall by physical entanglements with other polymers. A second, enzyme-inaccessible domain is more tightly held in the wall, probably by hydrogen bonding (along at least part of its length) to the microfibrils. The third domain, also inaccessible to endoglucanases, is very tightly associated with the microfibrils and probably consists of polymer domains trapped within the crystal structure.

Clearly it is possible for any single xyloglucan molecule to have regions of its polymer taking part in all three domains at any time. These different domains of xyloglucan may represent different sites of action for enzymes modulating wall extension.

An interesting observation in the composite pellicle formed between cellulose and matrix glycans by *Acetobacter*, is the drastic difference in mechanical properties between the composite and pure cellulose materials (Whitney *et al.*, 1999). As mentioned above, the cellulose pellicle normally produced by the bacteria is a dense mat of highly entangled microfibrils. Measurement of mechanical properties of this pellicle in uniaxial extension measurements reveal it to be a strong and stiff material. In contrast, the pellicle produced in the presence of xyloglucan is weaker and more extensible. In uniaxial extension measurements, the xyloglucan-containing pellicle exhibited about 20% of the strength of cellulose-only material. As well as being weaker, the composite was more extensible, extending by almost twice as much as the material without xyloglucan (in creep assays). Very similar properties were also reported in composites made with other polysaccharides, such as mannans (Whitney *et al.*, 2000).

The effects of matrix glycans on pellicle structure and material properties might tell us something of the role of these polysaccharides in cell walls. Rather than simply acting as cross-links to strengthen it, the cross-linking glycans may well enhance the extensibility of the cell wall. The presence of matrix glycans during cellulose synthesis probably results in the production of 'fuzzy fibrils' with long trailing ends of the glycans instead of the uncoated, partly crystalline surfaces of fibrils formed in their absence. This 'fuzziness' may help to hold microfibrils apart from one another. Somehow, the xyloglucans may prevent microfibril entanglements giving the wall a much looser structure. In this loose structure, the relatively weak cross-bridges may now also be of significance to the overall extensibility of the wall.

Whitney *et al.* (2000) have also shown that xyloglucan-containing composites made by *Acetobacter* are excellent substrates for the action of the wall-loosening enzyme, 'expansin'. Expansins were the first isolated plant cell wall protein shown to rapidly induce the long-term extension of this material when it is held under a constant physical load. Expansins are discussed in more detail later in this paper. The effects of expansin on *Acetobacter* cellulose composites are very similar to the effects on plant cell walls,

further supporting the validity of these materials for use as models of wall structure. These studies also showed that expansins were much less effective in inducing extension on composites made with shorter xyloglucans than those made with long polymers. In addition, it has been found that endoglucanase treatment of xyloglucan-containing composites results in increasing stiffness of the material, perhaps as the xyloglucan is digested by the enzyme (E. Chanliaud and M. Gidley, personal communication). Taken together, these observations suggest that, at least in this model system, smaller oligosaccharides can lead to stiffer materials. This observation goes against current thinking, which predicts that degradation of xyloglucan or other polymers will lead to more extensible walls.

Structural proteins

In addition to the cellulose-cross-linking glycan network, primary cell walls also contain small amounts of structural proteins that may be important during wall rigidification associated with cessation of cell expansion. Structural proteins, such as extensins, form a network in cell walls, and they were originally conceived to play a role in wall extension (Lampert, 1986). It is now generally accepted that this is unlikely as they are found in mono- and oligomeric forms in growing walls and appear only to be polymerised at the end of growth (Ye *et al.*, 1991). Arabinogalactan proteins (AGPs) have also been considered as potentially playing a role in wall extension. At present these glycoproteins are more generally considered to be signalling molecules (for review, see Gaspar *et al.*, 2001, this issue). There are reports that Yariv reagents, which are phloroglucinol-linked β -glycosides that specifically bind AGPs, can inhibit growth in cultured cells (Serpe and Nothnagel, 1993) and in *Arabidopsis* roots (Willats and Knox, 1996). The authors suggested that the inhibitory effect could be due to a direct effect of the Yariv reagent binding to AGPs and causing wall rigidification. We have investigated whether Yariv reagents can alter growth in cucumber hypocotyls and whether this was due to a direct effect on wall extensibility. The Yariv reagent can almost completely inhibit auxin-induced growth of cucumber hypocotyls (Figure 1A). However, direct application of Yariv reagent does not diminish the extensibility of the hypocotyls (Figure 1B), even though the dye was clearly taken up into the tissues (not shown). This suggests the effects of Yariv reagents, in this instance, are likely to be of a

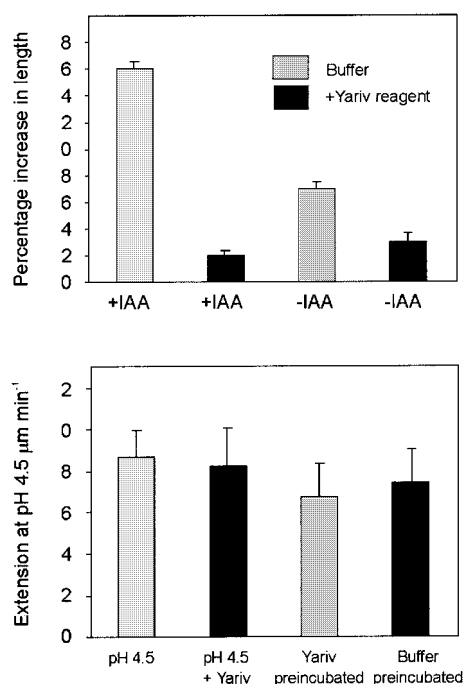


Figure 1. The effects of Yariv Reagent on IAA-induced growth and wall extension of cucumber hypocotyls. A. (top) Percentage increase in length of cucumber hypocotyls incubated in IAA, with and without Yariv reagents. Cucumber hypocotyls (*Cucumis sativus* cv. Burpee Pickler) were grown for 4 days in the dark at 28 °C. Sections (1 cm) were cut from the growing region just below the hook and floated for 1 h on a 5 mM KCl solution. Sections were then transferred to 5 mM KCl containing either 10 μM IAA only, 10 μM IAA with 30 μM Yariv phenylglycoside, no additive, or 30 μM Yariv phenylglycoside only. Sections were incubated in these solutions for 8 h and then re-measured along their axial length. Results are means and standard errors of 10 measurements. B (bottom). Extension of hypocotyl sections at pH 4.5. Apical 1 cm sections of hypocotyl were frozen, thawed and abraded, as described by McQueen-Mason *et al.* (1992). Sections were then either immediately suspended in an extensometer (McQueen-Mason *et al.*, 1992) under a constant load of 20 g in 50 mM sodium acetate pH 4.5 or in the same solution containing 30 μM Yariv phenylglycoside. Alternatively, the sections were pre-incubated for 30 min at room temperature in 50 mM sodium acetate pH 4.5 with or without 30 μM Yariv phenylglycoside, before being placed in the extensometer in 50 mM sodium acetate pH 4.5 as described above. Results are means and standard errors of 6 measurements.

longer-term nature rather than a direct physical result of binding AGPs in the hypocotyl walls.

Cell wall mutants

Genetic approaches to understanding wall structure and extension are becoming increasingly important, and already some important insights into the role of wall structure in normal plant growth have been attained. Reiter *et al.* (1997) screened populations of

EMS-mutagenized *Arabidopsis* for plants with altered cell wall compositions, and found 11 independent loci of 'mur' mutants (short for *murus*, which is Latin for 'wall') that were affected. Among these are some with clear effects on growth. For example, *mur1*, which has a defective GDP-mannose-4,6-dehydratase required for the synthesis of GDP-fucose, was characterised by a complete absence of fucose from shoot cell walls and was also slightly dwarfed (Zabackis *et al.*, 1996; see Reiter and Vanzin, 2001, this issue). The main effect appears to be on the mechanical properties of the primary cell walls. The terminal-fucosyl residue normally found in xyloglucan in these plants was partly replaced by the rare sugar L-galactose. It was originally believed that this alteration in xyloglucan structure might be responsible for the change in wall extension. However, fucose is found in other polymers such as complex pectins as well as in some glycans from glycoproteins, and the possibility that changes in some of these polymers are involved in the dwarf phenotype cannot be excluded.

A complimentary approach has been adopted by Nicol *et al.* (1998). In this instance, T-DNA-tagged populations of dwarf hypocotyl mutants were screened for defects in cell wall biogenesis. Some of these plants indeed have been shown to have altered wall compositions. One of these mutants, *korrigan* (*kor1*), showed severe cell expansion defects in all tissue types. Microscopic analysis of cell wall material revealed that these defects resulted from incorrect assembly of the cellulose/xyloglucan network (Nicol *et al.*, 1998). The corresponding *KOR* gene has been cloned and identified as a putative membrane-bound endo-(1 $\rightarrow$ 4) β -D-glucanase (EGase), a homologue of which was found associated with the ripening of melon fruit (Rose *et al.*, 1998). Although the precise role of this enzyme in cell wall assembly has yet to be elucidated, comparative studies of an EGase (*CelC*) from *A. tumefaciens* suggests that this enzyme may play a direct role in the synthesis of cellulose. In *celC* mutants an accumulation of lipid-linked (1 $\rightarrow$ 4) β -D-glucan oligosaccharides was detected, indicating that *celC* may be involved with cleavage and assembly of oligosaccharides used in the synthesis of nascent glucan chains (Matthysse *et al.*, 1995a, b). It remains to be established whether EGases are similarly involved in cellulose synthesis in plants.

In addition to mutants showing defects at the whole-plant level, plants have also been identified with organ- or cell-type-specific defects. Perhaps one of the most intriguing of these mutants are those of the *PRO*-

CUSTE (PRC) locus in *Arabidopsis*. Disruptions at this locus leads to cellulose defects which manifest as reduced hypocotyl elongation and increased radial expansion of root cells in dark-grown seedlings (Hauser *et al.*, 1995; Desnos *et al.*, 1996; Fagard *et al.*, 2000). The unusual aspect of this mutant is that the aberrant phenotype is light-dependent. Seedlings grown in the light showed no cell wall defects. Studies using double mutants with defective photoreceptors have shown that the light-dependent reversions of phenotype are phytochrome-dependent (Desnos *et al.*, 1996). These observations lead to speculation that PRC is involved in a novel dark-dependent pathway for cellulose synthesis in which, after phytochrome stimulation, *PRC* is down-regulated or its gene products become inactivated.

It seems clear, from the information gathered to date, that studies of these and other cell wall mutants will provide unique insights into the mechanisms underlying the synthesis and assembly of plant cell walls, as well as aspects of structure determining mechanical properties. For an in-depth review of this topic and related issues we recommend the article by Haigler *et al.* (2001, this issue).

The cell wall of grasses

So far, we have largely discussed wall structure and extension in the great majority of dicotyledonous and monocotyledonous cell walls are Type I (Carpita and Gibeaut, 1993). However, the cell walls of grasses and other related 'commelinoid' species that possess Type II walls show substantial differences in wall composition to those of all other angiosperms (Carpita and Gibeaut, 1993). Some of these differences, as well as the broad similarity, may offer insights into the molecular basis of wall extension. Type II cell walls are characterised as having relatively low levels of pectins and different matrix glycans than those of Type I cell walls. In Type II walls the major cross-linking glycan is glucuronoarabinoxylan, and this is generally believed to have a similar role to that played by xyloglucan in Type I walls. In addition to glucuronoarabinoxylan, a second matrix glycan, a mixed-linkage (1→3),(1→4) β -D-glucan, appears transiently during cell expansion. Most of this unbranched polymer consists of cellotriose and cellotetraose units connected by single (1→3) β linkages. This polymer appears at the onset of cell expansion and is rapidly metabolised during growth (Kim *et al.*, 2000). Because of this parallel appearance and metabolism during expansion,

this polymer has been proposed to play a direct biochemical and structural role in wall extension.

Wall loosening and expansion

Wall biosynthesis and polymer assembly

Clearly the deposition of new wall material is necessary to maintain wall strength and integrity during extension. Although it has been shown possible to maintain wall extension in the absence of deposition *in vitro* (Rayle *et al.*, 1970) it seems that the two cannot be uncoupled *in vivo*. It has been suggested that the deposition of new wall material might directly lead to wall extension by a process of intersusception. This hypothesis proposes that as new material enters the structure of the wall it permits existing components to slide relative to one another acting as a sort of integrating lubricant.

Little or no direct evidence has been shown for the induction of wall extension by newly secreted polymers. However, a pectin-rich polysaccharide fraction from cucumber hypocotyl cell walls can directly increase the extension of cucumber hypocotyls *in vitro* (Figure 2). These polysaccharides were extracted in CDTA from phenol-extracted walls and are unlikely to contain protein. In support of this effect not being protein based, the activity in these samples survived being heated to 100 °C. The simplest interpretation of these data is that the polysaccharides in this solution are inducing extension by integrating into the wall. An alternative interpretation is that the fraction, although dialysed, might contain residual CDTA, or might themselves serve as chelating agents. However, direct applications of CDTA during extension assays had no stimulating effect. These data support the possibility that wall deposition might itself directly induce wall extension. The molecular mechanisms by which these polysaccharides induce extension are not obvious, and this is an area requiring more work. The majority of this wall fraction is pectinaceous, but it represents a heterogeneous mixture of polymers and is likely to include some matrix glycans as well as pectins. It is worth noting that other wall polysaccharide fractions enriched in matrix glycans did not show this effect (data not shown), but these fractions had been extracted by harsh chemical treatments which might alter structural aspects of the polymers.

Given normal wall assembly there is good evidence that extensibility is directly modulated by activities in

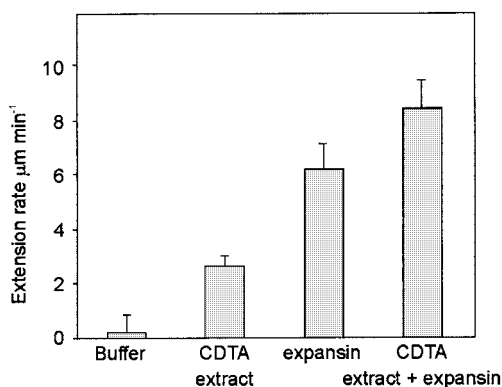


Figure 2. Effects of pectin-rich wall extracts on *in vitro* extension of cucumber hypocotyls. Cucumber seedlings were grown as described in Figure 1. The upper 3 cm of the hypocotyls was excised and homogenised in a Waring blender with an equal volume (to fresh weight) of wash buffer (25 mM Hepes, 2 mM EDTA, 2 mM DTT pH 6.8) and then washed 3 times this buffer. Wall residues were then extracted in wash buffer containing 1 M NaCl for 1 h at room temperature. The residues were then retained on a nylon filter and then suspended and washed in buffer-equilibrated phenol (pH 7.8). Wall residues were pelleted by centrifugation and then washed 3 times in large volumes of 95% ethanol to remove the phenol. Ethanol-washed wall residues were retained on a nylon filter and left to air-dry. These residues were then suspended in 50 mM CDTA and left to stir gently overnight. The extract was filtered through nylon mesh, dialysed against distilled water, and freeze-dried. CDTA extracts were dissolved at 50 mg/ml in 50 mM sodium acetate pH 4.5 for assays. Expansins were prepared from cucumber hypocotyls by ammonium sulfate precipitation of the 1 M NaCl extract as described by McQueen-Mason *et al.* (1992). Extension measurements were carried out as described in the legend to Figure 1. Data are averages and standard errors of 6 measurements.

the wall itself. It is important to bear in mind that most of the polymers in the cell walls of growing cells are not covalently connected to one another, and that the overall integrity of the wall is supported by large collections of non-covalent interactions between these polymers. This indicates that cell wall integrity and mechanical properties will largely be determined by three factors: the size of polymers in the wall, the degree of polymer entanglement, and the interactions among polymers. Changes in any of these three variables could, in theory, be a means of altering wall extensibility.

Most recent studies of cell wall loosening have generally focused on an enzymatic basis for cell wall extension. An exception to this is a novel wall-loosening mechanism proposed by Fry (1998). This mechanism involves the non-enzymatic cleavage of wall polysaccharides by millimolar amounts of L-ascorbate. The proposed mechanism involves the reduction of O_2 and Cu^{2+} by ascorbate in the wall to

form H_2O_2 and Cu^+ , which then react to form hydroxyl radicals ($\cdot OH$). These radicals then oxidatively cleave polysaccharide chains within the wall. Using an *in vitro* approach, Fry demonstrated that ascorbate-generated $\cdot OH$ could fragment xyloglucan under physiologically relevant conditions. As ascorbate, H_2O_2 and Cu^+ are naturally occurring wall components it is possible that $\cdot OH$ radicals could be generated *in vivo* and thus lead to wall loosening through the scission of wall polysaccharides. Recently, Schweikert *et al.* (2000), also demonstrated the potential for non-enzymic cleavage of cell wall polysaccharides. In this instance, $\cdot OH$ radicals are generated from O_2 by peroxidases. Whether these processes actually do occur *in vivo* and what contribution they make to cell extension remains to be established.

Wall-modifying enzymes

Expansins

Expansins were first identified in 1992 as mediators of acid-induced wall extension (McQueen-Mason *et al.*, 1992). That is, they catalyse cell wall extension, or 'creep', in response to a unidirectional extensive force. In addition to playing a key role in cell expansion and hence growth, expansins have since been ascribed several diverse roles in plant development, including leaf organogenesis (Fleming *et al.*, 1997), fruit softening (Rose *et al.*, 1997) and wall disassembly (Cho and Cosgrove, 2000). The general properties of expansins have been reviewed in several recent reviews, and we refer the reader to them (McQueen-Mason and Rochange, 1999; Cosgrove, 1999, 2000a, b). The aim of this section of the review is to focus specifically on the molecular aspects of expansins and how these relate to their biochemical mode of action.

The molecular cloning of two expansins from cucumber hypocotyls by Shcherban *et al.* (1995), opened the door to the identification of expansins from a wide-range of higher plants and organs, such as rice internodes (Cho and Kende, 1997a, b), tomato and strawberry fruits (Rose *et al.*, 1997; Civello *et al.*, 1999), cotton fibre (Shimizu *et al.*, 1997) and soybean cell cultures (Downes and Crowell, 1998). Analysis of the accumulating cDNAs has revealed that expansins have distinct and unique structures and can be classified into two subgroups, α and β , on the basis of substrate specificity and sequence similarities.

α -Expansins

α -Expansins were the first expansins to be characterised at both the biochemical and molecular level and, as such, more is understood about their biochemistry and physiology than about β -expansins. They comprise a large multigene family with 58 full-length or partial ESTs from 14 higher-plant species deposited in the GenBank database to date (for current listing, see <http://www.bio.psu.edu/expansins/>). This large number of isoforms within each gene family may reflect levels of control of wall loosening. In this case each isoform may act on different wall polymers and expression could be regulated by developmental or hormonal signals. Accumulated data support this notion. For example, McQueen-Mason and Cosgrove (1995) showed that two separate expansin isoforms purified from cucumber hypocotyls showed quite distinct effects on stress relaxation properties of hypocotyls and also showed small differences in pH optima. Cho and Kende (1997b, 1998) first demonstrated specific and distinct expression patterns of expansin transcripts in deep water rice. Subsequent studies demonstrated distinctive expression patterns in ripening tomato fruit (Brummell *et al.*, 1997, 1999; Rose *et al.*, 1997, 2000), leaf primordia initiation (Fleming *et al.*, 1997), and in xylem cells (Im *et al.*, 2000). Probably the most convincing evidence for isoform-specific expression and proposed function has recently been demonstrated by Cho and Cosgrove (2000). The authors showed that *AtEXP10* expression was localised to growing leaves and the base of leaf pedicels. Furthermore, morphological analysis of *AtEXP10* antisense inhibited plants indicated a corresponding localised function in these tissues.

Although α -expansin isoforms may have organ-specific expression patterns, they all share a highly conserved amino acid sequence, indicating a corresponding conservation of biochemical function. The primary transcripts of α -expansins characteristically encode a protein with three discrete domains. A signal peptide (typically 22–25 amino acids), which directs the nascent polypeptide into the ER/Golgi and is removed before the mature protein is secreted into the cell wall. A putative polysaccharide-binding domain (ca. 10 kDa) at the carboxyl terminus, which contains a series of conserved tryptophan residues and, resting between these two domains, a cysteine-rich region with limited, but significant, sequence similarity to family-45 endoglucanases (EG45-like domain). The mature protein has a molecular mass of 25–27 kDa

and does not appear to possess sites for *N*- or *O*-linked glycosylation.

β -Expansins

The major components of grass pollen are the Group 1 allergens (Esch and Klapper, 1989). These small glycoproteins share a weak sequence similarity to α -expansins (Shcherban *et al.*, 1995) and have been shown to induce cell wall expansion or 'creep' (Cosgrove *et al.*, 1997). It is believed that these proteins soften the stigma and stylar tissue *in vivo*, allowing the penetration of the pollen tube into the maternal tissue. Based on these studies Group 1 allergens and related sequences were designated as β -expansins, referring to their similar rheological effects on the cell wall as α -expansins, whilst indicating their sequence divergence. In common with α -expansins, β -expansins also comprise a multigene family. From sequences so far deposited in the TIGR database (<http://www.tigr.org>), it appears that β -expansins are more abundant in Type II walls (e.g. rice has 14 α -expansins and 15 β -expansins), than in Type I walls (e.g. *Arabidopsis* has 23 α -expansins and 3 β -expansins). Although it remains to be investigated thoroughly, these distribution patterns are at least consistent with apparent substrate preferences.

Expansin mode of action

Despite considerable knowledge of many aspects of expansin biochemistry, molecular biology and effects on wall rheology, the precise mechanism of how expansin makes the cell wall more extensible has yet to be resolved. Current models of expansin action are based on the interpretation of early studies of expansin biochemistry *in vitro* (McQueen-Mason *et al.*, 1992, 1993; McQueen-Mason and Cosgrove, 1994, 1995) and on similarities in protein sequence to bacterial cellulases (Davies *et al.*, 1995).

Only a limited (ca. 25%) amino acid identity exists between the α and β families. However, there are several conserved motifs within both expansin classes, which presumably have essential catalytic or structural roles, uniting the two expansin classes in a shared function. The most notable of these features are several paired cysteine residues in the N-terminus, a series of tryptophan residues at the C-terminus, and a conserved 'HFD' box in the central domain. We now examine these features in turn and explore how they might be functionally interpreted given the biochemical characteristics of expansin rheology.

The spacing between the C-terminal tryptophan residues shares similarity to that of the cellulose binding domains of some bacterial cellulases (Gilkes *et al.*, 1991; Hamamoto *et al.*, 1992). As α -expansins are observed to bind tightly to cell walls (unpublished data) it has been proposed that the C-terminal domain is involved with cellulose binding and serves to unite the catalytic domain with its substrate (Cosgrove, 1999). However, there are observations which question that tight binding is essential for expansin activity. For instance, the maize β -expansin does not bind strongly to the cell wall but nevertheless exhibits strong wall loosening activity. Alternative roles for the cellulose-binding domain (CBD) may still involve the anchoring of expansin within the cell wall, but in this case the anchor prevents expansins secreted by one cell influencing the growth of neighbouring cells.

The conserved cysteine residues within the N-terminal half of the expansin protein have similar spacing to cysteines in the chitin-binding domain of wheat germ agglutinin (Wright *et al.*, 1991). The cysteine residues in this lectin form a series of intramolecular disulfide bonds that stabilise the protein structure. It has been proposed that expansin may be folded and stabilised in an analogous manner (Shcherban *et al.*, 1995). The limited knowledge of expansin protein chemistry appears to substantiate the presence and importance of disulfide bonds for folding and concomitant activity. Recombinant α -expansins, expressed in either bacterial or baculovirus systems, require reducing agents in the extraction buffer to produce the same electrophoretic mobility as native expansin. Even under these conditions recombinant proteins consistently show far lower specific activity than native expansin and tend to form insoluble aggregates at higher protein concentrations, suggestive of improper bond formation and incorrectly folded proteins (V. Filatov and S. McQueen-Mason, unpublished data).

Perhaps the most intriguing molecular conservation between expansins is within the central domain. This region shares several characteristics of the catalytic core of family-45 endoglucanases and thus is believed to form the catalytic domain of expansin protein (Cosgrove, 1997). Although sequence similarity to these endoglucanases is slight, the residues which are conserved, the aspartate in the 'HFD' box and the second aspartate close to the N-terminus, are also the key residues at the catalytic site of the endoglucanases (Davies *et al.*, 1995). In addition, conservation of several pairs of cysteine residues within this domain

suggests that disulfide bonds would form and fold the protein enabling the two catalytic aspartates to reside on either side of a substrate binding groove. However, despite similarities in sequence expansins exhibit neither endoglucanase activity (McQueen-Mason and Cosgrove, 1995) nor related transglycosylase activity (McQueen-Mason *et al.*, 1993).

So how does expansin catalyse cell wall extension? Current hypotheses propose that the catalytic domain disrupts non-covalent bonds between wall polysaccharides, thus acting as a kind of 'molecular grease' that allows polymers to slide passed each other. This catalytic action may be facilitated through the cellulose binding domain, which would allow the protein to move along a single cellulose polymer, weakening the tethers of cross-linking glycans as it progresses. This model is consistent with the effect of expansins on the mechanical strength of paper (McQueen-Mason and Cosgrove, 1994), and the reversibility and time-dependence of expansin action (McQueen-Mason and Cosgrove, 1995). However, the question still remains as to how expansin catalyses the disruption of hydrogen bonds between the cellulose/matrix glycan polymers. The exploitation of recombinant proteins harbouring mutations in the putative catalytic residues should throw light on the elusive mechanism of cell wall loosening by expansin.

Substrate specificity has been examined in only one expansin isoform. Whitney *et al.* (1999), examined the activity of recombinant *CsExp1* on cellulose-composite materials made by *Acetobacter* cultures. This expansin displayed high activity on composites containing tamarind xyloglucan, but no activity was detected on similar composites made with glucomannan or galactomannan. This suggests a clear substrate specificity for xyloglucan. Binding studies with the same expansin showed that the protein had little binding affinity for xyloglucans in solution. The expansin showed low affinity for pure crystalline cellulose, but high affinity to complexes involving xyloglucan (or other matrix glycans) and cellulose. Expansin also showed higher binding affinity for swollen cellulose than for crystalline cellulose (McQueen-Mason and Cosgrove, 1995). These data suggest that the protein binds to the junction between crystalline cellulose and soluble glucan chains, whilst the activity data from the composites appears to indicate that the protein is discriminating in the types of polysaccharide it can displace from microfibrils.

Yieldin

There are only two classes of enzymes shown to directly modulate wall mechanical properties. Of these, expansins are the best known and best characterised. However, a new class of enzyme, referred to as 'yieldin', has been reported recently that also modulates wall extension *in vitro* (Okamoto-Nakazato *et al.*, 2000). Yieldins are characterised by their ability to lower the yield threshold for extension in cowpea hypocotyls. The yield threshold is the minimum force necessary to drive the extension of a material. In contrast, expansins catalyse the long-term extension (creep) of cell walls. Yieldins were purified to two bands on SDS-PAGE of around 30 kDa. The coding sequence for a yieldin has been obtained based on information from partial protein sequencing. The identity of the protein encoded by this gene has been confirmed by demonstrating that recombinant protein also has yieldin activity on *Vigna* hypocotyls. The predicted protein sequence of the cloned DNA reveals that yieldin has high amino acid similarity to acidic endochitinases. Although the recombinant protein possessed yieldin activity, it lacked chitinase activity. Given this lack of chitinase activity and the fact that it is generally accepted that plant cell walls do not contain chitin, it seems unlikely that chitin hydrolysis is the mechanism of yieldin action. Whether yieldin proves to be a hydrolytic enzyme or work by a more subtle mechanism remains to be seen.

Xyloglucan endotransglycosylases

The most abundant cross-linking glycan in dicot primary walls is xyloglucan. Given the primary role of this polysaccharide in cell wall architecture it is easy to imagine that factors that modify xyloglucan cross-linking would play a key role in cell wall loosening and expansion. Xyloglucan endotransglycosylases (XETs) were first identified and characterised by Fry *et al.* (1992) and Nishitani and Tominaga (1992). XETs predominantly display true transglycosylase activity, catalysing the cleavage of xyloglucan chains and grafting of the resulting half chain to the non-reducing end of a second xyloglucan chain. However, some species of XET can catalyse the transfer of xyloglucan to a water molecule and, as such, are simply endo-(1→4) β -D-glucanases. Although the exact mechanism of XET endotransglycosylase or endoglucanase activity has yet to be elucidated, a stable

covalent glycosyl-enzyme intermediate exists prior to transfer of the glycosyl moiety to its donor substrate (Sulova *et al.*, 1998), and this relatively stable intermediate can be exploited for biochemical purification of the enzyme (Steele and Fry, 1999). In addition to its potential ability to alter and loosen the cell wall matrix, studies have shown a strong correlation between XET expression and activity to areas of cell expansion (Fry *et al.*, 1992; Xu *et al.*, 1996; Visserberg *et al.*, 2000). Given these data it is somewhat surprising that, in contrast to expansins, XETs induce neither unilateral cell wall extension *in vitro* (McQueen-Mason *et al.*, 1993) nor do they have any synergistic interaction with expansins.

Several functions for XETs have been proposed, the most prominent of which is its role in wall biogenesis. As growing plant cells expand the walls do not become thinner, thus new wall material must be incorporated into the extending matrix. Nascent xyloglucan chains are secreted into the wall matrix and covalently linked to existing xyloglucan chains, maintaining the integrity of the wall architecture (Nishitani, 1997). It has been proposed that XETs participate in this process, although as yet the evidence for this is only indirect (Thompson *et al.*, 1997). XETs have also been ascribed roles in fruit ripening (Redgwell and Fry, 1993; Arrowsmith and de Silva, 1995; Schroder *et al.*, 1998; see also Brummell and Harpster, 2001, this issue) and flooding-induced aerenchyma formation (Saab and Sachs, 1996). Whatever the function of XETs probably the key-determining factor surrounding physiological aspects of this enzyme is substrate availability. If XET activity increases as xyloglucan is secreted into the cell walls, polymers will assemble around nascent cellulose microfibrils and wall biogenesis will occur. If xyloglucan is not available, XET activity will probably result in rearrangement and/or degradation of existing xyloglucan polymers. Thus, any presumptive physiological role must therefore take into account the complex interplay between XET activity and the levels of nascent xyloglucan secretion.

Recent advances in the molecular aspects of XETs have given further clues to the function of these enzymes *in vivo*. Systematic sequencing of plant genomes has revealed the existence of large XET and XET-related (XTRs) gene families (reviewed by Campbell and Braam, 1999b). Of the 14 species so far examined, 46 XET sequences have been identified, with 21 XETs or XTRs in the genome of *Arabidopsis* alone. All XETs and XTRs so far reported show a high degree (30–90%) of amino acid identity, suggesting a

shared biochemical function. XETs show greatest divergence at the C-terminus, and classification of XETs into 4 groups has been based on the relatedness of sequence in this region. However, all XETs sequences have several conserved features; a hydrophobic N-terminus which most likely acts as a signal peptide (Campbell and Braam, 1998; Rose *et al.*, 2000), a putative catalytic core which is directly preceded by N-linked glycosylation site, and, at the C-terminus, cysteine residues that have the potential to form two disulfide bonds.

The putative catalytic core features the amino acid motif DEIDFEFLG. This sequence shares significant similarity to the active site of *Bacillus* β -glucanases. *Bacillus* β -glucanases, like XETs, cleave (1 $\rightarrow$ 4) β linkages of polysaccharides within glucan backbones but differ from XETs in that their activity is purely hydrolytic. Recently, researchers have exploited the identity between *Bacillus* glucanases and XETs to target specific amino acid residues for site-directed mutagenesis. Campbell and Braam (1999a) have shown that altering the catalytic glutamate to a glutamine eliminates >98% of the enzyme activity, thus demonstrating that this residue is essential for catalysis. To date four proteins have been isolated that do not contain this highly conserved motif. Of these, one which provokes particular interest is the *Nasturtium* seed XET (NXG), which shows mostly hydrolytic activity not transglycosylase activity *in vivo*. This suggests that differences in activity result from slight structural changes in or around the catalytic site. However, in NXG the first phenylalanine residue is replaced with isoleucine and, therefore, substitution would maintain the non-polar, uncharged nature of the residues and is unlikely to affect the structure or catalytic ability of the active site.

Sequence divergence within the carboxyl terminal portions of XETs hints at differences in function of the corresponding isoenzymes. Campbell and Braam (1999a), have also shown subtle differences in the temperature, pH optima, and substrate specificity of four C-terminal divergent *Arabidopsis* XETs when activity was assessed *in vitro*. These subtle differences could translate into specific physiological roles *in vivo*. It has been speculated that the large XET family reflect delegation of a specific XETs to a target organ, tissue, or cell and, as an extension to this, the regulation of certain XET species by developmental or hormonal signals (Xu *et al.*, 1995, 1996).

Future research into XETs will undoubtedly aim to elucidate the precise cellular and physiological func-

tions of these enzymes and pinpoint their precise roles in modifying cell walls during plant growth and development.

Endoglucanases

In the 1970s biochemical studies were undertaken to examine the metabolism of wall polymers during extension. Labavitch and Ray (1974), showed that auxin-stimulated growth was accompanied by the release of soluble xyloglucan fragments from pea cell walls. The conclusion of these studies was that the release of xyloglucans resulted from biochemical activities in the wall associated with extension. Thus was born the hypothesis that the action of wall hydrolases, especially β -glucanases, might induce wall extension.

Subsequent studies have also revealed measurable decreases in the molecular mass of xyloglucans during growth in pea (Talbot and Ray, 1992), and azuki bean (Nishitani and Masuda, 1983). Inherent in the general hypothesis is that decreasing the molecular mass of a polymer will also decrease its viscosity. Decreasing the viscosity of major wall polymers will most likely decrease the stiffness of the wall, thus increasing the rate of extension. Considerable indirect support for the role of β -glucanases in wall extension has accumulated. Labrador and Nevins (1989) showed that treatment with β -glucanase could enhance elongation in maize coleoptiles. In addition, Inouhe and Nevins (1991) showed that antibodies raised against a maize glucanase could partially block auxin-induced growth. Similarly, Hoson *et al.* (1991) demonstrated that antibodies that bound xyloglucans could block the decrease in xyloglucan molecular mass, and diminish extension during auxin-induced growth in azuki beans. However, as in the case of XETs, there is no evidence that endoglucanases can directly modulate wall extensibility. In fact, Cosgrove and Durachko (1994) showed that β -glucanase treatment did not induce wall extension *in vitro* although it did weaken the wall.

It is now apparent that (1 $\rightarrow$ 4) β -D-glucanases comprise a multi-gene family in higher plants. Studies in tomato (Brummell *et al.*, 1997; Catalá *et al.*, 1997) have examined the expression patterns of various members of this family. Different isoforms have been shown to be expressed in ripening fruit and abscission zones, yet others are associated with cell expansion. Catalá *et al.* (1997) showed that the transcript of a glucanase gene associated with growing hypocotyls was present in both growing and mature

tissues, and that auxin induction of expression of this gene was much slower than the effects of auxin on growth. Certainly such an enzyme may be involved in xyloglucan turnover during growth although the pattern of its expression is not suggestive of an enzyme acting as a wall-loosening agent.

In summary, the evidence for a direct role for endo- β -glucanases in cell wall loosening is mixed. As previously discussed, the most convincing evidence for such a role in growth came from the *korrigan* mutant (Nicol *et al.*, 1998), but this particular gene appears involved in wall synthesis rather than loosening. The patterns of activity and expression of glucanases suggest that they certainly are involved in the metabolism xyloglucan and other matrix glycans during growth but a direct role in wall loosening awaits confirmation.

Wall rigidification

Once a cell has reached its final size, wall extension comes to a halt. In parallel with the cessation of growth the cell wall becomes more rigid, preventing turgor-induced extension. Wall rigidification could be brought about by three possible events: (1) the deposition of new wall polymers, (2) an increase in wall cross-linking activity, or (3) a halt in production of wall-loosening proteins, such as expansins. Evidence to date suggests that a combination of these events rather than a single factor leads to rigidification of the wall *in vivo*.

Expansins and wall rigidification

McQueen-Mason *et al.* (1992) showed that expansins extracted from growing regions of cucumber hypocotyls displayed strong expansin activity, whilst those from the base, where growth had stopped, exhibited no extractable activity. Northern blot analyses, with *CsExp1* used as probe, show the pattern of transcript accumulation in cucumber hypocotyls to be highest in the growing region and then gradually decline along the length of the hypocotyl (Figure 3). This pattern closely mirrors that of cell elongation and acid-induced expansion. Cosgrove and Li (1993), have also shown similar patterns of expansin-induced extension in oat coleoptiles. These data strongly suggest that the cessation of growth involves the progressive decrease in expansin as the wall matures. Contrary to this notion, McQueen-Mason (1995) showed that while expansin activity was probably sufficient to account for wall extension in the growing cells, the loss

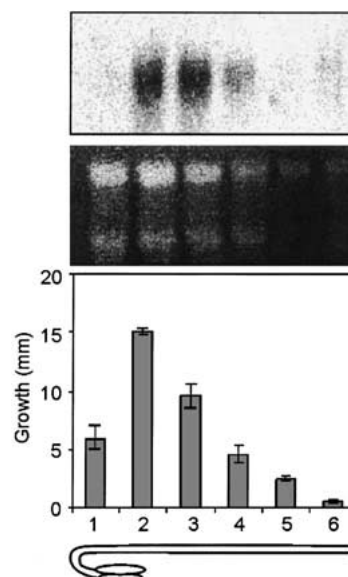


Figure 3. The distribution of *CsExp1* mRNA abundance in etiolated cucumber hypocotyls. A (top). Northern blot probed with *CsExp1*. B (middle). Ethidium bromide-stained denaturing gel. Cucumber (*Cucumis sativa* cv. Burpee Pickler) hypocotyls were germinated and grown in the dark for 4 days at 27 °C. The apical 3 cm including the apical hook was excised and cut into 6 mm × 5 mm sections such that the apical hook comprises the first 5 mm section. Sectioning was carried out under green light conditions and the sections frozen in liquid nitrogen immediately after cutting. Total RNA was extracted from the hypocotyl sections by hot SDS/phenol extraction and lithium chloride precipitation. RNA was separated by formaldehyde denaturing gel electrophoresis and transferred onto nylon membrane by capillary blotting. A DIG-labelled DNA probe was prepared from a full-length clone of *CsExp1* by random prime labelling. Blots were hybridised overnight in 250 mM Na₂HPO₄, 7% SDS at 65 °C and subsequently washed in 20 mM Na₂HPO₄, 5% SDS at 65 °C, followed by 20 mM Na₂HPO₄, 1% SDS at 65 °C. The probe was detected by a chemiluminescence enzyme immunoassay using a DIG Luminescent Detection Kit according to the manufacturers' instructions (Boehringer-Mannheim). For determination of the distribution of growth in cucumber hypocotyls, 4-day old dark-grown etiolated hypocotyls were marked with spots of oil paint at 5 mm intervals from the hypocotyl tip and grown for further 24 h in the dark; displacement of the marks was measured after 24 h, *n* = 30. All manipulations were carried out under green light conditions.

of expansin activity from the walls significantly lagged behind the cessation of growth. This suggests other events are important in wall rigidification and probably make it unresponsive to the diminishing level of expansins.

Modifications of the polymer networks

If loss of expansins is not on its own responsible for the decrease in wall extensibility, then alternative mechanisms must be considered. Potentially, rigid-

ification could involve modification of any of the polymer networks in the wall. Indeed, Carpita (1984) showed that arabinoxylan polymers become increasingly less branched in maturing maize coleoptiles permitting the polymer to form a tighter complex with cellulose. The removal of mixed-linked (1→4)β-D-glucans also mirrors cessation of growth in these walls. These changes in the polymer network could make the sites at which wall-modifying enzymes bind less accessible and thereby halt further extension of the cell wall.

Although the cellulose/matrix glycan network appears to determine the extensibility of the growing primary wall and to provide the target for wall loosening events, cross-linking of pectins or structural proteins, which form co-extensive networks, could constrain the extension of the cellulose/glycan network preventing cell expansion. Studies have shown that Type I cell walls are extensin and pectin-rich, whilst Type II cell walls are polyphenolic-rich (reviewed by Carpita and Gibeaut (1993). These pronounced differences in wall composition between grasses and dicots suggest that different mechanisms of primary wall rigidification might operate in these plants. There is considerable evidence that pectin cross-linking may be a primary wall rigidifying mechanism in dicots (Yamaoka *et al.*, 1993; McCann *et al.*, 1994), whereas in the grasses phenolic coupling between polysaccharides plays a key role in rigidification (Carpita and Gibeaut, 1993).

In Type I walls polygalacturonan is secreted into the primary wall in a highly methyl-esterified form (Kauss and Hassid, 1967). These methyl esters are introduced during pectin biosynthesis in the Golgi and mask the carboxyl groups of the galacturonyl residues. Subsequent to secretion into the wall, these methyl groups may be removed through the action of pectin methyl-esterases (PMEs) (Knox *et al.*, 1990).

Removal of methyl esters from polygalacturonan has two important effects. Firstly, this exposes the carboxyl group, thus increasing both the net charge of the polymer and increasing its capacity for cross-linking by cations, such as calcium. This can lead to stronger gelling of the pectins, which could in turn, be important both in rigidification as well as in strengthening cell-cell adhesion at the middle lamella. Secondly, many polygalacturonases have low activity against esterified pectins but high activity against the acidic forms. Hence, demethylation can render the pectins more labile to the action of hydrolases. This is clearly the case in fruit ripening, where antisense inhibition of PME leads to pectins that maintain higher degrees

of methyl esterification, resulting in decreased depolymerization of pectins by polygalacturonase (Hall *et al.*, 1993).

A number of studies have shown that PME activity shows an inverse correlation with growth rate in expanding tissues, suggesting it may be involved in wall rigidification. More direct evidence for such a role for PME came from some initial studies by McQueen-Mason and Cosgrove (1995), who demonstrated that the loss in sensitivity of cucumber hypocotyl walls to expansin action during wall rigidification probably resulted from PME activity. These authors also showed that incubating cell walls with exogenous PME could result in wall rigidification. Thus, it appears that pectin cross-linking may provide the primary means of wall rigidification in dicotyledons.

Peroxidases

The cross-linking of polymers by oxidative coupling through the action of peroxidases has long been thought to provide a route for wall rigidification. This might occur through the cross-linking/polymerisation of structural proteins, such as hydroxyproline-rich proteins (extensins) leading to a strong network in the wall. Alternatively, it might be through the cross-linking of polysaccharides through phenolic dimerisation. The grass arabinoxylans exist as feruloyl esters and can be cross-linked by peroxidase action (Brett *et al.*, 1999; Rizk *et al.*, 2000). These can subsequently enter free-radical condensation reactions in the wall leading to the formation of ferulate dimers and, hence, polysaccharide cross-links. These cross-links could provide an effective means of wall rigidification catalysed by peroxidases.

Several groups have shown that peroxidase activity in plants increases with the cessation of growth, indicating that the two phenomena are linked in some plants (Goldberg *et al.*, 1987; Tan *et al.*, 1992). However, in other plants there is no clear relationship between peroxidase activity and growth (Swoap *et al.*, 1993; Casal *et al.*, 1994). Schopfer (1996) reported that treatment with H₂O₂ leads to wall rigidification and to reduced growth in maize coleoptiles. He further demonstrated that these tissues contain considerable phenolic compounds in their cell walls and that the level of these was much higher in tissues where growth had ceased (Schindler *et al.*, 1997).

As mentioned earlier, plant cell walls contain considerable quantities of structural proteins. Some classes of these proteins, such as extensins, are poly-

merised in the wall to form a strong network. Such proteins are highly expressed in response to pathogen ingress and in parts of the plant subject to mechanical stress (Esquerré-Tugayé *et al.*, 1979; Showalter *et al.*, 1985). These proteins are also present as monomers in growing cell walls but cross-linked into the wall by peroxidase action after growth has ceased (Ye *et al.*, 1991), and, as such, this action could provide a means of wall rigidification. In support of this, Brownleader *et al.* (2000) report that treatment of tomato seedlings with extensin peroxidase inhibitors resulted in increased growth.

Concluding remarks

Advances in genetics, molecular biology and biochemistry have provided new tools to investigate both structural and functional components within the growing cell wall. With sophisticated molecular and immunological techniques we are now in a position to unravel the precise relationships of wall-modifying enzymes in the milieu of matrix polysaccharides. The plant genome project is now providing an ever-increasing wealth of data, allowing more efficient schemes for the study of cell wall mutants. In addition, the availability of transgenic technologies now allows us to manipulate the cell wall and shed light on the mechanisms of wall assembly and extension. Taken as a whole, these advances provide very exciting prospects in the study of the molecular basis of cell wall extension.

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