Feedback from the wall
Emma Pilling and Herman Höfte*

The ability of cells to perceive changes in the composition and mechanical properties of their cell wall is crucial for plants to achieve coordinated growth and development. Evidence is accumulating to show that the plant cell wall, like its yeast counterpart, is capable of triggering multiple signalling pathways. The components of the cell wall that are responsible for initiating these signal responses remain unknown; however, recent technological advances in cell wall analysis may now facilitate the identification of these components and accelerate the characterisation of changes that occur in cell wall mutants.

Introduction

The cell walls of higher plants provide mechanical strength, define cell shape and thus overall plant morphology, and protect against pathogen attack. The complex polysaccharide structure of wall must therefore combine strength with the plasticity that is required for cell expansion and plant growth. In addition to controlling the mechanical aspects of plant development, the cell wall must also posses inherent signalling properties. These properties are essential not only for the role of the cell wall in defence but also for the coordination of wall synthesis and expansion between adjacent cells, and thus the development of entire plant organs. Pien et al. [17] have already shown that the role of the cell wall in plant morphogenesis stretches far beyond supplying the physical constraints that control the direction of cell expansion. Local increases in the expression of expansin, a cell wall protein that facilitates growth by promoting wall loosening (see [2]), are sufficient to trigger the formation of an entire leaf, indicating that local changes in cell wall extensibility within the meristem set in motion the entire cascade of leaf development. Several recent studies have provided evidence that changes in wall composition can also be perceived by the cell, activate feedback-signalling networks, and thus provide a sensing mechanism through which growth responses can be co-ordinated or altered appropriately.

A role for the cell wall in signalling?

The recent characterisation of several cell wall mutants has revealed a causal link between cell wall synthesis and structure and the jasmonate (JA) and ethylene signalling pathways. These mutants, el1 (ectopic lignification1) and cev1 (constitutive expression of VEGETATIVE STORAGE PROTEINS1 [VSP1]), were originally isolated from screens for ectopic lignin production and defence responses [3,4]. Recently, further characterisation has revealed that the mutations responsible for these phenotypes lie within different highly conserved regions of the CELLULOSE SYNTHASE3 (CESA3) gene and, as expected, result in profound decreases in cellulose content [5,6]. The ectopic lignification of el1 is a phenotype common to cellulose-deficient mutants: similar lignin staining patterns have been observed in root swelling1 (rsw1), a temperature-sensitive CESA1 mutant; in plants with one of several mutant alleles of korrigan, which are mutated in an endo 1,4 β-glucanase that is involved in cellulose biosynthesis; in de-etiolated3 (det3), a mutant that exhibits reduced cell expansion and decreased levels of cellulose synthesis as a consequence of decreases in the activity of a vacuolar ATPase subunit [5,7]; and in wildtype plants that have been treated with the cellulose synthase inhibitor isoxaben [3,5]. Similarly, the constitutive expression of several JA- and ethylene-responsive genes, including VSP1 and PLANT DEFENSIN1.2 (PDF1.2), observed in both cev1 and el1 is also found in wildtype plants that have been treated with isoxaben or a second cellulose synthesis inhibitor 2,6-dichlorobenzonitrile (DCB). It appears that the induction of these defence-related genes, rather than

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**Abbreviations**

AGP: arabinogalactan protein
CESA3: CELLULOSE SYNTHASE3
cev1: constitutive expression of VSP1
el1: ectopic lignification1
elp1: ectopic deposition of lignin in pith1
FT: Fourier transform
GlcNAc: N-acetyl-D-glucosaminyl
IR: infra red
JA: jasmonate
OGA: oligogalacturonide
PDF1.2: PLANT DEFENSIN1.2
pmr6: powdery mildew resistant6
pom1: pom-pom1
sos5: salt overly sensitive5
VSP1: VEGETATIVE STORAGE PROTEIN1
WAK: wall-associated kinase
WSC1: CELL WALL INTEGRITY AND STRESS RESPONSE COMPONENT1
XG: xyloglucan

**Addresses**

Laboratoire de Biologie Cellulaire, INRA, Rte de Saint Cyr, 78026 Versailles cedex, France
*e-mail: hofte@versailles.inra.fr*

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defects in the regulation of the onset of secondary wall synthesis, is responsible for the ectopic production of lignin in cellulose-deficient plants. Indeed, induction of the lignification process in cellulose-deficient mutants and in wildtype plants treated with isoxaben is coordinated with the expression of \textit{VSP1} and \textit{PDF1.2}, and with that of the gene encoding cinnamoyl CoA-reductase \textit{CCR2}, an enzyme that catalyses the first committed step in the lignin biosynthetic pathway and is normally expressed during the defence response to pathogen invasion [5**,8].

**Cell wall signalling in yeast**

The studies described above demonstrate that modification of the plant cell wall is sufficient to activate signalling pathways, but little is known about the mechanisms by which changes in wall composition and mechanical properties are perceived. In contrast, the cell wall signalling pathway in yeast is well characterised and is induced by a variety of factors that affect wall integrity, including elevated temperature [9], osmotic shock [10] and the application of mating pheromones [11].

Two stress-sensing proteins have already been identified in yeast: \textit{WSC1} and \textit{MID2} are both type-I membrane proteins that contain small cytoplasmic tails, a single transmembrane region and extracellular domains that are rich in highly glycosylated Ser/Thr residues [12,13]. Extracellular signals that are perceived by \textit{WSC1} and \textit{MID2} are transmitted to the cytoplasm through the interaction of the cytoplasmic domains of these receptors with a guanine nucleotide exchange factor (GEF), termed \textit{ROM2} [14]. This \textit{WSC1}- and \textit{MID2}-mediated activation of the cell wall signalling network eventually results in the re-localisation of the actin cytoskeleton [15] and glucan synthase [16] away from the normal site of cell wall synthesis, the growing bud, to the periphery of the whole cell. The enzyme glucan synthase is responsible for the synthesis of \(\beta\),glucan, a major component of yeast cell walls. Thus, the redistribution of this enzyme ensures the rapid and universal repair of cell wall damage [17]. No homologues of \textit{MID2} and \textit{WSC1} have been found in plants, but members of a family of receptor-like Ser/Thr protein kinases that are tightly bound to the cell wall are likely candidates for receptors with a role in cell-wall-mediated signalling. Such wall-associated kinases (WAKs), which bind pectic polysaccharides [18], have already been implicated in the response to pathogens and are upregulated after exposure to jasmonic acid and ethylene [19]. In addition, the inducible expression of the \textit{WAK4} antisense has highlighted the role of WAKs in root elongation [20*].

**Which components of the cell wall trigger the signalling response?**

Numerous studies have shown that the complex polysaccharides of the cell wall provide a wealth of potential signalling molecules that are capable of regulating plant growth.

**Oligogalacturonide fragments**

Oligogalacturonide (OGA) fragments, consisting of between 2 and 20 \(\alpha\)-1,4-galactopyranosyluronic acid (GalA) residues, are derived from pectin by the degrading activity of pathogen-secreted or endogenous plant polygalacturonases and pectate lyases. OGAs are involved in a variety of signal transduction pathways that regulate normal plant growth and development and activate defence responses (reviewed in [21]). Short OGAs are capable of inducing the expression of both allene oxide synthase, an enzyme involved in the biosynthesis of jasmonates [22], and aminocyclopropane 1-carboxylic acid oxidase, an enzyme in the ethylene biosynthesis pathway [23]. These findings suggest that both the JA and ethylene defence responses can be induced by OGAs. The mechanism by which OGAs regulate normal plant development is less well understood but, in general, the ability of OGAs to regulate growth is thought to correspond to their inhibition of auxin action [24]. Recent studies of OGA- and auxin-related growth responses in intact cucumber seedlings and in soybean cell cultures suggest, however, that OGA signalling is mediated by a separate calcium-dependent pathway [25,26].

The powdery mildew resistant6 (pmr6) mutant, recently characterised by Vogel et al. [27*], is defective in a gene that encodes a pectate lyase-like protein. Indeed, Fourier transform (FT) infra red (IR) analysis suggested that the walls of this mutant contained alterations in pectic components. The defence response conferring resistance to pathogen attack in the pmr6 mutant is not mediated by either the JA or the ethylene signalling pathways, in contrast to this response in \textit{cer1} and \textit{ell1} and the mechanism of action of OGAs. No induction of \textit{PDF1.2} was observed in the pmr6 mutant, and the pathogen susceptibility of these mutants did not change in either the \textit{coronatine insensitive1} (col1; i.e. JA insensitive) or \textit{ethylene receptor1} (etr1; i.e. ethylene insensitive) backgrounds. The pectin-degrading activity of PMR6 is yet to be demonstrated, and the mechanism by which the pmr6 mutation confers increased resistance to pathogen invasion remains to be determined.

**Xyloglucans**

The xyloglucans (XGs) that decorate cellulose microfibrils and form cross-links between them are another source of growth-regulating oligosaccharides. Early work has shown that small xyloglucan-derived oligosaccharides play a role in the regulation of plant growth [28–30], an activity that depends on the presence of a terminal L-fucose [31,32]. The role of these molecules in regulating growth has recently been questioned, however, by the finding that mutation of the \textit{mur2} gene, which encodes fucosyltransferase (AtFUT1), decreases the levels of
fucosylated xyloglucan to less than 2% of that in the wildtype but causes no obvious developmental phenotype [33]. However, work conducted by Takeda et al. [34\textsuperscript{a}] confirmed that xyloglucan metabolism plays a key role in the control of cell elongation. The incorporation of whole XGs into the cell walls of pea stem segments suppressed cell elongation, whereas integration of short-XG-derived oligosaccharides had the opposite effect and accelerated growth. Newly synthesised XG polymers are incorporated into the wall by the grafting activity of xyloglucan endotransglycosylase (XET). An abundance of short XG fragments could saturate this XET grafting activity, thus reducing the average molecular weight of XGs (and hence the viscosity of wall matrix) and/or the extent of cross-linking between XGs and microfibrils. The production of a less viscous, more flexible wall would account for the acceleration in cell growth observed following the integration of short XG fragments.

Arabinogalactan proteins

In addition to XGs and pectins, the cell wall contains a variety of highly glycosylated proteoglycans — the arabinogalactan proteins (AGPs). AGPs have always been strong candidates to be mediators of cell–cell interactions and regulators of cell growth. They form a family of highly glycosylated extracellular proteoglycans, members of which have been implicated in various plant developmental processes, including root development and cell differentiation, fertilisation, pollen-tube growth, cell division and expansion, somatic embryogenesis and programmed cell death (reviewed in [35]). Microarray analysis has indicated that many AGPs also respond to biotic and abiotic stress [36]. Indeed, the recent isolation and characterisation of the salt overly sensitive5 (sos5) mutant appears to confirm a role for AGPs in abiotic stress responses [37\textsuperscript{**}]. The sos5 mutant was isolated in a screen for hypersensitive responses to salt stress. This mutant exhibits a defective cell wall and a cell-expansion phenotype only when grown under conditions of salt stress [37\textsuperscript{**}]. The sos5 mutation lies within a gene encoding a putative cell surface adhesion protein that has AGP-like and fasciclin-like domains, and a carboxy-terminal glycosylphosphatidylinositol (GPI) lipid anchor signal.

The exact mechanism through which AGPs could mediate cell wall signalling is unclear. One possibility is that the signalling capacity of AGPs could be associated with the cleavage of the GPI lipid anchor from the remainder of the AGP, which would generate both intracellular and extracellular signalling molecules. Cleaved AGPs, with their highly complex and variable glycan chains, are ideal candidates for diffusible signalling molecules. In fact, the mechanism through which AGPs regulate somatic embryogenesis is thought to involve the release of oligosaccharides from AGPs. AGPs are well-characterised promoters of somatic embryogenesis in carrot cell suspension cultures [38] and, interestingly, this promoting activity is further enhanced by incubation with carrot EP3 chitinase [39].

The supposed role of plant chitinases was originally confined to defence against pathogen invasion as their main substrate — chitin, a biopolymer of β-1,4-linked N-acetyl-D-glucosaminyl (GlcNAc) residues — is a major component of fungal cell walls but is absent from those of plants. The discovery that chitinases can also cleave the GlcNAc residues present in plant AGPs and thus promote somatic embryogenesis [39] suggests, however, that plant chitinases also play an important role in normal plant growth and development. The Arabidopsis orthologue of carrot EP3 chitinase (AtCHITIV) has recently been identified, but the pattern of AtCHITIV expression is not consistent with its having a role in somatic embryogenesis in Arabidopsis [40]. AtCHITIV is strongly expressed in root epidermal and tip-growing cells, where the characterisation of sos5 has already demonstrated the importance of AGP-like proteins in the regulation of cellulose synthesis and cell expansion. Perhaps even more convincingly, a second chitinase family member has recently been shown to be involved in cellulose biosynthesis. Like el1\textsuperscript{f}, the ectopic deposition of lignin in pitth1 (elp1) mutant (which is defective in a chitinase-like gene [AtCTL1]) displays abhorrent lignin formation; like cecl, elp1 overproduces ethylene [41\textsuperscript{*}]. Atctl1 is identical to pom-pom1 (pom1), a member of the CORE family of root expansion mutants that display an abnormally expanded root phenotype only under conditions that stimulate a rapid rate of root growth [42]. The pom1 mutation results in increased radial expansion [42] and an inhibition of cellulose synthesis [43\textsuperscript{*}], providing yet further evidence that chitinases may play an important role in cell wall signalling pathways.

The studies described above provide strong hints as to the identity of some of the components of the cell wall signalling pathway. Nevertheless, a thorough analysis of the changes in cell wall composition that occur in cell wall mutants is necessary before further insight can be gained into which cell wall components are key in triggering the signalling response. The recent advances in techniques for cell wall analysis that are described below will in no doubt be invaluable tools in conducting these studies.

Miniatrised analytical techniques for studying cell walls

Little is known about the cellular machinery that underlies cell wall synthesis and deposition. Mutants have been very instructive in elucidating the molecular machinery underlying these processes, but the analysis of cell wall changes remains a major bottleneck. Recently, major advances have been achieved in the miniaturisation of techniques for the analysis of polysaccharides and in the adaptation of micro-spectroscopic techniques to interpret complex mixtures of polymers found in the cell wall. Lerouxel and colleagues [44\textsuperscript{*}] described a strategy that is
based on the hydrolysis of crude wall preparations with specific hydrolases, and the subsequent analysis of the fragments using matrix-assisted laser desorption time of flight (MALDI-TOF) mass spectrometry (MS). Specifically, they used this technique for the analysis of XGs using a XG-specific endoglucanase [44*]. This endoglucanase released fragments with defined molecular weights that could be easily identified using MALDI-TOF MS. The technique is rapid and reproducible, and the amount of material that it requires could be scaled down to a single dark-grown Arabidopsis seedling. In principle, other polysaccharides — such as xylans, pectins and AGPs — could also be analysed in a similar manner, using the large number of polysaccharide-modifying enzymes that have been isolated from microorganisms to generate characteristic oligosaccharide fragments. Further miniaturisation of the technique is underway; for instance, for the study of specific cell-types isolated using laser-capture dissection of frozen tissue sections (M Pauly, personal communication).

Information on the chemical composition of cell walls can also be obtained using FT-IR spectroscopy. In the IR spectrometer, a source emits a beam of IR radiation that is passed through the sample. Chemical bonds absorb radiation from the beam at specific frequencies, and a plot of absorbance against frequency is generated. This absorbance spectrum contains information not only on the nature and abundance of specific chemical components, but also on the intermolecular interactions between these components. However, FT-IR spectra from total cell wall preparations are extremely complex and almost impossible to interpret visually, and so obtaining specific structural information from these spectra remains a major challenge.

Recently, our group reported on an alternative method that can be used to obtain information on the nature of the cell wall defects in mutants [43*]. Cluster analysis was carried out on FT-IR spectra sampled from the dark-grown hypocotyls of a large collection of Arabidopsis mutants with various cell wall defects. Information on the nature of the defects in novel samples could be deduced from their clustering with mutants that have known cell wall defects. FT-IR microscopy can also be used to obtain a chemical image of a tissue. Carpita et al. [45] scanned sections of maize coleoptiles, and could distinguish xylem, phloem and epidermal cell layers on the basis of their FT-IR spectra. The FT-IR technique has also been used to study the dynamic behaviour of polymers in walls of fully hydrated living onion epidermis cells to which a tensile stress was applied [46]. The results show that the cellulose-hemicellulose network reoriented as a result of the stress with a kinetic behaviour that was distinct from that of the pectin matrix. This finding confirms that the two polymer networks are independently organised in the cell wall. Further technological improvements continue to open new possibilities in this area: new FT-IR microscopes with multiple array detectors can be used to obtain one-shot two-dimensional images of an entire tissue, whereas chemical imaging can be used to follow changes in cell wall architecture during the growth and differentiation of specific cell types. All of these techniques will most likely become essential tools for many plant biologists, and will be vital in our pursuit of a clearer understanding of the changes in cell wall composition that trigger signalling pathways.

Conclusions

The discovery that the plant cell wall plays an integral role in multiple signalling pathways suggests that the cell wall properties of plants, like those of yeast, are constantly sensed to co-ordinate growth and defence responses. Virtually nothing is known about how changes in cell wall properties are perceived and how these signals are transduced. Feedback signalling may well involve stress sensors akin to WSC1 and MID2, which have been identified in yeast. Vital components of the cell wall signalling network in plants might include receptor kinases that are capable of binding to specific wall components, such as members of the WAK family; the release of oligosaccharides in response to certain conditions; and the activities of integral cell wall proteins such as AGPs. Obtaining a coherent picture from the initial findings discussed here is an impossible task. Nevertheless, these observations, in combination with new techniques for the analysis of cell wall composition, appear to open the door to further investigation of the role that the cell wall plays in feedback signalling during plant growth and development.

Note added in proof

Recently, further characterisation of the pmr4 mutant has suggested that callose, which is normally deposited at wound sites following pathogen invasion, may also be involved in feedback signalling from the wall. Surprisingly, Nishimura et al. [47**] showed that pmr4 mutants display increased resistance to pathogen attack, despite their lack of the callose synthase gene that is responsible for the production of callose in response to biotic and abiotic stress. The analysis of double mutants and DNA-chip experiments have revealed that the increased resistance of pmr4 is associated with the upregulation of the salicylic acid (SA) defence signalling pathway. Nishimura et al. suggest that, in wildtype plants, either the PMR4 protein or defence-related callose deposition function to negatively regulate SA-mediated defence responses.

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References and recommended reading

Papers of particular interest, published within the annual period of review, have been highlighted as:

• of special interest
**• of outstanding interest**


A mutation in a putative pectate lyase, an enzyme that hydrolyses pectins, leads to a pleiotropic phenotype that involves reduced cell size and increased resistance to powdery mildew in *Arabidopsis*. This resistance does not involve ethylene or jasmonic acid signaling. Comparison of the FT-IR spectra of mutants and wild-type plants suggests that the mutant has altered pectins. The mutant is fully susceptible to *Pseudomonas* and *Peronospora*, indicating that the putative pectate lyase is specifically required for compatible interactions with powdery mildew.


In vivo xyloglucan chains inhibit elongation. Hence, it is conceivable that xyloglucans promote cell elongation in isolated pea segments. In contrast, larger the action of the grafting enzyme xyloglucan endo-transglycosylase, Xyloglucan oligosaccharides, which are integrated into cell walls through the wall.

Arabidopsis thaliana lacks fucosylated xyloglucan because of a lesion in the wall. This AGP may play a role in the adhesion of the plasma membrane to the wall.

The authors present a procedure for the classification of cell walls in mutants and during normal plant development.

The authors of this paper describe the first arabinogalactan protein (AGP) mutant with a clearly defined phenotype. These mutant plants have increased sensitivity to salt stress, which causes the swelling of the cells in the elongation zone of the root. The wall architecture of these cells is perturbed and the plasma membrane is frequently detached from the wall. This AGP may play a role in the adhesion of the plasma membrane to the wall.

Arabidopsis SOS5 is allelic with mur1. This method has great potential for the characterisation of cell walls in mutants and during normal plant development.

Arabidopsis seedlings were used to analyse cell wall polysaccharides in samples as small as a single cell. This technique will simplify the analysis of cell wall dynamics of plant cell wall polysaccharides studied by Fourier-transform infrared spectroscopy. Plant Physiology 2002, 127:551-565.

Enzymatic fingerprinting using MALDI-TOF mass spectrometry can be used to analyse cell wall polysaccharides in samples as small as a single Arabidopsis seedling. This technique will simplify the analysis of cell wall mutants.

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