Irritable Walls: The Plant Extracellular Matrix and Signaling[1]

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CELL WALLS AFFECT CELL PHYSIOLOGY

The plant cell wall is a dynamic network of carbohydrates and proteins of enormous structural complexity that plays crucial roles in all aspects of plant life. Advances in molecular physiology and genetics have shed light on the relations between structure and function of various cell wall constituents. The application of compounds interfering with plant cell wall structure and biosynthesis as well as molecular genetics as a commonly used tool in plant science have generated several paradigms for the roles of various cell wall polymers in the living organism. Many mutants defective in cellulose, pectin, hemicellulose, lignin, or cell wall and cell surface proteins are available (Table I; Humphrey et al., 2007). Drugs interfering with cellulose biosynthesis, such as isoxaben (Scheible et al., 2001), thaxtomin A (Scheible et al., 2003), and 2,6-dichlorobenzonitrile (DCB; Peng et al., 2001), or β-glucosyl-Yariv dyes (βGlcY; Yariv et al., 1962) that specifically bind to arabinogalactan proteins (AGPs), are used to study the biological roles of specific cell wall polymers in wild-type plants and cell cultures. Phenotypes such as stunted growth, abnormal cellular shape, and altered tensile strength are suggestive of the cell wall's undisputed mechanical role (Willats and Knox, 1996; Fagard et al., 2000; Bouton et al., 2002; Ryden et al., 2003; Pena et al., 2004; Derbyshire et al., 2007). However, a second look at mutant physiology gives strong hints to a central regulatory network that monitors and controls cell wall performance and integrity (Somerville et al., 2004). Many mutants initially selected for altered disease or abiotic stress response or for constitutively expressing abiotic and biotic stress markers primarily affect cell wall biosynthesis (Table I; for review, see Pilling and Höfte, 2003). Other mutants reveal unexpected genetic interactions between different cell wall polymers (Bosca et al., 2006; Diet et al., 2006; Gille et al., 2009). Finally, nonadditive genetic interactions between cell wall defects and second site mutations in regulatory loci (Seifert et al., 2004; Hématy et al., 2007; Xu et al., 2008; Hamann et al., 2009) may pinpoint parts of the genetic system underlying cell wall performance and integrity control. In principle, this system detects functional and structural alterations in the extracellular matrix occurring throughout the normal life cycle of a plant and translates them into an appropriate corrective response. The alterations have to be detected by a specific sensorium that is most likely set to respond to deviations from a "correct" level. As in a multicellular plant, many types of cell walls exist and it can be expected that the control machinery including the correct level of set parameters underlie developmental control. Primary stimuli from the cell wall have to be transduced and integrated with other cues in order to bring about the due response, be it the repair of damaged structures or the modulation of mechanical properties in the cell wall itself or adaptive responses such as the production of antimicrobial metabolites or the restoration of osmotic balance. Such responses often involve a rapid modulation of already existing cellular machinery by posttranslational modifications as well as more long-term alterations of gene expression (Fig. 1). In most reviews on plant cell wall performance and integrity control, comparisons with the well-studied yeast pathway are invoked (Somerville et al., 2004; Humphrey et al., 2007; Hématy and Höfte, 2008; Hématy et al., 2009). Extrapolating from yeast (Levin, 2005), plant cells are expected to sense cell wall polymer structure as well as its mechanical performance that is closely linked to turgor pressure. Activated trans-plasma membrane sensor proteins might trigger a cascade of signal transduction events interwoven with other pathways that might modulate cellular functions by the activation or inhibition of specific transcription factors as well as by affecting posttranscriptional and posttranslational control of gene expression and protein function (Fig. 1). Hence, a symptomatic signature predicted for cell wall performance and integrity control is a drastic alteration in the expression of genes related to cell wall biosynthesis and remodeling (Fig. 1; Table I). There are no apparent plant orthologs to the proteins involved in yeast cell wall integrity control, leaving this area a painstaking but fascinating battleground for original discovery, and most present ideas on early events of "cell wall signaling" depend on interpolations and extrapolations from observations of cell

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Table I. *Indirect physiological alterations induced in cell wall-defective mutants or after transgenic or pharmacological interference with cell wall biosynthesis or structure*

↑, induction of; ↓, suppression of; as, antisense suppression; ASR, abiotic stress resistance; AXS, UDP-apiose/UDP-xylose synthase; CalS, callose synthase; CD, cell death, both programmed and necrotic; CE, cellulose; CesA, cellulose synthase catalytic subunit; CH, carbohydrates; DCB, 2,6-dichlorobenzonitrile; DR, disease resistance and reduced pathogen susceptibility; ET, ethylene; EXT, extensin; GM, glucomannan; GMD, GDP-D-mannose 4,6-dehydratase; HSR, hypersensitive to sugar; JA, jasmonic acid; PE, pectin; PME, pectin methyl esterase; SA, salicylic acid.

Mutant/Transgene/ Drug	Hypothetical Cell Wall Effect	Secondary Effect and Physiological Response	Reference
cesa3	Primary CE	DR; ↑ET, JA signaling; ↑VSP1 and PDF1.2 expression, THE1-dependent ↓growth, ↑lignin	Ellis et al. (2002); Cano-Delgado et al. (2003); Hématy et al. (2007)
cesa6		THE1-dependent ↓growth, ↑lignin, ↑callose	Hématy et al. (2007)
kobito		ABA insensitivity, Glc-dependent growth, Glc-insensitive germination	Brocard-Gifford et al. (2004)
elp1		THE1-dependent ↓growth, ↑lignin	Zhong et al. (2002); Hématy et al. (2007)
cesa7	Secondary CE	DR; ASR; ↓PE and XG in primary cell wall	Chen et al. (2005); Bosca et al. (2006); Hernandez-Blanco et al. (2007)
cesa4		DR	Hernandez-Blanco et al. (2007)
cesa8		DR; ASR; ↑ABA-inducible genes	Chen et al. (2005); Hernandez-Blanco et al. (2007)
pme3	PE	DR (cyst nematodes)	Hewezi et al. (2008)
pmr5		DR (independent of JA, SA, ET)	Vogel et al. (2004)
pmr6		DR (independent of JA, SA, ET)	Vogel et al. (2002)
AXSas		CD, cell wall thickening	Ahn et al. (2006)
pmr4	Lesion callose	DR; ↑SA pathway	Nishimura et al. (2003)
csla9	GM (?)	*Agrobacterium tumefaciens* resistance	Zhu et al. (2003)
mur3	XG	DR, ↑SA; HSR	Li et al. (2007); Tedman-Jones et al. (2008)
fla4	AGP	Salt-oversensitive root growth	Shi et al. (2003)
agp30		Resistant to ABA inhibition of germination	van Hengel and Roberts (2003)
agp17		*A. tumefaciens* resistance	Gaspar et al. (2004)
agp19		↓Cell division and elongation, chlorophyll	Yang et al. (2007)
xeg113	EXT arabinosylation	↓XGase response	Gille et al. (2009)
mur1	Fucosylated CH	HSR	Li et al. (2007)
mur4	Arabinosylated CH	HSR (suppressed by boric acid and *prl1* mutant)	Li et al. (2007)
rhm1	Rhamnosylated CH	↓*lrx1* phenotype in root hairs, ↑cell wall-remodeling genes	Diet et al. (2006)
βGlcY	AGP aggregation	Ca^{2+} influx, wound response-like transcript profile, CD	Gao and Showalter (1999); Roy et al. (1999); Guan and Nothnagel (2004); Pickard and Fujiki (2005)
Isoxaben	CE synthesis inhibition	↑PE biosynthetic genes, ↑defense-related genes, ↑JA, SA, ET synthesis, CD	Manfield et al. (2004); Hamann et al. (2009)
Thaxtomin A		↑Ca^{2+}, CD, ↓CE biosynthetic genes, ↑PE biosynthetic and cell wall-remodeling genes, ↑defense-related genes, ↑callose, ↑lignin	Duval et al. (2005); Errakhi et al. (2008); Bischoff et al. (2009); Duval and Beaudoin (2009); Meimoun et al. (2009)
DCB		↑Callose	Melida et al. (2009)

wall polymer mutants and more direct mechanistic studies of other signaling paradigms (Humphrey et al., 2007; Hématy and Höfte, 2008; Hématy et al., 2009) and on the serendipitous identification of novel signaling components (Kohorn et al., 1992; Xu et al., 2008).

THE SIGNALS

The cell wall provides countless potential sources of information. High-M_r cell wall matrix polymers such as hemicellulose, pectin, and glycoproteins display an enormous degree of structural complexity that is amenable to modulation during its biosynthesis and by remodeling in muro. One of the key areas of investigation in plant cell wall research is to establish the relationship between the structural complexity of cell wall polymers and their biological function. Therefore, it is vital to know if stimuli regarding the structural variation of cell wall polymers are perceived by specific receptors. Alternatively, the secondary consequences, such as altered mechanical stiffness or turgor pressure, might represent crucial signals. As in yeast (Levin, 2005), both scenarios are likely to play a role (Fig. 1). The modulation of responses to cellulose biosynthesis inhibition by osmotic support indicates that turgor sensors are likely to be involved in cell wall

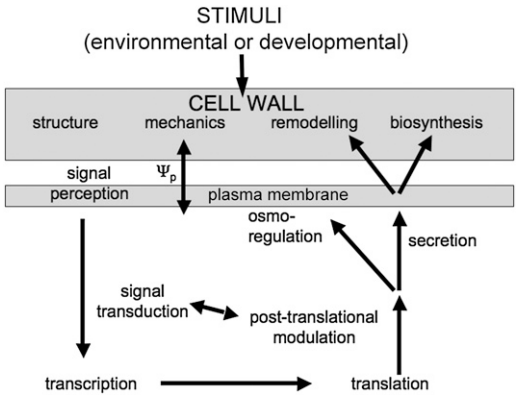

Figure 1. Components of the plant cell wall integrity and performance control system. Differentials in cell wall structure and mechanical performance are detected at the plasma membrane and translated into a compensatory response at the transcriptional and posttranslational levels to restore cell wall structure and performance to the "correct" parameters. Superimposed influences of cell cycle, cell type, cellular context, and developmental stage that determine the correct parameters have been deliberately omitted to emphasize the circular nature of this control system. Ψ_p, Turgor pressure.

stress signaling (Hamann et al., 2009). Historically, the typical cell wall signals are relatively low-M_r microbial degradation products such as pectin-derived oligogalacturonides (OGs; Galletti et al., 2009) and cellodextrins (Aziz et al., 2007) acting as potent elicitors of innate immunity. This class of elicitors has been termed host-associated molecular patterns (Galletti et al., 2009) or damage-associated molecular patterns (Zipfel, 2009), in analogy to pathogen-associated molecular patterns (PAMPs; for review, see Zipfel, 2009). It has been observed that cell wall carbohydrate fragments can also act as signals influencing developmental processes. Such bioactive oligosaccharides, whose activity can be quite specific, have been generally termed oligosaccharins (Albersheim et al., 1983). The biological activity of OGs depends on their degrees of polymerization, methylation, and conformation (compare with Cabrera et al., 2008), while side chain fucosylation of xyloglucan (XG)-derived oligosaccharins is important for their auxin-antagonizing effect (Fry et al., 1990). However, it is unclear at present if oligosaccharins act during normal development or if the responses such as modulation of growth regulator signaling (Fry et al., 1990; Bellincampi et al., 1996; Ferrari et al., 2008; Zabotin et al., 2009) represent an artificial adaptation to a perceived pathogen attack, as can be observed in numerous cell wall mutants.

Xylogen is a 50- to 100-kD highly glycosylated cell wall molecule that directly acts as an extracellular developmental signal (Motose et al., 2004). Xylogen promotes tracheid differentiation in vitro and, together with its paralogs, is required for normal vascular differentiation in planta. Xylogen is a hybrid glycosylphosphatidylinositol lipid (GPI)-anchored AGP and nonspecific lipid transfer protein (Motose et al., 2004). AGPs might act as paracrine and autocrine signals in many biological processes (for review, see Seifert and Roberts, 2007; see also article by Ellis et al., 2010). Their potential to bind to β-glycan polymers (for review, see Nothnagel, 1997) together with their plasma membrane localization via GPI anchors put them in a strategic position to mediate between cell wall polymers and cell signaling. However, available studies are too crude to annotate specific signaling roles to individual AGP species. Cell surface receptor binding to xylogen and most other cell wall-derived elicitors and potential signaling molecules remain to be identified. However, as discussed in the following section, pectic homogalacturonan and OGs are known to bind to specific cell surface receptors.

THE SENSORS

The Wall-Associated Kinase Family

Sensors for extracellular molecules consisting of an extracellular ligand-binding domain, a single transplasma membrane domain, and a cytosolic protein kinase domain have been termed receptor-like kinases (RLKs) and, with more than 610 genes in the Arabidopsis (*Arabidopsis thaliana*) genome, constitute the largest family of receptor-like proteins in plant genomes (Shiu and Bleecker, 2001a, 2001b). The binding of an extracellular ligand induces a conformational alteration leading to the activation of the protein kinase, thereby initiating a cascade of subsequent signal transduction events. Several RLKs are potentially involved in reporting sensing aspects of cell wall structure and function; however, only the subfamily of wall-associated kinases (WAKs) are known at present to directly bind to a cell wall carbohydrate. Its initially identified member, *WAK1* (Kohorn et al., 1992), binds to the cell wall extremely tightly and is specifically localized at the plasma membrane-cell wall interface (He et al., 1996). In plants, WAKs are covalently bound to pectic homogalacturonan (Wagner and Kohorn, 2001); however, they bind noncovalently to Ca^{2+}-cross-linked OGs in vitro (Decreux and Messiaen, 2005), pointing toward important endogenous factors involved in normal assembly of the WAK-pectin supramolecular structure. Genetic evidence implicates WAKs with cell elongation (Lally et al., 2001; Wagner and Kohorn, 2001; Kohorn et al., 2006b), tolerance and sensing for metals and minerals, respectively (Sivaguru et al., 2003; Hou et al., 2005), and pathogen resistance (Diener and Ausubel, 2005; Li et al., 2009). Without doubt, WAKs fulfill important biological roles, raising the stakes to uncover their mode of action. What are their physiological ligands and downstream substrates? *WAK2* is required for sugar-independent growth (Kohorn et al., 2006b), and the *wak2* growth phenotype is rescued by external sugar or sorbitol and by ectopic expression of Suc-6-P synthase, implicating the role of *WAK2* in normal

growth with sugar metabolism and osmotic control. Crucially, *WAK2* is required for the normal expression of vacuolar invertase, an enzyme releasing Glc and Fru from Suc. This might mean that WAK2 feeds cues regarding cell wall properties into the control module that maintains the correct balance of carbohydrates required for optimal growth both as energy source and as osmotically active compounds (Kohorn et al., 2006b). What might be the stimulus, and how could it be transduced to activate invertase transcription? The vacuolar invertase promoter is activated by the external addition of pectin to protoplasts in a *WAK2*-dependent fashion (Kohorn et al., 2009), indicating that the degree of pectin binding might determine WAK activity. The observation that factors influencing the Ca^{2+}-induced so-called egg-box conformation of pectin determine pectin binding to the WAK1 extracellular domain in vitro (Decreux and Messiaen, 2005; Cabrera et al., 2008) might mean that WAKs could sense pectin conformation in vivo. However, the nature of the interaction between WAKs and the cell wall is still somewhat ominous (Kohorn et al., 2006a). In fact, it appears that en route to the cell wall, WAK2 is retained in an insoluble complex in a secretory compartment related to the Golgi. Whether this is the cellular site where the WAK2-pectin association is formed remains to be investigated. Intriguingly, intracellular retention of WAK2 depends on L-Fuc biosynthesis that is required for rhamnogalacturonan II, XG, or AGPs but not for homogalacturonan, indicating the recruitment of a higher order structure into the WAK2-cell wall matrix polymer complex.

The Pro-Rich Extensin-Like Receptor Kinase Family

Another family of receptor kinase-like proteins recently implicated in cell wall signaling is termed PERK, as its 11 Arabidopsis members contain a Pro-rich region extracellular domain similar to extensin (EXT; Nakhamchik et al., 2004). EXTs are a group of Hyp-rich, rod-shaped extracellular proteins typically containing repeats of the Ser-$Hyp_{(3–5)}$ motif, with most Hyp residues typically being glycosylated by one to four Ara residues (Showalter, 1993). EXTs are mostly insoluble once secreted in the wall, cross-linked by di-isodityrosine bonds (Cannon et al., 2008), and ionically bound to pectin and AGPs by basic amino acids (Showalter, 1993). Due to the presence of Ser-$Hyp_{(3–5)}$ motifs sometimes interspersed with basic residues, PERKs might share some of the features of EXTs. However, peptide motifs previously implicated with di-isodityrosin-dependent cross-linking of EXTs are absent from the extracellular domain of PERKs. While transgenic alteration of PERK expression influences growth and cell wall deposition (Haffani et al., 2006), *PERK4* is specifically required for the abscisic acid (ABA)-dependent influx of Ca^{2+} and for normal ABA sensitivity in seeds and roots. The PERK4 protein is an active protein kinase localized at the plasma membrane, and its extraction from plant material is increased by pectinase treatment (Bai et al., 2009). The present data suggest that PERK4 might interact with cell wall polymers and also participate in ABA perception, potentially linking cell wall and growth regulator signaling at the receptor level as opposed to cross talk of signal transduction cascades. Additional roles of PERK genes remain to be investigated.

The *Catharanthus roseus*-Like Receptor-Like Kinase 1-Like Family

The group of *Catharanthus roseus*-like RLKs (CrRLK1L) contains 16 members in Arabidopsis. The *THESEUS1* (*THE1*), *FERONIA* (*FER*), and *HERKULES1* and *-2* (HERK) genes have recently been implicated with functions in cell wall integrity control and growth in a partially overlapping manner (Hématy et al., 2007; Guo et al., 2009). Two other members of the family, encoded by the *ANXUR1* and *ANXUR2* loci, suppress the premature rupture of germinated pollen tubes, a role not apparently related to elongation but potentially also related to cell wall integrity control (Boisson-Dernier et al., 2009; Miyazaki et al., 2009). Loss-of-function (LOF) mutations of *THE1* partially suppress the cell elongation defect and ectopic lignification in cellulose synthase-defective backgrounds while overexpression increases the responses, suggesting that *THE1* might be a component of cell wall function and integrity control (Hématy et al., 2007). Although the roles of *THE1*, *HERK1*, and *HERK2* for cell elongation appear to be largely overlapping, *FER* is essential for cell elongation, preventing the growth of *fer* full LOF mutants. Knockdown of *FER* results in stronger suppression of growth than in *the1 herk1 herk2* triple mutants, suggesting that FER might act as mandatory heterodimerization partner for other RLKs (Guo et al., 2009). To add to the genetic complexity, *FER* also plays an important role in the female gametophyte to restrict pollen tube growth (Escobar-Restrepo et al., 2007). Hence, at least two CrRLK1L genes, *THE1* and *FER*, can act both as repressors and promoters of cell elongation depending on the genetic background. It is tempting to explain this seemingly paradoxical behavior by the assertion that cell wall performance and integrity control acts not only when cell walls are damaged but also during unstressed normal development.

Leu-Rich Repeat Receptor Kinases

Containing at least 220 members in the Arabidopsis genome, leucine-rich repeat receptor kinases (LRR-RLKs) form the largest group among the superfamily of receptor-like kinases (Shiu and Bleecker, 2003). LRR-RLK genes encode receptors for ligands as diverse as brassinosteroid (Kinoshita et al., 2005) and bacterial peptide elicitor fls22 (Gomez-Gomez et al., 2001); however, most ligands for LRR-RLKs are unknown. *STRUBBELIG* (*SUB*) and *SUB RECEPTOR FAMILY* (*SRF*) are members of LRR-RLK family V that lack a

functional kinase domain (Eyuboglu et al., 2007; Fulton et al., 2009). Mutations in *SUB* and *SUB-LIKE MUTANT* (*SLM*) loci lead to comparable pleiotropic developmental defects and changes in transcript abundance of various genes related to cell wall biogenesis, growth regulator signaling, and abiotic stress. It was proposed that *SUB* and *SLM* loci might function in identical or convergent pathways potentially involved in cell wall integrity control (Fulton et al., 2009). Several SRFs were implicated in cell wall biosynthesis and function owing to their transcriptional cofluctuation with cell wall-related genes (Eyuboglu et al., 2007).

The family XIII LRR-RLK ERECTA (ER) is involved in many different aspects of development (for review, see van Zanten et al., 2009). Family XIII contains seven members, three of which, ER, ERECTA-LIKE1 (ERL1), and ERL2, act in an overlapping manner, as evidenced by the synergistic effect of *er*, *erl1*, and *erl2* LOF alleles (Shpak et al., 2003, 2004, 2005). Interestingly, *ER* is required for cell wall reinforcement during the wild-type defense response against a fungal pathogen and, in fact, for normal cell wall composition (Sanchez-Rodriguez et al., 2009). This phenotype suggests that ERECTA might be involved in cell wall performance and integrity control upstream of the regulation of cell wall structure. An alternative but not mutually exclusive interpretation is that ER might sense cell wall fragments released by pathogen attack (Sanchez-Rodriguez et al., 2009). In a reverse genetic investigation of family XIII of LRR-RLKs, the combination of LOF alleles of *FEI1* and *FEI2* was found to display Suc- and salt-sensitive cell expansion defects (Xu et al., 2008), reminiscent of various cell wall structural mutants such as *cobra* (Roudier et al., 2005), *sabre* (Aeschbacher et al., 1995), *rsw1* (Arioli et al., 1998), and *sos5* (Shi et al., 2003). Like *cobra* and *rsw1*, *fei1 fei2* double mutants suffer from severe deficiency in crystalline cellulose. Interestingly, *fei1 fei2 sos5* triple mutants display the same phenotype as the *fei1 fei2* and *sos5* parental lines. This nonadditive interaction was interpreted as *FEI1* and *FEI2* acting redundantly in the same pathway as *SOS5/FLA4* (Shi et al., 2003). It was found that the *fei1 fei2* phenotype is partially suppressed with the ethylene biosynthetic inhibitor α-amino-isobutyric acid and that both FEI proteins interact with the ethylene biosynthetic enzyme ACS5. These unexpected observations suggested that FEI proteins play a role in cell wall architecture, possibly as mediators between cell wall and signal transduction. 1-Aminocyclopropane-1-carboxylic acid might be produced locally by an activated FEI ACS complex and act as a novel second messenger. The physical relation between FEI1/2 and FLA4 remains to be studied; however, hypothetical binding between the protein-protein interaction fasciclin domain of FLA4 and the LRR domain of either of the FEI RLKs on the one hand and a carbohydrate-carbohydrate interaction between the FLA4 AG moiety and cell wall polymers on the other hand might physically connect protein-binding RLK and cell wall carbohydrates. Many LRR-RLK transcripts are coregulated with AGPs (G.J. Seifert, unpublished data), while several members are enriched in detergent-resistant membranes (Shahollari et al., 2004) that also contain GPI-anchored AGPs (Borner et al., 2005). Detergent-resistant membranes are generally hypothesized to represent biochemically distinct nanoscale membrane domains sometimes called lipid rafts (for review, see Lingwood and Simons, 2010). Hence, the speculative interaction between LRR-RLKs and GPI-anchored AGPs might be promoted by their subcompartmentalization into lipid domains.

Leguminous L-Type Lectin RLKs

A group of potential cell wall sensors are the leguminous L-type lectin RLKs (Bouwmeester and Govers, 2009) encoded by a large family of 46 Arabidopsis loci. Interestingly, several L-lectin RLKs were found to bind peptides containing the Arg-Gly-Asp (RGD) tripeptide (Gouget et al., 2006), a sequence present in various animal extracellular matrix proteins but so far not identified in any plant cell wall proteins. Addition of this peptide facilitates plasma membrane detachment during plasmolysis (Canut et al., 1998), implicating the identified RGD-binding RLKs in cell wall adhesion. It might be tempting to invoke binding of the extracellular lectin domain to cell wall carbohydrates; however, this seems unlikely, as sugar-binding residues in the identified proteins are insufficiently conserved (Bouwmeester and Govers, 2009). Hence, native ligands of RGD-binding L-lectin RLKs remain to be proposed.

Mechanosensitive Receptors and Ion Channels

As the plasma membrane is mechanically connected to the cell wall by turgor pressure, the perception of mechanical force at the plasma membrane-cell wall interface might be a global mechanism to report cell wall integrity and performance to the cell interior. An interesting hypothetical mechanism for how cell wall stress might be turned into a signal is deformation-dependent exposure of previously masked receptor recognition sites on cell wall polymers analogous to the animal extracellular matrix protein fibronectin (Monshausen and Gilroy, 2009). In principle, WAKs or other transmembrane receptor kinases might be activated according to this speculative concept. A more traditional type of turgor pressure gauge is the mechanosensitive (MS) ion channel, which opens in response to plasma membrane stretching or warping. Several Arabidopsis homologs to the prokaryotic and eukaryotic MscS/MSC1 MS channels form the MSL gene family. Although *MSL9* and *MSL10* are necessary for plasma membrane stretch-induced ion conductance in root protoplasts, a role of the *MSL* genes for plant growth and the specific substrate ion have yet to be established (Haswell et al., 2008). A locus involved in stretch-induced plasma membrane Ca^{2+} conductiv-

ity is *MCA1* and possibly its paralog *MCA2* (Nakagawa et al., 2007). Plant *MCA1* complements the yeast-lethal mutation *mid1* in a putative stretch-activated Ca^{2+} channel-encoding locus, and its expression in plant tissues is correlated with Ca^{2+} influx upon plasma membrane distortion and is required for root penetration into medium with heterogeneous mechanical properties. It is intriguing to speculate that alterations in mechanical cell wall properties during growth and abiotic stress might be sensed as a change in plasma membrane tension, thereby modulating transmembrane Ca^{2+} current. Both MSL and MCA proteins are the first candidates for molecular components of the plasma membrane MS ion channel system.

SIGNAL TRANSDUCTION

Phosphorylation Cascades

Surprisingly little is known on the molecular targets of receptor kinases plants (De Smet et al., 2009; Tor et al., 2009), although genetic evidence in many cases suggests signaling from receptor kinases to mitogen-activated protein kinase (MAPK) cascades (Nakagami et al., 2005). MAPK cascades act in the signal transduction of extracellular stimuli in all eukaryotes and consist of three types of protein kinases. In response to stimuli such as growth regulators, abiotic stress, oxidative stress, PAMPs, and developmental cues, an active MAPK kinase kinase (MAP3K) phosphorylates and thereby activates a MAPK kinase (MAP2K) that in turn activates a MAPK. Active MAPKs potentially phosphorylate various substrates such as transcription factors. Another level of protein kinases is understood to act on top of MAP3Ks. Because the number of MAPKs is relatively small, the specificity of signaling at the transcriptional level is an intriguing problem (for review, see Colcombet and Hirt, 2008).

There is accumulating evidence that the perception of pectin or pectin fragments by WAKs involves MAPK signaling. The immediate protein kinase substrates of WAK2 are unknown; however, *WAK2* is necessary for the rapid pectin-triggered activation of MAPK3 and for pectin-induced alterations in transcript levels, including activation of the vacuolar invertase promoter (Kohorn et al., 2009). Hypothetically, this might place WAK2 upstream of a MAPK cascade. Despite the dramatic influence of *WAK2* on the response to externally applied pectin in protoplasts, the *wak2* mutant phenotype is quite subtle (Kohorn et al., 2006b), invoking potential redundancy at the cellular or the whole plant level. Consistently, a dominant negative version of WAK2 causes a more dramatic growth phenotype that is synergistically enhanced by a *mapk3* LOF allele (Kohorn et al., 2006b). Families of transcripts influenced by pectin in a *WAK2*-dependent manner are transcription factors, defense related, involved in protein phosphorylation, or related to cell wall biosynthesis and remodeling. The transcript profile in response to pectin treatment of wild-type and *wak2* protoplasts displayed relatively few differences in comparison with short-term treatment of suspension cells with OGs (Moscatiello et al., 2006). One reason for this discrepancy might lie in the fact that protoplasts were kept with osmoprotectants while suspension cells were not. Osmotic stress might indirectly affect pectin conformation by mechanically stressing the primary cell wall and thereby synergistically activating WAKs in a pectin-dependent and an osmotic stress-dependent fashion. Hence, a possible scheme that can be crudely outlined from the available data might link pectin conformation/osmotic balance to WAK2/WAK1 activation, leading to the activation of MAPK3 (and possibly other MAPKs, such as its most closely related ortholog MAPK6) via unknown MAP3Ks and MAP2Ks and resulting in the activation of transcription factors regulating stress management, osmotic balance, and cell wall biosynthesis and remodeling. Generally, this cyclical sequence of events from sensing of cell wall structure to signal transduction to transcription to modulation of signal sensing, signal transduction, osmotic balance, cell wall biosynthesis, and remodeling constitutes an example of a regulatory module of cell wall integrity control as outlined in the introduction (Fig. 1).

Although the initial activation of MAPKs takes place within minutes and can be detected by phosphorylation of generic substrates, some MAPKs are also induced at the transcript level. Therefore, it is interesting that two independent studies addressing different aspects of cell wall integrity control, application of OGs (Moscatiello et al., 2006) or interference with AGP integrity by βGlcY (Guan and Nothnagel, 2004), found specific sets of MAPK cascade components transcriptionally up-regulated. *MAPK3*, *-5*, and *-11* and *MAP3K15* and *-16* were induced after 1 h of βGlcY treatment, while OG treatment for 2 h induced *MAPK3*, two isoforms of *MAPK4*, *MAP2K9*, and *MAP3K8* and *-19*. Although in both studies suspension cells were used and sampled at a similar time interval after the treatment, there might be technical explanations for the observed differences. However, it is tempting to speculate that in addition to the general stress response node represented by MAPK3/MAPK6, the activation of specific sets of MAPKs, MAP2Ks, and MAP3Ks is required for the precise tuning of the responses to different types of cell wall stresses.

An intracellular protein kinase potentially acting in cell wall stress signal transduction is *OXI1* (Rentel et al., 2004). *OXI1* transcript is induced by reactive oxygen species (ROS). It is also induced by cellulase treatment and transiently by wounding. Thirty minutes after the wounding stimulus, *OXI1* expression was induced; however, another 30 min later, it again reached a lower level. *OXI1* expression after hydrogen peroxide (H_2O_2) or cellulase treatment was more sustained, still increasing between 1 and 3 h of treatment. OXI1 kinase activity was also strongly stimulated by H_2O_2 and cellulase, peaking 5 min after H_2O_2

treatment and obtaining higher levels and a later occurring peak after treatment with cellulase. *OXI1* is required for full H_2O_2- and cellulase-stimulated activation of MAPK3 and MAPK6 (Rentel et al., 2004). The more efficient activation by cellulase compared with H_2O_2 might be due to multiple roles of cellulase in the stimulation process (Rentel et al., 2004), and these might be PAMPs in the cellulase peptide as well as host-associated molecular patterns such as the released cellodextrins. Third, damage to cellulose structure leading to turgor instability might be a trigger.

Ca^{2+} and ROS

Numerous small molecules such as protons and various other ions, ROS, nucleotides, lipids, and sugars can act as intracellular transmitters of information, or second messengers, often by binding to and reshaping specific transducer proteins such as calmodulin or hexokinase.

Drastic experimental interventions in cell wall integrity such as binding of βGlcY to AGPs trigger rapid elevation of intracellular Ca^{2+} (Roy et al., 1999; Pickard and Fujiki, 2005). Moreover, Ca^{2+} partially transmits the intracellular response to cell wall-derived OG elicitors (Moscatiello et al., 2006). On the other hand, the respiratory burst oxidase homolog isoform D (*AtRBOHD*), encoding an enzyme that produces superoxide anions, is required for the responses to cellulose biosynthesis inhibition (Hamann et al., 2009), and various inhibitors of ROS suppress cell death triggered by genetic and chemical interference with AGP structure (G.J. Seifert, unpublished data). Experimentally, far less tractable than genes, mRNAs, or proteins, the very dynamic intracellular fluxes of second messengers in response to specific structural stimuli coming in from the cell wall under natural conditions remain to be explored under less artificial conditions. Some understanding of how second messengers might be involved in cell wall signaling might be obtained from developmental models. Root hair initiation and growth have been studied physiologically and genetically. Hair initiation involves the selection of the appropriate position for the future hair close to the base of the trichoblast and cell wall remodeling to allow the formation of an outward bulge of precise shape and size. Hair growth requires the continuous tip-focused deposition of new cell wall material and subsequent cell wall remodeling at the flanks of the hair (Dolan, 2001; Carol and Dolan, 2002). Quite obviously, both the initiation and growth processes have to be responsive to the actual state of cell wall structure and stability. Interestingly, root hair growth follows an oscillatory pattern that might reflect bursts of cell wall expansion. Ca^{2+} and ROS together with extracellular pH oscillate in a manner similar to hair growth but trailing its peak by 4 and 8 s, respectively (Monshausen et al., 2007, 2008). One interpretation for this behavior is that a phase of rapid cell wall expansion is sensed at the level of the plasma membrane, causing an increase of intracellular Ca^{2+} followed by the production of ROS and alkalinization of the apoplast. The Ca^{2+} signal might limit cell wall expansion, preventing catastrophic failure. Consistently, suppression of both Ca^{2+} influx and ROS production can cause hair cells to burst (Foreman et al., 2003; Monshausen et al., 2008). Ca^{2+} fluxes might initially be stimulated by MS channels that respond to increased membrane stretch in a cell wall growth phase. As ROS and Ca^{2+} act in a positive feedback loop during root hair growth (Takeda et al., 2008), an increase of Ca^{2+} enhances ROS production that might vice versa increase intracellular Ca^{2+}, possibly by activating a hyperpolarization-activated Ca^{2+} channel (Foreman et al., 2003) until cell wall expansion is supplanted by cell wall rigidification. As opposed to previously discussed stress signaling pathways but consistent with the short time scale of the observed oscillations, this type of growth control might act rapidly on a posttranslational level. However, it is quite clear that cell wall signals also involve Ca^{2+} and ROS in a manner that depends on gene expression. One example is the addition of OGs to cultured cells, which induces Ca^{2+}-dependent as well as Ca^{2+}-independent alterations in the transcript profile (Moscatiello et al., 2006). Another example is the observation that responses in transcripts induced by H_2O_2 and cell wall stress show considerable overlap (Duval and Beaudoin, 2009).

Modulation by Sugar Signaling

Sugars such as Glc and Suc act as intercellular signals independently from their role in metabolism (for review, see Hanson and Smeekens, 2009). Sugar signaling is partially conserved among kingdoms and interacts with all major signaling networks such as growth regulator and light signal transduction in plants. Therefore, it is not surprising that sugar signaling also interacts with cell wall performance and integrity control. That the cell wall matrix structural mutants *mur4*, *mur1*, and *mur3* are sugar hyperresponsive suggested such a link (Li et al., 2007). External Glc or Suc is required for many responses to pharmacological inhibition of cellulose biosynthesis. Interestingly, metabolically inactive Glc and Suc analogs stimulated most of the Glc/Suc-dependent responses to cellulose biosynthesis inhibition, while Fru did not (Hamann et al., 2009). This behavior is reminiscent of the role of the *HEXOKINASE1* (*HXK1*) locus that mediates many responses to Glc, even in the absence of Glc-phosphorylating activity (Moore et al., 2003). *HXK1*-dependent sugar signaling modulates the signaling of numerous growth regulators (Moore et al., 2003); however, its role in cell wall performance and integrity control remains to be investigated. Another gene important for sugar signaling is *PLEIOTROPIC REGULATORY LOCUS1* (*PRL1*), which, similar to *HXK*, has a widespread regulatory role, influencing growth regulator response (Nemeth et al., 1998) and

innate immunity (Palma et al., 2007) and response to singlet oxygen (Baruah et al., 2009). Sugar hypersensitivity of *mur4*, assayed as inhibition of dark-grown hypocotyl elongation, depended on *PRL1* (Li et al., 2007). By contrast, only a minor proportion of genes differentially regulated in the *mur4* background was influenced by *PRL1*. This is consistent with the observation that many transcription factor genes, induced by interference with AGPs (Guan and Nothnagel, 2004) or by inhibition of cellulose synthesis (Duval and Beaudoin, 2009), are also induced by singlet oxygen, albeit independently of *PRL1* (Baruah et al., 2009). Taken together, this suggests that sugar signaling via *PRL1*, and possibly via *HXK1*, might modulate cell wall signaling without being essential.

Interactions with Growth Regulator Signaling and Other Factors

The isolation of cell wall mutants from forward genetic screens that were initially directed at other questions such as disease resistance mechanisms or sugar and growth regulator signaling was extremely important to highlight the integral role of cell wall structure in various aspects of signaling. Sustained defects in cell wall polymers often lead to the constitutive activation or modulation of numerous stress responses such as ethylene, jasmonate, or ABA-responsive genes and resistance to various pathogens (Table I). The unexpected diversity of observed responses might have several explanations that are not mutually exclusive: (1) diversity of plant materials; (2) differential sensitivity of cell types to chemicals or mutations; (3) diversity of cell wall stress stimuli, such as genetic or chemical inhibition with cellulose, hemicellulose, pectin, or AGPs; (4) combination with other relevant stimuli, such as light, water supply, or external carbohydrate and osmotically active compounds that modulate outcome; and (5) different time scopes of the various experiments, from relatively short-term drug experiments that can have immediate actions to mutant studies producing sustained effects. The significance of these individual parameters has not been systematically addressed so far. Moreover, it will be interesting to genetically dissect the role of growth regulator signaling pathways for the downstream responses such as transcriptional and posttranscriptional regulation of cell wall biosynthesis and remodeling.

THE OUTPUT

From the preceding discussion, it becomes clear that the typical fingerprint of an activated cell wall integrity and performance control machinery is the large-scale alteration of transcriptional activities related to cell wall biosynthesis and remodeling (Table I). However, most of what is known at present about the transcriptional regulation of cell wall biosynthesis or remodeling comes from developmentally regulated systems such as xylogenesis (Zhong et al., 2006; Demura and Fukuda, 2007; Lasserre et al., 2008; for review, see Zhong et al., 2007) or is based on the assumption that genes involved in a given metabolic process are (transcriptionally) coregulated (Brown et al., 2005; Persson et al., 2005). Evidence for post-translational modulation of cell wall biosynthesis is generally only circumstantial (Winter and Huber, 2000; Nuhse et al., 2004; Seifert, 2004; Jacob-Wilk et al., 2006). The identification of transcription factors and post-translational processes regulating cell wall biosynthesis and remodeling is an important field of future research.

PERSPECTIVES

In this article, we have reviewed the accumulating evidence for the involvement of the impact of structural alterations of the plant cell wall on many aspects of plant life. The mechanisms of how fluctuations in cell wall integrity and performance are monitored or how such stimuli are converted into appropriate control responses are expected to be highly complex, and only a few putative components have emerged. Among the different types of receptors, the group of WAK proteins provides the best understood paradigm for cell wall signaling. WAK binds to and is activated by OGs and pectin, leading to the activation of MAPK3 and to transcriptional alterations of cell wall biosynthesis and remodeling as well as stress signaling. Several other candidate receptors are in line to be further characterized for their extracellular ligands and intracellular substrates. The relation of MS ion channels of the MSL and MCA classes to cell wall performance and turgor sensing remains to be experimentally tested. The puzzle of how a limited set of MAPKs can effect the transcription of the correct set of cell wall-modifying and biosynthetic genes remains to be addressed, and cell wall structure-controlling transcription factors have yet to be identified. Because the life and walls of plant cells are far more complex than those of yeast cells, we expect future discoveries of many new receptors, signal transduction components, and transcriptional regulators involved in various aspects of cell wall performance and integrity control.

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