Cellulose-binding domains: cellulose associated-defensive sensing partners?

Bernard Dumas, Arnaud Bottin, Elodie Gaulin, Marie-Thérèse Esquerré-Tugayé

► To cite this version:

Bernard Dumas, Arnaud Bottin, Elodie Gaulin, Marie-Thérèse Esquerré-Tugayé. Cellulose-binding domains: cellulose associated-defensive sensing partners?. Trends in Plant Science, 2008, 13 (4), pp.160-4. 10.1016/j.tplants.2008.02.004 . hal-00320691

HAL Id: hal-00320691

<https://hal.science/hal-00320691>

Submitted on 11 Sep 2008

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.

Dumas et al.

**Cellulose Binding Domains: cellulose-associated defense sensing
partners?**

Bernard Dumas*, Arnaud Bottin, Elodie Gaulin and Marie-Thérèse Esquerré-Tugayé*

UMR 5546 CNRS-Université Paul Sabatier Toulouse III, Pôle de Biotechnologie
Végétale, 24 Chemin de Borde-Rouge, BP42617 Auzeville, 31326 Castanet-Tolosan,
France

*Corresponding authors: Dumas, B. (dumas@scsv.ups-tlse.fr), Esquerré-Tugayé, M.T.
(esquerre@scsv.ups-tlse.fr)

16

17 **Abstract**

18 The cellulose-binding domains (CBDs) of CBEL, the cellulose-binding elicitor lectin of
19 *Phytophthora*, are potent elicitors of plant defense responses. Induction of defense has also
20 been reported in various cellulose-deficient mutants of *Arabidopsis thaliana*. Based on these
21 observations, we propose a model linking cellulose alteration to defense induction. This
22 integrates the fast increase in cytosolic calcium recorded in response to CBEL, mechano-
23 stimulated calcium uptake mechanisms, and proteins that interact functionally with the
24 cellulose synthase complex. In this context, CBDs emerge as new tools to decipher the
25 signalling cascades that result from cell wall-cellulose perturbations.

|

26 Introduction

27 Owing to their strategic location surrounding the cell, plant cell walls play an important role
 28 in the outcome of plant–microorganism interactions. Understanding of the dialogue that takes
 29 place at this interface has progressed as a result of the improved biochemical and molecular
 30 knowledge of this cellular compartment. After the first report that plant cell walls contain
 31 proteins [1], the concept of the cell wall as a dynamic structure involved in signalling and
 32 defense has emerged, as notably illustrated by the ability of pectin-derived oligosaccharides to
 33 elicit defense responses [2], and by cell wall reinforcement by structural components
 34 following pathogen attack [3,4]. Perturbing the cell wall integrity by mechanical stress such
 35 as wounding often induces similar signalling and strengthening effects, thereby suggesting
 36 that the cell wall harbours its own surveillance system [5]. With the sequencing of a few plant
 37 genomes, particularly of *Arabidopsis thaliana*, the study of plant cell walls has now entered
 38 the genomic era, shedding new light on functional aspects of this important cell compartment.
 39 Several reviews of current knowledge about cellulose [6,7], and cell wall sugars and proteins
 40 as related to stress, have recently appeared [8,9].

41 Our interest in cellulose as a possible partner of the surveillance system has arisen from
 42 several reports linking cellulose to plant defense through the study of *Arabidopsis* cell wall
 43 mutants and microbial effectors. Thus, several mutants having defects in cellulose synthesis
 44 were shown to be more resistant to various pathogens [8,9]. The possibility that cellulose
 45 might be part of a sensing machinery was further supported by the finding that the cellulose-
 46 binding domains (CBDs) of the CBEL effector of *Phytophthora parasitica* are sufficient to
 47 elicit plant defense [10]. It is unlikely that this effect implies the enzymatic release of oligo- β -
 48 glucan elicitors from cellulose because CBEL and more generally CBDs are devoid of
 49 hydrolase activity. The presence of CBEL at the mycelium cell surface allows *Phytophthora*
 50 to adhere to cellulosic substrates [11].

Since altering as well as touching cellulose induce plant defense, one might wonder whether there is any crosstalk between the mechanisms underlying the response to these two different stresses. Hence this article focuses on the emerging links between the cellulose biosynthesizing machinery, plant defense, and CBDs as possible interactants.

Proteins associated with cellulose

Before reporting the few examples linking cellulose to defense sensing, we will give an overview of the genes and proteins that interact directly or indirectly with cellulose at the biosynthesis and structural levels.

Proteins of the cellulose biosynthesizing machinery.

The cell wall cellulose microfibrils consist of several chains of 1,4-linked β -D-glucose residues tightly assembled by hydrogen bonds. As a major metabolic, energy-demanding process in plants, the biosynthesis of cellulose must be tightly programmed and linked to a source of glucose monomers. The level of cellulose in the cell wall depends primarily on the activity of the cellulose synthase complex. After the discovery of the first cellulose synthase gene (*CESA*) encoding the catalytic subunit of the complex in cotton (*Gossypium hirsutum*) [12], genome sequencing data revealed that a large set of *CESA* genes, comprising at least 10 members, is present in the genome of *Arabidopsis*, and of other plants. CESA proteins are located within the plasma membrane, and share common structural features, notably conserved and more variable domains, and the D,D,D,QXXRW motif for substrate binding and catalysis. The phosphorylation status of cellulose synthase seems to be important for its activity [6].

In *Arabidopsis*, mutant complementation analysis has shown that *AtCESA1*, *AtCESA2*, *AtCESA3*, and *AtCESA6* are involved in cellulose biosynthesis of the primary cell wall

[6,7,13,14], whereas *AtCESA4*, *AtCESA7* and *AtCESA8* are expressed during secondary cell wall formation [6,7]. A number of *cesA* mutants, with mutations scattered along the whole sequence of these various genes, are available. They exhibit growth defects and a decrease in the cellulose content of the cell wall which is accompanied in a few cases by deposition *in muro* of weakly esterified pectin [15], of callose, or of lignin at ectopic sites [16].

Genetic screening based on phenotypes has led to the identification of several additional genes [17-19] encoding KORRIGAN (KOR) and the cell surface proteins KOBITO (KOB) and COBRA (COB), mutations in which lead to cellulose deficiencies (*kor*, *cob*) and mis-orientation of cellulose microfibrils (*kob*).

A necessary requirement for cellulose biosynthesis is the supply of the UDP-glucose substrate. Among enzymes that might fulfil this role, sucrose synthase (SuSy) has received special attention because it produces UDP-glucose and fructose from sucrose, and has been shown to be tightly associated with the deposition of cellulose in cotton fibers [20-22].

However, a clear picture of its involvement awaits further investigation [23].

As a major structural component of the cell wall, cellulose has to be correctly orientated to allow growth and optimal interactions with other polymers. A tight contact between the cellulose synthase machinery and cytoskeleton dynamics has long been claimed [24]. Indeed, confocal imaging has shown that isoxaben, an inhibitor of cellulose biosynthesis, alters the organisation of cortical microtubules [25], and alters alignment of microtubules with the cellulose synthases *AtCESA7* and *AtCESA6*, tagged respectively with the green (GFP:*AtCESA7*) and the yellow (YFP:*AtCES6*) fluorescent proteins [26,27]. Conversely, marked changes in YFP-CESA6 organisation are observed when the microtubules (MT) are disrupted using oryzalin. Even though the precise connection between cellulose synthase and microtubules is not definitively established, it is clear that cellulose synthase and microtubules do affect each other.

101

102 **In muro cellulose-binding proteins**

103   Once in the wall, the cellulose microfibrils are bound to hemicelluloses through hydrogen
 104   bonds, thereby contributing to the architecture of the cell wall. The protein expansin can
 105   loosen this architecture, probably by weakening the non-covalent adhesion between these
 106   polysaccharides [28] without hydrolysing them. It has been shown that downregulation of one
 107   gene encoding an α -expansin in *Petunia* reduces the amount of crystalline cellulose in the cell
 108   wall [29]. Cellulases (endo- β -1,4-glucanase, cellobiohydrolase, β -glucosidase) are another
 109   major category of plant and microbial enzymes that interact with cellulose, ultimately
 110   degrading cellulose to β -D-glucose. The presence of CBDs in some plant and microbial
 111   endocellulases participate in the efficacy of these enzymes by anchoring them to their
 112   substrate [30].

113   A search for microbial effectors has led to the isolation of a glycoprotein that is located in the
 114   inner and outer layers of the cell wall of *Phytophthora parasitica*, a pathogen of the tobacco
 115   plant *Nicotiana tabacum* [31]. Its protein moiety is composed of two direct cysteine-rich
 116   repeats connected by a linker. Each repeat contains a motif that closely resembles the fungal
 117   type I CBD consensus pattern found in cellulases from various fungi [32,33]. Further
 118   characterization showed that this molecule is able to bind to crystalline cellulose and to
 119   tobacco cell walls but does not possess any detectable hydrolytic activity on various
 120   polysaccharides [32]. Due to its elicitor effect and lectin-like activity, the molecule was
 121   named CBEL (for cellulose-binding elicitor lectin). CBEL is widespread in the genus
 122   *Phytophthora* [34]. In *Phytophthora*, it serves to organise polysaccharide (β -glucan)
 123   deposition in the cell wall, and it allows adhesion of the mycelium to cellulosic substrates
 124   [11].

CBDs are found in proteins of microbial (bacteria, fungi, oomycetes) and plant (endocellulases, expansin) origins [35,36], and are also encountered in non-enzymic effectors secreted by nematode pathogens during plant colonisation [37]. Although exhibiting different sizes depending on their origins, they share common structural features and aromatic amino-acid residues implicated in cellulose binding affinity [35,36]. The aromatic residues of the two CBDs of CBEL predicted to be surface-exposed and involved in cellulose binding were deduced from sequence alignment with the cellobiohydrolase I CBD from the fungus *Trichoderma reesei*. CBDs are thought to locally disrupt hydrogen bonding between cellulose chains, resulting in local destabilization of cellulose microfibrils [35,36]. Proteomic studies have shown that plant cell walls contain numerous proteins. A comparison of the patterns of wild-type and cellulose mutants might prove useful to uncover additional cellulose-interacting proteins.

Cellulose and associated defense sensing

Recent studies provide evidence that alteration of cellulose integrity is a warning signal to which the plant cell responds by activating defense pathways.

Cellulose synthase-associated sensing

Cellulose is the main load-bearing polymer of the plant cell wall to which it notably confers mechanical strength. Therefore, cellulose-deficient plants would be expected to be less resistant to pathogen ingress and abiotic stress. There are now several examples indicating that this is not the case.

The first contribution to this subject came from the report that a mutant (*cev*) of *Arabidopsis* showing constitutive expression of defense-related genes was mutated in the cellulose synthase gene *CESA3* [38]. Interestingly this mutant showed increased production of the

defense-related jasmonate (JA) and ethylene (ET) signalling molecules, and enhanced resistance to powdery mildew fungal diseases caused by *Erysiphe cichoracearum*, *Erysiphe oronti*, and *Oidium lycopersicum*. A clear link between cell wall alteration and signalling via JA and ET was supported by the observation that chemical inhibition of cellulose biosynthesis could reproduce the phenotype on wild-type plants, and that this phenotype i.e. *cev* was suppressed by mutations that interrupt the JA and ET pathways [39]. The notion that mutations of *CESA3* activate defense responses through JA and ET was soon confirmed independently [16] on the ectopic lignin mutant (*eli*) of *Arabidopsis*. In this case, the decrease in cellulose content was compensated by an activation of lignin biosynthesis, thereby indicating that cellulose perturbation has many cellular effects.

Mutations in cellulose synthase genes involved in secondary cell wall formation are further examples linking cellulose deficiencies to biotic and abiotic stresses. Thus, the *lew 2* mutant alleles of *AtCESA8* are more tolerant to drought and osmotic stress than wild-type plants, possibly as a result of the accumulation of soluble sugars, proline and abscisic acid (ABA) within the cell following inhibition of cellulose synthesis [40]. A role for ABA in signalling was later illustrated in *Arabidopsis* mutants of the three *CESA4*, *CESA7* and *CESA8* subunits. These mutants had reduced levels of secondary cell wall cellulose, but showed enhanced resistance to three fungal species (*Plectosphaerella cucumerina*, *Botrytis cinerea*, *Erysiphe cichoracearum*), and to the vascular bacterial pathogen *Ralstonia solanacearum* [41]. In-depth genetic and transcriptomic studies revealed that signalling was independent of JA, ET and SA (salicylic acid), and correlated with ABA-induced defense, particularly against *Ralstonia*.

In response to various stresses, plants synthesize and accumulate callose, a β -1,3-glucan polymer, in their cell walls. The *pmr4* mutant of callose synthase, another plasma membrane glucan synthase, was more resistant to the biotrophic pathogens *Erysiphe cichoracearum*,

Deleted: -

Erysiphe oronti, and *Peronospora parasitica*, an effect mediated by SA [42]. Since callose deposition normally participates in plant defense, this suggests that either deprivation of callose, or/and impairment of PMR4 behave as warning signals.

The above examples strongly support that defects in cell wall integrity are perceived by a surveillance system, which likely relies on a variety of sensor and receptor molecules.

Cellulose Binding domains (CBDs) as emerging partners

While studies of cellulose mutants were very fruitful in investigating the link between cellulose and defense responses, another approach could be the use of peptides or proteins with a disruptive activity on cellulose.

CBEL is a potent elicitor in the *Phytophthora* host plant tobacco, in which it induces local hypersensitive-like lesions, defense responses, and protection against subsequent infection with the oomycete [32]. It is also active on various non-host plants, notably on *Arabidopsis*. Using *Arabidopsis* mutants affected in the salicylic acid (SA), jasmonic acid (JA), and ethylene pathways (ET), it was shown that all three pathways are triggered by the elicitor. JA and ET are required for lesion formation, whereas induction of cell wall-associated defense proteins depends on SA [43]. *In planta* delivery of truncated or CBD-mutated versions of CBEL revealed that intact CBDs are essential for full elicitor and cellulose-binding activities [10]. Indeed, synthetic peptides corresponding to either CBD were sufficient to induce gene expression in tobacco, and to induce expression of a *GUS* reporter gene under control of the defense *PR1* gene promoter in transgenic *PR1::GUS Arabidopsis*. Interestingly, these CBDs did not provoke lesions following infiltration of tobacco and *Arabidopsis*. The fast (sec–min) and transient increase in cytosolic calcium that was observed upon incubation of tobacco-aequorin cells with CBEL required intact CBDs, and the presence of the cell wall. Whether other fungal type 1 CBDs are able to induce plant defense awaits further investigations. One

such example might be provided by the cellulase of *Trichoderma viride* whose membrane depolarization and other defense-related signalling effects are retained by the heat denaturated enzyme [44].

CBDs belong to a large superfamily of carbohydrate binding modules (CBMs) classified into more 40 different families, based on amino acid sequences, binding specificity, and structure. This large resource offers the opportunity to investigate precisely the cell wall sensing machinery since several of these CBMs specifically bind various cell wall components.

Cellulose and cell wall sensing: a proposed model

The above literature suggests that perturbation of the cellulose status, whether by mutation (*cesA*), adhesion (CBD), or chemical treatment (inhibitors), is a warning signal for the plant cell and leads to defense responses.

Is there a surveillance system common to such different stimuli? What do we know about the underlying mechanisms? A possible explanation is that they are perceived as a kind of mechanical stress. A similar situation is best documented in yeast, in which agents that cause cell wall stress, as well as mutations that impair cell wall biosynthesis, coactivate cell wall integrity (CWI) signalling and calcium signalling pathways [45], ultimately leading to cell wall biogenesis responses. Compared to yeast, what do we know of the mechanisms underlying cell wall sensing in plants? Could examining the responses induced by CBEL and CBDs provide answers?

In a proposed scenario, calcium would play a central role (Figure 1). Indeed the earliest effect related to cellulose perturbation recorded so far is a very fast increase (within the first minute) in cytosolic calcium in response to CBEL [10]. Interestingly, a plasma membrane protein named Mca1 that plays a crucial role in mechano-stimulated Ca^{2+} uptake was recently

characterized in *Arabidopsis* [46]. Another plasma membrane protein called THESEUS1 (THE1) that partly mediates growth inhibitory and ectopic lignin deposition phenotypes of the *prc1-1* mutant of *CESA6* of *Arabidopsis* [47] was also reported. THE1 is a receptor-like kinase which autophosphorylates, and requires a mutant *cesA6* background for its activation, suggesting that THE1 is involved in cellulose-sensing.

Knowledge of Mca1 now affords the possibility to check whether it mediates calcium entry in cellulose-deficient mutants and in response to CBEL and CBDs. In particular, does it interact with CESA and THE1? Whatever the case, increased cytosolic calcium might regulate phosphorylation processes, notably that of the THE1 and CESA proteins, leading to changes in their activity and, consequently, in the activity of their cell partners. One likely partner of CESA is SuSy, sucrose synthase, which catalyses the formation of UDPglucose, the substrate of CESA. Changes in its activity, *i.e.* glucose formation and/or sucrose consumption, might be perceived by a sugar-sensing machinery [48]. It was recently reported that sucrose can mediate priming of plant defense responses, and bring about broad-spectrum disease resistance in rice [49]. In the proposed model, a functional link between the CESA complex and the cytoskeleton MT might also be altered, as a result of the known depolymerisation of MT in the presence of increased calcium. MT depolymerisation has long been associated with defense responses against microbial invaders [50].

Additionally, or alternatively, the kinase activity of THE1 might be the starting point of a phosphorylation cascade leading to defense activation. Crosstalk between such a cascade and the JA, ET, SA, and ABA signalling pathways that are activated in response to cellulose perturbations can be predicted.

Based on this model, several questions emerge (Box 1). In particular, it will be interesting to know whether cellulose can relay information from external stimuli such as CBDs to intracellular signalling *via* cellulose synthase. The availability of *Arabidopsis* mutants and of

250 CBDs together with cell biology imaging technology provides us with powerful tools to probe
251 the proposed model.

252

253 **Conclusion**

254 Although the notion of cell wall integrity-sensing is not novel, the underlying mechanisms are
255 still largely unknown. The recent findings that cellulose alterations are perceived as warning
256 signals in *Arabidopsis* provide us with a powerful model system to study the surveillance
257 system of the cell. Future work will aim at identifying novel genes involved in cell wall
258 sensing. This might be accomplished by screening mutants for their response to CBD
259 peptides. Understanding responses to cell wall damages might contribute to improve the
260 resistance of plants to biotic stresses.

261

262

263

264 **Box 1. Outstanding questions**

- 265 • How general among known CBDs is the effect induced by the CBDs of CBEL?
- 266 • Besides cellulose, do the CBDs interact with other cell-surface components such as the
267 wall-associated kinases (WAKs) or other protein kinases?
- 268 • Is the cellulose-CBD interaction perceived directly by a Mca1-like protein, or through the
269 cellulose-synthesizing machinery?
- 270 • Does the chemical inhibition of cellulose synthase induce Ca^{2+} changes and associated
271 signalling events in a way similar to the CBDs?
- 272 • Can sucrose sensing and a CESA-MT link be involved in CBD-induced defense?

References

- 1 Lamport, D.T.A. and Northcote, D.H. (1960) Hydroxyproline in primary cell walls of higher plants. *Nature* 188, 665-666
- 2 Darvill, A. and Albersheim, P. (1984) Phytoalexins and their elicitors: A defense against microbial infection in plants. *Annu. Rev. Plant Physiol. Plant Mol. Biol.* 35, 243-275
- 3 Esquerré-Tugayé, M.T. (2006) Hydroxyproline-rich glycoproteins in plant-microbe interactions. In *The science and lore of the plant cell wall* (Hayashi, T., ed.), pp. 303-309, Brown Walker Press
- 4 von Röpenack, E. et al. (1998) Structural analyses and dynamics of soluble and cell wall-bound phenolics in a broad spectrum resistance to the powdery mildew fungus in barley. *J. Biol. Chem.* 273, 9013-9022
- 5 Humphrey, T.V. et al. (2007) Sentinels at the wall: cell wall receptors and sensors. *New Phytol.* 176, 7-21
- 6 Somerville, C. (2006) Cellulose synthesis in higher plants. *Annu. Rev. Cell Dev. Biol.* 22, 53-78
- 7 Joshi, C.P. and Mansfield, S.D. (2007) The cellulose paradox--simple molecule, complex biosynthesis. *Curr. Opin. Plant Biol.* 10, 220-226
- 8 Vorwerk, S. et al. (2004) The role of plant cell wall polysaccharide composition in disease resistance. *Trends Plant Sci.* 9, 203-209
- 9 Huckelhoven, R. (2007) Cell wall-associated mechanisms of disease resistance and susceptibility. *Annu. Rev. Phytopathol.* 45, 101-127
- 10 Gaulin, E. et al. (2006) Cellulose binding domains of a *Phytophthora* cell wall protein are novel pathogen-associated molecular patterns. *Plant Cell* 18, 1766-1777

- 298 11 Gaulin, E. *et al.* (2002) The CBEL glycoprotein of *Phytophthora parasitica* var-
 299 *nicotianae* is involved in cell wall deposition and adhesion to cellulosic substrates. *J.*
 300 *Cell Sci.* 115, 4565-4575
- 301 12 Pear, J.R. *et al.* (1996) Higher plants contain homologs of the bacterial *celA* genes
 302 encoding the catalytic subunit of cellulose synthase. *Proc. Natl. Acad. Sci. USA.* 93,
 303 12637-12642
- 304 13 Persson, S. *et al.* (2007) Genetic evidence for three unique components in primary
 305 cell-wall cellulose synthase complexes in *Arabidopsis*. *Proc. Natl. Acad. Sci. USA* 104
 306 (39), 15566-15571
- 307 14 Desprez, T. *et al.* (2007) Organization of cellulose synthase complexes involved in
 308 primary cell wall synthesis in *Arabidopsis thaliana*. *Proc. Natl. Acad. Sci. USA* 104
 309 (39), 15572-15577
- 310 15 Burton, R.A. *et al.* (2000) Virus-induced silencing of a plant cellulose synthase gene.
 311 *Plant Cell* 12, 691-706
- 312 16 Cano-Delgado, A. *et al.* (2003) Reduced cellulose synthesis invokes lignification and
 313 defense responses in *Arabidopsis thaliana*. *Plant J.* 34, 351-362
- 314 17 His, I. *et al.* (2001) Altered pectin composition in primary cell walls of *korrigan*, a
 315 dwarf mutant of *Arabidopsis* deficient in a membrane-bound endo-1,4 β -glucanase.
 316 *Planta* 212, 348-358
- 317 18 Pagant, S. *et al.* (2002) *KOBITO1* encodes a novel plasma membrane protein
 318 necessary for normal synthesis of cellulose during cell expansion in *Arabidopsis*.
 319 *Plant Cell* 14, 2001-2013
- 320 19 Roudier, F. *et al.* (2002) The COBRA family of putative GPI-anchored proteins in
 321 *Arabidopsis*. A new fellowship in expansion. *Plant Physiol.* 130, 538-548

- 322 20 Amor, Y. et al. (1995) A membrane-associated form of sucrose synthase and its
 323 potential role in synthesis of cellulose and callose in plants. *Proc. Natl. Acad. Sci.*
 324 *USA.* 92, 9353-9357
- 325 21 Haigler, C.H. et al. (2001) Carbon partitioning to cellulose synthesis. *Plant Mol. Biol.*
 326 47, 29-51
- 327 22 Ruan, Y.L. et al. (2003) Suppression of sucrose synthase gene expression represses
 328 cotton fiber cell initiation, elongation, and seed development. *Plant Cell* 15, 952-964
- 329 23 Coleman, H.D. et al. (2006) Up-regulation of sucrose synthase and UDP-glucose
 330 pyrophosphorylase impacts plant growth and metabolism. *Plant Biotechnol. J.* 4, 87-
 331 101
- 332 24 Baskin, T.I. (2001) On the alignment of cellulose microfibrils by cortical
 333 microtubules: a review and a model. *Protoplasma* 215, 150-171
- 334 25 Fisher, D.B. and Cyr, R.J. (1998) Extending the microtubule/microfibril paradigm.
 335 Cellulose synthesis is required for normal cortical microtubule alignment in elongating
 336 cells. *Plant Physiol.* 116, 1043-1051
- 337 26 Gardiner, J.C. et al. (2003) Control of cellulose synthase complex localization in
 338 developing xylem. *Plant Cell* 15, 1740-1748
- 339 27 Paredez, A.R. et al. (2006) Visualization of cellulose synthase demonstrates functional
 340 association with microtubules. *Science* 312, 1491-1495
- 341 28 Cosgrove, D.J. et al. (2002) The growing world of expansins. *Plant Cell Physiol.* 43,
 342 1436-1444
- 343 29 Zenoni, S. et al. (2004) Downregulation of the *Petunia hybrida* α -expansin gene
 344 *PhEXPI* reduces the amount of crystalline cellulose in cell walls and leads to
 345 phenotypic changes in petal limbs. *Plant Cell* 16, 295-308

- 346 30 Levy, I. et al. (2002) Modification of polysaccharides and plant cell wall by endo-1,4-
347 β -glucanase and cellulose-binding domains. *Biomol Eng.* 19, 17-30
- 348 31 Séjalon-Delmas, N. et al. (1997) Purification, elicitor activity, and cell wall
349 localization of a glycoprotein from *Phytophthora parasitica* var. *nicotianae*, a fungal
350 pathogen of tobacco. *Phytopathology* 87, 899-909
- 351 32 Villalba-Mateos, F. et al. (1997) Cloning and characterization of a cDNA encoding an
352 elicitor of *Phytophthora parasitica* var. *nicotianae* that shows cellulose-binding and
353 lectin-like activities. *Mol. Plant-Microbe Interact.* 10, 1045-1053
- 354 33 Gilkes, N.R. et al. (1991) Domains in microbial β -1,4-glycanases: sequence
355 conservation, function, and enzyme families. *Microbiol. Rev.* 55, 303-315
- 356 34 Judelson, H.S. (2007) Genomics of the plant pathogenic oomycete *Phytophthora*:
357 insights into biology and evolution. *Adv. Genet.* 57, 97-141
- 358 35 Hashimoto, H. (2006) Recent structural studies of carbohydrate-binding modules. *Cell*
359 *Mol Life Sci* 63 (24), 2954-2967
- 360 36 Shoseyov, O. et al. (2006) Carbohydrate binding modules: biochemical properties and
361 novel applications. *Microbiol. Mol. Biol. Rev.* 70, 283-295
- 362 37 Ding, X. et al. (1998) A secretory cellulose-binding protein cDNA cloned from the
363 root-knot nematode (*Meloidogyne incognita*). *Mol. Plant-Microbe Interact.* 11, 952-
364 959
- 365 38 Ellis, C. and Turner, J.G. (2001) The *Arabidopsis* mutant *cevl* has constitutively
366 active jasmonate and ethylene signal pathways and enhanced resistance to pathogens.
367 *Plant Cell* 13, 1025-1033
- 368 39 Ellis, C. et al. (2002) The *Arabidopsis* mutant *cevl* links cell wall signalling to
369 jasmonate and ethylene responses. *Plant Cell* 14, 1557-1566

- 370 40 Chen, Z. et al. (2005) Disruption of the cellulose synthase gene, *AtCesA8/IRX1*,
371 enhances drought and osmotic stress tolerance in *Arabidopsis*. *Plant J.* 43, 273-283
- 372 41 Hernandez-Blanco, C. et al. (2007) Impairment of cellulose synthases required for
373 *Arabidopsis* secondary cell wall formation enhances disease resistance. *Plant Cell* 19,
374 890-903
- 375 42 Nishimura, M.T. et al. (2003) Loss of a callose synthase results in salicylic acid-
376 dependent disease resistance. *Science* 301, 969-972
- 377 43 Khatib, M. et al. (2004) The CBEL elicitor of *Phytophthora parasitica* var. *nicotianae*
378 activates defence in *Arabidopsis thaliana* via three different signalling pathways. *New*
379 *Phytol.* 162, 501-510
- 380 44 Carden, D.E. and Felle, H.H. (2003) The mode of action of cell wall-degrading
381 enzymes and their interference with Nod factor signalling in *Medicago sativa* root
382 hairs. *Planta* 216, 993-1002
- 383 45 Levin, D.E. (2005) Cell wall integrity signaling in *Saccharomyces cerevisiae*.
384 *Microbiol. Mol. Biol. Rev.* 69 (2), 262-291
- 385 46 Nakagawa, Y. et al. (2007) *Arabidopsis* plasma membrane protein crucial for Ca^{2+}
386 influx and touch sensing in roots. *Proc. Natl. Acad. Sci. USA* 104, 3639-3644
- 387 47 Hematy, K. et al. (2007) A receptor-like kinase mediates the response of *Arabidopsis*
388 cells to the inhibition of cellulose synthesis. *Curr. Biol.* 17, 922-931
- 389 48 Rolland, F. et al. (2006) Sugar sensing and signaling in plants: conserved and novel
390 mechanisms. *Annu. Rev. Plant Biol.* 57, 675-709
- 391 49 Gomez-Ariza, J. et al. (2007) Sucrose-mediated priming of plant defense responses
392 and broad-spectrum disease resistance by overexpression of the maize pathogenesis-
393 related PRms protein in rice plants. *Mol. Plant-Microbe Interact.* 20, 832-842

394 50 Gross, P. *et al.* (1993) Translocation of cytoplasm and nucleus to fungal penetration
395 sites is associated with depolymerization of microtubules and defence gene activation
396 in infected, cultured parsley cells. *EMBO J.* 12, 1735-1744

Legend to figure 1

Figure 1. A model for signal transduction linking CBD perception and cellulose alterations to defense activation. (i) It is proposed that the fast increase in the cytosolic calcium induced by CBEL treatment is mediated by a permeable mechanosensing channel system that relies on Mca1 or on a Mca1-like protein. (ii) Activation of Mca1 would either occur directly in response to a changing physicochemical environment of the cell wall or through its interaction with a cellulose synthase-integrity sensor system (CESA, THE1). Mca1 and THE1 are two plasma membrane proteins related to Ca^{2+} influx (Mca1) and to cell wall integrity sensing (THE1) in *Arabidopsis*. (iii) Calcium might then activate phosphorylation processes, notably on the phosphorylable sites of CESA and THE1. (iv) Changes in CESA and THE1 activities would finally be the starting points of downstream signalling cascades leading to defense gene expression. Possible crosstalks with ethylene (ET), jasmonic acid (JA), salicylic acid (SA) and abscisic acid (ABA) signalling pathways that are induced by cellulose alterations are indicated.

Figure

FIGURE 1