Cell wall integrity maintenance in plants
Lessons to be learned from yeast?

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Key words: plant, cell wall integrity, biotic/abiotic stress response

The plant cell wall is involved in different biological processes like cell morphogenesis and response to biotic/abiotic stress. Functional integrity of the wall is apparently being maintained during these processes by changing structure/composition and coordinating cell wall with cellular metabolism. In *S. cerevisiae* a well-characterized mechanism exists that is maintaining functional integrity of the yeast cell wall during similar processes. During recent years it has become obvious that plants have evolved a mechanism to monitor and maintain functional integrity of their cell walls. However, our understanding of the mechanism is rather limited. The available evidence suggests that similar signaling cascades may be involved and particular protein activities may be conserved between plants and yeast. Here we review the available evidence briefly and highlight similarities between yeast and plants that could help us to understand the mode of action of the signaling cascades maintaining plant cell wall integrity.

The plant cell wall is involved in different biological processes such as cell morphogenesis and biotic/abiotic stress responses.1,2 Maintaining functional integrity of the cell wall during these different processes is essential. In *S. cerevisiae* a dedicated mechanism monitoring and maintaining functional integrity of the cell wall has been described.3,4 Although this specialized mechanism exists, the available data shows that both osmo- and mechano-perception mechanisms are also involved in cell wall integrity (CWI) maintenance in yeast.3,4 Recently, evidence has accumulated suggesting that a similar CWI maintenance mechanism exists in plants, and several excellent reviews have covered this area to some extent.3,6 However, it has become increasingly obvious that plant CWI maintenance may additionally involve osmo- and damage associated molecular pattern (DAMP)-perception.2,7 DAMPs are low-molecular weight molecules like oligogalacturonides (OGs) derived from plant cell walls named in analogy to pathogen associated molecular patterns (PAMP).8 They are thought to arise during exposure of cell walls to abiotic/biotic stress and possibly during cell morphogenesis. These observations suggest that the plant CWI maintenance mechanism could actually be just one component of a matrix of signaling cascades coordinating and tailoring cellular responses to maintain plant cell wall integrity during interaction with the environment and development.9 Combining knowledge derived from yeast and plant research, this review aims to highlight how different plant signaling cascades could interact to maintain functional plant CWI.

The Yeast Cell Wall Integrity Matrix

Three different sensor systems can monitor the functional integrity of the yeast cell wall and modulate responses to maintain CWI upon cell wall damage (CWD): The MID1 CCH1 based mechano-perception pathway; the high-osmolality glycerol (HOG) pathway and the CWI maintenance mechanism.3 The signal generated by the CCH1 MID1 complex upon membrane stretch is relayed via calcineurin and CRZ1 to activate response genes like the glucan synthase FKS2.10 FKS2 activity is additionally regulated by the CWI pathway. Two different sensors (SHO1; SLN1/YPD1/SSK1) perceive hyperosmotic stress and generate signals relayed to the MAPKinase HOG1.11,12 These signals lead to activation of the transcriptional response via SKN7.13 SKN7 also mediates responses induced by the plasma membrane localized CWI sensor MID2 through interaction with CRZ1.3,14 Sequence similarity between the different yeast CWI sensor proteins WSC1, 2, 3, MTL1 and MID2 is limited and they appear to be required during distinct biological processes.15 Their extracellular regions, formed by cysteine rich domains (CRD) and highly O-mannosylated serine/threonine rich (STR) domains, project antenna-like into the yeast cell wall.16 The CRD domain is considered capable of interacting with glucans thus linking the extracellular domain of the sensor closely to the cell wall.17 Biophysical evidence suggests that the STR domain has properties of a nanospring thus enabling it to translate any conformational change of the extracellular domain when triggered by strain on the cell wall or the membrane, to the cytoplasmic part of the sensor.18 The cytoplasmic region of the sensors interact with the GDP/GTP exchange factor ROM2 generating a signal that is translated via protein kinase C and a MAPkinase module that includes SLT2.19 Interestingly, the hyperosmotic stress activated HOG pathway interacts with the CWI pathway when induced by hypo-osmotic shock, thus modulating the response of the yeast cells to low pH, heat shock and zymolysis treatment.4 During the response to zymolase (an enzyme mix consisting mostly of β-1,3glucanase activity) treatment the molecular mechanism...
coordinating both signaling cascades involves the MAPKinases SLT2, HOG1 and the PTP2 phosphatase.\textsuperscript{20-22} In a SLT2 deficient strain PTP2 expression is not induced by zymolase treatment, the phosphorylation level of the HOG1 MAPKinase is increased and expression of several stress response genes is induced.\textsuperscript{20} To summarize; in yeast, three different signaling mechanisms monitor events (membrane stretch, CWD and osmo-stress) indicative of possible CWI impairment and mediate the responses to maintain CWI. In certain situations exemplified here by zymolase treatment different signaling mechanisms interact to modulate the response to a particular type of stress indicating a CWI signaling matrix exists in yeast.

**The Plant Cell Wall Integrity Maintenance Mechanism**

Evidence for the existence of a plant CWI maintenance mechanism has accumulated recently. A wide range of responses to different types of CWD has been described. Examples include enhanced pathogen resistance, ectopic lignin deposition, increased production of jasmonic acid, deposition of neutral cell wall sugars and changes in carbohydrate metabolism.\textsuperscript{23-28} WAKs have been shown to participate in the hydrolysis of homogalacturonan, PGs apparently release polysaccharides.\textsuperscript{30} During the hydrolysis of homogalacturonan, PGs apparently release OGs from the pectin matrix, (embedded in the cellulose-hemi-cellulose network). \textit{WALL-ASSOCIATED KINASE1} (WAK1) has been shown to reside in the plasma membrane, bind tightly to cell walls and respond to OGs derived from pectic polysaccharides.\textsuperscript{31,32} WAKs have been shown to bind covalently to pectic homogalacturonan in plants and non-covalently to Ca\textsuperscript{2+}-crosslinked OGs in culture.\textsuperscript{33,34} WAK2 has been implicated in turgor pressure-sensitive processes linking pectin perception with activation of an invertase that can modulate soluble sugar levels in planta, which in turn affects turgor pressure.\textsuperscript{35,36} These observations suggest that plant WAKs might be the functional analogs of the yeast CWI sensors monitoring the functional integrity of the plant cell wall through interaction with pectic polysaccharides. Downstream elements of the signaling cascade might be MAPKinases 3 and 6, but the signal transduction between these elements of cascade remains to be determined.\textsuperscript{36} BETWEEN US (THE), HERCULES (HERK1) and FERONIA (FER) belong to the \textit{Cataranthus roseus}-like RLK (CrRLK1L) family and have been implicated in CWI maintenance during development.\textsuperscript{37,39} HERK1 and FER affect cell elongation, but no CWD response phenotypes have been described to date.\textsuperscript{38} The seedlings exhibit defects in cell morphogenesis and CBI-induced lignin deposition suggesting that the same CWI maintenance mechanism may be active during both processes.\textsuperscript{37} While all these kinases have been implicated in CWI maintenance their specific functions and ligands remain to be characterized. To summarize, WAKs could represent the plant analogs of yeast CWI sensors. In the case of WAK2 the signals generated activate an invertase that can change soluble sugar levels, which in turn could affect turgor pressure.

Representative examples for the second group of sensors are \textit{MID1-COMPLEMENTING ACTIVITY} 1 and 2 (MCA1 and 2).\textsuperscript{40,41} These putatively stretch-activated, plasma membrane localized Ca\textsuperscript{2+}-channels can partially complement the mutant phenotype of a MID1 deficient yeast strain. \textit{mca1} Arabidopsis seedlings exhibit root growth defects, calcium influx in root cells upon mechano-stimulation is reduced and less ectopic lignin is deposited upon cellulose biosynthesis inhibition (CBI).\textsuperscript{39,40,41} CBI is a well-established method to cause highly specific cell wall damage by weakening the cellulose microfibril based exoskeleton, providing most of the structural support to a plant cell.\textsuperscript{42,43} These observations implicate \textit{MCA1} and Ca\textsuperscript{2+}-based signaling processes in the response to CWD. Furthermore, calcium signaling inhibitors prevent CBI-induced lignin and reactive oxygen species (ROS) and JA production in a concentration dependent manner.\textsuperscript{40} CBI-induced ROS signaling (OXII, \textit{rbohD}) mutants while being reduced in JA production enhanced.\textsuperscript{9} These observations suggest that THE is required for ROS biosynthesis and is not the only CWD sensor in Arabidopsis. In addition, it indicates that JA/ROS may form a negative feedback loop inhibiting each other’s production.\textsuperscript{9} The extent of CWD-induced lignin deposition seems to be modulated by JA/ROS signaling due to lignin being reduced in ROS signaling/production (\textit{OXII, rbohD}) mutants while being enhanced in JA signaling and biosynthesis mutants.\textsuperscript{9} To summarize JA, ROS and Ca\textsuperscript{2+}-based signaling mechanisms mediate the response to CWD in plants. Interestingly, Arabidopsis \textit{MCA1} is able to rescue the MIDI yeast mutant while also being required for CWD-induced lignin deposition in Arabidopsis.

The third group of sensors is exemplified by the \textit{ARABIDOPSIS HISTIDINE KINASES} (AHK1–3, AHK4/CRE1). They form part of a two-component system consisting of a histidine kinase acting as environmental sensor and a phosphor-relay system to translate the signal generated.\textsuperscript{45} The available literature has implicated these genes in cytokinin and osmo-perception as well as ABA-dependent abiotic plant stress responses.\textsuperscript{46,47} Expression of CRE1 in a SLN1 deficient yeast strain rescues the mutant phenotype if cytokinin is present.\textsuperscript{48} Previous work has shown that provision of osmotic support prevents ectopic lignification and
necrosis induced by cellulose inhibition. CBI also induces starch increases in Arabidopsis seedlings, which can be suppressed by osmotic support (Wormit et al. under review). These results implicate osmosensing in CWI maintenance, but do not clarify the specific function of turgor pressure in this context. Turgor pressure could function as an indicator of plant CWI or similarly, as in yeast, complement the activity of the CWI maintenance pathway. In ahk1, ahk4/cre1 and mca1 seedlings the CBI-induced starch increase is detectable. However, the osmotic suppression is not detectable in ahk4/cre1 and mca1 seedlings (Wormit et al. under review). These observations suggest that CRE1 and MCA1 but not AHK1 are mediating the observed osmotic support effect. Since the CBI-induced starch increases are detectable in both cre1 and mca1 seedlings, CWI perception itself is either occurring in parallel to turgor perception, or is redundantly specified. More importantly, the responses to CWD can be modulated by turgor pressure changes and CRE1 and MCA1 are required for this mechanism, as shown by the observed effects on starch levels in the mutant seedlings.

Conclusions

The available data suggest both design similarities and functional conservation between the yeast and plant cell wall integrity monitoring and maintenance systems. These are illustrated by the color-coding in Figure 1A and B of signaling cascades in yeast (oval) and plant cells (rectangular). Despite the CWI sensor proteins being well characterized in yeast, our understanding of the corresponding plant proteins is limited. The strongest candidates in plants to perform this function are DAMP receptors and WAKs. DAMP receptors are plant specific and nothing similar has been observed in yeast. They could represent an additional level of detection enabling plants to deal with biotic stresses that yeast cells do not experience. Based on this knowledge, it is likely that WAKs represent the group of plant proteins most similar to CWI sensors in yeast based on their mode of action and apparent biological activities. Data from WAK2 implicates turgor pressure perception in the modulation of the CWD response in plants. Previous work in yeast has shown that the CWI and the HOG signaling/osmosensing pathway regulate the response to zymolase treatment jointly. The effects of zymolase and CBI on yeast and plant cell walls are similar (breakdown of the load-bearing cell wall elements). The Arabidopsis protein CRE1 can functionally replace one of the two osmo sensors (SLN1) of the HOG pathway. Accordingly, it is intriguing that osmotic support can neutralize the effects of CBI on starch levels in Arabidopsis wild type seedlings but not in cre1 seedlings. Recent work has shown that the Arabidopsis MCA1 protein can complement a MID1 deficient yeast strain, and mca1 seedlings are impaired in CBI induced lignin deposition. These observations suggest that in yeast and Arabidopsis, a similar matrix of signaling cascades may mediate the response to impairment of CWI, and that protein activities are conserved to a certain degree between both species. From the current perspective this research area represents a novel, original approach to understand the mode of action of plant pathogen response mechanisms and environmental stress by placing the plant cell wall at the heart of initial perception, signaling and response to environmental and developmental stimuli.

Acknowledgments

The authors would like to thank Joe McKenna for critical reading of the manuscript and helpful comments. Work in the Hamann lab is supported by the BBSRC Sustainable Bioenergy Center, the Porter Institute at Imperial College and the Gatsby Charitable Foundation.
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