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Sensing environmental and developmental signals *via* cellooligomers

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ABSTRACT

Roots respond to a cocktail of chemicals from microbes in the rhizosphere. Infochemicals in nmol concentrations activate receptor-mediated signal pathways, which reprogram the plant responses to environmental changes. The microbial signals have to pass the cell wall to activate pattern recognition receptors at the surface of the plant plasma membrane. The structure of the cell wall is not only a barrier for the signaling molecules, but also changes permanently during growth and development, as well as in response to microbial attacks or abiotic stress. Recently, cellooligomers (COMs) were identified as novel chemical mediators in *Arabidopsis thaliana*, which inform the cell about the alterations in and around the cell wall. They can be of microbial and plant origin and represent novel invasion patterns (Cook et al., 2015). COMs initiate Ca^{2+}-dependent signaling events that reprogram the cell and adjust the expression and metabolite profiles as well as innate immunity in response to changes in their rhizosphere environment and the state of the cell wall. COMs operate synergistically with other signals or their recognition machineries and activates local and systemic responses in the entire plant. They also adjust the performance of the areal parts of the plant to signals perceived by the roots. Here, I summarize our current knowledge about COMs and propose strategies for future investigations.

1. Introduction

Roots respond to millions of chemicals from microbes in the rhizosphere. After perception, elaborate communication systems in the roots integrate the information for appropriate and balanced responses which result in pathogenic or mutualistic interactions (Jones and Dangl, 2006; Zipfel, 2008; van't Padje et al., 2016; Rasmann and Turlings, 2016; Zipfel and Oldroyd, 2017; Macho and Zipfel, 2014). Interaction of roots with microorganisms and recognition of chemical mediators often start at the cell surface. According to the Invasion Model proposed by Cook et al. (2015), plant immunity functions as a surveillance system to detect invasion of microbes into the host cells. Invasion patterns (IPs) perceived by IP receptors active IP-triggered responses which may be suppressed (e.g. by biotrophs) or ultilized (e.g. necrotrophs) to continue symbiosis. IPs can be microbe- or host-derived immunogenic ligands (Cook et al., 2015). Microbe-derived IPs may have to pass the plant cell wall before reaching the plasma membrane, host-derived ligands, e.g. generated by microbe-induced modifications of the host cell wall, may accumulate in the vicinity of the IP receptors at the plasma membrane. All microbes modify plant cell walls and thus potentially generate host-derived IPs according to their needs. Colonization by pathogenic microbes results in more cell wall damage than colonization by beneficial microbes which – besides damage – also induce re-arrangement of the cell wall or reprogramming of the entire cell to form new structures (Parniske, 2004). All these cell wall modifications might be associated with the generation of specific plant-derived IPs. Structural changes of the cell wall occur also upon exposure to abiotic stess (e.g. Rao and Dixon, 2017) or chemicals present in the environment, and during developmental processes such as growth, cell divison, elongation, differentiation or the initation of lateral root and root hair formation (Cosgrove, 2005; Boisson-Dernier et al., 2011; Kohorn, 2016; Franciosini et al., 2017). These changes in the cell wall structure caused by environmental and plant internal signals are perceived by the root cells, and the state and integrity of the wall activate appropriate local responses (Souza et al., 2017), but also inform distal parts of the plant *via* systemic signal propagation.

2. Arabidopsis plants perceive cellooligomers (COMs)

Recently, it was discovered that COMs are perceived by plant cells, in particular root cells from Arabidopsis, and induce signaling events which resemble those activated by other well-characterized IPs (Souza et al., 2017; Johnson et al., 2018). The plant sensing system responds to very low concentrations of COs in the vicinity of the plasma membrane suggesting sensitivitiy and, maybe, specificity. It has been proposed that COMs monitor the integrity and the current state of the cell wall

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Abbreviations: COM, cellooligomer; PARN, poly(A) ribonuclease; $[Ca^{2+}]_{cyt}$, cytoplasmic Ca^{2+}; IP, invasion pattern

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(Souza et al., 2017), since they are generated during cell wall degradation after pathogen attack. However, cellotriose is also released by the beneficial fungus *Piriformospora indica* (renamed *Serendipita indica*, Weiß et al., 2016) to establish a symbiotic interaction with the roots of the host (Johnson et al., 2018). Thus, it is likely that *S. indica* uses this ligand as a strategy to mimic cell wall damage. Since COMs can be of microbial and host origin, they might be good candidates to integrate information about the cell wall integrity with signals from the microbial rhizosphere.

COMs are short-chain β(1→4) linked D-glucose units originating from the primary carbon metabolism of all organisms, or from the breakdown of long-chain β-1,4-glucans such as cellulose. Numerous enyzmes of plant, bacterial and fungal origin have been described which synthesize COMs from short chain glucans by transglycosidation activities. Breakdown of cellulose, a polysaccharide consisting of a linear chain of hundreds to thousands of glucose units, is also important for the generation of COMs, since cellulose is the major constituent of the plant cell walls, but also found in cell walls of bacteria, algae and tunicates (Cumming et al., 2005, and ref. therein). The plant cell wall is degraded to COMs by plant or microbial glucosidases. Fungi do not contain cellulose in their cell walls, but secrete glucosidases into the apoplast which generate short chain β-1,4 glucans by the degradation of cellulose or other glucan polymers, e.g. from the plant cell wall (Karlsson et al., 2002; McCarthy et al., 2003; Liu et al., 2010; Chen et al., 2014). Many glucosidases of either plant or microbial origin posses also transglycosylation activities to add a glucose moiety to short chain COMs. Thus, fungal glucosidases with transglycosylation activities can synthesize cellotriose or other short chain COMs from cellobiose and glucose, i.e. COMs with an even or uneven number of glucose moieties in the intra- and extracellular space (Smaali et al., 2004; Zhao et al., 2015; Boudabbous et al., 2017; Mallek-Fakhfakh and Belghith, 2016; Guo et al., 2016). Some (saprophytic) fungi are known to produce cellobiose (Spanu et al., 2010; Glass et al., 2013). Suzuki et al. (2010) reported that cellotriose could also induce the expression of a cellobiohydrogenase gene in *Phanerochaete chrysosporium* which supports the idea that this COM may have signaling-inducing functions. Taken together, COMs can be generated *via* multiple biochemical pathways.

3. Integration of environmental and internal signals *via* COMs

One of the earliest plant response to COMs is a rapid and transient increase in the cytoplasmic Ca^{2+} ($[Ca^{2+}]_{cyt}$) elevation in the Arabidopsis root cells which reaches its maximum 90 s after the application of the stimulus (Souza et al., 2017; Johnson et al., 2018). Ca^{2+} responses are often monitored with the aequorin technology, which is based on light emission detected by a luminometer (Knight et al., 1993). The Ca^{2+} response cannot be induced by sugar monomers (Johnson et al., 2018) which indicates that plants have evolved a specific perception system for short chain β-1→4-glucans. Likewise, the COM-responsive *WRKY30* promoter responded only to cellobiose but not to disaccharides with different linkages (Souza et al., 2017).

All COMs are active in nmolar or μmolar concentrations, and a comparative analysis showed that cellotriose is more active in inducing $[Ca^{2+}]_{cyt}$ elevation in Arabidopsis roots than other COMs (n = 2, 4–7, Johnson et al., 2018). Cellotriose was originally identified as an effector molecule from *S. indica* (Johnson et al., 2018) which raises the question why a beneficial fungus without cellulose in its cell wall utilizes cellotriose for Ca^{2+} responses in plant roots. Cellotriose was isolated from sterile-grown *S. indica* cultures in the absence of a host (Johnson et al., 2018). Therefore, the fungus synthesizes and releases this chemical mediator in the absence of any symbiotic partner. *S. indica* has also a huge number of genes for cellulose-degrading enzymes in the genome (Zuccaro et al., 2011), presumably due to its saprophytic lifestyle (Lahrmann et al., 2013). These enzymes can cleave cellulose in the host cell wall once a contact is established, and they might contribute to the signaling although this has not been demonstrated yet. Moreover, the *S. indica* genome encodes glucosidases with putative transglycosylation capacities which allow synthesis of cellotriose from cellobiose and a glucose moiety. This suggests that *S. indica* possesses multiple possibilities to generate cellotriose in symbiotic interactions with plant cells.

The available data from Arabidopsis suggest that COM perception occurs more frequently in roots than in shoots (Souza et al., 2017; Johnson et al., 2018) indicating that it is preferentially involved in the recognition of below-ground information. *WRKY30*, a gene activated upon COM perception, exhibited constitutive expression in cotyledons, while expression in seedling roots was tightly regulated, being elicitor-dependent and undetectable in the absence of a stimulus (Souza et al., 2017). Since elevated expression of *WRKY30* by cellobiose is 20-fold less in the *mpk6* mutant, MAP kinase (MAPK) 6 plays an important role in the cellobiose signal transduction pathway leading to *WRKY30* expression (Souza et al., 2017). It is conceivable that COMs operate synergistically with more specific systems or elicitors to establish an appropriate response. Chitin activates immue responses in roots and shoots of Arabidopsis, and one of the responses induced by chitin is the stimulation of *RBOHD* (Respiratory Burst Oxidase Homologue D, a plant NADPH oxidase) expression and RBOHD activity for H_2O_2 production. COMs induces much less ROS production than chitin: e.g. cellotriose stimulates little ROS production which is dependent on RBOHD (Johnson et al., 2018) and cellobiose did not produce any ROS (Souza et al., 2017). Furthermore, the cellobiose signaling pathway appears to be independent of ROS formation, since stimulation of *WRKY30* expression by this COM is similar in WT and *rbohD rbohC* roots (Souza et al., 2017). These results are consistent with only a mild activation of defense-relatesd gene expression upon COM perception. The majority of genes which respond to chitin also respond to COMs, whereas the stimulation is often less (Souza et al., 2017; Johnson et al., 2018). However, numerous genes for proteins involved in primary metabolism, root development and growth regulation are only activated by the COM cellotriose, but not chitin (Johnson et al., 2018). Therefore, COMs have functions which differ from other IPs.

Chitin-stimulated *RBOHD* expression is stronger and faster if the roots are pretreated with cellotriose (Johnson et al., 2018). Likewise, Souza et al. (2017) showed that cellobiose pretreatment confers increased resistance to *Pseudomonas syringae* pv *tomato* DC3000 infection. Treatment with cellobiose, cellotriose, and cellotetraose elicited similar levels of *WRKY30* expression in Arabidopsis, but also other defense-related *WRKY* genes were activated. Cellobiose had an additive effect with other IPs on pattern-triggered immunity signaling (Souza et al., 2017). MAPK activation was amplified in combination treatments of cellobiose, chitooligomers, and flg22 (Souza et al., 2017). The authors suggest an independent mode of cellobiose perception and a quantitative amplification of the immune signaling cascade when additional stimuli are present. The amplification of defense signaling in response to the simultaneous perception of multiple stimuli may render a stronger immune response (Souza et al., 2017). These studies demonstrate that COM perception represents a novel surveillance system, preferentially active in roots, that synergistically operates with other stimuli (such as flg22, oligogalacturonides or chitooligomers) to inform the cell about the integrity of the cell wall (Souza et al., 2017; Johnson et al., 2018). Signaling systems informing the cell or the entire plant body about the integrity and state of the cell wall and events occuring in the apoplast have been often described (Pilling and Höfte, 2003; Vorwerk et al., 2004; Hématy et al., 2007, 2009; Cheung and Wu, 2011; Denness et al., 2011; Ramírez et al., 2011; Trouvelot et al., 2014; Bolouri Moghaddam and Van den Ende, 2012; Wolf et al., 2012). COMs as breakdown products of cell wall material or released by microbes might play a crucial role as apoplastic signals. Synergism and integration of the signal information, as well as discrimination between different stimuli can be easily achieved within the Ca^{2+} signaling network which is utilized by many IP-triggered immune responses.

Ca^{2+} as second messanger has the capability of integrating COM-induced signals with other diverse chemical information (Sarwat et al.,