Microreview

Enemy at the gates: traffic at the plant cell pathogen interface

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Summary

The plant apoplast constitutes a space for early recognition of potentially harmful non-self. Basal pathogen recognition operates via dynamic sensing of conserved microbial patterns by pattern recognition receptors or of elicitor-active molecules released from plant cell walls during infection. Recognition elicits defence reactions depending on cellular export via SNARE (soluble N-ethylmaleimide-sensitive factor attachment protein receptor) complex-mediated vesicle fusion or plasma membrane transporter activity. Lipid rafts appear also involved in focusing immunity-associated proteins to the site of pathogen contact. Simultaneously, pathogen effectors target recognition, apoplastic host proteins and transport for cell wall-associated defence. This microreview highlights most recent reports on the arms race for plant disease and immunity at the cell surface.

Introduction

As sessile organism plants cannot escape biotic and abiotic stresses and constantly have to defend against a vast array of pathogens. To cope with this threat, plants have developed several layers of defence (Lipka et al., 2005; Ham et al., 2007). Besides the cell wall, especially the plasma membrane (PM) is not only a passive penetration barrier but also acts as the central interface of pathogen recognition and defence. Analogue to innate immunity in animals (Ausubel, 2005), plant defence is induced by recognition of non-self patterns that are mostly conserved indispensable molecules of the attacking organism. To warrant recognition of pathogen-associated molecular patterns (PAMPs) or microbe-associated molecular patterns, plants encode a high number of potential pattern recognition receptors (PRR). PAMP recognition by PRRs induces a plethora of defence responses referred to as PAMP-triggered immunity (PTI) (Jones and Dangl, 2006). PTI-associated defence often manifests at the plant cell wall and is accompanied by rearrangement of the cytoskeleton, the development of specialized membrane domains, formation of callose containing cell wall appositions, and requires exocytic SNARE (soluble N-ethylmaleimide-sensitive factor attachment protein receptor) complexes (Bhat et al., 2005; Hückelhoven, 2007a; Kwon et al., 2008a; Lipka et al., 2008). Successful pathogens evolved mechanisms to evade recognition or to suppress PTI by interference with signalling or defence. Secretion of virulence effectors by the pathogens mediates suppression of PTI and leads to effector-triggered susceptibility (ETS) (Jones and Dangl, 2006; Zhou and Chai, 2008). Plants in turn have evolved resistance proteins that recognize these virulence effectors and induce effector-triggered immunity (ETI), which again can be suppressed by pathogen effectors (Jones and Dangl, 2006). Unlike mammals, where innate immunity is completed by adaptive immunity, plants rely on the innate immunity. However, plant innate immunity builds on slowly evolving PTI and quickly evolving ETI (Chisholm et al., 2006; Jones and Dangl, 2006).

Many of the processes, which determine success or failure of pathogens, take place at the cell surface and hence the apoplast constitutes a major battlefield of plant pathogen interactions. This microreview highlights most recent findings on cell wall-associated defence and how the plasma secretes defence compounds into the apoplast.

Effectors target recognition and transport

Substantial progress has been made in the identification of virulence effectors of bacterial plant pathogens and in the understanding of the mechanisms of ETS (Chisholm et al., 2006; Zhou and Chai, 2008). Gram-negative pathogenic bacteria secrete effector proteins via a type III secretion system into eukaryotic host cells. One of the most prominent bacterial effectors is Pseudomonas syringae pv. tomato AvrPto (Table 1). Recently, it was demonstrated that AvrPto directly targets the PRR kinases...
flagellin sensing 2 (FLS2) and EF-Tu receptor. Apparently, AvrPto suppresses PRR-mediated PTI by blocking receptor phosphorylation (Xiang et al., 2008). Effects on the cell wall-based defence are described for AvrPto and other bacterial effectors (Ham et al., 2007; Hückelhoven, 2007b; Zhou and Chai, 2008). Treatment of plants with bacterial effectors (Ham et al., 2007) leads to virulence and transmission of, for example, oomycetes (Table 1). RAR1-dependent suppression of callose deposition in planta rar1 mutants (Ham et al., 2008). Furthermore, heterologous expression of HopM1 effector Avr2 inhibits the tomato apoplastic papain-like proteases Pip1 and Rcr3, which were under divergent selection likely driven by co-evolution with fungal effectors (Table 1). RCR3 is also required for Avr2-triggered immunity via the resistance protein Cf-2 (Table 1). Because RCR3 is less strongly expressed, Pip1 might be the major virulence target whereas RCR3 seems to constitute a decoy for ETI (Shabab et al., 2008). Interestingly, the tomato-resistance protein PTO directly interacts with AvrPto and this interaction requires similar amino acid motifs as the FLS2–AvrPto interaction in ETS. PTO might thus also represent a decoy that competes with the viru-

Table 1. Examples of recently characterized proteins involved in apoplastic defence or pathogen virulence in this regard.

| Protein* | Organism | Virulence/defence | Reference mechanism |
|----------|----------|--------------------|---------------------|
| Pathogen effectors | | | |
| AvrPto | P. syringae pv. tomato | Blocking FLS2 kinase activity and downstream defence | Xiang et al. (2008) |
| AvrB | P. syringae pv. tomato | RAR1-dependent suppression of callose | Shang et al. (2006) |
| HopM1 | P. syringae pv. tomato | Host proteasome-mediated degradation of an ARF-GEF (ATMIN7) | Nomura et al. (2006) |
| ATR13 | Hyaloperonospora parasitica | Suppression of callose and oxidative burst | Sohn et al. (2007) |
| Avr2 | Cladosporum | Inhibition of tomato apoplastic papain-like proteases Pip1 | Shabab et al. (2008) |
| Host proteins MIN7 | Arabidopsis thaliana | ARF-GEF involved in callose deposition for basal resistance to P. syringae; target of HopM1 | Nomura et al. (2006) |
| RCR3 | Solanum lycopersicum | Papain protease as potential decoy for Avr2 in ETI | Shabab et al. (2008) |
| PR2 | Glycine max | Endogluccanase involved in elicitor-release from oomycete cell wall; acts in race-specific resistance | Graham et al. (2007) |
| THESEUS1 | A. thaliana | Rlk involved in cell wall survey; activates defence gene expression and lignification | Hématy et al. (2007) |
| RIC171 | Hordeum vulgare | Is recruited by powdery mildew fungus to the site of penetration and supports fungal entry | Schultheiss et al. (2008) |
| VAMP721/722 | A. thaliana | Forms a ternary SNARE complex with SYP121 and SNAP33 for defensive vesicle fusion at the plasma membrane | Kwon et al. (2008b) |
| SYP132 | Nicotiana benthamiana | SNARE protein required for secretion of PR1 | Kalde et al. (2007) |
| PEN3/PDR8 | A. thaliana | Plasma membrane transporter presumably involved in secretion of low-molecular-weight defence compounds | Stein et al. (2006) |

* Ordered by appearance in the text.
lence target FLS2 for effector binding and consequently mediates ETI (Xiang et al., 2008; Zhou and Chai, 2008).

Dynamics of non-self recognition

Plant PRRs are receptor-like proteins or receptor-like kinases (RLK) in the PM. Both possess either LysM- or leucine-rich repeat motifs in their extracellular domains, which bind PAMPs (Zipfel, 2008). The Arabidopsis RLK FLS2 is one of the best characterized PRRs in regard to biological relevance and downstream signalling (Gómez-Gómez and Boller, 2000; Altenbach and Robatzek, 2007; Zipfel, 2008). Arabidopsis fls2 mutants are more susceptible to virulent P. syringae pv. tomato and to non-adapted Pseudomonas species (Zipfel, 2008). Interestingly, transport of FLS2 appears to be crucial for receptor function. Endocytosis of FLS2 into intracellular vesicles was shown to be involved in the activation of defence responses. Receptor phosphorylation after flg22 application appears to be a key to endocytosis, because mutation of T867 in FLS2 blocked receptor transport and downstream signalling. Another motif that could be required for endocytosis of FLS2 is the possibly ubiquitinated PEST-like motif in the kinase domain (Robatzek et al., 2006). Inhibitor studies further suggested that the cytoskeleton is crucial for endocytosis (Robatzek et al., 2006; Altenbach and Robatzek, 2007). Ubiquitination may also subject FLS2 to protein degradation via multivesicular bodies (MVBs) and the endosomal sorting complex required for transport. Potential degradation of FLS2 could avoid hyperinduction of PTI (Robatzek et al., 2006; Altenbach and Robatzek, 2007). The recent finding of flg22-dependent interaction between FLS2 and BRI1-associated receptor kinase 1 and a reduced lateral diffusion of FLS2 in the membrane after flg22 binding further indicates involvement of protein complexes and/or accumulation of the activated receptor in specialized detergent-resistant microdomains (DRMs) in the PM (Ali et al., 2007; Chinchilla et al., 2007; Heese et al., 2007). Less is known for non-specific perception and signalling of fungal non-self. However, the LysM RLK chitin elicitor receptor kinase 1 of Arabidopsis has been identified as a top candidate for the major chitin PRR because it is required for chitin-responsive defence and for basal resistance to distinct fungal pathogens (Miya et al., 2007; Wan et al., 2008). One can await that chitin elicitor receptor kinase 1, when compared with FLS2-mediated signalling and defence regulation, will show commonalities and perhaps interesting differences.

The PAMP recognition is generally considered to operate in non-specific plant defence and usually does not elicit a hypersensitive cell death reaction (HR) (Jones and Dangl, 2006). However, there seem to be exceptions. A soybean endoglucanase (pathogenesis-related protein 2, PR-2) operates in elicitor release for RPS1-mediated race-specific resistance to Phytophthora sojae (Table 1). Genetic evidence supports that HR and generation of H₂O₂ also depend on biosynthesis of 5-deoxyisoflavonoids (Graham et al., 2007). Silencing of PR-2 abolishes the HR to avirulent P. sojae and to a cell wall glucan elicitor. In this scenario, elicitor-active glucan fragments in concert with recognized oomycete effectors might trigger race-specific resistance (Graham et al., 2007). It would be interesting to learn about the nature of these race-specific elicitors and whether they are indeed sufficient to trigger an HR and/or operate as PAMPs in other plant species. It is also not yet clear whether PR-2 might be an elicitor target or can directly operate in signalling.

Besides recognition of non-self molecules, plants have other mechanisms for early sensing of pathogen attack. The survey of cell wall integrity seems to constitute an alternative for perception of pathogen invasion (Hückelhoven, 2007b; Dumas et al., 2008). Recent reports support a link of proper cell wall synthesis and the control of defence. The cpr5 (constitutive expressor of pathogenesis related-genes 5) mutant displays alterations in the cell wall carbohydrate composition and in trichome development and is less susceptible to virulent P. syringae pv. maculicola and to Hyaloperonospora parasitica. Hence, aberrant cell wall constitution might be interpreted by the plant as a pathogen attack and cause a defence response (Bowling et al., 1997; Brininstool et al., 2008). Additionally, THESEUS1, a RLK normally expressed during cell elongation, was recently described to regulate lignification and defence-related gene expression in cellulose synthase ces6a mutants (Hématy et al., 2007; Table 1). Although these mutants show phenotypes in absence of pathogens, the principle of cell monitoring may allow for detection of non-self activity in the apoplast. Microbes alter cell wall structure by both, cell wall binding (Dumas et al., 2008) or cell wall-degrading factors. Transgenic expression of a fungal endo-polygalacturonase increased resistance in tobacco and Arabidopsis to Botrytis cinerea and P. syringae. Apparently, the enzyme releases endogenous elicitor-active oligogalacturonides, which antagonize auxin responses and induce defence reactions (Ferrari et al., 2008). However, the general interplay of cell wall biogenesis, cell wall repair and pathogen defence remains little understood.

Polarization and vesicle trafficking at the plant pathogen interface

One of the first reactions observed in pathogen-attacked cells is the reorganization and polarization of the cytoskeleton to the site of attack (Schmelzer, 2002; Hückelhoven, 2007b). Rapid transport of antimicrobial compounds, proteins and cell wall material to the plant pathogen interface is supported by the aggregation of...
peroxisomes, endoplasmic reticulum and Golgi bodies at the site of attack (Takemoto et al., 2003; Koh et al., 2005; Lipka et al., 2005; Eichmann and Hückelhoven, 2008). Subcellular polarization processes are not limited to organelles and vesicles. Attacked plant cells seem to form DRMs or lipid rafts in the PM where the fungus attempts to penetrate (Bhat et al., 2005; Lipka et al., 2008). Studies on DRMs revealed an enrichment of sphingolipids and sterols and an accumulation of proteins associated with signalling and response to biotic and abiotic stresses, cell wall metabolism and cellular trafficking (Mongrand et al., 2004; Morel et al., 2006; Lefebvre et al., 2007). Localization of specialized PM domains to pathogen penetration sites was supported by live cell imaging in Arabidopsis and barley. Arabidopsis PEN1 (penetration mutant 1 = AtSYP121, syntaxin of plants) and the orthologue barley syntaxin HvROR2 (required for mlo-specified resistance) are components of the vesicle-targeting machinery. They localize to sites of attack from powdery mildew fungi similarly as the synaptosome-associated protein 33 (AtSNAP33), the receptor-like susceptibility factor MILDEW LOCUS O and the ATP-binding cassette transporter AIPEN3 protein (Collins et al., 2003; Assaad et al., 2004; Bhat et al., 2005; Stein et al., 2006; Kwon et al., 2008b). Barley HvRIC171 [RHO of plant (ROP)-interacting, CDC42/RHO-interactive binding domain containing protein of 171 amino acids] binds the barley susceptibility factor HvRACB (a RHO-like plant GTPase, synonym: ROP) in planta and also shows an accumulation at the entry sites of the powdery mildew fungus Blumeria graminis f.sp. hordei (Table 1). An HvRACB-binding fragment of HvRIC171 exerts a dominant negative effect on fungal penetration, suggesting a role of peripheral protein accumulation in pathogen entry (Schultheiss et al., 2008). Although it has been shown that RACB influences host cell polarity and filamentous actin organization (Opalski et al., 2005), further studies are needed on RACB-mediated signalling and on how it is corrupted by virulent B. graminis.

The formation of DRMs is independent of the cytoskeleton but might involve the influence and interaction of flavonoids with proteins and lipids (Bhat et al., 2005; Tarahovsky et al., 2008). Recent results propose protein lipid modification as a mechanism for targeting proteins to DRMs. Lipid modification of Arabidopsis AtROP6 is dependent on the activation status of the protein. Activated AtROP6 is transiently S-acylated at a conserved cysteine residue-mediating accumulation in DRMs (Sorek et al., 2007; Hemsley and Grierson, 2008). This prompted us to image the localization of the constitutive activated GFP-ROP6 fusion protein (GFP-AtROP6G64L, Molendijk et al., 2001) in Arabidopsis during interaction with the powdery mildew fungus Golovinomyces orontii. The presumably acylated AtROP6 located to sites of fungal attack (Fig. 1), supporting presence of DRMs at the plant pathogen interface. Together, DRM formation at the site of pathogen attack provides the plant with the possibility of spatial and temporal organization of proteins for pathogen interaction. Focal accumulation of PM proteins that operate in PTI and ETS further suggests a crucial function for DRMs in plant pathogen interactions.

Protein secretory pathway genes have a critical function in the systemic acquired resistance. This was demonstrated by corresponding mutants with defects in acquired resistance (Wang et al., 2005). The role of vesicle transport and fusion proteins such as SNAREs in defence is further highlighted by the rapid phosphorylation of PEN1/AtSYP121 and AtSYP122 after treatment with flg22 and of NtSYP121 after recognition of the effector Avr9. Rapid phosphorylation might factor these proteins into signalling or in primed secretion (Heese et al., 2005; Benschop et al., 2007; Nühse et al., 2007). It would be interesting to learn whether bacterial effectors such as AvrPto interfere with the phosphorylation of SNAREs.

Fig. 1. Localization of GFP-AtROP6G64L at the site of fungal entry. Golovinomyces orontii invades an epidermal cell of Arabidopsis (entry sites are indicated with an arrow). The picture was generated 19 h after inoculation by confocal laser scanning microscopy. The GFP-tagged AtROP6G64L, was expressed from a dexamethasone-inducible promoter according to Molendijk et al. (2001). Picture was generated 28 h after induction by 30 μmol l⁻¹ dexamethasone on rosette leaves. (A) Transmission channel; (B) GFP-channel; (C) overlay of (A) and (B). Bar = 20 μm.
The SNARE proteins drive the fusion of vesicles with the target membrane. Vesicle fusion requires three distinct types of SNARE proteins that form a ternary complex (Kwon et al., 2008a; Lipka et al., 2008). Focal secretion of vesicle cargo to the site of fungal attack in Arabidopsis is likely mediated by the formation of a ternary SNARE complex of PEN1/AtSYP121, AtSNAP33 and the recently identified vesicle-associated membrane proteins (VAMP) AtVAMP721 and AtVAMP722 (Collins et al., 2003; Kwon et al., 2008b; Lipka et al., 2008; Table 1). This complex forms in vivo and presumably supports exocytosis, which is mechanistically similar to secretion via the immunological synapse in vertebrate T cells (Kwon et al., 2008a,b). The SNARE complex seems to participate also in important developmental processes because double mutants of AtVAMP721 and AtVAMP722 or of AtSYP121 and AtSYP122 show strong pleiotropic effects such as necrotic leaf lesions and dwarfism (Assaad et al., 2004; Zhang et al., 2007; Kwon et al., 2008b). VAMP722–GFP fusion proteins localize to vesicle-like structures of different size reminiscent of compound exocytosis in animals in which vesicles fuse to each other before they fuse to the PM (Kwon et al., 2008b). After inoculation with the non-adapted powdery mildew fungus Erisyphe pisi, the GFP-VAMP722-tagged structures move to the site of attack. Inducible co-silencing of the two VAMPs in Arabidopsis results in a higher penetration rate of non-adapted B. graminis, and the homo-/heterozygous double mutant VAMP721/−, VAMP722/− is hypersusceptible to virulent H. parasitica and G. orontii. This suggests an engagement of the same pathways in defence against host and non-host pathogens whereby the VAMPs appear to be active in defence responses to a broader range of pathogens than PEN1/AtSYP121 (Kwon et al., 2008b). In Nicotiana benthamiana, the SNARE NbSYP132 but not NbSYP121 is required for AvrPto-PTO-mediated ETI (Table 1). A crucial role of the actin cytoskeleton in cell wall penetration resistance was recently underlined because overexpression of barley actin depolymerization factor 3 breached complete mlo-mediated broad spectrum resistance and basal resistance to B. graminis (Miklis et al., 2007).

In contrast to proteins, low-molecular-weight secondary metabolites may use transporter pathways for export from pathogen-attacked cells. The Arabidopsis PEN3 gene was identified in a genetic screening for defective non-host penetration resistance of Arabidopsis to B. graminis f.sp. hordei and acts in a PEN1/AtSYP121-independent secretion mechanism (Stein et al., 2006; Lipka et al., 2008). PEN3 codes for the PM resident ATP-binding cassette transporter AIPDR8, and there is genetic evidence that PEN3/AIPDR8 acts together with the peroxisome-associated glycosyl hydrolase PEN2, which is also crucial for penetration resistance (Table 1). This suggests the secretion of unknown PEN2-derived catalytic products via PEN3/AIPDR8 (Lipka et al., 2005; 2008; Stein et al., 2006).
Concluding remarks

The plant apoplast is a space for arms race in plant–microbe interactions. Plants recognize and counteract both molecular patterns and activity of pathogens in their cellular periphery. For defence, they employed several independent pathways of secretion of antimicrobial and structural compounds. Microbial virulence effectors may particularly target mechanisms of transport and secretion for suppression of cell wall-associated defence. Recently, major progress has been made in understanding bacterial and oomycete effector functions (Birch et al., 2008; Zhou and Chai, 2008), whereas knowledge of fungal effectors is still limited with some exceptions. Therefore, identification of fungal effectors together with further studies on virulence/compatibility mechanisms likely will facilitate understanding of apoplastic remodelling. This will contribute to our knowledge of how plants generally function and how they evolved innate immunity.

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