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Tapping into molecular conversation between oomycete plant pathogens and their hosts

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Abstract Several plant pathogenic oomycetes have been under investigation using modern molecular approaches. Genome sequencing and annotations are underway or near to completion for some of the species. Pathogen-associated molecular pattern molecules (PAMPs) and effector molecules perform inter- and intracellular tasks as adaptation factors and manipulators of the defence network. Hundreds of secreted putative effectors have been discovered and conserved molecular patterns such as RXLR and EER motifs have been identified and used for classifications. PAMPs and effectors are recognized directly or indirectly by the pattern recognition receptors at the cell surface including receptor-like kinases and receptor-like proteins, and/or by nucleotide binding site–leucine rich repeat proteins within the cytoplasm. The current knowledge of effectors, immune receptors and the defence network, will help us understand the 'intricate genetic dance' between the oomycete pathogens and their hosts. This review concentrates on the recent findings in oomycete-plant interactions.

Keywords PAMPs · Effector · Oomycete · Receptor · Biotrophy

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Introduction

The oomycetes include a unique group of biotrophic and hemibiotrophic plant pathogens including *Plasmopara viticola* (grapevine downy mildew), *Albugo candida* (white rust), *Bremia lactucae* (lettuce downy mildew), *Hyaloperonospora arabidopsis* (downy mildew on *Arabidopsis*, formerly *Hyaloperonospora parasitica*; Göker et al. 2004) and *Phytophthora infestans* (potato and tomato late blight; Kamoun 2003; Hardham 2007). These pathogens establish intimate relations with their hosts by forming haustoria during the infection, which are well known structures used for obtaining nutrients from the plant, redirecting host metabolism and suppressing host defence in biotrophy (Hahn and Mendgen 2001; Voegele and Mendgen 2003; O'Connell and Panstruga 2006). *Bremia lactucae*, *P. viticola* or *P. infestans* have a significant economic importance in agriculture (Agrios 1997). *Hyaloperonospora arabidopsis* in *Arabidopsis thaliana* has been developed as an important model system to study plant–microbe interactions (Holub 2008). In addition, *A. candida* provides an alternative model on *Arabidopsis*; however, it has not been fully explored despite interesting characteristics such as the suppression of *R*-gene mediated and non-host resistance mechanisms to allow the growth of a second parasite like *H. arabidopsis* and causing a hormonal imbalance, which induces 'green island' formation (Cooper et al. 2002; Holub 2006, 2008).

Understanding the mechanisms of microbial pathogenesis and plant–microbe interactions has motivated plant pathologists for a long time. In nature, plants are generally resistant to most pathogens due to their innate ability to recognize pathogen-derived molecules and to mount a series of carefully orchestrated and highly evolved defence responses. Much of the progress in the field of molecular plant–pathogen interactions has been led by the research on prokaryotic bacterial plant pathogens. This may have been due to the fact that bacterial pathogens have the ability to secrete effectors including avirulence proteins with their type III secretion system into the cytoplasm of their host plant cell (Van den Ackerveken et al. 1996; Mudgett and Staskawicz 1998; Chisholm et al 2005). However, in the last few years, significant progress has also been made in the understanding of interactions between eukaryotic pathogens, including oomycetes, ascomycetes and basidiomycetes, and their host plants (Allen et al. 2004; Catanzariti et al. 2006; Shen et al. 2007). Results from these studies led to the establishment of a general consensus on plant-microbe interactions, which is that; (a) pathogens have pathogen associated molecular pattern molecules (PAMPs) and effector molecules that modulate the immune system (Kamoun 2006; Lotze et al. 2007); (b) the plant innate immune system is a collection of subsystems that carry out distinct functions in the host's defence; (c) the cell surface receptors or pattern recognition receptors (PRRs) and cytoplasmic receptors or nucleotide binding site-leucine rich repeat (NB-LRR) proteins play a significant role in the detection of these PAMPs and effectors (Chisholm et al. 2006), and (d) effector molecules are virulence factors and have the ability to suppress the immune system of the plant (Bos et al. 2006; Jones and Dangl 2006; Fig 1).

Recent genomic studies on the oomycete pathogens including *Phytophthora sojae, Phytophthora ramorum*, *P. infestans* and *H. arabidopsis* (Win et al. 2007; Whisson et al. 2007) have revealed hundreds of hypothetical effectors that are secreted into the apoplast or the cytoplasm of the host plants. This review focuses on the contributions of recent findings to the understanding of oomycete pathogenesis with emphasis on PAMPs, effectors and receptors rather than repeating the existing reviews on gene-for-gene interactions.

PAMPs play a significant role in pathogenesis and trigger the innate immune system

These molecules were originally described as microbial elicitors and could be present in pathogenic and non-pathogenic microorganisms. They are unique to microbes, invariant among the given class of microorganisms and seen as foreign molecules by plants. They are important for microbial fitness and are able to elicit innate immune responses in a non-cultivar specific manner. Their conserved nature makes it difficult for the pathogen to avoid recognition through adaptive evolution of these molecules (Hahn 1996; Medzhitov and Janeway 2002; Ingle et al. 2006; Medzhitov 2007).

Several bacterial PAMPs including flagellin and Ef-Tu have been identified and studied in detail (Felix et al. 1999; Zipfel et al. 2006). The majority of PAMP studies on oomycete pathogens have been carried out with *Phytophthora* species. For example, studies on *P. parasitica* var. *nicotianae* have identified the cell wall elicitor protein cellulose binding elicitor lectin (CBEL), which enables the pathogen to attach to the host cell and contains two cellulose-binding domains 1 and 2. When recombinant CBEL is expressed in *Escherichia coli* and the protein injected into tobacco (*Nicotiana tabacum*) leaves, activation of the defense gene expression and formation of necrotic lesions have been observed. In addition, CBEL production *in planta* induced necrosis and synthetic peptides derived from CBEL activated the defence response in tobacco and *A. thaliana* leaves, indicating that these molecules have the necessary molecular patterns to be recognized by the innate immune system of plants (Gaulin et al. 2002, 2006).

Another molecule that has the characteristics of a PAMP is the β-glucan obtained from the cell wall of the soybean oomycete pathogen *P. sojae* (formerly known as *Phytophthora megasperma* f. sp. *glycinea*, Sharp et al. 1984). Treatment of the soybean cell suspension cultures with β-glucan has been shown to induce defence reactions including an increase in the cytosolic calcium concentration, the production of reactive oxygen species (ROS), and the activation of genes such as two mitogen-activated protein kinases (MAPKs) and one MAPK kinase, which play a role in signal transduction (Mithofer et al. 2001; Yamamizo et al. 2006; Daxberger et al. 2007).

Pep-13 is also a cell wall product from the soybean pathogen *P. sojae* and considered to be a PAMP. Initial

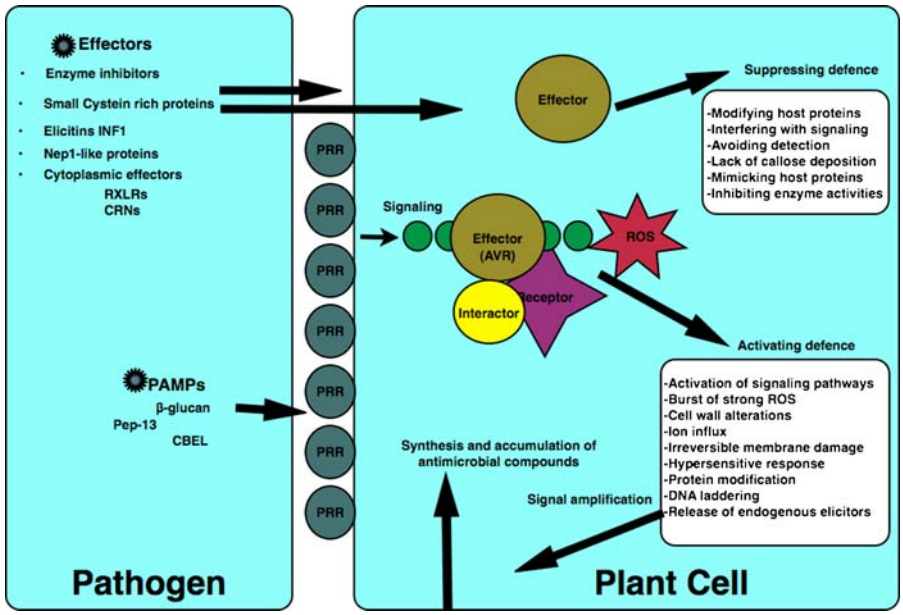

Fig. 1 Pathogen-associated molecular pattern molecules (*PAMPs*) and effector molecules help pathogen adapt to its niche. Oomycete pathogens including *P. infestans*, *B. lactucae*, *A. candida* and *H. arabidopsis* signal their presence with PAMPs such as β-glucan or Pep13. Recognition of these PAMPs by yet unidentified cell surface receptors (or pattern recognition receptors) activates a signalling cascade leading to innate immune responses including a small burst of reactive oxygen species (*ROS*). These pathogens have effector molecules, such as enzyme inhibitors, small cysteine rich proteins and RXLR type cytoplasmic effectors, which are encoded by the pathogen genome and are delivered into the apoplast or cytoplasm of the plant cell. These effectors are usually virulence factors and have the capability of suppressing the plant's immune response by modifying host proteins, interfering with signalling and inhibiting enzyme activities. However, similar to PAMPs, these effectors (which are then termed as AVR proteins) could also be recognized directly or indirectly by RLK/RLP type receptors at the cell surface or NB-LRR type receptors within the cytoplasm. Recognition of AVRs triggers a defence response including activation of signalling pathways, generation of a strong ROS, cell wall alterations, irreversible membrane damage, protein modifications, DNA laddering, hypersensitive response and the release of endogenous elicitors. These local defence responses are further amplified by secondary signalling. Antimicrobial compounds are synthesised within and at the neighbouring cells and accumulate at the infection sites. RLK, receptor-like kinase; RLP, receptor-like protein; NB-LRR, nucleotide binding site-leucine rich repeat proteins

studies with this molecule have been carried out with parsley cells, which are not normally a host for this pathogen. Activation of complex defence responses including ion influxes and effluxes, generation of oxidative burst, elevated expression of defense-related genes, and phytoalexin formation have been reported (Nurnberger et al. 1994). Later, Brunner et al. (2002) showed that the Pep-13 pattern is conserved in all *Phytophthora* species including *P. infestans* and forms part of the cell wall calcium-dependent transglutaminase (TGase) enzyme. However, it is still not clear what type of role TGase plays in the fitness of the pathogen. When cells of a susceptible host plant such as potato have been used in experiments with Pep-13, responses similar to those reported in parsley cells have been observed. Although, Brunner et al. (2002) reported the absence of TGase-related transcripts from other oomycete pathogens such as *H. arabidopsis* and *Pythium*, bioinformatic investigation into the recently available genome sequences of *H. arabidopsis* showed at least five copies of TGase elicitor precursor (M. Tör, unpublished).

One of the main reasons why PAMPs have been reported only from *Phytophthora* species could be the life-style of these pathogens, being easy to grow in axenic culture without contaminants from the host plants. As new and refined techniques are developed for the identification of new PAMPs, we would expect to see more from other oomycetes particularly the obligate biotrophs.

If the PAMPs are triggering a defence response in both a susceptible host and resistant non-host plants, the important question then arises as to why these defence responses are not sufficient to stop pathogen invasion. One possible explanation might be that the

PAMP-activated defence responses including ROS are weak and the pathogen can tolerate them. Another and more likely answer may be the ability of these pathogens to use other molecules in the apoplast or within the cytoplasm of the host cell to suppress or manipulate the immune system of plants for their own purpose.

Effectors are adaptation factors and manipulators of the defence network

Earlier physiological, biochemical and classic genetic studies in plant–pathogen interactions have concentrated on understanding pathogenicity determinants and disease resistance genes. When the modern techniques of molecular genetics were applied to analyse the pathogen, especially bacteria, important pathogenicity factors including strong attachment of bacteria to the host cell and hydrolytic enzymes, such as pectinases and cellulases, that facilitate pathogen invasion into host tissues, were identified. Studies on avirulence proteins in bacteria led to the discovery of the trafficking of effectors from the pathogen into host cells via the Type III secretion system. These molecules were found to bind to a protein and thereby alter the activity of that protein (Mudgett and Staskawicz 1998). This finding helped the establishment of a common link in the mechanisms of pathogenicity between plant and animal pathogens. It has also brought a change in our thinking. Rather than killing the host cell from outside, pathogens inject effector proteins as virulence factors into the host cell to adapt to a particular niche (Medzhitov 2007) and manipulate it for its own purpose (Xiao et al. 2007). When these effectors are somehow recognized by the cytoplasmic receptors, they are termed avirulence (AVR) proteins (Jones and Dangl 2006). Furthermore, it also promoted the question whether effector trafficking could also be observed in eukaryotes, such as oomycete or fungal pathogens (Birch et al. 2006; Ellis et al. 2006). The identification of the effector proteins, their function and their corresponding molecular targets in the host has been a challenge for the scientists working on oomycete pathogens.

In the past few years, genome sequencing and annotations, genome mapping and associated genetic studies have led to the categorization of effectors into apoplastic and cytoplasmic groupings. This is not surprising because: (a) host plants have defence-related proteins including glucanases, chitinases and proteases that are secreted outside the cell for protection; (b) the pathogens have intercellular hyphae with which they invade the apoplastic region, and it is expected that they deliver apoplastic effectors to inhibit or escape plant enzymes, (c) they form haustoria within individual cells and from which they can deliver cytoplasmic effectors, and (d) localization studies of plant receptor proteins have provided vital clues to the whereabouts of the recognition sites of PAMPs and effectors.

The majority of apoplastic and cytoplasmic effectors have signal peptides and the strategies for their identification, classification, characteristic properties and species of origin have been well documented (Kamoun 2006). Currently known apoplastic effectors include enzyme inhibitors, members of the NEP1-like protein family and small cysteine-rich proteins (Qutob et al. 2006; Kamoun 2006). We should also expect to find that some effectors are delivered to the apoplast but function within the cytoplasm after being brought into the cell through endocytosis or membrane trafficking.

Recently, cloning of four *Avr* genes, *Avr1b-1*, *ATR13* and *ATR1*^{NdWs} and *Avr3a* from three oomycetes, *P. sojae, H. arabidopsis* and *P. infestans*, respectively (Shan et al. 2004; Allen et al. 2004; Rehmany et al. 2005; Armstrong et al. 2005) has enabled the identification of common conserved regions including the N-terminal RXLR (for arginine (Arg), any amino acid, leucine (Leu), Arg) and EER (for glutamine (Glu), Glu, Arg.) motifs (Fig. 2). These motifs along with the signal peptide have been used in bioinformatic studies to analyse the available sequence data and identify a class of RXLR cytoplasmic effectors from the oomycete plant pathogens (Kamoun 2007; Morgan and Kamoun 2007). Bhattacharjee et al. (2006) were quick to explore the resemblances of these two motifs to that (RXLXE/D/Q) used for translocation of the malaria parasite (*Plasmodium*) into host erythrocytes (Hiller et al. 2004) and they demonstrated the function of the *P. infestans* RXLR motif in the *Plasmodium* system. In addition, Bos et al (2006) used the *P. infestans* Avr3a effector and demonstrated that the C-terminal-half activated the *R*-gene mediated resistance and suppressed INF1-induced cell death in tobacco (*Nicotiana benthamiana*) plants. These findings

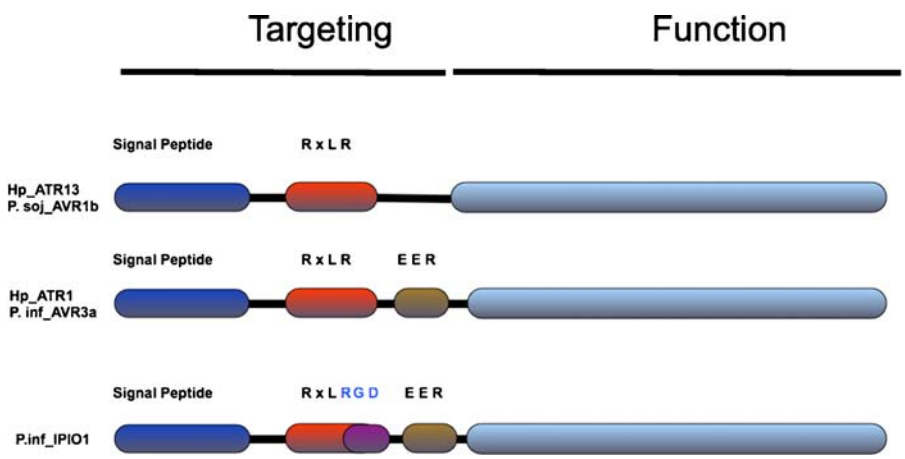

Fig. 2 Predicted structures of RXLR type oomycete effectors. RXLR family effectors from *P. infestans*, *P. sojae* and *H. arabidopsis* have targeting and functional domains. These proteins have a signal peptide and an RXLR motif (for Arg, any amino acid, Leu, Arg). Detailed analyses of the genome sequences of *P. sojae*, *P. ramorum*, and *H. arabidopsis* showed that the majority of these RXLR proteins have also an EER motif (Win et al. 2007). Some of the effectors with and without EER motif are shown. *Hyaloperonospora arabidopsis* ATR1NdWsB (311aa) and *P. infestans* AVR3a (147aa) have the EER motif, whereas *H. arabidopsis* ATR13 (187aa) and *P. sojae* AVR1b (138aa) do not. *Phytophthora infestans* IPI-O1 protein has the EER motif but also has the RGD motif that overlaps with the RXLR motif. In these effectors, the targeting domains are involved in secretion of the effectors out of the pathogens and translocation into the plant cell and are usually well conserved. The C-terminal is responsible for manipulating the host immune system

suggest that, similar to bacterial effectors, oomycete effectors also play a role in altering signalling in the defence network during their adaptation into the given niche. In addition, the N-terminal region of these effectors that contains the signal peptide, RXLR and EER motifs has been shown to be responsible for the delivery of the effectors in the elegant studies by Whisson et al. (2007). Using *Avr3a* from *P. infestans*, Whisson et al. (2007) fused the N-terminal region containing the RXLR and EER motifs, to the GUS protein and showed that the GUS protein could be delivered from the pathogen into the host cell. In addition, *P. infestans* failed to deliver the Avr3a or Avr3a-GUS fusion into the host cell when the RXLR and EER motifs were modified. However, silencing of *Avr3a* in *P. infestans* has not been carried out, which would be a key experiment to reveal whether this effector has a role in pathogenicity.

Although a great deal of information has been accumulating on these effectors, the method of their transmission from extracellular space into the host cytoplasm is still not clear. Morgan and Kamoun (2007) have proposed that RXLR binding proteins, chaperons or translocons, originating either from the pathogen or plant, may be required for the delivery of these effectors into the host cytoplasm.

Phytophthora infestans IPI-B and *IPI-O* genes are expressed at an early stage of the infection (Pieterse et al. 1994) and IPI-O1 belongs to the RXLR family of effector proteins. In addition to the signal peptide, RXLR and EER motif, IPI-O1 has an RGD tripeptide, which overlaps with the RXLR motif (Fig. 2). The RGD motif has been described as a cell adhesion motif found in several mammalian extracellular matrix proteins and has been proposed to reduce plant defence responses by disrupting adhesions between the cell wall and plasma membrane (Senchou et al. 2004). Furthermore, detailed studies showed that RGD-containing proteins could be ligands for some of the receptor-like kinase type cell surface receptors described below (Gouget et al. 2006).

Bacterial flagellin is a ligand for the receptor-like kinase (RLK)-type pattern recognition receptor flagellin-sensitive 2 (FLS2) and the ligand-stimulated receptor endocytosis is a kind of trafficking at the plasma membrane (Robatzek et al. 2006; Robatzek 2007). It can be proposed that at least some of the RXLR family effectors may be translocated into the

extrahaustorial matrix with the help of RXLR and EER motifs and physically interact with cell surface receptors through the RGD motif as was shown with IPI-O1 (Gouget et al. 2006) and could be internalized by these receptors as was observed with FLS2.

Effectors often undergo diversifying selection as a result of an 'arms race' with the host organism. This has been well documented with studies on *H. arabidopsis* ATR13 and ATR1^{NdWsB} effector proteins and effectors from bacterial pathogens such as *Pseudomonas syringae* (Allen et al. 2004, Rehmany et al. 2005; Guttman et al. 2006). Recent work on RXLR effectors from *P. sojae, P. ramorum*, and *H. arabidopsis*, showed that positive selection is mainly on the C-terminal region, which is responsible for the function (Win et al. 2007).

Recognition and beyond

Cell biological research of oomycete–plant interactions has entered a new phase with the identification of hundreds of putative effector molecules. Since both PAMPs and effectors are foreign molecules to the host plant, we need to address several questions including; (a) whether the effectors from oomycete pathogens mimic the host plant protein as seen with the bacterial effector AvrPtoB (Abramovitch et al. 2006), (b) whether all the molecules with the same motif, such as RXLR, function as effectors, (c) which of these effector molecules are suppressors or activators of the immune response and which microbial patterns are recognized by the plant's sensor mechanism.

Although the oomycete effector delivery system is different from that of bacteria, nematodes and aphids, the end result, recognition of these effectors and activation of the defence response, would probably use similar mechanisms. It has been well established that plants have sensors at the cell surface and within the cytoplasm (Fig. 3). PRRs localised at the cell surface play a significant role in connecting the cell wall, plasma membrane and cytoskeleton. They are also major players in the perception and transmission of external signals. They include several classes such as polygalacturonase inhibitor-like proteins, receptor-like proteins (RLPs) and, RLKs (Shiu et al. 2004; Fritz-Laylin et al. 2005). Some of these PRRs have been shown to recognize PAMPs such as Ef-Tu (Zipfel and Felix 2005) and effector molecules such as AvrXa21 (Lee et al. 2006) from bacterial pathogens. Until now, no PRR that recognizes an oomycete PAMP has been identified. However, reports are emerging that Pep-13 could be recognized in parsley by an RLK-type receptor (Altenbach and Robatzek 2007). In addition, β-glucan elicitor (GE) from *P. sojae* has been used to identify a receptor protein. However, although a GE-binding protein (GEBP) was purified from the membrane fraction of soybean root cells, no signal peptide or transmembrane domain was identified. Nevertheless, immunolocalization assays indicated that the GEBPs are localized in the plasma membrane of root cells (Umemoto et al. 1997). This suggests that the GEBP may be part of a protein complex localized to the membrane, which somehow interacts with other membrane bound proteins including PRRs.

An *Arabidopsis* RLK with a lectin domain recognizes IPI-O1, an RXLR type effector protein with RGD motif (Fig. 2) from *P. infestans* (Gouget et al. 2006). This is an interesting finding since there is no report of an *Arabidopsis* lectin RLK orthologue from tomato or potato to suggest that IPI-O1 is recognized in tomato or potato, and secondly, the effector is from *P. infestans* for which *Arabidopsis* is a non-host. Thus, the immunity triggered by this effector may be that of non-host resistance, which would be a fascinating piece of *data*.

The expression level of *Arabidopsis* wall-associated kinase 1, an RLK-type PRR, increases when plants are challenged with the Hiks1 isolate of *H. arabidopsis* (Eulgem et al. 2007), which is a further confirmation that some of these plasma membrane–cell wall interacting PRRs are involved in signal transduction. Alterations of expression levels of the PRRs in *Arabidopsis* have also been reported in studies with Nep1-like protein (Qutob et al. 2006). Similarly, we have investigated the publicly available microarray databases on *H. parasitica–Arabidopsis* interactions and observed increased and decreased levels of expression in some of these PRRs (N. Holton and M. Tör, unpublished data). *Arabidopsis* Brassinosteroid insensitive 1-associated receptor kinase 1 (BAK1) is involved in the regulation of the containment of microbial infection-induced cell death. When *bak1* mutants were challenged with several compatible and incompatible isolates of *H. arabidopsis*, reduced sporulation of the pathogen was observed (Kemmerling et al. 2007) indicating that cell surface receptors may also play a role in compatibility.

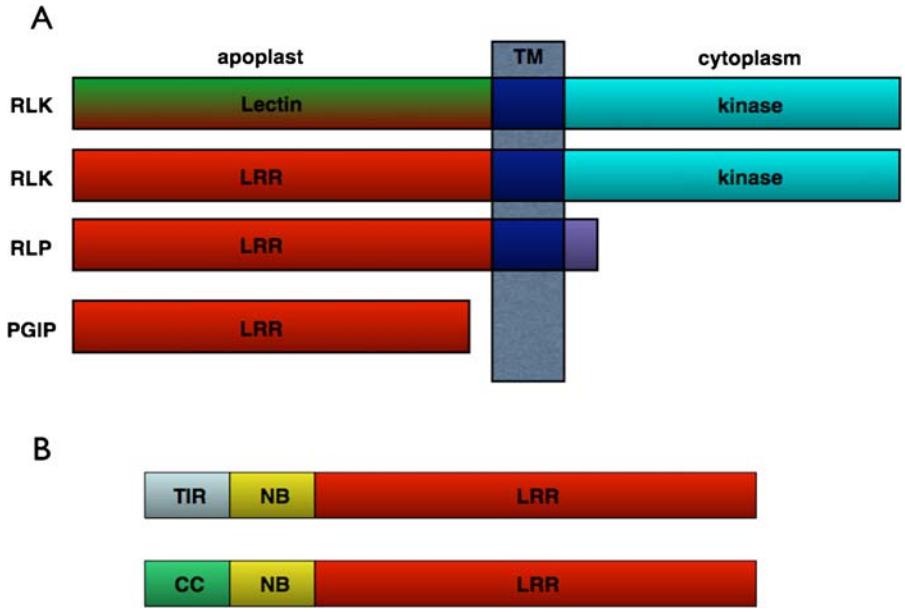

Fig. 3 Canonical domain structures of cell surface and cytoplasmic receptors. *A* Cell surface receptors show variation in their domains. Receptor-like kinases (*RLKs*) have varying types of extracellular domains such as leucine-rich repeat (*LRR*) or lectin-type, followed by a transmembrane spanning region (*TM*) and a cytoplasmic kinase domain. Receptor-like proteins (*RLPs*) are similar to RLKs but do not have the cytoplasmic kinase domains. Instead, they have a short cytoplasmic tail. Polygalacturonase inhibitor proteins have an LRR domain and are totally extra cellular. These cell surface receptors are also known as Pattern Recognition Receptors as they have been implicated in the recognition of PAMPs. To date, only one Lectin RLK type receptor from *Arabidopsis* (Gouget et al. 2006), has been implicated to play a role in oomycete–plant interactions. *B* Cytoplasmic receptors show variation at their N-terminal. These proteins have a central nucleotide binding (*NB*) region and an LRR domain at their C-terminal. The N-terminal region shows variations and either has a *TIR* domain, resembling the cytoplasmic signalling domain of the *T*oll and *I*nterleukin 1 transmembrane *r*eceptors (referred to henceforth *TIR-NB-LRR* genes) or has a *c*oiled-*c*oil domain, (referred to as *CC-NB-LRR* genes). Most of the receptors that are involved in the recognition of oomycete pathogens are cytoplasmic and include *Arabidopsis RAC1* (Borhan et al. 2004) for recognition of *A. candida*, *Arabidopsis RPP1*, *RPP4*, *RPP5* and *RPP13* for *H. arabidopsis* (Tör et al. 2003), potato *R3a* (Huang et al. 2005) for *P. infestans* and lettuce *RGC2B* (Shen et al. 2002) for *B. lactucae*

Another mode of direct and indirect effector detection and recognition takes place within the cytoplasm by NB-LRR proteins (Fig. 3). Traditionally, the genes encoding these proteins have been known as disease resistance genes or *R*-genes and form one of the largest gene families within the plant kingdom. Several members of the R-proteins that provide resistance to oomycete pathogens have been identified or cloned. For example, *RPP* and *RAC* genes from *Arabidopsis* confer resistance to isolates of *H. arabidopsis* and *A. candida* (Tör et al. 1994, 2003; Holub 2001; Borhan et al. 2004), *DM3* and *RGC2* gene clusters in lettuce confers resistance to *B. lactucae* (Shen et al. 2002; Wroblewski et al. 2007), *R1* and *R3a* in potato provide resistance to *P. infestans* (Ballvora et al. 2002; Huang et al. 2005). Domain structures of these proteins (Fig. 3) are known (Tör et al. 2003) and the ways in which they activate the immune response are beginning to emerge. Recent findings suggest that although these NB-LRR proteins are residents of the cytoplasm, the majority of them have a nuclear localization signal (Meyers et al. 2003). Some of them including barley MLA, tobacco N and *Arabidopsis* RPS4, have been shown to move into the nucleus and it has been proposed they activate defence expression by de-repressing basal defence through association with a WRKY transcription factor (Dangl 2007; Shen and Schulze-Lefert 2007; Shen et al. 2007). Two *Arabidopsis* NB-LRR proteins RPP2a and RPP2b are required for the recognition of Cala2 isolate of *H. arabidopsis* (Sinapidou et al. 2004). In this case, it

will be fascinating to elucidate whether both NB-LRR proteins travel together between cytoplasm and the nucleus to activate the immune system.

Recognition of PAMPs or effector molecules activate the signalling cascade and major building blocks of the defence network including transcription factors, kinases, components of proteolysis or innate immunity such as EDS1, SGT1, RAR1 and NDR1, which have been identified from *Arabidopsis* or from plants that are hosts to oomycete pathogens (Tör et al. 2002, 2003; Eulgem et al. 2007, Takahashi et al. 2007). With the identification of putative effectors, it should now be possible to investigate which one of these signalling components are the targets for suppression.

Physiological changes as a result of the recognition of the oomycete PAMPs and effectors include ion influx, formation of wall apposition around haustoria, hypersensitive response, formation of ROS, synthesis of phytoalexins and PR proteins and production of salicylic acid. These have been well documented elsewhere (Hardham 2007).

Role of PAMPs and effectors in biotrophy

A common denominator for the important oomycete pathogens is the biotrophic phase in their life cycles. While *H. arabidopsis, B. lactucae* and *A. candida* are obligate biotrophs and cause minimum injury to their hosts, *P. infestans* and *P. sojae* are hemi-biotrophs being biotrophic for the initial stage of up to 36 h after inoculation and subsequently becoming necrotrophic killing the host tissue to consume the cell content (Grenville-Briggs and van West 2005). One of the most distinguishing features of the biotrophic phase in these pathogens, as well as some of the fungal pathogens including powdery mildews and rusts, is the formation of haustoria, which are used in nutrient acquisition (Catanzariti et al. 2007, Voegele et al. 2001). Recent studies on the flux–rust interaction identified effector molecules such as AvrL567, AvrM, AvrP4, and AvrP123 within the haustorium, indicating that haustoria act as reservoirs for effector molecules during the infection process (Catanzariti et al. 2006).

Using a viral-based expression system, Qutob et al. (2002) identified a necrosis-inducing protein (PsojNIP) from *P. sojae* and proposed that this protein plays a significant role in the transition from biotrophy to necrotrophy. However, complementation of this study by the down regulation of this gene to show that it is involved in biotrophy has yet to be reported. Molecular studies carried out with *H. arabidopsis* infecting *Arabidopsis* helped the identification of several putative pathogen genes that are expressed *in planta* and are involved in membrane or cell wall biosynthesis, amino acid metabolism, osmoregulation, phosphorylation and protein secretion (Bittner-Eddy et al. 2003) or in housekeeping roles (van der Biezen et al. 2000). Similar molecular studies coupled with proteomics carried out with *P. infestans* showed that the amino acid biosynthesis in both pathogen and the host increases during the infection. In addition, energy consumption, and elevated metabolism are required at the initial stage of biotrophy (Guo et al. 2006; Grenville-Briggs and van West 2005).

Since data on PAMPs and effector molecules from oomycetes have been accumulating, their role in the establishment of biotrophy rather than as the activator of immunity can be re-evaluated. Although flagella on zoospores of *A. candida* and *P. infestans* provide motility for the establishment of biotrophy and are an important part of the structure, no PAMP associated with these flagella has yet been identified. Attachment of these pathogens to the host cell wall is important in the early stage of infection for initiation of appressoria and haustoria. In this regard, the role of PAMPs such as cell wall binding proteins cannot be ignored.

The major players for the establishment of biotrophy will undoubtedly be the effector molecules. Working on the expression of RXLR and EER motif-containing effectors from *P. infestans*, Whisson et al. (2007) divided these effectors into three groups according to the stage of infection at which they are induced; during pre-infection, throughout infection and during biotrophy only. Silencing of those effectors induced pre-infection and during the biotrophic phase would help to understand the contribution of effectors towards biotrophy.

Although expression of some effectors such as ATR13 from *H. arabidopsis* has been found to be present in spores (Allen et al. 2004), it is not yet known whether the effector protein is localised in the spore (Rebecca Allen, personal communication). When working on an incompatible *H. arabidopsis*–*Arabidopsis* interaction, we observed that in most cases the resistance response is triggered after the

formation of haustoria (Tör et al. 2002) indicating that the pathogen is able to develop the necessary structures such as appressoria and intercellular hyphae and establish a limited biotrophy before recognition. These findings, along with those from *P. infestans* infections indicate that (a) some effectors are delivered from hyphae and appressoria into the apoplast (see above) and are used by the pathogen as pioneering molecules to suppress the initial innate immune response and adapt the pathogen to the surrounding niche; and (b) other effectors that are delivered through haustoria into the cytoplasm may be used for diverting nutrients towards the pathogen.

The question as to whether the pathogen is solely responsible for initiating biotrophy is one of the central problems in the interactions between obligate pathogens and their host plant. If the pathogen has PAMPs and effectors to establish a compatible interaction, what is the contribution of the host plant in the compatibility? A great deal of information on defence responses and disease resistance is available. However, the knowledge on 'susceptibility' is very limited. Until now, a few host genes required for susceptibility have been isolated through mutant screens and subsequent genetic analysis. Some examples of these include *POWDERY MILDEW RESISTANT 4* genes (Vogel and Somerville 2000; Vogel et al. 2002, Nishimura et al. 2003) and oomycete *DOWNY MILDEW RESISTANT* genes in *Arabidopsis* (van Damme et al. 2005).

Concluding remarks and future prospects

In the last few years, genome sequencing of several oomycete plant pathogens, including *H. arabidopsis*, *Phytophthora capsici*, *P. infestans*, *P. sojae*, and *P. ramorum* has been carried out and annotations are underway (Tyler et al. 2006). Molecular genomic studies, including large-scale expressed sequence tag sequencing or generating genomic libraries, are also being carried out with other oomycete pathogens including *Bremia*. Arrival of new technologies such as use of Solexa machines should be a great help in these studies. For those species where sequence information is available, bioinformatics studies are being carried out to identify putative effector molecules and classify them according to their functional locations (apoplastic or cytoplasmic), mode of actions, (e.g. enzymatic or transcription factor), their motifs or domains (RXLR, RGD). These studies should also consider whether these effectors are constitutive or are induced *in planta*. Although we are in the middle of stock counting and cataloguing these effectors, we have seen some excellent studies towards functional analysis with a few of the known effector molecules such as the RXLR family members from different oomycete species (Allen et al. 2004, Shan et al. 2004, Rehmany et al. 2005; Sohn et al. 2007). In general, it is assumed that the effector response depends on the pathogen type. Therefore, the initial studies on the known oomycete effectors can be used as a starting point to launch large-scale, high throughput effector analyses to uncover whether there are common lines of communication between oomycete pathogens and their host plants.

Several laboratories around the world are adopting the bacterial type III secretion system to study these oomycete effectors on a large scale (Sohn et al. 2007). This technique may be very suitable for the study of cytoplasmic effectors, but, other techniques should also be adopted to investigate the effectors that are delivered to the apoplast.

Results obtained from high throughput studies that concentrate on individual effectors should be compared and contrasted with those obtained from native, pathogen-delivered effectors. A given pathogen would deliver multiple effectors some of which would act as suppressors of the others. Therefore, pathogen delivery should not be ignored, and if necessary the same effector should be put back into the same pathogen with a known tag and investigated further to obtain a clear picture.

Secretion and translocation of the RXLR type effectors have been attributed to the N-terminal of these effectors (Win et al. 2007). However, it would be interesting to find out which plant proteins, if any, at the cell surface are involved in the endocytosis or transmission of these effector proteins into the cytoplasm of the plant cell.

Micro-array studies have been employed to investigate the plant's defence network and to understand the modulation of signalling in plants by pathogens. However, in the next few years, we should also expect to see microarray studies on these oomycete pathogens. A great deal of information should then be obtained about how the host plant can modulate gene expression in the pathogen genome. A systems

biology approach can then be used to look at the interaction from both the pathogen and the host's side.

Although effectors are receiving much attention in current investigations, the next few years should also bring more publications on oomycete PAMPs. These would be particularly useful in the elucidation of non-host resistance.

Another area of great importance is the genetic manipulation of these oomycete pathogens. Although *Phytophthora* species can be transformed and subjected to genetic manipulations, routine genetic transformation methods have not been established for the obligate species. In the next few years, we expect to see the development of different stable transformation methods for these pathogens including *Bremia*, *Hyaloperonospora* and *Albugo*. Development of the RNA interference method to silence these effectors within the pathogen may be an alternative way to stable transformation. It would then be possible to study pathogen genetics.

Although development of new technologies is vital to investigate the interactions between oomycete pathogens and their hosts, the ultimate aim of these studies, in the longer term, should be the development of intelligent systems to control economically important crop pathogens.

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References

Abramovitch, R. B., Janjusevic, R., Stebbins, C. E., & Martin, G. B. (2006). Type III effector AvrPtoB requires intrinsic E3 ubiquitin ligase activity to suppress plant cell death and immunity. *Proceedings of the National Academy of Sciences of the United States of America*, *103*, 2851–2856.

Agrios, G. N. (1997). *Plant pathology*. San Diego: Academic.

Allen, R. L., Bittner-Eddy, P. D., Grenville-Briggs, L. J., Meitz, J. C., Rehmany, A. P., Rose, L. E., et al. (2004). Host-parasite coevolutionary conflict between *Arabidopsis* and downy mildew. *Science*, *306*, 1957–1960.

Altenbach, D., & Robatzek, S. (2007). Pattern recognition receptors: From the cell surface to intracellular dynamics. *Molecular Plant–Microbe Interactions*, *20*, 1031–1039.

Armstrong, M. R., Whisson, S. C., Pritchard, L., Bos, J. I., Venter, E., Avrova, A. O., et al. (2005). An ancestral oomycete locus contains late blight avirulence gene *Avr3a*, encoding a protein that is recognized in the host cytoplasm. *Proceedings of the National Academy of Sciences of the United States of America*, *102*, 7766–7771.

Ballvora, A., Ercolano, M. R., Weiss, J., Meksem, K., Bormann, C. A., Oberhagemann, P., et al. (2002). The *R1* gene for potato resistance to late blight (*Phytophthora infestans*) belongs to the leucine zipper/NBS/LRR class of plant resistance genes. *Plant Journal*, *30*, 361–371.

Bhattacharjee, S., Hiller, N. L., Liolios, K., Win, J., Kanneganti, T. D., Young, C., et al. (2006). The malarial host-targeting signal is conserved in the Irish potato famine pathogen. *PLoS Pathogens*, *2*, e50.

Birch, P. R., Rehmany, A. P., Pritchard, L., Kamoun, S., & Beynon, J. L. (2006). Trafficking arms: Oomycete effectors enter host plant cells. *Trends in Microbiology*, *14*, 8–11.

Bittner-Eddy, P. D., Allen, R. L., Rehmany, A. P., Birch, P., & Beynon, J. L. (2003). Use of suppression subtractive hybridization to identify downy mildew gene expressed during infection of *Arabidopsis thaliana*. *Molecular Plant Pathology*, *4*, 501–507.

Borhan, M. H., Holub, E. B., Beynon, J. L., Rozwadowski, K., & Rimmer, S. R. (2004). The *Arabidopsis* TIR-NB-LRR gene *RAC1* confers resistance to *Albugo candida* (white rust) and is dependent on *EDS1* but not *PAD4*. *Molecular Plant-microbe Interactions*, *17*, 711–719.

Bos, J. I., Kanneganti, T. D., Young, C., Cakir, C., Huitema, E., Win, J., et al. (2006). The C-terminal half of *Phytophthora infestans* RXLR effector AVR3a is sufficient to trigger R3a-mediated hypersensitivity and suppress INF1-induced cell death in *Nicotiana benthamiana*. *Plant Journal*, *48*, 165–176.

Brunner, F., Rosahl, S., Lee, J., Rudd, J. J., Geiler, C., Kauppinen, S., et al. (2002). Pep-13, a plant defense-inducing pathogen-associated pattern from *Phytophthora* transglutaminases. *EMBO Journal*, *21*, 6681–6688.

Catanzariti, A. M., Dodds, P. N., Lawrence, G. J., Ayliffe, M. A., & Ellis, J. G. (2006). Haustorially expressed secreted proteins from flax rust are highly enriched for avirulence elicitors. *Plant Cell*, *18*, 243–256.

Catanzariti, A. M., Dodds, P. N., & Ellis, J. G. (2007). Avirulence proteins from haustoria-forming pathogens. *FEMS Microbiology Letters*, *269*, 181–188.

Chisholm, S. T., Dahlbeck, D., Krishnamurthy, N., Day, B., Sjolander, K., & Staskawicz, B. J. (2005). Molecular characterization of proteolytic cleavage sites of the *Pseudomonas syringae* effector AvrRpt2. *Proceedings of the National Academy of Sciences of the United States of America*, *102*, 2087–2089.

Chisholm, S. T., Coaker, G., Day, B., & Staskawicz, B. J. (2006). Host-microbe interactions: Shaping the evolution of the plant immune response. *Cell*, *124*, 803–814.

Cooper, A. J., Woods-Tör, A., & Holub, E. B. (2002). *Albugo candida* (White rust) suppresses resistance to downy mildew pathogens in *Arabidopsis thaliana*. Proceedings 6th Conference of EFPP, *Plant Protection Science*, *38* (Special issue), 474–476.

Dangl, J. L. (2007). Plant science. Nibbling at the plant cell nucleus. *Science*, *315*, 1088–1089.

Daxberger, A., Nemak, A., Mithofer, A., Fliegmann, J., Ligterink, W., Hirt, H., et al. (2007). Activation of

members of a MAPK module in beta-glucan elicitor-mediated non-host resistance of soybean. *Planta*, *225*, 1559–1571.

Ellis, J., Catanzariti, A. M., & Dodds, P. (2006). The problem of how fungal and oomycete avirulence proteins enter plant cells. *Trends in Plant Science*, *11*, 61–63.

Eulgem, T., Tsuchiya, T., Wang, X. J., Beasley, B., Cuzick, A., Tör, M., et al. (2007). EDM2 is required for RPP7-dependent disease resistance in *Arabidopsis* and affects RPP7 transcript levels. *Plant Journal*, *49*, 829–839.

Fritz-Laylin, L. K., Krishnamurthy, N., Tör, M., Sjolander, K. V., & Jones, J. D. (2005). Phylogenomic analysis of the receptor-like proteins of rice and *Arabidopsis*. *Plant Physiology*, *138*, 611–623.

Felix, G., Duran, J. D., Volko, S., & Boller, T. (1999). Plants have a sensitive perception system for the most conserved domain of bacterial flagellin. *Plant Journal*, *18*, 265–276.

Gaulin, E., Jauneau, A., Villalba, F., Rickauer, M., Esquerre-Tugaye, M. T., & Bottin, A. (2002). The CBEL glycoprotein of *Phytophthora parasitica* var *nicotianae* is involved in cell wall deposition and adhesion to cellulosic substrates. *Journal of Cell Science*, *115*, 4565–4575.

Gaulin, E., Drame, N., Lafitte, C., Torto-Alalibo, T., Martinez, Y., Ameline-Torregrosa, C., et al. (2006). Cellulose binding domains of a *Phytophthora* cell wall protein are novel pathogen-associated molecular patterns. *Plant Cell*, *18*, 1766–1777.

Gouget, A., Senchou, V., Govers, F., Sanson, A., Barre, A., Rouge, P., et al. (2006). Lectin receptor kinases participate in protein–protein interactions to mediate plasma membrane–cell wall adhesions in *Arabidopsis*. *Plant Physiology*, *40*, 81–90.

Göker, M., Reithmuller, A., Voglmayr, H., Weiss, M., & Oberwinkler, F. (2004). Phylogeny of *Hyaloperonospora* based on nuclear ribosomal internal transcribed spacer sequences. *Mycological Progress*, *3*, 83–94.

Grenville-Briggs, L. J., & van West, P. (2005). The biotrophic stages of oomycete-plant interactions. *Advances in Applied Microbiology*, *57*, 217–243.

Guo, J., Jiang, R. H., Kamphuis, L. G., & Govers, F. (2006). A cDNA-AFLP based strategy to identify transcripts associated with avirulence in *Phytophthora infestans*. *Fungal Genetics and Biology*, *43*, 111–123.

Guttman, D. S., Gropp, S. J., Morgan, R. L., & Wang, P. W. (2006). Diversifying selection drives the evolution of the type III secretion system pilus of *Pseudomonas syringae*. *Molecular Biology and Evolution*, *23*, 2342–2354.

Hahn, M. G. (1996). Microbial elicitors and their receptors in plants. *Annual Review of Phytopathology*, *34*, 387–412.

Hahn, M., & Mendgen, K. (2001). Signal and nutrient exchange at biotrophic plant fungus interfaces. *Current Opinion in Plant Biology*, *4*, 322–327.

Hardham, A. R. (2007). Cell biology of plant–oomycete interactions. *Cellular Microbiology*, *9*, 31–39.

Hiller, N. L., Bhattacharjee, S., van Ooij, C., Liolios, K., Harrison, T., Lopez-Estrano, C., et al. (2004). A host-targeting signal in virulence proteins reveals a secretome in malarial infection. *Science*, *306*, 1934–1937.

Holub, E. B. (2001). The arms race is ancient history in *Arabidopsis*, the wildflower. *Nature Reviews Genetics*, *2*, 516–527.

Holub, E. B. (2006). Evolution of parasitic symbioses between plants and filamentous microorganisms. *Current Opinion in Plant Biology*, *9*, 397–405.

Holub, E. B. (2008). Natural history of *Arabidopsis thaliana* and oomycete symbioses. *European Journal of Plant Pathology*, (in press).

Huang, S., van der Vossen, E. A., Kuang, H., Vleeshouwers, V. G., Zhang, N., Borm, T. J., et al. (2005). Comparative genomics enabled the isolation of the *R3a* late blight resistance gene in potato. *Plant Journal*, *42*, 251–261.

Ingle, R. A., Carstens, M., & Denby, K. J. (2006). PAMP recognition and the plant–pathogen arms race. *Bioessays*, *28*, 880–889.

Jones, J. D., & Dangl, J. L. (2006). The plant immune system. *Nature*, *444*, 323–329.

Kamoun, S. (2003). Molecular genetics of pathogenic oomycetes. *Eukaryotic Cell*, *2*, 191–199.

Kamoun, S. (2006). A catalogue of the effector secretome of plant pathogenic oomycetes. *Annual Review of Phytopathology*, *44*, 41–60.

Kamoun, S. (2007). Groovy times: Filamentous pathogen effectors revealed. *Current Opinion in Plant Biology*, *10*, 358–365.

Kemmerling, B., Schwedt, A., Rodriguez, P., Mazzotta, S., Frank, M., Qamar, S. A., et al. (2007). The BRI1-associated kinase 1, BAK1, has a brassinolide-independent role in plant cell-death control. *Current Biology*, *17*, 1116–1122.

Lee, S. W., Han, S. W., Bartley, L. E., & Ronald, P. C. (2006). Colloquium review. Unique characteristics of *Xanthomonas oryzae* pv. *oryzae* AvrXa21 and implications for plant innate immunity. *Proceedings of the National Academy of Sciences of the United States of America*, *103*, 18395–18400.

Lotze, M. T., Zeh, H. J., Rubartelli, A., Sparvero, L. J., Amoscato, A. A., Washburn, N. R., et al. (2007). The grateful dead: Damage associated molecular pattern molecules and reduction/oxidation regulate immunity. *Immunological Reviews*, *220*, 60–82.

Medzhitov, R. (2007). Recognition of microorganisms and activation of the immune response. *Nature*, *449*, 819–826.

Medzhitov, R., & Janeway, C. A. (2002). Decoding the patterns of self and non-self by the innate immune system. *Science*, *296*, 298–300.

Meyers, B. C., Kozik, A., Griego, A., Kuang, H., & Michelmore, R. W. (2003). Genome-wide analysis of NBS-LRR-encoding genes in Arabidopsis. *Plant Cell*, *15*, 809–834.

Mithofer, A., Fliegmann, J., Daxberger, A., Ebel, C., Neuhaus-Url, G., Bhagwat, A. A., et al. (2001). Induction of H(2)O (2) synthesis by beta-glucan elicitors in soybean is independent of cytosolic calcium transients. *FEBS Letters*, *508*, 191–195.

Morgan, W., & Kamoun, S. (2007). RXLR effectors of plant pathogenic oomycetes. *Current Opinion in Microbiology*, *10*, 332–338.

Mudgett, M. B., & Staskawicz, B. J. (1998). Protein signaling via type III secretion pathways in phytopathogenic bacteria. *Current Opinion in Microbiology*, *1*, 109–114.

Nishimura, M. T., Stein, M., Hou, B. H., Vogel, J. P., Edwards, H., & Somerville, S. C. (2003). Loss of a callose synthase results

in salicylic acid-dependent disease resistance. *Science*, *301*, 969–972.

Nurnberger, T., Nennstiel, D., Jabs, T., Sacks, W. R., Hahlbrock, K., & Scheel, D. (1994). High affinity binding of a fungal oligopeptide elicitor to parsley plasma membranes triggers multiple defense responses. *Cell*, *78*, 449–460.

O'Connell, R. J., & Panstruga, R. (2006). Tete a tete inside a plant cell. Establishing compatibility between plants and biotrophic fungi and oomycetes. *New Phytologist*, *171*, 699–718.

Pieterse, C. M., van West, P., Verbakel, H. M., Brasse, P. W., van den Berg-Velthuis, G. C., & Govers, F. (1994). Structure and genomic organization of the *ipiB* and *ipiO* gene clusters of *Phytophthora infestans*. *Gene*, *138*, 67–77.

Qutob, D., Kamoun, S., & Gijzen, M. (2002). Expression of a *Phytophthora sojae* necrosis-inducing protein occurs during transition from biotrophy to necrotrophy. *Plant Journal*, *32*, 361–373.

Qutob, D., Kemmerling, B., Brunner, F., Kufner, I., Engelhardt, S., Gust, A. A., et al. (2006). Phytotoxicity and innate immune responses induced by Nep1-like proteins. *Plant Cell*, *18*, 3721–3744.

Rehmany, A. P., Gordon, A., Rose, L. E., Allen, R. L., Armstrong, M. R., Whisson, S. C., et al. (2005). Differential recognition of highly divergent downy mildew avirulence gene alleles by *RPP1* resistance genes from two *Arabidopsis* lines. *Plant Cell*, *17*, 1839–1850.

Robatzek, S. (2007). Vesicle trafficking in plant immune responses. *Cellular Microbiology*, *9*, 1–8.

Robatzek, S., Chinchilla, D., & Boller, T. (2006). Ligand-induced endocytosis of the pattern recognition receptor FLS2 in *Arabidopsis*. *Genes and Development*, *20*, 537–542.

Senchou, V., Weide, R., Carrasco, A., Bouyssou, H., Pont-Lezica, R., Govers, F., et al. (2004). High affinity recognition of a *Phytophthora* protein by Arabidopsis via an RGD motif. *Cellular and Molecular Life Sciences*, *61*, 502–509.

Shan, W., Cao, M., Leung, D., & Tyler, B. M. (2004). The *Avr1b* locus of *Phytophthora sojae* encodes an elicitor and a regulator for avirulence on soybean plants carrying resistance gene *Rps1b*. *Molecular Plant–Microbe Interactions*, *17*, 394–403.

Sharp, J. K., Valent, B., & Albersheim, P. (1984). Purification and partial characterization of a beta-glucan fragment that elicits phytoalexin accumulation in soybean. *Journal of Biological Chemistry*, *259*, 11312–11320.

Shen, Q. H., & Schulze-Lefert, P. (2007). Rumble in the nuclear jungle: Compartmentalization, trafficking, and nuclear action of plant immune receptors. *EMBO Journal*, *26*, 4293–4301.

Shen, K. A., Chin, D. B., Arroyo-Garcia, R., Ochoa, O. E., Lavelle, D. O., Wroblewski, T., et al. (2002). *Dm3* is one member of a large constitutively expressed family of nucleotide binding site-leucine-rich repeat encoding genes. *Molecular Plant–Microbe Interactions*, *15*, 251–61.

Shen, Q. H., Saijo, Y., Mauch, S., Biskup, C., Bieri, S., Keller, B., et al. (2007). Nuclear activity of MLA immune receptors links isolate-specific and basal disease-resistance responses. *Science*, *315*, 1098–1101.

Shiu, S. H., Karlowski, W. M., Pan, R., Tzeng, Y. H., Mayer, K. F., & Li, W. H. (2004). Comparative analysis of the receptor-like kinase family in Arabidopsis and rice. *Plant Cell*, *16*, 1220–1234.

Sinapidou, E., Williams, K., Nott, L., Bahkt, S., Tör, M., Bittner-Eddy, P., et al. (2004). Two TIR:NB:LRR genes are required to specify resistance to *Peronospora parasitica* isolate Cala2 in *Arabidopsis*. *Plant Journal*, *38*, 898–909.

Sohn, K. H., Lei, R., Nemri, A., & Jones, J. D. (2007). The downy mildew effector proteins ATR1 and ATR13 promote disease susceptibility in *Arabidopsis thaliana*. *Plant Cell*, *19*, 4077–4090. DOI 10.1105/tpc.107.054262.

Takahashi, Y., Nasir, K. H., Ito, A., Kanzaki, H., Matsumura, H., Saitoh, H., et al. (2007). A high-throughput screen of cell-death-inducing factors in *Nicotiana benthamiana* identifies a novel MAPKK that mediates INF1-induced cell death signaling and non-host resistance to *Pseudomonas cichorii*. *Plant Journal*, *49*, 1030–1040.

Tör, M., Holub, E. B., Brose, E., Musker, R., Gunn, N., Can, C., et al. (1994). Map positions of three loci in *Arabidopsis thaliana* associated with isolate-specific recognition of *Peronospora parasitica* (downy mildew). *Molecular Plant-microbe Interactions*, *7*, 214–222.

Tör, M., Gordon, P., Cuzick, A., Eulgem, T., Sinapidou, E., Mert, F., et al. (2002). *Arabidopsis* SGT1b is required for defence signaling conferred by several downy mildew (*Peronospora parasitica*) resistance genes. *Plant Cell*, *14*, 993–1003.

Tör, M., Yemm, A., & Holub, E. (2003). Role of proteolysis in *R*-gene mediated defence in plants. *Molecular Plant Pathology*, *4*, 287–296.

Tyler, B. M., Tripathy, S., Zhang, X., Dehal, P., Jiang, R. H., Aerts, A., et al. (2006). *Phytophthora* genome sequences uncover evolutionary origins and mechanisms of pathogenesis. *Science*, *313*, 1261–1266.

Umemoto, N., Kakitani, M., Iwamatsu, A., Yoshikawa, M., Yamaoka, N., & Ishida, I. (1997). The structure and function of a soybean beta-glucan-elicitor-binding protein. *Proceedings of the National Academy of Sciences of the United States of America*, *94*, 1029–1034.

Van Damme, M., Andel, A., Huibers, R. P., Panstruga, R., Weisbeek, P. J., & Van den Ackerveken, G. (2005). Identification of *Arabidopsis* loci required for susceptibility to the downy mildew pathogen *Hyaloperonospora parasitica*. *Molecular Plant-microbe Interactions*, *18*, 583–592.

Van den Ackerveken, G., Marois, E., & Bonas, U. (1996). Recognition of the bacterial avirulence protein AvrBs3 occurs inside the host plant cell. *Cell*, *87*, 1307–1316.

van der Biezen, E. A., Juwana, H., Parker, J. E., & Jones, J. D. G. (2000). cDNA-AFLP display for the isolation of *Peronospora parasitica* genes expressed during infection in *Arabidopsis thaliana*. *Molecular Plant–Microbe Interactions*, *13*, 895–898.

Voegele, R. T., & Mendgen, K. (2003). Rust haustoria: Nutrient uptake and beyond. *New Phytologist*, *159*, 93–100.

Voegele, R. T, Struck, C., Hahn, M., & Mendgen K. (2001). The role of haustoria in sugar supply during infection of broad bean by the rust fungus *Uromyces fabae*. *Proceedings of the National Academy of Sciences of the United States of America*, *98*, 8133–8138.

Vogel, J., & Somerville, S. (2000). Isolation and characterization of powdery mildew-resistant *Arabidopsis* mutants. *Proceedings of the National Academy of Sciences of the United States of America*, *97*, 1897–1902.

Vogel, J. P., Raab, T. K., Schiff, C., & Somerville, S. C. (2002). PMR6, a pectate lyase-like gene required for powdery mildew susceptibility in *Arabidopsis*. *Plant Cell*, *14*, 2095–2106.

Whisson, S. C., Boevink, P. C., Moleleki, L., Avrova, A. O., Morales, J. G., Gilroy, E. M., et al. (2007). A translocation signal for delivery of oomycete effector proteins into host plant cells. *Nature*, *450*, 115–118.

Win, J., Morgan, W., Bos, J., Krasileva, K. V., Cano, L. M., Chaparro-Garcia, A., et al. (2007). Adaptive evolution has targeted the C-Terminal domain of the RXLR effectors of plant pathogenic oomycetes. *Plant Cell*, *19*, 2349–2369.

Wroblewski, T., Piskurewicz, U., Tomczak, A., Ochoa, O., & Michelmore, R. W. (2007). Silencing of the major family of NBS-LRR-encoding genes in lettuce results in the loss of multiple resistance specificities. *Plant Journal*, *51*, 803–818.

Xiao, F., Giavalisco, P., & Martin, G. B. (2007). *Pseudomonas syringae* type III effector AvrPtoB is phosphorylated in plant cells on serine 258, promoting its virulence activity. *Journal of Biological Chemistry*, *282*, 30737–30744.

Yamamizo, C., Kuchimura, K., Kobayashi, A., Katou, S., Kawakita, K., Jones, J. D., et al. (2006). Rewiring mitogen-activated protein kinase cascade by positive feedback confers potato blight resistance. *Plant Physiology*, *140*, 681–692.

Zipfel, C., & Felix, G. (2005). Plants and animals: A different taste for microbes. *Current Opinion in Plant Biology*, *8*, 353–360.

Zipfel, C., Kunze, G., Chinchilla, D., Caniard, A., Jones, J. D., Boller, T., et al. (2006). Perception of the bacterial PAMP EF-Tu by the receptor EFR restricts *Agrobacterium*-mediated transformation. *Cell*, *125*, 749–760.