Journal of Experimental Botany Advance Access published February 9, 2009

Journal of Experimental Botany, Page 1 of 8
doi:10.1093/jxb/ern353

Journal of Experimental Botany
www.jxb.oxfordjournals.org

REVIEW ARTICLE

Towards understanding the virulence functions of RXLR effectors of the oomycete plant pathogen *Phytophthora infestans*

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Received 2 November 2008; Revised 25 November 2008; Accepted 28 November 2008

Abstract

Plant pathogens establish infection by secretion of effector proteins that may be delivered inside host cells to manipulate innate immunity. It is increasingly apparent that the ubiquitin proteasome system (UPS) contributes significantly to the regulation of plant defences and, as such, is a target for pathogen effectors. Bacterial effectors delivered by the type III and IV secretion systems have been shown to interact with components of the host UPS. Some of these effectors possess functional domains that are conserved in UPS enzymes, whilst others contain novel domains with ubiquitination activities. Relatively little is known about effector activities in eukaryotic microbial plant pathogens. Nevertheless, effectors from oomycetes that contain an RXLR motif for translocation to the inside of plant cells have been shown to suppress host defences. Annotation of the genome of one such oomycete, the potato late blight pathogen *Phytophthora infestans*, and protein–protein interaction assays to discover host proteins targeted by the RXLR effector AVR3a, have revealed that this eukaryotic plant pathogen also has the potential to manipulate host plant UPS functions.

Introduction

Plants face a constant barrage, below and above ground, from plant pathogenic microorganisms such as bacteria, fungi, and oomycetes. In all cases, pattern recognition receptors in plant cell membranes can detect widely conserved secreted or surface-displayed pathogen-associated molecular patterns (PAMPs) and orchestrate appropriate defence responses that prevent colonization and infection. Such PAMPs include flagellin, lipopolysaccharide, and elongation factor Tu from Gram-negative bacteria; chitin, β-glucan and ergosterol from fungi; and the CBEL protein family, required for adhesion to cellulose, and PEP-13, a peptide motif within a secreted 42 kDa transglutaminase enzyme, from oomycetes (Nürnberger and Lipka, 2005; Ingle *et al.*, 2006). The wide range of defences induced by PAMP recognition is collectively termed PAMP-triggered immunity (PTI) (Jones and Dangl, 2006). PTI is effective in preventing invasion by the vast majority of microorganisms with which plants come into contact.

To establish infection, pathogens must evade, suppress, or otherwise manipulate PTI. This may be achieved by the secretion of proteins called effectors which act outside or inside plant cells to target and perturb signalling, regulatory or mechanistic processes associated with defence. Understanding the molecular bases of such effector-triggered

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susceptibility is a major focus of research in plant pathology (Jones and Dangl, 2006). Gram-negative bacterial pathogens deliver effectors to the inside of plant cells by means of a type III secretion system (T3SS). Many T3SS-delivered effectors have been shown to target and manipulate specific host defence-associated proteins and display a range of enzymatic activities, including protease, kinase, phosphatase, and E3 ubiquitin ligase (Chisholm *et al.*, 2006; Desveaux *et al.*, 2006; Grant *et al.*, 2006; Block *et al.*, 2008). By contrast, our knowledge of how eukaryotic pathogen effectors function is scant, although secreted inhibitors of proteases and glucanases from both fungi and oomycetes have been shown to target, respectively, defence-associated host proteases and glucanases in the plant apoplast (Chisholm *et al.*, 2006; Kamoun, 2006; Shabab *et al.*, 2008).

When PTI is suppressed by a pathogen to establish disease, a second layer of resistance can be activated following direct or indirect detection of effectors by resistance (R) proteins [effector-triggered immunity (ETI)]. The prevailing model for indirect interaction follows the 'Guard Hypothesis', in which R proteins monitor key defence-associated host proteins for perturbations triggered by effector activity. Effectors and R proteins are fast-evolving, reflecting an evolutionary 'battle' between interacting pathogen and host to, respectively, evade or maintain recognition (Dangl and Jones, 2001; Jones and Dangl, 2006). Most bacterial T3SS effectors studied to date are detected by a corresponding R protein found in specific host genotypes (Desveaux *et al.*, 2006; Grant *et al.*, 2006). Effectors that are detected by R proteins are termed avirulence (Avr) proteins.

Identification of avirulence genes and knowledge of effector functions in bacteria–plant interactions has expanded considerably in recent years, but much less is known about either in eukaryotic microbial plant pathogens. Nevertheless, a small number of *Avr* genes have been identified in fungi and oomycetes (Birch *et al.*, 2006, 2008; Kamoun, 2006; Ellis *et al.*, 2007*a*, *b*) that encode proteins which are recognized intracellularly and so are presumed to be delivered to the inside of host cells. All of the oomycete *Avr* genes identified to date encode proteins with a signal peptide, followed by the motif RXLR and often a stretch of acidic amino acids ending with the motif EER (Birch *et al.*, 2008). The RXLR–EER twin peptide motif has been shown to be required for delivery/translocation of these effectors inside host plant cells (Fig. 1) (Whisson *et al.*, 2007; Dou *et al.*, 2008*a*; Grouffaud *et al.*, 2008). Hundreds of potential RXLR effector-encoding genes reside in the genomes of sequenced oomycete plant pathogens, revealing a wealth of gene candidates for studying both the establishment of infection and the elicitation of plant defences (Whisson *et al.*, 2007; Win *et al.*, 2007; Birch *et al.*, 2008; Jiang *et al.*, 2008). This fast-emerging field of plant pathology research will be reviewed below.

A second rapidly developing field of study concerns pathogen effector-mediated manipulation of host proteins involved in ubiquitination. It has become increasingly

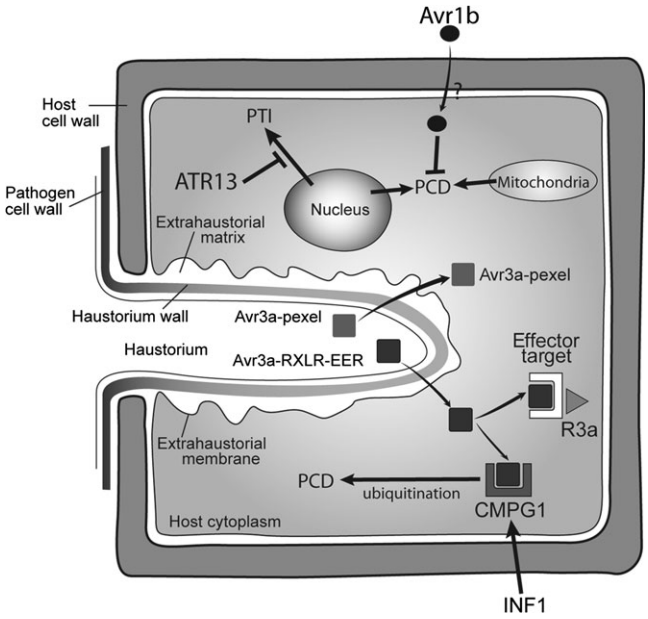

Fig. 1. RXLR effector translocation and function. Avr3a delivered inside plant cells from the *Phytophthora infestans* haustorium is dependent on the RXLR–EER motifs (Whisson *et al.*, 2007). However, delivery of Avr3a by *P. infestans* can also be achieved following replacement of the RXLR–EER-encoding sequences with the Pexel host targeting motif from *Plasmodium falciparum* effectors (Grouffaud *et al.*, 2008). Avr1b from *P. sojae* has been shown to enter plant cells in the absence of the pathogen, suggesting that the formation of a haustorium is not essential for RXLR effector delivery (Dou *et al.*, 2008*a*). The RXLR effector ATR13, from *Hyaloperonospora parasitica*, upon delivery into the host cell, suppresses key components of PAMP-triggered immunity (PTI) (Sohn *et al.*, 2007). Avr1b has been shown to suppress host programmed cell death (PCD) involving the mitochondrial BAX protein (Dou *et al.*, 2008*b*). Avr3a, upon delivery, potentially interacts with at least two proteins: one involved in R3a-mediated recognition, and one involved in INF1-mediated PCD. One Avr3a interactor, CMPG1, is an ubiquitin E3 ligase required for INF1-mediated cell death (Gonzales-Lamothe *et al.*, 2006).

apparent that ubiquitination is a major regulatory contributor to many metabolic and developmental processes in plants, including mechanisms leading to disease resistance (Dreher and Callis, 2007). Evidence has emerged that bacterial plant pathogens target host ubiquitination to facilitate disease (Angot *et al.*, 2007; Block *et al.*, 2008). Here, these recent developments will be reviewed and the potential considered for similar effector-mediated perturbations of the host by eukaryotic oomycete plant pathogens such as *P. infestans*.

Oomycete RXLR effectors reveal an ancient means of host cell entry

Six oomycete avirulence effectors have been reported to date: Avr1b (Shan *et al.*, 2004) from the soybean pathogen *Phytophthora sojae*; ATR1 (Allen *et al.*, 2004) and ATR13

(Rehmany *et al.*, 2005) from the *Arabidopsis* pathogen *Hyaloperonospora arabidopsidis* (formerly *Peronspora parasitica*); and Avr3a (Armstrong *et al.*, 2005), Avr4 (van Poppel *et al.*, 2008), and Avr-blb1 (Vleehouwers *et al.*, 2008) from the potato and tomato pathogen *P. infestans*. All six proteins possess the RXLR motif which has since been shown to be required for the effectors Avr3a (Whisson *et al.*, 2007) and Avr1b (Dou *et al.*, 2008*a*) to traverse the plant host cell plasma membrane (Fig. 1).

Using *P. infestans* transformants expressing Avr3a fused to monomeric red fluorescent protein, or the signal peptide and RXLR–EER domains fused to β-glucuronidase, Whisson *et al.* (2007) showed that this effector was delivered from biotrophic structures called haustoria, which are in intimate contact with the plant cell membrane, to the inside of the host cell (Fig. 1). Both Whisson *et al.* (2007) and Dou *et al.* (2008*a*) demonstrated the requirement of both RXLR and EER motifs for, respectively, AVR3a and Avr1b effector delivery. Whisson *et al.* (2007) showed that replacement of the motifs with amino acids KMIK–DDK, conserving the physicochemical properties of the motif, nevertheless prevented effector delivery. Dou *et al.* (2008*a*) demonstrated that the amino acids R at position 1 and L at position 3 of the RXLR motif are critical for translocation.

The twin peptide RXLR–EER motif is similar in sequence and relative location to the PEXEL or host-targeting motif, RXLXE/D/Q, found in effector proteins of malaria parasites (Hiller *et al.*, 2004; Marti *et al.*, 2004). This motif is required for effectors secreted by *Plasmodium* spp. to enter animal host blood cells, suggesting that it fulfils an equivalent function to the RXLR–EER motif. Indeed, a region of AVR3a containing the RXLR–EER domain was shown to be sufficient to translocate GFP inside erythrocytes (Bhattacharjee *et al.*, 2006; Haldar *et al.*, 2006). In reciprocal experiments, both Dou *et al.* (2008*a*), using DNA bombardment, and Grouffaud *et al.* (2008), using *P. infestans* transformants, have shown that replacement of the RXLR–EER domain in oomycete effectors with the host-targeting domain from *Plasmodium falciparum* effectors retains their ability to be delivered inside host plant cells (Fig. 1). Thus the RXLR–EER and RXLXE/D/Q translocation signals are functionally equivalent and, whether they are the products of divergent or convergent evolution, imply an ancient mechanism of host cell entry that is common to plant and animal cells. The precise pathways and constituents underlying this mechanism remain to be elucidated, but evidence suggests that translocation can occur in the absence of the pathogen (Fig. 1) (Dou *et al.*, 2008*a*), perhaps favouring the exploitation of host endocytic processes (Birch *et al.*, 2008). The experimental system described by Dou *et al.* (2008*a*) provides the means to directly support or challenge this hypothesis.

RXLR effectors possess virulence functions

The delivery of RXLR effectors inside plant cells where they can be detected by R proteins, many of which have been proposed to 'guard' host defence-associated proteins, is consistent with the hypothesis that they perform important functions in establishing colonization and promoting disease. Indeed, this is the case for bacterial T3SS effectors, which have been shown to manipulate a number of components of PTI, and to suppress programmed cell death (PCD) that is often associated with ETI or PTI (Chisholm *et al.*, 2006; Desveaux *et al*, 2006; Grant *et al.*, 2006; Block *et al.*, 2008).

The form of AVR3a (K80I103) from *P. infestans* that is recognized by the potato R3a protein was shown also to suppress cell death triggered by the *P. infestans* PAMP INF1 in *Nicotiana benthamiana*. Both R3a-mediated recognition and suppression of INF1-mediated cell death were independent of the N-terminal RXLR domain (Bos *et al.*, 2006), consistent with the hypothesis that domains downstream of the RXLR domain are involved in effector function (Win *et al.*, 2007). More recently, AVR1b from *P. sojae* has been shown to suppress PCD (Fig. 1) triggered by the pro-apoptotic BAX protein in yeast and in plant species that are distantly related, suggesting that it targets a highly conserved host PCD mechanism (Dou *et al.*, 2008*b*). Again, PCD suppression was independent of the N-terminal signal peptide and RXLR translocation domains. Dou *et al.* (2008*b*) proposed the existence of conserved K, W, and Y motifs in the C-terminal halves of AVR1b, AVR3a, and a large number of RXLR effectors predicted in the genomes of *Phytophthora* spp., and showed that mutation of key residues in the W and Y domains of AVR1b abolished cell death suppression. They hypothesized that these domains are thus critical for the functions of these RXLR effectors.

Sohn *et al.* (2007) demonstrated that delivery of the *H. parasitica* effectors ATR1 and ATR13 into host cells via the T3SS of *Pseudomonas syringae* pathovar. *tomato* (*Pst*) enhanced virulence in susceptible plants. They reported that T3SS-mediated delivery of ATR13 from a *Pst* ΔCEL mutant restored its ability to suppress callose deposition, a key marker of PTI (Fig. 1). Thus, taken together, the results of Bos *et al.* (2006), Sohn *et al.* (2007), and Dou *et al.* (2008*b*) indicate that RXLR effectors, in addition to triggering ETI in plant genotypes possessing cognate *R* genes, also contribute to virulence through the suppression of PTI and ETI.

Ubiquitination is required for plant defence

Ubiquitination is a fundamental eukaryote-specific protein modification system involved in many processes, including transcriptional regulation, signal perception and transduction, cell cycle progression, and responses to biotic and abiotic stresses (Kerscher *et al.*, 2006). It involves the conjugation of single or multiple ubiquitin molecules onto a target protein. Generally speaking, monoubiquitination alters the localization or activity of a protein, whereas polyubiquitination modifies protein properties, or marks the protein for degradation via the 26S proteasome. Polyubiquitin chains can be linked by one of their seven lysine (K)

residues in ubiquitin. K48-linked ubiquitin chains are associated with degradation, whereas K63-linked chains, whilst also sometimes designating degradation, can be associated with modified protein activity or trafficking (Pickart and Fushman, 2004). In addition to ubiquitination, the fates and activities of cellular proteins can be modified by conjugation to other small ubiquitin-related molecules, such as SUMO (small ubiquitin-like modifier).

Ubiquitination involves a cascade of interacting enzyme activities. Ubiquitin is first activated by an E1 (ubiquitin-activating enzyme), involving ATP-dependent activation of ubiquitin resulting in an Ub–E1 thiolester via its active site cysteine residue. Two E1 proteins are encoded by the *Arabidopsis* genome. Thioester-linked ubiquitin is transferred to a cysteine residue in an E2 ubiquitin-conjugating enzyme, of which there are at least 37 in *Arabidopsis*. The morphology (e.g. K48, K63) of polyubiquitin chain linkages is dependent on the particular E2-conjugating enzyme. E3 ubiquitin ligase enzymes, of which there are >2000 in *Arabidopsis*, recruit target proteins for ubiquitination and, as such, are responsible for substrate-specificity. There are two main groups of E3s based on the component domains that interact with E2s: those that contain a RING (Really Interesting New Gene) domain protein and those that contain a HECT (Homologous to E6-AP C-Terminus) domain protein. RING E3s directly and covalently attach the C-terminus of ubiquitin from an E2 to a lysine residue on the target protein, whereas HECT E3s form a thioester intermediate with ubiquitin via their active site cysteine residue before transferring it to the substrate. There are more E3s in plant genomes, such as *Arabidopsis*, than found in any other eukaryotes, indicating the major roles ubiquitination is likely to play in plant regulatory processes (Downes and Vierstra, 2005). Once the fate of an ubiquitinated target is met, ubiquitin can be removed for re-use by de-ubiquitinating enzymes.

Ubiquitination has been shown to contribute to disease resistance, most notably through the regulation of defences orchestrated by signalling molecules such as auxin, giberellin, abscisic acid, jasmonic acid, and ethylene (Dreher and Callis, 2007, and references therein). Plant responses to biotic stresses or defence-associated signalling molecules have been shown to include transcriptional up-regulation of a number of E3 ligases (Zeng *et al.*, 2006) and, moreover, of the two E1 ubiquitin-activating enzymes (Takizawa *et al.*, 2005), indicating likely involvement of the ubiquitin proteasome system (UPS) in defence. More recently, Goritschnig *et al* (2007) used a *suppressor of npr1-1 constitutive 1* (*snc1*) mutant, which shows constitutive activation of defence responses in the absence of pathogen challenge, to investigate downstream signalling components. The mutation *mos5*, with a deletion in one of the two *Arabidopsis* E1 ubiquitin-activating enzymes (*UBA1*), suppresses *snc1*-mediated constitutive defence. Mutation in the other E1 protein *UBA2* does not have the same effect, indicating a possible differential involvement of these enzymes in plant defence.

A number of E3 ubiquitin ligases are required for PCD during *R* gene-mediated plant responses to pathogens. A study of *Avr9-Cf9 rapidly elicited* (*ACRE*) genes revealed three putative E3 ligases that were up-regulated early in this gene-for-interaction. One of these (*ACRE189*/*ACIF1*) encodes an F-box protein required for the HR triggered by a range of pathogen elicitors (van den Burg *et al.*, 2008). The other two, *ACRE276*/*PLANT U-BOX17* (*PUB17*) and *ACRE74*/*CMPG1*/*PUB20*/*21*, encode members of a plant-specific U-box ARMADILLO repeat class of E3 ligases (Gonzales-Lamothe *et al.*, 2006; Yang *et al.*, 2006). In addition to being required for the cell death mediated by a number of R proteins, all three are required for HR triggered by the putative PAMP elicitor INF1 from *P. infestans*.

In contrast to the three E3 ligases described above that are required for activation of defence, Trujillo *et al.* (2008) have shown that a group of three homologous U-box E3 ligases, PUB22, PUB23, and PUB24, are required for repression of PTI. A *pub22*/*pub23*/*pub24* triple mutant showed de-repressed and impaired down-regulation of responses triggered by PAMPs, suggesting that the roles of these E3 ligases may be to 'switch off' PTI once its effect has been achieved.

Ubiquitination is used and targeted by bacterial plant pathogen effectors

Many animal and plant pathogenic bacteria utilize either T3SS or T4SS effectors to manipulate the host's ubiquitination system. The timing, functions, and concentrations of effectors can be controlled by programming them for degradation by the host UPS at key stages of infection. Effectors can also suppress the actions of UPS components associated with the innate immune system, or they can target the degradation of host proteins by mimicking UPS enzyme functions (Angot *et al.*, 2007). All of these different roles have been attributed to effectors from animal pathogenic bacteria. Some of the interactions of bacterial plant pathogen effectors with the host UPS will be briefly reviewed here.

The first demonstration of a prokaryote effector that interacts with a host UPS involved the plant pathogen *Agrobacterium tumefaciens*. The T4SS in *A. tumefaciens* is required for translocation of effectors and for DNA into eukaryotic cells. One T4SS effector, virF, contains a conserved F-box domain with the potential to interact with E3 ubiquitin ligase components (Schrammeijer *et al.*, 2001). VirF has been shown to interact with the plant virE2-interacting protein 1, promoting its nuclear proteasome-dependent degradation and, indirectly, the degradation of VirE2, possibly playing a role in uncoating the T-complex by removing VirE2 molecules prior to T-DNA insertion into the host genome (Tzfira *et al.*, 2004). This was the first indication that searches for conserved ubiquitination-associated domains could reveal effectors that influence the host UPS.

The genome of *Ralstonia solanacearum* contains a family of seven T3SS effectors containing an N-terminal F-box motif and a leucine-rich repeat that have been termed

GALA proteins. GALA proteins have been shown to interact with *Arabidopsis* SKP1-like proteins in a manner reminiscent of plant F-box proteins. Deletion of all seven *GALA* genes is required to attenuate virulence, indicating that there is functional redundancy between them (Angot *et al.*, 2006).

The *Pseudomonas syringae* T3SS effector HopM1 is required for full virulence and is involved in suppression of cell wall-associated defences. HopM1 mediates the proteasome-dependent degradation of the *Arabidopsis* protein AtMIN, which is required for cell wall-mediated defences. The authors postulated that HopM1 acts as an adaptor-mediating recognition of AtMIN7 by the plant UPS (Nomura *et al.*, 2006). In contrast to the examples above, HopM1 is not known to contain any domain conserved in eukaryotic UPS-associated proteins.

Two additional T3SS effectors of *P. syringae*, AvrPto and AVRPtoB, trigger Pto-dependent HR in certain host plants. Intriguingly, AvrPtoB suppresses cell death triggered by AvrPto and a range of other elicitors. The C-terminal portion of AvrPtoB contains a domain with the activity and structural similarity of an E3 ligase (Jansusevic *et al.*, 2006). This activity has been reported to cause the UPS-dependent degradation of Fen kinase, a host protein that activates the plant innate immune response. The authors hypothesized that AvrPtoB has evaded activating resistance by acquiring an E3 ligase activity (Rosebrock *et al.*, 2007). This activity is not required for an additional function of AvrPtoB—suppression of PTI—indicating that it is a multifunctional protein, possibly with more than one host target.

Analysis of the effector complement of prokaryotes has revealed effectors with conserved ubiquitination-associated domains that can readily be annotated, and effectors with novel domains that nevertheless possess ubiquitination enzymatic activities. Thus, both genome annotation and direct experimental investigation of effector classes, such as the oomycete RXLRs, are required to reveal the potential interactions of eukaryotic microbial pathogen effectors with the host UPS.

Do eukaryotic oomycete plant pathogens have the potential to manipulate host ubiquitination processes?

Whereas it is clear that prokaryotic pathogens both utilize and manipulate the plant host UPS to facilitate disease, such roles have yet to be reported for eukaryotic microbial plant pathogens, such as oomycetes and fungi. Potentially secreted components of the ubiquitin proteasome pathway, including an S-phase kinase-associated protein 1 (SKP-1), RING-H2 (Gao *et al.*, 2003), and novel ubiquitin extension proteins (Tytgat *et al.*, 2004), have been reported for plant parasitic nematodes, although their roles in parasitism have not been elucidated.

Annotation of eukaryotic pathogen genome sequences reveals a blueprint of the genes encoding proteins with conserved domains that contribute to ubiquitination. Such information, in combination with searches for the presence of signal peptides, indicating secretion from the pathogen, can reveal those proteins with the potential to interact with host defence components (Torto *et al.*, 2003). A search for ubiquitination-associated genes from the *P. infestans* genome (http://www.broad.mit.edu/annotation/genome/phytophthora_infestans/Home.html) revealed all of the expected components of the ubiquitination machinery (Table 1), including two E1-activating enzymes (similar to what is found in plants), 18 putative E2-conjugating enzymes, and 65 E3 ligases, 34 of which are HECT domain and 31 of which are of the RING domain class. All ubiquitination-associated protein sequences were submitted to SignalP 3.0 and, intriguingly, four HECT domain E3 ligases were found to have a potential signal peptide for secretion. Expression analysis of these genes is being undertaken to see if they are induced during infection. The absence of other potentially secreted components of the *P. infestans* UPS suggests that these E3 ligases may interact with host E2-conjugating enzymes to affect the ubiquitination of either plant or pathogen target proteins during disease development. This hypothesis is under investigation.

None of the predicted RXLR effectors from *P. infestans* contains conserved domains associated with ubiquitination, indicating that if they interact with host UPS components, they must do so using a domain that has not yet been identified as conserved for this function. The *P. infestans* genome contains >400 putative RXLR–EER class effectors (Whisson *et al.*, 2007; Jiang *et al* 2008), and systematic analyses of potential host protein interactors, through yeast-2-hybrid (Y2H) and co-immunoprecipitation assays, for example, are required to investigate whether they target the plant UPS. Recently, both the *AVR3aKI* and *AVR3aEM* alleles from *P. infestans* have been used as bait to identify host proteins interacting with their products from a potato–*P. infestans* Y2H prey library. The library was constructed by combining RNA prepared from 15 h post-inoculation, in the early biotrophic phase, and from 72 h post-inoculation, within the necrotrophic phase of

Table 1. Annotation of ubiquitin-associated genes in the *P. infestans* genome

Enzyme annotation	No. of genes within the *P. infestans* genome
E1 ubiquitin activating enzyme	2
E2 ubiquitin conjugating enzyme	18
E3 U-box ubiquitin ligase	5
E3 RING ubiquitin ligase	5
E3 F-box ubiquitin ligase	16
E3 cullin	5
E3 HECT ubiquitin ligase	34
Ubiquitin specific protease	22
SUMO activating enzyme SAE1	2
SUMO activating enzyme SAE2	1
SUMO conjugating enzyme	1
SUMO ligase	1
SUMO protease	5

a compatible (susceptible) interaction. A number of host proteins were identified in the screens, the majority of which interact with both forms of AVR3a. One of these was the U-box E3 ubiquitin ligase CMPG1 (Fig. 1) (JIB Bos, M Armstrong, EM Gilroy, RM Taylor, PC Boevink, A Sadanandom, S Kamoun, PRJ Birch, unpublished results).

Whereas the AVR3aKI form is both recognized by R3a and suppresses INF1-mediated cell death, the AVR3aEM form does neither (Bos *et al.*, 2006). Recently, it has been shown that deletion of the C-terminal tyrosine, at position 147, whilst not affecting R3a recognition of AVR3aKI, abolishes its ability to suppress INF1-mediated cell death (Bos *et al.*, 2009), separating these two attributes of AVR3aKI and supporting the hypothesis that the effector interacts with more than one host protein (Fig. 1). The Y147 deletion also abolishes AVR3a interaction with the host protein CMPG1. As stated earlier, CMPG1 is one of three U-box E3 ubiquitin ligases that is required for INF1-mediated cell death (Gonzales-Lamothe *et al.*, 2006). Further work is needed to investigate the nature of the interaction between AVR3a and CMPG1. What are the biochemical and biological consequences of the interaction? When and where in the host cell does it occur during infection? What is the significance of the amino acid differences in AVR3aKI and AVR3aEM in relation not only to R3a-mediated recognition, but also suppression of CMPG1-mediated cell death?

Conclusions

Ubiquitination is an important regulatory contributor to many processes in plants, including disease resistance. Consequently, it has been targeted for manipulation by effectors from prokaryotic pathogens. Some of these effectors, delivered by either T3SS or T4SS in plant pathogenic bacteria, contain domains with conserved ubiquitination functions. These have presumably been acquired by horizontal gene transfer, as ubiquitination is a eukaryote-specific regulatory system. In other cases, bacteria have evolved effector domains that are absent in eukaryotes but which nevertheless interact with, and in cases enzymatically modify, components of the host UPS. Our knowledge of effector functions in eukaryotic plant pathogens is relatively minimal. However, recently, hundreds of candidate effectors containing the RXLR–EER motif for translocation inside plant cells have been identified in oomycete genomes, offering the potential to investigate the ways in which these eukaryotes manipulate host plant defences. None of the RXLR effectors contain domains that are conserved in proteins of the UPS. Nevertheless, annotation of the *P. infestans* genome has revealed members of the E3 ubiquitin ligase family that may be secreted, and could thus interact with host UPS enzymes to trigger, suppress, or otherwise modify ubiquitination. Moreover, protein–protein interaction studies to seek the targets of the RXLR effector AVR3a have revealed that it interacts with the plant E3 ligase CMPG1, which is required for cell death triggered by a range of pathogen-derived stimuli. The AVR3aKI form is able to suppress such cell death, indicating a possible rationale for this interaction. Further yeast-2-hybrid screens and co-immunoprecipitation assays may reveal additional members of the RXLR effector complement that interact with, and manipulate, components of the host plant UPS.

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