



ELSEVIER

Available online at www.sciencedirect.com

SciVerse ScienceDirect

Current Opinion in
Plant Biology

Oomycetes, effectors, and all that jazz

Tolga O Bozkurt¹, Sebastian Schornack¹, Mark J Banfield² and
Sophien Kamoun¹

Plant pathogenic oomycetes secrete a diverse repertoire of effector proteins that modulate host innate immunity and enable parasitic infection. Understanding how effectors evolve, translocate and traffic inside host cells, and perturb host processes are major themes in the study of oomycete–plant interactions. The last year has seen important progress in the study of oomycete effectors with, notably, the elucidation of the 3D structures of five RXLR effectors, and novel insights into how cytoplasmic effectors subvert host cells. In this review, we discuss these and other recent advances and highlight the most important open questions in oomycete effector biology.

Addresses

¹ The Sainsbury Laboratory, Norwich Research Park, Norwich NR4 7UH, United Kingdom

² The Department of Biological Chemistry, John Innes Centre, Norwich Research Park, Norwich NR4 7UH, United Kingdom

Corresponding author: Kamoun,

Sophien (sophien.kamoun@tsl.ac.uk)

Current Opinion in Plant Biology 2012, 15:1–10

This review comes from a themed issue on
Biotic interactions
Edited by Ken Shirasu and Pamela Ronald

1369-5266/\$ – see front matter
© 2012 Elsevier Ltd. All rights reserved.

DOI 10.1016/j.pbi.2012.03.008

Introduction

In recent years, the field of plant–microbe interactions has coalesced around a general model. All major classes of molecular players both from plants (surface and intracellular immune receptors) and microbes (pathogen associated molecular patterns [PAMPs] and effectors) have now been revealed [1,2]. Within the context of host–pathogen interactions, ‘effectors’ are molecules, typically proteins, secreted by pathogens that manipulate host cell processes for their own benefit. In plants, effectors can be targeted to the apoplast (the space outside plant cell membranes) or translocated into the host cell (cytoplasmic effectors). It is widely accepted that effectors are deployed mainly to suppress host defences, but they are also likely to have other, as yet ill-defined, roles in remodelling processes such as host metabolism, that could provide nutrients for pathogen growth. However, on resistant plant genotypes, effectors can become a liability for the pathogen. Plants monitor the presence

of effectors using receptors encoded by disease resistance (*R*) genes, which can activate plant immunity in response to pathogens and restrict their growth [1,3,4]. The co-evolutionary dynamics between pathogens and plants have resulted in structurally and functionally diverse families of effectors and immune receptors that are encoded by rapidly evolving genes. Understanding the mechanisms by which effectors perturb plant processes and modulate immunity is central to the study of plant–microbe interactions.

The oomycetes are an important class of filamentous eukaryotic pathogens that secrete effectors to promote infection and colonization of plant tissue. The availability of genome sequences for some of the most important plant pathogenic oomycetes, including *Pythium* [5], *Phytophthora* [6–8], *Albugo* [9,10], and *Hyaloperonospora* [11] species have significantly advanced the field by enabling the cataloguing of putative effectors. These genome-wide repertoires revealed unexpectedly large and diverse classes of effectors indicating that oomycetes have evolved sophisticated pathogenicity mechanisms. Individually, some of these pathogens are the causative agents of devastating crop diseases of both historical and current socio-economic importance (e.g. potato late blight and the Irish potato famine, sudden oak death, soybean root rot); many also cause disease on model plants such as *Nicotiana benthamiana* or *Arabidopsis thaliana*, which makes them tractable pathosystems for the study of effectors in the laboratory.

In this review, we discuss some of the most recent advances and highlight the most important open questions in oomycete effector biology. We also refer readers to other recent reviews on the subject [12–15].

Effector repertoires: more and more

The first oomycete genome sequences determined uncovered effector secretomes much more complex than expected, with several hundred proteins presumably dedicated to perturbing plant cell physiology [6,8]. Two large classes of cytoplasmic effectors, RXLR and CRN proteins, could be reliably predicted by the occurrence of conserved motifs in an N-terminal region that follows the signal peptide. However, these predictions may have underestimated the diversity of the effector secretome. The identification of new effector genes revealed sequence variations on the RXLR consensus in downy mildews. *Hyaloperonospora arabidopsidis* ATR5, an effector that mediates avirulence on *Arabidopsis* plants

2 Biotic interactions

with the RPP5 resistance protein, lacks the canonical RXLR motif (RVRN instead of RVLN in related effectors), although it shares overall similarity to typical RXLR domains and still carries a conserved EER motif [16[•]]. Genome sequencing of the cucurbit downy mildew, *Pseudoperonospora cubensis*, revealed secreted proteins with similarity to typical RXLR effectors, such as ATR1 and AVR3a, but with an R to Q substitution in the first residue of the motif (QXLR) [17]. Overall, these findings suggest that variants of the RXLR motif might be frequent and that effector mining exercises should take this new information into account.

The phylogenetic distribution of RXLR and CRN effectors also revealed some surprises. New genome sequences of pathogenic oomycete species did not simply reveal additional members of the established classes but uncovered new families of secreted proteins. The RXLR effectors turned out to be restricted to the Peronosporales clade, specifically *Phytophthora* and downy mildews. RXLR effector genes are not overrepresented in the genomes of *Pythium ultimum* and species outside the Peronosporales, and are probably missing in these pathogens [5,9[•],10,18]. By contrast, the host-translocated CRN effectors are ubiquitous and have been detected in all examined plant pathogenic oomycetes [18]. However, the CRN family is dramatically expanded in *Phytophthora infestans* (196 genes, 255 pseudogenes), whereas most other oomycetes have relatively fewer CRN genes, with *P. ultimum* having only 18 [5,6]. Unbiased computational analyses of newly sequenced genomes revealed novel families of secreted proteins to be enriched in *P. ultimum*, *Albugo laibachii* and *Albugo candida* [5,9[•],10]. These include secreted proteins with conserved N-terminal sequences, namely the YxSL[RK] motif in *P. ultimum* and CHXC in *Albugo*, that were proposed to function as translocated effectors in these species [5,9[•]].

Host-translocation of oomycete effectors: the plot thickens

One central question in oomycete research is how cytoplasmic effectors are delivered inside host cells. Following secretion, cytoplasmic effectors need to cross the host cell membrane, a process that is thought to require translocation domains defined by specific sequence motifs, such as RXLR [19–21], LXLFLAK [6,18], and CHXC [9[•]]. The mechanisms of translocation are best studied in the RXLR-type effectors, which are thought to be predominantly secreted via haustoria, specialized hyphal structures that invaginate the host membrane [20]. RXLR effectors carry an N-terminal motif that is similar in sequence, position, and function to the host cell-targeting signal (PEXEL/HT motif) required for translocation of proteins from animal parasitic plasmodia into red blood cells [22,23]. In a noted yet controversial paper, Kale *et al.* [24] proposed that the RXLR domain functions in binding the phospholipid phosphatidylinositol-3-phosphate (PI3P). This activity

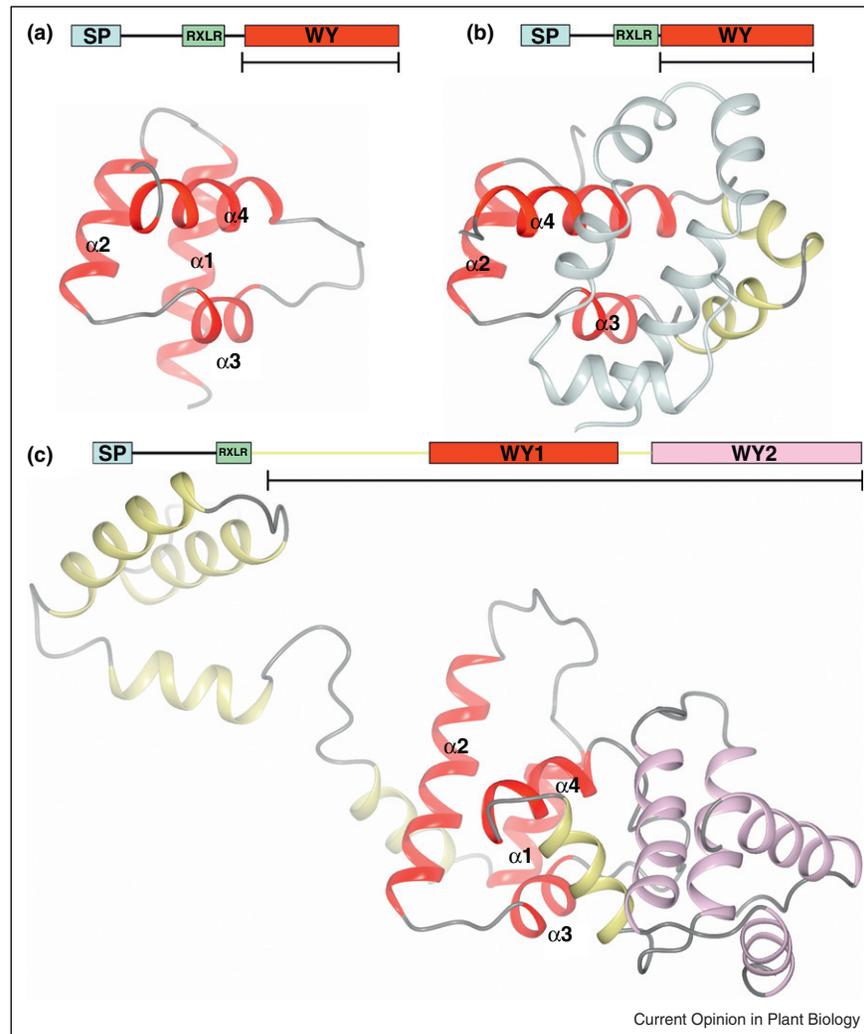
was proposed to enable pathogen-independent entry of RXLR effectors into plant cells via binding abundant outer surface PI3Ps [24]. Kale *et al.* then extended this model to effector proteins from diverse plant pathogenic fungi, such as the rust fungi, which have been proposed to carry highly degenerate RXLR-like motifs that also bind PI3Ps to mediate host cell entry [24,25]. However, this model has not been universally accepted by the oomycete and fungal pathogen communities, due partly to lack of reproducibility of the PI3P binding experiments [26,27,28^{••}] (see also review by P.N. Dodds in this issue). For instance Gan *et al.* [26] could not demonstrate that the flax rust effectors AvrM and AvrL567 require PI3P binding for cell entry. Yaeno *et al.* [28^{••}] were not able to reproduce the RXLR leader to PI3P binding experiments using three RXLR effectors AVR3a, AVR3a4, and AVR1b. Interestingly, Yaeno *et al.* [28^{••}] discovered that PIP binding was not a property of the RXLR region, but rather of the C-terminal effector domain. PIP binding is important for *in planta* stability of AVR3a and as a consequence affects the virulence activity of this effector. Given that host-translocation activity has been ascribed to the N-terminal RXLR domain [20,21], PIP binding to the AVR3a C-terminus should not have any relevance to host-translocation.

The mechanisms of RXLR effector entry into plant cells remain unclear and under debate. It is possible that oomycetes have evolved multiple mechanisms to translocate effector proteins inside host cells. Recently, Wawra *et al.* [29[•]] showed that the oomycete fish pathogen *Saprolegnia parasitica* translocates SpHtp1 via binding tyrosine-*O*-sulphate displayed on the host cell surface. SpHtp1 failed to bind phospholipids and the inhibitor inositol-1,4-diphosphate did not prevent cell entry [29[•]]. Multiple mechanisms of host-cell targeting have also been proposed for the malaria parasite *Plasmodium falciparum*. The PEXEL/HT motif is specifically cleaved within the parasite endoplasmic reticulum (ER) and is subsequently N-acetylated before secretion and targeting into red blood cells via a specific pathogen-encoded translocon [30,31]. Recently, this model was challenged by the finding that the PEXEL motif mediates binding to PI3Ps within the parasite ER, thereby enabling sorting of host-translocated proteins [32]. Nonetheless, unlike what has been suggested for oomycete RXLR effectors, PEXEL/HT effectors are not known to be taken up in the absence of the pathogen, and a pathogen-encoded translocation machinery, whether PI3Ps, chaperones or translocon proteins, appears to be essential for host cell entry.

3D structures of RXLR effectors: take five

The last year has seen the publication of the first 3D structures of oomycete RXLR effectors (Figure 1). To date, five structures have been published: AVR3a4 and AVR3a11 from *Phytophthora capsici* [28^{••},33^{••}]; PexRD2 from *Phytophthora infestans* [33^{••}]; and ATR1 and ATR13

Figure 1



Crystal structures of the C-terminal regions of RXLR-type oomycete effectors. Schematic representations showing the modularity and structures of (a) AVR3a11 (AVR3a4 adopts essentially the same structure), (b) PexRD2 and (c) ATR1. The black bars represent the regions of the protein sequence that were crystallized. The 3 α -helices of the WY domain in each structure are shown in red and labelled $\alpha2$, $\alpha3$ and $\alpha4$; for AVR3a11 and ATR1 the N-terminal helix that forms the 4-helix bundle is also shown in red ($\alpha1$). For clarity, in (b), helices not part of the core WY domain are shown in yellow and the second PexRD2 monomer of the homodimer is in grey. In (c), the second WY 4-helix repeat is shown in pink; helices preceding the WY regions, and a linker helix, are shown in yellow.

from *H. arabidopsidis* [34^{••},35]. Each of the studies investigated structure/function relationships in the C-terminal region of the effectors, the region associated with the biochemical activity of the proteins inside plant cells [12]. The structure of the N-terminal region, which includes the RXLR-dEER translocation domain, could not be elucidated and in the case of AVR3a4 was shown to be un-structured [28^{••}]. *In silico* analyses of other RXLR effectors suggest that the RXLR-dEER leader most probably adopts a disordered conformation, possibly an important molecular feature for function in host cell translocation.

A striking finding emerged from the structures of the AVR3a homologues, PexRD2 and ATR1. These proteins share a conserved alpha-helical protein fold, termed the WY domain [33^{••},36], a composite of the previously defined W and Y motifs [6,37]. This structural conservation could not be confidently predicted from sequence comparisons. Further, the structure of ATR1 was shown to contain two tandem WY domains, linked by an additional helix [34^{••}]. Some RXLR effectors are predicted to carry up to 11 tandemly-repeated WY domains [33^{••}]. Computational analyses suggest that at least 44% of annotated *Phytophthora* RXLR effectors and 26% of

4 Biotic interactions

annotated *H. arabidopsidis* RXLR effectors contain the WY domain [33^{••}]. The occurrence of the WY fold appears to be linked to evolution of biotrophy and the emergence of RXLR effectors in the Peronosporales given that the WY domain signature could not be detected outside this lineage [36]. It is important to note that RXLR effectors show extensive sequence diversity and not all of them are predicted to have WY domain folds. One example is ATR13, which does not appear to harbour the WY domain fold [35]. In summary, it appears that the WY domain has evolved in a large number of RXLR effectors to function as a conserved but adaptable protein fold that supports diversification of effectors. This in turn may support the gain of new effector functions and evasion of host immunity pathways.

Structure and function of RXLR effectors: poker face

Effectors with avirulence activities are under selective pressure to evade recognition by immune receptors while retaining their virulence activities. The 3D structures of RXLR effectors revealed that functionally important and polymorphic residues locate to the surface of the structures [28^{••},33^{••},34^{••},35]. For instance, the structures allowed the mapping of *H. arabidopsidis* ATR1 and ATR13 residues involved in the recognition conferred by RPP1 [34^{••},38] and RPP13 [35], the Arabidopsis resistance proteins that sense these proteins. Interestingly, residues involved in recognition of ATR1 (Emoy2, Maks9 and Cala2 alleles) by RPP1-NdA and RPP1-WsB are different and are distributed across different protein surfaces, suggesting multiple sites of interaction are important for activity [34^{••}]. By contrast, critical residues for recognition of ATR13 (Emoy2 and Emco5 alleles) by RPP13-Nd were co-localized in the structure, pointing to a single region of the protein surface that mediates avirulence activity [35].

Molecular models of *P. infestans* AVR3a, based on the structures of its homologues AVR3a4 and AVR3a11 revealed that residues 80 and 103, which are crucial for recognition by the potato immune receptor R3a [39], map to the same face of the four-helix bundle [28^{••},33^{••}]. In addition, these structures identified a positively-charged surface patch, largely formed from Lys residues of the N-terminal α -helix [28^{••},33^{••}]. Structure-informed mutagenesis of *P. infestans* AVR3a based on the AVR3a4 structure revealed that this C-terminal region was sufficient for AVR3a binding to PIPs [28^{••}]. These mutants had reduced stability *in planta* suggesting that phospholipid binding is important for AVR3a accumulation inside host cells to suppress immunity. By contrast, mutations affecting PIP binding did not alter recognition by R3a [28^{••}].

RXLR effectors dimers: two of a kind

Structure/function analysis of PexRD2 has shown that this effector adopts a homodimer of WY domains *in vitro*, and oligomerizes *in planta* [33^{••}]. This suggests that

oligomerization may provide an additional mechanism for structural and functional diversification of RXLR effectors. This finding raises intriguing questions about the extent to which RXLR effectors form homo-oligomers or hetero-oligomers once they are delivered inside plant cells.

A Nudix hydrolase effector: lonely stranger

The majority of RXLR effectors, including the RXLR-WY class, have little similarity, at the primary amino acid sequence level, to known proteins and many are thought to be too small to encode enzymatic activities. One exception is the *Phytophthora sojae* RXLR effector AVR3b, which carries a Nudix motif that mediates ADP-ribose and NADH pyrophosphorylase activity [40^{••}]. Mutagenesis analyses revealed that the Nudix motif is important for AVR3b virulence function but not for the activation of the resistance protein Rps3b [40^{••}]. AVR3b might mimic plant Nudix hydrolases, which are known to act as negative regulators of plant immunity [40^{••}].

Effectoromics screens: just can't get enough

The sheer number of predicted effector genes in oomycetes makes it challenging to assign them activities and functions. The community's answer to this daunting challenge has been to establish high-throughput *in planta* expression screens of candidate effectors, an approach coined 'effectoromics' [41–43]. In a pioneering effectoromics study, Vleeshouwers *et al.* screened an infection-ready library of computationally predicted RXLR effectors of *P. infestans* on late blight resistant *Solanum* genotypes representing eight different species [41]. The screen revealed AVRblb1, the RXLR effector that is recognized by the *Solanum* resistance proteins Rpi-blb1, Rpi-sto1, and Rpi-pta1. In a related study, the same effector library was co-expressed with the cloned *Solanum bulbocastanum* resistance protein Rpi-blb2 in *N. benthamiana* resulting in the identification of AVRblb2 [43]. More recently, Rietman and colleagues expanded the *P. infestans* RXLR effector library to 270 candidates (Rietman H, thesis 2011). Extensive screening of wild potatoes (*Solanum* spp.) using *Agrobacterium tumefaciens* transient expression (agroinfiltration) revealed a total of 11 new putative avirulence effectors (Rietman H, thesis 2011). A common finding across these studies is that most effector-plant interactions did not result in specific hypersensitive response cell death. A similar result was obtained by Fabro *et al.* with ~60 *H. arabidopsidis* RXLR effectors delivered to 12 ecotypes of Arabidopsis using the Type III secretion system of the bacterial plant pathogen *Pseudomonas syringae* [44]. Except for the previously known ATR13, none of the tested *H. arabidopsidis* RXLR effectors triggered macroscopic cell death indicating that avirulence activity towards the examined set of Arabidopsis ecotypes is relatively rare [44]. A similar finding was obtained by Goritschnig *et al.* [45] who screened 83 A.

thaliana ecotypes with 18 candidate RXLR effectors from *H. arabidopsidis* only to discover a single new effector–R protein interaction (ATR39–RPP39).

In contrast to effectoromics screens for activation of immunity, screens for virulence activities yielded divergent findings [43,44,46]. Screens for suppressors of the cell death induced by the INF1 elicitor, a *P. infestans* secreted protein with features of PAMPs, resulted in 3 positives out of 62 *P. infestans* RXLR effectors [43] versus 43 of 49 *P. sojae* RXLR effectors [46]. The difference between the two studies is striking given that they both used the same *A. tumefaciens*-mediated expression assay in *N. benthamiana* [43,46]. Also, a rather high frequency (35 out of 62) of *H. arabidopsidis* RXLR effectors were found to suppress callose deposition, a component of PAMP-triggered immunity [44]. Such high frequencies of positives are intriguing in our view given that they imply identical and redundant defence suppression activities for most (70–88%) of the tested *P. sojae* and *H. arabidopsidis* effectors. Future follow-up experiments will determine how robust the reported activities are. As a word of caution, such high frequencies of cell death suppression resemble those obtained with screens for suppression of the cell death induced by the mouse protein BAX [46]. However, suppression of BAX cell death is probably an artefact of heterologous expression system given that BAX-induced cell death can be readily suppressed following activation of the unfolded protein response (UPR) [47]. UPR is frequently triggered by heterologous protein expression *in planta* indicating that suppression of BAX cell death is probably unrelated to immunity (S. Schornack, unpublished).

Subcellular localization of effectors: where do you think you're going

Direct microscopic observations of oomycete effectors inside plant cells have proven difficult to achieve, partly because translocated effectors are probably rather dilute in the plant cytoplasm. Whisson *et al.* [20] expressed a fusion between the signal peptide and RXLR domain of AVR3a to *Escherichia coli* β -glucuronidase (GUS) in *P. infestans*, and detected GUS activity only in potato cells in contact with haustoria. However, this experiment has not been convincingly reproduced. In *Magnaporthe oryzae*–rice interactions, such experiments are prone to false positives due to breakage of the plant membrane and resultant localization of proteins inside damaged host cells [48,49]. Therefore, many in the oomycete community investigate subcellular localization of effectors using heterologous expression inside plant cells, particularly transient expression in the model plant *N. benthamiana* [18,50,51^{••},52^{••}]. These experiments revealed that several oomycete effectors target the host cell nucleus similar to bacterial plant pathogen effectors [17,18,52^{••}]. Remarkably, unrelated CRN proteins from different oomycete species localize to the host cell nucleus, pointing to a

common but still unknown nuclear function of this class of effectors [18]. In another study, Caillaud *et al.* [52^{••}] visualized 49 fluorescently tagged RXLR effectors of *H. arabidopsidis* inside plant cells and discovered that one third accumulate in the nucleus, while another third localize to the nucleus and cytoplasm [52^{••}]. The remaining proteins mainly targeted various host membranes, including the endoplasmic reticulum. One RXLR effector, HaRxL17, associates with the plant tonoplast (Figure 2) and confers enhanced susceptibility to *H. arabidopsidis* [52^{••}]. Bozkurt *et al.* [51^{••}] discovered that the *P. infestans* RXLR effector AVRblb2, which enhances susceptibility, targets the plant plasma membrane and that this localization is essential for AVRblb2 virulence function (see below and Figure 2). Overall, these studies indicate that oomycete effectors have evolved to target a variety of subcellular compartments inside plant cells.

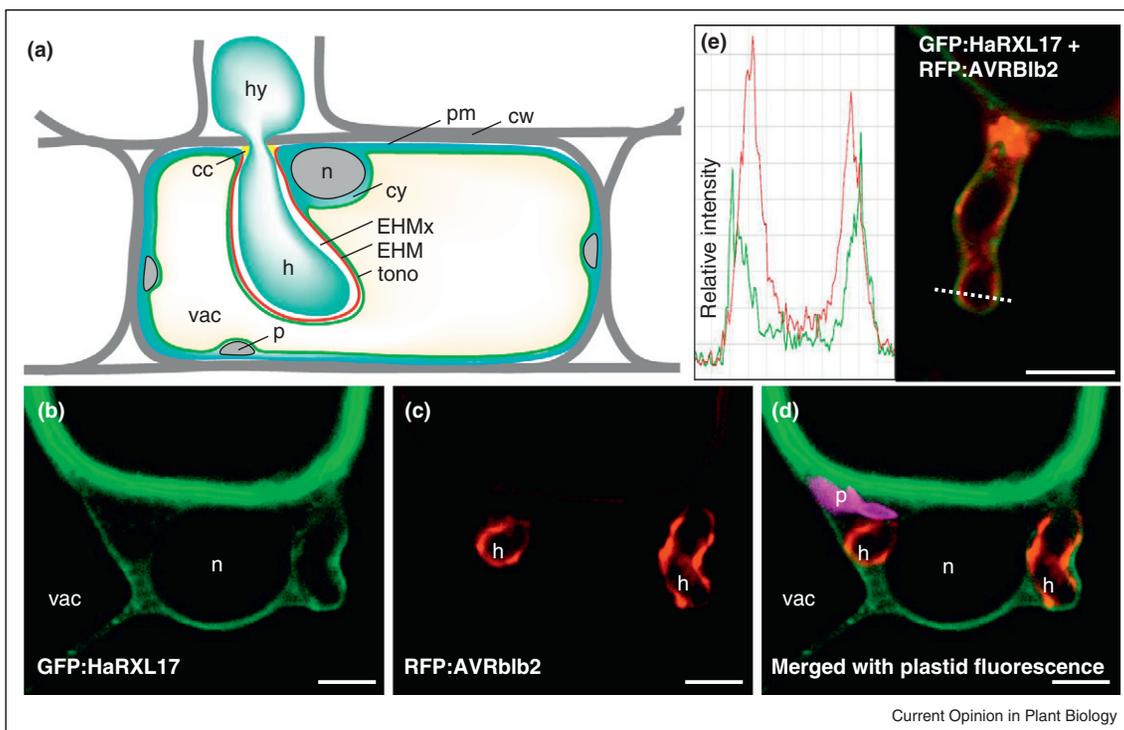
Host targets of effectors: candy everybody wants

The identification of effector host targets is crucial for understanding how effectors enhance virulence and how they are recognized by host immune receptors. It can also lead to the discovery of novel components of plant immunity. Until recently, our knowledge of the targets of oomycete effectors was limited to apoplastic effectors. *Phytophthora* species counteract secreted host hydrolytic enzymes by deploying an arsenal of apoplastic effectors with inhibitory activities against these enzymes [53–57]. *P. infestans* deploys Kazal-like and cystatin protease inhibitors to target secreted host serine and cysteine proteases, respectively [55–57]. Interestingly, the tomato defence cysteine protease RCR3 is not only inhibited by the *P. infestans* effectors EPIC1 and EPIC2B but also by the effector AVR2 of the fungal plant pathogen *Cladosporium fulvum* [56]. This study was a first indication that phylogenetically unrelated pathogens have evolved to target common host defence components [56]. Recently, a large-scale yeast two-hybrid (Y2H) screen for effector targets of both *H. arabidopsidis* RXLR effectors and *P. syringae* Type III effectors also highlighted a set of 18 common host target proteins [58]. However, *in planta* validation of these interactions and detailed functional studies to understand the biological relevance of the candidate host targets remain to be performed.

The last two years have seen new insight into how particular RXLR effectors target host proteins to suppress plant immunity. The *P. infestans* RXLR effector AVR3a binds a host E3 ubiquitin ligase CMPG1, a protein previously shown to be required for the plant cell death response induced by the PAMP-like secreted protein INF1 elicitor [59]. Bos *et al.* found that wild-type AVR3a, but not an inactive mutant, stabilizes CMPG1 *in planta* to mediate suppression of INF1-induced plant cell death [59]. Recently, the *P. infestans* RXLR effector AVRblb2 has been shown to interfere with focal plant immunity at

6 Biotic interactions

Figure 2



Membrane compartments in a haustorium-containing (haustoriated) plant cell. **(a)** Schematic representation of a haustoriated plant cell highlighting the various membranes. **(b–e)** Localization of two RXLR effectors, *H. arabidopsidis* HaRXL17 [52^{**}] and *P. infestans* AVRblb2 [51^{**}], in haustoriated *Nicotiana benthamiana* cells infected with *P. infestans*. In this system, GFP:HaRXL17 localizes to the tonoplast and plasma membrane but not to the EHM (b). By contrast, RFP:AVRblb2 associates with the EHM (c). Both effectors have separate subcellular distribution maxima and highlight two adjacent membranes (d, e). Hy, oomycete hypha; h, haustorium; n, nucleus; p, plastids; vac, vacuole; cy, cytoplasm; pm, plasma membrane; cw, cell wall; EHMx, extrahaustorial matrix; EHM, extrahaustorial membrane, tonoplast; and cc, callosic collar, bar = 5 μm.

the haustorial interface (see also below). Using an *in planta* co-immunoprecipitation screen for targets of RXLR effectors [60], AVRblb2 was found to associate with the plant immune cysteine protease C14 [51^{**}]. AVRblb2 contributes to pathogen virulence by preventing trafficking of C14 into the apoplast. Interestingly, C14 is also targeted by the *P. infestans* apoplastic effectors EPIC1 and EPIC2 indicating that components of the plant immune system can be targeted by distinct effectors at different host subcellular compartments [51^{**},57].

Extrahaustorial membrane composition: you're so different

Over the course of an infection, biotrophic oomycetes associate closely with plant cells. In the Peronosporales, the haustoria are a key feature of biotrophy. These spherical or digit-like hyphal protrusions penetrate host cells, allowing a clear distinction between infected and noninfected cells. In infected 'haustoriated' cells, the host membrane remains intact but is invaginated by the invading hyphae and becomes the extrahaustorial membrane (EHM) (Figure 2). Formation of the EHM requires biogenesis of novel host cell membrane material, a process

that is poorly understood. Recently, Lu *et al.* [61[•]] found that the EHM that surrounds *P. infestans* and *H. arabidopsidis* haustoria differs from the remaining plant plasma membrane in lacking membrane proteins, such as aquaporin or a calcium transporter, a feature previously reported for fungal haustoria [62,63]. EHM exclusion of plasma membrane resident plant proteins appears to be a selective process since pattern recognition receptors are selectively absent depending on the oomycete species and host system [61[•]]. Moreover, membrane-associated proteins that do not carry hydrophobic transmembrane stretches, such as a potato Remorin (StRem1.3) or Arabidopsis Synaptotagmin 1 (SYT1), are not excluded from the EHM [61[•]]. It will be interesting to determine the extent to which the exclusion of membrane proteins from the EHM is driven by pathogen effectors and whether oomycetes have evolved to purge the EHM of particular membrane proteins as a counter-defence strategy (Box 1).

Perihaustorial accumulation of effectors: wrapped around your finger

Plant cells generally respond to mechanical penetration with a spatially confined cell-autonomous response that

Box 1 A common mechanism between animal and plant parasites for exclusion of host proteins from parasite-induced membranes?

Divergent eukaryotic pathogens have been proposed to share common mechanisms of pathogenicity [68]. This might apply to the exclusion of host proteins from parasite-induced membranes, such as the parasitophorous vacuolar membrane that surround apicomplexan parasites and the oomycete-induced extrahaustorial membrane (EHM). Apicomplexan mammalian parasites employ a set of dedicated proteins to invade host cells. The apical Rhoptry apparatus secretes RON proteins, some of which integrate into the host envelope to anchor the parasite [69]. A ring-like moving junction is formed between parasite and host plasma membranes while the apicomplexan is invaginating into the host cell and finally gets accommodated inside a host derived parasitophorous vacuole (PV). Remarkably, although the PV envelope originates from the host plasma membrane it lacks several membrane-integral host proteins whereas GPI-anchored host proteins are still detectable. It is believed that pathogen mediated modulation of the host cytoskeleton might affect membrane protein anchoring. However, only a significantly concentrated host cytoskeleton at penetration sites, but no direct parasite protein–host actin interaction has been reported to date.

Two recent studies described a similar phenomenon for the EHM triggered by oomycete pathogens. Several plant transmembrane domain containing proteins could not be detected in the extra-haustorial membrane (EHM) that envelops oomycete haustoria [52**,61*]. Interestingly, membrane proteins that associate or integrate via anchors other than transmembrane domains, such as remorins or synaptotagmin, could still be detected at the EHM. Oomycete haustoria also display actin filament accumulation [52**,64]. However, the mechanisms underlying this observation remain unknown and pathogen-encoded host transmembrane proteins that modulate the EHM composition have not been reported from oomycetes. Future research will distinguish the extent to which oomycete pathogens, and their effectors, inhibit lateral diffusion of plasma membrane proteins or block secretory vesicles during EHM biogenesis [61*].

includes focal accumulation of organelles and secretory compartment for targeted deployment of defence compounds [61,64–66]. Other host dynamic relocalizations might be promoted by the pathogen (see review by Beck *et al.* in this issue). Recently, studies on oomycete effectors started to shed light on the underlying molecular mechanisms that define focal responses to pathogen penetration in haustoriated cells. A technical breakthrough has been the ability to perform live-cell imaging of haustoriated plant cells that express fluorescent-tagged effector proteins [51**,52**] (Figure 2). It was found that effectors could be used as molecular probes to dissect cellular dynamics at the haustorial interface. For instance, the plasma membrane associated effector AVRblb2 of *P. infestans* was shown to intensely accumulate around haustoria in penetrated plant cells, suggesting that it targets processes affected by dynamic relocalization [51**]. The tonoplast associated HaRxL17 effector of *H. arabidopsidis* was also found to localize around haustoria in infected Arabidopsis cells suggesting that the tonoplast membrane associates closely with the EHM [52**] (Figure 2). Overall, these studies raise the exciting prospect of elucidating

how localized effector activity alters dynamic cellular processes at the haustorial interface.

Outlook: the best is yet to come

As this article illustrates, research on oomycetes and their effectors has come a long way in recent years. Nonetheless, despite significant advances there are still important gaps in our knowledge of oomycete effectors and their roles in host interactions. There are a number of critical questions about effector function, trafficking and evolution that need to be addressed. What are the spatial and temporal aspects of effector deployment? Are the effectors deployed in a regulated fashion from growing intercellular hyphal tip or haustoria? What novel and exciting insights can we learn from the 3D structures of different classes of effectors such as CRNs? What types of host macromolecules do effectors target and how do they interact? How do effectors adapt to these host target molecules? To what extent do oomycete pathogens remodel the EHM? How do oomycete effectors evolve new functions? Can the effectors traffic from cell-to-cell? Finally, there is an urgent need to shed some light on the mechanisms of effector host-translocation and resolve the conflicting models that have been put forward, particularly with regards to the roles of phospholipid and tyrosine-*O*-sulphate binding [27,29*].

To conclude, we acknowledge the remarkable progress of the field and we hope that the basic knowledge amassed on oomycete effector biology will lead to novel and effective strategies for plant disease resistance as recently discussed by Vleeshouwers *et al.* [42,67].

Acknowledgements

We thank Marie-Cecile Caillaud and Jonathan D. G. Jones for providing biomaterials, and Serdar Kebabcilar for inspiration. The authors are funded by the Biotechnology and Biological Sciences Research Council (BBSRC, UK) and the Gatsby Charitable Foundation. TOB also received a Marie Curie Re-integration Grant.

References and recommended reading

Papers of particular interest, published within the period of review, have been highlighted as:

- of special interest
- of outstanding interest

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.pbi.2012.03.008.

1. Dodds PN, Rathjen JP: **Plant immunity: towards an integrated view of plant-pathogen interactions.** *Nat Rev Genet* 2010, **11**:539–548.
2. Martin F, Kamoun S (Eds): *Effectors in Plant Microbe Interactions.* Wiley-Blackwell; 2012.
3. Chisholm ST, Coaker G, Day B, Staskawicz BJ: **Host-microbe interactions: shaping the evolution of the plant immune response.** *Cell* 2006, **124**:803–814.

8 Biotic interactions

4. Jones JD, Dangl JL: **The plant immune system.** *Nature* 2006, **444**:323-329.
5. L vesque CA, Brouwer H, Cano L, Hamilton JP, Holt C, Huitema E, Raffaele S, Robideau GP, Thines M, Win J *et al.*: **Genome sequence of the necrotrophic plant pathogen *Pythium ultimum* reveals original pathogenicity mechanisms and effector repertoire.** *Genome Biol* 2010, **11**:R73.
6. Haas BJ, Kamoun S, Zody MC, Jiang RH, Handsaker RE, Cano LM, Grabherr M, Kodira CD, Raffaele S, Torto-Alalibo T *et al.*: **Genome sequence and analysis of the Irish potato famine pathogen *Phytophthora infestans*.** *Nature* 2009, **461**:393-398.
7. Raffaele S, Farrer RA, Cano LM, Studholme DJ, MacLean D, Thines M, Jiang RH, Zody MC, Kunjeti SG, Donofrio NM *et al.*: **Genome evolution following host jumps in the Irish potato famine pathogen lineage.** *Science* 2010, **330**:1540-1543.
8. Tyler BM, Tripathy S, Zhang X, Dehal P, Jiang RH, Aerts A, Arredondo FD, Baxter L, Bensasson D, Beynon JL *et al.*: ***Phytophthora* genome sequences uncover evolutionary origins and mechanisms of pathogenesis.** *Science* 2006, **313**:1261-1266.
9. Kemen E, Gardiner A, Schultz-Larsen T, Kemen AC, Balmuth AL, Robert-Seilaniantz A, Bailey K, Holub E, Studholme DJ, Maclean D *et al.*: **Gene gain and loss during evolution of obligate parasitism in the white rust pathogen of *Arabidopsis thaliana*.** *PLoS Biol* 2011, **9**:e1001094.
- This paper describes the Illumina sequencing of the genome and transcriptome of the *Arabidopsis* parasite *Albugo laibachii*. A novel effector class with a CHXC host translocation motif was discovered expanding the diversity of host translocation motifs in oomycetes.
10. Links MG, Holub E, Jiang RH, Sharpe AG, Hegedus D, Beynon E, Sillito D, Clarke WE, Uzuhashi S, Borhan MH: **De novo sequence assembly of *Albugo candida* reveals a small genome relative to other biotrophic oomycetes.** *BMC Genomics* 2011, **12**:503.
11. Baxter L, Tripathy S, Ishaque N, Boot N, Cabral A, Kemen E, Thines M, Ah-Fong A, Anderson R, Badejoko W *et al.*: **Signatures of adaptation to obligate biotrophy in the *Hyaloperonospora arabidopsidis* genome.** *Science* 2010, **330**:1549-1551.
12. Schornack S, Huitema E, Cano LM, Bozkurt TO, Oliva R, Van Damme M, Schwizer S, Raffaele S, Chaparro-Garcia A, Farrer R *et al.*: **Ten things to know about oomycete effectors.** *Mol Plant Pathol* 2009, **10**:795-803.
13. Stassen JH, Van den Ackerveken G: **How do oomycete effectors interfere with plant life?** *Curr Opin Plant Biol* 2011, **14**:407-414.
14. Thines M, Kamoun S: **Oomycete-plant coevolution: recent advances and future prospects.** *Curr Opin Plant Biol* 2010, **13**:427-433.
15. Tyler BM: **Entering and breaking: virulence effector proteins of oomycete plant pathogens.** *Cell Microbiol* 2009, **11**:13-20.
16. Bailey K, Cevik V, Holton N, Byrne-Richardson J, Sohn KH, Coates M, Woods-Tor A, Aksoy HM, Hughes L, Baxter L *et al.*: **Molecular cloning of ATR5(Emoy2) from *Hyaloperonospora arabidopsidis*, an avirulence determinant that triggers RPP5-mediated defense in *Arabidopsis*.** *Mol Plant Microbe Interact* 2011, **24**:827-838.
- This paper describes the cloning of the ATR5 effector, which triggers RPP5-mediated resistance. Interestingly, ATR5 has a divergent sequence (GRVR) at the position of the canonical RXLR motif while it still retains the EER motif. This implies that the number of candidate RXLR-like host-translocated proteins in the secretome of oomycete parasites might be larger than expected.
17. Tian M, Win J, Savory E, Burkhardt A, Held M, Brandizzi F, Day B: **454 Genome sequencing of *Pseudoperonospora cubensis* reveals effector proteins with a QXLR translocation motif.** *Mol Plant Microbe Interact* 2011, **24**:543-553.
18. Schornack S, van Damme M, Bozkurt TO, Cano LM, Smoker M, Thines M, Gaulin E, Kamoun S, Huitema E: **Ancient class of translocated oomycete effectors targets the host nucleus.** *Proc Natl Acad Sci USA* 2010, **107**:17421-17426.
19. Rehmany AP, Gordon A, Rose LE, Allen RL, Armstrong MR, Whisson SC, Kamoun S, Tyler BM, Birch PR, Beynon JL: **Differential recognition of highly divergent downy mildew avirulence gene alleles by RPP1 resistance genes from two *Arabidopsis* lines.** *Plant Cell* 2005, **17**:1839-1850.
20. Whisson SC, Boevink PC, Moleleki L, Avrova AO, Morales JG, Gilroy EM, Armstrong MR, Grouffaud S, van West P, Chapman S *et al.*: **A translocation signal for delivery of oomycete effector proteins into host plant cells.** *Nature* 2007, **450**:115-118.
21. Dou D, Kale SD, Wang X, Jiang RH, Bruce NA, Arredondo FD, Zhang X, Tyler BM: **RXLR-mediated entry of *Phytophthora sojae* effector Avr1b into soybean cells does not require pathogen-encoded machinery.** *Plant Cell* 2008, **20**:1930-1947.
22. Bhattacharjee S, Hiller NL, Liolios K, Win J, Kanneganti TD, Young C, Kamoun S, Haldar K: **The malarial host-targeting signal is conserved in the Irish potato famine pathogen.** *PLoS Pathog* 2006, **2**:e50.
23. Birch PR, Rehmany AP, Pritchard L, Kamoun S, Beynon JL: **Trafficking arms: oomycete effectors enter host plant cells.** *Trends Microbiol* 2006, **14**:8-11.
24. Kale SD, Gu B, Capelluto DG, Dou D, Feldman E, Rumore A, Arredondo FD, Hanlon R, Fudal I, Rouxel T *et al.*: **External lipid PI3P mediates entry of eukaryotic pathogen effectors into plant and animal host cells.** *Cell* 2010, **142**:284-295.
25. Plett JM, Kempainen M, Kale SD, Kohler A, Legue V, Brun A, Tyler BM, Pardo AG, Martin F: **A secreted effector protein of *Laccaria bicolor* is required for symbiosis development.** *Curr Biol* 2011, **21**:1197-1203.
26. Gan PH, Rafiqi M, Ellis JG, Jones DA, Hardham AR, Dodds PN: **Lipid binding activities of flax rust AvrM and AvrL567 effectors.** *Plant Signal Behav* 2010, **5**:1272-1275.
27. Ellis JG, Dodds PN: **Showdown at the RXLR motif: serious differences of opinion in how effector proteins from filamentous eukaryotic pathogens enter plant cells.** *Proc Natl Acad Sci USA* 2011, **108**:14381-14382.
28. Yaeno T, Li H, Chaparro-Garcia A, Schornack S, Koshiba S, Watanabe S, Kigawa T, Kamoun S, Shirasu K: **Phosphatidylinositol monophosphate-binding interface in the oomycete RXLR effector AVR3a is required for its stability in host cells to modulate plant immunity.** *Proc Natl Acad Sci USA* 2011, **108**:14682-14687.
- One of the three papers on the 3D structure of RXLR effectors that were published concurrently in 2011. This paper is particularly notable for failing to reproduce the phospholipid binding of the RXLR domain reported by [24]. By contrast, the C-terminal effector domain was found to have the capacity to bind phosphatidylinositol monophosphate. See [27] for a commentary on the topic.
29. Wawra S, Bain J, Durward E, de Bruijn I, Minor KL, Matena A, Lobach L, Whisson SC, Bayer P, Porter AJ *et al.*: **Host-targeting protein 1 (SpHtp1) from the oomycete *Saprolegnia parasitica* translocates specifically into fish cells in a tyrosine-O-sulphate-dependent manner.** *Proc Natl Acad Sci USA* 2012, **109**:2096-2101.
- The most recent paper on mechanisms of effector translocation inside host cells adds a new twist to the story. Whether tyrosine-O-sulphate binding will also apply to classical RXLR effectors remains to be determined.
30. Russo I, Babbitt S, Muralidharan V, Butler T, Oksman A, Goldberg DE: **Plasmepsin V licenses *Plasmodium* proteins for export into the host erythrocyte.** *Nature* 2010, **463**:632-636.
31. Boddey JA, Hodder AN, Gunther S, Gilson PR, Patsiouras H, Kapp EA, Pearce JA, de Koning-Ward TF, Simpson RJ, Crabb BS *et al.*: **An aspartyl protease directs malaria effector proteins to the host cell.** *Nature* 2010, **463**:627-631.
32. Bhattacharjee S, Stahelin RV, Speicher KD, Speicher DW, Haldar K: **Endoplasmic reticulum PI(3)P lipid binding targets malaria proteins to the host cell.** *Cell* 2012, **148**:201-212.
33. Boutemy LS, King SR, Win J, Hughes RK, Clarke TA, Blumenschein TM, Kamoun S, Banfield MJ: **Structures of *Phytophthora* RXLR Effector Proteins: a conserved but adaptable fold underpins functional diversity.** *J Biol Chem* 2011, **286**:35834-35842.
- The 3D crystal structures of two *Phytophthora* RXLR effectors (PexRD2 and AVR3a11) are described. Unexpectedly, these two effectors, despite having less than 20% sequence identity, share a common core helical fold, which enables a significant level of plasticity.

34. Chou S, Krasileva KV, Holton JM, Steinbrenner AD, Alber T, Staskawicz BJ: **Hyaloperonospora arabidopsidis ATR1 effector is a repeat protein with distributed recognition surfaces.** *Proc Natl Acad Sci USA* 2011, **108**:13323-13328.
- This paper describes the 3D structure of the ATR1 effector and highlights surface-exposed amino acid residues that are crucial for recognition by cognate R protein RPP1.
35. Leonelli L, Pelton J, Schoeffler A, Dahlbeck D, Berger J, Wemmer DE, Staskawicz B: **Structural elucidation and functional characterization of the *Hyaloperonospora arabidopsidis* effector protein ATR13.** *PLoS Pathog* 2011, **7**:e1002428.
36. Win J, Krasileva KV, Kamoun S, Shirasu K, Staskawicz BJ, Banfield MJ: **Sequence divergent RXLR effectors share a structural fold conserved across plant pathogenic oomycete species.** *PLoS Pathog* 2012, **8**:e1002400.
37. Jiang RH, Tripathy S, Govers F, Tyler BM: **RXLR effector reservoir in two *Phytophthora* species is dominated by a single rapidly evolving superfamily with more than 700 members.** *Proc Natl Acad Sci USA* 2008, **105**:4874-4879.
38. Krasileva KV, Dahlbeck D, Staskawicz BJ: **Activation of an *Arabidopsis* resistance protein is specified by the in planta association of its leucine-rich repeat domain with the cognate oomycete effector.** *Plant Cell* 2010, **22**:2444-2458.
39. Bos JI, Chaparro-Garcia A, Quesada-Ocampo LM, McSpadden Gardener BB, Kamoun S: **Distinct amino acids of the *Phytophthora infestans* effector AVR3a condition activation of R3a hypersensitivity and suppression of cell death.** *Mol Plant Microbe Interact* 2009, **22**:269-281.
40. Dong S, Yin W, Kong G, Yang X, Qutob D, Chen Q, Kale SD, Sui Y, Zhang Z, Dou D et al.: ***Phytophthora sojae* avirulence effector Avr3b is a secreted NADH and ADP-ribose pyrophosphorylase that modulates plant immunity.** *PLoS Pathog* 2011, **7**:e1002353.
- This paper reports the cloning and characterization of the Avr3b effector from the soybean parasite *P. sojae*. Avr3b is recognized by the soybean R gene *R3b*. In contrast to bacterial Type III secretion effectors, oomycete effectors typically lack enzymatic activities. However, Avr3b is the first example of an oomycete RXLR effector encoding a protein with an enzymatic activity (Nudix hydrolase).
41. Vleeshouwers VG, Rietman H, Krenek P, Champouret N, Young C, Oh SK, Wang M, Bouwmeester K, Vosman B, Visser RG et al.: **Effector genomics accelerates discovery and functional profiling of potato disease resistance and *Phytophthora infestans* avirulence genes.** *PLoS ONE* 2008, **3**:e2875.
42. Vleeshouwers VG, Raffaele S, Vossen JH, Champouret N, Oliva R, Segretin ME, Rietman H, Cano LM, Lokossou A, Kessel G et al.: **Understanding and exploiting late blight resistance in the age of effectors.** *Annu Rev Phytopathol* 2011, **49**:507-531.
43. Oh SK, Young C, Lee M, Oliva R, Bozkurt TO, Cano LM, Win J, Bos JI, Liu HY, van Damme M et al.: **In planta expression screens of *Phytophthora infestans* RXLR effectors reveal diverse phenotypes, including activation of the *Solanum bulbocastanum* disease resistance protein Rpi-blb2.** *Plant Cell* 2009, **21**:2928-2947.
44. Fabro G, Steinbrenner J, Coates M, Ishaque N, Baxter L, Studholme DJ, Korner E, Allen RL, Piquerez SJ, Rougon-Cardoso A et al.: **Multiple candidate effectors from the oomycete pathogen *Hyaloperonospora arabidopsidis* suppress host plant immunity.** *PLoS Pathog* 2011, **7**:e1002348.
45. Goritschnig S, Krasileva KV, Dahlbeck D, Staskawicz BJ: **Computational prediction and molecular characterization of an oomycete effector and the cognate *Arabidopsis* resistance gene.** *PLoS Genet* 2012, **8**:e1002502.
46. Wang Q, Han C, Ferreira AO, Yu X, Ye W, Tripathy S, Kale SD, Gu B, Sheng Y, Sui Y et al.: **Transcriptional programming and functional interactions within the *Phytophthora sojae* RXLR effector repertoire.** *Plant Cell* 2011, **23**:2064-2086.
47. Watanabe N, Lam E: **BAX inhibitor-1 modulates endoplasmic reticulum stress-mediated programmed cell death in *Arabidopsis*.** *J Biol Chem* 2008, **283**:3200-3210.
48. Mosquera G, Giraldo MC, Khang CH, Coughlan S, Valent B: **Interaction transcriptome analysis identifies *Magnaporthe oryzae* BAS1-4 as Biotrophy-associated secreted proteins in rice blast disease.** *Plant Cell* 2009, **21**:1273-1290.
49. Khang CH, Berruyer R, Giraldo MC, Kankanala P, Park SY, Czymbek K, Kang S, Valent B: **Translocation of *Magnaporthe oryzae* effectors into rice cells and their subsequent cell-to-cell movement.** *Plant Cell* 2010, **22**:1388-1403.
50. Boevink PC, Birch PR, Whisson SC: **Imaging fluorescently tagged *Phytophthora* effector proteins inside infected plant tissue.** *Methods Mol Biol* 2011, **712**:195-209.
51. Bozkurt TO, Schornack S, Win J, Shindo T, Ilyas M, Oliva R, Cano LM, Jones AM, Huitema E, van der Hoorn RA et al.: ***Phytophthora infestans* effector AVRblb2 prevents secretion of a plant immune protease at the haustorial interface.** *Proc Natl Acad Sci USA* 2011, **108**:20832-20837.
- This paper describes an RXLR effector, AVRblb2, secreted by *P. infestans* that accumulates intensely around haustoria and contributes to virulence by preventing secretion of a host immune protease. This work indicates that effectors can be used as molecular probes to dissect focal immune responses and potentially unravel the diversity of secretory vesicles and their cargo.
52. Caillaud MC, Piquerez SJ, Fabro G, Steinbrenner J, Ishaque N, Beynon J, Jones JD: **Subcellular localization of the *Hpa* RXLR effector repertoire identifies a tonoplast-associated protein HaRxL17 that confers enhanced plant susceptibility.** *Plant J* 2012, **69**:252-265.
- This study reports a screen for subcellular localizations of *H. arabidopsis* (*Hpa*) RXLR effectors inside plant cells. Interestingly, a tonoplast associated RXLR effector HaRxL17 was found to localize around the EHM during interactions between *Hpa* and *Arabidopsis* confirming that the two membranes can occur in close proximity inside haustoriated cells.
53. Rose JK, Ham KS, Darvill AG, Albersheim P: **Molecular cloning and characterization of glucanase inhibitor proteins: coevolution of a counterdefense mechanism by plant pathogens.** *Plant Cell* 2002, **14**:1329-1345.
54. Tian M, Huitema E, Da Cunha L, Torto-Alalibo T, Kamoun S: **A Kazal-like extracellular serine protease inhibitor from *Phytophthora infestans* targets the tomato pathogenesis-related protease P69B.** *J Biol Chem* 2004, **279**:26370-26377.
55. Tian M, Win J, Song J, van der Hoorn R, van der Knaap E, Kamoun S: **A *Phytophthora infestans* cystatin-like protein targets a novel tomato papain-like apoplastic protease.** *Plant Physiol* 2007, **143**:364-377.
56. Song J, Win J, Tian M, Schornack S, Kaschani F, Ilyas M, van der Hoorn RA, Kamoun S: **Apoplastic effectors secreted by two unrelated eukaryotic plant pathogens target the tomato defense protease Rcr3.** *Proc Natl Acad Sci USA* 2009, **106**:1654-1659.
57. Kaschani F, Shabab M, Bozkurt T, Shindo T, Schornack S, Gu C, Ilyas M, Win J, Kamoun S, van der Hoorn RA: **An effector-targeted protease contributes to defense against *Phytophthora infestans* and is under diversifying selection in natural hosts.** *Plant Physiol* 2010, **154**:1794-1804.
58. Mukhtar MS, Carvunis AR, Dreze M, Epple P, Steinbrenner J, Moore J, Tasan M, Galli M, Hao T, Nishimura MT et al.: **Independently evolved virulence effectors converge onto hubs in a plant immune system network.** *Science* 2011, **333**:596-601.
59. Bos JI, Armstrong MR, Gilroy EM, Boevink PC, Hein I, Taylor RM, Zhendong T, Engelhardt S, Vetukuri RR, Harrower B et al.: ***Phytophthora infestans* effector AVR3a is essential for virulence and manipulates plant immunity by stabilizing host E3 ligase CMPG1.** *Proc Natl Acad Sci USA* 2010, **107**:9909-9914.
60. Win J, Kamoun S, Jones AM: **Purification of effector-target protein complexes via transient expression in *Nicotiana benthamiana*.** *Methods Mol Biol* 2011, **712**.
61. Lu YJ, Schornack S, Spallek T, Geldner N, Chory J, Schellmann S, Schumacher K, Kamoun S, Robatzek S: **Patterns of plant subcellular responses to successful oomycete infections reveal differences in host cell reprogramming and endocytic trafficking.** *Cell Microbiol* 2012 doi: 10.1111/j.1462-5822.2012.01751.x.

10 Biotic interactions

This study highlights differences between oomycete pathogens in remodelling the EHM inside infected plant cells.

62. Koh S, André A, Edwards H, Ehrhardt D, Somerville S: **Arabidopsis thaliana** subcellular responses to compatible *Erysiphe cichoracearum* infections. *Plant J* 2006, **44**:516-529.
63. O'Connell RJ, Panstruga R: **Tete a tete inside a plant cell: establishing compatibility between plants and biotrophic fungi and oomycetes.** *New Phytol* 2006, **171**:699-718.
64. Takemoto D, Hardham AR: **The cytoskeleton as a regulator and target of biotic interactions in plants.** *Plant Physiol* 2004, **136**:3864-3876.
65. Takemoto D, Jones DA, Hardham AR: **Re-organization of the cytoskeleton and endoplasmic reticulum in the Arabidopsis pen1-1 mutant inoculated with the non-adapted powdery mildew pathogen, Blumeria graminis f. sp. hordei.** *Mol Plant Pathol* 2006, **7**:553-563.
66. Eschen-Lippold L, Landgraf R, Smolka U, Schulze S, Heilmann M, Heilmann I, Hause G, Rosahl S: **Activation of defense against Phytophthora infestans in potato by down-regulation of syntaxin gene expression.** *New Phytol* 2012, **193**:985-996.
67. Rietman H, Bijsterbosch G, Cano LM, Lee H-R, Vossen JH, Jacobsen E, Visser RGF, Kamoun S, Vleeshouwers VGAA: **Qualitative and quantitative late blight resistance in the potato cultivar Sarpo Mira is determined by the perception of five distinct RXLR effectors.** *Mol Plant Microbe Interact* 2012, doi:10.1094/MPMI-01-12-0010-R.
68. Haldar K, Kamoun S, Hiller NL, Bhattacharje S, van Ooij C: **Common infection strategies of pathogenic eukaryotes.** *Nat Rev Microbiol* 2006, **4**:922-931.
69. Besteiro S, Dubremetz JF, Lebrun M: **The moving junction of apicomplexan parasites: a key structure for invasion.** *Cell Microbiol* 2011, **6**:797-805.