Structure and Function of RXLR Effectors of Plant Pathogenic Oomycetes

William Morgan, Jorunn Bos, Catherine Bruce, Minkyoung Lee, Hsin-Yen Liu, Sang-Keun Oh, Jing Song, Joe Win, Carolyn Young, and Sophien Kamoun

1 Introduction

A diverse number of plant pathogens, including bacteria, fungi, oomycetes and nematodes, secrete effector proteins to different cellular compartments of their hosts to modulate plant defense circuitry and enable parasitic colonization (Birch et al., 2006; Chisholm et al., 2006; Kamoun, 2006; O′Connell and Panstruga, 2006). The current paradigm in the study of plant–microbe interactions is that unraveling the molecular function of effectors is central to a mechanistic understanding of pathogenicity. Indeed, significant progress has been made in elucidating the virulence functions of bacterial effectors and the biochemical activities that enable these proteins to perturb host defense processes and facilitate pathogenicity (Chisholm et al., 2006). In contrast, little is known about the biology of effectors of eukaryotic plant pathogens. Nonetheless, this area of research is progressing rapidly as illustrated by the recent identification of effectors from the flax rust and barley powdery mildew fungi (Catanzariti et al., 2006; Dodds et al., 2004; Ridout et al., 2006), the oomycetes *Phytophthora* and *Hyaloperonospora* (Allen et al., 2004; Armstrong et al., 2005; Rehmany et al., 2005; Shan et al., 2004), as well as root-knot nematodes (Huang et al., 2006a; Huang et al., 2006b).

Oomycetes form a distinct group of eukaryotic microorganisms that includes some of the most notorious pathogens of plants (Kamoun, 2003). Research on oomycete effectors has accelerated in recent years due in great part to the availability of resources stemmed from genomics. The emerging picture is complex and fascinating. Oomycetes are now thought to secrete hundreds of effector proteins belonging to two classes that target distinct sites in the host plant (Birch et al., 2006; Kamoun, 2006; Tyler et al., 2006). Apoplastic effectors are secreted into the plant extracellular space, whereas cytoplasmic effectors are translocated inside the plant cell, where they target different subcellular compartments (Birch et al., 2006; Kamoun, 2006). Several apoplastic effectors have been determined

W. Morgan
Department of Plant Pathology, Ohio State University Ohio Agricultural Research and Development Center, Wooster, OH, USA

AQ1

J.P. Gustafson (ed.), *Genomics of Disease*,

to function as inhibitors of host enzymes, such as proteases and glucanases (Rose et al., 2002; Tian et al., 2005; 2004). They are thought to contribute to counter-defense by disabling host enzymes that accumulate in response to pathogen infection. In contrast, the biochemical activities of cytoplasmic effectors remain poorly understood. Oomycete cytoplasmic effectors have been discovered first through their avirulence (Avr) function, i.e. their ability to trigger hypersensitive cell death on specific host genotypes that carry particular disease resistance (*R*) genes (Allen et al., 2004; Armstrong et al., 2005; Rehmany et al., 2005; Shan et al., 2004). The function of these effectors in plants that do not carry the cognate *R* genes remains largely unknown (Kamoun, 2006).

This review summarizes recent findings on the structure and function of the RXLR class of oomycete effectors (Birch et al., 2006; Kamoun, 2006). These effectors function inside host cells and are characterized by a highly conserved region defined by the invariant sequence RXLR. This review will cover two main topics of RXLR effector research: trafficking and function.

2 The RXLR Sequence Defines a Conserved Domain of Oomycete Avr Proteins

Four oomycete *Avr* genes, *ATR1*^{NdWsB} and *ATR13* from the downy mildew *Hyaloperonospora parasitica*, *Avr1b-1* from the soybean pathogen *Phytophthora sojae*, and *Avr3a* from *P. infestans*, have been cloned recently (Allen et al., 2004; Armstrong et al., 2005; Rehmany et al., 2005; Shan et al., 2004). The R proteins that target ATR1^{NdWsB}, ATR13, and AVR3a^{KI} belong to the intracellular class of NBS-LRR (nucleotide binding site and leucine-rich repeat domain) proteins, suggesting that recognition of these Avr proteins occurs inside the plant cytoplasm (Allen et al., 2004; Armstrong et al., 2005; Rehmany et al., 2005). Indeed, when directly expressed *in planta* by transient transformation, AVR3a^{KI}, ATR1^{NdWsB}, and ATR13 did not require a signal peptide sequence to trigger hypersensitivity, and are therefore recognized inside the plant cytoplasm (Allen et al., 2004; Armstrong et al., 2005; Rehmany et al., 2005). How these effectors are translocated into host cells during infection is unknown but a conserved sequence centered on the RXLR motif might be implicated (Birch et al., 2006; Kamoun, 2006; Rehmany et al., 2005) (Fig. 1). All four oomycete Avr proteins carry a signal peptide followed by the RXLR region, which occurs within the N-terminal ca. 60 amino acids of these proteins (Rehmany et al., 2005). So far, definite elucidation of the function of the RXLR region has not been obtained, but the prevailing hypothesis is that RXLR functions in delivery of the effectors inside host cells (Birch et al., 2006; Kamoun, 2006; Rehmany et al., 2005). Indeed, this motif is similar in sequence and position to a host cell-targeting signal (HT/Pexel motif) that is required for translocation of proteins from malaria parasites (*Plasmodium* species) into the cytoplasm of host red blood cells (Hiller et al., 2004; Marti et al., 2004). Interestingly, the cellular biology of *Plasmodium* infection of host blood cells shares some commonalities

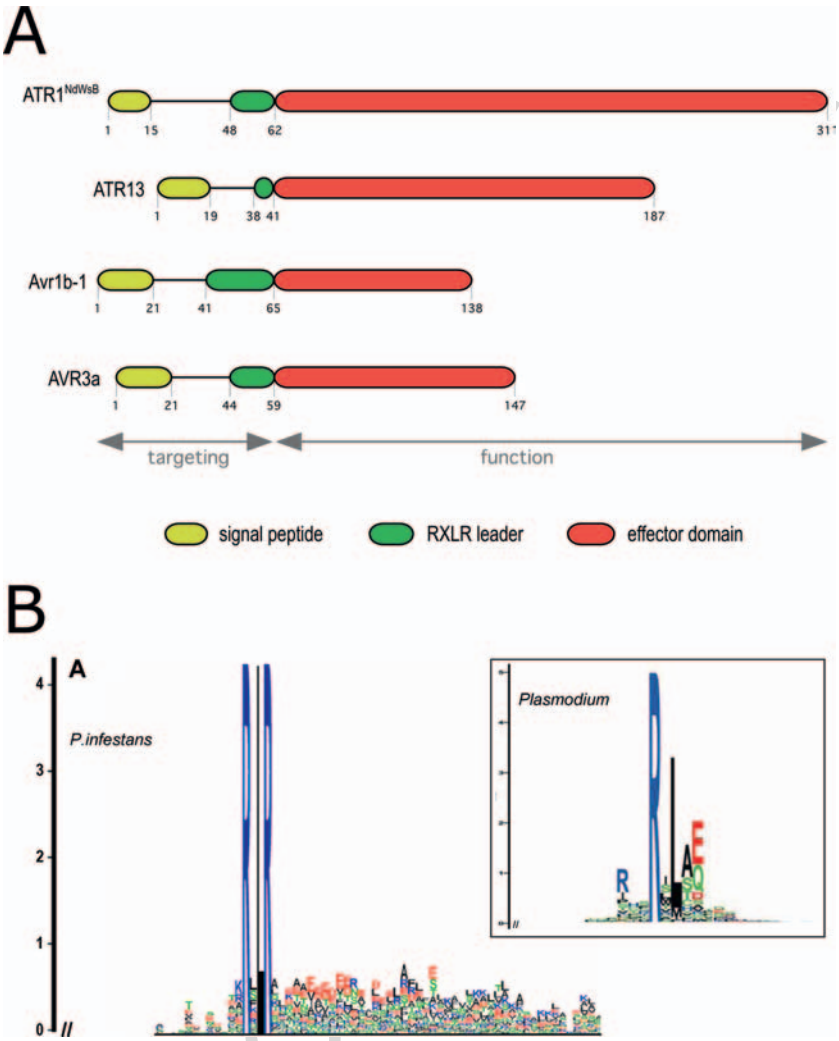

Fig. 1 (**A**)Domain organization of cytoplasmic RXLR effectors. Schematic drawings of ATR1^{NdWsB} and ATR13 of *Hyaloperonsopora parasitica*, Avr1b-1 of *Phytophthora sojae*, and AVR3a of *P. infestans*. The numbers under the sequences indicate amino-acid positions. The highlighted RXLR domain includes the RXLR sequence itself and the downstream dEER sequence. The *gray arrows* distinguish the regions of the effector proteins that are involved in secretion and targeting from those involved in effector activity. (**B**) Similarity between the RXLR motif of oomycetes and the HT/Pexel motif of *Plasmodium falciparum*. Sequence logos were derived from *P. infestans* and *P. falciparum* effector proteins. Adapted from Bhattacharjee et al. (2006)

with oomycete infection structures like haustoria (Bhattacharjee et al., 2006). During the blood stages of infection, *Plasmodium* invades mature erythrocytes and develops within a parasitophorous vacuolar membrane (PVM) derived from an invagination of the erythrocyte membrane. While residing within the PVM, the

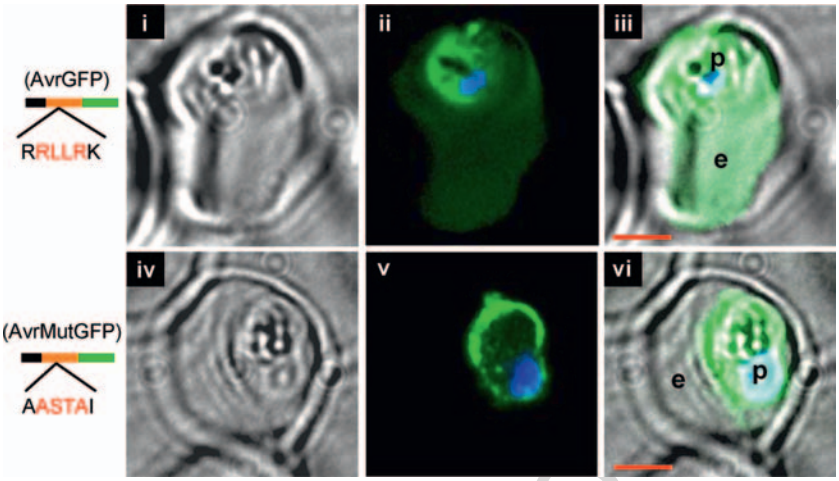

Fig. 2 The *Phytophthora infestans* AVR3a RXLR leader region mediates the export of the green fluorescent protein (GFP) from the *Plasmodium falciparum* parasite to the host erythrocyte. Erythrocytes expressing wild-type or mutated RXLR region of AVR3a (residues 21 to 69) fused to a *P. falciparum* signal peptide and GFP. Panels **ii** and **v** represent fluorescence images, **i** and **iv** bright field images, and **iii** and **vi** merged images. Parasite (p), erythrocyte (e), Hoechst stained nucleus (*blue*), scale bar represents 2 μm. Adapted from Bhattacharjee et al. (2006)

AQ2

parasite exports effector proteins to the erythrocyte resulting in reprogramming of the invaded blood cell. Thus, both *Plasmodium* and oomycetes need to translocate effector proteins across a host-derived membrane during colonization of host cells suggesting that the conserved sequence motif might be required to overcome this comparable hurdle (Bhattacharjee et al., 2006). Although direct demonstration that the oomycete RXLR domain is a host-targeting signal has not been reported, a number of experiments that are consistent with this hypothesis have been performed. The next three sections describe these experiments.

The discovery that host-targeted proteins from *Phytophthora* and *Plasmodium* share a positionally conserved sequence begged the examination of functional conservation. Bhattacharjee et al. (2006) demonstrated that the *P. infestans* AVR3a^{KI} RXLR leader sequence is sufficient to mediate the export of the green fluorescent protein (GFP) from the *Plasmodium falciparum* parasite to the host red blood cell (erythrocyte) (Fig. 2).

3 The *Phytophthora* RXLR Domain Mediates Host Targeting in *Plasmodium*

Mutations in the RXLR consensus abolished export. In addition, the RXLR region of PH001D5, another candidate effector identified computationally from *P. infestans* sequences, was also functional in *Plasmodium*. Interestingly, regions upstream and downstream of the RXLR motif were required for host targeting, suggesting that

the RXLR sequence defines a ca. 30 amino acid leader sequence. Thus the targeting domain extends beyond the RXLR sequence. Consistent with this view, sequence biases were observed in the regions flanking RXLR, particularly a high rate of E/D residues following RXLR. In summary, these findings suggest that plant and animal eukaryotic pathogens share similar secretory signals for effector delivery into host cells (Bhattacharjee et al., 2006). At this stage, it is unclear whether this functional conservation reflects conserved export machinery between these divergent eukaryotes (see below for further discussion on this topic). However, the functional analogy with bacterial type III secretion system is remarkable. In both cases, export signals are highly conserved across unrelated pathogen species but the effector secretome that is delivered to the host is highly divergent (Bhattacharjee et al., 2006).

4 The RXLR Domain Is Not Required for Effector Activities

The view that the RXLR region functions in translocation into host cells suggests that RXLR effectors are organized into two main functional domains (Kamoun, 2006) (Fig. 1). The first domain encompassing the signal peptide and RXLR leader functions in secretion and targeting, while the remaining C-terminal domain carries the effector activity. This model predicts that the RXLR region should not be required for activity when the effector is expressed inside host cells. Indeed, Bos et al. (2006) recently showed that mutation of *P. infestans* AVR3a^{KI} RXLR sequence into AXAA did not interfere with induction of R3a hypersensitivity when the protein is directly expressed in *N. benthamiana* leaves. In fact, deletion analyses of AVR3a^{KI} showed that the C-terminal 75-amino acid, which excludes the RXLR region but includes the two polymorphic amino acids K^{80} and I^{103} that are mutated in the nonfunctional allele, was sufficient for avirulence function when expressed directly inside plant cells (Bos et al., 2006). These findings are consistent with the view that the N-terminal region of AVR3a^{KI} and other RXLR effectors is involved in secretion and targeting but is not required for effector activity.

5 The C-Terminal Region of RXLR Effectors Is Typically More Polymorphic than the Signal Peptide and RXLR Domains

Higher levels of polymorphisms, particularly non-synonymous substitutions, have been detected in the C-terminal regions of RXLR effectors than in the signal peptide and RXLR leader region. For example, the C-terminal regions of *H. parasitica* ATR1 and ATR13 exhibit higher levels of non-synonymous polymorphisms than the N-terminal regions, suggesting that the effector activity is localized to the C-terminal domain (Allen et al., 2004; Rehmany et al., 2005). Also, two out of the three polymorphic residues between the two *Avr3a* alleles of *P. infestans*, amino acids 80 and 103, are located in the C-terminal effector domain (Armstrong et al., 2005). The observation that these effectors are under diversifying selection is consistent with the view that pathogen effectors with avirulence functions are

caught in a coevolutionary arms race with host factors, particularly their cognate R genes (Allen et al., 2004). Signatures of selection are expected in regions involved in effector activity rather than targeting. Thus, the observation that the RXLR domain is less polymorphic than the C-terminal region of RXLR proteins is consistent with the view that it is not exposed to selection pressure by host defenses and that it functions in targeting.

6 Can RXLR Effectors Enter Host Plants in the Absence of the Pathogen?

Whether RXLR effectors require pathogen machinery or structures (e.g., haustoria) to enter plant cells is currently unclear. In our laboratory, we have failed so far to trigger R3a hypersensitivity using recombinant AVR3a^{KI} proteins (unpublished data). Shan et al. (2004) reported that infiltration of *P. sojae* RXLR effector Avr1b-1, produced in *Pichia pastoris*, into Rps1b soybean leaves resulted in cell death. However, it is unknown (although highly likely) whether Rps1b is a cytoplasmic protein and confirmation of *Avr1b-1* activity by *in planta* expression inside soybean cells has not been reported yet. Clearly, it would be highly informative to test Avr1b-1 recombinant proteins mutated in the RXLR sequence using the infiltration assay of Shan et al. (2004).

The issue of host translocation is also relevant to cytoplasmic effectors of fungal pathogens. In their work with Avr proteins of the flax rust fungus *Melampsora lini*, Catanzariti et al. (2006) concluded that the avirulence protein AvrM enters plant cells in the absence of the pathogen. AvrM is a secreted protein with a canonical signal peptide that is recognized inside flax cells in an *M* gene dependent manner. In addition, *Agrobacterium tumefaciens*-mediated expression of a full-length AvrM construct in flax plants carrying the *M* gene resulted in hypersensitive cell death. Interestingly, similar constructs carrying the ER retention sequence HDEL were unable to trigger cell death. The authors concluded from this experiment that AvrM first exits plant cells into the apoplast and then reenters through an unknown process (Catanzariti et al., 2006). We are not in absolute agreement with this interpretation and are of the opinion that this experiment is inconclusive in evaluating whether AvrM can enter plant cells in the absence of the pathogen. An equally plausible explanation is that the signal peptide carrying AvrM is translocated back from the ER into the cytosol through the well-established retrograde transport pathway (Brandizzi et al., 2003; Di Cola et al., 2001). In such case, the HDEL motif would retain the protein into the ER and prevent retrograde translocation. In summary, although the experiments of Catanzariti et al. (2006) suggest that AvrM needs to shuttle through the ER, perhaps to achieve maturation, infiltration experiments with purified AvrM proteins are necessary to obtain conclusive evidence as to the ability of this protein to enter host cells in the absence of the pathogen. Such data has been obtained conclusively with ToxA, a host-selective toxin produced by the plant pathogenic fungus *Pyrenophora tritici-repentis*. Manning and Ciuffetti (2005) demonstrated elegantly that a recombinant ToxA protein

tagged with GFP internalizes inside sensitive wheat cells, where it localizes to cytoplasmic compartments and chloroplasts. This implies that effector proteins can translocate inside host cells in the absence of pathogen machinery, probably using host-derived machinery. Host-translocation of ToxA is likely to occur via receptor-mediated endocytosis, and might implicate the RGD sequence that is identical to a cell adhesion motif of mammalian extracellular matrix proteins (Manning and Ciuffetti, 2005).

In our own work with $AVR3a^{KI}$, we noted that high levels of expression of a full-length construct resulted in R3a-dependent hypersensitivity (Bos et al., 2006). These experiments are not particularly informative since they could be equally explained by (1) mis-targeting of the protein to the cytoplasm or (2) secretion of $AVR3a^{KI}$ followed by re-entry of the protein inside the plant cell resulting in R3a activation. Also, in other experiments, attempts to exploit this full-length $AVR3a^{KI}$ to evaluate the role of the RXLR motif in translocation inside host cells were inconclusive since a RXLR to AXAA mutant retained the ability to elicit R3a response (Bos et al., 2006).

7 A Model for RXLR Effector Delivery into the Host

The process through which the RXLR leader sequence might function in host targeting of effectors remains unknown. Many key questions still need to be addressed. What is the export machinery of effectors in eukaryotic pathogens? Is it derived from the pathogen or are the effectors exploiting host transport systems? Is the export machinery conserved between oomycetes and *Plasmodium*? Despite these persisting questions, some reasonable assumptions about the translocation process can be made. For instance, it seems sensible to break down the export process into two steps (Bhattacharjee et al., 2006). First, the effectors are secreted outside the pathogen cell through the general secretory pathway and endoplasmic reticulum (ER) type signal peptides. Then, the secreted effectors are transported across a host-derived membrane, most likely the haustorial membrane, via the RXLR leader. In the GFP export experiments of Bhattacharjee et al. (2006), constructs with a mutated RXLR sequence accumulated GFP outside the parasite but within the parasitophorous vacuole suggesting that the main function of the RXLR leader consists of transport across this host-derived membrane.

Here, we propose a model for effector delivery (illustrated in Fig. 3). The model is based on the fact that mechanisms of protein transport across membranes follow canonical processes involving recurrent themes (Wickner and Schekman, 2005). We propose that host translocation of the effectors via the RXLR leader involves at least a RXLR leader binding protein, one or more chaperones, and a translocon, which could be of either pathogen or plant origin. Translocation into host cells initiates with the RXLR binding protein recruiting secreted mature effectors in coordination with the chaperones. The effector cargo is then transferred to a translocon embedded in the haustorial membrane, and is then released across the membrane into the plant cytosol. The chaperones are important for maintaining the folding state of the transported effectors both prior and after transit through the translocon.

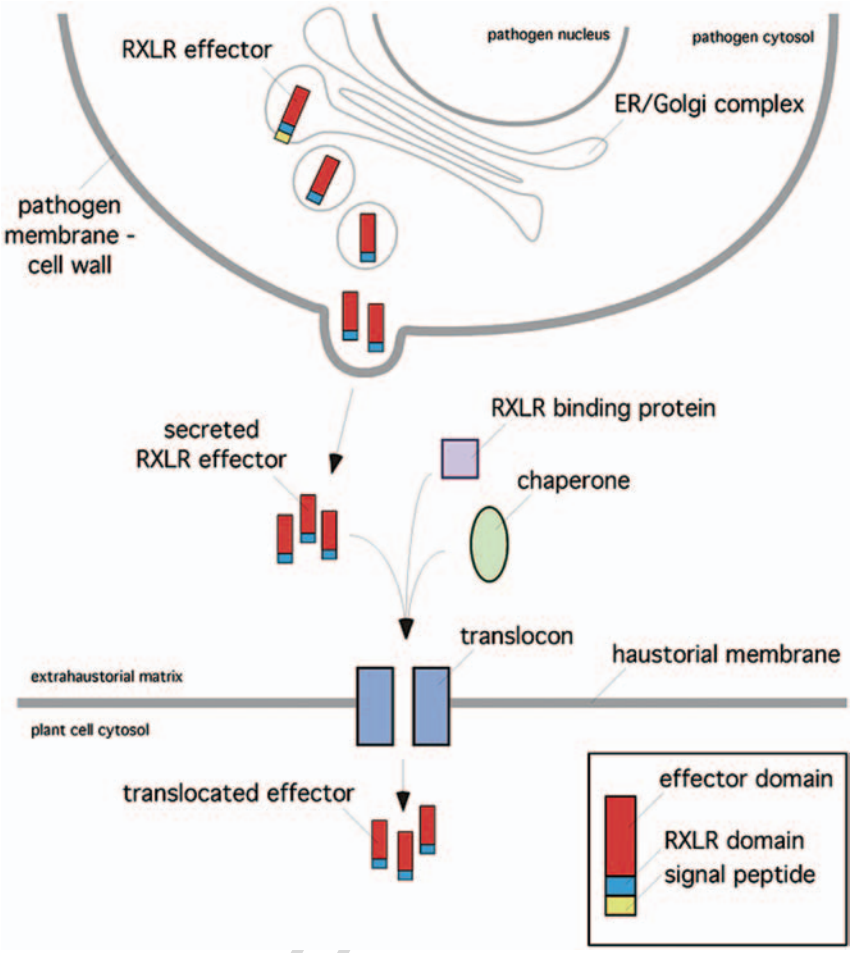

Fig. 3 A hypothetical model for RXLR effector secretion and delivery into host cells (see text for details)

At this point, this model is highly speculative. But our purpose is to outline a useful working model to serve as a hypothesis generator and help guide future research. Indeed, the model suggests immediate research avenues that would shed light on the translocation process, for instance the identification of RXLR binding proteins.

8 Virulence Functions of RXLR Effectors

The virulence function of RXLR effectors, i.e. their activity in plants that do not carry cognate *R* genes, remains in great part unknown. Bos et al. (2006), in an effort to assign virulence-related functions to AVR3a, discovered that AVR3a^{KI} suppresses

the hypersensitive cell death induced by the major *P. infestans* elicitin INF1 in *Nicotiana benthamiana*. The cell death suppression activity of AVR3a^{KI} exhibited some level of specificity. AVR3a^{KI} did not suppress the cell death induced by other *P. infestans* effectors, like PiNPP1 and CRN2, which elicit distinct and antagonistic cell death signaling pathways compared to INF1 (Kanneganti et al., 2006). The biological relevance of this activity of AVR3a^{KI} is unknown but could be significant considering that suppression of innate immunity is a widespread function of plant pathogen effectors, particularly the type III secretion system (TTSS) effectors of bacterial phytopathogens (Espinosa and Alfano 2004). AVR3a^{KI} could interfere with the avirulence activity of INF1 or other unidentified effectors that trigger hypersensitivity using similar pathways as INF1 (Bos et al., 2006). Future work is needed to clarify these issues and determine whether cell death suppression is a common function among RXLR effectors.

9 Outlook: Too Many Effectors, Too Little Time

Considering that five oomycete species, *H. parasitica*, *P. capsici*, *P. infestans*, *P. ramorum*, and *P. sojae*, are undergoing genome sequencing and annotation, we are moving rapidly toward genome-wide catalogues of RXLR effectors. Already it is evident that the RXLR effector secretome of plant pathogenic oomycetes is much more complex than expected, with perhaps several hundred proteins dedicated to manipulating host cells (Kamoun, 2006; Tyler et al., 2006). Recent estimates of RXLR effectors based on the draft genome sequences of *P. sojae* and *P. ramorum* range from ca. 150 to 350 per genome (Bhattacharjee et al., 2006; Tyler et al., 2006). The task of tackling the study of so many effectors is daunting. One of the challenges is to establish "effectoromics" approaches, or global studies of effector function and activity. Ultimately, comprehensive understanding of RXLR effector activities and the perturbations they cause in plants is a precondition for understanding the molecular basis of oomycete pathogenesis and disease.

Acknowledgments We thank collaborators and colleagues in the oomycete field for useful discussions and insight. This work was supported by NSF Plant Genome Research Program grant DBI-0211659. Salaries and research support were provided, in part, by State and Federal Funds appropriated to the Ohio Agricultural Research and Development Center, The Ohio State University.

References

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

Armstrong, M.R., Whisson, S.C., Pritchard, L., Bos, J.I., Venter, E., Avrova, A.O., Rehmany, A.P., Bohme, U., Brooks, K., Cherevach, I., Hamlin, N., White, B., Fraser, A., Lord, A., Quail, M.A., Churcher, C., Hall, N., Berriman, M., Huang, S., Kamoun, S., Beynon, J.L., and Birch, P.R., 2005, An ancestral oomycete locus contains late blight avirulence gene *Avr3a*, encoding

a protein that is recognized in the host cytoplasm, *Proc. Natl. Acad. Sci. USA* **102**: 7766–7771.

Bhattacharjee, S., Hiller, N.L., Liolios, K., Win, J., Kanneganti, T.D., Young, C., Kamoun, S., and Haldar, K., 2006. The malarial host-targeting signal is conserved in the Irish potato famine pathogen. *PLoS Pathog.* **2**:e50.

Birch, P.R., Rehmany, A.P., Pritchard, L., Kamoun, S., and Beynon, J.L., 2006, Trafficking arms: oomycete effectors enter host plant cells, *Trends Microbiol.* **14**:8–11.

Bos, J.I., Kanneganti, T.D., Young, C., Cakir, C., Huitema, E., Win, J., Armstrong, M.R., Birch, P.R., and Kamoun, S., 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 J.* in press.

Brandizzi, F., Hanton, S., DaSilva, L.L., Boevink, P., Evans, D., Oparka, K., Denecke, J., and Hawes, C., 2003, ER quality control can lead to retrograde transport from the ER lumen to the cytosol and the nucleoplasm in plants, *Plant J.* **34**:269–281.

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

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

Di Cola, A., Frigerio, L., Lord, J.M., Ceriotti, A., and Roberts, L.M., 2001, Ricin A chain without its partner B chain is degraded after retrotranslocation from the endoplasmic reticulum to the cytosol in plant cells, *Proc. Natl. Acad. Sci. USA* **98**:14726–14731.

Dodds, P.N., Lawrence, G.J., Catanzariti, A.M., Ayliffe, M.A., and Ellis, J.G., 2004, The Melampsora lini AvrL567 avirulence genes are expressed in haustoria and their products are recognized inside plant cells, *Plant Cell* **16**:755–768.

Espinosa, A., and Alfano, J.R., 2004, Disabling surveillance: bacterial type III secretion system effectors that suppress innate immunity, *Cell Microbiol.* **6**:1027–1040.

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

Huang, G., Allen, R., Davis, E.L., Baum, T.J., and Hussey, R.S., 2006a, Engineering broad root-knot resistance in transgenic plants by RNAi silencing of a conserved and essential root-knot nematode parasitism gene, *Proc. Natl. Acad. Sci. USA*, in press.

Huang, G., Dong, R., Allen, R., Davis, E.L., Baum, T.J., and Hussey, R.S., 2006b, A root-knot nematode secretory peptide functions as a ligand for a plant transcription factor, *Mol. Plant Microbe Interact.* **19**:463–470.

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, *Annu. Rev. Phytopathol.* **44**:41–60.

Kanneganti, T.D., Huitema, E., Cakir, C., and Kamoun, S., 2006, Synergistic interactions of the plant cell death pathways induced by *Phytophthora infestans* Nepl-like protein PiNPP1.1 and INF1 elicitin, *Mol. Plant Microbe Interact.* **19**:854–863.

Manning, V.A., and Ciuffetti, L.M., 2005, Localization of Ptr ToxA Produced by *Pyrenophora tritici-repentis* Reveals Protein Import into Wheat Mesophyll Cells, *Plant Cell* **17**: 3203–3212.

Marti, M., Good, R.T., Rug, M., Knuepfer, E., and Cowman, A.F., 2004, Targeting malaria virulence and remodeling proteins to the host erythrocyte, *Science* **306**:1930–1933.

O′Connell, R.J., and Panstruga, R., 2006, Tete a tete inside a plant cell: establishing compatibility between plants and biotrophic fungi and oomycetes, *New Phytol.* **171**:699–718.

Rehmany, A.P., Gordon, A., Rose, L.E., Allen, R.L., Armstrong, M.R., Whisson, S.C., Kamoun, S., Tyler, B.M., Birch, P.R., and Beynon, J.L., 2005, Differential recognition of highly divergent downy mildew avirulence gene alleles by *RPP1* resistance genes from two Arabidopsis lines, *Plant Cell* **17**:1839–1850.

Ridout, C.J., Skamnioti, P., Porritt, O., Sacristan, S., Jones, J.D., and Brown, J.K., 2006, Multiple avirulence paralogues in cereal powdery mildew fungi may contribute to parasite fitness and defeat of plant resistance, *Plant Cell* **18**:2402–2414.

Rose, J.K., Ham, K.S., Darvill, A.G., and Albersheim, P., 2002, Molecular cloning and characterization of glucanase inhibitor proteins: coevolution of a counterdefense mechanism by plant pathogens, *Plant Cell* **14**:1329–1345.

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

Tian, M., Benedetti, B., and Kamoun, S., 2005, A Second Kazal-like protease inhibitor from *Phytophthora infestans* inhibits and interacts with the apoplastic pathogenesis-related protease P69B of tomato, *Plant Physiol.* **138**:1785–1793.

Tian, M., Huitema, E., da Cunha, L., Torto-Alalibo, T., and Kamoun, S., 2004, A Kazal-like extracellular serine protease inhibitor from *Phytophthora infestans* targets the tomato pathogenesis-related protease P69B, *J. Biol. Chem.* **279**:26370–26377.

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

Wickner, W., and Schekman, R., 2005, Protein translocation across biological membranes, *Science* **310**:1452–1456.

Chapter-12

Query No.	Page No.	Line No.	Query
AQ1	161	45	Please provide e-mail address.
AQ2	164	Figure 2	'2um' has been changed to '2μm'. Is this OK?
AQ3	170	09	Please update
AQ4	170	27	Please update.