MINIREVIEW

Avirulence proteins from haustoria-forming pathogens

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Abstract

A major insight that has emerged in the study of haustoria-forming plant pathogens over the last few years is that these eukaryotic biotrophs deliver suites of secreted proteins into host cells during infection. This insight has largely derived from successful efforts to identify avirulence (*Avr*) genes and their products from these pathogens. These *Avr* genes, identified from a rust and a powdery mildew fungus and three oomycete species, encode small proteins that are recognized by resistance proteins in the host plant cytoplasm, suggesting that they are transported inside plant cells during infection. These Avr proteins probably represent examples of fungal and oomycete effector proteins with important roles in subverting host cell biology during infection. In this respect, they represent a new opportunity to understand the basis of disease caused by these biotrophic pathogens. Elucidating how these pathogen proteins gain entry into plant cells and their biological function will be key questions for future research.

Introduction

Eukaryotic pathogens have evolved numerous strategies to gain nutrients from plants, but arguably one of the most specialized is that of the obligate biotrophic fungi and oomycetes, which feed only from living plant cells. These pathogens are completely dependent on their host plant for their growth and propagation and consist of a diverse range of species from taxonomically distinct groups: the oomycete downy mildews, and the fungal powdery mildews (Ascomycetes) and rusts (Basidiomycetes). A distinguishing feature of these obligate biotrophs is the formation of a specialized structure called the haustorium. During infection, the pathogen penetrates the cell wall and invaginates the plasma membrane of a host cell where it develops an haustorium. The haustorium therefore remains separated from the host cell cytoplasm, surrounded by the haustorial wall, a region known as the extrahaustorial matrix and the extrahaustorial membrane, derived from the invaginated host plasma membrane (Fig. 1). Haustoria appear to play an essential role in nutrient acquisition (Hahn & Mendgen, 2001) and there is evidence to suggest they are involved in the redirection of host metabolism and the suppression of host defenses (Voegele & Mendgen, 2003). It is also thought that the haustorium is a hub of cellular communication allowing important information exchange between the host and the pathogen for the establishment of a successful biotrophic relationship (Heath, 1997; Voegele & Mendgen, 2003), but as yet the nature of these communication processes remains obscure. Some hemibiotrophic fungi and oomycetes, such as *Phytophthora*, also form haustoria, but in contrast to obligate biotrophs these pathogens initiate host cell death during a later necrotrophic stage of infection.

Collectively, haustoria-producing pathogens include some of the most destructive plant parasites, causing huge economic losses in important agricultural industries and also environmental destruction in natural ecosystems. Nonetheless, little is known about the molecular basis of pathogenicity in this important class of plant pathogens, mainly due to the fact that most of these organisms are difficult to culture and thus are not easily amenable to molecular genetic approaches. Recently, a number of avirulence proteins have been identified from haustoria-forming fungi and oomycetes (Table 1) that are targets of recognition

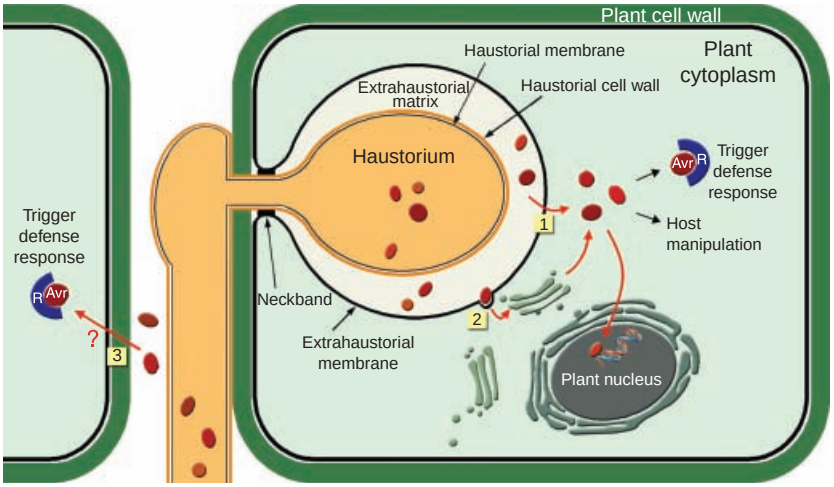

Fig. 1. The host–haustorium interface. Schematic diagram of a rust haustorium within a host cell showing the extrahaustorial membrane and the extrahaustorial matrix, which is thought to be a discrete compartment due to the presence of the neckband, note that this feature is absent from oomycete haustoria. Effector proteins are secreted from the haustoria into the extrahaustorial matrix. A subset of proteins are further transported into the host cell, either directly crossing the extrahaustorial membrane (1) or through vesicles into the host endomembrane system (2). Once inside the host cytoplasm, effectors may alter host metabolism and defence pathways. Those effectors that are recognized by resistance gene products (R) are known as avirulence proteins (Avr) and trigger a defense response. Other effectors may be further targeted to host organelles such as the nucleus to alter host transcription. Effectors secreted from the hyphae into the apoplast may also enter plant cells via an unknown mechanism (3), and when recognized by a resistance protein, trigger a defense response.

Table 1. Avirulence proteins identified in haustorial pathogens

Avr	Size (aa)	Predicted signal peptide	Cysteine-rich	Pathogen	Host plant	Corresponding R gene class*	Reference
Avr1b	138	Yes	No	*P. sojae*	Soybean	Unknown	Shan *et al.* (2004)
Avr3a	147	Yes	No	*P. infestans*	Potato	CC-NBS-LRR	Armstrong *et al.* (2005)
ATR13	187	Yes	No	*H. parasitica*	*Arabidopsis*	CC-NBS-LRR	Allen *et al.* (2004)
ATR1^{NdWsB}	311–324	Yes	No	*H. parasitica*	*Arabidopsis*	TIR-NBS-LRR	Rehmany *et al.* (2005)
AvrL567	150	Yes	No	*M. lini*	Flax	TIR-NBS-LRR	Dodds *et al.* (2004)
AvrM	314–377	Yes	No	*M. lini*	Flax	TIR-NBS-LRR	Catanzariti *et al.* (2006)
AvrP4	95	Yes	Yes	*M. lini*	Flax	TIR-NBS-LRR	Catanzariti *et al.* (2006)
AvrP123	117	Yes	Yes	*M. lini*	Flax	TIR-NBS-LRR	Catanzariti *et al.* (2006)
Avr-a10	286	No	No	*B. graminis*	Barley	CC-NBS-LRR	Ridout *et al.* (2006)
Avr-k1	177	No	No	*B. graminis*	Barley	Unknown	Ridout *et al.* (2006)

*Domain structures of the corresponding resistance gene products are indicated as follows: CC, coiled-coil; NBS, nucleotide binding site; LRR, leucine-rich repeat; TIR, Toll and interleukin 1 receptor homology.

by resistance proteins, components of the plant innate immunity system. All these avirulence proteins are active inside host cells, leading to the conclusion that these pathogens can direct transport of some proteins into the host cytoplasm during infection. In this review, the nature of these avirulence proteins, the evidence for their translocation and possible transport mechanisms are discussed.

Avirulence proteins from haustoria-forming pathogens

Like many other microorganisms that parasitize plants, infection by biotrophic fungi and oomycetes can be thwarted by a host-resistance (*R*) gene-dependent defense response. *R* genes encode receptor-like proteins that recognize specific pathogen proteins, known as avirulence (Avr) proteins. This recognition triggers a defense response characterized by the rapidly induced necrosis of host cells at the site of infection, referred to as a hypersensitive response (HR), which restricts the colonization of the pathogen. Many Avr proteins have been identified from a number of plant pathogens including viruses, bacteria and fungi. It is generally thought that these proteins are pathogenicity effectors with positive roles in establishing infection, which the plant has evolved to recognize as an indicator of invasion. For instance, pathogenic bacteria use the

well-characterized type III secretory system to direct effector proteins into plant cells, where they act to influence host transcription or target specific host proteins for degradation, and many of these effectors are also recognized as Avr products by host R proteins (Lahaye & Bonas, 2001; Espinoza & Alfano, 2004). However, it was only recently that the first *Avr* genes from haustoria-forming pathogens were cloned and characterized. Among the first of these was *AvrL567*, from the flax rust fungus *Melampsora lini* (Dodds *et al*., 2004). This gene was identified through the use of cDNA subtraction to generate a library enriched for rust genes expressed during infection, and segregation analysis of candidate genes in a rust family containing multiple *Avr* loci. *AvrL567* encodes a small secreted protein that is expressed in haustoria. Indeed, during rust *R* gene-dependent resistance, the HR is observed in plant cells containing emerging haustoria (Coffey & Allen, 1983; Kobayashi *et al*., 1994; Heath, 1997). These observations led to a more focused screen for flax rust *Avr* gene candidates based on the purification of intact haustoria from infected plant cells and identification of genes encoding secreted proteins. This approach resulted in the isolation of the *AvrM*, *AvrP123* and *AvrP4* genes, which also encode small secreted proteins expressed in flax rust haustoria (Catanzariti *et al*., 2006).

Currently, there are four avirulence genes that have been isolated from oomycete pathogens; *Avr1b-1* from *Phytophthora sojae* (Shan *et al*., 2004), *Avr3a* from *Phytophthora infestans* (Armstrong *et al*., 2005), and *ATR13* and $ATR1^{NdWsB}$ from *Hyaloperonospora parasitica*, the downy mildew of *Arabidopsis* (Allen *et al*., 2004; Rehmany *et al*., 2005). *Avrlb-1* and $ATR1^{NdWsB}$ were isolated by a map-based cloning approach, while enrichment for genes specifically expressed in the host through cDNA subtraction and cosegregation with *Avr* loci led to the cloning of *ATR13*. The *Avr3a* gene from *P. infestans* was isolated as an EST with similarity to *Avr1b-1* after searching for genes encoding secreted proteins, a similar approach to that used in the cloning of *AvrM*, *AvrP123* and *AvrP4* from flax rust. Like the rust *Avr* genes, and indeed like most of the *Avr* genes that have been identified from nonhaustoria-forming fungal plant pathogens (Rep, 2005), the oomycete *Avr* genes also encode small proteins with N-terminal signal peptides to direct them to the endoplasmic reticulum (ER) secretory pathway.

For most of these genes, confirmation of an avirulence function was obtained using biolistic or *Agrobacterium*-mediated expression in host plants and observation of an *R* gene-dependent necrotic response. This response occurred when the Avr proteins were expressed as cytoplasmic proteins (i.e. without the signal peptide), implying that recognition by the cognate R protein takes place inside the plant cell. In support of this finding, the R proteins that recognize these pathogen products all belong to the cytoplasmic class of proteins containing the nucleotide binding site and leucine-rich repeat (NBS; LRR) domains. Furthermore, the flax rust AvrL567 proteins directly interact with the corresponding L5 and L6 resistance proteins of flax (Dodds *et al*., 2006). However, intracellular recognition means that during infection these rust and oomycete Avr proteins must somehow enter host cells.

Two *Avr* genes, *Avr-a10* and *Avr-k1*, have now been isolated by map-based cloning from the ascomycete pathogen *Blumeria graminis* pv. *hordei* (*Bgh*), which causes powdery mildew infections on barley (Ridout *et al*., 2006). In contrast to the rust and oomycete proteins, these Avr proteins are not predicted to contain a secretion signal peptide, but nevertheless are recognized by intracellular barley resistance proteins when transiently expressed *in planta*. Ridout *et al*. (2006) suggest that they may be secreted from the fungus by a nonendomembrane pathway. Although the expression pattern of these genes has not been determined, *Bgh* develops an aerial mycelial network on the outside of the leaf with only the haustoria in direct contact with host cells suggesting that, as for flax rust, these structures are the site of Avr protein expression and translocation into host cells. One important question now is how do these fungal- and oomycete-translocated proteins gain access to the plant cytoplasm across the host plasma membrane?

Translocation of avirulence proteins into host cells

The expression of the rust Avr proteins in haustoria and the concomitance of HR induction with haustoria formation suggest that haustoria play a critical role in delivering effector proteins into the infected host cell. This hypothesis is supported by localization studies of secreted proteins from the bean rust pathogen, *Uromyces fabae*. Kemen *et al*. (2005) showed that a small secreted protein that is specifically expressed in bean rust haustoria, *Uf*-RTP1, enters host cells during infection. Immunolabelling of tissue sections from infected bean leaves with antibodies raised to *Uf*-RTP1 detected this protein within the extrahaustorial matrix, and as infection progressed, it accumulated within the cytoplasm of the infected host cell and in the host nucleus. This is consistent with the presence of a predicted nuclear localization signal, and suggests that this rust protein is targeted to the host nucleus and may have a role in influencing host gene expression during infection. Interestingly, several other bean rust proteins secreted from the haustoria were only detected within the extrahaustorial matrix, suggesting that only a subset of secreted proteins are translocated into host cells (Kemen *et al*., 2005), and implying that the protein transport mechanism acts selectively.

The modified environment of the haustorium–plant cell interface may facilitate the entry of secreted pathogen proteins into host cells. The extrahaustorial membrane is

thought to be newly synthesized during the growth of the haustorium and, although contiguous with the plant plasma membrane, differs in a number of physical and biochemical characteristics, which can vary between different rust and host species (Harder & Chong, 1991; Perfect & Green, 2001). This membrane provides a distinct separation between the pathogen and host, which may be important for the establishment of a biotrophic association (Perfect & Green, 2001; Voegele & Mendgen, 2003). The extracellular matrix appears to exist as a discrete compartment. Rust fungi have a distinct thickened region midway along the neck of the haustoria (the narrowed region at the site of host cell penetration; Fig. 1), known as the neckband, which is thought to seal the extrahaustorial matrix from the plant apoplast (Harder & Chong, 1991). The powdery mildews are also known to form neckbands yet they appear to be absent from haustoria-forming oomycetes (Perfect & Green, 2001), with the exception of *Albugo candida*, which develops a neckband-like structure that differs from those formed by fungi (Coffey, 1983; Soylu, 2004). However, in several oomycete species a distinct electron-dense region has been observed between the haustorium wall and the plant callose deposition, often referred to as the collar or papilla, which occurs at the site of cell penetration. It has been suggested that this substance may create a seal analogous to the neckbands present in fungi (Mims *et al.*, 2004). The formation of a discrete, compartmentalized region would perhaps also allow control of the exchange of signals and nutrients.

An intriguing observation in several rust species is the presence of long tubular extensions of the extrahaustorial membrane that reach into the host cell cytoplasm (Harder & Chong, 1991; Mendgen *et al.*, 1991; Mims *et al.*, 2002). These extensions have been observed in close contact with host ER and dictyosomes, suggesting the exchange of materials between the extrahaustorial membrane and the inner membrane systems of the plant cell. Furthermore, during rust infection of daylily leaves by *Puccinia hemerocallidis*, Mims *et al.* (2002) observed electron-dense deposits in the extrahaustorial matrix that extend into these tubular elements and the appearance of budding from the end of these elements resembling vesicle bodies. They also observed that these tubular extensions attached to flattened cisternae in the host. This continuity could allow access to the host endomembrane system and may provide a means for protein trafficking into the host cells. Ultrastructure studies of downy mildew-infected *Arabidopsis* also showed vesicular activity at the host–haustorium interface; however, only very small extensions into the host cell from the extrahaustorial matrix were noted (Mims *et al.*, 2004). The *Arabidopsis* RPP1A protein, which confers downy mildew resistance, is associated with the ER and/or Golgi membranes through an N-terminal hydrophobic peptide (Weaver *et al.*, 2006), hence it is possible that this resistance protein can intercept a cognate Avr protein using the endomembrane system to gain entry into the host cell.

Although the haustorium–host interface offers a modified environment that may be conducive to protein transfer, rust effector proteins may also have intrinsic plant cross-membrane transport mechanisms. *In planta* expression of full-length rust *Avr* genes, with the inclusion of the signal peptide leader sequence, also results in an *R* gene-dependent HR (Dodds *et al.*, 2004; Catanzariti *et al.*, 2006). This observation could be the result of small amounts of the Avr proteins accumulating in the cytoplasm due to aberrant or inefficient processing of the signal peptide. However, addition of an ER retrieval signal to AvrM inhibited the necrotic response (Catanzariti *et al.*, 2006), suggesting that this protein successfully enters the secretory pathway *in planta* and can subsequently enter the host cytoplasm in the absence of the pathogen, either from the apoplast or from one of the later compartments of the endomembrane secretory system such as the Golgi or transport vesicles. This may reflect a transport system involving the observed continuum between the extrahaustorial membrane extensions and the endomembrane system (Mims *et al.*, 2002). In addition, the AvrP4 protein induces a much stronger recognition reaction when expressed as a secreted protein in flax, probably due to the enhanced folding efficiency of this cysteine-rich protein in the ER (Catanzariti *et al.*, 2006). Again this implies entry of the correctly folded protein into the cytoplasm after it has been cotranslationally inserted into the endomembrane system during transient expression. Another secreted fungal protein, the PtrToxA toxin from the wheat tan spot pathogen *Pyrenophora tritici-repentis*, was found to enter plant cells from the apoplast in the absence of the pathogen but only in toxin-sensitive wheat genotypes (Manning & Ciuffetti, 2005). Uptake of this protein is thought to be due to endocytosis mediated by an unidentified receptor encoded by the dominant toxin sensitivity gene, *Tsn1*, which may recognize an RGD motif on the surface of the ToxA protein.

Oomycete avirulence proteins contain a host-targeting signal

A notable feature among all Avr proteins from oomycete pathogens is a conserved, novel sequence motif located just downstream of the signal peptide, and recent evidence suggests that in oomycete pathogens this motif acts as a specific host-targeting signal (Birch *et al.*, 2005; Rehmany *et al.*, 2005). This signal is absent from the flax rust effectors, indicating that oomycetes and rust fungi utilize different transfer mechanisms. The motif consists of the sequence RxLR followed by an E/D-rich domain and is strikingly similar to a host-targeting signal found in proteins secreted by the malaria parasite (*Plasmodium falciparum*).

Plasmodium falciparum develops inside a parasitophorous vacuole within an erythrocyte and, like haustorial cells, remains separated from the host cell cytoplasm by a membrane derived from the invagination of the host membrane during invasion of the erythrocyte. During infection, proteins secreted by *P. falciparum* enter the parasitophorous vacuole, and those containing the N-terminal sequence motif RxLxE/Q are further transported across the parasitiphorous vacuolar membrane (Hiller *et al.*, 2004; Marti *et al.*, 2004). The role of this signal sequence was confirmed by the visualization of *P. falciparum*-expressed fluorescently tagged proteins within the cytoplasm of infected erythrocytes in a motif-dependent manner. This assay was recently used by Bhattacharjee *et al.* (2006) to show that the RxLR oomycete motif also functions as a host-targeting signal in *P. falciparum*, providing strong evidence that these eukaryotic microorganisms share a conserved translocation mechanism for effector proteins.

As was found for *Plasmodium* species, bioinformatics analysis on several *Phytophthora* species found the RxLR motif in several hundred secreted proteins, suggesting these pathogens deliver a large and complex set of proteins into host cells, which are likely to have important roles in pathogenesis-related processes (Bhattacharjee *et al.*, 2006). Intriguingly, the motif appears to be absent from secreted oomycete proteins that are cysteine-rich, implying oomycetes secrete two classes of effectors with different target sites; those that are translocated into host cells via the RxLR-dependent mechanism including the Avr proteins, and those that function in the apoplast, which are often cysteine-rich such as the protease inhibitors identified from *P. infestans* (Kamoun, 2006). An important question that remains is how do the *P. falciparum* and oomycete host-targeting signals mediate host cell entry? It was shown that additional amino acids flanking the core conserved residues of the motifs are also required for host cell uptake and was therefore suggested that the leader sequence may allow adequate spacing between the host-targeting signal and the effector protein, and perhaps forms a small (25–30 aa) globular domain (Bhattacharjee *et al.*, 2006).

Role of host-translocated effectors in fungal and oomycete infection

The translocation of specific pathogen proteins into host cells implies a distinct purpose in promoting disease through manipulation of the host cell biology. Analagously, most Avr proteins identified from bacteria are disease effectors that are directly secreted into plant cells via the type III secretion system (Lahaye & Bonas, 2001). Some of these may act as transcription factors while others target host proteins for degradation or phosphorylation, and in some cases it is this modification of a host protein target that is recognized by the corresponding resistance protein, a situation described in the 'guard hypothesis' (Dangl & Jones 2001). However, as yet there are few clues to the precise roles of the putative effector proteins from fungi and oomycetes, and understanding their biological function remains a crucial question. There is now some emerging evidence for positive roles of these proteins in infection. For instance, Avr3a can inhibit the necrotic response induced by the *P. infestans* INF1 elicitor in *Nicotiana benthamiana* (Bos *et al.*, 2006), suggesting a role in suppression of host-resistance responses. This suppression was specific for INF1, as no effect on HR induction by the PiNPP1 and CRN2 elicitors of *P. infestans* was observed. Transient expression of Avr-a10 and Avr-k1 in barley epidermal cells enhances the successful penetration of *Bgh* haustoria, again suggesting an effector function of these proteins in promoting pathogen infection. The nuclear localization of *Uf*-RTP1 suggests that it may function to influence host gene expression (Kemen *et al.*, 2005). A functional nuclear localization signal has also been found in a *P. infestans*-predicted secreted protein containing the RxLR motif (Birch *et al.*, 2005) and in several bacterial Avr proteins with putative roles in host transcriptional regulation (Lahaye & Bonas, 2001; Deslandes *et al.*, 2003). Pathogen effectors that localize to the host nucleus may be transcription factors that induce changes in host gene expression, possibly a common tactic among diverse plant pathogens.

All the rust and mildew Avr proteins described to date have 'novel' sequences with no significant sequence similarity to proteins of known function, so it is difficult to predict their biochemical functions. Only two of these proteins show some distinguishing features. AvrP123 is a cysteine-rich (10 Cys) protein containing a Kazal serine protease inhibitor signature sequence, and AvrP4 has six cysteines that are spaced according to the consensus of an inhibitor cysteine knot structure commonly found in toxins and inhibitors of receptors or proteases (Pallaghy *et al.*, 1994). Two Kazal-like protease inhibitors have also been identified in *P. infestans*, and act on an apoplastic protease in tomato (Tian *et al.*, 2004, 2005), while a cysteine-knotted structure is found in the extracellular avirulence protein Avr9 from the fungal pathogen *Cladosporium fulvum* (Vervoort *et al.*, 1997; van den Hooven *et al.*, 2001), although its biological activity is not known. The cysteine-rich nature of these two flax rust Avr proteins stands in contrast to the other host-translocated Avr proteins, as this feature is more characteristic of the apoplastic effector proteins of fungal pathogens that colonize the intercellular space of the plant tissue (Rep, 2005). These strictly extracellular pathogens, such as *C. fulvum*, secrete a range of cysteine-rich effector proteins that function in the apoplast of their host. For instance, the *C. fulvum* Avr2 protein inhibits an apoplastic cysteine protease (Rooney *et al.*, 2005) while Avr4 protects fungal

cell walls against plant chitinases (van den Burg *et al.*, 2006). Disulfide bonds are thought to be critical for the structure and stability of these proteins in this protease-rich environment (van den Ackerveken *et al.*, 1993; Joosten *et al.*, 1997; van den Burg *et al.*, 2003). Consistent with their apoplastic location, the *C. fulvum* Avr proteins are recognized by transmembrane R proteins with extracellular LRR domains. On the other hand, the Avr-Pita protein of *Magnaporthe grisea* is a cysteine-rich metalloprotease that is recognized inside plant cells by the Pita NBS-LRR resistance protein (Jia *et al.*, 2000). Although they do not form haustoria, hyphae of *M. grisea* do penetrate host plant cells and this fungus may also directly translocate proteins into the plant cytoplasm. Thus the presence of disulfide bonds in AvrP4, AvrP123 and AvrPita may be important for their structure and function rather than providing resistance to apoplastic proteases. An alternative possibility is that AvrP123 and AvrP4 do function in the apoplast, and their host–cell uptake may represent an aspect of the host-resistance response. These proteins may be recognized by an extracellular receptor that mediates their internalization and subsequent recognition by the corresponding NBS-LRR resistance proteins. In a possibly analogous fashion, the *Cf-4* resistance gene in tomato encodes a transmembrane protein with an extracellular LRR domain, which recognizes the *C. fulvum* Avr4 protein: however, a second intracellular NBS-LRR protein has been identified, which is necessary for Avr4-induced HR (Gabriëls *et al.*, 2006). Endocytosis of an extracellular receptor has also been implicated in basal disease resistance against bacteria. The FLS2 plasma membrane-bound LRR kinase receptor of *Arabidopsis* binds to the flg22-epitope of bacterial flagellin (Chinchilla *et al.*, 2006) to trigger innate immune responses and mediate FLS2 internalization (Robatzek *et al.*, 2006). Similarly, ToxA internalization is also thought to be mediated through the binding and endocytosis of a wheat receptor protein (Manning & Ciuffetti, 2005).

The lack of cysteine residues in most of the Avr proteins identified from haustoria-forming pathogens may suggest that these proteins are not exposed to the protease-rich environment of the apoplast. The effectors, after secretion from the haustoria, would enter the extrahaustorial matrix and this may be a less hostile environment for proteins. So far there has been no localization and only limited expression data for these effectors, and although many are only expressed *in planta*, this does not rule out their expression and secretion from infection hyphae, or other intercellular structures, into the apoplast. Furthermore, *Avr3a* and *AvrM* are also expressed during germination of *Phytophthora* cysts and flax rust spores, respectively. The expression of *Avr3a* declines after appressoria formation and peaks again 24 h after infection, coinciding with the start of the biotrophic stage and haustoria formation, suggesting that this effector is not intended for the apoplast. Interestingly, expression of this gene is greatest 72 h after infection, the onset of the necrotrophic stage for this pathogen (Armstrong *et al.*, 2005).

Perspectives

The recent cloning of the *Avr* genes described in this review provides a significant advance in the study of haustoria-forming plant pathogens. The effectors they encode, all of which are recognized intracellularly by cognate R proteins, represent an exciting opportunity to investigate the molecular interactions involved in biotrophic plant disease and address new questions such as how do these proteins enter host cells and what is their function? Unlike bacteria, no specialized translocation apparatus has yet been discovered, although the haustorial cell and unique interface it shares with the host may provide a modified environment to facilitate the translocation of effectors across the host membrane. However, more information on the nature of the haustoria/host interface, on the expression and localization of these proteins and ultimately on the transport mechanism is required. Research into how small proteins enter plant cells may uncover novel and very different mechanisms, as is evident with the oomycete host-targeting signal motif, which is absent from the fungal effectors. Furthermore, this translocation appears to be selective, acting only on defined sets of pathogen proteins, suggesting host-targeted effectors have positive roles in establishing infection. Future research to reveal the biochemical function and host targets of these avirulence proteins may provide significant clues as to how these pathogens manipulate their host to achieve biotrophic growth.

As for bacterial pathogens, where complete sets of delivered type III effectors have been identified, uncovering the repertoire of translocated effectors in haustoria-forming pathogens and classifying their functions would greatly assist the understanding of plant pathogen interactions and their associated disease. Advances in sequencing technologies and bioinformatics are facilitating this pursuit. Leading the way is the current genome sequencing and annotation of several oomycete pathogens including two completed *Phytophthora* species (Tyler *et al.*, 2006), which has allowed sets of proteins to be identified based on the host-targeting signal. The sequencing of the *Ustilago maydis* genome has also provided significant insights into the infection strategies of this biotrophic fungus (Kämper *et al.*, 2006). During infection, *U. maydis* hyphae penetrate host cells and, like haustoria, are surrounded by an invaginated host cell plasma membrane. The genome sequence has revealed several clusters of secreted proteins that are essential for proliferation of the fungus inside the host. It will be intriguing to see if these proteins function inside plant cells and ultimately to

determine how they function to promote disease. Genome sequencing of two rust species, *Puccinia graminis* f. sp. *tritici* and *Melampsora medusae*, is currently underway and should reveal further information on the repertoire of secreted proteins in these pathogens. Comparative genomics studies with these and other biotrophs may elucidate common strategies used by fungal and oomycete pathogens to cause disease in plants.

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