Journal of
Experimental
Botany
www.jxb.oxfordjournals.org

REVIEW ARTICLE

Focal accumulation of defences at sites of fungal pathogen attack

William Underwood[1] and Shauna C. Somerville[1,2,*]

[1] *Energy Biosciences Institute, 130 Calvin Hall, Mail Code 5230, University of California, Berkeley, CA 94720, USA*

[2] *Department of Plant and Microbial Biology, University of California, Berkeley, CA 94720, USA*

Abstract

Plants resist attack by haustorium-forming biotrophic and hemi-biotrophic fungi through fortification of the cell wall to prevent penetration through the wall and the subsequent establishment of haustorial feeding structures by the fungus. While the existence of cell wall-based defences has been known for many years, only recently have the molecular components contributing to such defences been identified. Forward genetic screens identified *Arabidopsis* mutants impaired in penetration resistance to powdery mildew fungi that were normally halted at the cell wall. Several loci contributing to penetration resistance have been identified and a common feature is the striking focal accumulation of proteins associated with penetration resistance at sites of interaction with fungal appressoria and penetration pegs. The focal accumulation of defence-related proteins and the deposition of cell wall reinforcements at sites of attempted fungal penetration represent an example of cell polarization and raise many questions of relevance, not only to plant pathology but also to general cell biology.

Key words: Actin, disease resistance, immunity, MLO, papilla, PEN1, PEN2, PEN3, powdery mildew.

Introduction

Plants exhibit what is referred to as non-host resistance to the majority of potential pathogens (Heath, 2000). Non-host resistance is defined as resistance in which an entire plant species is resistant to all genotypes of a given pathogen species and recent evidence suggests that this temporally durable resistance is conditioned by at least two layers of defence response (Thordal-Christensen, 2003; Lipka *et al.*, 2005). The layers that account for non-host resistance can be broadly categorized as pre- and post-penetration defences, based on their engagement and efficacy at different stages of the infection sequence. After landing on a leaf of a prospective host plant, fungal spores must germinate and, for those fungi that penetrate directly into the epidermis, subsequently penetrate the cuticle and cell wall of the underlying epidermal cell to gain access to plant resources such as nutrients and water. Biotrophic fungi that do not directly penetrate the plant epidermis typically germinate on plant surfaces, enter via stomates or other natural openings, and subsequently penetrate mesophyll cell walls to gain access to nutrients. The waxy cuticle and thick cell wall surrounding plant epidermal cells serve as preformed and passive barriers to invasion by pathogens such as fungi. However, many fungi have evolved specialized infection structures known as appressoria to facilitate the breach of the cuticle and cell wall through mechanical and/or enzymatic means (Howard, 1997; Pryce-Jones *et al.*, 1999). It is at this critical juncture, attempted penetration of the cell wall, that the first line of inducible plant defences is called into action. Non-adapted fungi, those that cannot cause disease on any member of a selected plant species, generally exhibit some ability occasionally to overcome this first layer of defence, albeit at a very low frequency compared to adapted pathogens. The conidiospores that overcome penetration resistance and successfully develop haustoria, feeding structures in close contact with a plant-derived extrahaustorial membrane, are subjected to a second layer of defence characterized by the hypersensitive response (HR)-like programmed cell death (PCD) of invaded host cells (Thordal-Christensen, 2003; Lipka *et al.*, 2005). This article will focus primarily on penetration resistance against biotrophic powdery mildew fungi and, particularly,

* To whom correspondence should be addressed. E-mail: ssomerville@berkeley.edu

on newly discovered molecular components of this first layer of defence against fungal pathogens.

Molecular components of penetration resistance

It has been known for many years that plants assemble cell wall appositions, known as papillae, in the paramural space subtending the cell wall at sites of attempted fungal penetration. Papillae have been found to contain callose, phenolic compounds, lignin, reactive oxygen species, and proteins and are thought to act as a physical barrier to halt penetration by the fungal penetration pegs (Aist, 1976; McLusky *et al.*, 1999). Until recently, studies of penetration resistance associated with papilla formation have been primarily descriptive in nature. Recently, however, forward genetic screens have identified a number of molecular components that contribute to the ability of *Arabidopsis* to resist penetration by the non-adapted powdery mildew fungus *Blumeria graminis* f. sp. *hordei* (*Bgh*), a pathogen of barley (Collins *et al.*, 2003; Lipka *et al.*, 2005; Stein *et al.*, 2006). Ethyl methane sulphonate (EMS)-mutagenized *Arabidopsis* populations were screened for mutants that allowed increased frequencies of penetration by *Bgh*. Successful penetration by *Bgh* resulted in whole-cell autofluorescence and encasement of the nascent haustorium in callose, which could be viewed microscopically after staining inoculated tissue with the fluorescent dye aniline blue (Stein *et al.*, 2006) or by visualizing whole cell autofluorescence (Collins *et al.*, 2003). From these mutant screens, several *penetration* (*pen*) mutants allowing higher frequencies of *Bgh* entry into epidermal cells were recovered. Three of these mutants, designated *pen1*, *pen2*, and *pen3*, have been characterized and the corresponding genes identified.

PEN1 defines a defence pathway associated with vesicle-mediated secretion

Map-based cloning revealed that *PEN1* encodes one of 24 *Arabidopsis* syntaxins, designated SYP121 (Sanderfoot *et al.*, 2000; Collins *et al.*, 2003). Syntaxins, also known as tSNARES (target SNAP receptor), participate in vesicle fusion events through the formation of ternary SNARE complexes with a corresponding VAMP (vesicle associated membrane protein) and a SNAP25 homologue. *ROR2* (*REQUIRED FOR mlo RESISTANCE 2*), a barley (*Hordeum vulgare*) homologue of *PEN1*, was found to contribute to broad spectrum powdery mildew resistance conferred by loss of function at the *MLO* locus and to basal penetration resistance to *Bgh* in susceptible barley encoding a functional MLO (Freialdenhoven *et al.*, 1996; Collins *et al.*, 2003). Functional fusions of PEN1 and ROR2 to GFP and YFP, respectively, were found to localize at the plasma membrane and to accumulate at sites of attempted penetration by appressoria of both

adapted and non-adapted powdery mildew species (Collins *et al.*, 2003; Bhat *et al.*, 2005; Fig. 1A).

The predicted function of PEN1 and ROR2 and their presence in the plasma membrane, particularly at sites of attempted fungal penetration, strongly suggest a role in targeted trafficking of secretory vesicles to the site of papilla formation. Consistent with a role in papilla deposition, the frequency of papilla formation in *pen1-1* mutants in response to *Bgh* was reduced at early time-points (10, 12, and 14 h), corresponding to a ~2 h delay in papilla deposition (Assaad *et al.*, 2004). A strong correlation between timing of papilla deposition and penetration success is evident, as resistance of *mlo* mutants is correlated with the rapid deposition of papillae, whereas enhanced susceptibility to penetration in *pen1-1* is correlated with delayed papilla deposition (Skou *et al.*, 1984; Assaad *et al.*, 2004). Thus, Assaad and colleagues speculate that the delay in papilla formation in *pen1* mutants is the primary cause of reduced penetration resistance.

Unexpectedly, GFP-PEN1 was found in the apparent interior of the papilla structure in addition to the plasma membrane subtending the papilla (Assaad *et al.*, 2004). Recently, electron micrographs of papillae formed by *mlo5* mutant barley in response to *Bgh* challenge revealed the presence of membrane-like structures and multivesicular bodies associated with or appearing within papilla structures (An *et al.*, 2006). The presence of GFP-PEN1 in such membrane structures may explain the observation of GFP-PEN1 within papillae. In addition, the reduced appearance of large (~1 μm) H_2O_2-containing vesicles was reported at *Bgh* penetration sites on barley *ror2 mlo5* double mutants compared to *mlo5* single mutants (Collins *et al.*, 2003). This finding is in contrast to what would be expected if ROR2 and PEN1 participate only in the fusion of secretory vesicles to the PM. Such a model for ROR2/PEN1 function would predict an increased accumulation of secretory vesicles in the vicinity of the PM rather than a reduction of such vesicles. Collins *et al.* (2003) hypothesize that ROR2 may also
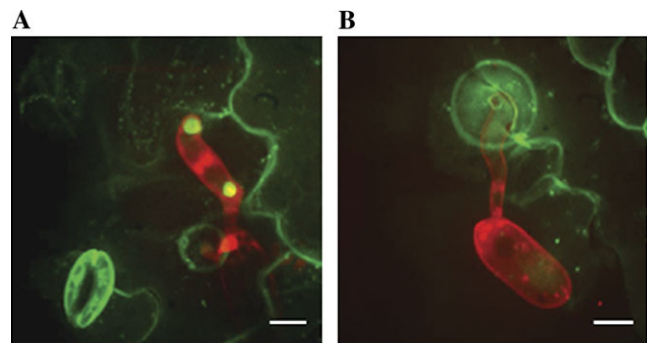
Fig. 1. Z-projected confocal micrographs depicting focal accumulation of GFP-PEN1 (A) and PEN3–GFP (B). Propidium iodide-stained *Blumeria graminis* f. sp. *hordei* appressoria and conidiospores appear red. Scale bars represent 5 μm.

participate in homotypic vesicle fusion to generate the large vesicles frequently observed near fungal penetration sites. Consistent with this hypothesis, GFP-PEN1 was found to localize to endomembrane compartments ~1 µm in diameter in the vicinity of papillae formed at *Bgh* penetration sites (Assaad *et al*., 2004).

Despite the delayed timing, *pen1* mutants were able to deposit papillae that were morphologically indistinguishable from those of wild-type plants, suggesting that at least one other syntaxin can substitute for PEN1 in the process of papilla formation, albeit with reduced efficiency (Assaad *et al*., 2004). The most closely related syntaxin to PEN1 in the *Arabidopsis* genome is SYP122, a syntaxin for which transcript levels are dramatically upregulated in response to pathogen challenge and that was found to be rapidly phosphorylated in response to perception of the bacterial flagellin-derived peptide flg22 (Nühse *et al*., 2003*a*; Assaad *et al*., 2004). Interestingly, the *pen1 syp122* double mutants developed spontaneous necrotic lesions and showed elevated levels of salicylic acid (SA) and *PR1* transcript (Assaad *et al*., 2004). Triple mutants impaired in the synthesis or perception of SA showed a partial rescue, suggesting additional, and as yet undefined, roles for PEN1 and SYP122 in the modulation of both SA-dependent and -independent cell death (Zhang *et al*., 2007). Surprisingly, *pen1 syp122* mutants were no more susceptible to *Bgh* penetration than *pen1* single mutants (Assaad *et al*., 2004), suggesting that SYP122 does not contribute significantly to papilla deposition or, alternatively, that further disruption of papilla deposition is not sufficient to allow more frequent *Bgh* entry.

Most recently, the corresponding *Arabidopsis* VAMP and SNAP25 homologue proteins that participate in SNARE complex formation with PEN1 were identified. SNAP33 and members of the VAMP72 family of vSNARES were found to immunoprecipitate in complexes with PEN1 (Kwon *et al*., 2008*a*). The conditional reduction of VAMP721 and VAMP722 transcript levels using an inducible RNAi construct targeted to the two closely-related VAMPs also resulted in an increased frequency of *Bgh* penetration, confirming a role for these VAMPs in penetration defence (Kwon *et al*., 2008*a*). YFP-SNAP33 was found to accumulate at the sites of attempted *Bgh* penetration, where it co-localized with CFP-PEN1. GFP-VAMP722, on the other hand, showed a distinct localization to vesicle-like structures near the penetration sites (Kwon *et al*., 2008*a*).

PEN2 and PEN3 participate in a pathway distinct from PEN1 to limit powdery mildew penetration

The *pen2* mutation occurs in a member of the *Arabidopsis* family 1 glycosyl hydrolyse group of enzymes (Lipka *et al*., 2005). These enzymes are thought to participate in the hydrolysis of *O*- or *S*-glycosidic bonds of complex carbohydrates or glycosylated metabolites. Mutation of a predicted catalytic residue prevented complementation by the mutant protein, suggesting that enzymatic activity is essential for PEN2 function in limiting powdery mildew penetration (Lipka *et al*., 2005). A functional PEN2–GFP fusion protein was found to localize to peroxisomes, an organelle that shown to preferentially accumulate at the sites of attempted powdery mildew penetration (Koh *et al*., 2005; Lipka *et al*., 2005). The probable enzymatic activity of PEN2 and its localization to peroxisomes suggest that PEN2 may participate in the enzymatic processing of a compound(s) that may contribute to penetration resistance, potentially through antifungal activity (Lipka *et al*., 2005). Double mutant analysis revealed that *pen1 pen2* mutants allow a higher frequency of *Bgh* penetration than either single mutant, suggesting that PEN1 and PEN2 function in separate pathways contributing to penetration resistance (Lipka *et al*., 2005). Interestingly, penetration success of another non-adapted powdery mildew, *Erysiphe pisi*, was enhanced ~2-fold (55% compared to 25% in WT) in the *pen1* mutant and was greatly enhanced to over 80% in the *pen2* mutant (Lipka *et al*., 2005). The penetration success of *E. pisi* on the *pen1 pen2* double mutants was indistinguishable from *pen2*. Taken together, these data suggest that the contribution of PEN2-mediated defences to penetration resistance against *E. pisi* is significantly greater than that of PEN1-mediated defences and that defences conferred by PEN1 are insufficient to limit *E. pisi* entry in the absence of PEN2. In addition, disruption of those defences associated with SA-mediated PCD in *pad4 sag101* double mutants, in combination with the disruption of penetration resistance conferred by PEN2, led to the formation of microcolonies and the successful conidiation by *Bgh* on the *pen2 pad4 sag101* triple mutant and to disease development by *E. pisi* that was indistinguishable from that of the adapted powdery mildew *Golovinomyces orontii* on wild-type *Arabidopsis* (Lipka *et al*., 2005). These results suggest that non-host resistance of *Arabidopsis* to *E. pisi* is conferred primarily by defences associated with only two pathways, the pathway involving PEN2 to limit fungal entry, and the SA-mediated pathway involving SAG101 and PAD4 leading to PCD. However, *Arabidopsis* non-host resistance to *Bgh* is more complex requiring, in addition, PEN1 and as yet uncharacterized components.

The third characterized *pen* mutant, *pen3*, was found to be affected in the *Arabidopsis* ATP-binding cassette (ABC) transporter PDR8. ABC transporters collectively facilitate the transfer of a vast array of substrates across cellular membranes and participate in numerous cellular processes. A PEN3–GFP fusion protein localized to the plasma membrane in uninoculated leaves and, like PEN1, showed strongly focused accumulation at sites of attempted penetration by *Bgh* (Stein *et al*., 2006; Fig. 1B).

Unexpectedly, *pen3* mutants were more resistant to the adapted *Arabidopsis* powdery mildew, *Golovinomyces cichoracearum*, and displayed chlorosis and cell death after inoculation with *G. cichoracearum* (Stein *et al.*, 2006). Similarly, *pen3* mutants showed HR-like cell death upon inoculation with the oomycete *Phytophthora infestans* and the bacterial pathogen *Pseudomonas syringae* pv. *tomato*, suggesting that PEN3 may play a role in interactions with numerous plant pathogens (Kobae *et al.*, 2006). Enhanced resistance to *G. cichoracearum* was associated with the up-regulation of the SA pathway and mutations disrupting SA biosynthesis or signalling suppressed cell death and the resistance of *pen3* mutants to *G. cichoracearum* (Stein *et al.*, 2006). Interestingly, mutation of *PEN2* in *pen2 pen3* double mutants partially suppressed the chlorosis and cell death observed in response to inoculation with *G. cichoracearum* and partially restored susceptibility to the adapted powdery mildew. These results led to the development of a model in which PEN3 exports a poten-tially fungi-toxic compound that is processed by PEN2 in peroxisomes. It is proposed that the intracellular accumula-tion of the PEN2 product, and, potentially, other toxic compounds exported by PEN3, leads to the activation of the SA pathway in *pen3* mutants, resulting in enhanced resistance to *G. cichoracearum* (Stein *et al.*, 2006).

PEN3 has also been found to be rapidly phosphorylated in response to perception of defence elicitors such as flg22 and fungal xylanase, suggesting a potential role for elicitor-induced phosphorylation in PEN3 activation (Nühse *et al.*, 2003*b*, 2007; Benschop *et al.*, 2007). Most recently, PEN3 (PDR8) was proposed to participate in tolerance to the heavy metals lead (Pb) and cadmium (Cd; Kim *et al.*, 2007). *pen3* mutants or RNAi lines grew poorly on medium containing Pb or Cd and accumulated higher levels of Cd, whereas plants overexpressing *PEN3* showed enhanced tolerance to Pb and Cd and reduced accumulation of Cd. It is not yet clear whether PEN3′s roles in defence and heavy metal tolerance are somehow related, or simply reflect a broad substrate specificity or promiscuity of transport for PEN3.

The mystery of MLO

MLO is probably the most well-studied player in non-host resistance to powdery mildew fungi. MLO appears to be a negative regulator of defences contributing to powdery mildew resistance, with mutations at the *MLO* locus of barley conferring broad spectrum resistance to most known isolates of the barley mildew *Bgh* (Jørgensen, 1992; Piffanelli *et al.*, 2004). The phenomenon of *mlo*-based resistance to powdery mildew was initially thought to be unique to barley. However, *Arabidopsis* homologues of barley MLO were recently identified that play a similar negative regulatory role in defence against powdery mildew fungi and, most recently, loss of function at the

tomato (*Solanum lycopersicum* var. *cerasiforme*) *MLO* locus *SlMLO1* was found to confer resistance to the powdery mildew *Oidium neolycopersici* (Consonni *et al.*, 2007; Bai *et al.*, 2008).

Arabidopsis encodes 15 genes with high similarity to barley MLO. Mutation of *AtMLO2* was found to confer partial resistance to *G. orontii* that was characterized by reduced penetration success and conidiation (Consonni *et al.*, 2007). The observed partial resistance was found to be due to functional redundancy among *Arabidopsis* MLO homologues as *Atmlo2 Atmlo6 Atmlo12* triple mutants displayed full resistance to *G. orontii*. Interestingly, *mlo2 pen1* double mutants allowed a near wild-type frequency of *G. cichoracearum* penetration, but no significant increase in conidiation. Like PEN1 and barley ROR2, an MLO–YFP fusion protein was found to accumulate at penetration sites in barley and MLO and AtMLO2 were found physically to interact with ROR2 and PEN1, respectively (Schulze-Lefert, 2004; Bhat *et al.*, 2005; Panstruga, 2005). These results suggest that MLO may negatively regulate defences mediated by PEN1/ROR2 through direct physical interaction with these syntaxins. In addition, *Atmlo2 pen2* and *Atmlo2 pen3* double mutants also allowed wild-type frequencies of *G. cichoracearum* penetration, and unlike *Atmlo2 pen1* mutants, displayed a significant increase in *G. cichoracearum* conidiation (Consonni *et al.*, 2007). These results suggest a role for the PEN2/PEN3 pathway in both pre- and post-penetration defences, possibly via continued poisoning of the fungal haustorium through secretion of toxic compounds.

These results also implicate MLO in the modulation of both PEN1 and PEN2/PEN3 pathways leading to penetra-tion resistance. A model depicting the involvement of the PEN proteins and MLO in resistance to powdery mildew is presented in Fig. 2. The three characterized *pen* mutants appear to represent proteins involved in carrying out the resistance response, rather than signalling components; whereas, MLO may play a regulatory role in modulating defence activation. In this model, the PEN3 transporter exports the product of the PEN2 enzymatic activity (X) and additional, as yet undefined, defence compound(s) (Y). The PEN1/SNAP33 complex mediates fusion of VAMP721/VAMP722-carrying Golgi-derived vesicles at the plasma membrane. These vesicles are presumed to contain materi-als for the construction of the nascent papilla. Finally, MLO exerts a negative effect on both the PEN1 and PEN2/3 pathways through an unknown mechanism(s).

Role of cytoskeletal components in penetration resistance

Numerous processes associated with penetration resistance are predicted to involve cytoskeletal components such as actin filaments and microtubules. These include delivery of secretory vesicles to sites of papilla deposition, and
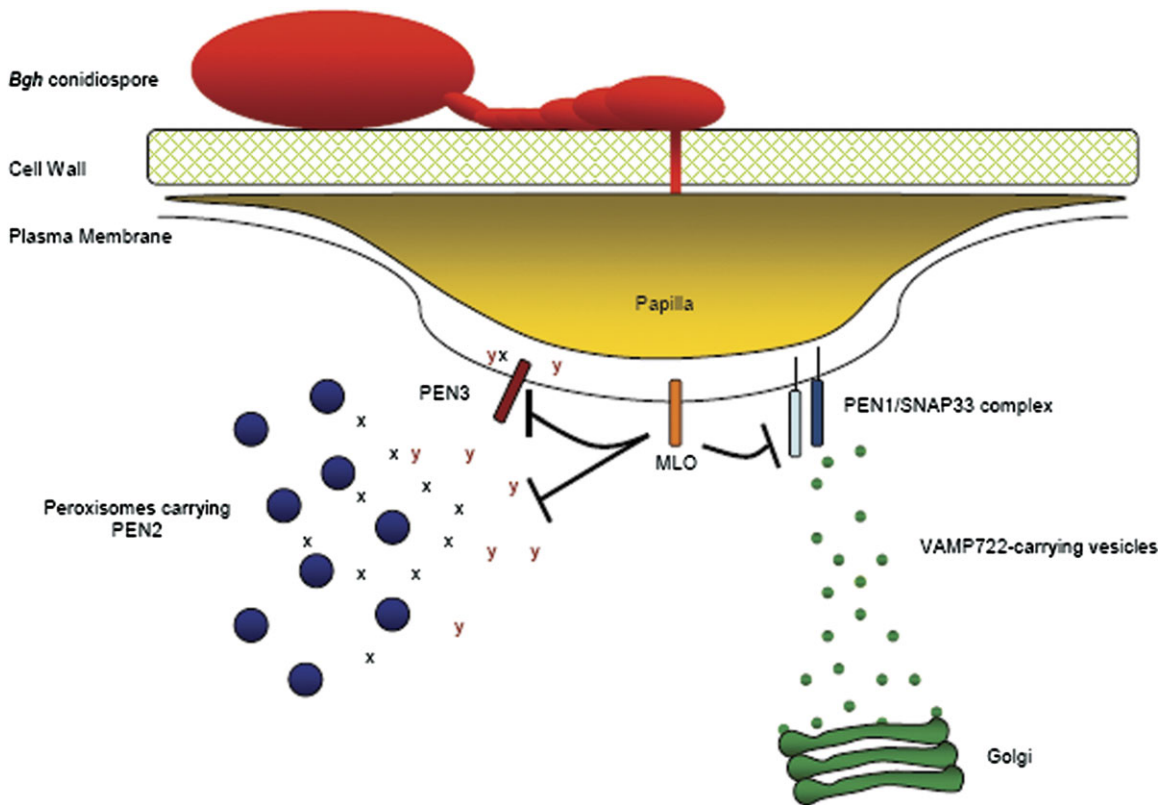
Fig. 2. Model depicting the roles of the PEN proteins and MLO in resistance to penetration by powdery mildew fungi. X and Y represent putative PEN3 transport substrates.

cytoplasmic streaming and migration of organelles such as peroxisomes, Golgi, and the nucleus to the site of interaction with the fungus. Consistent with a predicted role for cytoskeletal components in these processes, pharmacological and genetic interference with actin filaments disrupts penetration resistance, leading to increased penetration frequency by various powdery mildew species in several experimental systems (Kobayashi *et al*., 1997*a*, *b*; Yun *et al*., 2003).

In addition, an intact actin cytoskeleton is also required for full *mlo*-mediated resistance to powdery mildew (Miklis *et al*., 2007). Actin filaments have frequently been found to show significant polarization toward sites of attempted fungal penetration and the extent of such actin polarization has been correlated with success or failure of penetration resistance (Opalski *et al*., 2005; Shimada *et al*., 2006). Interestingly, genetic disruption of actin filaments in barley through overexpression of the actin depolymerizing factor HvADF3 did not alter the focal accumulation of MLO-YFP or YFP-ROR2 at penetration sites, despite completely disrupting peroxisome motility (Bhat *et al*., 2005). Similarly, in *Arabidopsis*, GFP–PEN1 accumulation at penetration sites is not affected by treatment with the actin disrupting agent cytochalasin E (Underwood and Somerville, unpublished results). These results suggest that PEN1/ROR2 and MLO are recruited to penetration sites through actin-independent mechanisms. By contrast, focal accumulation of PEN3–GFP at sites of attempted *Bgh* penetration is disrupted by cytochalasin E treatment (W Underwood and SC Somerville, unpublished results), suggesting at least two potentially distinct mechanisms for the recruitment of defence-related proteins to the sites of interaction with invading fungi.

While experimental evidence strongly supports a pivotal role for the actin cytoskeleton in penetration resistance, the role of microtubules is not as clear. Disruption of microtubules in some systems was found to confer a modest increase in fungal penetration efficiency, whereas in other systems, similar disruption of microtubules was found to have no effect (Kobayashi *et al*., 1997*a*; Takemoto *et al*., 2003). In addition, the disruption of microtubules did not affect *mlo*-mediated resistance (Miklis *et al*., 2007). In general, microtubules appear to have little or no role in defences leading to penetration resistance.

Parallels with other biological systems

Dramatic membrane polarization and focal accumulation of proteins is not unique to plant penetration resistance to powdery mildew fungi. The processes of membrane polarization, focal recruitment of specific proteins, formation

of lipid raft-like, sterol rich membrane domains (Bhat *et al.*, 2005), and targeted vesicle-mediated secretion in penetration resistance are strikingly similar to the processes involved in the formation of the immunological synapse in vertebrate immunity (recently reviewed in Kwon *et al.*, 2008*b*). Similar to the formation of the immune synapse, invasion of human erythrocytes by the malarial parasite *Plasmodium falciparum* involves the recruitment of lipid raft membrane domains to the site of the junction between parasite and host cell (Murphy *et al.*, 2006). It is thought that the host cell membrane is substantially remodelled ultimately to form the parasitophorous vacuole that accommodates the parasite within the erythrocyte cell (Haldar and Mohandas, 2007). Such a process of recruitment of host components, remodelling of host membranes, and subsequent accommodation of the parasite within a host membrane-derived structure parallels the accommodation of powdery mildew haustoria within plant epidermal cells. Similarly, invasion of intestinal cells by the intracellular parasite *Cryptosporidium parvum* involves the clustering of lipid raft-like membrane domains to the point of contact between the host cell and the parasite (Nelson *et al.*, 2006). Remodelling of actin filaments within the host cell toward the site of contact with the invading parasite also plays a role in the entry of *C. parvum* into host cells (Bonnin *et al.*, 1999; Elliott and Clark, 2000; O'Hara and Lin., 2006). However, the mechanisms of recruitment of lipid microdomains and specific proteins to these specialized membrane structures remain enigmatic.

Future perspectives

The development of advanced microscopy techniques including the use of fluorescent tags and probes, together with the isolation of mutants impaired in the ability to resist fungal penetration, opens up a wealth of possibilities for probing the molecular and cell biological aspects of cell wall-based defences, the studies of which have been primarily descriptive until very recently. Along with new possibilities arise fascinating new questions, summarized in Box 1. Understanding the mechanisms underlying the substantial cell polarization and focal accumulation of proteins and lipid raft-like domains to sites of fungal invasion will contribute not only to studies of invasive phytopathogens, but to general cell biology as cell polarization plays a crucial role in many biological processes.

A question of great significance to host–pathogen interactions is what determines the outcome of the interaction, disease development or resistance? Successful powdery mildews are able both to overcome penetration resistance and to avoid the second line of defence associated with HR-like cell death. This may be due to a lack of elicitors that would be perceived by the plant or, alternatively, to active suppression of the cell death

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Box 1.   *Outstanding questions in non-host resistance to powdery mildew*

(i) How are PEN1, PEN3, MLO, and other components of penetration resistance recruited to sites of interaction with the invading fungus?

(ii) How are virulent fungi able to overcome or circumvent cell wall-based defences?

   – Resistance to secreted antimicrobial compounds?

   – Greater efficiency/speed at degrading cell wall polymers?

   – Concealment of pathogen-associated molecular patterns?

   – Manipulation of host defences by effector proteins?

(iii) How do papillae associated with penetration resistance compare to morphologically similar collar-like structures associated with compatible intractions in which haustoria are formed?

(iv) How do papilla structures assembled in response to bacterial invasion relate to analogous (and microscopically indistinguishable) structures assembled at fungal penetration sites? Are some components of papilla assembly and function used for defence against both types of pathogens?

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response by effector proteins. The identification of R–Avr pairs in the powdery mildew system, such as *Bgh* AVR_{a10} and barley MLA10 and the fact that at least some barley R proteins are located within the plant cytoplasm strongly support the notion that powdery mildews translocate effector proteins into plant cells (Ridout *et al.*, 2006). In contrast to the recent cataloguing of effector proteins from several phytopathogenic bacteria, the number and nature of powdery mildew effectors remain enigmatic, as do the mechanisms of their translocation into plant cells. The identification and characterization of effector proteins from powdery mildews will be a significant step forward toward understanding how fungal pathogens manipulate plant cells.

Finally, plants assemble morphologically similar papilla-like structures in response to numerous pathogens including both fungi and bacteria. It is currently not clear whether these structures serve similar defence roles in the context of different classes of pathogens and whether plant cells utilize similar or identical components and mechanisms to deposit these structures.

The study of penetration resistance of plant cells to fungal pathogens represents an interesting interface between plant pathology and cell biology and future insights into the mechanisms underlying cell wall-based defences should contribute significantly to both areas of research.

Acknowledgements

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