ELSEVIER

The role of secreted proteins in diseases of plants caused by rust, powdery mildew and smut fungi

Jeffrey G Ellis, Peter N Dodds and Gregory J Lawrence

Five unrelated avirulence (Avr) gene families have been cloned from flax rust and barley powdery mildew, fungal pathogens that make close contact with living host plant cells using specialized feeding structures called haustoria. Transgenic expression studies indicate Avr proteins are recognized by disease resistance (R) proteins within host cells, which suggests that Avr proteins are transported via an as yet unidentified route from the fungus to the host during infection. Recognition of flax rust AvrL567 proteins is by direct R-Avr protein interaction. Virulence effector functions have been demonstrated for barley powdery mildew Avr proteins Avra10 and Avrk1. Mildew resistance triggered by Avra10 in barley involves association of the cognate barley R protein Mla10 and transcriptional repressor proteins, including HvWRKY2, in the host nucleus. High amplitude defence gene expression has a dual dependence on transcriptional de-repression induced by specific R-Avr protein recognition and additionally, activation signals initiated by host perception of general pathogen molecules.

Addresses
Commonwealth Scientific and Industrial Research Organisation, Division of Plant Industry, GPO Box 1600, Canberra ACT 2601, Australia

Corresponding author: Ellis, Jeffrey G. (jeff.ellis@csiro.au)

Current Opinion in Microbiology 2007, **10**:326–331

This review comes from a themed issue on
Fungi
Edited by Jim Kronstad

Available online 14th August 2007

1369-5274/$ – see front matter

DOI 10.1016/j.mib.2007.05.015

Introduction

The current 'spirit of the times' in molecular plant pathology, eloquently outlined in a recent review [1], is that evolution of a plant–pathogen disease system involves a complex move–countermove scenario. In step 1, evolution of a plant pathogen from a non-pathogenic ancestor involves the acquisition of molecules called effectors that function to blunt host basal resistance responses that are induced by common microbe-specific molecules, known as pathogen associated molecular patterns (PAMPs). These molecules are recognized by host receptors as 'non-self' and trigger a low-level defence response. Step 2 is evolution by the host plant of 'effector detectors', more commonly known as resistance (R) proteins that specifically recognize pathogen effectors and then trigger strong host defences. When effector proteins are recognized by polymorphic host resistance proteins they are also called avirulence proteins. In step 3, the pathogen's evolutionary countermove is envisaged to be more complex and can involve either modification of effectors to escape R protein recognition but retain virulence function, or loss and replacement of old effector repertoires with new ones. An additional flourish that can be added to step 3 by quintessential pathogens is the evolution of inhibitor proteins that either directly or indirectly block recognition of effectors by R proteins. Much of the experimental support for this theory comes from the study of bacterial pathogens of plants. In this review we consider how recent advances in the study of a group of fungal plant pathogens, rusts, powdery mildews and smuts, sit with this evolutionary perspective. These fungi all share a common extracellular lifestyle that involves penetration of plant cell walls and intimate contact with living host plant cell membranes as a means of acquiring nutrients from, and potentially suppressing defences of, living plant cells [2]. These pathogens are of major importance in agriculture and forestry and an in depth understanding of the biology of their interaction with host plants is a foundation stone of continued world food security.

Flax-flax rust interactions

Rusts are basidiomycete fungi that cause disease in many plant species. Genetic studies of the interaction between the flax plant (*Linum usitatissimum*) and flax rust (*Melampsora lini*) have identified about 30 flax resistance (R) genes, which occur as series of closely linked or allelic genes at 5 loci, and about 30 'corresponding' flax rust avirulence (Avr) genes that are mostly dispersed in the flax rust genome. These studies defined the specific genetic interactions of R-Avr genes that trigger 'gene-for-gene' rust resistance. These studies have also identified a second class of rust genes called 'Inhibitor of avirulence' (I) genes that inhibit resistance responses of certain R-Avr gene interactions [3], consistent with the evolutionary step 3 outlined in the introduction. Flax R genes encode cytoplasmic nucleotide binding site leucine rich repeat (NBS-LRR) proteins and recently flax rust Avr genes were cloned from 4 rust Avr loci, *AvrL567*, *AvrM*, *AvrP123* and *AvrP4* [4••,5•].

The *AvrL567* locus [4••,6••], cloned by mapping candidate genes expressed during rust infection, contains from one to four paralogs, depending on the rust strain, and among a sample of 6 strains, 12 *AvrL567* sequence variants were identified with more than 90% nucleotide

identity. These genes each contain open reading frames that encode 150 amino acid polypetides with highly conserved predicted 23 residue N-terminal secretion signals. The 127 residue mature secreted proteins are highly variable with 25% of the amino acid positions having one or more polymorphisms. Analysis of the gene sequences indicated that they have undergone selection for diversity during evolution. Expression of the genes is induced during rust infection and occurs in rust haustoria, the specialized rust feeding structure within infected host cells.

In the absence of flax rust transformation, confirmation of avirulence gene function of *AvrL567* variants was achieved by transient and stable Agrobacterium-mediated transformation of flax plants of different genotypes of the *L* rust resistance locus. Avirulence function was indicated by R gene-dependent leaf necrosis in transient assays and seedling inviability and stunting in transgenic plants [4••,6••]. In transient assays the 12 variants fell into two classes: 7 'avirulence' forms that induced necrosis on one or more of the *L5*, *L6* and *L7* genotypes and 5 'virulence' forms that did not induce necrosis [6••]. For example, *AvrL567-A* expression induced leaf necrosis in *L5*, *L6* and *L7* plants (hence the *AvrL567* designation) but not in *L*, *L1*, *L2*, *L3*, *L4*, *L8*, *L9* and *L10* genotypes. Avirulence activity of *AvrL567-D* was distinguished from the other variants by induction of necrosis in *L6* and *L7* leaves but not *L5*. *AvrL567-J* was distinguished by induction of necrosis in *L5*, *L6*, *L7* and additionally in *L6L11RV* leaves that carry an in vitro constructed *L6-L11* chimeric gene. There was a complete association between the specific induction of necrosis in the transient assay and induction of resistance by a rust strain. For example rust strains carrying *AvrL567-A*, and strains homozygous for *AvrL567-C* are avirulent and virulent, respectively to *L6* plants [6••].

Avirulence forms of *AvrL567* gave a stronger reaction when expressed without the N-terminal signal peptide [4••]. This indicates recognition of the Avr protein by the R protein occurs within the host cytoplasm, consistent with the proposed cytoplasmic location of the R protein, and suggests that the Avr protein is secreted by the rust and taken up by the host cell during rust infection. Furthermore, interactions between L resistance and AvrL567 avirulence proteins in yeast two hybrid assays matched the necrosis reactions in transient expression assays. For example, L6 but not L5 interacts with AvrL567-D in yeast, and co-expression of L6 but not L5 with AvrL567-D induces necrosis in flax leaves. Furthermore, the L6L11RV protein interacts with only AvrL567-J in yeast and only this Avr protein induces necrosis in *L6L11RV* flax leaves. The virulence forms that do not induce plant necrosis do not interact with resistance proteins in yeast. The correspondence between protein interaction and the induction of necrosis indicates that direct R-Avr protein interaction is the basis for recognition specificity [6••].

Further flax rust avirulence genes were isolated among genes expressed in haustoria and encoding secreted proteins [5•]. Among 429 different genes, 21 were predicted to encode secreted proteins and, in addition to a second isolation of *AvrL567*, there were three new Avr genes, *AvrM*, *AvrP4* and *AvrP123* among the 21 selected genes. The 4 groups of avirulence proteins are all unrelated, contain no motifs indicative of function and within each group, are highly variable between flax rust isolates. No related proteins, including other fungal Avrs, occur in current data bases, including the recently released wheat stem rust genome sequence (http://www.broad.mit.edu/annotation/genome/puccinia_graminis), except for one protein encoded by a poplar leaf EST (genbank: BI127090) highly related to AvrM (blastP score 63, E = 4e-17) which is probably derived from leaf infection with one of the *Melampsora* sp. rusts of poplar.

Powdery mildew

Powdery mildews are a large group of ascomycete haustorium-forming fungal pathogens of plants. In the barley (*Hordeum vulgare*) and barley powdery mildew (*Blumeria graminis hordei*) interaction over 80 resistance genes have been identified, 28 mapping to the *Mla* locus from which 6 highly related NBS-LRR genes have been cloned [7••]. In a recent breakthrough [8••], two Avr genes, *Avra10* and *Avrk1*, recognized by *Mla10* and *Mlk* resistance genes respectively, have been cloned from barley mildew by map based cloning. *Avra10* and *Avrk1*, located within in a 30 kbp region of the mildew genome and with open reading frames of 861 and 534 base pairs, respectively, share 60% sequence identity over 344 base pairs near the 3′ end of the genes but are unrelated elsewhere in the gene. Genomic DNA blotting with the conserved region of *Avrk1* detects multiple fragments and hybridization to one genome equivalent in BAC clones lead to an estimate of approximately 30 *Avrk1* homologs in the *Blumeria* genome, compared to the one to 5 copies in any of the 4 cloned flax rust Avr gene groups. Whether these paralogs are clustered or dispersed in the genome is not reported. The predicted 287 and 178 residue protein products of these genes share a core region of 113 residues with approximately 60% and 52% amino acid similarity and identity, respectively. In further contrast to most other identified fungal and oomycete Avr proteins, the mildew Avr genes do not encode N-terminal secretory signals. Nevertheless, cytoplasmic expression of these Avr proteins using biolistic transient transformation assays suggests R-Avr recognition occurs in the cytoplasm, like flax–flax rust R-Avr proteins [8••].

Smut fungi

Smut fungi include a large group of basidiomycete biotrophic pathogens of plants that are particularly important in cereal production and have great advantages over rusts and mildews in molecular genetic studies because of the ease of culturing smut fungi on defined media and

consequently ease of molecular transformation [9]. In *Ustilago* species, the pathogen does not form haustoria but makes close contact with living host cells via intracellular hyphae that invaginate the host cell membrane. Major gene resistance to smut fungi occurs in wheat and barley and avirulence genes have been genetically mapped in the barley pathogen *Ustilago hordei* [10]. The most advanced molecular studies have centered on the maize smut pathogen *Ustilago maydis* [9], which along with *Magnaporthe grisea* (rice blast, see Talbot, this volume) is one of the most extensively studied plant fungal pathogens at the molecular level. Interestingly, no major gene resistance to smut has been identified in the maize gene pool and consequently no avirulence genes have been described in this species. An exciting advance has been the complete annotation of the 20.5 Mbp of the *U. maydis* genome, which provides the first opportunity to examine the genes of a biotrophic fungal plant pathogen [11••]. Consistent with the biotrophic lifestyle, the gene complement for toxin synthetic pathways and cell wall degrading enzymes is low compared with sequenced genomes of necrotrophic fungal pathogens, which kill and consume host cells. In respect of the major advances in identifying pathogenically relevant secreted proteins (secretome) in rusts and powdery mildews described above and oomycete plant pathogens (Kamoun this volume), the secretome of *U. maydis* has provided some fascinating preliminary results. 426 secreted proteins were predicted, 70% of which have no ascribed molecular function (as is the case for flax rust, powdery mildew and oomycete Avr proteins) and of these 66% have no close counterparts outside of *U. maydis*. Among these genes, 18.6% are arranged in 12 clusters of 3–26 genes containing from 1 to 5 different gene families, at least some of which were expressed during host infection but not in culture medium. Each of these clusters was examined for its role in fungal virulence. Complete deletions of each cluster were made and mutants inoculated into maize plants. Deletion of 5 of the 12 clusters had an observable effect, with 4 deletions reducing and one increasing virulence. However the effect of these mutations on the essential first step in this organism's pathogenicity, host epidermal penetration, was not reported. Importantly none of the deletions affected growth on artificial growth medium. Whether any of these secreted proteins enter the host cell is not known.

Are proteins secreted by rust, mildew and smut fungi, including Avr proteins, effectors?

The mutation data for *U. maydis* secreted proteins provide preliminary evidence for a virulence function of these proteins. Data for barley mildew also indicates a virulence effector role for Avra10 and Avrk1 in reducing host basal resistance. Barley epidermal cells (with no effective R genes) co-bombarded with a GFP reporter and plant expression adapted Avr genes support more mildew infections in the GFP-expressing cells than the control GFP-empty vector co-bombarded cells [8••]. Only circumstantial evidence for effector roles of flax rust Avr proteins is available. For example, *AvrL567* genes have undergone selection for diversity, and virulence alleles of *AvrL567* are expressed during infection and encode stable full length proteins that differ from the avirulence forms only in putative surface exposed residues [6••]. These data strongly suggest that the flax rust proteins have been selected to avoid recognition while maintaining an unidentified effector function consistent with evolutionary step 3 outlined in the introduction.

How do Avr proteins enter host cells?

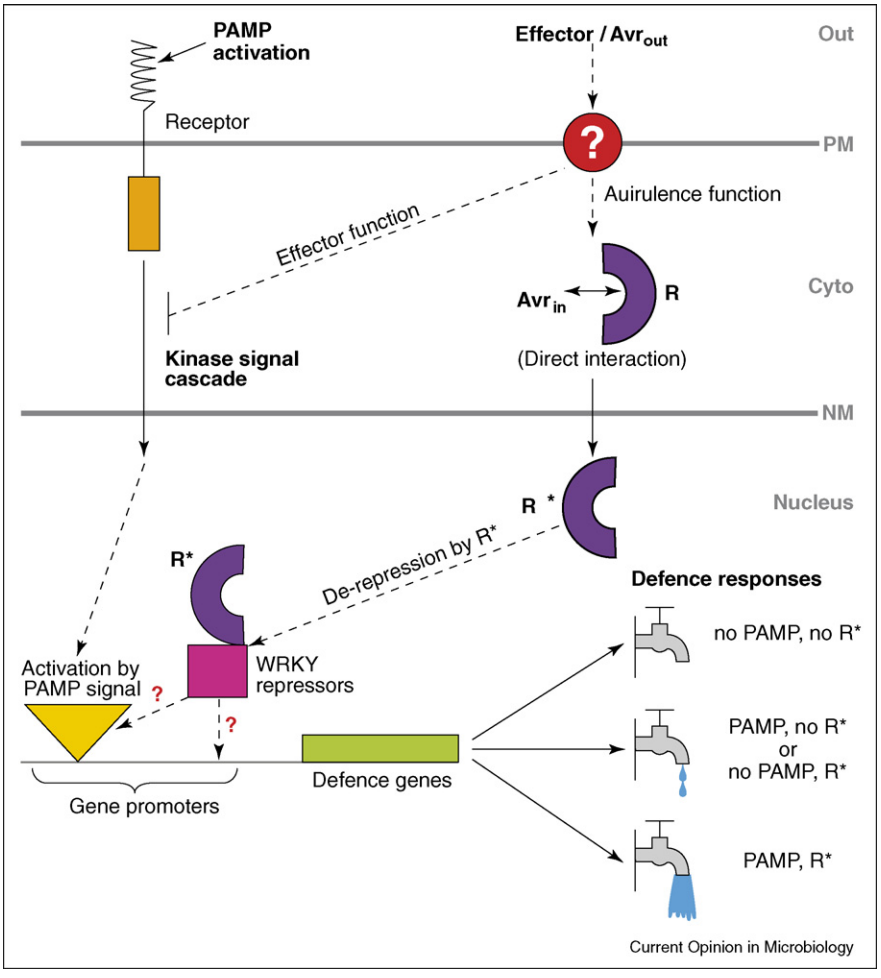
Avr proteins secreted by oomycetes carry a conserved motif that potentially signals uptake by host plant cells via an unknown route (Kamoun, this volume). Although no equivalent signals have been detected, the observations that flax rust and barley powdery mildew Avr proteins expressed in the cytoplasm of host cells induce defence responses dependent on the presence of cytoplasmic R proteins provides indirect evidence that these proteins enter the host cell. Direct observation of uptake by plant cells of a secreted rust protein expressed in haustoria is provided by studies of the 220-residue glycoprotein RTP1p from broad bean rust (*Uromyces fabae*) [12•]. This protein, which has a nuclear localization signal, was detected by immunofluorescence microscopy in the host plant cytoplasm and nucleus. The function of RTP1 is unknown and no avirulence role has been reported. Close homologs occur in other legume rusts including *Uromyces striatus* [12•], *Uromyces appendiculatus* (genbank: EH303509, blast score 120, Expect = 7e-26) and soybean rust *Phakopsora pachyrhizi* (genbank: EH221860, blast score 99.0, Expect = 3e-19) and are also detected in the recently released wheat stem rust genome (http://www.broad.mit.edu/annotation/genome/puccinia_graminis) but not any other species including *U. maydis*, suggesting this protein is rust-specific(J. Ellis, unpublished). Importantly, a second rust protein, PIG15p, also secreted from bean rust haustoria, is not localized in the host cell, which indicates a process that discriminates between rust secreted proteins that are/are not destined for transport to the host [12•].

Experiments indicate the likely route of uptake of the flax rust AvrM protein is via a host encoded system and probably not a specialized rust secretory system analogous to the type three secretion system used by bacterial pathogens [5•]. The secreted form of AvrM, expressed in plant cells, triggers resistance responses that are inhibited by addition of a C-terminal HDEL endoplasmic reticulum retention signal. Mutation to the non-active retention signal HDDL or removal of the secretory peptide, both predicted to cause retention in the cytoplasm, restores AvrM avirulence activity. The data imply that in this transgenic system the secreted AvrM protein re-enters the host cytoplasm in the absence of the rust

fungus. A host uptake system for a 117-residue protein toxin, ToxA, secreted into the apoplast of wheat by the necrotrophic fungal pathogen *Pyrenophora tritici-repentis* is encoded by the polymorphic *Tsn1* gene in wheat [13,14]. The uptake of this and the AvrM protein are the first documented transfer of extracellular proteins across the plant membrane [13]. Cloning of *Tsn1* will provide insight into mechanisms in plants for transport of proteins from the apoplast across the plasma membrane.

In contrast to flax rust Avr genes and oomycete Avr genes, the barley mildew genes do not encode proteins with N-terminal secretion signals and it has been proposed that either alternative secretion signals exist for these proteins, or that like the AVR-ACE1 avirulence protein in *Magnaporthe grisea*, the barley mildew proteins may have enzymatic activity that produces a secreted small molecular weight secondary product that triggers R gene dependent defence responses [8••]. However, no enzymatic

Figure 1



Model for host defence gene activation by biotrophic fungal pathogens based on current understanding of the flax–flax rust system [6••] and host–powdery mildew systems [7••]. Pathogen associated molecular patterns (PAMPs), (also elaborated by non-pathogen microbes) are detected by transmembrane receptors that activate kinase signal cascades leading to expression of defence genes [17]. Host transcription repressors (WRKY repressors) dampen defence responses possibly by direct interaction with host gene promoters, presumably to prevent host damage ('chronic inflammatory responses') that would result from overinduction by ubiquitous non-pathogen microbial species. The result is low-level (basal) resistance. [In the absence of PAMPs, similar defence expression can occur in plants carrying weak auto-active R gene alleles (15).] Host-adapted, extra-cellular fungal pathogens produce effector/Avr proteins that enter the host cytoplasm by unidentified uptake mechanisms (?). One postulated effector function is to dampen PAMP-induced host defence to the advantage of the pathogen. Recognition (direct in the flax-rust system, unknown in the mildew system) of internalised pathogen effector/Avr proteins by polymorphic host resistance (R) proteins triggers R protein activation (*R), either in the cytoplasm or nucleus [R* can also be achieved in the absence of Avr proteins by autoactive R gene mutations (15)]. For the mildew system (7), nuclear localized *R interferes with WRKY repressor functions (mechanisms unknown) through direct R-WRKY interactions to allow high level activation of defence genes in response to the residual, effector-dampened PAMP signals. The result is high-level R protein-associated resistance. Other abbreviations: PM, plasma membrane, cyto, cytoplasm, NM, nuclear membrane. Tap flows depict defence pathway fluxes.

activity is predicted from the amino acid sequences of the mildew AVRs and the avirulence activity of the Avr genes expressed transiently in barley cells would require this enzyme to be functional in the production of a secondary ligand in both mildew and barley cells. Alternatively, perhaps the mRNA of these Avr genes is secreted to the host cell?

How do Avr proteins trigger resistance?

This is a big question in molecular plant pathology and recent work provides a major insight [7••]. Transient expression in barley leaf epidermal cells of the barley mildew Avra10 protein, cognate Mla10 resistance protein and transcriptional repressor HvWRKY2 induced nuclear interaction between Mla10 and the HvWRKY2. Experimental data supports the model (Figure 1) that low level expression of PAMP-induced basal defence genes is massively amplified by an Avr-activated, nuclear localized, Mla10-HvWRKY2 interaction. Consequently it is postulated that the difference between basal and R gene dependent defence is simply the level of expression of the same pathway. Whether this R-gene dependent induction of mildew resistance involves direct interaction between Mla10 and Avra10, as is the case in recognition of AvrL567 in flax, has not been reported. This model perhaps explains earlier observations with auto-active flax rust resistance genes. Flax plants expressing certain auto-active alleles of *L6* (equivalent to R^* in Figure 1) express higher levels of defence genes than wildtype (and a dwarf phenotype) in the absence of the pathogen. Moreover, they are resistant to strains of rust that lack the cognate *AvrL567* gene (i.e. strains virulent to wild-type L6 plants) and undergo hypersensitive cell death resistance (HR) [15]. HR may be the consequence of a massive increase of the defence gene flux induced by PAMPs elaborated by the normally virulent rust stain.

Perspectives

The availability of the full genome sequence and relatively routine procedures for transformation and gene disruption in *U. maydis* now provide exciting new opportunities to understand the molecular basis of biotrophy. Of particular interest will be whether any of the proteins secreted during infection enter the host cells and how they affect virulence. Unlike *U. maydis*, rusts and mildews cannot yet be simply and reliably transformed for expression and gene replacement analysis and remedying this situation will be crucial for ascribing function to genes identified from genome sequences. Also, given the importance of small-secreted proteins for virulence/avirulence, careful annotation of fungal genomes will be required to accurately identify this commonly under annotated class of genes [16]. The major questions to be addressed are how do fungal effector proteins enter host cells and what are their effector functions?

References and recommended reading

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

- • of special interest
- •• of outstanding interest

1. Jones JG, Dangl JL: **The plant immune system**. *Nature* 2006, **144**:323-329.

2. Hahn M, Mendgen K: **Signal and nutrient exchange at biotrophic plant-fungus interfaces**. *Curr Opin Plant Biol* 2001, **4**:322-327.

3. Lawrence GJ, Mayo GME, Shepherd KW: **Interactions between genes controlling pathogenicity in the flax rust fungus**. *Phytopathology* 1981, **71**:12-19.

4. •• Dodds PN, Lawrence GJ, Catanzariti A-M, Ayliffe MA, Ellis JG: **The *Melampsora lini AvrL567* avirulence genes are expressed in haustoria and their products are recognized inside plant cells**. *Plant Cell* 2004, **16**:755-768.

This paper describes the cloning of the first avirulence gene from a haustorium forming fungus and demonstrates that it is expressed in haustoria and its product recognised within the host cell by the corresponding resistance protein, implying that rust fungi transfer proteins to host cells.

5. • Catanzariti A-M, Dodds PN, Lawrence GJ, Ayliffe MA, Ellis JG: **Haustorially expressed secreted proteins from flax rust are highly enriched for avirulence elicitors**. *Plant Cell* 2006, **18**:243-256.

In this paper, four out of 21 genes expressed in flax rust haustoria are identified as different flax rust avirulence genes. Experiments involving transgenic expression of Avr M and its derivatives suggest the existence of a host-encoded system for protein uptake.

6. •• Dodds PN, Lawrence GJ, Catanzariti A-M, Teh T, Wang C-IA, Ayliffe MA, Kobe B, Ellis J: **Direct protein interaction underlies gene-for-gene specificity and coevolution of the flax resistance genes and flax rust avirulence genes**. *Proc Natl Acad Sci U S A* 2006, **103**:8888-8893.

This paper demonstrates that gene-for-gene specificity in the flax–flax rust interaction is based on direct interaction between rust resistance and flax rust avirulence proteins and supports the view that an evolutionary 'arms race' drives diversification of plant resistance and pathogen avirulence/effector genes in biotrophic interactions.

7. •• Shen Q-H, Saijo Y, Mauch S, Biskup C, Bieri S, Keller B, Seki H, Ulker B, Somssich I, Schulze-Lefert P: **Nuclear activity of MLA immune receptors links isolate-specific and basal disease-resistance responses**. *Science* 2007, **315**:1098-1103.

The authors show that for the Mla resistance gene in barley, there is no long cytoplasmically localized signal transduction pathway between Avr protein perception by the R protein and defence gene activation but rather a short pathway that involves direct interaction between a nuclear localized and AVR-activated R protein and WRKY transcriptional repressors. This provides the first real insight into how R proteins activate defence responses and that these defence responses are simply overexpression of basal responses.

8. •• Ridout CJ, Skamnioti P, Porritt O, Sacristan S, Jones JDG, Brown JKM: **Multiple avirulence paralogues in cereal powdery mildew fungi may contribute to parasite fitness and defeat of plant resistance**. *Plant Cell* 2006, **18**:2402-2414.

By identifying the Avr gene corresponding to the Mla resistance gene this paper has removed a major limitation in the full examination of the molecular basis of major gene resistance to barley powdery mildew.

9. Basse CW, Steinberg G: ***Ustilago maydis*, model system for analysis of the molecular basis of fungal pathogenicity**. *Molec Plant Pathol* 2004, **5**:83-92.

10. Linning R, Lin D, Lee N, Abdennadher M, Gaudet D, Thomas P, Mills D, Kronstad JW, Bakkeren G: **Marker-based cloning of the region containing the UhAvr1 avirulence gene from the basidiomycete barley pathogen *Ustilago hordei***. *Genteics* 2004, **166**:99-111.

11. •• Kämper J, Kahmann R, Bölker M, Ma LJ, Brefort T, Saville BJ, Banuett F, Kronstad JW, Gold SE, Muller O *et al.*: **Insights from the genome of the biotrophic fungal plant pathogen Ustilago maydis**. *Nature* 2006, **444**:97-101.

The first full genome sequence of a biotrophic plant fungal pathogen that is amenable to simple agar plate based growth and manipulation has opened the door for a full description of how this class of fungi (biotrophs) live in 'pretend harmony' with their hosts and avoid host recognition as 'non self'.

12. • Kemen E, Kemen AC, Rafiqi M, Hempel U, Mendgen K, Hahn M, Voegele RT: **Identification of a protein from rust fungi transferred from haustoria into infected plant cells**. *Mol Plant Microbe Interact* 2005, **18**:1130-1139.
The immuno-localization of a secreted rust protein in the cytoplasm and nucleus of a host cell infected with a rust haustorium provides the first direct demonstration of fungus to host protein transfer.

13. Manning VA, Ciuffetti LM: **Localisation of Ptr ToxA produced by *Pyrenophora tritici-repentis* reveals protein import into wheat mesophyll Cells**. *Plant Cell* 2005, **17**:3203-3212.

14. Haen K, Lu H, Freisen T, Faris J: **Genomic targeting and high resolution mapping of the Tsn1 gene in wheat**. *Crop Sci* 2004, **44**:951-962.

15. Howles P, Lawrence GJ, Finnegan J, McFadden H, Ayliffe MA, Dodds PN, Ellis JG: **Autoactive alleles of the Flax *L6* rust resistance gene induce non-race-specific rust resistance associated with the hypersensitive response**. *MPMI* 2005, **18**:570-582.

16. Lease K, Walker JC: **The Arabidopsis unannotated secreted peptide database, a resource for plant peptidomics**. *Plant Physiol* 2006, **142**:831-838.

17. Asai T, Tena G, Plotnikova J, Willmann MR, Chiu WL, Gomez-Gomez L, Boller T, Ausubel FM, Sheen J: **MAP kinase signalling cascade in Arabidopsis innate immunity**. *Nature* 2002, **415**:977-983.