MOLECULAR BIOLOGY OF PLANT PATHOGENS 2007

University of Bath, UK   11th – 12th September, 2007

TUESDAY 11th SEPTEMBER	
11.00 – 12.30	**REGISTRATION**
12.30 – 13.30	**LUNCH**
SESSION 1A – PATHOGENICITY 1 (Chair – Sarah Gurr)	
13.30 - 13.55	**A putative protein kinase C is a virulence determinant in *Magnaporthe grisea*** Solange A Mateo Montalcini, *University of Oxford*
13.55 - 14.20	**Pathogenicity of *Fusarium graminearum* and *F. culmorum* on wheat ears** Andrew Beacham, *Rothamsted Research*
14.20 – 14.45	**Investigating the roles of reactive oxygen species during *Mycosphaerella graminicola* infection of wheat** Siân Deller, *Rothamsted Research*
14.45 – 15.00	**The translocation of *Phytophthora* effectors and their manipulation of host plant disease resistance.** Severine Grouffaud, *Scottish Crop Research Institute / University of Aberdeen*
15.00 – 15.30	**TEA / COFFEE**
SESSION 1B - PATHOGENICITY 2 (Chair – Kim Hammond-Kosack)	
15.30 – 15.45	**Are glycolipid-anchored surface proteins involved in pathogenesis in *Magnaporthe grisea*?** Maeve Price, *University of Oxford*
15.45 – 16.00	**Molecular analysis of sterol C14α-demethylase gene (*CYP51*) from azole resistant and sensitive *Podosphaera fusca* isolates** F. J. López-Ruiz, *John Innes Centre*
16.00 – 16.25	**Comparative analysis of amino acid assimilation pathways in *Pseudomonas* using hypergraphs** Aziz Mithani, *University of Oxford*
16.25 – 17.00	**The Annual MBPP Food and Drink Seminar** **'Soft fruit - hard liquor'** Leighton Pritchard & Dave Cooke, *SCRI*

WEDNESDAY 13th SEPTEMBER	
SESSION 2A – DEFENCE 1 (Chair – Paul Birch)	
09.00 – 09.25	**The efficacy and biosafety of nematode control using synthetic, chemodisruptive peptides expressed transgenically in plants** Dong Wang, *University of Leeds*
09.25 – 09.50	**Characterisation of potato genes that respond to parasitism by *Globodera pallida*** Jamie Smith, *University of Leeds*
09.50 – 10.15	**How bacteria use acidic overcoats to hinder detection by innate plant defences** Kate Morrissey, *University of Bath*
10.15 – 10.30	**Ubiquitination and plant pathology** Ros Taylor, *University of Glasgow / SCRI*
10.30 – 11.00	**Tea / Coffee**
SESSION 2B – DEFENCE 2 (Chair – Matt Dickinson)	
11.00 – 11.15	**The roles of Cathepsin B-like proteases in plant disease resistance** Hazel McLellan, *University of Edinburgh*
11.15 – 11.40	**Exploring resistance to fusarium ear blight** Sarah Lee, *Rothamsted Research*
11.40 – 12.05	**AtBTB1 is a negative regulator of the hypersensitive response in *Arabidopsis thaliana*** Joelle Mesmar, *University of Glasgow*
12.05 – 12.20	**The tobacco Ubiquitin-specific protease NtCDD1 positively regulates cell death during plant disease resistance.** Richard A. Ewan, *University of Glasgow*
12.20 – 12.30	**Other MBPP Business**
12.30–14.00	**LUNCH FOR BSPP MEETING DELEGATES / DEPARTURE**
14.00 onwards	**BSPP PRESIDENTIAL MEETING**

ABSTRACTS

SESSION 1A – PATHOGENICITY 1

A putative protein kinase C is a virulence determinant in *Magnaporthe grisea*

Solange A Mateo Montalcini, Pari Skamnioti and Sarah Gurr
Plant Sciences, University of Oxford

Magnaporthe grisea is the causal agent of rice blast disease. The fungus elucidates a specialised infection structure, the appressorium, to directly penetrate the plant cuticle. This melanized dome-shaped cell is produced following the perception and integration of a number of host-derived cues, such as the presence of a hard, hydrophobic surface and cutin monomers, via various signal transduction cascades. Indeed, much attention has focused on studying signal relay and its pivotal role in appressorium formation.

In *Colletotrichum trifolii*, the causal agent of alpha alpha anthracnose, a lipid induced protein kinase (LIPK) is thought to be involved in the detection of the cutin monomer plant signal and appressorium development has been identified in *C. trifolii*, the causal agent of alpha alpha anthracnose. We have identified a putative homologue of the *CtLIPK* in *M. grisea*, and have determined by qrt-RTPCR that its transcript is up-regulated during appressorium formation and penetration. Targeted gene replacement of the *MgLIPK* gives a mutant with reduced fitness and an unusual phenotype; reduced colony pigmentation and detergent wettability, and severely reduced conidiation. The mutant is less pathogenic than wild type Guy11 on barley and produces multiple appressoria on artificial surfaces. Application of exogenous cAMP (IBMX) partially restores the WT phenotype on artificial surfaces but did not improve pathogenicity, suggesting that *MgLIPK* might be involved in later stages of infection. I shall present the data gathered thus far.

Pathogenicity of *Fusarium graminearum* and *F. culmorum* on wheat ears

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Fusarium ear blight (FEB) is a highly destructive disease that affects cereal crops and has now been reported in most wheat growing areas of the world [1]. The main causal agents of FEB are the hemibiotrophic filamentous ascomycetes *Fusarium graminearum* and *F. culmorum* (http://www.leatherheadfood.com/eman2/fsheet3_5.asp European Mycotoxin Awareness Network). As well as causing grain damage and yield reduction, many *Fusarium* species produce trichothecene mycotoxins (such as deoxynivalenol) that present a serious health hazard to humans and animals [2]. Using the datasets of the Pathogen-Host Interactions Database (www.phi-base.org), developed and maintained by scientists at Rothamsted Research, combined with our new chromosome visualisation software, MycoMap, we have identified a region of *F. graminearum* chromosome 1 possessing a cluster of verified pathogenicity gene homologues. Analysis of this target cluster region with a number of bioinformatics tools has indicated the degree of conservation of the region across closely and less closely related fungal species. Gene expression pattern, repeated element and predicted protein property investigations are allowing a thorough analysis of the evolution the region. Targeted deletion using the split marker technique of genes in the cluster, beginning with the pathogenicity gene homologue neutral trehalase (*NTH1*), will allow an investigation of the role of individual genes in *F. graminearum* pathogenicity. By this combined bioinformatics and reverse genetics approach the function of the entire cluster should emerge.

References

1. Parry, D.W., Jenkinson, P., McLeod, L. (1995) Plant Pathology **44**: 207-238.
2. Gang, G., Miedaner, T., Schuhmacher, U., Schollenberger, M., Geiger, H. H. (1998) Phytopathology **88**: 879-884.

Investigating the roles of reactive oxygen species during *Mycosphaerella graminicola* infection of wheat

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Mycosphaerella graminicola (anamorph *Septoria tritici*) is a hemibiotrophic fungal pathogen of wheat leaves. Infection reduces crop yield via the appearance of chlorotic / necrotic lesions, which reduce the photosynthetically active leaf area. In the early stages of infection, following penetration through stomata, the fungus grows in the leaf intercellular space without producing any specialised feeding structures or causing visible disease symptoms. In the later infection stages, fungal biomass increases, hyphal nutrition becomes necrotrophic and localised host programmed cell death occurs (1). Septoria leaf blotch disease is currently regarded as the most economically damaging disease of wheat in the UK and Western Europe.

Microscopy with reactive oxygen species (ROS)-specific stains has shown that hydrogen peroxide and superoxide are present in infected leaves, and that levels of ROS increase with the appearance of disease symptoms (1,3,4). In particular ROS are present on and within the asexual fruiting bodies, pycnidia. Microarray expression profiling of *M. graminicola* during infection of susceptible wheat genotypes has shown a number of genes, many of which have functions that are ROS-associated, that have greatly increased expression as disease progresses and symptoms become visible (1,2,3).

This project aims to better understand the molecular basis of the involvement of ROS in the disease-causing ability of *M. graminicola* on wheat. Candidate genes have been selected for functional analysis based either upon literature or their transcriptional up-regulation *in planta* during disease symptom formation. The recent availability of the sequenced genome of *M. graminicola* has enabled targeted *Agrobacterium*-mediated gene deletion to be used for investigating fungal genes involved in the production of ROS or the oxidative stress response. Results will be presented describing the progress made in the functional characterisation of these genes to evaluate their roles in plant infection.

1. Keon, J., Antoniw, J., Carzaniga, R., Deller, S., Hammond-Kosack, K., & Rudd, J. (2007) *MPMI*, **20**, 178-193.
2. Keon, J., Antoniw, J., Rudd, J., Skinner, W., Hargreaves, J., & Hammond-Kosack, K. (2005) *Fungal Genetics and Biology* **42,** 376-389.
3. Keon, J., Rudd, J. J., Antoniw, J., Skinner, W., Hargreaves, J., & Hammond-Kosack, K. (2005) *Molecular Plant Pathology* **6,** 527 - 540.
4. Shetty, N.P., Kristensen, B.K., Newman, M.A., Møller, K., Gregersen, P.L., & Jørgensen, H.J.L. (2003) *Physiological and Molecular Plant Pathology* **62**, 333-346

This project receives financial support from Syngenta and BBSRC. Rothamsted Research receives grant aided support from the BBSRC.

The translocation of *Phytophthora* effectors and their manipulation of host plant disease resistance.

Severine Grouffaud, Paul Birch, Stephen Whisson and Pieter van West
Scottish Crop Research Institute / University of Aberdeen

Like bacteria and fungi, oomycetes deliver effector proteins into the host cell during infection, but the translocation mechanism used by eukaryotic plant pathogens remains unknown. However, in oomycetes this process may depend on a short conserved amino acid sequence located near the signal peptide of many secreted proteins. Surprisingly, this motif, termed RXLR, is very similar to the host cell targeting-signal that is found in virulence proteins from the malaria parasite *Plasmodium falciparum.* Recently, the SCRI Phytophthora laboratory identified the avirulence gene *Avr3a* from the late blight pathogen *P. infestans*, and showed that the effector protein AVR3a was recognized by the product of resistance gene *R3a* in the host cytoplasm, triggering the hypersensitive response in the plant (Armstrong *et al*., 2005).

During the first part of this PhD project, the specificity of the RXLR motif is going to be investigated by transforming *P. infestans* with various *Avr3a* constructs, in which the translocation motif has been replaced with alternative sequences, from two other known avirulence genes from the oomycete *Hyaloperonospora parasitica*, and also with the malarial host-targeting signal. If the alternative sequence is functionally similar to the native one, then the transformation of a virulent strain *P. infestans* with such a construct should generate avirulent transformants.

SESSION 1B – PATHOGENICITY 2

Are glycolipid-anchored surface proteins involved in pathogenesis in *Magnaporthe grisea*?

Maeve Price and Sarah Gurr
Department of Plant Sciences, Oxford

The ascomycete fungus *Magnaporthe grisea* causes one of the world's most devastating diseases of rice. Since rice is the staple food crop in many countries, it is of paramount importance to learn more about the molecular basis of *M. grisea* infection in order to develop both resistant rice cultivars and to unmask specific targets for fungicides. Microarray analysis of a dataset derived from *M. grisea* conidia and germlings differentiating on artificial surfaces (Dean et al 2005 Nature 434 980-986) identified a cluster of 98 genes upregulated during pathogenesis (Lees et al 2007 J Comp. Biol 14 72-78). I used bioinformatics to ascribe putative "functions and structures" to the proteins encoded by these genes, with the aim of identifying candidate genes likely to be (a) unique to pathogenic fungi and (b) vital for pathogenesis. Based on these data I have selected two genes, MGG08370 and MGG11861, for targeted gene knockout, using a split marker approach (Fairhead *et al.* 1996). Both genes are homologous to the GAS1 gene, required for pathogenesis in *Candida albicans* and *Fusarium oxysporum* (Saporito-Irwin *et al.* 1994; Caracuel *et al.* 2005), and for maintenance of cell shape in *Saccaromyces cerevisiae* (Popolo and Vai 1999). Quantitative real time RT-PCR shows MGG08370 and MGG11861 are upregulated in conidia, placing them at the initiation of pathogenesis. Based on their homology to GAS1 it is likely that they are involved in cell wall biosynthesis and/or morphogenesis. In this talk, I shall describe the data thus far, speculate about the potential roles of GAS1 and its homologues and about how the two *M. grisea* GAS1 homologues may contribute to pathogenicity.

Molecular analysis of sterol C14α-demethylase gene (*CYP51*) from azole resistant and sensitive *Podosphaera fusca* isolates

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Cucurbit powdery mildew caused by *Podosphaera fusca* is one of the most important limiting factors for cucurbit production worldwide. With the recent spread of resistance to strobilurins (QoIs), reliable control is mainly dependent upon inhibitors of sterol C14α-demethylase (DMIs). To date, strains with reduced sensitivity to DMIs are widespread and additional applications of these chemicals are needed to maintain control. DMIs target cytochrome P450 (*CYP51*), so variation in this protein could explain variation for DMI sensitivity. To test this, the *CYP51* gene was isolated and sequenced from a small set of isolates which differed in their responses to DMI fungicides in populations of *P. fusca* in Spain. A transposon-like element upstream the promoter and amino acid substitution in the coding sequence were found. The relationship between these changes and DMI resistance in a larger set of isolates is currently being investigated.

Comparative analysis of amino acid assimilation pathways in *Pseudomonas* using hypergraphs

Aziz Mithani, Arantza Rico[†], Rachel Jones[†], Gail Preston[†] and Jotun Hein
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We present a tool for prediction and comparative analysis of metabolic pathways by representing metabolic networks as directed hypergraphs. A hypergraph is a generalisation of a graph where an edge (reaction) may connect any number of vertices (metabolites). Representing relationships between metabolites using hypergraphs makes it possible to enumerate multiple reactions connecting metabolites, and to predict possible pathways between metabolites or groups of metabolites. We demonstrate the tool by performing *in silico* predictions of amino acid assimilation pathways in *Pseudomonas* and comparing the results of these analyses to experimental data generated for genome-sequenced strains of *Pseudomonas*. Our results show that amino acids that are rapidly assimilated as carbon or nitrogen sources are generally predicted to have short pathways between the amino acid and the TCA cycle or ammonia. We can identify strain-specific differences in predicted assimilation pathways that are likely to account for strain-specific differences in amino acid assimilation. We have also identified discrepancies between experimental data and *in silico* analyses, which are likely to reflect gaps and errors in current annotations of metabolic pathways. For example, *Pseudomonas fluorescens* PfO-1 has been shown to assimilate alanine, but pathway predictions indicate that there are no short reaction paths from alanine to ammonia. Pathway predictions based on the KEGG reference pathway suggest that alanine-using *Pseudomonas* strains may contain an as yet unidentified aminotransferase, which catalyses conversion of alanine to pyruvate and an amino acid. By performing *in silico* comparison of metabolic networks we can identify differences in assimilation pathways that distinguish strains of *Pseudomonas syringae* from their non-pathogenic counterparts. This tool can be adapted for a wide range of applications ranging from genome-genome comparisons to genome-'X'ome (X: metabolome, transcriptome, proteome) comparisons and from predicting knock-out/insertion effects to understanding host-pathogen interactions and investigating toxic effects.

SESSION 2A – DEFENCE 1

The efficacy and biosafety of nematode control using synthetic, chemodisruptive peptides expressed transgenically in plants

Dong Wang, *University of Leeds*

Nematode location and invasion of plant roots relies on chemoreception. Synthetic peptides can disrupt these processes after their retrograde transport into certain nematode chemoreceptive sensillae and their neurons. The targets of two distinct peptides are acetycholinesterase and nicotinic acetylcholine receptors. Our aims are to define if such peptides can control nematodes when expressed in plants and to determine the extent of any adverse effects of the approach on non-target organisms. This biotechnology relies on the continual presence of a low level of peptide sufficient to inhibit chemoreception in the root and rhizoplane. It involves much lower levels than used for acetycholinesterase inhibition by pre-plant soil application of a nematicide such as aldicarb. Our preference is for a nicotinic receptor-binding peptide. We have shown a fluorescent form of this peptide passes up amphidial neurons to their cell bodies and synapses. We are now defining the specificity of the effect and any consequences for non-targeting soil nematodes and micro-organisms. Significant inhibition of infection in occurs in glasshouse trials when plant parasitic nematodes challenge peptide-expressing plants. The work is now defining the value of promoters that restrict peptide expression to regions of the root where nematodes invade. Peptides expressed by plant roots may provide a replacement biotechnology for conventional nematicides providing efficacy and biosafety can be assured.

Characterisation of potato genes that respond to parasitism by *Globodera pallida*

Jamie Smith,
University of Leeds

Cyst nematodes are plant endoparasites that invade roots and induce the formation of a feeding site termed a syncytium. They induce the syncytium by introducing secretions into an initial feeding cell close to the xylem tissue followed by recruitment of surrounding cells into the feeding site by progressive cell wall degradation and cytoplasmic fusion. Current methods of nematode control involve pesticides which are harmful to mammal, bird and insect species. As a result many of these products are being removed from the market. The aim of this study is to identify potato genes that are differentially regulated upon infection with the cyst nematode *G. pallida* and to determine their importance. Various genes of interest have been selected based on microarray studies of *Arabidopsis thaliana* roots challenged with the cyst nematode *Heterodera schachtii.* RT-PCR is being undertaken to determine if these genes are similarly expressed in infected potato roots. Future work will involve the cloning of the promoters of these genes into GUS reporter vectors and subsequent transformation into potato to confirm these expression patterns.

How bacteria use acidic overcoats to hinder detection by innate plant defences

Shazia N. Aslam[1], Kate L. Morrissey[1], Robert W. Jackson[1], Marc R. Knight[2], Delphine Chinchilla[3], Thomas Boller[3], Gitte Erbs[4], Tina Tandrup Jensen[4], Mari-Anne Newman[4] and Richard M. Cooper[1].

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Many studies have implicated extracellular polysaccharides (EPS) secreted by bacterial pathogens in pathogenicity and fitness. However, the precise function of these molecules is unknown. Polyanionic EPSs, such as alginate (*Pseudomonas syringae*), xanthan (*Xanthomonas campestris*) and amylovoran (*Erwinia amylovora*) bind divalent cations, especially the key signaling ion, calcium. We hypothesized that, following invasion of host intercellular spaces, Ca^{2+} ions stored in the plant cell wall are sequestered by EPS thus hindering calcium influx to the cytosol which in turn prevents initiation of defences. We have shown with aequorin-transformed *Arabidopsis* that pure EPSs can suppress calcium influx associated with recognition of flagellin and other PAMPs such as EF-Tu. After EPS treatment of *Arabidopsis* cells, we could still measure specific binding activity of radiolabelled flg22 to FLS2 receptor, suggesting that EPSs do not block non-specifically binding of elicitors to the cell surface. An EPS knockout mutant of *X. campestris* triggers more rapid and greater calcium influx than its respective wild type. Defence genes (monitored by real time PCR) are induced by PAMPs and by EPS-defective mutants, but expression is also suppressed by EPS supplied in pure forms or produced from wild type cells. In contrast to PAMPs, most EPSs were not able to trigger oxidative burst and apoplast alkalinisation. Interestingly, calcium-saturated xanthan but not free xanthan triggered defence genes *PR1* and *PDF 1.2*, suggesting suppression of PAMP activity only by the free form. The widespread production of these ion-binding acidic polymers by diverse bacterial pathogens and mutualists suggests that EPSs play a key role in establishing compatibility by hindering bacterial recognition.

Ubiquitination and Plant Pathology

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Plants are constantly exposed to a variety of biotic stresses (i.e. pathogen infection and insect herbivory). To survive these attacks, plants have developed molecular

mechanisms to perceive the threat posed by a pathogen and trigger signals to manifest adaptive responses with proper physiological and morphological changes. We are aiming to understand at a mechanistic level, how whole plants interact with microbial pathogens and initiate defence responses. Although genetic approaches have shown that various resistance genes activate signal transduction pathways little is known of how this is achieved biochemically. By focusing on the ubiquitin-proteasome system we will target key biochemical events which bring about defence signaling during biotic stress conditions. The project is aimed at identifying important ubiquitinated negative regulators of molecular mechanisms controlling pathogen stress signaling. This will potentially lead to the discovery of novel genes and their roles in stress signaling pathways.

SESSION 2B – DEFENCE 2

The roles of Cathepsin B-like proteases in plant disease resistance

Hazel McLellan[1], Eleanor Gilroy[2], Ingo Hein[2], Petra Boevink[2], Paul Birch[2] & Gary Loake[1]
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The hypersensitive response (HR) in plants is a form of programmed cell death (PCD), which is under genetic control. Apoptosis in animals is also a form of PCD and it is thought that some mechanisms may be conserved between the HR and apoptosis; particularly with respect to the involvement of caspase-like enzymes and other proteases. Cathepsin B is a non-caspase cysteine protease which was identified in a screen in *Solanum tuberosum* for genes up-regulated in a compatible HR against the oomycete *Phytophthora infestans*. Previous work has demonstrated that silencing *Cathepsin B (CTB)* in *Nicotiana benthamiana* greatly reduced the HR and consequently increased susceptibility to non-host pathogens and compromised resistance (*R*)-gene mediated protection.

Further work carried out using GFP and mRFP protein fusions has localised *Nb*CTB to the apoplast where there is potential for it to interact with pathogen effectors. Also, overexpressed, active *Nb*CTB has been isolated from the apoplast giving us a system to assay the effects of various inhibitors and pathogen effectors on CTB activity. *Arabidopsis* has three homologues of the potato and tobacco *CTB* genes. T-DNA insertion knockout lines have been isolated for each of these genes. As these genes have >80% similarity and functional redundancy is thought to occur, double and triple knockout lines as well as plants over-expressing *CTB* have been constructed and are currently being analysed. Preliminary results indicate that *Arabidopsis CTB* genes may be involved in cell death and defence signalling mediated by coiled-coil- nucleotide binding site-leucine rich repeat (*CC-NBS-LRR*) & Toll interleukin-1 receptor (*TIR*)-*NBS-LRR R*-genes. In contrast, these proteases do not seem to be required for basal disease resistance.

Exploring resistance to fusarium ear blight

Sarah Lee and Kim Hammond-Kosack
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Fusarium ear blight (FEB), caused principally by the fungal pathogens *Fusarium graminearum* and *Fusarium culmorum*, is a devastating disease of wheat[1]. The problems caused by ear infection are two fold: firstly, shrivelling of grain causes a reduction in yield and quality and secondly, the accumulation in the grain of *Fusarium* trichothecene mycotoxins, primarily deoxynivalenol (DON) and its acetylated derivates 3-ADON and 15-ADON and nivalenol (NIV), results in a reduction in quality and is a concern for food safety. Control of the disease is difficult. The use of resistant cultivars is now considered to be the best control option[2]. In this project, hexaploid wheat genotypes from around the world have been screened in field trials over two years for resistance to FEB. Harvested grain from the trial was analysed using gas chromatography-mass

spectrometry to assess the quantity of DON mycotoxin present. Genotypes which showed reduced disease symptoms and/or mycotoxin accumulation are now being analysed further under controlled environment conditions. The infection biology in the more resistant genotypes is being investigated in two ways. Firstly, the measurement of a DON breakdown product DON-3-glucoside[3] will establish whether the mycotoxin is being broken down *in planta*. Secondly, using transgenic isolates of *Fusarium graminearum* producing the reporter protein β-glucuronidase (GUS), the route of infection can be further explored.

1. US Wheat and Barley Scab Initiative Website – http://www.scabusa.org 2. Parry, D.W., *et al*. (1995). *Plant Pathology*, **44**, 207-238. 3. Lemmens, M., *et al*. (2005). *MPMI*, **18**, 1318-1324.

AtBTB1 is a negative regulator of the hypersensitive response in *Arabidopsis thaliana*

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Plants have evolved numerous defence systems that prevent infections caused by phytopathogenic bacteria. Resistance depends on a local triggering of a set of defence reactions and cell death around the infection site, a process known as the hypersensitve response (HR). The ubiquitin-proteasome system controls many signal transduction pathways including plant defence through targeted protein degradation. It was recently shown in our lab that the E3 ubiquitin ligase AtPUB17 is involved in disease resistance in *Arabidopsis thaliana* (Yang et al., 2006). In a yeast-two-hybrid screen we isolated AtBTB1, a potential interactor of PUB17. AtBTB1 is a BTB/POZ domain protein containing a MATH domain at its C-terminus. AtBTB1 knock out plants have a lesion mimic phenotype, suggesting that AtBTB1 may be a negative regulator of cell death.

The tobacco Ubiquitin-specific protease NtCDD1 positively regulates cell death during plant disease resistance

Richard A. Ewan**,** Craig Carr, Elizabeth O' Donnell, Ari Sadanandom,
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The hypersensitive cell death response (HR) and disease resistance are intimately connected and crucial in plant defence against pathogens. Although Resistance (R) proteins are known to be important players in pathogen recognition little is known of the molecular mechanisms downstream of this recognition event leading to hypersensitive cell death. The Ubiquitin-Proteasome system is a key regulatory mechanism in many plant signaling pathways and a number of E3 ubiquitin ligase have been implicated in disease resistance specified by multiple R-genes. We have now identified a Ubiquitin-specific Protease (NtCDD1) that positively regulates Avr9/Cf9 elicited hypersensitive cell death in tobacco. We silenced *NtCDD1* in solanaceous species to demonstrate its role in disease resistance against a fungal and viral pathogens. The target proteins deubiquitinated by NtCDD1 represent key regulators of the plant HR. This data represents a first example linking regulatory deubiquitination to disease resistance signaling in plants.

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