

### PS4.1

#### **$\beta$ -1,3-glucan synthase of the maize anthracnose fungus *Colletotrichum graminicola* is essential at specific stages of pathogenesis**

Ely Oliveira-Garcia, [Holger B. Deising](#)

*Martin-Luther-University Halle-Wittenberg, Interdisciplinary Center for Crop Plant Research, Betty-Heimann-Str. 3, D-06120 Halle (Saale), Germany.*

Covalently cross-linked  $\beta$ -1,3-glucan and chitin are the most prominent and morphogenetically relevant carbohydrate polymers of fungal cell walls. In most filamentous fungi several chitin synthases, but only a single  $\beta$ -1,3-glucan synthase contribute to forming the glucan-chitin core. While the role of individual chitin synthase genes in pathogenic development has been analyzed in several plant pathogenic fungi, functional analyses of  $\beta$ -1,3-glucan synthase genes, encoding the catalytic subunit of the  $\beta$ -1,3-glucan synthase complex, is lacking.

We investigated the role of the  $\beta$ -1,3-glucan synthase gene (*GLS1*) in infection structures of the maize pathogen *Colletotrichum graminicola*. Infection assays with a *GLS1:eGFP* replacement strain, in combination with aniline blue fluorochrome-staining, showed that massive  $\beta$ -1,3-glucan synthesis occurs in conidia, appressoria and necrotrophic hyphae, but, surprisingly, not in biotrophic hyphae. As targeted deletion of *GLS1* was lethal, we employed RNA interference (RNAi) to generate transformants gradually differing in *GLS1* transcript abundance. Appressoria of RNAi strains had reduced turgor pressure, elastic, inefficiently melanized cell walls, and many of these infection cells exploded spontaneously. Due to loss of appressorial adhesion, penetration of intact maize leaves did not occur and normally shaped biotrophic primary hyphae formed on the maize cuticle. In wounded leaves, only necrotrophic hyphae were found, as indicated by strains carrying biotrophy- and necrotrophy-specific promoters controlling expression of *eGFP*. Necrotrophic hyphae formed by RNAi strains in the host tissue were severely distorted, hyper-melanized, and unable to cause spreading disease. Our studies suggest that *GLS1* is essential in appressoria and fast growing necrotrophic, but not in biotrophic hyphae of *C. graminicola*.

## PS4.2

### Effectors and Biotrophic Invasion by the Rice Blast Fungus, *Magnaporthe oryzae*

Barbara Valent<sup>[1]</sup> Mihwa Yi<sup>[1]</sup> Martha C. Giraldo<sup>[1]</sup> Chang Hyun Khang<sup>[1,2]</sup> Melinda Dalby<sup>[1]</sup> Kirk Czymmek<sup>[3]</sup> Mark Farman<sup>[4]</sup>

<sup>1</sup>Department of Plant Pathology, Kansas State University, Manhattan, Kansas, U.S.A.; <sup>2</sup>Department of Plant Biology, University of Georgia, Athens, Georgia, U.S.A.; <sup>3</sup>Department of Biological Sciences and Delaware Biotechnology Institute, Newark, Delaware, U.S.A.; <sup>4</sup>Department of Plant Pathology, University of Kentucky, Lexington, Kentucky, U.S.A.

Blast disease, caused by the haploid ascomyceteous fungus *Magnaporthe oryzae*, remains a major disease of rice, and wheat blast has emerged as a threat to global wheat production since it was first identified in Brazil in 1985. To cause disease, *M. oryzae* sequentially invades living rice cells using intracellular invasive hyphae (IH) that are enclosed in host-derived extracellular-hyphal membrane. In each subsequently colonized host cell, IH initially grow as filamentous hyphae and then switch into pseudohyphal-like bulbous hyphae that proliferate throughout the host cell. Fluorescently-labeled avirulence effectors are secreted from the fungus and accumulate in the biotrophic interfacial complex (BIC), which forms at the filamentous IH tip and remains beside the first differentiated bulbous IH cell as the IH continues to grow. Effectors that show preferential BIC localization are translocated into the rice cytoplasm, suggesting that BICs are a staging center for effector delivery into the host cell. In addition to known effectors, 80 biotrophy-associated-secreted (BAS) proteins localize to BICs. Twenty-six of these BAS proteins are translocated into the cytoplasm of invaded rice cells. Additionally, 25 translocated effector or BAS proteins move ahead into neighboring rice cells before invasion by the fungus, presumably to prepare these host cells before invasion. Two translocated proteins naturally accumulate in rice nuclei and six others accumulate where the IH crossed the rice cell wall into neighboring cells. This talk will focus on current understanding of effector secretion into BICs and translocation into rice cells, and on effector sequences that mediate these processes.

## PS4.3

### Downy mildew effectors and their activity in the host plant

Stan Oome<sup>[1,2]</sup> Joost Stassen<sup>[1]</sup> Adriana Cabral<sup>[1]</sup> Ruslan Yatusevich<sup>[3]</sup> Jane Parker<sup>[3]</sup> Guido van den Ackerveken<sup>[1,2]</sup>

<sup>1</sup>Plant-Microbe Interactions, Utrecht University, Padualaan 8, 3584 CH, Utrecht, The Netherlands. <sup>2</sup>Centre for BioSystems Genomics (CBSG), Wageningen, The Netherlands. <sup>3</sup>Department of Plant-Microbe Interactions, Max Planck Institute for Plant Breeding Research, Cologne, Germany

Downy mildews are obligate biotrophic pathogens belonging to the oomycetes. Each downy mildew species is highly specialized on a particular host plant with which it has strongly co-evolved. This is evident from the secretomes of these oomycetes that contain numerous species-specific proteins, many of which are candidate effector proteins that could interfere with plant life in order to create a favourable environment for pathogen infection. We are studying effectors of the Arabidopsis downy mildew *Hyaloperonospora arabidopsidis*, in particular apoplastic effectors that act extracellularly in plant tissue and RXLR effectors that are host-translocated and therefore have their presumed activity inside plant cells. We will report on the progress of the analysis of apoplastic effectors belonging to the group of necrosis- and ethylene-inducing proteins and glucose-6 phosphate epimerases, and on the immune-suppressive activity of RXLR effectors. The mode-of-action of several RXLR effectors is being uncovered by identification of interacting host-target proteins and their immunity-related activity. These studies are revealing the mechanisms by which downy mildews interfere with host cell processes. In addition, knowledge on effectors will aid breeding for resistance to oomycetes in crops. We have made a first step towards application by identifying RXLR effectors in the lettuce downy mildew *Bremia lactucae* that are now being used to select lettuce lines with new resistance specificities.

#### PS4.4

##### **SpHtp1 from the oomycete *Saprolegnia parasitica* shows fish cell-specific entry and tyrosine-O-sulphate-dependent import**

Stephan Wawra<sup>[1]</sup> Judith Bain<sup>[2]</sup> Elaine Durward<sup>[1]</sup> Irene de Bruijn<sup>[1]</sup> Kirsty L. Minor<sup>[1]</sup> Stephen C. Whisson<sup>[3]</sup> Andy J. Porter<sup>[2]</sup> Paul R.J. Birch<sup>[3]</sup> Chris J. Secombes<sup>[4]</sup> Pieter van West<sup>[1]</sup>

<sup>1</sup>Aberdeen Oomycete Laboratory, UK <sup>2</sup>University of Aberdeen, UK <sup>3</sup>The James Hutton Institute, Dundee, UK <sup>4</sup>Scottish Fish Immunology Research Centre, University of Aberdeen, UK

The eukaryotic oomycetes, or water moulds, contain several species that are devastating pathogens of plants and animals. During infection, oomycetes translocate effector proteins into host cells, where they interfere with host defence responses. For several oomycete effectors (e.g. the RxLR-effectors) it has been shown that their N-terminal polypeptides are important for delivery into the host. We found that the N-terminus of the putative RxLR-like effector SpHtp1 from the fish pathogen *Saprolegnia parasitica* shows host cell specific translocation. The translocation process can be blocked by enzymatic desulfation of cell surface proteins, by sulfotransferase inhibitors and antibodies specifically recognising tyrosine-O-sulphate. The quantitative analysis of these inhibitory effects suggests that the uptake process for SpHtp1 requires binding to tyrosine-O-sulfate modified cell surface molecules. No evidence was found that the SpHtp1 translocation involves phospholipid binding, as has been reported for RxLR-effectors from plant pathogenic oomycetes. Here we show a novel effector translocation route based on tyrosine-O-sulfate binding, which could be highly relevant for a wide range of host-microbe interactions.

#### PS4.5

##### **Calnexin complex is involved in the establishment of fungal biotrophy in *Ustilago maydis***

Alfonso Fernandez-Alvarez, Alberto Jimenez-Martin, Miriam Marin-Menguiano, Alberto Elias-Villalobos, Jose I Ibeas

*Universidad Pablo de Olavide, Sevilla, Spain*

Protein N-glycosylation consists in the addition of an oligosaccharide core of acetilglucoseamines, glucoses and mannoses to the nascent N-glycoproteins in the Endoplasmic Reticulum (ER). Later, N-glycoproteins undergo maturation processes catalysed by two glucosidases and three mannosidases, which remove specific sugar residues. The  $\alpha$ -glucosidase II has been previously described as required for virulence in the corn smut fungus *Ustilago maydis* (Schirawsky, et al., 2005). Here, we characterize the role of the  $\alpha$ -glucosidase I,  $\beta$ -glucosidase II and  $\alpha$ -mannosidase during the pathogenic development of *U. maydis* by using genetic and biochemical approaches. Interestingly, we have observed that the maturation of glucoses but not mannoses is crucial for virulence. Using a 2D-DIGE proteomic screening to identify putative substrates of  $\alpha$ -glucosidase I, we have found that the protein disulfide isomerase Pdi1, a calnexin partner at the ER quality control system, has a different electrophoretic mobility in N-glycosylation mutant cells. This system is highly conserved in eukaryotic cells, although poorly known. We show now that the ER quality control system for N-glycoproteins is functional in the basidiomycete *U. maydis* and that it is specifically required for the establishment of the biotrophic state between this fungus and its host.

#### PS4.6

##### **Functional analysis of candidate effector proteins by Host-Induced Gene Silencing in *Blumeria graminis* sp. *hordei***

Clara Pliego<sup>[1]</sup> Daniela Nowara<sup>[2]</sup> Giulia Bonciani<sup>[1]</sup> Dana Gheorghe<sup>[1]</sup> Patrick Schweizer<sup>[3]</sup> Laurence Veronique Bindschedler<sup>[4]</sup> Rainer Cramer<sup>[4]</sup> Pietro D. Spanu<sup>[1]</sup>

<sup>1</sup> Department of Life Sciences, Imperial College London, South Kensington Campus, London SW7 2AZ, UK <sup>2</sup> Institute of Plant Genetics and Crop Plant Research, 06466-Gatersleben, Germany <sup>3</sup> Leibniz-Institute of Plant Genetics and Crop Plant Research, 06466-Gatersleben, Germany <sup>4</sup> Department of Chemistry, University of Reading, Whiteknights Campus, Reading, RG6 6AS, UK

The powdery mildew fungus *Blumeria graminis* is one of the most significant pathogens of cereal crops. In this obligate biotroph haustoria are responsible for nutrient uptake and are hypothesized to deliver effectors which modulate disease development. We selected a panel of fifty *Blumeria* Candidate Effector proteins (BECs) focussing on small proteins detected specifically in haustoria and predicted to be secreted. We then screened them to detect effector action by Host-Induced Gene Silencing on barley. This uses a transient assay system based on bombardment of epidermal cells with RNAi constructs. Seven out of the fifty BECs caused a significant effect in the reduction in the percentage of conidia capable of forming haustorium. The strongest effects were obtained for BEC1011 and BEC1054, two related genes that are part of a small gene family. Complementation analysis of these two effectors by transient over-expression of synthetic homologs resistant to RNAi demonstrated no cross-silencing. BEC1011 appeared to interfere with host cell death. Three BECs, BEC1005, BEC1054 and BEC1019 had domains reminiscent of a glucosidase, an RNase and a protease, respectively. We are currently testing whether these proteins display the actual enzymatic activity. The outcome of this research will lay the foundation for a detailed understanding of the molecular mechanisms underlying disease in the cereal mildews.

#### PS4.7

##### **Elucidating the response of wheat to the exposure of *Stagonospora nodorum* effectors**

Lauren Du Fall, Peter Solomon

Plant Sciences, The Australian National University, Canberra ACT, Australia 0200

The dothideomycete *Stagonospora nodorum* is a necrotrophic fungal pathogen of wheat and is the causal agent of *Stagonospora nodorum* blotch (SNB)<sup>1</sup>. This disease is responsible for over \$100 million of yield losses in Australia annually. Recent studies have shown that this fungus produces a number of effector proteins that are internalised into host cells of susceptible wheat cultivars. The mechanism by which these effectors induce tissue necrosis in susceptible hosts is yet to be fully elucidated. We have applied a metabolomics approach to elucidate the cellular processes leading to disease and provide insight into the mode-of-action of these effectors. Gas chromatography-mass spectrometry analysis of primary polar metabolites has been undertaken on tissue extracts and apoplastic fluid from SnToxA infiltrated wheat. Results illustrate widespread perturbations in primary metabolism and reveal the first direct evidence of an increase in energy production in response to a pathogen effector. To further understand the host response to SnToxA at the secondary metabolism level, samples were also analysed using liquid chromatography-mass spectrometry. Our data indicate SnToxA causes an increase in defence-related secondary metabolites. The effect of these metabolites on *Stagonospora nodorum* growth and sporulation in vitro and in planta is currently under investigation. These complementary approaches have provided a novel insight into the contribution of the SnToxA effector protein to SNB in wheat.

1 Oliver, R. P. & Solomon, P. S. New developments in pathogenicity and virulence of necrotrophs. *Current Opinion in Plant Biology* (2010).

#### **PS4.8**

##### **How do mobile pathogenicity chromosomes collaborate with the core genome?**

Charlotte van der Does, Martijn Rep  
*University of Amsterdam*

In the tomato pathogen *F. oxysporum* f. sp. *lycopersici*, most known effector genes reside on a pathogenicity chromosome that can be exchanged between strains through horizontal transfer. As a result, this fungus has sub-genomes with different evolutionary histories: the conserved core genome and the mobile 'extra' genome. Interestingly, expression of the effectors on the mobile genome requires Sge1, a conserved transcription factor encoded in the core genome. Also, a transcription factor on the mobile chromosome, Ftf1, is associated with pathogenicity (de Vega-Bartol et al. 2011). Random insertion of an effector-promoter GFP reporter construct revealed that the position in the genome has a strong influence on the level of expression. Furthermore, in all transformants tested, expression could no longer be induced, but was constitutive. To discover how Sge1 and Ftf1 control gene expression from the core genome and the mobile chromosome(s), their targets will be identified using ChIPseq: sequencing of DNA fragments to which Sge1 binds and RNAseq: in depth-sequencing of transcripts, comparing standard culture conditions to *in planta*-mimicing conditions. Additional components necessary to express effector genes on mobile chromosomes will be identified *via* insertional mutagenesis.

### **Sunday 1 April**

#### **Parallel session 5: Mitochondria**

#### **PS5.1**

##### **Functional analysis of ERMES and TOB (SAM) complex components in *Neurospora crassa*.**

Frank E. Nargang, Sebastian W.K. Lackey, Jeremy G. Wideman  
*Department of Biological Sciences, University of Alberta, Edmonton, Alberta T6G 2E9*

The *Neurospora crassa* TOB complex (topogenesis of beta-barrel proteins), also known as the SAM complex (sorting and assembly machinery), inserts a subset of mitochondrial outer membrane proteins into the membrane—including all beta-barrel proteins. The TOB core complex contains Tob55, Tob38, and Tob37. Each of these proteins is essential for viability of *N. crassa*. The TOB holo complex contains an additional protein called Mdm10. Deficiency of any of these proteins results in impaired assembly of beta-barrel proteins. Lack of Mdm10 also results in a phenotype of enlarged mitochondria. The Mdm10 protein has also been shown to be a member of the ERMES complex (endoplasmic reticulum-mitochondria encounter structure) in *Saccharomyces cerevisiae*. The complex is thought to function in lipid and calcium exchange between the two organelles. Other members of the ERMES include the Mdm12, Mmm1, and Gem1 proteins. We have shown that deficiency of Mdm12 or Mmm1 also results in the presence of enlarged mitochondria and impaired beta-barrel protein assembly into the mitochondrial outer membrane while lack of Gem1 does not. The possibility that different domains of the proteins of the TOB and ERMES complexes are responsible for specific interactions and functions is currently being explored.