Workshop II

Fungal-Plant Interactions
Chair: Henriette Giese

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STRATEGIES FOR FUNCTIONAL ANALYSES OF FUNGAL PATHOGENESIS RELATED GENES

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The study of Plant-Fungal Interactions has entered a new era since whole genome sequences of plants and pathogenic fungi has been made public. The Initiative of the Whitehead Institute has made possible comparative genetics of diverse fungi with different lifestyles and may well be the factor that leads to the determination of the function of many novel genes. This is particularly true for the obligate parasites such as *Blumeria graminis* where no efficient transformation system is yet available to carry out functional analyses. For known function genes it offers the possibility to directly address the predicted processes in relation to pathogenicity.

Transformation technology has facilitated not only functional analyses by silencing or over expression but also *in planta* analyses of how the fungus spreads inside the plant. Different GFP constructs combined with confocal microscopy allow the study of control of gene expression *in planta* to correlate gene activity and pathogenicity. Combination of sequence information and transformation facilitates the use of surrogate hosts to study the function of novel genes from an obligate pathogen. Gene disruptions by transfomation and selection for particular phenotypes combined with sequence information is also very powerful tool for the identification of pathogenesis related genes.

Examples will be given to illustrate the use of GFP to study the infection processes of *Fusarium culmorum* in barley roots. The use of mutagenesis combined with the knowledge of the *F. graminearum* genome sequence to identify the gene cluster encoding the polyketide aurofusarin will be shown. Finally the identification of a *Blumeria graminis* pathogenesis related gene family and the use of a surrogate host will be described.

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TRANSCRIPTOMICS AND METABOLOMICS FORM AN INTEGRATED APPROACH TO UNRAVEL THE SYMBIOTIC INTERACTION BETWEEN PERENNIAL RYEGRASS AND NEOTYPHODIUM LOLII.

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Perennial ryegrass (Lolium perenne), an agriculturally important pasture grass is typically associated with Neotyphodium lolii, a symptomless seed borne endophytic fungus. These endophyte-grass associations display prolonged field persistence over their endophyte free equivalents. This is presumably as a consequence of the endophyte-induced protection from insect and mammalian herbivory, as well as to some abiotic stresses. In the case of N. lolii, ergot alkaloids (ergovaline), the pyrrolopyrazine alkaloid peramine, and indole-diterpenoids (lolitrem) are fungal secondary metabolites with proposed plant protective roles. Other uncharacterised endophyte secondary metabolites may also play vital roles in this association, in addition to grass metabolites, that may be induced or repressed by the presence of endophyte. A detailed analysis of the metabolome and transcriptome of the perennial ryegrass-N. lolii association is currently under investigation using the approaches of metabolomics and transcriptomics. Endophyte infected perennial ryegrass will be compared with endophyte free. Preliminary metabolite data has been generated for sugars, phenolics, alkaloids and amino acids from both endophyte infected and endophyte-free perennial ryegrass. Microarray expression data will be linked with the metabolic profile of the N. lolii-grass symbiosis in order to develop metabolite pathway models. These models will ultimately enable us to connect enzymes with metabolites and enable us to identify the biosynthetic function of genes. Several non-ribosomal peptide synthetase (NRPS) genes with unknown function have been identified in N. lolii. These enzymes are commonly associated with secondary metabolite biosynthetic pathways in filamentous fungi and are involved in the production of many important bioactive peptides, such as antibiotics and toxins. We intend to use metabolomics and transcriptomics together to analysis ryegrass plants infected with endophyte strains mutated in these genes. Progress towards this goal has been accomplished by generating endophyte gene knockouts of two non-ribosomal peptide synthetases (NRPSs).

THE REGULATION OF TARGET GENES OF AT LEAST TWO SIGNALLING CASCADES VIA THE G ALPHA SUBUNIT BCG1 OF BOTRYTIS CINEREA IN THE INTERACTION WITH PHASEOLUS VULGARIS

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The G alpha subunit BCG1 plays an important role during the infection of host plants by Botrytis cinerea (1). Δbcg1-mutants are able to conidiate, to penetrate host tissue and to produce small primary lesions. However, in contrast to the wild-type, the mutants completely stop invasion of plant tissue at this stage; secondary lesions have never been observed. Suppression sub-tractive hybridization (SSH) was used to identify fungal genes whose expression on the host plant is specifically affected in bcg1 mutants(2). Among the 22 differentially expressed genes we found those which were predicted to encode proteases, enzymes involved in secondary me-tabolism and those encoding cell wall-de-grading enzymes. All these genes are highly expres-sed during infection in the wild-type but not in the mutant. However, the genes are expressed in both the wild-type and the mutant under certain con-ditions in vitro. In order to show if all BCG1-controlled genes are regulated via the cAMP signalling pathway, we analyzed their expression in adenylate cyclase (BAC) mutants (3). Unexpectedly, most of the BCG1-controlled genes are still expressed in bac mutants in planta, suggesting that BCG1 is involved at least in one additional signalling cascade beside cAMP-depending pathway. Only four of the genes (e.g. a xylanase-encoding gene) are indeed regulated by BCG1 and BAC via the cAMP pathway. Reporter gene assays with promoters of one gene from the cAMP and one gene from the yet unknown signalling pathways will give new insights to the BCG1 regulatory network and the corresponding transcription factors.
In a second SSH approach, 1,500 clones were screened for those that are specifically in-duced by the wild-type during the infection of bean leaves(2). Eleven of the twenty two BCG1-controlled genes were also found in the in planta SSH library. Therefore, SSH technology can be success-fully applied to identify target genes of signalling pathways and differentially in planta expressed genes.

(1) Schulze Gronover C, Kasulke D, Tudzynski P and Tudzynski B (2001) The role of G protein alpha subunits in the infection process of the gray mold fungus Botrytis cinerea. Molecular Plant-Mi-crobe Interactions 14: 1293-1302

(2) Schulze Gronover C, Schorn C, and Tudzynski, B (2004) Identification of Botrytis cinerea genes up-regulated during infection and controlled by the G alpha subunit BCG1 using suppression subtractive hybridization (SSH). Molecular Plant-Microbe Interactions (in press)

(3) Klimpel A, Schulze Gronover C, Williamson B, Stewart JA and Tudzynski B (2002) The adenylate cyclase (BAC) in Botrytis cinerea is required for full pathogenicity. Molecular Plant Pathology 3: 439-450

SIMULTANEOUS SILENCING OF MULTIPLE GENES IN THE APPLE SCAB FUNGUS VENTURIA INAEQUALIS BY EXPRESSION OF CHIMAERIC INVERTED REPEATS

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Post transcriptional gene silencing is a mechanism of suppressing gene expression in a sequence specific manner. This naturally occurring phenomenon of RNA interference (RNAi) has been used by researchers for gene function analysis in plants, fungi and animals. There are currently only three reports of RNAi in filamentous fungi, including Neurospora crassa (1), Cryptococcus neoformans (2) and Magnaporthe oryzae (3).
Here we report on gene silencing in the apple scab fungus Venturia inaequalis, a highly pigmented filamentous fungus which derives its olive green phenotype from DHN melanin (4). We have identified EST gene sequences involved in the DHN melanin biosynthesis pathway in this fungus by sequence similarity with other published and characterised fungal genes. Agrobacterium vectors were created to transform the fungus with hairpin constructs of the scytalone dehydratase (SD), Trihydroxynaphtalene reductase (THN) and GFP genes. The constructs were transformed to wild type and GFP-expressing V. inaequalis. Silencing of these three genes independently resulted in cultures with an obvious phenotype that was in agreement with the proposed function of the gene product of interest. In addition, co-silencing of the GFP and THN genes was observed when the GFP-expressing V. inaequalis was transformed with a chimaeric GFP-THN construct. Phenotypic observations have been supported with Real Time PCR and Northern analysis. THN-silenced transformants appear to maintain their pathogenicity on apple. The possibility to achieve simultaneous silencing of multiple genes in a single transformation opens new perspectives for gene function analysis.

(1) Romano N and Macino G (1992) Molecular Microbiology 6: 3343-3353
(2) Liu H, Cottrell TR, Pierini LM, Goldman WE and Doering TL (2002). Genetics 160: 463-470
(3) Kadotani N, Nakayashiki H, Tosa Y and Mayama S (2003) Molecular Plant Microbe Interactions 9: 769-776
(4) Bassam SE, Benhamou N and Carisse O (2002) Canadian Journal of Microbiology 48: 349-358

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Session: Fungal-plant interactions

ELUCIDATING THE ROLE OF THE F-BOX PROTEIN FRP-1 IN PATHOGENESIS OF FUSARIUM OXYSPORUM.

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During evolution, plants have developed effective ways to defend themselves against microbial invasion. A microbial pathogen has to break through these defences in order to colonize the plant. To investigate the genetic basis of this ability of pathogens, we use the interaction between the soil-borne fungus Fusarium oxysporum f.sp. lycopersici and its host tomato as a model system. Using insertional mutagenesis of F. oxysporum, an F-box protein called Frp1 was found to be required for pathogenicity. It was shown that this protein, like other F-box proteins, binds to Skp1, a subunit of the E3 complex. This complex is involved in the ubiquitinylation of proteins recruited by F-box proteins, marking them for degradation by the proteasome. To unravel the function of Frp1, other interacting proteins will be searched for, in particular ones that are recruited for degradation. To do so, a yeast two-hybrid screening using only the C-terminal part of Frp1 will be carried out. Another approach is the identification of interacting proteins by tandem affinity purification (TAP) and mass-spectrometry. TAP-tagged Frp1 will be generated and used to fish interacting proteins from an extract of Fusarium oxysporum. Interacting proteins will then be analysed for their role in pathogenicity.

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WORKSHOP FUNGAL-PLANT INTERACTIONS

CELL WALL BIOGENESIS AND PATHOGENICITY IN *FUSARIUM OXYSPORUM*

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Fusarium oxysporum f.sp. *lycopersici* is a soilborne pathogen that causes vascular wilt on tomato plants. Six chitin synthase (*chs*) genes have been identified up to now from its genome. Fungal chitin synthases participate in the biogenesis of the cell wall and are essential for the maintenance of cell shape, prevention of lysis, and take part in conidiation and regulation of the uptake of substances from the fungal environment (Roncero, Curr. Genet. 2003). In this plant pathogenic fungus a class V chitin synthase (ChsV) mediates resistance to plant defence compounds, such as the tomato phytoanticipin α-tomatine. This antifungal compound belongs to the saponin family, secondary metabolites produced by plants that provide a preformed chemical barrier against pathogens, and significantly inhibited hyphal growth of a loss-of-function *chsV* mutant (*chsV*) (Madrid *et al*., Mol. Microbiol. 2003). *F. oxysporum* f.sp. *lycopersici* and other tomato pathogens produce extracellular enzymes known as tomatinases, which deglycosylate α-tomatine to yield less toxic derivatives (Roldán-Arjona *et al*. Mol. Plant-Microbe Interact. 1999). To establish the molecular basis for the increased sensitivity shown by the Δ*chsV* mutant to α-tomatine, a constitutive copy of the tomatinase gene obtained by fusion of the coding region to the *Aspergillus nidulans gpdA* promoter has been introduced by genetic transformation into a Δ*chsV* mutant background. On the other hand, mutants harbouring an interrupted copy of the *FoTom1* allele are being isolated by targeted gene replacement of the wild type allele. In addition, a second chitin synthase class V gene (*chsV$_b$*), identified for being adjacent to the previously described *chsV* gene, has been isolated from a *F. oxysporum* genomic library. Currently targeted disrupted *chsV$_b$* mutants are being constructed and their physiological and pathotypical behaviour will be determined. All these mutants are expected to shed light on the cell wall biogenesis in *F. oxysporum* and the relationship between fungal cell wall integrity and pathogenicity.