IX
PLANT PATHOGENS AND THEIR MECHANISMS

Chair:

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Genome-wide RNA expression analysis during conidial maturation and germination in the filamentous fungus, *Fusarium graminearum*

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The fungal plant pathogen, *F. graminearum*, causes Fusarium head blight disease of wheat and barley. To understand the early infection cycle of this organism, we monitored the RNA expression profiles in newly formed spores (macroconidia), in maturing spores and during the early stages of spore germination using the 18 K probe set, *F. graminearum* Affymetrix GeneChip. Surprisingly high expression levels of thousands of genes were found in spores, structures presumed to be metabolically quiescent. Positive signals for 6,384 probe sets were detected in newly formed spores and 2,916 probe sets were detected in spores that had been aged for ten days (detection p value <0.001), indicating that spores are metabolically active. Newly formed spores differentially accumulate RNAs corresponding to proteins responsible for peroxisomal β-oxidation and key components of the glyoxylate cycle as well as gluconeogenesis. Many genes encoding catabolic enzymes involved in glucose production, such as enzymes for degrading long-chain carbohydrates, also are expressed in young spores. A total of 1,257 probe sets were up-regulated more than 2-fold during spore maturation including genes predicted to be involved in detoxification, cell wall synthesis, and autophagocytosis. Several genes encoding putative transmembrane transporters were preferentially or uniquely expressed during spore maturation, suggesting their involvement with detection of spore activation signals or in uptake of nutrients in early germination stages. Upon spore activation, a total of 2,149 probe sets were up- or down-regulated more than 2-fold during the first two hours. Spore activation was associated with high expression levels of genes involved in protein synthesis, transcription, and basic primary metabolism. Inference from gene expression profiles at different spore developmental stages may be used to develop hypotheses for the mechanisms controlling the basic processes of spore maturation, dormancy and activation.

Microarrays meet pathogenicity: Unravelling the secrets of pathogenic development in *Ustilago maydis*

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In the phytopathogenic fungus *Ustilago maydis*, plant infection is initiated by fusion of two haploid, yeast-like sporidia. The resulting dikaryon grows as a filament on the plant surface, however, only the tip cell is filled with cytoplasm. Cell proliferation is stalled until the fungus has successfully penetrated the plant epidermis. The key regulator for the switch from saprophytic to biotrophic growth is a complex of the two homeodomain proteins bE and bW that are encoded by the *b*-mating type locus.

To get insight into the processes that precede plant infection, we have performed a microarray analysis of *U. maydis* cells grown on the plant surface, comparing a pathogenic strain carrying an active bE/bW heterodimer with a non-pathogenic wild type strain. We identified 328 differentially expressed genes, some of which have putative functions in plant cell wall degradation, transport or transcriptional regulation. Interestingly, 41 % of genes induced in the pathogenic strain encode *U. maydis* specific proteins, most of which are predicted to be secreted. We presume that these proteins have distinct functions during infection, such as surface attachment, host recognition, or the suppression of plant defense reactions.

Four of the induced genes encode for putative transcription factors. Of particular interest is Biz1, a C2H2 zinc finger protein. Strains deleted for *biz1* show no obvious phenotype during the saprophytic growth stage, but are unable to penetrate the plant epidermis, resulting in a complete loss of pathogenicity. Microarray analysis revealed that *biz1* is both required and sufficient for the regulation of 20 genes induced in the pathogenic strain *on planta* that could account for the penetration defect in *Δbiz1* strains. Systematic deletion analysis of the *biz1* dependent genes led to the identification of *pst1*, encoding a potentially secreted *U. maydis* specific protein, as a novel pathogenicity factor. However, in contrast to the *Δbiz1* strain, the *Δpst1* strain is still able to penetrate the plant surface, but subsequently fails to invade and colonize the plant. Thus, *biz1* appears not only to be a regulator for genes required for plant penetration, but also for genes with impact on pathogenicity at later stages.

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Genome environment is instrumental in evolution towards virulence at the *AvrLm1* avirulence locus of *Leptosphaeria maculans*

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The Dothideomycete *Leptosphaeria maculans* is the most damaging disease of oilseed rape (*Brassica napus*) worldwide. Genetic studies demonstrated the occurrence of gene-for-gene interactions in the *L. maculans* / *B. napus* system. In this model, avirulence (Avr) proteins produced by the pathogen directly or indirectly activate plant defense responses, such as the hypersensitive response, upon recognition mediated by matching plant resistance (R) proteins. To date nine avirulence genes (*AvrLm1-9* genes) have been identified in the pathogen and the corresponding nine resistance genes (*Rlm1-9 genes*) were identified in the host plant. In France, disease control relies mainly on the use of disease-resistant cultivars. The *Rlm* genes effectively control the disease as long as the corresponding avirulent allele (*AvrLm*) dominates in the pathogen population. However, *L. maculans* has the ability to very rapidly adapt to the selection pressure exerted by a novel resistance gene as exemplified by the 3-year evolution towards virulence at the *AvrLm1* locus in French field conditions. Here, we investigated the molecular mechanisms responsible for the gain of this new virulence in French field isolates. The *AvrLm1* avirulence gene was recently cloned and shown to be a solo gene within a 269 kb non-coding, heterochromatin-like region consisting of mosaics of degenerated repeats. We fully or partly sequenced the *AvrLm1* genomic region in one avirulent and two virulent isolates. The gain of virulence was linked in both cases with a 260 kb deletion of a chromosomal segment spanning *AvrLm1* and deletion breakpoints were identical or similar for both the virulent isolates. Among 191 field isolates analyzed, a similar large deletion leading to chromosome length polymorphism was evidenced by multilocus haplotype analysis in 90% of the virulent isolates. Furthermore, deletion breakpoints were strongly conserved in all these virulent isolates leading to the hypothesis that a strong constraint in the genome environment is instrumental in generating a unique event of chromosomal rearrangement leading to virulence towards *Rlm1* plants.

PPT1 - a virulence factor of *Fusarium graminearum* - encodes phosphopantetheinyltransferase required for post-translational activation of polyketide synthases and non-ribosomal peptide synthases

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Plant pathogenic fungi frequently produce secondary metabolites with a suspected role in plant disease. Many of these compounds are produced by polyketide synthases (PKSs) or non-ribosomal peptide synthases (NRPSs), both of which require posttranslational attachment of the prosthetic group 4′-phosphopantetheine to the respective acyl-carrier or peptidyl-carrier domains. We have identified the *Fusarium graminearum* (*Gibberella zeae*) gene encoding phosphopantetheinyltransferase (*PPT1*). Disruption of this gene, which is equivalent to a loss of all PKSs and NRPSs leads to slightly reduced growth on PDA plates and dramatically reduced virulence on wheat. In contrast, inactivation of individual PKS and NRPS genes predicted in the fully sequenced Fusarium graminearum genome so far led to only minor virulence effects. Attempts do identify which gene or redundant set of genes downstream of *PPT1* are responsible for the virulence phenotype, and steps towards developent of a screening method for PPT1-inhibitors will be described. (Supported by the Austrian Genome Programme GEN-AU and the Christian Doppler Society).

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Horizontal transfer of a fungal virulence gene controlling host specificity

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The *ToxA* gene of the ascomycete fungal pathogen *Pyrenophora tritici-repentis* controls specificity of the interaction with its host, wheat. Host genotypes carrying dominant alleles of *Tsn1* are susceptible to isolates of the pathogen expressing *ToxA*. Isolates of *P. tritici-repentis* lacking *ToxA* cause significantly reduced symptoms on lines containing *Tsn1*. *Stagonospora* (*Phaeosphaeria*) *nodorum* is a related pathogen, which also infects wheat. Whole genome sequence analysis of *S. nodorum* revealed the presence of a gene that is nearly identical to *ToxA*, called *SnToxA*, adjacent to a transposase-like gene. *SnToxA* was expressed especially during early infection of wheat. Disruption of *SnToxA* by gene replacement resulted in strains that produced significantly reduced disease on wheat lines carrying *Tsn1*.

ToxA genes from a world wide collection of isolates of *P. tritici-repentis* and *S. nodorum* were sequenced. The *P. tritici-repentis* sequences were found to be identical to each other and very similar to the *SnToxA* sequence present in a West Australian isolate of *S. nodorum*. The *S. nodorum* sequences were highly variable and showed an excess of non-synonymous codon changes over synonymous, suggesting diversifying selection. In addition to *ToxA*, the transposase gene and flanking sequence comprising approximately 10.25 kb was present in *Pyrenophora tritici-repentis*. These findings suggest that the *ToxA* has recently been horizontally transferred from *S. nodorum* to *P. tritici-repentis* resulting in the evolution of significantly more virulent of the pathogen.

Divergent role of signaling pathways in development and pathogenicity of *Mycosphaerella graminicola*

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We used extensive EST resources and the recently released unannotated genome sequence of *Mycosphaerella graminicola* IPO323 in a comprehensive functional analysis of signaling pathways. We functionally characterized 10 genes encoding three mitogen-activated protein kinases, *MgHog1, MgFus3, MgSlt2,* the p21-activated kinase, *MgSte20*, the regulatory and catalytic subunits of protein kinase A, *MgTpk2, MgBcy1*, three Ga proteins, *MgGpa1*, *MgGpa2*, *MgGpa3*, and the Gb protein *MgGpb1*. *In planta* analyses revealed that eight of these genes are required for pathogenicity, but in a different stages of infection process. The dimorphic switch from yeast-like to filamentous growth was impaired in *MgHog1* mutants that consequently could not initiate infectious germ tubes. *MgFus3* mutants do not recognize stomata and thus are impaired in penetration. *MgSlt2* mutants penetrate successfully but are unable to establish invasive growth. *MgTpk2* and *MgBcy1* mutants colonized the mesophyll but cannot differentiate pycnidia in the substomatal cavities and therefore fail to fructify.

Detailed *in vitro* screens showed that *MgGpa2*, *MgSte20,* and *MgBcy1* are dispensable for the dimorphic transition from yeast-like to filamentous growth. *MgGpa1* negatively regulates filamentation, but *MgHog1, MgGpb1*, *MgGpa3* and *MgTpk2* positively regulate this process. *MgGpb1* negatively regulates anastomosis as disruption resulted in abundant highly uncharacteristic cell fusions in germ tubes and hyphae leading to a dense compact phenotype. Interestingly, exogenous cAMP restored the phenotype of the wild type IPO323 in the *MgGpb1* and *MgGpa3* mutants but not in the *MgGpa1* mutants, indicating a stimulating function for *MgGpb1* and *MgGpa3* and an inhibitory function for *MgGpa1* in the regulation of the cAMP pathway. We conclude that *M. graminicola* is an excellent model to study signaling in relation to pathogenesis and dimorphism.

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Reduced virulence of a glucose oxidase producing strain of *Fusarium graminearum* on wheat

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The *Aspergillus niger* glucose oxidase encoding gene (*goxA*) was cloned behind the *Trichoderma reesei* xylanase 1 promoter and transformed into the plant pathogen*Fusarium graminearum* PH1. Glucose oxidase production of the new strain has been demonstrated on several carbon sources, with autoclaved green wheat spikes as the best inducer for this expression system. Growth rates of the transgenic strain on xylose and autoclaved green wheat spikes were comparable with the wild type.

Glucose oxidase production in plants or by fungi in the rhizosphere has previously been demonstrated to enhance the systemic resistance of plants to pathogens. Glucose oxidase catalyzes the oxidation of glucose to glucono-lactone with hydrogen peroxide as a by-product. H_2O_2 is known to be involved in the oxidative burst of plants which causes the activation of a systemic resistance. To test the transgenic *Fusarium* strain for altered virulence, wheat was inoculated with spores of the wild-type or the transgenic strain, respectively. The spread of the fungi within the spike was monitored visually and by a recently developed quantitative real-time PCR assay for infected plant material. The wild type strain colonized the entire spike after 16 days post inoculation. The glucose oxidase producing strain completely failed to spread over the spike even after more than three weeks. Quantitative real-time PCR failed to detect more fungal DNA than provided by the initial inoculum at any time point after inoculation. Results of co-infection experiments in wheat with the glucose oxidase producing strain and the wild-type strain on the same spike will be presented.

Throughout or studies we designed a functional expression system for *Fusarium*, which can be activated by either autoclaved plant material or during the infection process on wheat spikelets. Additionally, we demonstrated a significant reduction of virulence by production of glucose oxidase during the infection process.

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