PS04-225

The direct protein-protein interaction results in the arms race co-evolution between *Magnaporthe oryzae AVR-Pik* and rice *Pik*

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Between pathogen and host, antagonistic interactions impose strong reciprocal selection on each organism, leading to the development of arms race evolutionary dynamics. However, studies on specific recognition and co-evolution between resistance (R-) gene and avirulence (AVR-) gene are still limited. Here we show that *AVR-Pik* of *Magnaporthe oryzae*, the rice blast pathogen, and cognate rice R-gene *Pik* exhibit high levels of DNA polymorphisms causing amino acid changes. We found a tight recognition specificity of *AVR-Pik* alleles by different *Pik* alleles. Pik is composed of two kinds of CC-NBS-LRR, Pik1 and Pik2. We found that AVR-Pik physically interacts with the N-terminal coiled-coil domain of Pik1 in yeast 2-hybrid assay as well as in *in-planta* co-immunoprecipitation assay. Furthermore, this binding specificity corresponds to the recognition specificity between AVR-Pik and Pik alleles. These data suggest that the direct protein-protein interaction results in the arms race co-evolution between *AVR-Pik* and *Pik*.

PS04-226

Arabidopsis WRKY18- and WRKY40-regulated host responses in plant immunity

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Transcriptional reprogramming represents a vital component of the overall host defense machinery triggered in response to phytopathogen challenge. Recently, we showed that simultaneous mutation of two WRKY-type transcription factors, WRKY18 and WRKY40, rendered otherwise susceptible wild type *Arabidopsis* plants resistant towards the biotrophic powdery mildew pathogen *Golovinomyces orontii*. This resistance was accompanied by an imbalance in JA/SA signaling, exaggerated expression of certain defense genes, and elevated camalexin levels (Pandey et al., TPJ 64, 912, 2010). Our current studies are focused on determining the signaling pathways in which WRKY18 and WRKY40 act, and in identifying direct targets of these two transcription factors. Data will be presented showing that SA is essential for resistance towards *G. orontii* in the *wrky18 wrky40* background but that additional biochemical pathways are also required. Moreover, whereas WRKY18 and WRKY40 act as negative regulators of basal defense towards *G. orontii* this is not the case for other tested powdery mildews. Thus, their loss-of-functions do not confer broad-spectrum resistance towards these powdery mildew fungi. Interestingly, WRKY18 and WRKY40 also act as positive regulators of RPS4-mediated resistance as *wrky18 wrky40* double mutants were found to be strongly susceptible towards *Pseudomonas syringae* DC3000 bacteria expressing the *avrRPS4* effector gene. This response appears to be highly specific since it was not observed with bacteria expressing other *avr* genes.

PS04-227

Necrosis and ethylene-inducing peptide-like proteins of the obligate biotrophic oomycete *Hyaloperonospora arabidopsidis*; Contradictio in Terminis?

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The obligate biotrophic pathogen *Hyaloperonospora arabidopsidis* expresses several Necrosis and ethylene-inducing peptide (Nep1)-Like Proteins (NLPs) during infection of *Arabidopsis*. In the *H. arabidopsidis* genome, we found that 12 of a total of 14 *NLP* genes form a species-specific cluster when compared with other oomycete *NLP* genes, suggesting this class of effectors has recently expanded. As NLPs are best known for their phytotoxicity it is surprising that this obligate biotrophic pathogen has an expanded *NLP* gene family. Contrary to most of the studied *NLP* genes, none of the *HaNLP*s causes necrosis when expressed *in planta*. Even HaNLP3, which is most similar to necrosis-inducing NLP proteins of other oomycetes and which contains all amino acids that are critical for necrosis-inducing activity, did not induce necrosis. Chimeras constructed between HaNLP3 and the necrosis-inducing PsojNIP protein demonstrated that most of the HaNLP3 protein is functionally equivalent to PsojNIP, except for an exposed domain that prevents the induction of necrosis. The early expression and species-specific expansion of the *HaNLP* genes is suggestive of an alternative function of noncytolytic NLP proteins during biotrophic infection of plants. We will report on our advances in analyzing *Arabidopsis* lines expressing different *HaNLP*s. As the *Arabidopsis* plants constutitively expressing *HaNLP*s show a severe phenotype, we have also created inducible *HaNLP3* lines to study the effects of these proteins on host cell processes.

PS04-228

***Verticillium* manipulates RNA silencing to suppress host immunity**

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RNA silencing is the regulation of gene expression based on the accumulation of sequence-specific small RNAs (sRNAs) that target messenger RNAs (mRNAs) resulting in their degradation. Several genes controlling RNA silencing in plants have been identified. The plant RNA silencing pathway mediates plant immunity against viruses and bacteria.Previous data from our laboratory indicate that fungus *Verticillium dahliae* also targets the plant RNA silencing pathway, presumably by secreted effectors, to suppress host defence (1). How *Verticillium* manipulates the RNA silencing pathway to suppress host immunity is still unknown. We are using the model plant Arabidopsis that is a host of *Verticillium* to unravel the role of RNA silencing in *Verticillium* wilt disease. We plan to identify the secreted *Verticillium* effectors and the Arabidopsis components that play a role in RNA silencing and are essential for *Verticillium* wilt disease. We are currently identifying *Verticillium* regulated mRNAs and sRNAs of the host, and *Verticillium* effectors that target host RNA silencing by combining transcriptomics, sRNA profiling, and effector screening. The obtained results will be presented. (1) Ellendorff U, Fradin EF, de Jonge R, Thomma BP. (2009) RNA silencing is required for Arabidopsis defence against Verticillium wilt disease. *J Exp Bot.*; 60 (2):591-602.

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COM1* encodes a novel component of the spliceosome to regulate conidium development and virulence in *Magnaporthe oryzae

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