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Pathogen-informed strategies for sustainable broad-spectrum crop resistance

Workshop on cellular dynamics of effector action and recognition

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ABSTRACTS

Oral presentations

Host cell membrane trafficking in response to powdery mildew attack

Hans Thordal-Christensen

University of Copenahagen, Denmark

We study host membrane trafficking related to two essential stages of powdery mildew invasion of leaf epidermal cells. In the first stage involving penetration resistance, a syntaxin mediating membrane fusion defines a pathway that executes this defence. We have identified an ARF-GTPase and an AFR guanine nucleotide exchange factor (GEF) to be functioning on the same penetration resistance pathway. At the second stage of invasion, the fungus establishes a haustorium inside the host cell. Using fluorescent membrane dyes and marker fusion proteins, we have been able to suggest the nature of the plant derived extrahaustorial membrane. The powdery mildew fungus expresses approximately 500 secreted effector proteins, and we currently aim at identifying such components that influence these host-pathogen interaction associated membrane trafficking processes.

The plant membrane protein REM1.3 remorin accumulates in discrete extrahaustorial membrane domains marked by RXLR effector AVRblb2 and enhances susceptibility to Phytophthora infestans

Tolga O. Bozkurt[1,5], Annis Richardson[1,2], Yasin F. Dagdas[1], Sébastien Mongrand[3], Sophien Kamoun[1], Sylvain Raffaele[1,4]

[1] The Sainsbury Laboratory, Norwich Research Park, NR4 7UH Norwich, UK
[2] John Innes Centre, Norwich Research Park, NR4 7UH Norwich, UK
[3] Laboratoire de Biogenèse Membranaire UMR 5200 CNRS - Université Bordeaux Segalen – INRA, F-33883 Villenave d'Ornon Cedex, France
[4] Laboratoire des Interactions Plantes-Microorganismes (LIPM), UMR441 INRA – UMR2594 CNRS, F-31326 Castanet-Tolosan, France
[5] Imperial College London, Department of Life Sciences, Exhibition Road, London SW7 2AZ

Filamentous plant pathogens such as the oomycete, *Phytophthora infestans* associate with plant cells through specialized infection structures termed haustoria to deliver effector proteins or uptake nutrients. Haustorium is separated from the plant cell by a host-derived membrane known as the extrahaustorial membrane (EHM), with unknown origin and composition. The mechanisms underlying the biogenesis of this host pathogen interface are unknown. Remarkably, several plasma membrane localised proteins are excluded from the EHM but the remorin REM1.3, a plant membrane associated protein, accumulates around *P. infestans* haustoria. Here, we analysed cellular dynamics of REM1.3 during *P. infestans* infection. Super resolution microscopy revealed distinct domains labelled by REM1.3 and *P. infestans* effector AVRblb2. Moreover, SYT1 synaptotagmin, another previously identified perihaustorial plant protein, localized to subdomains, which are mainly not labelled, by REM1.3 and AVRblb2. Functional characterization of REM1.3 revealed that it is a susceptibility gene that promotes infection by *P. infestans*. This activity, and REM1.3 recruitment to the EHM, requires REM1.3 membrane binding domain. Our results implicate REM1.3 EHM micro-domains in plant susceptibility to an oomycete pathogen and support the view that diverse host endomembrane pathways contribute to the formation of the EHM.

Xanthomonas translucens – do type III and particulary TAL effectors contribute to pathogenicity?

Céline Pesce[1,2], Claude Bragard[2], Ralf Koebnik[1]

[1] UMR 186 Résistance des Plantes aux Bioagresseurs, 911 Avenue Agropolis, 34394 Montpellier, France
[2] Earth and Life Institute, Applied Microbiology Phytopathology, Université Catholique de Louvain la Neuve, 1348 Louvain-la-Neuve, Belgium

To cause disease, most xanthomondas depend on a highly conserved type III secretion system (Hrc system) which translocates type III effectors (T3Es) into target host cells. A specific family of T3Es, called Transcription Activator-Like (TAL) effectors, modulates plant gene expression upon binding to target boxes in the promoter regions of plant genes. By mimicking eukaryotic transcription factors, TAL effectors induce certain plant gene(s) whose activities are required to establish a susceptible state of the host towards infection.

Xanthomonas translucens (*Xt*) is responsible of Bacterial Leaf Streak (BLS), the most common bacterial disease of small grain cereals. This disease has been reported at diverse locations worldwide and received increased attention in recent years. BLS of barley and wheat is a seed-borne disease and thus a constraint for international germplasm exchange. Seemingly, bacteria enter through the stomata and multiply in the parenchyma. Yet, the infection route is not known in detail. Therefore, we use fluorescent reporters to study which tissues are affected during the infection process. Since virtually nothing is known about pathogenicity factors of *Xt*, we wish to find key virulence determinants involved in the interaction with the host plant. To this end, several candidate genes (*hrcT*, *tal*) were knocked out and mutants were characterized by pathogenicity assays. In parallel, *tal* genes were cloned and sequenced in order to identify candidate susceptibility genes in barley.

Characterization of host cell responses and root-knot nematode effectors involved in the formation of multinucleate and hypertrophied feeding cells

Bruno Favery

INRA – UNSA – CNRS Sophia Antipolis, France

Root-knot nematodes are obligate endoparasites able to infect almost all cultivated plants worldwide. These nematodes maintain, for weeks, a biotrophic relationship with their hosts by inducing the differentiation of root cells into hypertrophied and multinucleate feeding cells. Nematode effectors produced in the esophageal glands and injected within host cells thought the stylet certainly play a role during infection to manipulate plant cell morphogenesis and physiology, and promote nematode establishment. In our study of both partners of the interaction, we characterized key plant cytoskeleton regulators involved in giant cell ontogenesis and showed that giant cells result from cell division without complete cytokinesis. Our identification of nematode effectors was based on proteome, transcriptome and genome analysis. We identified parasitism genes that are specifically expressed in nematode oesophageal glands and demonstrated their secretions in planta using immunolocalisation. I will focus on the cell biology approaches we developed to study these effectors and the giant cells formation.

Microscopic analysis of syncytia induced by potato cyst nematode *Globodera rostochiensis* in wild-type and *ERabp1*- or *NGB*-silenced tomato plants

Dabrowska-Bronk J., Wisniewska A., Fudali S., Sobczak M., **Baranowski L.,** Czarny M., Morgiewicz K., Medrzycka A., Matuszkiewicz M., Wroblewski T., Bartoszewski G., Filipecki M.

Warsaw University of Life Sciences, Poland

Potato cyst nematodes induce a specific feeding structure (syncytium) in host plant roots. The microscopic analysis of syncytia induced by *Globodera rostochiensis* in T2 transgenic plants with silenced expression of *ERabp1* (auxin binding protein) or *NGB* (GTP-binding protein) genes revealed several ultrastructural differences in comparison to syncytia induced in roots of wild-type tomato plants. In *ERabp1*-silenced plants the syncytial cytoplasm was usually less electron opaque. They contained also less endoplasmic reticulum (ER) structures. Presence of hypertrophied nuclei, intact plastids and mitochondria, and small vacuoles and vesicles resembled typical syncytium. However, older syncytia contained granular and plasmolysed cytoplasm with degraded organelles. Syncytia induced in *NGB*-silenced plants usually contained strongly electron translucent cytoplasm with only a few ER structures present in the syncytial elements, but no degeneration of the syncytial cytoplasm, mitochondria or plastids occurred during all developmental stages of syncytia. In syncytia induced in *ERabp1*- and *NGB*-silenced plants the nuclei acquired regular round shape instead of becoming amoeboid as in control plants. Syncytia induced in roots of *ERabp1*-silenced plants revealed features of delayed hypersensitive response whereas no such features were observed in syncytia induced in roots of *NGB*-silenced plants.

Infection modes of *Plasmopara halstedii*, the agent of sunflower downy mildew disease and characterization of pathogenicity effectors

Quentin Gascuel, Yves Martinez; Luis Buendia, Marie-Claude Boniface, Nicolas Blanchet, Patrick Vincourt, Magalie Pichon; Laurence Godiard.

Laboratoire des Interactions Plantes-Microorganismes (LIPM), INRA-CNRS, INRA Auzeville, F-31326 Castanet-Tolosan, France.

Plasmopara halstedii is an obligate biotroph oomycete causing downy mildew disease on sunflower, an economically important cultivated crop. Disease symptoms observed in fields, plant dwarfism, leaf bleaching, sporulation and production of infertile flowers impair strongly seed yield. *P.halstedii* pathotypes are defined by their divergent virulence profiles in a set of sunflower differential hosts carrying different *Pl* Resistance genes, not yet cloned. Number of pathotypes increased to 16 during the last 25 years in France, concomitantly with the breakdown of *Pl* resistance loci used in fields. Studying disease infection mechanisms and pathogen molecular determinants is a prerequisite for deciphering plant sustainable resistance.

We set up infection conditions on sunflower plants grown in hydroponic cultures that mimic field plant symptoms, to follow life cycle and infection modes of *P. halstedii*. A couple of downy mildew resistant and susceptible near isogenic sunflower lines was infected in these conditions, in order to get easy access to the infected organs including roots. Scanning Electron Microscopy (SEM) and light microscropy were used to investigate *P. halstedii* life cycle *in planta* during incompatible and compatible interactions: penetration in plant tissues, colonization, haustoria production and dissemination structures.

In parallel, sequencing of cDNA from *P.halstedii* spores and sunflower infected tissues was performed in order to identify molecular determinants of pathogenicity. 100 putative RXLR and CRN type effectors potentially expressed in sunflower infected tissues were found by *in silico* approaches. Transient expression in sunflower of selected effectors fused to GFP is currently done to study their functional role *in planta*.

Systematic plant sub-cellular localization of Ralstonia solanacearum type III effectors

Cazalé A.C., Genin S and Peeters N.

Laboratoire des intéractions plantes microorganismes (LIPM), UMR CNRS-INRA 2594/441, F-31320 Castanet-Tolosan, France

Ralstonia solanacearum, the causal agent of bacterial wilt disease, infects more than two hundred species including economical important crops among which many Solanacae. The type III secretion system plays a major role in its pathogenicity. A total of 70 type III effectors have been identified in the reference strain GMI1000, with around 30 present in all sequenced strains and thus representing the "core" effector set of the Ralstonia species complex (Peeters et al., 2013).

Experimental evidences indicates that there is substantial functional overlap among this repertoire. In order to get insights into the functions of this large set of effectors, we have defined a systematic functional approach using in planta transient expression assays to study their localization in the plant cell. The first results show that many of the effector-RFP fusions are localized in both the nucleus and the cytoplasm except for two that are exclusively nuclear-localized. In the future we want to use this information to test whether functional redundancy could be associated with overlapping plant sub-cellular localization.

In this presentation I will present our progress in determining the sub-cellular localization of all core type III effectors and highlight other avenues of research aimed at understanding their contribution to pathogenicity.

Phytophthora capsici effectors target the host nucleus to promote virulence

Edgar Huitema

James Hutton Institute, United Kingdom

Plant-Microbe interactions are complex associations that feature recognition of Pathogen Associated Molecular Patterns by the plant immune system and dampening of subsequent responses by pathogen encoded secreted effectors. With large effector repertoires identified in sequenced microbial genomes, some of which travel inside plant cells, much attention now centers on the identification of the targets and understanding their roles in immunity or disease. The Phytophthora intracellular effector repertoire encodes a large class of proteins that translocate into host cells and target the host nucleus. Recent functional studies have implicated the CRN protein family as an important class of diverse effectors that target distinct sub-nuclear compartments and modify host cell signaling. Given the size and diversity oomycete effector repertoires, detailed studies on function and dynamic localization patterns may help unveil novel nuclear processes required for infection. Here I will report on the identification and characterization of nuclear effectors activities in plants. Our results suggest diverse

functions which can be exploited to identify novel processes, required for immunity or susceptibility, in the nucleus.

Functional study of lectins in plant parasitic nematodes

Silke Nowak

Ghent University, Belgium

Meloidogyne graminicola is the most harmful rootknot nematode in rice and in the last 10 years, infections became more problematic, mainly due to the increased aerobic rice cultivation. We are examining the distribution and function of secreted lectins as potential effectors in plant parasitic nematodes (PPN). At this moment a functional analysis is being performed on 2 lectins from *M. graminicola*: UK41 and UK42. Both lectins were found through EST sequencing of J2's. They both have a signal peptide, show expression in the subventral glands and are specific for PPN. (Co)localization in *Nicotiana benthamiana* epidermal cells showed nuclear localisation of UK42. UK41 is possibly localized in a kind of vesicles, however the identity of these vesicles is currently unknown. Co-infiltration of *N. benthamiana* leaves with these 2 effectors and R/Avr pairs has been used to reveal suppression of 'effector triggered immunity'. No suppression has been found with the R/Avr pairs used so far. Therefore more pairs need to be tested.

Transgenic rice lines overexpressing these nematode lectins have been generated, these plants are currently forming seed and will be used for infection experiments. RNAi lines would be useful for functional analysis too.
An expression system for high production of the lectins in BY2 cells is being set up. After purification, the lectins will be used in a pull-down to find interacting proteins and a glycan array to identify the sugar affinities of the lectins.
Combining the results of these experiments, we hope to gain more insight in the functions and targets of these secreted lectins and their potential role in immune regulation.

Root-knot nematodes may manipulate intracellular trafficking to promote infection

Utermark, J. & **Gleason, C.A**.

Georg-August-Universität Göttingen, Germany.

The root knot nematode *Meloidogyne hapla* is a devastating plant pathogen on many of the world's main food crops. During infections the phytonematode uses a stylet to secrete an array of effector proteins to modify plant cells and ingest nutrients to support its growth and development. To identify the effector complement of this pathogen we have used a bioinformatic analysis of the known root-knot nematode secretome and genome(s). We identified several novel proteins from the nematode and we focused our work on an effector protein specific to *M. halpa* and with no homology to known proteins. Gene expression profiles in various nematode life-stages revealed that the gene's transcript is highly up-regulated during the early invasion stages of the host–pathogen interaction. We also have evidence to suggest that ectopic expression of this nematode gene in Arabidopsis enhances susceptibility to nematodes and a bacterial pathogen. Moreover, an *in situ* analysis showed that the candidate is synthesized in the esophageal gland cells of the nematode, indicating a potential stylet-

translocation into the plant tissue. Intriguingly, subcellular localization of this tagged protein in plants indicated that it is associated with the endoplasmic reticulum. To link the observed phenotypes with the molecular function, we have found that this nematode protein can interact with a member of the prenylated Rab acceptor 1 (PRA1) domain protein that is involved in vesicle-mediated protein transport. By using biochemistry approaches and cell biology techniques, we hope to develop new insights into how this nematode gene can function as an effector and how it may to negate host defense responses by hijacking the regulation of host vesicle trafficking.

An atypical effector of Blumeria graminis targets barley the RHO GTPase RACB for entry into epidermal cells of barley

Ralph Hueckelhoven

Technical University Munich, Germany

Plants and their pathogens are engaged in a constant battle. Non-adapted pathogens fail at the first line of basal plant defense whereas adapted pathogens can trigger susceptibility by secretion of effector proteins. Plants facing this challenge evolve R-proteins that recognize effector activity leading to re establishment of resistance and a consequent hide-and-seek for both sides. The understanding of effector proteins from Blumeria graminis f.sp. hordei (Bgh), the causal agent of powdery mildew on barley, is just at the beginning. We identified Bgh ROP-interacting peptide1 (ROPIP1) as a potential effector manipulating barley susceptibility factor RACB, which is a Rho of plants (ROP) small GTPase. RACB is required for full susceptibility of barley against Bgh and acts as a molecular switch in cell polarity and cell expansion inter alia by influencing cytoskeleton dynamics. The microtubule-associated ROP GTPase activating protein (MAGAP1) is an antagonist to RACB in susceptibility of barley towards Bgh and likely inactivates RACB. ROPIP1 function might be in stabilizing RACB activity by interfering with MAGAP1 mediated regulation at microtubules. Susceptibility may be triggered via ROPIP1 for the ingrowth of a haustorium, the central feeding organ of Bgh, into barley epidermal cells.

The *Xanthomonas campestris* type III effector XopJ proteolytically degrades proteasome subunit RPT6 to inhibit proteasome-mediated turnover of NPR1

Suayib Üstün

Leibniz-Institute of Vegetable and Ornamental Crops, Germany

XopJ is a type III effector protein from *Xanthomonas campestris* pv. *vesicatoria* that interferes with plant immune responses. Previous work reported that XopJ targets the proteasomal subunit RPT6 *in planta* to suppress proteasome activity. Mutation of the catalytic triad as well as of the N-terminal myristoylation motif in XopJ abolished its proteasome-inhibiting ability, suggesting that enzymatic activity and membrane anchoring are required for proteasome inhibition. XopJ-mediated manipulation of the host proteasome interferes with salicylic acid (SA)-dependent defense response to attenuate onset of necrosis. However, it was not clear how XopJ acts mechanistically to inhibit the proteasome and SA-signaling. Co-expression and BiFC studies reveal that only membrane-localized XopJ is able to recruit RPT6 to punctuate structures at the plant plasma membrane being reminiscent of lipid rafts. Further analysis of the RPT6-XopJ complex shows that once localized to the membrane, RPT6 is

degraded by XopJ *in planta*, dependent on its myristolyation motif and catalytic activity. In vitro activity measurement using a generic substrate demonstrates that XopJ displays protease activity. Transient co-expression of effector and target protein in *N. benthamiana* suggests that XopJ proteolytically degrades RPT6 to inhibit the proteasome assembly and hence its activity. XopJ-mediated suppression of the proteasome function interferes with the proteasomal turnover of NPR1, the master regulator of SA responses and thus leads to the accumulation of ubiquitinated NPR1. These data suggest that XopJ acts as a protease to degrade RPT6 leading to malfunction of the proteasome and hence resulting into a reduced proteasome-mediated turnover of NPR1.

The plant exocyst as an expeller of pathogens

E.J.R. Overdijk, Y. Du, K. Bouwmeester, T. Ketelaar and F. Govers.

Laboratories of Phytopathology and Cell Biology, Wageningen University, The Netherlands

Recently, we have shown that the RXLR effector AVR1 of the late blight pathogen *Phytophthora infestans* targets exocyst subunit SEC5 in the host plant potato, thereby suppressing plant defence. The exocyst is a protein complex involved in exocytosis by mediating initial vesicle docking to the plasma membrane. In plants, the exocyst is poorly studied, but known to be essential for viability. Exocytosis is one of several cellular processes playing an essential role in plant defence and therefore more insight in its exact functioning could help to improve plant immunity.

Our main goal is to understand the role of exocyst-mediated exocytosis in immunity. For that purpose we investigate how AVR1-mediating SEC5 targeting affects the cellular dynamics of this exocyst-mediated exocytosis. We hypothesize that the plant exocyst is involved in (I) targeted exocytosis of defence-related compounds towards the site of pathogen attack and/or (II) the insertion of defence-related membrane proteins into the plasma membrane prior to pathogen attack. We use advanced imaging technologies to image exocyst-mediated exocytosis events to mechanistically study the effect of pathogen attack and the presence of effector AVR1 on exocyst-mediated vesicle docking. We will also use proteomics to analyse whether the presence of AVR1 disturbs the exocytosis of defense-related proteins

This multidisciplinary project provides insight into the function of the plant exocyst in defence and reveals how pathogen effectors affect the localization and cellular dynamics of their host targets.

Ustilago maydis induced tumor formation in maize requires organ-specific effector proteins

Gunther Döhlemann

Max-Planck-Institute for Terrestrial Microbiology, Germany

Ustilago maydis infects all aerial organs of its host plant maize to establish a biotrophic interaction which ultimately results in formation of plant tumors. Host infection depends on effector proteins, which are secreted by the fungus throughout the infection process. Given the fundamental differences between the maize organs that are infected by U. maydis, we hypothesized that the fungus deploys organ specific effectors. To this end, we performed a candidate gene approach based on transcriptional regulation and sequence divergence of effector genes. This approach identified a set of U. maydis effectors, which contribute to virulence in an organ-specific manner. One of these effectors is See1

(Seedling efficient effector 1), which shows strictly leaf-specific expression and function. U. maydis deletion mutants for see1 are fully virulent in ears and anthers, but fail to induce tumor expansion in maize seedling leaves. Using 5-ethynyl-2-deoxyuridine (EdU), we could show that Δsee1 mutants fail to induce mitotic activity of infected plant cells, while host cell division in leaves is strongly induced U. maydis wild type. To understand the organ-specific role of See1, its interaction with plant proteins is investigad. Molecular genetics, imaging and biochemical analyses are used to unravel how See1 triggers tumorigenesis specifically in maize leaves.

Cell biology studies of RXLR effector(-target) functions

Petra Boevink

James Hutton Institute, United Kingdom

Phytopthora infestans has a remarkably large array of effector proteins that it deploys to infect plants. The largest class of these contain the RXLR motif, which is required for translocation of the effectors into plant cells. Using criteria such as expression patterns, phenotypes in pathogenicity assays, and the nature of the putative host target proteins determined by in-depth yeast-2-hybrid screening, we have prioritised a subset of RXLR-containing effectors for further investigation. Cell biology is an integral part of our drive to understand the activities of effectors in host cells. I will discuss the different approaches we have used to study our priority effectors, including where they localise to and the effects of mis-localising them; detecting and measuring interactions with putative targets inside plant cells and the modification of target proteins by effectors. I will provide cautions about the use and interpretation of these different approaches.

Effector delivery by the appressoria and biotrophic hyphae of *Colletotrichum*

Richard O'Connell[1], Jochen Kleemann[2], Guillaume Robin[1], Hiroyuki Takahara[3], Linda Rincon[2]

[1]INRA-BIOGER, Thiverval-Grignon, France
[2]Max-Planck Institute for Plant Breeding Research, Cologne, Germany
[3]Ishikawa Prefectural University, Ishikawa, Japan

Colletotrichum higginsianum causes anthracnose disease on cruciferous plants, including *Arabidopsis*. It uses a hemibiotrophic infection strategy, involving formation of several specialized cell types. After melanized appressoria puncture host surfaces, bulbous biotrophic hyphae develop inside living host cells, surrounded by a modified host plasma membrane; finally, the fungus switches to destructive necrotrophy, associated with thin filamentous hyphae. The *C. higginsianum* genome encodes 365 putative secreted effectors (ChECs), of which 97 are expressed *in planta*. Transcriptome sequencing revealed waves of ChEC gene expression, suggesting different sets of effectors function at each infection stage. Wave 1 and 2 ChECs are expressed in appressoria before and during penetration; Wave 3 ChECs are expressed in biotrophic hyphae after penetration, and Wave 4 ChECs are induced at the necrotrophy switch. To localize ChECs during infection, they were expressed as C-terminal fusions with mRFP under their native promoters and visualized by confocal microscopy and TEM-immunogold labelling. Wave 2 effectors ChEC6 and ChEC36 are focally secreted by appressoria before penetration through the 200 nm penetration pore, suggesting appressoria are organs for effector delivery, in addition to their role in penetration. Wave 3 effectors ChEC34 and ChEC89 accumulate in novel compartments called 'interfacial bodies' on the hyphal surface, and also diffuse into the plant

cell wall, but are not detectable in the cytoplasm of infected cells. The chitin-binding LysM effector ChEC90 similarly localizes to the biotrophic interface. However, transient expression *in planta* of 22 ChECs as N-terminal fusions with GFP revealed four are targeted to the plant nucleus, suggesting some effectors may be translocated into host cells

CRN13 effectors from a plant pathogenic oomycete and an amphibian pathogenic fungus target DNA

Diana Ramirez-Garcés, **Laurent Camborde**, Michiel J.C. Pel, Hélène San-Clemente, Bernard Dumas, Elodie Gaulin

Laboratoire de Recherche en Sciences Végétales (LRSV), UM5546 CNRS-Univ Toulouse III, Pôle de Biotechnologie Végétale, 31326 Castanet-Tolosan ; France

Microbial pathogens translocate effectors inside host cells to subvert cellular functions and suppress immune responses. Oomycetes, which are fungal-like eukaryotic microorganisms that cause some of the most destructive plant diseases in the world, secrete a group of effector proteins called CRN (Crinkler). CRNs are modular proteins with a conserved N-terminus, which is characterized by the presence of an LFLAK translocation motif, and a highly diverse C-terminal effector domain. Although many CRNs have been identified almost no data is available on CRN functions and targets. CRNs were thought to be oomycete specific until the recent identification of CRNs in the genome of the fungal amphibian pathogen *Batrachochytrium dendrobaditis* (*Bd*). We have characterized AeCRN13 from *Aphanomyces euteiches*, a root pathogen of legumes, and its *Bd* ortholog BdCRN13. We found that both proteins localize in the nuclei of plant and amphibian cells where they induce cell-death and developmental abnormalities. Additionally, we demonstrated that both CRN13s are able to bind DNA *in vitro.* Altogether, this work reveals that cytotoxic translocated effectors produced by unrelated plant and animal pathogens bind DNA to interfere with host cell development.

Complex regulation of the defence-related Arabidopsis transcription factor AtMYB30: from the plant cell to bacterial effectors

Daniel Marino, Joanne Canonne, Solene Froidure, Alain Jauneau, Cecile Pouzet, Dominique Roby and **Susana Rivas**

INRA/CNRS, Laboratoire des Interactions Plantes-Microorganismes (LIPM), UMR441, F-31326 Castanet-Tolosan, France.

The MYB transcription factor AtMYB30 was previously identified as a positive regulator of Arabidopsis defense and associated cell death (HR) responses to bacterial pathogens. We recently showed that AtMYB30 is targeted by the *Xanthomonas* type III effector XopD resulting in suppression of AtMYB30-mediated plant defences and underlining the crucial role played by AtMYB30 in the regulation of plant disease resistance. In addition, the activity of AtMYB30 is tightly controlled by plant cells. First, in the presence of AtMYB30, the secreted phospholipase *At*sPLA2-□ is partially relocalized to the nucleus where the two proteins interact, leading to repression of AtMYB30-mediated HR and defences. These data highlight the importance of cellular dynamics for defence-associated gene regulation in plants. Second, new data show that the RING-type E3-ubiquitin ligase protein MIEL1 (AtMYB30-Interacting E3 Ligase1) interacts with AtMYB30 in the plant cell nucleus, leading to AtMYB30 ubiquitination and proteasomal degradation. As a result, MIEL1 negatively regulates AtMYB30-mediated transcriptional activation and Arabidopsis defence and HR responses.

Finally, AtMYB30 is additionally regulated through its interaction with a protease of the subtilase family. The existence of separate, nonredundant, negative regulatory mechanisms that temporally and spatially regulate the activation of AtMYB30-mediated HR will be discussed.

Phytophthora palmivora interactions with roots of symbiosis model plants

Sebastian Schornack

The Sainsbury Laboratory, United Kingdom

We are exploring the extent to which beneficial symbiosis mechanisms intersect with pathogen infection. Filamentous pathogens and symbiotic microbes both colonise plant roots and form intracellular accommodation structures, termed haustoria and arbuscules, respectively. We study the oomycete Phytophthora palmivora which causes diseases in roots of leguminous symbiosis model plants Medicago truncatula and Lotus japonicus. We identified symbiosis mutants with similar or contrasting effects on P. palmivora root infection. We utilise genome-wide association mapping in M. truncatula to identify genes contributing to root or leaf invasion by P. palmivora. Extending our screening to monocot species revealed a dominant genetic trait supporting P. palmivora root colonisation. We use P. palmivora isolates secreting fluorescent effectors to study processes involved in formation of intracellular haustoria and compare them to arbuscular mycorrhiza fungi. From genome data we identified functionally conserved avirulence genes between different Phytophthora species. This enables exploitation of established disease resistance genes to control P. palmivora.

TAL effectors as toolbox to visualize host cells and tissues targeted by *Xanthomonas oryzae* during infection of susceptible rice

Tu Tuan Tran*, Jens Boch° and **Boris Szurek***

*UMR 186 IRD-UM2-Cirad 'Résistance des Plantes aux Bioagresseurs', BP 64501, 34394, Montpellier Cedex 5, France;
°Department of Genetics, Martin Luther University Halle-Wittenberg, Weinbergweg 10, D-06120, Halle (Saale), Germany;

Bacterial plant pathogenic Xanthomonads translocate TAL (Transcription Activator-Like) effectors into plant cells to function as specific plant transcription factors via a novel programmable DNA-binding domain. Several TAL effectors from rice-pathogenic *Xanthomonas oryzae* pv. *oryzae* (*Xoo*) strains constitute important virulence factors, but the role of target induced genes is poorly understood. The *Xoo* TAL effectors AvrXa7 and TalC, from strains PXO86 and BAI3 that originate from the Philippines and Burkina Faso respectively, direct expression of the rice gene *SWEET14* as a common target. The induction of *SWEET14* or its homolog *SWEET11* is essential for disease development and it was recently shown that both genes encode sugar transporters, which might play a role in the nutrition of pathogens. Presumably, *Xoo* TALs upregulate these genes in parenchyma vascular cells, thus leading to an increase of sugar concentration in the xylem which is the ecological niche of *Xoo*. With the objectives of better characterizing i) the types of host cells and tissues challenged by *Xoo* (and *Xoc*) during infection and ii) the kinetic of target genes induction, we took advantage of TAL effectors inherent potential as transcription activator, to analyze TAL-responsive *GUS* reporter rice lines. Preliminary results highlight the interest of such approach to get insight into the histology of host cells and tissues challenged with *Xoo* or *Xoc*, which is a mesophyll-restricted pathogen causing the bacterial leaf streak of rice.

Functional characterization of avirulence genes in *Leptosphaeria maculans*: linking 3-D structure, functional characteristics and evolution

Karine Blondeau[1], Françoise Blaise[2], Marc Graille[1], Shiv D. Kale[3], Juliette Linglin[2], Bénédicte Ollivier[2], Audrey Labarde[1], Noureddine Lazar[1], Guillaume Daverdin[2], Marie-Hélène Balesdent[2], Brett M. Tyler[4], Thierry Rouxel[2], Herman van Tilbeurgh[1] and **Isabelle Fudal[2]**

[1]IBBMC, Université Paris-Sud, CNRS-UMR8619, Orsay, France.
[2]INRA, UR 1290 BIOGER-CPP, Thiverval-Grignon, France.
[3]Virginia Bioinformatics Institute, Virginia Tech, Blacksburg, Virginia, USA.
[4]Center for Genome Research and Biocomputing, Oregon State University, Corvallis, Oregon, USA.

Leptosphaeria maculans is an ascomycete causing stem canker of oilseed rape. To date, four *L. maculans* avirulence genes were cloned by our team and our objective is to elucidate their involvement in pathogenicity through their structural and functional characterization and the determination of their interactants. *AvrLm4-7* confers a dual specificity of recognition by two resistance genes (*Rlm4* and *Rlm7*) and is strongly involved in fungal fitness. The AvrLm4-7 protein was produced in *Pichia pastoris* and its crystal structure determined. It revealed the presence of four disulfide bridges and no close structural analogs could be identified. Translocation assays in oilseed rape roots and transient expression in tobacco leaves showed that AvrLm4-7 is translocated into plant cells in the absence of the pathogen and targeted to the cytoplasm. Translocation necessitates the presence of a RAWG motif located in a loop as part of a positively charged region and also the presence of a well-conserved stretch of amino acids (R/N)(Y/F)(R/S)E(F/W) in the C-terminal part of the protein. Loss of recognition of *AvrLm4-7* by *Rlm4* is due to mutation of a single glycine to arginine residue located in a loop of the protein. Loss of recognition by *Rlm7* is governed by three point mutations targeting residues either located in the (R/N)(Y/F)(R/S)E(F/W) motif or close to the glycine involved in *Rlm4*-mediated recognition. Using a particle bombardment assay, we determined that recognition by Rlm4 and Rlm7 occurred into the cytoplasm of plant cells. Finally, a yeast two-hybrid screen was performed and possible plant targets will be discussed.

Localization and function analysis of Type III effector RipG7 of *Ralstonia solanacearum*

Keke Wang, Philippe Remigi, Ilona Kars, Ton Timmers, Stephane Genin and Nemo Peeters

Laboratoire des intéractions plantes microorganismes (LIPM), UMR CNRS-INRA Castanet-Tolosan, France

The soil-borne pathogen *Ralstonia solanacearum* naturally infects plant roots and causes bacterial wilt disease in over 200 plant species, especially *solanaceous* plants. A main pathogenicity determinant of *Ralstonia solanacearum* is type III secretion system (T3SS). *R. solanacearum* use T3SS to inject a large repertoire of effectors into plant cells. Among these effectors, RipG (formerly named "GALA") is a family of seven effectors in GMI1000 that have homologies with plant F-box proteins. RipG7 is an essential effector among the GALA genes for the virulence on *Medicago truncatula* and also involved in the pathogenicity on tomato and *Arabidopsis*.

In our effort to understand the function of this type III effector we wanted to better characterise its localization both at the root tissue level during infection and at a sub-cellular level after injection into plant cells.

Our data showed that RipG7 is localized both in the cytoplasm and the nucleus of infected cells where it can probably form SCF complexes with the plant resident protein SKP1-like. We also showed that nuclear localization is sufficient for the function. Furthermore, the possibility to detect RipG7 with a specific antibody also allowed us to start to investigate which cells of the plant root are actually subjected to injection by this type III effector.

Pattern recognition receptors: From the cell surface to endosomal trafficking

Silke Robatzek

The Sainsbury Laboratory, United Kingdom

It is now well established that plants deploy receptors at the plasma membrane as primary sensors of potentially pathogenic microbes. Receptor transportation to and retrieval from the plasma membrane depends upon the dynamic secretory and endocytic trafficking network and provide mechanisms, by which the presence of the receptors at the plasma membrane is regulated. We investigate the spatio-temporal dynamics of the Arabidopsis receptor kinases FLAGELLIN SENSING 2 (FLS2), EF-Tu RECEPTOR (EFR) and Pep1 RECEPTOR 1 (PEPR1), and the Cf-4 receptor-like protein from tomato. I will describe a common, ligand-induced endosomal pathway of these receptors and the involvement of the co-receptor BRI1-ASSOCIATED KINASE 1 (BAK1) in this process. Of note, in the case of FLS2, we could show that its ligand is co-internalized with the receptor. Focusing on late endosomal sorting, I will present results showing that FLS2 is a cargo of the ESCRT machinery and internalized inside multivesicular bodies for vacuolar degradation. These studies also suggest a role of FLS2 endosomal trafficking in stomatal immunity. To further study receptor-mediated endocytosis we explored clathrin, and I will describe recent results showing clathrin-mediated endocytosis of FLS2 and its involvement in downstream signaling. Taken together, we use live-cell imaging combined with functional studies to understand the dynamic changes involved in the interaction between plants and microbes at the plasma membrane.

Identification of plant proteins targeted by oomycete RXLR effectors using *in planta* co-immunoprecipitation

Joe Win, Martin Schattat, Ahmed Abd-El-Haliem, Tolga O. Bozkurt, Yasin Dagdas, Lilliana M. Cano, Jan Sklenar, Alex Jones, Jack Vossen, and Sophien Kamoun

The Sainsbury Laboratory, Norwich, NR4 7UH, UK

Oomycete plant pathogens secrete RXLR effectors that are translocated inside plant cells to alter plant cellular processes to establish infection. This study aims to identify both the target and function of oomycete effectors *in planta*. We selected 68 putative effectors including the validated oomycete RXLR effectors and used the high-expression vector pJL-TRBO, a binary plasmid containing a modified *Tobacco mosaic virus* to express FLAG-tagged mature oomycete effectors. Effector constructs were delivered into the leaves of *Nicotiana benthamiana* by agroinfiltration and effectors expressed under the viral coat protein promoter. Leaves were harvested 2-3 days post-infiltration and total proteins extracted. Effector proteins and their host targets were co-immunoprecipitated (co-IP) with anti-FLAG resins under non-denaturing conditions. Bound proteins were washed, specifically eluted with 3X FLAG peptides, separated by SDS-PAGE and visualized by colloidal Coomassie blue staining. Protein bands corresponding to each *in planta* effector Co-IP were excised, digested with

trypsin and identified by LC-MS/MS peptide ion spectrum matching against *N. benthamiana* proteome database. We have expressed 75 effectors in total and 53 to sufficient levels for co-IP and subsequent MS identification of precipitated proteins. We confirmed more than 20 effector-plant protein associations. We found that one family of effectors was associated with some components of vesicle trafficking machinery and affected the formation of endosomal membrane compartments. We will discuss these findings and their implications on plant-oomycete interactions.

Towards a better understanding of TNL receptor activation triggered by *Ralstonia solanacearum* PopP2 effector

Clémentine Le Roux[1], Alain Jauneau[2], Gaëlle Huet[3], Laurent Camborde[4] and **Laurent Deslandes[3]**.

[1]Dept. Plant-Microbe Interactions, Max Planck Institute for Plant Breeding Research, Cologne, Germany
[2]Laboratory of Plant-Microbe Interactions, UMR CNRS-INRA 2594-441, Chemin de Borde Rouge, F-31326 Castanet-Tolosan, France
[3]Plateforme Imagerie TRI FR3450, Centre INRA de Toulouse, Pôle de Biotechnologie Végétale, 31326 Castanet-Tolosan, France
[4]Laboratoire de Recherche en Sciences Végétales (LRSV), UMR 5546 UPS/CNRS, Pôle de Biotechnologies Végétales, 24, chemin de Borde Rouge, B.P. 42617 Auzeville, 31326 Castanet-Tolosan, France.

Plants have evolved intracellular receptors to recognize pathogen effectors and trigger a robust immune response (ETI). Arabidopsis TNL (Toll/Interleukin1-Nucleotide Binding-Leucine rich repeat) immune receptors RRS1-R (Resistance to *Ralstonia solanacearum*1) and RPS4 (Resistance to *Pseudomonas syringae*4) function as a dual resistance system able to recognize, among other effectors, *Ralstonia solanacearum* PopP2. PopP2, a YopJ family member, physically associates with RRS1-R, promotes its nuclear accumulation and displays an acetyltransferase activity required for RRS1-R-mediated immunity. To understand better the molecular bases of TNL activation, we are focusing on the characterization of protein complexes engaged with PopP2 within the nucleus. We will present data showing proteins-protein and protein-DNA interactions monitored by FRET-FLIM in plant cells.

Complex interactions between rice NB-LRR proteins govern the recognition of magnaporthe oryzae effectors

Stella Césari[1, 2, 3] , Hiroyuki Kanzaki[4] , Tadashi Fujiwara[5] , Maud Bernoux[3],Véronique Chalvon[1], [2], Yoji Kawano[5], Ko Shimamoto[5], Peter Dodds[3], Ryohei Terauchi[4], **Thomas Kroj**[1], [2]

[1] INRA, UMR BGPI, F-34398 Montpellier, France.
[2] CIRAD, UMR BGPI, F-34398 Montpellier, France.
[3]CSIRO Plant Industry, Canberra, ACT 2601, Australia.
[4] Iwate Biotechnology Research Center, Kitakami, Iwate 024-0003, Japan.
[5] Laboratory of Plant Molecular Genetics, Nara Institute of Science and Technology, 8916-5 Takayama, Ikoma, Japan

Plant resistance proteins of the class of nucleotide-binding and leucine-rich repeat domain proteins (NB-LRRs) are immune sensors which recognize pathogen-derived molecules termed avirulence (AVR) proteins. We show that RGA4 and RGA5, two NB-LRRs from rice, interact functionally and physically to mediate resistance to the fungal pathogen *Magnaporthe oryzae* and accomplish different functions in AVR recognition. RGA4 triggers an AVR-independent cell death that is repressed in the presence of RGA5 in both rice protoplasts and *Nicotiana benthamiana*. Upon recognition of the pathogen effector AVR-Pia by direct binding to RGA5, repression is relieved and cell death occurs. RGA4 and RGA5 form homo- and hetero-complexes and interact through their coiled-coil domains.

Functional analysis indicates that RGA4 acts outside the nucleus. Localization studies in rice protoplast and *N. benthamiana* suggest that RGA4 and RGA5 are excluded from the nucleus. Recent advances in the investigation of the localization of RGA4 and RGA5 will be presented.

Form and place; what is the relation between conformation and subcellular localization for the NB-LRRs Rx1 and Gpa2?

Erik Slootweg, Rikus Pomp, Jan Roosien, Jaap Bakker, Aska Goverse

Laboratory of Nematology, Wageningen University, Wageningen,, The Netherlands

The highly homologous NB-LRR resistance proteins Rx1 and Gpa2 consists of several domains with distinct functions in pathogen recognition, intermolecular interactions and signalling. The C-terminal half of the leucine-rich repeat domain(LRR), for example, is the main determinant of pathogen recognition specificity, whereas the nucleotide-binding domain alone is sufficient for the induction of a cell-death response.

The three main domains of Rx and Gpa2; their N-terminal coiled coil, central nucleotide-binding domain and C-terminal LRR, all interact and can even form a functional protein when coexpressed as separate polypeptides. Interestingly, some of these interactions are released in the presence of avirulent pathogen effectors indicative of conformational changes that play a role in the regulation and activation of the R protein. Slight mismatches between the interacting domains can lead to strong autoactivation or loss-of-function.

Rx1 and Gpa2 are distributed between the nucleus and cytoplasm and for Rx we could show that shifting this distribution to either subcellular compartment reduces its ability to initiate resistance against PVX. The finding that the individually expressed domains have strikingly different cellular distributions and that the introduction of a mutation in Rx that would disrupt nucleotide-binding leads to a strong reduction in the nuclear localized Rx points to a link between conformation, activational state and subcellular localization. We hope to gain a further understanding of the functioning of resistance proteins by studying this link using in vivo spectroscopic techniques in combination with biochemical analyses of the domain interactions and cell fractionation assays.

Localisation and re-localisation of R3a following perception of Avr3a from Phytophthora infestans

Paul Birch

University of Dundee, United Kingdom

The avirulence effector AVR3a exists in two forms in nature: AVR3aKI is recognised by R3a, whereas AVR3aEM evades recognition. Using confocal microscopy we have shown that R3a, which cytoplasmic in the absence of effectors, is re-localised to late endosomes in the presence of AVR3aKI, but not AVR3aEM. We have mutated R3a, by error-prone PCR and PCR-shuffling, to gain recognition of AVR3aEM. This gain of recognition is accompanied by a gain of re-localisation to late endosomes. Through studies of AVR3a targets in plant cells, we are investigating the potential contributions of these to the recognition and re-localisation process.

Emerging role of SGT1 as a regulator of NB-LRR receptors' nucleocytoplasmic partitioning

Rafał Hoser, Małgorzata Lichocka, Marek Żurczak, Jacek Hennig and **Magdalena Krzymowska***

Institute of Biochemistry and Biophysics, Polish Academy of Sciences, Poland

Plant Nucleotide-Binding (NB) and Leucine-Rich Repeat (LRR) - receptors mediate effector triggered immunity. There are two major classes of the NB-LRRs involved in this process, Toll-Interleukin Receptor (TIR)-NB-LRR and Coiled Coil (CC)-NB-LRR proteins. Recent reports show that at least some of the NB-LRRs of both classes are localized to the cytoplasm and nucleus and that the proper equilibrium between these pools is required to establish full resistance. This implies that nucleocytoplasmic shuttling of the receptors has to be tightly regulated. We have recently shown that SGT1, a protein known so far to control stability and activity of NB-LRRs, facilitates nuclear import of N protein, which belongs to TIR-NB-LRR class of the receptors. We proposed also that nucleocytoplasmic partitioning of N can be finely tuned by phosphorylation of SGT1. Here we show, that subcellular localization of Rx, a CC-NB-LRR type protein, tracks the position of the ectopic variants of SGT1 in the cell. This suggests that SGT1 might have a general role in maintaining nucleocytoplasmic balance of NB-LRR receptors. This issue as well as differences in N - and Rx - systems will be presented.

Subcellular localization of *Phytophthora infestans* RXLR effector AVR1 and its cognate resistance protein R1

Yu Du, Klaas Bouwmeester & Francine Govers.

Laboratory of Phytopathology, Wageningen University, The Netherlands

Phytophthora infestans is a devastating plant pathogen that causes late blight on potato and tomato. To colonize host plants, *P. infestans* secretes effectors that can modulate host defence. Well-known are the RXLR effectors, which are able to translocate into host cells to manipulate the cell machinery. However, to counteract the pathogen potato has a set of immune receptors known as nucleotide-binding leucine-rich repeat (NLR) proteins that confer resistance against *P. infestans*. NLR-conferred resistance is mediated by recognition of RXLR effectors, with each NLR protein (or R protein) having its own cognate RXLR effector (or AVR protein). The mechanisms underlying NLR-mediated resistance are still poorly understood. In this study we focussed the *P. infestans* RXLR effector AVR1 and its cognate potato NLR R1 and addressed the question in which subcellular compartment effector perception and defence activation takes place. We determined the subcellular localization of both AVR1 and R1. We also fused Nuclear Localization Signals (NLS) and Nuclear Export Signals (NES) to R1 and AVR1, as well as mutated NLS and NES, and used these constructs for artificial subcellular targeting of R1 and AVR1. This allowed us to determine the subcellular localization that is required to elicit R1-mediated immunity and AVR1-mediated host defence suppression

ABSTRACTS

Posters

Regulate and be regulated: tight control of plant defence responses by the Arabidopsis transcription factor MYB30

Pierre Buscail, Daniel Marino, Joanne Canonne, Alain Jauneau, Cecile Pouzet, Dominique Roby and Susana Rivas

Laboratoire des interactions plantes micro-organismes (LIPM), UMR CNRS-INRA, FRANCE

Plant defence responses are often associated to the development of the so-called hypersensitive response (HR), a form of programmed cell death that confines the pathogen to the inoculation site. The sharp boundary of the HR suggests the existence of efficient mechanisms that control cell death and survival. The transcription factor MYB30 is a positive regulator of Arabidopsis defence and HR responses against bacterial pathogens. MYB30 appears to modulate cell death-related lipid signaling by enhancing the synthesis of sphingolipid-containing very long chain fatty acids after bacterial inoculation.

Plant and animal pathogenic bacteria inject type III effectors (T3Es) into host cells to suppress host immunity and promote successful infection.We have shown that MYB30 is targeted by the bacterial T3E XopD, resulting in suppression of MYB30-mediated plant defences and underlining the crucial role played by MYB30 in the regulation of plant disease resistance. In addition, the activity of MYB30 is tightly controlled inside plant cells through protein-protein interactions and posttranslational modifications. Our recent work identified a protease of the subtilase family (SBT) as a MYB30-interacting partner in yeast. Interestingly, we have shown that the *SBT* transcript is alternatively spliced, leading to the production of two distinct gene products that encode either a secreted (SBT-1) or a nycleocytoplasmic (SBT-2) protein. The specific interaction between MYB30 and SBT-2 was confirmed *in planta* using the FRET-FLIM technique. Importantly, analysis of *sbt* mutant Arabidopsis plants identified SBT as a negative regulator of HR and defence responses to bacterial inoculation. The implications of these findings will be discussed.

Molecular components of XopAC effector recognition at the plasma membrane.

Brice Roux, Endrick Guy, Nicolas Denancé, Martine Lautier, Emmanuelle Lauber, Matthieu Arlat and **Laurent Noël**

Laboratoire des interactions plantes micro-organismes (LIPM), UMR CNRS-INRA 2594/441, F-31326 Castanet-Tolosan, FRANCE.

The Gram-negative bacterium *Xanthomonas campestris* pv. *campestris* (Xcc) is the causal agent of black rot of *Brassicaceae*. *Xcc* colonizes first plant xylem vessels and presumably injects type III effectors in neighbouring parenchyma cells to suppress plant immunity. In Arabidopsis, the type III effector XopAC is able to inhibit the receptor-like cytoplasmic kinase (RLCK) BIK1 by uridylylation to supress PTI. We demonstrated that the XopAC-triggered ETI is dependent on at least two other RLCK of the same subfamily, namely PBL2 and RIPK. Interestingly, PBL2, RIPK and XopAC localize at the plasma membrane. While most RLCK are known to be myritoylated/palmitoylated, the molecular basis of XopAC membrane anchoring remains elusive. Importantly, a loss of interaction between XopAC and these RLCKs correlates with a cytoplasmic localization of XopAC. These results

suggest that RLCK mediate relocalization of XopAC to the plasma membrane where XopAC-triggered ETI happens. The latest progress in this project will be presented.

Incidence of *meloidogyne incognita* esophageal protein expression in host plants and interaction networks with host proteins.

Grossi De Sa, Maira[1], Vignols, F [1,3], Lambou, K [1.],Mezzalira Im [2.], Beneventi M [2], Petitot As [1], Grossi-De-Sa, Mf [2], Fernandez D [1]

[1] *Institut de Recherche pour le Développement - UMR 186 IRD-UM2 &* [3] *CNRS*
[2] *Embrapa Recusos Geneticos e Biotecnologia*

Root-knot nematodes (RKNs) release effector proteins from their esophageal gland cells into host roots tissues. Whether interactions between *M. incognita* proteins (MSPs) and host proteins govern plant susceptibility to the nematode remain to a key question. To gain insight into the function of MSPs in the plant cells, we performed histological analyses of *Nicotiana tabaccum* plants expressing individual selected MSPs and further infected by *M. incognita*. Overexpression of two MSPs induced an accelerated giant cell formation time-course and an increase of nematode infectivity suggesting that these proteins could play a critical role in parasitism success. Sub-cellular localization analyses of these proteins fused to the GFP marker protein in onion cells showed that MSPs are targeted to the nucleus and in the cytosol. In order to check whether these effectors target host proteins, we are currently performing yeast-two hybrid binary interaction assays, first studying possible interactions with Arabidopsis highly connected cellular hub proteins (Mukhtar et al, Science 2011). Data obtained should significantly widen our knowledge about host-nematode interaction and open new strategies for nematode control.

The *Ustilago* core effector Pep1 and its role in biotrophy of smut fungi

Hemetsberger C.[1, 2], Herrberger C.[2], Mueller A.[2], Matei A.[2], Hensel G.[3], Kumlehn J. [3], Thines M. [4], Hueckelhoven R[1]., Doehlemann G[2].

[1]Lehrstuhl für Phytopathologie, Technische Universität München, Emil-Ramann-Str. 2, D-85350 Freising-Weihenstephan,Germany
[2]Max Planck Institute for Terrestrial Microbiology, Karl-von-Frisch Str. 10, D-35043 Marburg, Germany
[3]Leibniz Institute of Plant Genetics and Crop Plant Research Gatersleben, Plant Reproductive Biology, D-06466 Gatersleben, Germany
[4]Biodiversity and Climate Research Centre, Senckenberganlage 25, D-60325 Frankfurt am Main, Germany

The secreted fungal effector Pep1 is essential for successful penetration of the host epidermis and establishment of biotrophic interaction in the *Ustilago maydis* / maize pathosystem.
Deletion of *pep1* does not affect saprobic growth, however pathogenic development is arrested after the penetration attempt and a strong plant immune response is elicited [1]. We have shown that Pep1 acts as direct inhibitor for apoplastic plant peroxidases and therefore has the ability to suppress the oxidative burst, a primary immune response of the host plant, enabling fungal colonization [2].
In addition, functional conservation of Pep1 among the different smut fungi *U. maydis* and *U. hordei* was documented [1]. To investigate a possible conservation of Pep1 in other pathogens, genomes of related smut species were screened for *pep1* orthologs. Indeed, Pep1-ORFs could be detected in a variety of smuts. Sequence analysis of these orthologs revealed a remarkable degree of structural conservation of Pep1, indicating that this effector might play a fundamental role in virulence of

biotrophic smut fungi. Here we show that Pep1 function is also conserved among different pathosystems, even across the monocot / dicot border of host plants, classifying it as basal core effector. Furthermore, we studied the influence of Pep1 on another, non smut-related pathosystem, i.e. the barley powdery mildew *Blumeria graminis f. s. hordei* to assess its significance as suppressor of early plant defense.

A partial duplicate of the susceptibility-related E3 ligase *PUB15* mediates powdery mildew resistance in barley and wheat

Jeyaraman Rajaraman[1], Dimitar Douchkov[1], Stefanie Lück[1], Jörn Klinkenberg[2], Götz Hensel[1], Caroline Höfle[3], Ralph Hückelhoven[3], Marco Trujillo[2], Jochen Kumlehn[1], and Patrick Schweizer[1] .

[1] Leibniz Institute of Plant Genetics and Crop Plant Research (IPK), Germany
[2] Leibniz Institute of Plant Biochemistry (IPB), Halle (Saale), Germany
[3] Lehrstuhl für Phytopathologie, Technische Universität München (TUM), Germany

The U-box E3 ligase is a key regulatory component of plant ubiquitination machinery affecting almost all aspects of plants growth and development. Because of its central role in degradation and modification of proteins, E3 ligases are also preferred targets of pathogen-encoded effectors for manipulation of host's defense responses. Plants on the other hand have evolved several strategies to avoid such manipulations and thereby to arrest the growth of pathogens. Here we report on a partial gene duplicate with a probable function as a decoy for a functional E3 ligase which may be an effector target.
Using a high-throughput transient-induced gene silencing screen we identified a partial gene duplicate of the *HvPUB15* U-box/armadillo-repeat E3 ligase designated as *HvARM1* (for *barley Armadillo 1*) affecting the interaction with the biotrophic powdery mildew fungal pathogen. Functional characterization of *HvARM1* by transient silencing and stable RNAi in barley resulted in enhanced susceptibility to *Blumeria graminis f.sp. hordei (Bgh).* Furthermore, transient over-expression in barley and wheat triggered resistance to Bgh. *HvPUB15* and *HvARM1* were also found to physically interact with the plastid-localized barley homologue of *TaToxABP1* (for *wheat toxin A binding protein 1*) and with *HvClpS1* (for *Clp-protease adaptor S*) in Y2H assay. Transient silencing and overexpression of *HvToxABP1* revealed this putative *HvPUB15*-client protein as susceptibility-related plant factor for *Bgh* interaction. Putting together, our data suggests a neo-functionalized role of *HvARM1* as a factor functioning as a decoy for the susceptibility-related *HvPUB15* protein, which might be involved in regulating downstream plastid-localized responses to support powdery mildew infection. .

ADDITIONAL POSTER TITLES

(see above for abstract summary)

Celine Pesce *(Institute of Research for Development, Montpellier, France)*
Xanthomonas translucens **– do type III and particulary TAL effectors contribute to pathogenicity?**

Bruno Favery *(INRA Sophia Antipolis, France)*
Characterization of host cell responses and root-knot nematode effectors involved in the formation of multinucleate and hypertrophied feeding cells

Lukasz Baranowski *(Warsaw University Of Life Sciences, Poland)*
Microscopic analysis of syncytia induced by potato cyst nematode *globodera rostochiensis* in wild-type and *ERABP1*- or *NGB*-silenced tomato plants

Anne-Claire Cazale *(INRA Toulouse, France)*
Systematic plant sub-cellular localization of *ralstonia solanacearum* type iii effectors

Suayib Üstün *(Leibniz Institute Of Vegetable & Ornamental Crops, Germany)*
The *xanthomonas campestris* type iii effector XOPJ proteolytically degrades proteasome subunit RPT6 to inhibit proteasome-mediated turnover of NPR1

Elysa Overdijk *(Wageningen University, The Netherlands)*
The plant exocyst as an expeller of pathogens

Joe Win *(The Sainsbury Laboratory, United Kingdom)*
Identification of plant proteins targeted by oomycete RXLR effectors using *in planta* co-immunoprecipitation

Yu Du *(Wageningen University, The Netherlands)*
Subcellular localization of *phytophthora infestans* RXLR effector AVR1 and its cognate resistance protein R1

Thomas Rey (The Sainsbury Laboratory, Cambridge University)
Mining Medicago genetic ressources to identify common elements of colonization by pathogens and symbionts

List of Participants

Participant Name	Institution	Country	Email Address
Lukasz Baranowski	Warsaw University of Life Sciences	Poland	*lukasz_baranowski@sggw.pl*
Paul Birch	University of Dundee	United Kingdom	*paul.birch@scri.ac.uk*
Petra Boevink	The James Hutton Institute	United Kingdom	*petra.boevink@hutton.ac.uk*
Pierre Buscaill	INRA Toulouse	France	*pierre.buscaill@toulouse.inra.fr*
Laurent Camborde	CNRS Toulouse	France	*camborde@lrsv.ups-tlse.fr*
Anne-Claire Cazalé	INRA Toulouse	France	*accazale@toulouse.inra.fr*
Laurent Deslandes	CNRS Toulouse	France	*laurent.deslandes@toulouse.inra.fr*
Gunther Döhlemann	Marburg University	Germany	*doehlemann@mpi-marburg.mpg.de*
Yu Du	Wageningen University	Netherlands	*yu.du@wur.nl*
Bruno Favery	INRA Sophia-Antipolis	France	*bruno.favery@sophia.inra.fr*
Isabelle Fudal	INRA Versailles-Grignon	France	*fudal@versailles.inra.fr*
Quentin Gascuel	INRA Toulouse	France	*quentin.gascuel@toulouse.inra.fr*
Cynthia Gleason	Georg-August-Universität Göttingen	Germany	*cgleaso@uni-goettingen.de*
Laurence Godiard	INRA Toulouse	France	*laurence.godiard@toulouse.inra.fr*
Aska Goverse	Wageningen University	Netherlands	*aska.goverse@wur.nl*
Maira Grossi de Sa	IRD Montpellier	France	*maira.grossi-de-sa@ird.fr*
Christoph Hemetsberger	Technical University Munich	Germany	*c.hemetsberger@tum.de*
Ralph Hueckelhoven	Technical University Munich	Germany	*hueckelhoven@wzw.tum.de*
Edgar Huitema	University of Dundee	United Kingdom	*edgar.huitema@hutton.ac.uk*
Thomas Kroj	INRA Montpellier	France	*thomas.kroj@supagro.inra.fr*
Magdalena Krzymowska	Institute of Biochemistry & Biophysics, PAS	Poland	*krzyma@ibb.waw.pl*
Laurent Noël	CNRS Toulouse	France	*laurent.noel@toulouse.inra.fr*
Silke Nowak	Ghent University	Belgium	*silke.nowak@ugent.be*
Richard O'Connell	INRA Versailles-Grignon	France	*richard.oconnell@versailles.inra.fr*
Elysa Overdijk	Wageningen University	Netherlands	*elysa.overdijk@wur.nl*
Nemo Peeters	INRA Toulouse	France	*nemo.peeters@toulouse.inra.fr*
Céline Pesce	IRD Montpellier	France	*celine.pesce@ird.fr*
Jeyaraman Rajaraman	IPK Leibniz	Germany	*rajaraman@ipk-gatersleben.de*
Thomas Rey	The Sainsbury Laboratory	United Kingdom	*thomas.rey@slcu.cam.ac.uk*
Susana Rivas	LIPM Toulouse	France	*susana.rivas@toulouse.inra.fr*
Silke Robatzek	The Sainsbury Laboratory	United Kingdom	*robatzek@tsl.ac.uk*
Sebastian Schornack	The Sainsbury Laboratory	United Kingdom	*sebastian.schornack@slcu.cam.ac.uk*
Erik Slootweg	Wageningen University	Netherlands	*erik.slootweg@wur.nl*
Boris Szurek	IRD Montpellier	France	*boris.szurek@ird.fr*
Hans Thordal-Christensen	University of Copenhagen	Denmark	*htc@life.ku.dk*
Suayib Üstün	IGZ EV Leibniz	Germany	*uestuen@igzev.de*
Keke Wang	INRA Toulouse	France	*keke.wang@toulouse.inra.fr*
Joe Win	The Sainsbury Laboratory	United Kingdom	*joe.win@tsl.ac.uk*