Meetings

Effectors, effectors *et toujours des* effectors

22nd New Phytologist Symposium: Effectors in plant–microbe interactions, INRA Versailles, France, September 2009

Despite their diversity, microbes seem to have evolved similar strategies to interact with plants. One such strategy involves the production of effectors that were first identified in bacteria as proteins injected into plant cells by a specific molecular syringe. Since then, effectors have been identified in a variety of eukaryotic microbes and are now defined as molecules, produced by bio-aggressors, pathogens and symbionts during infection, that act on plant cells (Hogenhout *et al.*, 2009). The study of effectors is a rapidly expanding research field that benefits from recent advances in microbial and plant genomics. The 22nd New Phytologist Symposium aimed to support this research field by bringing together, in Versailles, scientists from around the world who were working on different aspects of plant–microbe interactions over a wide range of pathosystems (viruses, bacteria, fungi, oomycetes, nematodes and insects). The Symposium was timely and popular, even though it took place just a few months after the XIV International Congress on Molecular Plant–Microbe Interactions in Quebec, which had a strong emphasis on effector biology (Walton *et al.*, 2009). Indeed, this specialized New Phytologist Symposium translated this mainstream research on microbial effectors into lively discussion sessions.

The conference was covered with a live twitter feed (#NPS09 tag, at http://tinyurl.com/y877xoy). Here, we present some of the highlights, with a special mention of three posters that were selected for awards.

'…fungal symbionts use proteins secreted in host plant tissues to interact with their hosts, extending the importance of effectors from pathogens to beneficial microbes.'

Bacterial effectors act on a wide range of plant cellular processes

Pioneering research on bacterial effectors has revealed that their type III molecular syringe consists of a complex structure derived from the flagellum. Guy Cornelis (Biozentrum, Basel, Switzerland) presented a full three-dimensional structure of this complex and showed that it is composed of proteins with auto-assembly properties. This machinery allows specific bacterial proteins to be injected into plant cells through membranes and cell walls. Novel bacterial proteins injected through this mechanism were identified and included effectors with multiple functional protein domains, suggesting that there are a large number of possible targets in plant cells, as reported in a runner-up poster (poster 63: Plant proteins targeted by the GALA type III effector family from the pathogenic bacterium *Ralstonia solanacearum*. P. Remigi, I. Kars, S. Genin, C. Boucher and N. Peeters). Indeed, a wide range of plant proteins targeted by bacterial effectors were identified (kinase, NBS-LRR, ubiquitin ligase, RNA-binding protein and enzymes from the micro-RNA pathway). Additionally, Ulla Bonas (Martin-Luther-University Halle-Wittenberg, Germany) described how the effector, AvrBs3, acts on DNA by binding to plant promoters and functioning as a transcriptional activator. AvrBs3 was discovered to bind DNA through a unique mechanism, following a code for sequence recognition that depends on the number of repeats of the protein and on the sequence of two hypervariable residues (Boch *et al.*, 2009). Bacterial secondary metabolites were also shown to act as effectors, with Syringolin A acting as a proteasome inhibitor, as reported in a runner-up poster (poster 50: The small effector molecule Syringolin A inhibits the plant proteasome *in vivo* and during infection. J. J. Misas-Villamil, I. Kolodziejek, J. Clerc, M. Kaiser, M. Verdoes, H. Overkleeft, B. Schellenberg, R. Dudler and R. A. L. van der Hoorn). The evolutionary genomics of bacterial effectors was also boosted by the increased number of available bacterial genomes, revealing a high genetic diversity within effector families from related strains/species (Stephan Genin, LIPM, Toulouse, France; Brian Stackawicz, University of California-Berkeley, USA).

Jim Alfano (University of Nebraska, USA) reported how large-scale *in planta* functional screens have revealed that a large number of *Pseudomonas syringae* type III effectors inhibit plant defenses (Guo *et al.*, 2009). Greg Martin (Cornell University, NY, USA) discussed AvrPtoB, another

cell death-suppressing type III effector of *P. syringae*. AvrPtoB targets the tomato protein Bti9, a receptor-like protein kinase orthologous to the *Arabidopsis thaliana* chitin receptor Cerk1. Hairpin silencing of Bti9 in tomato enhanced susceptibility to bacteria. AvrPtoB was previously known to interact with the resistance protein Pto. Martin suggested that Pto may have evolved as a decoy of Bti9, the potential operative target of AvrPtoB (Van der Hoorn & Kamoun, 2008).

Translocation of eukaryotic microbe effectors into plant cells: towards conserved mechanisms

In eukaryotic pathogens, effectors are secreted in plant tissues through classical secretion machineries. These effectors can diffuse in the plant apoplast or enter into plant cells through largely unknown mechanisms. Several presentations have illustrated how research on effectors from oomycetes has brought some clarity to this topic (Dodds *et al.*, 2009). Indeed, genome-wide analyses of these eukaryotic filamentous organisms related to brown algae have shown that they carry a large number of gene families encoding candidate effectors with either the RxLR-dEER motif (~550 in the potato blight pathogen *Phytophthora infestans*) or the LFLAK motif (Crinkler family ~200 members in *P. infestans*) (Haas *et al.*, 2009). Sophien Kamoun (The Sainsbury Laboratory, UK) reported that the Crinkler effectors have a wider species distribution (Peronosporales to Saprolegniales) than the RxLR family (Peronosporales). Expansion of these multigenic families was observed in all plant pathogenic species including, as reported by Jim Beynon (University of Warwick, UK), *Hyaloperonospora arabidopsidis*, a biotrophic oomycete pathogen. Brett Tyler (Virginia Bioinformatics Institute, USA) reported significant progress on the mechanisms of translocation of RxLR effectors into plant cells. Experimental evidence was presented for the rapid and efficient uptake of RxLR proteins into plant and human cells through a phosphatidyl-inositide phosphate-facilitated process. This process requires the RxLR-dEER motif that is located after the signal peptide (the N-term of the mature protein) and that resembles the PEXEL translocation motif of *Plasmodium* effectors. Significant progress was also made in understanding the biochemical activities of RxLR effectors. Paul Birch (Scottish Crop Research Institute, UK) reported that *P. infestans AVR3a* targets CMPG1, an E3 plant ligase, as well as Sec5, a plant protein involved in exocytosis.

Translocation into plant cells of effectors from plant pathogenic fungi was also shown in several cases, namely Pwl2 from *Magnaporthe oryzae*, AvrL567 from *Melamspora lini* and ToxA from *Pyrenophora tritici*. According to Brett Tyler (Virginia Bioinformatics Institute, USA), some fungal effectors carry degenerate RxLR-like motifs. When tested using a soybean root-translocation assay designed for oomycete effectors, RxLR motifs from three fungal effectors (AvrL567, Six1 and AvrLm6) proved sufficient to translocate green fluorescent protein (GFP) into plant cells. These results enable the identification of candidate effectors and thus potentially open up a whole new research area in fungal effectomics. These findings also imply that different pathogens use similar strategies to deliver their proteins into host cells. Genome-wide analysis of fungal effectors has also revealed either their biased localization in transposon-based AT-rich regions of the *Leptosphaeria maculans* genome, as discussed by Thierry Rouxel (BIOGER-INRA, Grignon, France), or their expansion in the *Cladosporium fulvum* genome (Pierre de Wit, Wageningen University, the Netherlands). Pierre de Wit also reported biochemical activities, such as binding to chitin and inhibition of proteases, of some *C. fulvum* apoplastic effectors (De Wit *et al.*, 2009). Also, as reported for bacteria, fungal secondary metabolites were shown to act as effectors, either as toxins facilitating infection or as avirulence signals recognized by resistant plants (Marc-Henri Lebrun, BIOGER-INRA, Grignon, France).

Do effectors from symbiotic bacteria and fungi play a role in these interactions?

The genomes of plant symbiotic bacteria also contain a small number of effector-encoding genes injected through a type III molecular syringe, as reported by William Deakin (Université de Genève, Switzerland). These effectors have either positive or negative effects on symbiosis. Indeed, the same bacterial effector was shown to act positively on symbiosis during the interaction with *Medicago truncatula*, while it was detrimental during the interaction with *Phaseolus vulgaris*, making the overall response more complex. Francis Martin (INRA-Nancy, France) described how the genomes of symbiotic fungi also carry genes encoding effectors, as observed in the ectomycorrhizal symbiont *Laccaria bicolor* (Martin *et al.*, 2008). Genome analysis of *L. bicolor* revealed a large number of genes encoding small secreted proteins that were overexpressed during symbiosis. The most highly expressed effector during symbiosis (MiSSP7) specifically accumulates in fungal cells colonizing the root apoplasm. Silencing of this gene dramatically reduced fungal root colonization, demonstrating, for the first time, that a fungal effector is required for symbiosis. *Glomus intraradices*, an endomycorrhizal symbiotic fungus, also produces effectors that are secreted and translocated into the root cell nucleus, as illustrated in the talk of Natalia Requena (University of Karlsruhe, Germany). Expression of this protein in plant root facilitated the establishment of symbiosis. These results were also presented in a poster that received the First Prize during this meeting (poster 37: A putative novel effector family in arbuscular mycorrhizal fungi. S. Kloppholz and N. Requena). Overall, these presentations clearly demonstrate

that fungal symbionts use proteins secreted in host plant tissues to interact with their hosts, extending the importance of effectors from pathogens to beneficial microbes.

Effectors in plant insect–nematode interactions: an emerging field

Effectors are not limited to microbial pathogens as they have also been identified in insects and worms. Richard Hussey (North Carolina State University, USA) described how plant-associated nematodes inject peptides into host plant cells that act on plant transcription or that mimic plant-signaling peptides, such as Clavata 3 (Davis *et al.*, 2008). A genome-wide survey of nematode genome sequences confirmed the presence of gene families encoding a variety of candidate effector proteins. Gerald Reeck (Kansas State University, USA) reported that insects, such as aphids, also secrete effectors into their saliva, allowing them to be injected into plant cells when the insect pricks host tissues with its stylus (Mutti *et al.*, 2008).

Conclusions

The study of effectors is an emerging and unifying theme across plant-associated microbes. The symposium strengthened the view that effectors play a pivotal role in plant–microbe interactions. Indeed, both symbiotic and pathogenic microbes appear to mobilize a rich assortment of effectors to manipulate host plant cells. The symposium was useful in bringing together researchers who have been studying effectors for a long time and have reached the point of detailed biochemistry, with other researchers who have only recently discovered effectors in the genome of their favourite plant-associated microbe. One take-home message of this meeting was the fact that the concept of effectors has clearly expanded to symbionts and plant-associated nematodes and insects. This promises a future rich in exciting discoveries and insights into plant–microbe interactions.

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Marc-Henri Lebrun[1]* and Sophien Kamoun[2]

[1]BIOGER, UMR INRA-APT, Campus Agroparistech, 78850 Thiverval Grignon, France; [2]The Sainsbury Laboratory, Colney Lane, Norwich, NR4 7UH, UK
(*Author for correspondence:
email marc-henri.lebrun@bayercropscience.com)

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Soil respiration across scales: towards an integration of patterns and processes

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In view of a rapidly changing climate system there has been a growing interest in the role of ecosystems in the global carbon (C) cycle. Considerable uncertainties still exist concern-