
Preface

Over the past decade a major paradigm shift has taken place, from studying disease resistance in plants to investigating the roles that plant pathogen effectors play in suppressing, triggering, or otherwise manipulating plant defenses. Effectors are secreted proteins or other molecules that can act either inside or outside plant cells. Many effectors are thought to be required for suppressing Pattern-Triggered Immunity (PTI), the front line of inducible plant defense. However, they can also be the targets for resistance proteins, leading to the activation of Effector-Triggered Immunity (ETI), making them central players in dictating the outcomes of plant–pathogen interactions. Effectors and their functions are being studied in their own right. However, they are emerging as major tools to dissect host defense pathways and as primary targets to develop new screens for host disease resistance genes. Effectors may also play other important roles in determining the success of a pathogen that are not necessarily related to suppression of host defenses. For example, effectors may be important for manipulation of the host metabolism to provide food to the pathogen. This is the case for the biotrophic plant-parasitic nematodes, which need to induce the formation of large and complex feeding structures in order to obtain nutrients, but is also likely to be true for other plant pathogens.

The past 3 years have seen an explosion in available plant pathogen genome sequences, revealing the blueprints for host interactions and the repertoires of effectors needed to overcome host immunity. Increased access to high-throughput sequencing platforms means that this pattern will continue and that generating genome and transcriptome sequences, and all the subsequent benefits of access to functional genomics approaches, will be achievable for most pathogens. Genomics has driven effector searches in eukaryotic pests and pathogens (such as fungi, oomycetes, nematodes, aphids). New bioinformatic methods for genome assembly, annotation, comparison and mining have emerged, indicating the phenomenal dynamics in genome evolution, and flooding labs with effector candidates to study interactions and functions. Chapter 1 (Cock and Pritchard) describes a Galaxy platform and workflows to identify candidate effectors from genome sequences, and Chapter 2 (Reid and Jones) describes approaches to identify effector candidates from expression data, applied to nematode pests. Chapter 3 (Saunders et al.) presents methods to analyze and visualize genome architecture, indicating gene-rich and -sparse regions. It allows researchers to portray patterns of gene expression, nucleotide polymorphisms, and the relative locations of effector candidates in the context of overall genome architecture. Chapter 4 (Pritchard and Broadhurst) takes a timely and cautionary look at the statistics of candidate effector prediction, and provides strategies to assist in improving design and evaluation of effector classifiers.

New cell biology approaches have been developed to image the molecular processes underlying plant–pathogen interactions. Chapter 5 (Beck et al.) presents a high-throughput method to visualize the earliest stages of PTI and, in particular, to quantify the dynamics of endocytic trafficking following activation of pattern recognition receptors. Cell biology is also an increasingly important tool to study effector delivery, subcellular localization, and

interactions with host target proteins. In Chapter 6, Boevink et al. describe the use of bimolecular fluorescence complementation to study *in planta* interactions, or close proximity, between pathogen effector proteins and their “target” proteins in the host cell. Chapter 7 (Takemoto and Jones) describes a rapid procedure for particle bombardment-mediated transient expression of fluorescently tagged proteins in leaf epidermal cells. This procedure is applied to investigate subcellular localization of resistance proteins, and to identify associated targeting signals. The method also lends itself to detection of pathogen effector protease activities directed against target proteins in the plant cell and analysis of protease recognition sites within these target proteins. In Chapter 8 (Garnica and Rathjen) a method is presented for rapid purification of fungal haustoria, structures from which effectors are delivered inside plant cells. They describe a new technique which combines initial gradient purification of haustoria with flow-sorting based on labeling of haustoria with fluorescent Concanavalin A.

To study effector functions, methods have been developed to mutate them, or manipulate their expression, spawning techniques to study the effects of such changes on both host and pathogen performance. Chapter 9 (Elling and Jones) describes the use of RNA interference (RNAi) to knock down the expression of specific effector candidate genes in plant-parasitic cyst and root-knot nematodes in order to investigate their impact on host interactions. Plant-mediated RNAi is described in Chapter 10 (Coleman et al.) as an approach to knock down expression of candidate effectors in aphids, and Chapter 11 (Rodriguez et al.) presents a method to identify effectors that, when transiently expressed *in planta*, have an impact on aphid performance. Chapter 12 (Tomé et al.) provides a method to quantify colonization of plant material by an obligate biotrophic oomycete (*Hyaloperonospora arabidopsidis*; *Hpa*) pathogen, which lends itself to evaluation of the contributions of *Hpa* effectors to pathogenicity. Chapter 13 (Ayliffe et al.) describes a general approach to quantify fungal colonization (applied to the wheat pathogen *Puccinia graminis* f.sp. *tritici*), based upon the specific binding of the plant lectin wheat germ agglutinin to fungal chitin.

New methods are presented to study the functions of defense-associated proteins in plant hosts. Zhang and Thomma (Chapter 14) describe the methodology for *Tobacco rattle virus* (TRV)-based VIGS in *Nicotiana tabacum*. Following coexpression of the tomato immune receptor Ve1 and the corresponding *Verticillium* effector Ave1 they show how the VIGS approach can be used as a rapid system for assessing the requirement of candidate plant genes for Ve1-mediated immune signaling. Hong and van der Hoorn (Chapter 15) describe the use of “click-chemistry” to profile serine hydrolase activities in the apoplast of *Nicotiana benthamiana* challenged with *Pseudomonas syringae* p.v. *tomato* DC3000.

There is considerable interest in finding host targets of pathogen effectors as this helps to develop an understanding of how these proteins promote host susceptibility and disease. Steinbrenner et al. (Chapter 16) present a rapid co-immunoprecipitation protocol to identify effector–host protein complexes *in planta*. To explore the roles of effectors in suppressing the earliest events in PTI, Fraiture et al. (Chapter 17) describe a medium-throughput method to identify effectors that prevent activation of mitogen-activated protein kinases and upregulation of early marker genes in tomato mesophyll protoplasts.

As effector targets are identified, and effector functions are revealed, structural analysis of effectors in relation to function is an emerging area aimed at determining the detailed molecular basis of how these proteins manipulate host processes. Hughes and Banfield (Chapter 18) present a medium-throughput protocol for expression testing oomycete RXLR effectors in *Escherichia coli*, followed by methods to purify and

crystallize soluble effector protein. The methods help investigators to fully assess *E. coli* as a host for soluble protein production before considering alternative hosts for heterologous protein expression.

Effector availability has spurred the development of new approaches to screen for durable disease resistance genes in host plants. This, in turn, has promoted the conception of techniques to rapidly accelerate *R* gene discovery. Du and Vleeshouwers (Chapter 19) draw on their extensive experience of “effectoromics” in recent years to share tips, do’s, and don’ts of effector transient expression in host germplasm to seek responses indicative of ETI. Kanzaki et al. (Chapter 20) describe a rice protoplast cell death assay to identify candidate effectors based on their avirulence activities from *Magnaporthe oryzae*, and Upadhyaya et al. (Chapter 21) present the use of a bacterial type III secretion system to assay the functions of, and responses to, fungal effectors in cereals. Finally, Jupe et al. (Chapter 22) detail the use of “capture arrays” to annotate resistance genes in plant genomes, and to accelerate the discovery of resistance genes in combination with bulked segregant analysis.

In conclusion, this volume covers a breadth of new techniques (bioinformatics, cell biology, protein structural, biochemical, and functional assays) developed to identify and characterize effectors and to study their impacts on host immunity and their role in pathogen biology. It presents protocols to identify avirulence and resistance genes and new methods to investigate the roles of effector targets and other defense-associated proteins in plant immunity.

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Plant-Pathogen Interactions

Methods and Protocols

Birch, P.; Jones, J.; Bos, J. (Eds.)

2014, XIII, 306 p. 57 illus., 43 illus. in color., Hardcover

ISBN: 978-1-62703-985-7

A product of Humana Press