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ARABIDOPSIS: A RICH HARVEST 10 YEARS AFTER COMPLETION OF THE GENOME SEQUENCE

Arabidopsis and the plant immune system

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SUMMARY

Understanding the fundamental mechanisms of plant disease resistance is of central importance to sustainable agriculture and human health. Use of the model plant *Arabidopsis thaliana* has resulted in an explosion of information regarding both disease resistance and susceptibility to pathogens. The last 20 years of research have demonstrated the commonalities between Arabidopsis and crop species. In this review, commemorating the 10th anniversary of the sequencing of the Arabidopsis genome, we will address some of the insights derived from the use of Arabidopsis as a model plant pathology system.

Keywords: Arabidopsis, genome, pathology, model system.

OVERVIEW

The ability to sequence entire genomes has revolutionized biology. Since the sequencing of *Haemophilus influenzae* in 1995, hundreds of prokaryotes and dozens of eukaryotes have been sequenced (Chain *et al.*, 2009). One of the first eukaryotes to have its genome sequenced was *Arabidopsis thaliana*, which at the time was a relatively young model organism, originally promoted for its power as a genetic system in the 1980s (Rédei, 1975; Estelle and Somerville, 1986). That original reference genome sequence of Arabidopsis accession Col-0 (AGI 2000) has proven to be a key enabling factor in the acceleration of essentially all aspects of plant biology. Here, to mark the 10th anniversary of the sequencing of the Arabidopsis genome, we discuss highlights from the relatively short history of Arabidopsis–pathogen interactions.

In the mid-1980s, as Arabidopsis gained momentum as a model plant species, it was not an obvious system in which to study plant–pathogen interactions. The accepted wisdom of the time was that Arabidopsis had no pathogens. Originally, Arabidopsis was considered by some to be 'contrived', with 'largely contrived pathogens' (Keen, 1990). Progress in plant pathology as a discipline in the decades preceding the 1980s was diffused across crop plant species, with a disease-by-disease approach to systems that lacked the basic tools that Arabidopsis researchers today take for granted. With the benefit of 20 years of hindsight, it is clear that Arabidopsis has been an excellent model for answering fundamental questions in molecular plant–microbe interactions (Table 1). Over that time, a focus on Arabidopsis has contributed immensely to explaining the evolution and underlying mechanisms of disease resistance and susceptibility. As with all good models, much of what we have learned studying Arabidopsis pathology has been generalizable to plant pathosystems of nearly all kinds. Yet, Arabidopsis is not a perfect model for studying all host–microbe interactions. For example, Arabidopsis has not been found to be a host for important microbes such as the rust-causing fungal pathogens. In this regard, although traditional plant pathology studies perhaps lacked the concerted research effort required to define molecular generalities, those traditional efforts did generate a fabulous wealth of both host genetic diversity and pathogen isolates, and an understanding of the genetics governing their interactions. These provide a rich resource for application of the generalities derived from a focus on Arabidopsis.

We hope to illuminate in this review some of the key findings made using Arabidopsis, with reference to other

Table 1 A timeline of important events in plant pathology

Approximately 10 000 BP	Domestication of crops, first agricultural pathogen (Brown *et al.*, 2009)
1846	Irish potato famine
1884	Koch's postulates
1907	Disease resistance can be inherited as dominant, Mendelian genes (Biffen, 1907)
1942	Gene-for-gene hypothesis (Flor, 1947)
1975	'Arabidopsis as a Genetic Tool' published (Rédei, 1975)
1981	Arabidopsis as a viral host (Balazs and Lebeurier, 1981)
1984	Cloning of the first avirulence gene from bacteria (Staskawicz *et al.*, 1984)
1986	Transformation of Arabidopsis (Lloyd *et al.*, 1986)
1986	'The mutants of Arabidopsis' published (Estelle and Somerville, 1986)
1988	Arabidopsis as a bacterial host (Tsuji and Somerville, 1988)
1990	Arabidopsis as a fungal host (Koch and Slusarenko, 1990)
1990	Arabidopsis genome project started
1990	Avirulence genes can promote virulence (Kearney and Staskawicz, 1990)
1993	First *R* gene cloned: *Pto* of tomato (Martin *et al.*, 1993)
1994	*RPS2* cloned: first Arabidopsis *R* gene (Bent *et al.*, 1994; Mindrinos *et al.*, 1994)
1994	*NPR1* described, molecular dissection of SAR begins (Cao *et al.*, 1994)
1996	Enhanced disease susceptibility genetic screen (Glazebrook *et al.*, 1996)
1996	Type-III effectors act inside the plant cell (Gopalan *et al.*, 1996; Leister *et al.*, 1996; Van den Ackerveken *et al.*, 1996)
1997	*Escherichia coli* genome sequenced (Blattner *et al.*, 1997)
1997	*NDR1*, a gene required for *R*-gene function cloned (Century *et al.*, 1997)
1998	*RPM1* on the PM; first localization of a NB-LRR (Boyes *et al.*, 1998)
1999	*RAR1*, a gene required for *R*-gene function cloned (Shirasu *et al.*, 1999)
1999	Fitness costs associated with NB-LRR function (Stahl *et al.*, 1999)
1999	*PAD4* and *EDS1*, genes required for R-gene function cloned (Falk *et al.*, 1999; Jirage *et al.*, 1999)
2000	First plant pathogenic bacterium sequenced: *Xylella* (Simpson *et al.*, 2000)
2000	Human genome sequenced
2000	Arabidopsis genome sequenced (AGI, 2000)
2000	2010 project begins
2000	MAP kinases regulate defenses – *MPK4* cloned (Petersen *et al.*, 2000)
2000	PAMP receptors: *FLS2* cloned (Gomez-Gomez and Boller, 2000)
2001	Details and mechanistic expectations of guard hypothesis published
2001	*SID2*/*EDS16* cloned
2001	Characterization of *WRKY* gene family in Arabidopsis begins
2001	*PBS1* cloned, proposal for its effects on RPS5 consistent with the guard hypothesis (Swiderski and Innes, 2001)
2002	*RIN4* cloned; proposal for its effects on RPM1 consistent with guard hypothesis (Mackey *et al.*, 2002)
2003	Support for guard hypothesis: RIN4 cleaved by AvrRpt2 (Mackey *et al.*, 2003)
2003	Non-host mutant screens (Collins *et al.*, 2003)
2003	PBS1 is cleaved by AvrPphB; recognition of modified self (Shao *et al.*, 2003)
2003	*Pseudomonas syringae* DC3000 genome is sequenced (Buell *et al.*, 2003)
2003	Effectors can suppress basal resistance (Hauck *et al.*, 2003)
2003–2004	NB-LRRs require a co-chaperone complex (Schulze-Lefert, 2004)
2005	EDS1/PAD4/SAG101 complexes (Feys *et al.*, 2005)
2005	Secretory pathway required for SAR (Wang *et al.*, 2005)
2006	Small RNAs involved in bacterial defense (Navarro *et al.*, 2006)
2007	Twenty Arabidopsis strains re-sequenced (Clark *et al.*, 2007)
2007	NB-LRRs can function in the nucleus (Burch-Smith *et al.*, 2007; Shen *et al.*, 2007; Wirthmueller *et al.*, 2007)
2007	Immune system incompatibility as a means to reproductive isolation (Bomblies *et al.*, 2007)
2009	Sequencing of 1001 Arabidopsis Genomes announced (Weigel and Mott, 2009)
2009	*Phytophthora* genome sequenced (Irish potato famine) (Haas *et al.*, 2009)
2010	Last 2010 projects to be funded

systems where appropriate. We hope to convince the reader that after 20 years of determined effort, our understanding of the plant immune system is now detailed enough to contemplate the rational deployment of it to combat disease in a sustainable manner. The examples highlighted here are by no means exhaustive, and reflect the authors' interests to some degree; we refer to recent reviews to guide the reader to deeper reading.

ARABIDOPSIS EMERGES AS A PLANT–PATHOGEN MODEL

One of the first challenges in the study of plant–pathogen interactions in Arabidopsis was the misperception that there 'weren't any pathogens' of this weed. Early attempts to falsify this dogma relied on testing pathogens that were known to infect cruciferous relatives of Arabidopsis. Cauliflower mosaic virus and the bacterial pathogen *Xanthomo-*

nas campestris pv. *campestris* were among the first pathogens found to infect Arabidopsis (Balazs and Lebeurier, 1981; Tsuji and Somerville, 1988). In 1991, researchers began assaying different inbred accessions of Arabidopsis, and found considerable variation in disease resistance and susceptibility among them following inoculation with strains of the bacterial pathogen *Pseudomonas syringae* (Debener *et al.*, 1991; Dong *et al.*, 1991; Whalen *et al.*, 1991). Importantly, they found that some of this variation resulted from the recognition of specific bacterial avirulence genes, *avrRpt2* and *avrRpm1*, which were capable of restricting the growth of an otherwise virulent *P. syringae* isolate. This was the first step in identifying *avr-R* gene pairs in Arabidopsis, and opened the door to using the strengths of Arabidopsis to dissect the key genetic paradigm in plant pathology: the gene-for-gene hypothesis (Flor, 1947).

Early researchers also went to the field and found that there were, in fact, pathogens capable of infecting Arabidopsis in the wild (Koch and Slusarenko, 1990; Holub, 2001, 2006). The two resulting pathosystems are built around the co-adapted oomycete parasites *Hyaloperonospora arabidopsidis* and *Albugo candida*. Finally, another Arabidopsis pathosystem was discovered fortuitously. The first strain of powdery mildew found to infect Arabidopsis (*Erysiphe cichoracearum*, UCSC) blew into a glasshouse at UC Santa Cruz (Adam and Somerville, 1996). These organisms are obligate biotrophs that cannot be axenically cultured. As such, they are host-adapted to a large extent. It has proven important to compare their modes of pathogenesis with pathogens of broader host range (Schulze-Lefert and Panstruga, 2003). Of particular note, it may be the case that evolutionary pressure particular to obligate, co-evolved biotrophic pathogens has driven a different mode of recognition than that operating against pathogens of broader host range (Dodds *et al.*, 2006). The application of genomics-era tools to a focused effort using Arabidopsis to study disease and disease resistance has spilled over into an enlightening unraveling of the genomes of all of its pathogens, and their relatives, that infect a range of crop species. A natural expansion of the development of Arabidopsis as a model for studying plant disease resistance has been the application of genomics and evolutionary biology tools to the study of its oomycete parasites, along with the investigation of related pathogens like the late blight oomycete *Phytophthora infestans* (Haas *et al.*, 2009).

The early emphasis on establishing these pathosystems in Arabidopsis focused on interaccession variation, in particular the identification of resistant and susceptible inbreds, and the use of interaccession F_2 populations to map resistance. The pre-genome (and post-genome) era of Arabidopsis biology was, of course, powered by forward genetics. Both were driven by the need to define genetically tractable systems as a prelude to positional cloning of the causal alleles of genes required for disease resistance. Interestingly, the early focus on natural variation in the analyses of disease resistance foreshadowed one of the current trends in studying plant–pathogen interactions: the use of natural variation to understand the evolution and potential fitness costs of particular immune system alleles (Stahl *et al.*, 1999; Nordborg *et al.*, 2005; Bomblies *et al.*, 2007; Jeuken *et al.*, 2009; Todesco *et al.*, in press). It goes perhaps without saying that all of the breakthroughs alluded to in the preceding two paragraphs were driven by advances in high-throughput sequencing, using techniques unimaginable to researchers in the 1980s.

The biggest accomplishment of these early projects was the positional cloning of several disease resistance, or *R* genes, as defined by Flor. The first positionally cloned *R* gene, *Pto*, was isolated from tomato in 1993. *Pto* encodes a cytoplasmic, membrane-tethered, serine-threonine kinase (Martin *et al.*, 1993). In hindsight, based on the structure of the many subsequently cloned *R* genes, it appears that Pto is not a stereotypical disease resistance protein. Researchers soon cloned the Arabidopsis *R* genes *RPS2* and *RPM1* (Bent *et al.*, 1994; Mindrinos *et al.*, 1994; Grant *et al.*, 1995), and the tobacco *N* and flax *L* genes (Whitham *et al.*, 1994; Lawrence *et al.*, 1995). The deduced proteins defined a structurally related class called NB-LRR after their characteristic nucleotide-binding and leucine-rich-repeat domains (Staskawicz *et al.*, 1995). NB-LRR proteins have since been found to be ubiquitous in seed-bearing plants as well as in mosses (Akita and Valkonen, 2002). Furthermore, it was quickly established, mostly via the analysis of chimeric NB-LRR genes from the flax–flax–rust pathosystem, that the LRR domains conferred recognition specificity (Ellis *et al.*, 1999). Researchers soon identified genes required for the function of these *R* genes, the first being *NDR1*, which encodes a novel plasma membrane-localized protein required for *avrB*-triggered resistance. *NDR1* was shown to be required for *R* genes recognizing both bacteria and oomycetes, indicating a conserved signaling mechanism existed for at least some *R* genes (Century *et al.*, 1997). Subsequently, *PAD4* and *EDS1*, genes with sequence similarity to lipases, were also identified as being required for multiple *R*-gene pathways (Falk *et al.*, 1999; Jirage *et al.*, 1999). Thus, by the late 1990s, a focus on Arabidopsis, along with the development of tools for the study of disease resistance in other reasonably tractable crop species that additionally benefited from superb plant disease resistance genetics resources (e.g. flax and tomato), had led to the first major conceptual breakthrough in molecular plant pathology: that disease resistance to all classes of microbial plant pathogens and insects could be conferred by a related set of NB-LRR proteins that acted as molecular receptors. Even at these early stages, with the gene-for-gene hypothesis largely validated, subtleties within the system were beginning to become evident. For example, the surprising fact that the *R* gene *RPM1* could identify at least two sequence-unrelated *avr* genes would have to be explained (Grant *et al.*, 1995).

The NB-LRR proteins were predicted to be intracellular, and have since been found to reside in a variety of pre-stimulation compartments, including the plasma membrane (Boyes *et al.*, 1998). Separated by the cell wall and plasma membrane, it was unclear how an NB-LRR protein might recognize pathogen molecules to trigger disease resistance. In 1996, researchers showed that bacterial virulence factors, termed effector proteins, acted inside host cells, and could be recognized there (as so-called avirulence proteins) by the NB-LRR proteins (Gopalan *et al.*, 1996; Leister *et al.*, 1996; Van den Ackerveken *et al.*, 1996). It later became clear that most bacterial effectors function within the plant cell and are translocated by a conserved molecular mechanism: the type-III secretion system (TTSS) (He *et al.*, 2004). An understanding of the co-regulation of effectors with the TTSS system (Huynh *et al.*, 1989) led to the high-throughput identification of many bacterial type-III effectors (Chang *et al.*, 2005). More recently, it has become clear that many fungal and oomycete effectors also function within the cell (Allen *et al.*, 2004; Win *et al.*, 2007). In contrast to the TTSS-delivery of bacterial effectors, effectors from fungal and oomycete pathogens are likely to be delivered by entirely different mechanisms: surprisingly, one is related to the delivery of malarial effectors into mammalian cells (reviewed by Kamoun, 2006; Panstruga and Dodds, 2009). Defining the mechanism(s) by which fungal and oomycete effectors are trafficked through the haustorial matrix and into the host cell remains one of the most important problems in the field.

Collectively, the effector suites deployed by a pathogen to specific sites of action inside the host cell contribute significantly to that pathogen's virulence. Pathogen effectors are directed to many host cell compartments where they presumably act on one or more targets to dampen, delay and impede host defense responses. By contrast, if only one of the many effectors delivered by a given pathogen into the host is recognized by the action of an NB-LRR protein, then the host triggers a rapid and high-amplitude output response that stops pathogen growth. Hence, recognition of pathogen effectors inside the host cell, mediated by NB-LRR proteins, leads to effector triggered immunity (ETI; Chisholm *et al.*, 2006; Dangl and Jones, 2001; Jones and Dangl, 2006).

Plants also can recognize the presence of conserved pathogen molecules, collectively termed pathogen- or microbial-associated molecular patterns (PAMPs/MAMPs). Recognition of these elicitors is mediated by high-affinity cell surface receptors. In 2000, FLS2, a leucine-rich repeat receptor-like kinase (LRR-RLK), was identified as a pattern recognition receptor (PRR) for a peptide fragment derived from the bacterial protein flagellin (flg22), through a forward genetic screen (Gomez-Gomez and Boller, 2000). This was a critical finding as it placed decades of research on elicitors that are not highly variable across pathogen strains into context. The activation of a largely overlapping set of defense genes following treatment of Arabidopsis cells with different PAMPs/MAMPs (Navarro *et al.*, 2004), and the demonstrable disease resistance mediated by PRRs (Zipfel, 2008; Boller and He, 2009), led to the concept that PAMP-triggered immunity (PTI) was a first line of defense in all plants that acted against the majority of microbes (Dangl and Jones, 2001; Chisholm *et al.*, 2006; Jones and Dangl, 2006). The machinery that generates PTI presents the major line of targets for pathogen effectors. Hence, plants apparently evolved specific NB-LRR-mediated ETI to counter virulence mechanisms that weakened the PTI to a point that allowed pathogenicity.

Thus, three major conceptual breakthroughs were in place at about the time the reference Arabidopsis Col-0 genome was finished: (i) at least some of the very large LRR-RLK protein family in Arabidopsis serve as specific PAMP receptors, whereas others serve as cell–cell communication receptors; (ii) pathogens deliver virulence factors to the inside of eukaryotic host cells; and (iii) NB-LRR proteins are a dedicated class of intracellular receptors, although the issue of whether they detected pathogen molecules directly or indirectly was not clear. At that time, and still, there were no other credible loss-of-function NB-LRR phenotypes, except for the loss of specific, effector-triggered disease resistance (ETI).

AN IMMEDIATE IMPACT OF THE ARABIDOPSIS GENOME SEQUENCE (2000)

The reference Col-0 genome sequence enabled a gene inventory related to plant defense against pathogens. In contrast to the other sequenced genomes of the day (*Haemophilus influenzae*, *Saccharomyces cerevisiae*, *Drosophila melanogaster* and *Caenorhabditis elegans*), Arabidopsis stood out for the relatively high proportion of genes in gene families (AGI 2000). One of the larger gene families in Arabidopsis encoded the NB-LRR proteins. What emerged was a clear picture of the diversity and distribution of NB-LRR genes throughout the Arabidopsis genome. There are approximately 150 NB-LRR proteins in Arabidopsis, many of which are distributed in clusters (Meyers *et al.*, 2003), as had been predicted from mapping the interaccession variability in disease resistance responses to *Hyaloperonospora* (Holub and Beynon, 1996). The availability of the sequences of NB-LRR clusters in Arabidopsis, *Solanum lycopersicum* (tomato) and, through the use of degenerate PCR markers, a variety of additional plants paved the way for several studies that demonstrated how these key molecules of the plant immune system evolve. In essence, examples of all the tricks of DNA sequence recombination were discerned: unequal meiotic crossovers to generate duplications and contractions in gene family number, gene conversion tracts within particular NB-LRR genes with donor sequences from paralogous gene family members, and intra- and inter-NB-LRR recombination events were all observed (Meyers *et al.*, 2003; Kuang *et al.*, 2008). The most important findings

from these studies showed that NB-LRR genes undergo diversifying selection at specific amino acid residues throughout the LRR domains, consistent with the notion that the LRR determines specificity (Michelmore and Meyers, 1998).

Potentially revealing oddities were also noted in the Col-0 genome. For example, the discovery of an NB-LRR protein with an additional WRKY TF domain provided a testable Rosetta hypothesis, linking NB-LRR receptor activity to a pathology-related TF family involved in the transcriptional reprogramming that accompanies defense responses (AGI 2000; Pandey and Somssich, 2009). The NB-LRR-WRKY protein called RRS1 was, in fact, shown to mediate naturally variable disease resistance to *Ralstonia* (Deslandes *et al.*, 2002), and works in concert with a canonical Toll/interleukin-1 receptor-like (TIR)-NB-LRR protein called RPS4 to mediate disease resistance to a fungal pathogen (Narusaka *et al.*, 2009). Consistent with the Rosetta hypothesis posed by RRS1, other NB-LRR proteins have been shown to translocate into the nucleus, where at least one of them interacts with a WRKY TF to regulate defense responses (Burch-Smith *et al.*, 2007; Shen *et al.* 2007; Wirthmueller *et al.*, 2007, Garcia and Parker, 2009).

Many of the other previously identified defense proteins were found to be members of gene families in the Col-0 genome. It was immediately obvious that LRR-transmembrane Ser/Thr kinases were another large and diverse gene family in the Arabidopsis genome (Shiu and Bleecker, 2001). At the time only FLS2 had been shown to have a role in defense as a PAMP receptor (see above); since then, EFR1 and CERK1 (a LysM-transmembrane kinase) have also been shown to be required for the response to the presence of specific pathogen molecules, an EF-Tu-derived peptide and chitin, respectively (Zipfel *et al.*, 2006; Miya *et al.*, 2007).

Other gene families noted at the time of the Col-0 genome release included homologs of pathology-related genes in other species, such as the seven-transmembrane protein MLO of *Hordeum vulgare* (barley), the PTO kinase from tomato and the reactive oxygen-producing NADPH-oxidases. Later work would demonstrate that many of these genes have important roles in defense in Arabidopsis (Consonni *et al.*, 2006; Torres *et al.*, 2005). In contrast, another interesting observation was the lack of clear homologs of many of the mammalian cell death-related genes, such as the Bcl2/Bax family and caspases. This was an indication that at the least the process of cell death in plants was driven by different types of proteins. Subsequent studies have focused on potential metacaspases (Uren *et al.*, 2000), proteases with casapse-like activity (Elbaz *et al.*, 2002; Hatsugai *et al.*, 2004, 2009), and conserved proteins required for autophagy as a means of cell death regulation in Arabidopsis and other plants (Hofius *et al.*, 2009).

Phenotypic dissection of disease resistance using the genome was, of course, hugely facilitated by the other functional genomics tools developed within the US National Science Foundation's Arabidopsis 2010 project and other programs like it around the world. The major tool enabled by the genome sequence was the high-throughput production of an essentially saturating collection of location-verified T-DNA insertion lines (Alonso *et al.*, 2003). It is difficult to underestimate the importance of this mutant collection, and others like it, which is now the backbone of biological process dissection in Arabidopsis. Although the frequency of cases of functional overlap (commonly misidentified as 'redundancy') in essentially all processes studied were underestimated before the completion of the Col-0 genome, it is still plausible to make multi-mutants that knock-out entire gene families of branches thereof in order to reveal a mutant phenotype (Hua and Meyerowitz, 1998). One useful response to functional overlap was to engineer constitutive promoters reading outwards from T-DNA borders to identify gain-of-function and loss-of-function phenotypes (Weigel and Nordborg, 2005). Here, the rapidity with which transgenic Arabidopsis can be made, and their sites of insertion sequenced, renders solutions to many defense-related problems technically feasible.

High-throughput, microarray-based analysis of gene expression was invented just prior to the sequencing of the Arabidopsis genome (Schena *et al.*, 1995). With the sequencing of the Col-0 genome, arrays expanded from small-scale boutique cDNA arrays into a whole-genome technology from Affymetrix based on photolithography (Lipshutz *et al.*, 1999). These technologies enabled diverse studies, including: defining the transcriptome during systemic acquired resistance (Maleck *et al.*, 2000); assigning putative functions for stress-responsive transcription factors (Chen *et al.*, 2002); and exploring the quantitative nature of responses to both virulent and avirulent bacteria (Tao *et al.*, 2003). The widespread use of Affymetrix whole-genome microarrays as a community standard has resulted in a huge volume of publicly available, directly comparable data. Community tools like Genevestigator (Zimmermann *et al.*, 2004) allow third parties to leverage old data into new hypotheses. Examining gene expression across different treatments, tissues types or mutant backgrounds has been a productive way to exploit this massive data pool.

The technical impacts of the Col-0 reference genome sequence, and tools generated to exploit it, are hard to overstate. Before the genome sequence, the final step of a forward genetic screen was typically a laborious positional cloning, often plagued by problems such as chimeric YAC clones or a myriad of other problems. Positional cloning of genes with robust loss-of-function phenotypes in Arabidopsis (at least in the sequenced accession Col-0) became trivial with the release of the genome sequence (Lukowitz *et al.*, 2000), and with the release of a large number of single-nucleotide polymorphisms (SNPs) between Col-0 and the common mapping partner L*er* (Jander *et al.*, 2002). This

facilitated mapping, and anticipated the depth of ecotypic sequence variation that would soon be available in the next few years when a re-sequencing project provided tens of thousands of SNPs across 20 commonly used and naturally diverse ecotypes (Clark *et al.*, 2007). These tools foreshadow the use of increasingly cheap DNA sequencing technologies to perform association mapping relevant to disease resistance (Weigel and Mott, 2009). As stated above, the rate-limiting step now becomes high-throughput, robust, disease phenotyping. This is critical, as new sequencing techniques now provide the ability to isolate mutations of interest by whole-genome sequencing of mutants and/or segregating F_2 mapping populations (Schneeberger *et al.*, 2009; Cuperus *et al.*, in press). Hence, it will now become even more feasible to design and carry out deep forward genetic screens, and then to simply sequence the resulting mutants to identify causal genes.

ARABIDOPSIS PATHOLOGY IN THE MODERN ERA (2000–PRESENT)

The number of papers published on Arabidopsis plant pathology has skyrocketed since the late 1990s (Figure 1). Thus, any discussion of the impact of the genome sequence on this field will be incomplete. Some of the more important stories, such as anti-viral small-RNA systems will be covered in depth elsewhere in this issue. We have chosen to highlight a few examples where a combination of previous genetics, a finished genome and orthogonal knowledge from traditionally studied plant–microbe interaction systems was particularly helpful in opening new research doors and answering previously unresolved questions.

UNDERSTANDING PAMP RECEPTOR BIOLOGY

As noted above, plants can respond to many purified pathogen molecules: PAMPs/MAMPs. Following the identification of FLS2 as a flagellin peptide receptor (Gomez-Gomez and Boller, 2000), a compelling speculation was that at least a subset of the large LRR-RLK class of proteins could encode a variety of PAMP receptors. A good example of using genomic resources to tackle a problem is the cloning of the PAMP receptor EFR1 (Zipfel *et al.*, 2006). These authors found that EF-Tu, another bacteria-derived elicitor, elicited similar defense responses as flg22 when measured either biochemically or by whole-genome microarray. Intriguingly, flg22 treatment increased the number of binding sites for EF-Tu, whereas EF-Tu did the same for FLS2. Hypothesizing that the putative EF-Tu receptor should share many characteristics with FLS2, the authors narrowed their search to LRR-RLKs similar to FLS2. The microarray experiments indicated that FLS2 and the EF-Tu receptor triggered transcription of a shared set of roughly 100 of the 610 RLKs in the Col-0 genome. The authors collected homozygous insertion mutants for this set of RLKs and tested them for EF-Tu-based growth inhibition. The *fls2* mutant and all of the T-DNA lines tested, except one, were responsive to EF-Tu. The non-responsive mutant has an insertion in an RLK gene responsible for the perception of EF-Tu, termed *EFR1*. One would assume that this approach will be generally applicable to the identification of the receptors responsible for recognizing orphan PAMPs/MAMPs.

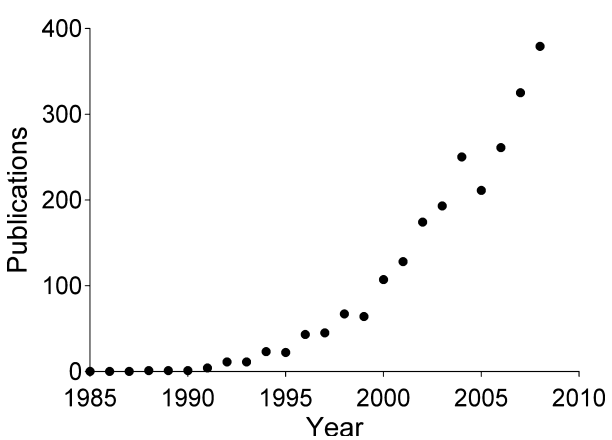

Figure 1. Use of Arabidopsis to study plant–pathogen interactions has rapidly expanded since the sequencing of the Arabidopsis genome. Data retrieved from Medline using the search: ['YEAR' (publication date)] AND (Arabidopsis) AND (defense or disease or pathology).

FLS2 has structural similarity to BRI1, another LRR-RLK identified as a receptor for the plant hormone brassinolide. Intriguingly, the co-receptor for BRI1, BAK1, is also required for PAMP perception by FLS2. FLS2 and BAK1 form an elicitor-dependent complex in both Arabidopsis and *Nicotiana benthamiana* (Heese *et al.*, 2007). It appears that BAK1 may be required for the perception of many LRR-RLK-sensed signals. Consistent with a central function in PAMP perception, multiple bacterial effectors target BAK1 and are able to disrupt the formation of the FLS2/BAK1 complex (Shan *et al.*, 2008). CERK1, a chitin co-receptor (see above) is also targeted by a bacterial effector (Gimenez-Ibanez *et al.*, 2009). The genome sequence will now enable the reverse genetic dissection of PAMP/MAMP receptor function by designing and using a functional testing matrix of LRR-kinase mutants and candidate PAMPs/MAMPs from a wide spectrum of microbial pathogens.

REFINING OUR UNDERSTANDING OF NB-LRR FUNCTION

Much of the basic genetic framework for how NB-LRR mediated recognition of pathogen effectors shapes plant–pathogen interactions has remained unchanged since the original gene-for-gene hypothesis (Flor, 1947). The idea that NB-LRRs act as intracellular receptors for the classically defined 'avirulence factors' is generally accepted. However, the way we think about the perceived signal has changed. Early models typically proposed that avirulence proteins were ligands that were directly bound by *R*-encoded receptors. It was unclear at the time what the primary function of avirulence genes was:

'we generally do not understand the function of avirulence genes in pathogens that harbor them, but their role in recognition by plants is likely gratuitous' (Keen, 1990). Subsequently, several direct Avr-NB-LRR interactions have indeed been found (Tang *et al.*, 1996; Jia *et al.*, 2000; Dodds *et al.*, 2006); however, direct interactions are difficult or impossible to detect in several other cases.

A new hypothesis was proposed whereby the molecular outcome of these virulence functions could be 'monitored' by NB-LRR proteins, and that pathogen effector manipulation of host targets generated 'modified self' signals that were subsequently responsible for the induction of NB-LRR-dependent defense responses. This idea was first proposed as an explanation for the apparent oddity of the AvrPto-Pto gene-for-gene system (van der Biezen and Jones, 1998). Several important predictions were subsequently proposed to formalize the guard hypothesis (Dangl and Jones, 2001; Jones and Dangl, 2006). The first was that indirect perception could explain a scenario where a relatively small number of NB-LRR genes (approximately 150) in Arabidopsis must ostensibly protect the plant from all possible relevant pathogen-encoded molecules. By comparison, a receptor system based on direct recognition of a single ligand (as predicted by Flor) would probably need to be much larger to 'cover' the structural space available to pathogen-encoded molecules. Thus, in the extended guard hypothesis, the effective NB-LRR number could be limited to in essence the number of possible host targets of effector action. One consequence of this prediction was that a single NB-LRR protein could recognize the presence of multiple, unrelated effectors if those effectors targeted and modified the same host target. This is the case, for example, with the RPM1 NB-LRR protein that 'guards' RIN4 when the latter is targeted by either AvrB or AvrRpm1 (Mackey *et al.*, 2002). A second prediction is that multiple NB-LRR proteins might evolve to monitor the integrity of the same host target of effector action. This is also true, as RPS2 is activated by effector-dependent manipulation of RIN4, in this case cleavage of RIN4 by AvrRpt2 (Axtell and Staskawicz, 2003; Mackey *et al.*, 2003). Hence, at least three sequence-unrelated effectors from *P. syringae* (and maybe more; see Luo *et al.*, 2009) target RIN4. A third prediction is that divergent pathogens should evolve effectors that target critical host machinery, consistent with data demonstrating that a non-canonical tomato R protein, called Cf-2, and its associated effector target, Rcr3, can be targeted by both fungal and oomycete effectors (Kruger *et al.*, 2002; Song *et al.*, 2009).

A further extension of the guard hypothesis has recently been presented as the decoy model (van der Hoorn and Kamoun, 2008). Under the decoy model, a host protein that mimics the real virulence target of an effector can act as a decoy to titrate effector function away from productive targets. A critical, and as yet untested, aspect of this model with respect to effector-triggered immunity is that a true 'decoy' should have no additional cellular function in the absence of its co-functional NB-LRR protein. Hence, as RIN4, for example, has an NB-LRR-independent function in PAMP-triggered immunity (Kim *et al.*, 2005), it cannot be merely a decoy. As more cases of R protein and associated host targets of effector action are defined, this debate is likely to become more interesting.

ZIG-ZAG, ZIG: A UNIFYING CONCEPTUAL SUMMATION

The zig-zag model, a product of years of work by many labs, attempts to synthesize PAMP perception, PTI, effector/virulence factor function and NB-LRR-based disease resistance (Jones and Dangl, 2006). The outcome of any plant–pathogen interaction lies on a continuum between susceptibility and resistance. In the zig-zag model, PAMP perception initiates PTI, which is the primary defense response limiting pathogen growth. This is the first 'zig' towards resistance. PTI acts as a generic, broad-range defense response, as it is triggered by conserved microbial elicitors. In an effort to suppress PTI, pathogens of all types have evolved effector/virulence proteins that act to block PAMP/MAMP-triggered signaling and/or defense output. This effector-triggered susceptibility (ETS) pushes the interaction towards host susceptibility (the 'zag'). In response to the ETS, plants have evolved ETI, a largely NB-LRR-based recognition of the 'modified-self' by-products of ETS (another 'zig'). This attack-and-response can conceptually occur iteratively with multiple rounds of ETS, followed by recognition, resulting in ETI. The final outcome of the interaction then depends on the sum total of (PTI − ETS) + ETI.

ARABIDOPSIS ENABLES COMPARISONS WITH OTHER PLANT SYSTEMS

Arabidopsis has been an exemplary tool with which to test hypotheses about defense responses in other plant species. Even before the Col-0 genome was sequenced it was clear that NB-LRR proteins are ubiquitous in land plants. Immediately after the sequencing of the Col-0 genome, researchers realized that Arabidopsis could serve a useful purpose, for example, in tomato functional genomics (Mysore *et al.*, 2001). Arabidopsis is useful for trans-species functional assays, much in the way that *Agrobacterium*-mediated transient gene expression assays in *N. benthamiana* are used as a complement to Arabidopsis transgenic plants. Synteny is observed between Arabidopsis, *Medicago* and *Glycine max* (soybean) (Mudge *et al.*, 2005). The genome sequencing of *Oryza sativa* (rice) demonstrates that a large percentage of rice genes (approximately 70%) have a close relative in Arabidopsis (Paterson *et al.*, 2005). However, it seems that NB-LRR proteins are structurally divergent between species. For example, the monocot genomes don't contain TIR-NB-LRRs, which are common in Arabidopsis, but rather contain exclusively non-TIR-NB-LRRs, such as coiled-coil (CC)-NB-LRR proteins (Bai *et al.*, 2002; Monosi *et al.*, 2004).

Important genes in plant defense that were laboriously identified in less tractable species have also been found to have orthologous functions in Arabidopsis. Barley has long been a model for studies of resistance to the obligate fungal pathogen powdery mildew (Schulze-Lefert and Panstruga, 2003). The *rar1* mutant was originally identified in 1988 because many of the NB-LRR *Mla* genes are functionally *RAR1*-dependent (Jørgensen, 1988). *RAR1* was positionally cloned from Barley in 1999 in a technical tour de force, and a homolog in Arabidopsis was immediately apparent (Shirasu *et al.*, 1999). Genetic screens (Muskett *et al.*, 2002; Tornero *et al.*, 2002) in Arabidopsis identified *rar1* null mutants, and most of the downstream mechanistic experiments have been performed in Arabidopsis (Azevedo *et al.*, 2002; Holt *et al.*, 2005; Hubert *et al.*, 2009). The domain structure of RAR1 allowed a prediction to be made that it would work together with SGT1 (Azevedo *et al.*, 2002). Subsequent analyses support the hypothesis that RAR1, SGT1b and cytosolic HSP90 proteins operate together as a cytosolic co-chaperone for NB-LRR function (Schulze-Lefert, 2004; Shirasu, 2008). It is clear in this case that a focus on Arabidopsis as a model, combined with the judicious use of available genetic material from other species, led to an important generalization regarding the need for cytosolic co-chaperones to fine-tune NB-LRR function. Moreover, this work in plant innate immunity explicitly guided subsequent studies in the mammalian innate immune system (see below).

A pair of genetic screens looked at the interaction of Arabidopsis and powdery mildew. The first screen defined genes that were required for the compatible interaction between Arabidopsis and the powdery mildew *E. cichoracearum* (Vogel and Somerville, 2000). The mutants recovered reflect generalities of the plant immune system, and its manipulation by pathogens. Powdery mildew resistant 2 (PMR2) was identified as a member for the MLO family of seven transmembrane proteins originally described in barley (Consonni *et al.*, 2006). Barley *mlo* mutants confer recessive and durable disease resistance to all isolates of barley powdery mildew, *Blumeria graminis* f. sp. *hordei*. Hence, it is likely that MLO encodes a protein required by an ancient powdery mildew fungus virulence mechanism. A second Arabidopsis locus identified in this screen, *PMR4*, encodes the callose synthase responsible for the pathogen-induced callose response, a ubiquitous plant defense response at sites of attempted penetration. Unexpectedly, loss-of-function alleles of PMR4 result in disease resistance caused by the activation of SA-dependent defense responses (Nishimura *et al.*, 2003).

The second screen (for *pen*, or 'penetration' mutants) identified loci required for non-host resistance to *B. graminis* f. sp. *hordei*. This screen uncovered a set of proteins acting together in the secretion of antimicrobial compounds that are also conserved across the barley–Arabidopsis divergence. PEN1 encodes a PM-resident SNARE protein (SYP121) that mediates the fusion of vesicles to secrete a set of undefined antimicrobials (Collins *et al.*, 2003). A related SNARE, SYP132, has been shown to be required in tobacco for resistance to bacterial pathogens (Kalde *et al.*, 2007). PEN2 is a glucosyl hydrolase that is involved in the synthesis of glucosinolate-derived secondary metabolites (Bednarek *et al.*, 2009). The anti-fungal activity of these compounds indicates that glucosinolates have a broader defensive role beyond their well-established role as anti-herbivory agents. The third mutant from this screen, PEN3, encodes an ABC transporter of unknown specificity (Stein *et al.*, 2006). It is intriguing to speculate that PEN3 is responsible for the export of PEN2-dervied antimicrobial compounds. Consistent with this idea, cell death in the *pen3* mutant observed upon infection with a compatible powdery mildew is suppressed by the *pen2* mutant (Stein *et al.*, 2006). This putative PEN2/PEN3 pathway is distinct from the PEN1 pathway, given the additive double mutant phenotype (Lipka *et al.*, 2005). The PEN proteins, and PMR4-synthesized callose, all localize to sites of penetration by powdery mildew (Meyer *et al.*, 2009); intriguingly, this localization may be through diverse mechanisms (Underwood and Somerville, 2008).

Many of the proteins identified from the PMR/PEN screens point to the importance of secretion in defense, which is predictable based on the extracellular nature of most plant pathogens. The definition of the defense secretome is still ongoing, using a variety of approaches. There is a huge upregulation of the secretory pathway in response to infection. The NPR1 protein has been extensively characterized as a master regulator of pathogenesis-related (PR) protein expression, and it is strictly required for the systemic defense response known as SAR (Dong, 2004). By looking for direct targets of NPR1, Dong *et al.* demonstrated that many ER-resident, secretion-related genes are upregulated as part of SAR, presumably to handle increased traffic of the co-regulated and secreted PR genes (Wang *et al.*, 2005). Mutation of these secretory genes resulted in both a defect in defense secretion after induction (assayed by the marker protein PR1) and an impairment in resistance against a bacterial pathogen (Wang *et al.*, 2005). Complementary approaches have used proteomics to define both the secretory defense output and to define how pathogens can modify that output, presumably contributing to pathogen growth (Kaffarnik *et al.*, 2009). Although secreted pathogenesis-induced proteins were first identified decades ago (Van Loon and Van Kammen, 1970), the contents, mechanism and specificity of the defense secretome remain largely mysterious.

PLANT HORMONES AND DEFENSE

The interaction between plant hormone signaling and plant pathology is complex and intertwined. Genetic screens in Arabidopsis have defined many of the pathways involved in the synthesis, perception and effect of plant hormones

(reviewed in Santner and Estelle, 2009). Many of these hormones were initially recognized for their (often complicated) effects on growth and development; however, it has become clear that plant hormone signaling plays a major role in determining the outcome of plant–pathogen interactions (Grant and Jones, 2009). The best-characterized defense hormones include salicylic acid (SA), jasmonic acid (JA) and ethylene. Critical components of the SA pathway were revealed via genetic screens in Arabidopsis using pathology-based reporter genes or screens for enhanced disease susceptibility (Cao *et al.*, 1994; Dewdney *et al.*, 2000). Arabidopsis genetics has proven invaluable both for dissecting the SAR pathway at the NPR1 node, as discussed above, and in directly testing hypotheses about the nature of systemic signaling in other plant systems (Attaran *et al.*, 2009). These pathways interact in a complex manner demonstrated by the antagonism of SA and JA, as well as the synergism between JA and ethylene (Glazebrook, 2005).

The importance of hormone signaling pathways in defense is underscored by the efforts that pathogens undertake to modulate plant hormone pathways or to produce phytohormones. Critical components of the JA signaling pathway were defined by isolation of mutants insensitive to the pathogen-derived phytohormone coronatine (Feys *et al.*, 1994). Coronatine is a major virulence factor for some bacterial phytopathogens, and is implicated in the opening of stomates to allow entry into the mesophyll (Melotto *et al.*, 2006). The JA receptor was recently identified to be COI1, an F-box protein required for response to both coronatine and JA (Katsir *et al.*, 2008; Yan *et al.*, 2009). The structural similarity between coronatine and jasmonic acid has long been noted, but more recently it became clear that coronatine is actually a structural mimic of JA-isoleucine, the active form of JA (Staswick *et al.*, 2002). JA-isoleucine mediates a physical interaction between COI1 and the JAZ family of transcriptional repressors, leading to ubiquitination of JAZ, followed by its degradation and the derepression of JA-responsive genes (Katsir *et al.*, 2008; Yan *et al.*, 2009). This mechanism is, in essence, shared with the auxin receptor TIR and the Aux/IAA transcription factors: the hormone mediates the ubiquitination, and resulting degradation, of a transcriptional repressor (Dharmasiri *et al.*, 2005).

PAMP-triggered immunity has unexpected links to hormone biology. ABA, traditionally described as the abiotic stress hormone, is required for stomatal closure in response to drought stress. Melotto *et al.* found that as part of the PTI response to flagellin, plants close their stomata to prevent entry of bacteria into the leaf apoplast. This aspect of PTI requires ABA, an ABA-responsive kinase and the production of nitric oxide (Melotto *et al.*, 2006). This response is disabled by coronatine, and bacteria that lack coronatine have a reduced ability to colonize the mesophyll. The bacterial effector HopAM1 has been shown to induce hypersensitivity to ABA, so it is possible that bacterial effectors and toxins are working together to regulate ABA biology in the plant (Goel *et al.*, 2008). Auxin has also been implicated in PAMP-triggered responses to bacterial pathogens. As part of PTI, the perception of flagellin leads to the induction of a specific small RNA called mir393 (Navarro *et al.*, 2006). The target of mir393 is the auxin receptor TIR, resulting in the inability to express auxin-responsive genes and increased resistance to pathogens (Navarro *et al.*, 2006). Intriguingly, many bacteria produce auxin, potentially to promote pathogenesis. Fungal plant pathogens also make plant hormones to influence their hosts. The plant hormone gibberellic acid (GA) was originally isolated from the fungal plant necrotroph *Gibberella fujikuroi*, the cause of the 'foolish seedling' disease of rice, where infected plants undergo excessive growth (Bomke and Tudzynski, 2009). GA has also recently been shown to regulate defenses to *Pseudomonas* via degradation of the DELLA plant growth repressors (Navarro *et al.*, 2008). These stories appear to have no end, so pathogen evolution continues to provide a deep toolbox with which to dissect basic plant processes.

THE PLANT IMMUNE SYSTEM AND HUMAN IMMUNOLOGY INFORM EACH OTHER

Mammalian and plant innate immune systems share several features, at least some of which are driven by convergent evolution (Ausubel, 2005). Mammalian analogs of plant NB-LRR proteins, known as NLR proteins, are important intracellular innate immune receptors (Franchi *et al.*, 2009). The mammalian NLR proteins are structurally similar to plant NB-LRR proteins: they contain a central nucleotide binding domain (NB) and a C-terminal leucine-rich-repeat domain (LRR). Other related receptors, such as Apaf-1, contain a C-terminal WD-40 domain rather than an LRR domain. The N terminus of some NLR proteins contains a caspase-activating and recruitment domain (CARD), which potentially links them with processes of caspase-mediated cell death. The N terminus of NLR proteins is modular, and is found attached to pyrin and bir domains in other family members. NB-LRR and NLR proteins both require the same set of chaperones (SGT1 and HSP90), indicative of common evolutionary history, convergence onto a common mechanistic constraint or both (Mayor *et al.*, 2007; da Silva Correia *et al.*, 2007). Mutations in NLR proteins are associated with auto-immune disorders, such as Crohn's disease (Hugot *et al.*, 2001).

The function of mammalian NLRs as intracellular receptors is analogous with plant NB-LRR proteins. Some NLR proteins recognize bacterial peptidoglycan elicitors, whereas others recognize indirect effects of immune signals, such as uric acid. Upon activation by elicitor, negative regulation by the LRR is relaxed, and the proteins oligomerize through their NB domain. This oligomerization can result in the activation of molecules recruited to the N-terminal

domain (Ting *et al.*, 2006). In the case of NALP1, oligmerization was shown to result in the activation of caspase-1 (Faustin *et al.*, 2007). By analogy, protein activity regulated by oligomerization may also occur in plant NB-LRRs. Domains of the CC-NB-LRR protein RPS5 have been shown to make homo- and heteromultimers before activation (Ade *et al.*, 2007). Similar mulitmers are seen with different domains of the NB-LRR protein Rx, and these are disrupted by the addition of elicitor (Moffett *et al.*, 2002). On an intermolecular scale, oligomerization of the TIR-NB-LRR N protein has been shown to occur in the presence of elicitor (Mestre and Baulcombe, 2006). At this point it is unclear if the N termini of plant NB-LRRs are also functioning as adaptors to bring together and activate other proteins. Defining the precise mechanism(s) of NB-LRR activation is a major current challenge.

THE BRIGHT FUTURE OF ARABIDOPSIS AS A REFERENCE SPECIES FOR PLANT–PATHOGEN INTERACTIONS

The study of plant–microbe interactions (and plant biology more generally) is poised to enter a new multigenome era. This is driven by rapid improvements in inexpensive high-throughput sequencing, and is, importantly, occurring on 'both sides' of several Arabidopsis pathology systems. On the host side, the 1001 genomes project promises an expanding view of the allelic diversity and population structure of key protein families, like NB-LRRs and PRRs (described in this issue by Weigel). High-density SNP mapping is already allowing association mapping to identify disease resistance-related loci (Aranzana *et al.*, 2005). On the pathogen side, there is an ongoing explosion of bacterial, fungal and oomycete genomes (Kamper *et al.*, 2006; Collmer *et al.*, 2009; Haas *et al.*, 2009). Once exclusively the domain of large genomics labs, bacterial genomes are now being sequenced and assembled by relatively small groups (Reinhardt *et al.*, 2009; Studholme *et al.*, 2009). We can anticipate that several strains of all of the major bacterial, fungal and oomycete pathogens of Arabidopsis will be sequenced within the next year. Moreover, it is expected that most major fungal pathogens of crops will also be sequenced. This will drive a new era in the identification of a vast repertoire of effectors and their host targets, and a detailed knowledge of virulence mechanisms, across all of plant biology.

At this point, the rate-limiting step for exploiting the flood of genomic sequences is, as noted above, the ability to perform high-throughput pathology-related phenotyping. The traditional method of quantifying plant–pathogen interactions has been measuring the *in planta* growth of the pathogen though laborious coring, grinding and dilution plating. Fan *et al.* provided one useful solution to this problem by generating recombinant *P. syringae* DC3000 that contains a chromosomal copy of the *luxCDABE* operon from *Photorhabdus luminescens* (Fan *et al.*, 2008). The resulting strain is constitutively bioluminescent, and requires no exogenous substrate. The authors screened the *P. syringae*-derived luminescence of 100 ecotypes of Arabidopsis, and identified two quantitative trait loci (QTLs) associated with basal resistance. This sort of high-throughput phenotyping will soon be linked to association mapping to identify novel loci required to restrict bacterial growth.

As always, important unanswered questions remain. Although many of the important players have been identified through forward genetic screens, there are basic events about which we know surprisingly little. Much of the signaling and many of the actual signal transduction players for both PTI and ETI remain unknown. It appears that there are many orphan RLKs. How many PAMPs are plants able to sense? What are the outputs of PTI? Can we catalog the contents of the vesicle-based defense response? Are there vesicles loaded for an anti-fungal response, and a different set loaded for antibacterial defense?

Mechanistically, it is still unclear how NB-LRR-mediated recognition of pathogens leads to the downstream activation of hypersensitive cell death. Do NB-LRR proteins function in, or connect directly to, PTI machinery? Will NB-LRR activation in plants be accompanied by generalizable changes in oligomerization? Will certain NB-LRR proteins exhibit ligand-dependent oligomerization, while others display ligand-dependent disruption of resting state heteromers? Are unknown players recruited to an oligomerized NB-LRR plant resistasome, as is seen with animal NLR proteins? Further downstream of recognition, what are the critical events that lead to transcriptional reprogramming and host cell death? Are these two phenomena spatially separated within the cell? How is pathogen growth stopped?

At a higher level, there are also many questions about the evolution of these interactions. It's clear that looking within a given interaction there is a history of attack and response. It appears that many different pathogen effectors can target the same host proteins. How common is this trend and how generalizable are these foci to other plants? We predict that many of the signaling nodes targeted by pathogen effectors are shared across plant evolution. As with PAMP receptors, there is also a surplus of orphan NB-LRRs: how many of them have novel guardees?

Exploitation of Arabidopsis to advance our understanding of the plant immune system has come a long way in a short time. Arabidopsis was chosen as a model organism mainly for its benefits as a genetic tool, not for a history as a pathology model. Despite this lack of history, and because of its genetic power, Arabidopsis research has transformed plant pathology. Most of the basic knowledge that we have about PAMP perception and PTI, NB-LRR-based disease resistance and ETI, vesicle transport and polarized cellular defense responses, transcriptional output networks, and the interplay between disease resistance and hormone pathway signaling have all been greatly accelerated by the power of Arabidopsis forward genetics and genomic resources. It is

probably fair to say that the cream has been skimmed from this model system, and that major breakthroughs in molecular plant pathology will now be harder won. This is as it should be when successful fields mature. It is also probably fair to say that some of next great frontiers in molecular plant pathology will be the deployment of paradigms and molecules gleaned from the application of Arabidopsis to plant pathology over the last 20 years into crop systems. And it is certainly important to say emphatically that continued focus on Arabidopsis, from exploitation of its natural diversity to more sophisticated, deep forward genetic screens, will continue to drive our overall mechanistic understanding of how the plant immune system works, and how pathogen virulence mechanisms combat it.

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