Review

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Orchestration of plant defense systems: genes to populations

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Research over the past decades has made immense progress in identifying some genes and mechanisms underlying plant defense against biotic organisms. The recent movement towards systems biology approaches has increased mechanistic knowledge, revealing a need for understanding how all the genes and mechanisms integrate to create a response to any given biotic interaction. This begins with evidence that diverse molecular patterns converge, suggesting that the plant perceives signals not the interacting species. These signals then coordinate across regulatory networks via molecular interactions and cause non-cell autonomous responses in neighboring and systemic cells. Finally, the identification of transporters is showing that plant defenses are harmonized across tissues and even show the potential for coordination across individuals within a population.

Plant defense systems: pieces, mechanism, and biology

Throughout their life, plants interact with a diverse array of other organisms. Indeed, we do not truly know the number of bacteria, fungi, nematodes, insects, and other organisms that a plant encounters during its life [1]. Even without specific catalogs of interacting organisms, we know that plants have the capacity to sense and respond to almost any biotic interaction. With the advent of molecular biology and genomics, great progress has been made over the past 30 years in identifying the individual genes responsible for these biotic interactions, including genes that enable a plant to sense other organisms and to transmit this information, and resistance mechanisms to counter pathogens and pests, as well as other mechanisms to aid beneficial organisms.

The recent advent of systems biological approaches has increased the rate of identifying specific genes and, more importantly, has aided in the identification of networks and mechanisms underlying the defense biology of plants [2]. Numerous systems biology studies have shown that plant biotic interactions represent a highly integrated system where information must flow at different molecular (gene, transcript, and metabolite) and morphological (cellular, tissue, and whole-plant) levels to generate the proper response to any given organism interacting with the plant [3–6]. These studies have also identified strong similarities in the mechanisms underlying how a plant responds to any organism [7–10]. In this review, I focus on how plant systems biology is being used to reveal how plants integrate and coordinate their biotic defenses across numerous molecular and morphological scales to generate a response. Given the similarities across interactions with biotic organisms, I do not differentiate between interacting classes of organism but discuss how mechanisms integrate to handle an environment wherein a plant interacts simultaneously with innumerable organisms.

Specificity of plant systems responses

Plants encounter a vast array of bacteria, fungi, nematodes, insects, mammals, and other organisms. Although most biotic interactions have no net impact on the plant, some are negative (e.g., pathogens and herbivores) and some are beneficial (e.g., mycorrhizal or commensal insects). However, all tested interactions lead to altered plant gene regulation, frequently with similar sets of genes responding, regardless of whether the organism is a pathogen, commensal, or neutral [11]. This is largely explained by the presence of molecules in the different organisms that are detected by plant receptors to cause downstream regulatory responses within the plant [12]. Frequently, these molecules are called pathogen-associated molecular patterns (PAMPs), microbe AMPs (MAMPs), molecular AMPs (MAMPs), metabolic AMPs (MAMPs), or herbivore AMPs (HAMPs). This nomenclature makes the explicit or implicit hypothesis that the molecule in question provides specific information to the plant about the organism with which it is interacting. For instance, a HAMP would be considered to inform the plant that it is interacting with an herbivore rather than an aphid. However, with each new molecule–receptor pair found, the specificity of information provided by these interactions is becoming fuzzy. For example, a recent study measured the transcriptomic and physiological response of *Arabidopsis* (*Arabidopsis thaliana*) to the laying of an insect egg on its surface. The transcriptome and physiological responses were similar to those elicited by PAMP signaling, suggesting that the plant does not reserve the PAMP signaling system for just pathogens [10]. This raises the question: how specific is the regulatory system response of a plant to a precise biotic

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Box 1. Plant defense against biotic organisms

The supplementary movie (see the supplementary material online) shows a simplified schematic of the multicellular aspect of plant defense against biotic organisms. In this movie, the plant combines the information from multiple signals (AMPs) to induce the production of a defense metabolite via a metabolon. This metabolite then moves from the leaves to the roots to stimulate the production of another metabolon that transforms the compound into the active defense compound, which then moves back to kill or deter the attacking insect. Animation by Kimberly Falk, Jena, Germany (http://www.moveslikenature.com).

interaction? More simply, can the regulatory machinery of a plant detect the specific identity of every interacting organism? Although some studies indicate that there is specialization, these studies typically have not tested multiple organisms (i.e., multiple lepidopterans) involved in the same interaction because of the cost of transcriptomics studies involving replication [13,14].

Transcriptomics has been used to test the precision of interactions using multiple species from the same class of interaction. Measuring the transcriptomic response of *Arabidopsis* to infection by the bacteria *Pseudomonas syringae* and *Escherichia coli* showed significant overlap in the set of genes responding to the two organisms [11]. In this study, the largest differences in transcriptome responses were between different genotypes of *P. syringae* [11]. One potential explanation is that the two pathogens have similar PAMPs but the effectors within *P. syringae* lead to greater differentiation in the response [11]. Similarly a transcriptomic analysis of *Arabidopsis* responses to diverse *Botrytis cinerea* genotypes showed that the wild type plant had similar responses to genotypes of the same pathogen. However, doing the same transcriptomic experiment on jasmonate knockout plants showed that the transcriptomic responses were different to the diverse pathogen genotypes. This suggests that the methyl jasmonate pathway helped to constrain these different responses potentially by being a point of convergence linking different perception signals [15]. Together, these results suggest that a plant does not have regulatory responses that are specialized to a unique organism. Instead, each regulatory response may be the combined result of all the signals passing between the plant and the specific organism (Box 1). This model would suggest that diverse organisms could stimulate the same regulatory response if they provide the same signals to the plant. Support for this hypothesis comes from transcriptomic analysis of *Arabidopsis*–insect interactions where the plant responds to the type of herbivory, chewing versus sucking, but not the specific species of chewing or sucking insect or the specialization of the insect on the plant species [8]. More comparative transcriptomic studies testing how a common reference plant(s) respond(s) to multiple species within a bacterial, fungal, or insect guild and multiple guilds are required to test how specific the regulatory response of a plant may or may not be [16].

Temporal architecture of plant–biotic regulatory systems

One of the main aims of studies on plant defense systems is to identify regulatory networks by conducting a fine-scale time course using transcriptomics [17,18]. The goal is to determine the timing of regulatory events under the assumption that there is a discrete series of processes that can be ordered. This time-course approach has been applied to plant biotic interactions by measuring the *Arabidopsis* transcriptome every 2 h over a 48-h period after infection with *B. cinerea* [7]. Although these time-course data provided some resolution regarding the order of events after infection, the most striking observation was that most regulated transcripts changed expression within an 8-h window, starting at approximately 18 h after infection, with a smaller pulse of changes occurring at 12 h after infection [7]. This pattern of pulsed gene expression changes suggests that the interaction between *Arabidopsis* and *Botrytis* represents a change between two regulatory steady states, uninfected and infected, rather than a continuously changing temporal system [7]. Using computational modeling, the authors identified key regulatory genes required for initiating this change in steady states, TGA1A-Related gene 3 (*TGA3*) and NAC domain containing protein 055 (*ANAC055*). Mutations in both genes altered resistance to *Botrytis*, indicating that they have important roles in the interaction. Time-course analysis of the interaction between barley (*Hordeum vulgare*) and the powdery mildew *Blumeria graminis* also identified a pulsed nature to the transcriptome regulation and linked similar time points with the system switching between resistance responses [19].

Together, these time-course studies suggest that plant biotic interactions are not best described as continuous regulatory changes that begin at the time of infection. Alternatively, regulatory networks controlling plant biotic interactions may have transition states that differentiate between alternate stable gene expression patterns. This could be similar to the bistability seen in some bacterial developmental and environmental response systems, whereby a system of rapid temporal steps leads to near instantaneous transitions from one steady state to another [20,21]. However, given the breadth of different biotic organisms with which a plant interacts, it seems more likely that plant regulatory networks have multiple steady states. Each different steady state may represent evolutionarily optimal defense responses to major pathogens or herbivores, or mixes thereof. This potential for the biotic regulatory machinery of a plant to sample across multiple potential steady states is supported by the observation from massive Yeast-2-hybrid studies measuring the interaction of the *Arabidopsis* proteome that a large amount of the regulatory machinery potentially physically interacts within the plant [22,23]. Testing whether plant biotic interactions are marked by multiple steady states instead of a quantitative continuum would require more fine-scale time courses as well as the inclusion of multiple biotic organisms simultaneously.

Integration of plant defenses into the organism

The abiotic environment (e.g., light, nutrients, and water) determines the maximal potential growth of the plant and, hence, it has long been theorized that any energy devoted to responding to biotic attacks necessarily takes away from this maximal potential for growth and reproduction

[24–26]. Although the costs of defense mechanisms and responses are predicted to be fairly large, with glucosinolate defense metabolites requiring a theoretical 15% of photosynthetic carbon [27], identifying the concurrent growth consequence of these costs has been challenging [28,29]. One potential explanation is that these costs are overcome by selection ameliorating their trade-offs [30]. The simplest way to manage these costs would be a high level of integration between the biotic and abiotic response pathways of plants, enabling optimal partitioning of resources between growth and defense [31]. Recent systems work has started to identify the mechanistic basis of this integration across biotic and abiotic response pathways [32,33].

A central component of the ability of a plant to integrate abiotic fluctuations is the coordination of plant metabolism by the circadian clock. This enables the optimal use of energy by timing metabolism with diurnal solar oscillations [34,35]. In addition to abiotic integration, the circadian clock has recently been shown to control directly the expression of genes crucial for resistance to biotrophic pathogens, explaining a long-held plant pathology observation of the critical role of the time of infection in determining successful resistance [6,36,37]. Similarly, circadian clock proteins directly interact with key components of the jasmonate signaling pathway and alter its responsiveness to biotic stimuli [38]. The regulatory effect of the clock on both the salicylate and jasmonate pathways controls differential responses to insects and pathogens, leading to altered defense metabolite accumulation and, ultimately, altered virulence and herbivory [6,39]. Similar mechanistic regulatory connections between pathways typically related to biotic responses (e.g., salicylate and jasmonate) have been identified with key growth or abiotic pathways (e.g., abscisic acid and gibberellic acid) [32,40–43]. It is likely that these molecular connections between regulatory pathways are simply the first steps in the identification of a regulatory system for which it is likely to be challenging to separate one pathway from another.

Integration of defenses into plant regulation

The above research has focused on how regulatory pathways might interact in ways that modulate defense and growth. However, this is not the only direction of integration because evidence is accumulating that the downstream resistance mechanisms are also modifying their upstream regulator networks. For example, using a time course of transcriptomic responses to infection with the necrotrophic pathogen *B. cinerea* showed that this infection dampened circadian oscillations in *Arabidopsis* core clock genes without altering the phase [7]. More directly, the use of mutants that alter flux through the flavonoid metabolic pathway causes transcriptional changes in the jasmonic regulatory network via an as yet unknown mechanism [44]. Similarly, altering the function of a biosynthetic enzyme required to make a specific glucosinolate defense metabolite can change the circadian clock phase by 1 h [45]. Furthermore, this biosynthetic enzyme has also been linked to regulatory changes in the upstream MYB transcription factors, which can directly influence plant central metabolism via the nitrate and sulfate pathways [46–48]. Although it is tempting to dismiss these results as merely the flap of the wings of a butterfly in a chaotic metabolic network, plant defense metabolites can directly bind dozens of proteins and alter their functions [49]. Functional analysis of plant defense metabolites has largely been performed in studies of human nutrition; however, it is logical to presume that plant defense chemicals can also affect protein function in the organism within which they evolved. Future work should test the frequency with which output resistance metabolites have crucial yet unrecognized potential to influence the upstream regulatory networks.

Whole-plant regulatory systems begin at the cell

Another intricacy in the integration of plant biotic defense systems is that the pathogen or herbivore typically initiates the attack at a defined location on the plant. These localized interactions lead to many potentially cell autonomous regulatory events. A transcriptomic analysis of the *Arabidopsis–Golovinomyces orontii* interaction using laser microdissection of specifically infected cells highlighted a series of highly local responses that are likely to be important for both resistance at the specific site of infection as well as the ability for the pathogen to capture energy from the plant to grow and reproduce [50]. These responses included changes in primary metabolism as well as host cell ploidy that were shown to control the resulting disease interaction, although this highlights the difficulty of ascribing a change to direct resistance versus the attempts of the pathogen to grow and reproduce using the energy of the plant [50,51]. A similar level of highly local cell autonomous responses has been found in the interaction between a cyst nematode and soybean (*Glycine max*) [52–54]. Thus, cell autonomous responses are a key aspect of plant defense systems.

However, the local cell autonomous events do not function in isolation and instead lead to local responses that occur within the uninfected parts of the tissue containing the interaction. Transcriptomics studies of localized *B. cinerea* infections revealed that the whole leaf shows a rapid response to the germination of as few as 20 spores in a single mm^2 of the leaf [55]. Transcriptional responses to the localized droplet infection were found in the leaf up to 1 cm away from the point of infection [55]. The ability of a leaf to show a systemic response to a localized infection was reinforced by the observation that leaves show massive transcriptional and proteomic reprogramming even when >99% of the tissue is not in contact with the pathogen [7,15,56]. Furthermore, the transcriptomic and metabolic data revealed that the transcriptional responses differed as distance from the local infection increased, with direct defenses being locally induced and more systemic resistance-related responses occurring within the leaf at a greater distance from the point of infection [55,57]. Thus, the cell autonomous responses in the infected cells lead to broader local responses.

Beyond these localized responses, the plant also uses regulatory and communication systems to transport signals from this highly localized interaction and modulate distal plant tissues. This systemic response is best known from systemic acquired resistance, where infection of one

leaf leads to increased resistance in another leaf [58]. However, systemic signaling during plant–biotic interactions can involve almost any plant tissue interacting with almost any herbivore or pathogen (see [26,59–64] and the movie in the supplementary material online). Thus, when thinking of the systems response of a plant to a biotic interaction, it is necessary to transition from the single cell to the local cellular community to the whole plant because all morphological levels of a plant appear to be integrated into a single response system.

Whole-plant defense mechanisms and transport

In animals, this coordination across tissues is mediated by both the neural network as well as the vascular transport of signals. Plants do not have neurons and, hence, most known systemic signals are chemicals that require transporters to get the signal out of the cell in which it is produced and into the vasculature to proceed to the cell in which it functions [65–68]. This suggests that transport processes are key to understanding how the plant can integrate its defense mechanisms and responses across the multiple scales required to coordinate its response properly to diverse biotic interactions. To date, only the TIR1 and EDS5 transporters for the auxin and salicylic acid regulatory compounds are known [69,70]. Thus, exciting progress is being made in the understanding of transport systems involved in direct defense mechanisms involving both active transporters and potentially the role of plasmodesmata.

Co-expression of candidate genes with a fluorescence resonance energy transfer glucose sensorled to the identification of SWEETs, key sugar efflux carriers required to move sugars out of the cell, in plants and animals [71]. These proteins were recognized as targets of avirulence proteins produced by plant pathogens, and it was hypothesized that they enabled the pathogen to reprogram the plant to export sugar for use by the pathogen in a cell autonomous manner [71]. Recent work has extended this analysis to show that these same transporters are required to load carbohydrate into the phloem [72]. This observation and the finding that plant cells respond to changes in sugar availability suggests that alteration of key SWEET functions by a pathogen cause systemic changes in the plant by altering global carbon partitioning and sink–source relations.

In addition to local transport processes, key direct metabolic defenses, such as nicotine in tobacco (*Nicotiana tabacum*), require long-distance transport to move from the site of synthesis to the site of the biotic attack (see [26,73] and the movie in the supplementary material online). In the case of nicotine, this is transport from the root directly to the leaf under attack. This secondary metabolite transport is specifically induced by the plant–biotic interaction and requires both local and systemic responses [74,75]. Thus, these secondary metabolite transporters are potentially targets of systemic regulation that help to facilitate local resistance; however, their identity is as yet unknown.

Recently, one of the first defense metabolite transporters was found using an *in vitro* library of *Arabidopsis* transporters that was fed glucosinolate defense compounds. This analysis found three transporters, glucosinolate transporter (*GTR*)*1, GTR2*, and *GTR3*, that could specifically transport glucosinolates [4]. These transporters represent a *Brassica*-specific gene family whose members are part of a broader set of transporters, including the key plant nitrate transporters. This suggests that the defense metabolite transporters, *GTR1, GTR2*, and *GTR3*, have evolved from transporters involved in central metabolic functions. Importantly, a double mutant abolishing *GTR1* and *GTR2* leads to the loss of glucosinolates within the seed and hyperaccumulation within the leaf, suggesting that they have a role in regulating the long-distance transport of defense compounds [4]. Micrografting this transport mutant onto different biosynthesis genotypes showed that *GTR1* and *GTR2* mediate long-distance transport of glucosinolates from the root to the shoot [5]. Unlike nicotine, this long-distance transport also works in reverse to take shoot glucosinolates to the root. It remains to be tested whether the GTRs or other unidentified glucosinolate transporters are regulated to control the transport of defensive glucosinolates from sites of synthesis to the site of an active biotic attack. This transporter library approach should be a powerful means of rapidly identifying transporters for other key defense metabolites [4].

Population-level systems

The above observations show that an individual plant integrates its response to biotic interactions across signal networks and tissues to coordinate fully a defense response. This idea is being extended to suggest that plants have the capacity to coordinate across individuals within a local population. At the level of foliar communication, methyl jasmonate and other volatiles can move between plants and can be used to provide regulatory cues to plants of what other individuals in their environment are exposed to [76]. In addition to foliar communication using volatiles, roots also appear to enable communication between plants. In rice (*Oryza sativa*), the root systems of two plants appear to have the capacity to recognize and respond to each other, potentially with self- versus nonself-recognition [77]. Similar results have been observed in other species, suggesting that communication between root systems is widespread in the plant kingdom [78–81]. Furthermore, recent results have suggested that mycorrhizal fungal connections between plants enable information to be passed from one individual plant to another [82]. In this instance, the information communicated between plants specifically related to plant biotic relations, specifically aphid infestation. Together, these results suggest that at least some rudimentary signaling occurs between individual plants. This indicates that any signal integration that occurs at the whole-plant level may extend to the population level to optimize the defensive response of the population in a manner that is different from an isolated individual [83].

Concluding remarks and future perspectives

Despite the progress that has been made in understanding how plant biotic interactions integrate across a plant, we have still barely begun to explore this topic. In this review, I have highlighted only a few dimensions that frame the space in which this integration must occur: temporal, regulatory networks, interacting species, morphological levels, and individuals to populations. However, other

dimensions that are also likely to have an important role in this integration include genetic variation, abiotic environment, and life history. More crucially, in each instance, the studies described largely only queried a single dimension and did not attempt factorial analysis. For instance, how do different regulatory networks integrate with each other across a time course when simultaneously confronted with an insect and a bacterium? Combining different dimensions of integration for plant biotic interactions imparts significant pressure on experimental design and finances, given the required expansion of sample numbers. This is particularly challenging with systems biological and genomics approaches; however, there are efforts underway to begin enabling these studies to be performed [84,85]. Regardless of the difficulties inherent in studying integration across all these levels, this is where the plant exists in its natural environment. Thus, to understand truly how a plant fully integrates its response to biotic interactions, we will have to move into these higher-level interaction studies using modern systems biological approaches. These efforts should enable us to realize the efforts laid by the groundwork of the past decades and to begin to link the molecular pieces to the mechanisms and fully dissect plant biotic interactions.

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Appendix A. Supplementary data

Supplementary data (movie) associated with this article can be found at doi:10.1016/j.tplants.2014.01.003.

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