HHS Public Access

Author manuscript

CRC Crit Rev Plant Sci. Author manuscript; available in PMC 2020 December 18.

Published in final edited form as:

CRC Crit Rev Plant Sci. 2020 ; 39(1): 72–100. doi:10.1080/07352689.2020.1757829.

The Lifecycle of the Plant Immune System

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Abstract

Throughout their life span, plants confront an endless barrage of pathogens and pests. To successfully defend against biotic threats, plants have evolved a complex immune system responsible for surveillance, perception, and the activation of defense. Plant immunity requires multiple signaling processes, the outcome of which vary according to the lifestyle of the invading pathogen(s). In short, these processes require the activation of host perception, the regulation of numerous signaling cascades, and transcriptome reprograming, all of which are highly dynamic in terms of temporal and spatial scales. At the same time, the development of a single immune event is subjective to the development of plant immune system, which is co-regulated by numerous processes, including plant ontogenesis and the host microbiome. In total, insight into each of these processes provides a fuller understanding of the mechanisms that govern plant-pathogen interactions. In this review, we will discuss the “lifecycle” of plant immunity: the development of individual events of defense, including both local and distal processes, as well as the development and regulation of the overall immune system by ontogenesis regulatory genes and environmental microbiota. In total, we will integrate the output of recent discoveries and theories, together with several hypothetical models, to present a dynamic portrait of plant immunity.

Keywords

Defense; development; environment; pathogens; plant immunity; signaling; system; virulence

I. An introduction to plant biotic interactions

In natural ecosystems, most plants are resistant to most pathogens, a phenomenon whose mechanism is undoubtedly one of the holy grails in plant pathology-to understand and harness the ability of a plant to respond to, and successfully defend against, pathogen invasion (Staskawicz, 2001). Indeed, the abundance of host, pathogen, and climatic diversity provides a rich source of broad-spectrum resistance, the result of which is a naturally selected balance of genetically diverse plant and pathogen/pest populations. Therefore,

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epidemics in ecosystems are rare, and when they do occur, they are typically restricted to a specific geographical region, climate, or a combination of both. In the case of natural resistance, typically referred to as non-host resistance, the breadth of genetic diversity represented in the host population is often sufficient to limit infection(s), resulting in the evolution of what is referred to as nonadapted pathogens (Stam *et al.*, 2014). Conversely, the selection for and enrichment of pathogens that are adapted to their host has resulted in the establishment of ecosystems where pathogen virulence and disease are more often the norm than the exception.

Plants begin and end their lifecycles in a single geographical location; however, the environment around plants is in constant flux. In response to these changes, and ultimately, to survive and thrive, plants must sense, respond, and adapt to an endless barrage of external perturbations, such as biotic and abiotic threats. Thus, it is not surprising that an emerging theme in plant pathology is the contribution and influence of the environment on immune system maturation. Herein, while we periodically invoke the concept of environmental impact on the plant immune system, we will not delve too deeply into this body of work, as the complexity and breadth of research in this area is broad, fast moving, and requires a dedicated narrative to appropriately cover this topic. Rather, we point the reader to several recent comprehensive reviews on this topic (Chappelka and Grulke, 2016; Morris *et al.*, 2017; Cheng *et al.*, 2019; Corredor-Moreno and Saunders, 2020).

To successfully respond to and defend against biotic threats, plants have evolved highly complex pathogen defense systems, or surveillance networks, which function in a manner similar to the innate immune systems of humans. The defense signaling acts cooperatively with numerous cellular processes, and together, the sum of these interactions imparts the ability to recognize a vast array of biotic threats (e.g., pathogens, pests, and viruses) and distinguish self from nonself (Sanabria *et al.*, 2008). As described in greater detail below, underpinning the function and activity of the plant immune system is a complex network of preformed and inducible signaling processes, which provides unfettered access to both external and internal (i.e., systemic) cues.

In this review, we describe the events, in a broadly temporal fashion, that encompass the lifecycle of the plant immune system. From perception and recognition, to the multitude of signaling events that require the plant cell to either respond or not to pathogenic microbes, our understanding of the activation and attenuation of immune signaling remains incomplete. Moreover, we highlight recent studies describing the molecular-genetic processes that define how the plant immune system matures and during its development, how it integrates into a multitude of host signaling processes that regulate plant development and response to the environment. To do this, we primarily focus on the dynamics of plant immune signaling as a function of time and scales; from pathogen perception and virulence, to the activation of local and distal defense signaling. In short, we aim to describe and illustrate the development, activation, and ultimate function of the immune system as an extension of whole plant physiology, growth, development, and reproduction.

II. Pathogen invasion

A. Host invasion by filamentous pathogens

During fungal colonization of plants, the transition from external to internal growth and proliferation begins with germination of a spore and formation of the penetration-specialized architecture, the appressoria (Yi and Valent, 2013; Ryder and Talbot, 2015). In short, this process enables “forced entry”, or direct penetration, of the outer physical barriers of the plant, a common yet diverse invasion strategy among filamentous pathogens (see Figure 1). For instance, the model foliar powdery mildew pathogen *Golovinomyces orontii* uses its appressoria to forcibly invade into leaf epidermal cells by breaking the cuticle and cell wall (Braun *et al.*, 2019). Such a strategy is also common in soilborne pathogens, including, for example, *Phytophthora sojae*, which invades the roots of soybean (Fawke *et al.*, 2015). Alternatively, pathogens do not necessarily need to directly penetrate into a live cell at the onset of the interaction; case-in-point, the oomycete pathogen *Hyaloperonospora arabidopsidis* (Coates and Beynon, 2010) penetrates the cuticle and grows into the apoplast, the space between the junction of two pavement cells (Underwood, 2012), which potentially benefits the pathogen by delaying the full engagement with plant immune system. As a point of strategy, while the “forced entry” model greatly expands the opportunity for filamentous pathogens to successfully invade the host, the “passive entry” mechanism (e.g., through natural openings or wounds) presents less of a challenge to the pathogen as a function of reduced physical barriers and defense response. One of such examples is the invasion of *Colletotrichum* species, which causes anthracnose diseases. Outside plant, they generate nonpenetrative appressoria, from which undifferentiated germ-tubes extend and search for stomata to enter, resulting in host colonization via intercellular hyphae development (Latunde-Dada *et al.*, 1999).

Of the numerous systems that have advanced our understanding of the processes underpinning appressorium-mediated penetration, the interaction between rice and the fungal pathogen *Magnaporthe grisea* represents one of the better understood examples of this virulence mechanism. As demonstrated using a combination of genetics-, cell biology-, and classical plant pathology-based methods, *M. grisea* initiates appressorium development upon the perception of the hydrophobic leaf surface environment, in combination with contact of the wax cuticle (Ryder and Talbot, 2015; Anjago *et al.*, 2018). Upon contact and assessment of the leaf surface environment by the developing fungus, physical penetration is mediated by the establishment and maintenance of cellular turgor pressure, which comes from elevated concentrations of glycerol in the appressoria, as well as a semi-permissive melanin barrier at the host-fungal interface (Chang *et al.*, 2014; Ludwig *et al.*, 2014). During this stage of infection, appressorial growth and development is facilitated by the assembly of a condensed septin-actin network, a mechanism hypothesized to enhance mycelia growth and trafficking during the maturation of pathogen infection (Van Ngo and Mostowy, 2019). In addition to the early stages of fungal development and infection, the pathogen secretes a battery of virulence-associated enzymes to promote infection, including cutinases (Kebdani *et al.*, 2010; Auyong *et al.*, 2015), cellulases (Kebdani *et al.*, 2010; Van Vu *et al.*, 2012), and pectinases (Kebdani *et al.*, 2010), which target host cell wall components to promote further ingress. In total, the integrity of the whole penetration-facilitating system of filamentous

pathogen is a pre-requisite of successful and efficient invasion. Indeed, mutants with reduced turgor pressure or an absence of cell wall degrading enzymes display reduced penetration capabilities (Skamnioti and Gurr, 2007; Auyong *et al.*, 2015; Paccanaro *et al.*, 2017; Tang *et al.*, 2018).

B. Bacterial pathogen invasion

In the case of phytopathogenic bacteria, the transition from epiphytic/saprophytic growth to infection is hypothesized to be induced by external signals, including those emanating from the host, abiotic environment, and the microbial community (i.e., microbiome composition, quorum sensing, etc.) (Baker *et al.*, 2010; Leonard *et al.*, 2017; Xin *et al.*, 2018). In the case of leaf-attached bacterial colonies, communities may persist as noninfective entities as a consequence of low surface humidity. Such "dormancy" on the host surface is mediated by a humidity-regulated quorum sensing system that inhibits the transition to an infection phase, as indicated by bacterial mobility, exopolysaccharide production, and pathogen secretion system maturation (Quiñones *et al.*, 2005; Dulla and Lindow, 2008; Cheng *et al.*, 2016). Once the stimulus is perceived by potential pathogenic microorganisms, as described in the case of the model bacterial phytopathogen *P. syringae* (Ortiz-Morea *et al.*, 2016), the bacteria enters infection phase. In short, this process coincides with the rapid expression of core pathogenesis regulons, including *hrp*/*hrc*, *hrpA*, *hrpL*, and *hrpR*. In turn, this leads to the activation of signaling associated with the production of key virulence factors, including toxins (Brooks *et al.*, 2005; Baker *et al.*, 2010; Geng *et al.*, 2012), and the induction of signaling responsible for the production of the type III secretion system (TTSS) (Tang *et al.*, 2006). In another example for soilborne bacteria, *Ralstonia solanacearum* perceives oleanolic acid (Wu *et al.*, 2015) and ferulic acid (Zhang *et al.*, 2017) as critical host-released virulence inducive signals, potentially via PrhA-PrhR receptor complex. This is significant, because these compounds are directly released into the soil matrix, and *R. solanacearum*, like other soilborne pathogens, may induce transitions to pathogenesis and gain higher virulence before host invasion.

In the case of bacterial pathogens, bypassing the surface barrier via natural opening or wound is necessary. Among the best characterized modes of phytobacterial invasion is through stomata, the opening between two guard cells that functions as the site of transpiration and gas exchange. For nearly 30 years, research in the area of plant-pathogen interactions has led to the accumulation of strong evidence demonstrating that a range of pathogens, including bacteria and fungi, utilize stomata as points of host entry. However, it is only within the past decade or so that our understanding of the molecular mechanisms underpinning this interaction has been realized (Melotto *et al.*, 2006; Melotto *et al.*, 2017). Although stomatal aperture is dominantly regulated by light and the internal circadian clock (Hubbard and Webb, 2015), plants can also activate stomatal closure outside of the standard circadian rhythms of daylight hours in response to pathogen infection (Melotto *et al.*, 2006). To counter the defense-induced stomata closure, pathogens, such as *P. syringae*, have evolved mechanisms to re-open stomata through the process of defense hormone mimicry to ensure successful and efficient invasion (Geng *et al.*, 2014). This paradigm, the back-and-forth of virulence and defense through stomatal gating, is referred to as stomatal immunity

(Melotto *et al.*, 2017) and represents a new battle-ground in the field of plant-pathogen interactions and immune signaling.

C. Virulence in advance of entry

As a foundation describing molecular plant-pathogen interactions during host immune signaling and defense, it is important to clarify the status of pathogen virulence in advance of host infection. Current models portray pathogen virulence, in the most generalizable terms, as a process activated upon host contact. In this context, and herein, we too will define contact between a pathogen and the apoplast or living cell as "time zero" in the chronology of the activation of plant immunity. This leads to an essential question related to the entire process of plant immunity - *are pathogens already capable (*i.e., *competent) of interfering with immune signaling at time zero*? For bacteria, as discussed above, they are capable of entering the infection phase and activating the effector/toxin secretion systems before time zero. For filamentous pathogens, spore germination, *per se*, is a hallmark of the initiation of the infection phase, which activates a virulent secretome before penetration (Kleemann *et al.*, 2012). Hence, it is reasonable to hypothesize that pathogens have already obtained the ability to inhibit the impending defense response by host before confronting with plant immune system. This temporal advance is critical for pathogenesis because it ensures that the secretion of effectors or other defense-inhibitory compounds to host is, at a minimum, simultaneous with pathogen perception, if not in advance.

III. Temporal development of local immunity

A. The timing of local immune activation

In plants, local immunity describes a fundamental concept founded on the basic principle that an independent live cell is immuno-totipotent, or possessing the full capability of the immune response, independent of additional signal input(s) from other host-associated components (Verdeil *et al.*, 2007). Previous work has generalized a canonic model to describe local immunity with two primary nodes, namely PAMP-triggered immunity (PTI) and effector-triggered immunity (ETI) (Thomma *et al.*, 2011). PTI, as mechanism of basal defense, is activated following host perception of microbial PAMPs (pathogen associated molecular patterns), the conserved organismal motifs required for the survival and lifestyle of the microbe (e.g., flagellin, chitin). As an immune response that best illustrates the initiation and integration of complex host signaling and innate immunity, PTI follows a classic cellular signaling model comprised of receptors, cascades, and defense executors. Here, we describe the temporal function(s) of each of these three components (i.e., receptors, cascades, and defense executors) as they correlate with the initiation of basal defense signaling (Figure 2).

Plant pattern recognition receptors (PRRs) perceive a wide range of elicitors, including pathogen-derived cell wall/membrane components (e.g., peptidoglycan, chitin), pathogen-associated proteins (e.g., flagellin, effectors), and host-derived danger associated molecular patterns (DAMPs; e.g., cuticle) (Ziv *et al.*, 2018). Though diverse, these receptors share certain features: they are single-transmembrane receptor-like kinases or receptor-like proteins, containing a leucine-rich repeat (LRR), LysM, EGF-like, or lectin domain for

ligand binding within the apoplast (Boutrot and Zipfel, 2017). For most cases, evidence supports a general mechanism wherein a core receptor and their associated kinases form the primary receptor complex, in association with additional regulators, mediate pathogen recognition and the initiation of downstream signaling. Herein lies one of the key remaining questions: *How fast does PRR activation occur following pathogen perception?*

While no technical approaches currently exist which can directly measure the speed of PRRs activation, this question can be answered by correlating the timing of measurable downstream outputs, such as the generation of apoplastic reactive oxygen species (ROS) - one of the earliest measurable defense responses (Torres *et al.*, 2006; Lehmann *et al.*, 2015). In Arabidopsis, the PTI-triggered apoplastic ROS burst is generated by respiratory burst oxidase homolog protein D (RBOHD) (Kadota *et al.*, 2015), a plasma membrane (PM)-associated NADPH oxidase that generates H_2O_2 as secondary signaling messenger. As one example of the link to PRR signaling complex activation, RBOHD is phosphorylated and activated by BIK1 (*Botrytis*-induced kinase 1), a core signaling kinase within the FLS2 (flagellin sensitive 2)-associated PRR complex (see Figure 2; Kadota *et al.* 2014). As an indicator of the timing of this response, the rate of ROS accumulation (i.e., d[ROS]/dt) reaches saturation at approximately 3 min after flg22 (elicitor that activates FLS2) stimulation (Nühse *et al.*, 2007), with complementary data demonstrating a maximum accumulation approximate 25 min following *P. syringae* infection (Smith and Heese, 2014). Taken together, the initial activation of PRRs occurs within the first few minutes following pathogen perception (Figure 3); the rapidity of this process further illustrates the role of ROS as second messenger in downstream immune signaling, including regulating Ca^{2+} influx.

Once PAMP recognition and PRR associated signaling events are activated, the immune signal is handed off to downstream signaling processes, which serves to not only amplify the initial signal, but importantly, functions as a mechanism to regulate signaling specificity and the activation of defenses that are appropriate to the nature of the stimulus. To accomplish this, plants utilize a complex series of phosphorylation-dependent signaling cascade, the best characterized of which include mitogen-activated protein kinases (MAPK) (Meng and Zhang, 2013) and calcium-dependent protein kinase (CPDK; aka CPK) relays (Singh *et al.*, 2017). To date, one of the best characterized signaling pathways is flg22-triggered PTI, wherein MAPKs are rapidly activated following phosphorylation by FLS2-associated signal regulators - a cascade from MAPKKK3/5 to MAPK3/4/6 (see Figure 2; Mithoe and Menke, 2018). As an illustration of the rapidity of this process, it has been demonstrated that flg22-induced signaling occurs within ~5 min following ligand perception as determined by MAPK3/4/6 phosphorylation; maximal phosphorylation is believed to peak at ~30 min post elicitation (see Figure 3; Frei dit Frey *et al.*, 2014).

Simultaneous with MAPK cascading, CPK-dependent signaling is induced by Ca^{2+} influx, a process that is initiated by gated Ca^{2+} channel(s) downstream of PRRs (also discussed in Section IV). While direct evidence is largely absent which describes the dynamic status of CPK phosphorylation during PTI, the influx of cytosolic Ca^{2+} can be used as an indirect index of CPK activity. Indeed, the accumulation of cytosolic Ca^{2+} obtains maximum speed (i.e., $d[Ca^{2+}]_{cyt}/dt$) in 1 min after elicitor treatment; the Ca^{2+} concentration reaches the peak in 3 min post-treatment (Qi *et al.*, 2010). Because CPKs are directly activated by elevated

concentration of cytosolic Ca^{2+} without intermediate kinases, and CPKs, Ca^{2+} channels, and RBOHD form a positive feedback loop (see Figure 2 and 4), the activation of CPKs is supposed to be slightly faster than MAPKs. This hypothesis is supported by evidence demonstrating that the rice *Os*CPK18 functions as direct upstream regulator of *Os*MAPK5 (Xie *et al.*, 2014), which further indicates that CPK-MAPK cross-talk is involved in PTI signaling. In total, these data support a model whereby MAPKs and CPKs work synergistically (Tena *et al.*, 2011), yet nonredundantly (Mehlmer *et al.*, 2010; Li *et al.*, 2018), as pro-immune activators.

Following MAPK and CPK signaling, the next step is the activation of defense executor proteins, a process leads to nuclear-based transcriptional reprograming (Li *et al.*, 2016), induction of defense hormone accumulation and signaling (Verma *et al.*, 2016), cytoskeleton/organelle remodeling (Li and Day, 2019), regulation of the secretome and cell wall/apoplast composition (Bellincampi *et al.*, 2014; Gupta *et al.*, 2015), and cellular motion (e.g. stomatal closure (Arnaud and Hwang, 2015). As key outputs of defense, the development of each of these cellular processes can be briefly categorized into two distinct phases. The first phase is the fast, pretranscriptional defense responses, which are often directly activated as a by-product of the basal immune signaling cascade. For example, RBOHD, described above, activates a robust ROS burst in the apoplast via the direct activation of PRR complex assembly and activation (Kadota *et al.*, 2014; Kadota *et al.*, 2015); as noted above, the timing of this response is detectable within 3 min of elicitation. Similar rapid signaling responses are also observed in the case of PM-associated ion channels (Jeworutzki *et al.*, 2010). As another example of rapid signaling through PTI executor, PAMP-triggered actin remodeling illustrates the integration of PRR function with broader signaling platforms, as illustrated by the detection of changes in microfilament remodeling within 5–15 min following PTI elicitation (Henty-Ridilla *et al.*, 2014; Li *et al.*, 2015). While the full mechanism(s) underpinning this response is unknown, we posit that it involves the regulation of actin depolymerizing factors (ADFs) by cytosolic kinases at the downstream of PRRs, as well as H_2O_2 and phosphatidic acid (Porter et al., 2012; Li *et al.*, 2015; Li *et al.*, 2017; Li and Staiger, 2018; Li and Day, 2019). Although still largely hypothetical, this model is in agreement with an abundance of data describing fast responses mediated by changes in actin filament organization, including the activation of downstream immune signaling processes.

In order to initiate a large-scale and long-term output of defense, signaling next proceeds to the phase of transcriptional activation of sustained and robust defense processes (Lewis *et al.*, 2015). As indicated by its classification, the foundation of this stage of immunity lies in the activation of stress-responsive transcription factors and the gene networks under their control. In brief, key regulators of this includes AP2/ERF, bHLH, bZIP, MYB, NAC, and WRKY (Tsuda and Somssich, 2015). Here, as a result of MAPK activation (described above), phosphorylation of defense transcription factors by MAPK significantly contributes to plant immunity. For example, in response to necrotrophic fungal pathogen *Botrytis cinereal*, MAPK3/6 phosphorylates WRKY33 within 0–12 h post-infection (hpi), which has been shown to regulate the overall resistance signaling within 6–24 hpi (Mao *et al.*, 2011). Interestingly, as an example of the dynamic control and specificity of signaling, the *WRKY33* mRNA is upregulated in response to flg22 or HrpZ (a bacterial elicitor) elicitation

at ca. 1 hpi and subsequently downregulated at 4 hpi (eFP Browser, Winter *et al.*, 2007), suggestive of a negative feedback loop to control signaling (Liu *et al.*, 2015). As such, a single PAMP treatment does not necessarily reflect the true dynamics of TF activity, because pathogens possess multiple elicitors (e.g., PAMPs, effectors) that result in the stimulation of various synergistic signaling cascades. In this regard, the overall dynamics and pattern of defense-induced transcription cannot be measured exclusively by the early (ca. minutes to hours) events, but rather, must be evaluated over the duration of the interaction, which can last days or longer. Thus, as illustrated in Figures 2 and 3, the activation of immunity is not a sequential series of events, but rather, represents a complex network of processes, each of which can be activated or attenuated multiple times during the host-pathogen interaction.

B. The activation of ETI

Effector-triggered immunity (ETI), is a robust and sustained immune response activated following perception of pathogen-secreted effector proteins. As an additional layer of the immune surveillance platform, ETI resembles PTI in many regards, such as the involvement of MAPK signaling cascades (Lu et al., 2018) and defense gene activation. However, distinct from PTI, ETI results in the activation of an apoptosis-like cell death (aka hypersensitive response (HR); Balint-Kurti, 2019), a fast process hypothesized to result in an abrogation of pathogen proliferation. As estimated by the dynamics of electrolyte leakage (indicating cell death) during HR, full intensity of ETI occurs within 2–6 h after inoculation of avirulent (containing effectors that triggers ETI) bacterial pathogens (Mackey *et al.*, 2002; Mackey *et al.*, 2003). Similar to such dynamic pattern, the transcriptome reprograming during ETI reaches the maximum speed during the same period of time (Mine *et al.*, 2018), indicating that ETI is a fast-acting immune response that may overcover the development of basal defense.

In terms of its mechanism, ETI relies on the function of host resistance (R) proteins to survey the cell for perturbations, through what is referred to as the Guard Hypothesis (Van der Biezen and Jones, 1998). As highlighted in reviews by Dangl and Jones (Jones and Dangl, 2006) and Chisholm *et al.* (Chisholm *et al.*, 2006), the Guard Hypothesis posits that R protein “guards” another host derived protein (guardee), and when a guardee is modified (e.g., cleaved, phosphorylated, etc.) by a pathogen-secreted effector, its associated R protein recognize such modification and triggers downstream signaling. Most of R protein belongs to nucleotide-binding site leucine-rich repeat (NB-LRR or NLR) protein family, which is also the best studied R protein architecture (Monteiro and Nishimura, 2018). According to their distinguishing feature of the structure and activity, NLR proteins have been historically divided into two subgroups, based on the amino-terminal presence of either a coiled-coil (CC) domain or a domain with similarity to the Toll/interleukin-1 receptor (TIR) family of proteins. In total, different domains (e.g., TIR/CC, NB, and LRR) of NLR provide abundant interaction interfaces, which not only supports intramolecular interactions that inhibit NLR activation at the absence of corresponding effector, but also serves as intermolecular adapters to form NLR heterodimers that regulates ETI with higher order of flexibility (Sukarta *et al.*, 2016). Directly related to this feature is the genetic evidence that certain extra downstream NLR(s) may be commonly required for ETI activation mediated by various NLRs (Adachi *et al.*, 2019). This leads to a helper-sensor model where a “sensor” NLR

(sNLR; Bernoux *et al.*, 2014) perceives the existence of avirulent effector and activates a "helper" NLR (hNLR; Bonardi *et al.*, 2011), which next processes ETI signaling pathway. Such model potentially explains why NLRs form heterodimer and why ETI is mediated by various R protein shares a unique pattern. However, one of the key questions that remains in ETI is: *How does activated NLR protein function in ETI initiation?*

Recently, a series of publications offers a mechanistic insight into the biochemical function of activated NLR proteins. To explore the topic, Wang and colleagues (Wang *et al.*, 2019a; Wang *et al.*, 2019b) inspected the protein structure of activated NLR ZAR1 (HOPZ-activation resistance-1) through a combined approach of cryo-EM based modeling and analysis. In brief, the study demonstrated that ZAR1 presents a pentameric-like structure, forming a general funnel shape within the PM (Dangl & Jones 2019; Wang *et al.*, 2019a). Related to its function to activate programed cell death, this conformation is easily associated to mammalian inflammasome complexes, which serve as a cytosolic catalytic center to activate downstream apoptosis (Sharma and Kanneganti, 2016). However, the PM-localized ZAR1 differs from cytosolic inflammasomes with respect to its subcellular localization, which suggests a distinguishing function of ZAR1 as a massive channel that mediates influx of apoplast components (including Ca^{2+}) and leakage of cytosol and trigger the downstream signaling of ETI. Interestingly and related to this, co-expression of NAIP (inflammasome structure protein that resembles sNLR and recognize animal PAMP), $RPS4^{TIR}$-NLRC4 (inflammasome structure protein that resembles hNLR, and fused with TIR domain of RPS4), and corresponding PAMP (conceptually equal to "effector" in plant immunity) in *N. benthamiana* can trigger HR-like symptoms (Duxbury, 2016), which suggests that formation of the inflammasome in plants is sufficient to trigger ETI, yet the downstream signaling events in animals and plants may vary.

A general picture of ETI can be further clarified if the sensor-helper model is combined with the hypothesis that NLR functions as a PM localized channel. As described by Jubic and colleagues (Jubic *et al.*, 2019), while some NLR, such as ZAR1, can both perceive effector activity and form up an active pentamer channel on the PM, other NLRs (i.e., absolute sNLR) does not have the second capability due to the lack of corresponding interaction interfaces or PM localization. In this case, an hNLR to be activated by sNLR is required for the assembly of PM-localized channel to activate ETI. Howbeit, it is still not clear whether the vast material transport mediated by the NLR channel is the major process responsible for ETI. Since some NLRs have additional biochemical activity, such as regulating TFs in the nucleus (Sun *et al.*, 2020), it is possible that activated NLR can initiate several relatively independent signaling pathways the synergistically contributes to the development of ETI.

C. Local immune attenuation: rebalancing growth versus defense

The energy distribution of growth versus defense requires a constant balancing of signaling processes, including the simultaneous activation and attenuation of processes that share considerable overlap. Quite obviously, plant defense signaling following pathogen perception requires the rapid engagement and activation of a broad range of immune signaling processes, as described above. At the same time, in the absence of pathogens, plants redirect a considerable amount of energy to processes which downregulate immune

signaling (Huot *et al.*, 2014; Karasov *et al.*, 2017). Thus, the attenuation of immune signaling is a critical process of self-defense which likely evolved as a mechanism to protect the host from the ill effects of hyperactivated defenses that down-regulates growth.

In contrast to the events associated with the activation of pro-immune signaling, our current knowledge of con-immune signaling is relatively limited. What we do know, however, is that much like immune activation signaling, MAPK cascade also plays an essential role in this process and represent one of the best characterized con-immune signaling mechanisms known. In Arabidopsis, a well-illustrated example of immune attenuation lies in our understanding of signaling mediated by MAPK3/6, which is activates its own inhibitory, MAPK phosphatase 1/2 (MKP1/2; Jiang *et al.*, 2018). In a detailed and elegant series of temporal gradient analyses focusing on the dynamics of MKP1 activity, it was revealed that MKP1 phosphorylation by MAPK6 is saturated at ~10 min following PAMP treatment, resulting in the stabilization of MKP1 and an increase in MPK1 protein levels (Jiang *et al.*, 2017a). Corelated to this observation, *MPK1* and *MKP2* mRNAs are nominally upregulated (< 2-fold) in response to biotic stress perception; we surmise that this illustrates a relatively low impact of transcriptional regulation on MPK abundance. Besides MAPKs *per se*, MKPs may actually dephosphorylates a wide spectrum of immune signaling substrates, as evidenced by the fact that MKP1 regulates thousands of MPK6-independent pro-immune transcriptions within 90 min after flg22 elicitation (Jiang *et al.*, 2017b). Moreover, and consistent with the role for MPK1/2 as broad regulators of immune attenuation, it is noteworthy that previous work has demonstrated that MPK1/2 are negative regulators of defense against biotrophic (e.g., *R. solanacearum*) and hemi-biotrophic (e.g., *P. syringae*) pathogen, whereas MKP2 is a positive regulator against necrotrophic (e.g., *B. cinerea*) pathogens (Lumbreras *et al.*, 2010; Anderson *et al.*, 2011). Taken together, these data illustrate that defense attenuation facilitates host immunototipotency against full spectrum of pathogens.

In addition to MKPs, other protein phosphatases (i.e., PP2A/Cs) also function as known contributors of counteracting kinase activity in immune signaling, and as such, play a substantial role in immune attenuation (Withers and Dong, 2017). For example, recent work has demonstrated that a group of PP2Cs (i.e., HAI1/2/3) quench MAPK3/6 downstream of flg22 triggered ABA signaling - a key virulence mechanism utilized by pathogens to manipulate immune signaling (Mine *et al.*, 2017). Likewise, Arabidopsis AP2C1 (aka PP2C25) dephosphorylates MAPK4/6, which modulates JA- and SA-associated immune signaling. In similar mechanisms, additional kinases also regulate the activation of PP2A/Cs, including the key PTI signaling regulators CPK6, BIK1, and BAK1 (Brandt *et al.*, 2012; Segonzac *et al.*, 2014; Couto *et al.*, 2016). However, evidence indicating phosphatase targets of other immune signaling components, such as receptors, enzymes, channels, and TFs, is still lacking, illustrating a general knowledge gap in the breadth of engagement by the mechanism of immune attenuation.

Another important mechanism for immune signaling attenuation is the degradation of immune signaling components, a process that is typically mediated via the ubiquitin-proteasome system (UPS) (Nandi *et al.*, 2006). In brief, UPS functions through the enzymes E1, E2, and E3, among which E1 and E2 energize and load ubiquitin onto the ubiquitin ligase E3, while E3 determines ubiquitination target specificity (Sharma *et al.*, 2016). As a

common mechanism in plant immune signaling, several well-characterized examples of ubiquitin-mediated attenuation exist. For example, FLS2 is targeted by the U-box E3 ligases PUB12/13, resulting in the degradation of FLS2 following flg22 stimulation (Lu *et al.*, 2011). As an illustration of the specificity and rapidity of this response, it was further demonstrated that physical association of PUB13 with FLS2 is initiated at ca. 30 seconds post FLS2 activation, indicating that PUB12/13 promotes rapid quenching of immunity. In contrast, LYK5, a membrane-associated receptor kinase responsible for chitin perception, is also targeted by PUB13, but the activation of LYK5 results in its dissociation from PUB13 and enhances LYK5 accumulation (Liao *et al.*, 2017). In another example, the immune kinase BIK1 is ubiquitinated by U-box E3 ligases PUB25/26, but such process is inhibited by the hetero-trimeric G-protein complex XLG2/3-AGB1-AGG1/2 when BIK1 is inactive (Liang *et al.*, 2016). Upon activation of BIK1 (i.e., in response to PTI elicitation), the XLG2/3-AGB1-AGG1/2 inhibitory complex dissociates, releasing unblocked BIK1 for UPS mediated-degradation (Liang *et al.*, 2016; Wang *et al.*, 2018).

Given that the proteasome exists within the cytosol, nucleus, and vacuole, it is a reasonable assumption that free, soluble, proteins are targeted to the proteasome via simple diffusion processes. However, this is not the case of PM-associated proteins, which are typically anchored through a variety of mechanisms, including transmembrane domains, post-translational modification, as well as via association with PM-resident components. In this regard, PM-associated immune signaling components, such as FLS2, BAK1, SERK1, CERK1, LYK5, PERP1, and SlCf-4, all require endocytosis-based mechanisms as a means to regulate recycling and or degradation (Claus *et al.*, 2018). In a general sense, the constitutive endocytosis of membrane components serves as a recycling mechanism to ensure that immunity is maintained in signaling-competent state. As a mechanism describing the naïve and activated recycling of immune receptors, the example of flg22-triggered FLS2 endocytosis is one of the best characterize models (Robatzek *et al.*, 2006; Mbengue *et al.*, 2016). As observed, following flg22 elicitation, the majority of FLS2 (GFP-tagged FLS2) was internalized from the PM to cytosolic vesicles within 20–40 min following flg22 treatment. As an illustration of immune attenuation and the regulation of PTI, during this same time, *de novo* synthesized FLS2 was not replenished at the PM. Instead, a marked induction in *FLS2* transcription was observed, indicating that PRR endocytosis is an approach of immune regulation, in support of UPS, to maintain the equilibrium of immune signaling.

As a final example, the negative regulation of pro-immune transcription represents a key component of the defense signaling network attenuation. Just as immune activation requires the induction of TF-mediated gene expression, so does immune attenuation. Among the best example(s) of this process is illustrated by the activity of the plant-specific family of WRKY transcription factors, known for their broad roles in signaling processes associated with both abiotic and biotic stress (Tsuda and Somssich, 2015; Hussain *et al.*, 2019). For example, Arabidopsis WRKY18 and WRKY40 are rapidly induced following *P. syringae* DC3000 and *G. orontii* perception, yet they function as synergistic negative regulators of resistance in response to both pathogens (Xu *et al.*, 2006; Pandey *et al.*, 2010). Using a series of ChIP-seq and RNA-seq approaches, it was further revealed that WRKY18 and WRKY40 possess broad transcriptional regulatory (presumably inhibitory) functions over defense genes during

the early activation of PTI (Birkenbihl *et al.*, 2017). Taken together, these studies indicate that certain "WRKY sub-regulatory networks" may serve as a mechanism to prevent over-induction of immunity, through balancing the in/activation of transcription following pathogen perception. Related to this hypothesis, Moore and colleagues (Moore *et al.*, 2011) provide a similar network perspective, proposing a transcription pulse model to describe transcription cascading in plant immunity. In short, this posits that the expression of TFs in different temporal nodules display consecutive cyclical bursts, with sharp up- and down-regulated oscillations over the course of the lifecycle, a process regulated in part by UPS-mediated degradation of transcription activators in the nucleus. Indeed, such a mechanism is required for the degradation of activated NPR1, as well as the enrichment of pro-immune TF inhibitors, including MYC2-induced *JAZ* expression.

IV. Distal immune signaling

A. Distal immune signaling varies by pattern and pathway

In parallel to the activation of local immune signaling, plants also employ long-distance signaling as a mechanism to prime defense activation in advance of pathogen proliferation. This strategy, to "nip it in the bud", functions to halt pathogen spread via the mobilization of a core, evolutionarily conserved, class of highly specific signaling molecules. Once mobilized, these signals activate defense responses in distal uninfected cells and tissues, which reduces secondary pathogen invasion, proliferation, and disease (see Figure 4). As a consequence, noninfected cells are primed to enter a pro-immune status. This process, referred to as systemic acquired resistance (SAR; (Durrant and Dong, 2004; Shine *et al.*, 2019)), provides protection against a broad range of pathogens, including bacteria, fungi, and viruses.

Following pathogen perception, a broad spectrum of distal immune signaling is activated, which can be categorized into two basic forms. The first, the electrical wave, mediated by self-feedback ion fluxes that resemble the neural transmission networks found in animals (Leybaert and Sanderson, 2012). In brief, this type of signal travels along the charged PM and requires the operation of a regulated channel transport between the apoplast, cytosol, endoplasmic reticulum, and tonoplast. While a detailed mechanism of intercellular transmission of the electric wave is not fully defined, it is hypothesized that Ca^{2+} influx plays a dominant role and that plasmodesmata (PD) is significantly involved in the intercellular transmission of electric wave (see Figure 4, (C); Choi *et al.*, 2017). Indeed, evidence in support of this is described in a recent study indicating that blockage of PD inhibits Ca^{2+} waves through mesophyll cells, yet not the vasculature (Toyota *et al.*, 2018). Based on the robustness and speed of this signal, it is reasonable to hypothesize that the Ca^{2+} wave represents the first phase of long-distance signaling in response to biotic stress perception.

The second class of immune signal that has been described is broadly classified as messenger molecules, including hormones (e.g., SA, JA), RNA (Kehr and Kragler, 2018; Huang *et al.*, 2019), proteins, and peptides (Segonzac and Monaghan, 2019). These signal molecules, which transmits by themselves, are distinguished from electric waves that stimulates membrane potential without transporting molecules to distal cells. As such, the

long-distance messengers transmit signals with high specificity, robustness, and durability, at the expense of speed. Moreover, each of these characteristics determines their biological function to induce and maintain the second phase of distal immunity, when massive pathogen inhibitory molecules are synthesized.

To facilitate the activation and spread of distal signals, there are two transmission pathways in plant tissues - symplastic and apoplastic (Conde *et al.*, 2011; Notaguchi and Okamoto, 2015; Canales *et al.*, 2018). In the symplastic pathway, immune activation signals move within the symplast, the space on the interior of the plasma membrane, comprised of the plant cytosol and endomembrane system, an intercellular network connected by plasmodesmata (PD). In the case of the apoplastic pathway, the signal moves beyond the fringe of the PM, within the apoplast - the space between the PM and the cell wall - wherein solutes diffuse freely. However, intercellular signaling within local parenchyma (mesophyll) has limited speed (Toyota *et al.*, 2018), potentially because the cell wall impedes the diffusion efficiency of signaling molecules within the apoplast. In addition, PD have reduced ion pools and small apertures, both of which diminish electrical signal transmission.

To accelerate the speed of distal immune signaling transmission, plants have evolved the use of the vascular system, particularly the phloem, for immune system activity. Among these signaling conduits, sieve tubes, the conducting cells in phloem, are wire-like cylinders that are joined in tandem with shared cytosol (symplast) and interconnected by multiple sieve poles. As subcellular structures required for intercellular communication, sieve poles originate from PD during development of the phloem, and are distinguished by a diameter of approximately 10–25-fold greater than PD themselves (Heo *et al.*, 2014). Hence, within sieve tubes, electrical signals can be transmitted along the PM of the symplast with extremely minor reductions in speed due to reduced gaps between cells (see Figure 4, (D)). Similar to electrical waves, messenger molecules can diffuse at high speeds inside the sieve tube without physical impediment of the cell wall (De Schepper *et al.*, 2013). As a general result, systemic signals usually travel as fast as 100–1000 mm/s across the vasculature (Choi *et al.*, 2016; Choi *et al.*, 2017), a speed that surpasses that of the spreading pathogen. Interestingly, the fastest speed recorded for vasculature mediated transmission in plants was the cold-shock signal following ice touch in *Aloe vera*, which was recorded at 132 m/s (Volkov *et al.*, 2007), comparable to myelinated neurons.

B. Calcium: the vanguard of long distance immune signaling

Unlike neural exon networks in animals where ion influx is modulated by voltage-gated channels, current evidence supports that the plant Ca^{2+} electrical wave is driven by PM-associated ligand-gated channels (Leybaert and Sanderson, 2012). Once pathogen invasion is perceived locally, systemic signaling is initiated by the activation of PRR-associated signaling complexes and followed by ROS accumulation and Ca^{2+} influx. While a detailed mechanism is not yet fully described, current knowledge supports a model whereby ROS generation and Ca^{2+} influxes are partially interdependent, and function synergistically in support of immune signaling amplification (see Figure 4, (B)). Data in support of this mechanism includes the observation that the *rbohd* mutant is capable of inducing Ca^{2+} influx, yet lacks the sustained (i.e., second burst) signaling response (Ranf *et al.*, 2011). *Vice*

versa, in the absence of Ca^{2+}, RBOHD can still be phosphorylated by BIK1 but not CPKs (Kadota *et al.*, 2014), which results in dampened activity of RBOHD in the local (Beneloujaephajri *et al.*, 2013; Miller *et al.*, 2009). Therefore, the initial Ca^{2+} influx cannot be fully attributed to the activation of a hypothetical Ca^{2+} channel activated by H_2O_2, either directly or indirectly. Instead, the most likely mechanism is that Ca^{2+} influx is initiated by an unknown PRR signaling component which directly activates Ca^{2+}-channels, a process in functions in parallel with H_2O_2-triggered Ca^{2+} influx (Yuan *et al.*, 2017). Interestingly, channels gated by Ca^{2+}-derived signals, as a positive-feedback loop, may be responsible the majority of PAMP-triggered Ca^{2+} influx, such as CNGC2/4 (cyclic nucleotide-gated channel 2/4), the calmodulin (CaM)-gated channels activation by flg22 elicitation (Tian *et al.*, 2019).

For distal Ca^{2+} signal transmission, however, activation of channels strictly requires certain patterns of “loop-feedback”, because there is no activated PRR complex to “ignite the calcium spark”. In this process, H_2O_2 is presumably a significant signaling mediator (Miller *et al.*, 2009). While the detailed mechanism linking H_2O_2 and Ca^{2+} remains largely unknown, one potential signaling pathway is via HPCA1, an H_2O_2-activated receptor-like kinase that contributes to the activation Ca^{2+} channels (Wu *et al.*, 2020). As described in a classical model describing distal Ca^{2+} signaling, H_2O_2 generated by RBOHD defuses to adjacent region and activates Ca^{2+} influx, which next leads to the activation of specific CPKs and RBOHD (by CPK). In such a mechanism, the Ca^{2+} signal is transmitted the distal cells and tissues, thus forming a Ca^{2+} electric wave (see Figure 4). This model is supported by evidence demonstrating that the *rbohd* mutant has a severely dampened Ca^{2+} wave in the root-shoot transmission (Evans *et al.*, 2016). Related to this, vacuolar ion channel TPC1 (two-pore channel 1) is identified as an essential gate regulating Ca^{2+} cytosolic influx from tonoplast during distal Ca^{2+} signaling (Choi *et al.*, 2014b). Since it is dually-gated by both Ca^{2+} ligand and voltage indispensably (Guo *et al.*, 2016), it may function to amplify Ca^{2+} influx following the initial influx mediated by H_2O_2.

In addition to H_2O_2, glutamate has been identified as another key messenger molecule for Ca^{2+}-mediated long-distance signaling. It is demonstrated that the distal Ca^{2+} wave, naturally triggered by mechanical damage, requires glutamate receptor like (GRL) family proteins GRL3.3/3.6, and simultaneously, generates wave-like apoplastic glutamate accumulation (Toyota *et al.*, 2018). Because, plant GRLs are broadly classified as amino acid gated ion channels (Forde and Roberts, 2014), it is reasonable to hypothesize that GRLs (such as GRL3.3/3.6 in this case) and an unknown glutamate release mechanism establish a ROS-like loop-feedback system to deliver Ca^{2+} distant signal. However, its relationship with H_2O_2-Ca^{2+} loop is uncertain: they may work independently but synergistically, or dramatically in tandem.

While our understanding of the function and mechanisms underpinning the transmission of Ca^{2+} waves is growing, we are still just scratching the surface in terms of the downstream signaling components following the arrival of Ca^{2+} waves in distal cells/tissues. For example, numerous Ca^{2+}-signaling cascades associated with calmodulin (CaM), CaM-like (CML), CPKs, calcineurin B-like protein (CBL)-interacting protein kinase (CIPK), and Ca^{2+}/calmodulin-dependent protein kinase (CCaMK) suggest a requirement for both transcriptional-dependent and independent defense responses (Marcec *et al.*, 2019). This

includes but is not limited to regulation of TF activity (Bredow and Monaghan, 2019), induction of SA synthesis (Guerra *et al.*, 2020; Wang *et al.*, 2009; Wang *et al.*, 2011), and cross-talk with second messengers (e.g., ROS and NO) (Marcec *et al.*, 2019). However, a critical question remains: *how does plant distinguish specific type* Ca^{2+} *signals with diverse output, while using the shared* Ca^{2+}*-mediated mechanism?* As a developing foundation which illustrates this complexity, the "Ca^{2+} signature model" offers a plausible hypothesis to describe signal origination and specificity (Yuan *et al.*, 2017; Marcec *et al.*, 2019). In brief, it is hypothesized that distal Ca^{2+} signals may differ in terms of oscillation dynamics, thus supporting a mechanism whereby Ca^{2+} signals possess an "identity" that is unique to their downstream effect(s). In support of this model, the pattern of the Ca^{2+} wave contributing to several immune processes has already been identified using mathematical approaches (Lenzoni *et al.*, 2018; Liu *et al.*, 2020).

C. Raging defense hormones

As a classic example of long-distance mobile signaling molecules in plants, hormones are not only critical regulators of growth and development, but also play integral roles in stress signaling, including in response to environmental (i.e., abiotic), bacterial, fungal, viral, and insect stimuli. Of the numerous host-derived defense signaling molecules associated with plant immunity, SA and JA are arguably the 2 best characterized hormones required for defense activation in response to a range of pests and pathogens. In simplest terms, SA biosynthesis and activity is triggered following biotrophic pathogen (e.g., bacteria) invasion, while JA is essential for the activation of defense to necrotrophic pathogens and pests. While current models describing defense signaling in plants often bifurcate based on SA- and/or JA-dependent modes of signaling, there is a growing body of literature that describes roles for additional plant hormones in both abiotic and biotic signaling. Indeed, the ethylene (ET), abscisic acid (ABA), brassinosteroids (BR), cytokinins (CKs), auxin (AUX), gibberellins (GAs) and strigolactones (SLs) also contribute to the regulation of plant immunity. However, for sake of brevity, we primarily focus on SA and JA, and thus, point the reader to several recent reviews that cover the role(s) of additional plant hormones in response to pathogen and pest perception (e.g., (Berens *et al.*, 2017; Burger and Chory, 2019)).

1. Salicylic acid: biosynthesis, regulation, and accumulation—Foundational work in our understanding of the regulation of SA-mediated pathogen defense was first provided by Wildermuth *et al.*, who demonstrated that in Arabidopsis the majority (ca. 90%) of SA utilized for plant defense signaling is generated by isochorismate synthase 1 (ICS1) (Wildermuth *et al.*, 2001). In the chloroplast, ICS1 converts chorismate into isochorismate - a product of the shikimate pathway and a common intermediate compound of primary and secondary metabolism. Next, isochorismate is exported from chloroplast by EDS5, a member of the multidrug and toxin extrusion (MATE) transporter family (Serrano *et al.*, 2013). In cytosol, isochorismate is then converted into sochorismate-9-glutamate by the acyl-adenylate/thioester-forming protein PBS3, and finally transformed to SA spontaneously or catalyzed by EPS1, an isochorismate-9-glutamate pyruvoyl-glutamate lyase (Rekhter *et al.*, 2019; Torrens-Spence *et al.*, 2019). Similar to the biological significance of ICS1, PBS3 and EDS5 also play essential roles in SA signaling, as mutation of either of them results in significant reductions in SA accumulation and signaling associated with local and systemic

defense activation. However, according to current knowledge, ICS1 is the rate-limiting enzyme and hence, the dominant regulatory site for pathogen-induced SA accumulation. Upon immune signaling activation, *ICS1* expression is directly induced by pro-immune TF SARD1 and CBP60g, which activates SA biosynthesis (Wang *et al.*, 2011; Sun *et al.*, 2015). Subject to this mechanism, significant SA accumulation, as well as an increase in *ICS1* expression can be detected 4 h after induction of ETI in local leaves (Wang *et al.*, 2015; Liu *et al.*, 2016), and 36 hpi in distal leaves following bacterial infection (Návarová *et al.*, 2012).

2. Salicylic acid: signaling and defense activation—Pathogen-induced SA production and transport from the chloroplast leads to the activation of both local and distal defense signaling processes. In response to the accumulation of SA, the NPR (nonexpressor of pathogenesis related genes) family of proteins, including NPR1, NPR3, and NPR4, serve as SA co-receptors and dominantly regulates SA-mediated defense (Ding *et al.*, 2018; Backer *et al.*, 2019). In brief, NPR1 generally serves as an activator of SA signaling. When SA binds to the C-terminal transactivating domain of NPR1, it disassociates from the N-terminal autoinhibitory domain, which enables NPR1 to activate defense genes in a TF-like manner. On the other hand, NPR3/4 is hypothesized to serve as general negative regulators. At the absence of SA, NPR3/4 act as repressors that inhibit key pro-immune genes, such as WRKY70, SARD1 and TFs in TGA protein family. Upon pathogen perception, induced SA binds to NPR3/4 and inhibits their repressor activity, which acts in parallel with NPR1 to up-regulate defense genes in a stringent manner of control (Ding *et al.*, 2018; Fu *et al.*, 2012; Wu *et al.*, 2012).

3. SA-mediated defense signaling and systemic acquired resistance—While PTI and ETI describe immune processes within infected regions, SAR imparts protection to distal and noninfected sites. As a function of distal immune signaling, and by definition, SAR describes the priming of systemic defense following local activation of immunity, whereby SA is hypothesized as one of the key messenger molecules to transmit the signal. As a first description of the molecular mechanisms underpinning SAR, the induction of a suite of genes (i.e., *pathogenesis-related* (*PR*) genes) following pathogen perception and the accumulation of SA provided a set of genetic markers whose expression patterns correlated with the onset of defense signaling, including in association with the activity of SA (Linthorst and Van Loon, 1991). Thanks to this early work, a body of knowledge describing the function and transmission of other SAR messengers has emerged, including the role of pipecolic acid (Pip) and its derivative N-hydroxy-pipecolic acid (NHP) (Hartmann and Zeier, 2018). As candidates for distal immune signal transmission, both Pip and NHP accumulate in systemic leaves as early as 24 h post-inoculation, suggesting a role for these 2 molecules in SAR and immunity. As evidence in support, mutation of critical biosynthetic enzymes required for NHP production, namely ALD1 (AGD2-like defense response protein 1) and SARD4 (SAR deficient 4), causes deficiency in Pip production, with concomitant reductions in SAR. (Hartmann and Zeier, 2018). Further studies demonstrated that mutation of *FMO* (flavin-containing monooxygenase), which catalyzes conversion of Pip into NHP, results in compromised SAR in plants challenged with *P. syringae* and oomycete pathogen *Hyaloperonospora arabidopsidis* (Hartmann *et al.*, 2018b). Interesting, the *fmo1* phenotype could be rescued by addition of exogenous NHP, but not Pip, further demonstrating the role

for FMO in the SAR, as well as reinforcing the hypothesis that NHP may in fact be the bioactive signal.

4. Jasmonic acid: synthesis, perception, and signaling—Like SA, biosynthesis of JA also occurs within the chloroplast, and requires the conversion of galactolipids to 12-oxophytodienoic acid (OPDA), an intermediate step in the generation of JA by jasmonoyl isoleucine conjugate synthase1 (JAR1) (Ruan *et al.*, 2019). As an inducer of JA biosynthesis, the polypeptide systemin plays an indispensable role in JA-mediating signaling following wounding, including damage resulting from insect herbivory and necrotrophic fungal pathogen infection (Campos *et al.*, 2014). As an illustration of the speed of the JA-induced response, a rapid induction in JA marker genes (e.g., *PDF1.2*) has been observed as early as 15 min following wounding in both local and distal leaves (Manners *et al.*, 1998). Interestingly, preceding the transcriptional activation of the JA response, increases in the levels of JA-Ile have been observed as early as 5 min post-elicitation, a response that is sustained up at ca. 6 h in local leaves following elicitation. In systemic leaves, JA-Ile levels increased rapidly (ca. 5 min post-elicitation) yet had levels that were substantially reduced as compared to local leaves. Likewise, systemic levels of JA-Ile were also diminished, with reductions as early as ~1 h post-elicitation (Schuman *et al.*, 2018).

5. JA perception—Once JA-Ile is synthesized and properly localized within the immune-activated cell, it is recognized by the receptor COI1 (coronatine insensitive 1; (Sheard *et al.*, 2010)), together with the co-receptor JAZ (JA ZIM domain). As a complex, COI1 and JAZ mediate the downstream signaling of defense through a highly complex, yet elegant, series of events. In short, JAZ proteins are comprised of a N-terminal ZIM domain and a C-terminal Jas domain, which facilitate JA-Ile binding to the receptor. First, the COI1 protein forms a pocket which accommodates JA-Ile with high affinity. Following substrate recognition, the conserved degron motif at N-terminus of the Jas domain found in JAZ, forms a loop to trap JA-Ile into the COI1 pocket, with the C-terminal region of Jas provides a helical structure for COI1 docking (Sheard *et al.*, 2010). Once fully docked, the perception of JA-Ile results in JAZ degradation, via the activity of the SCF^{COI1} ubiquitin ligase and the 26S proteasome system, which initiates a series of transcription de-repression events, including the activation of MYC2, an essential TF responsible for the activation of JA-regulated genes (Withers *et al.*, 2012). At the same time, MYC2 stimulates the expression of its downstream repressors (e.g., MTB1/2/3; Liu *et al.*, 2019), which act as an elegant negative feedback loop to attenuate this signaling cascade. Interestingly, and as a mechanism illustrating the antagonistic relationship between JA and SA (noted above), biotrophic pathogens (i.e., SA-dependent), such as *P. syringae*, utilize secreted effectors that manipulate SA-mediated immunity via modulation of the JA signaling pathway. For instance, the *P. syringae* effector HopZ1a promotes JAZ1 degradation in a COI1-dependent manner through the activation of JA signaling, leading to a suppression of SA-mediated immunity via down-regulation of *ICS1* (Gimenez-Ibanez *et al.*, 2014). Similarly, additional recent studies have demonstrated pathogen effector-mediated manipulation of SA defense via targeting of JAZ-associated function and JA signaling processes (Jiang *et al.*, 2013; Gimenez-Ibanez *et al.*, 2016), illustrating the both the complexity and connectivity of SA and JA signaling in plants.

D. Danger, danger

In addition to a critical role for defense hormones as signaling molecules following pathogen perception and infection, plants also utilize the recognition of self-derived molecules to activate distal defense signaling. The compounds, damage associated molecular patterns (DAMPs), refer to a class of plant-derived signaling molecules that accumulate as a result of pathogen infection, cell injury, and/or the activation of death signaling (Hou *et al.*, 2019). Similar in concept to SAR, DAMPs mediate immune priming in distal cells and tissues, and similar to the recognition of PAMPs, DAMP perception requires plasma membrane-localized receptors, whose activation leads to the initiation of similar signaling cascades (e.g., MAPK, Ca^{2+}) and transcriptional reprograming.

Broadly, DAMPs encompass peptides, ATP, host-derived proteins released from damaged cells, and degraded cell wall polysaccharides. As one of the most diverse group of DAMPs, peptide signaling molecules have been well-explored, and in total, illustrates not only defense mechanisms that plants employ to defend against pathogen invasion, but also highlight the evolution and adaptation of pathogens to subvert host defense processes (Heil and Land, 2014; Hirakawa *et al.*, 2017). Among the numerous peptide-based DAMPs identified in plants, the activity of systemin and PEP represent classic examples of wound-induced signaling molecules (Savatin *et al.*, 2014). The first DAMP identified was systemin, an 18-amino acid peptide that accumulates in the apoplast in response to wounding and/or insect damage (Pearce *et al.*, 1993). As a host-derived activator of defense responses, systemin is derived from prosyste-min, a ~200 amino acid precursor that accumulates in the cytosol following wound response activation. Not surprisingly, systemin perception is mediated by a plasma membrane associated receptor (Scheer and Ryan, 1999). Once perceived, receptor binding to systemin results in the rapid induction of JA biosynthesis, which as described above, activates systemic defense signaling in response to fungal pathogenesis and insect herbivory (Wang *et al.*, 2018a; Zhang *et al.*, 2020).

Pep1, another well characterized DAMP, is a 23 amino acid bioactive signaling molecule derived from a larger “propeptide” (i.e., 92 amino acid PROPEP1), whose accumulation leads to the activation of defense signaling, including the generation of H_2O_2 and regulation of JA-responsive genes (Huffaker *et al.*, 2006). In Arabidopsis, it has been demonstrated that transformation of Pep1 from PROPEP1 in cytosol is catalyzed by the cysteine protease metacaspase 4 (MC4), which is activated by wound-induced Ca^{2+} influx (Hander *et al.*, 2019). Once released, Pep1 binds the co-receptors PEPR1 and PEPR2 (PEPR1/2) of adjacent cells, which in turn leads to the activation of defense signaling (Krol *et al.*, 2010; Yamaguchi *et al.*, 2010). Not surprisingly, PEPR1 forms a complex with the LRR kinase BAK1, a critical component of many PRR complexes, further illustrating the connectivity among different immune signaling pathways (Yamada *et al.*, 2016). Such similarity is also embodied in the dynamics of protein recycling. Like FLS2, the PERP1 complex is internalized for degradation in a clathrindependent manner ca. 20 min after exogenous application of Pep1; however, recycling of PERP1 and FLS2 utilize different trafficking pathways (Ortiz-Morea *et al.*, 2016; Mbengue *et al.*, 2016).

In addition to peptide-based elicitors of damage perception, other types of host-derived signaling molecules also function as elicitors of wound-induced defense signaling (Li *et al.*,

2020). One of such DAMPs is extracellular adenosine 5-triphosphate (eATP), which is perceived by the lectin receptor kinase DORN1 (DOes not Respond to Nucleotides; (Choi *et al.*, 2014a). At the downstream, the activation of DONR1 leads to many PTI-like signaling processes, which include Ca^{2+} influx, ROS/NO generation, MAPK phosphorylation, and transcriptional regulation of genes involved in SA and JA signaling (Jewell *et al.*, 2019; Wang *et al.*, 2018b). Similarly, and as a second example of nonpeptide derived elicitor, the co-factor NAD^+ also plays an important role as a host-derived elicitor of immune signaling, which serves as indicator of cell damage in plant (Wang *et al.*, 2017). Like eATP, NAD^+ perception also results in the activation of PTI-like responses, yet those specific to SA-dependent defense processes. Not surprisingly, NAD^+, together with its receptor LecRK-1.8, a PM-localized kinase and homolog of DORN1, is required for SAR (Luo *et al.*, 2017).

V. The development of the plant immune system

A. Age related resistance and immune system development

The ontogenesis of an organism is an amazing biological process. Indeed, as organismal systems grow and mature, “new” signals are activated and “old” processes are attenuated; collectively, this dynamic signaling landscape highlights the concept of organismal development. In the context of an integrated system, we would argue that much of the plant immune system is invisible, because, as described above, the immune system is associated with and connected to nearly all processes within the living cell. Unlike the immune system of animals, which possesses a narrowly defined, yet highly differentiated immune surveillance network, the plant immune system is not specifically differentiated based on cell and/or tissue type (Handley *et al.*, 2005). In this vein, the development and maturity of the plant immune system is virtually indistinguishable from the development and maturation of the organism itself.

For the sake of comparison, we propose two general perspectives to illustrate the development of the plant immune system. First, in temporal scales, the chronological age of a plant, as well as the developmental stage of growth, is positively corelated with the robustness of pathogen resistance (Hu and Yang, 2019). In this regard, the robustness of immune signaling is known to increase from early developmental stages to reproductive stages, after which time, the fitness/robustness of the immune system is reduced, presumably as a function of host senescence (Eichmann and Schafer, 2015; Haffner *et al.*, 2015). During this process, discernable patterns of age-related immunity can be described as: (1) transitions in immune robustness at each developmental checkpoint in plant growth (Rusterucci *et al.*, 2005); and (2) the accumulation of immunity as a feature of organ maturation (Ficke *et al.*, 2002; Gadoury *et al.*, 2003). Taken together, it is also tempting to hypothesize that the biological significance of age-related immunity may have its foundation in the energy tradeoff of immunity vs reproduction. If true, this would suggest a mechanism which reinforces the necessity to protect reproduction by investing energy to immunity during development. In support of this hypothesis, a recent ecogenetic study reveals that two strategies are adapted by Arabidopsis natural population to secure their reproduction: an extension of the vegetative stage for higher energy gain to invest a robust immune system, or

a shortened vegetative stage for fast reproduction to avoid pathogen infection and death (Glander *et al.*, 2018).

Second, and as a function of spatial scales, the robustness of immune system increases from early to late developed organs. To illustrate this concept, we present a simple disease symptom record when Arabidopsis is inoculated with *P. syringae* (Figure 5, (A)). As shown, an obvious trend emerges whereby late developed leaves have increasingly lower disease symptom development (i.e., enhanced resistance). Indeed, this phenomenon is further supported by several studies, including recent work which demonstrated that juvenile rosettes, adult rosettes, and cauline leaves from 8-week-old Arabidopsis plants have dramatically different levels of resistance against both *P. syringae* and *S. sclerotiorum* (Hu and Yang, 2019; Kus *et al.*, 2002); a similar observation has also been observed using *N. benthamiana* and tobacco (Xu *et al.*, 2018). At a mechanistic level, age-related enhancements in immunity in mature organs require the function of key defense hormone signaling components, as evidenced by compromised resistance in adult and cauline leaves in the hormone biosynthesis mutants *sid1* (SA), *jar1* (JA), and *aba1* (abscisic acid) (Wilson *et al.*, 2017). As a step to further describe this mechanism at a transcriptional level, we analyzed publicly available mRNA datasets derived from Arabidopsis leaves of different developmental stages and observed that mRNA expression profiles of immune-related genes show certain corelated patterns to the morphogenesis order of different leaf samples (Figure 5, (B)). In short, this correlation suggests the involvement of a shared upstream signaling nodule that regulates plant morphogenesis and age-dependent immunity in an integral and synergistic manner.

B. Autologous genetic regulation controls immune system development

Similar to the development of any organ/system during ontogenesis, the development of the plant immune system is mediated by autologous transcriptional regulation. So far, studies focusing on a group of microRNA, miR156/157, have revealed a spatiotemporal regulatory network over the maturation process of plant immune system. At upstream, miR156/157 is regulated by the age-sensitive CDK8-MED12/13 mediator complex (Gillmor *et al.*, 2014); at downstream, miR156/157 target TFs in the SPL (Squamosa-promoter binding protein-like) family and inhibit their expression (Preston and Hileman, 2013). Since SPLs directly and indirectly regulate expression of defense genes (see Figure 6), the miR156/157-SPLs signaling module plays central role in regulating the development of the plant immune system, thereby shaping age-related immunity (Zheng *et al.*, 2019).

For example, miR156-SPL9 contributes to resistance against *P. syringae* during early vegetative stages through the regulation of defense genes and ROS accumulation (Yin *et al.*, 2019). As one of the best characterized mechanisms in behind, miR156-SPL9 controls FLS2 and basal defense through regulating miR172. In brief, miR172, promoted by SPL9 (and maybe SPL10/15 redundantly; Wu *et al.*, 2009), inhibits two FLS2-repressive TFs, TOE1/2. Thus, while miR159 decreases in seedlings from day 2 to 6, the miR172 is upregulated for 7 folds, which eliminates TOE1/2 transcript by 65% and therefore increases FLS2 transcript by 7 folds (Zou *et al.*, 2018), leading to the immune maturation of seedlings. Besides miR172, the miR156/157-SPLs module also regulates other components of immunity

through the lifetime of plant, including facilitating JA signaling by stabilizing JAZ3 from UPS mediated degradation (Gaquerel and Stitz, 2017; Mao *et al.*, 2017) and contributing to the ETI mediated by TIR-NB-LRR protein N and RPS4 (Padmanabhan *et al.*, 2013).

Interestingly, SPLs are considered critical regulators of organ morphogenesis rather than having exclusive roles as defense genes (Ye *et al.*, 2019). This is significant, as it offers a unique perspective to understand the relationship between plant development and immunity - instead of antagonistic signaling modules (in terms of energy tradeoff) or relatively independent processes, they are synergistic pathways subject to a common upstream signaling center (i.e., miR156/157-SPLs). In accordance with this perspective, many essential genes in charge of plant vegetative/reproductive morphogenesis at the downstream of miR156/157-SPLs can also regulate the defense genes simultaneously, such as LEAFY (Yamaguchi *et al.*, 2009) and SOC1 (Lee and Lee, 2010), which contribute to PTI (Winter *et al.*, 2011) and SA signaling (Wilson *et al.*, 2017), respectively. Hence, plant morphogenesis and immune maturation are merely two sides of a coin named “development”.

Furthermore, this regulatory framework enables an advanced strategy to bypass certain antagonistic tradeoffs when confronting biotic stresses, by dynamic replacements of dominant signaling pathway at deferent developmental stages. For example, older plants possess dampened JA signaling-associated processes because a high level of SPL9 stabilizes JAZs. However, these plants also show robust resistance against insects, potentially due to an abundance of glucosinolates accumulated in leaves (Gaquerel and Stitz, 2017). As a result, such mechanism, which is subject to the JA/SA antagonism paradigm, would enable a high dynamic range to mobilize SA-mediated immunity, thus maintaining broad levels of resistance. The next step in advancing our understanding of these mechanisms is to reveal how plants utilize a dynamically developing immune system to overcome what is typically a “zero sum game” with respect to antagonistic tradeoffs.

C. The role of beneficial microbial associations on the plant immune system

Plants serve as host to numerous microorganisms originated from a variety of sources, including via aerosols, animals, rain, and soil (Müller *et al.*, 2016). There are two primary interfaces for microbial interaction: above-ground (aka phyllosphere) and below-ground (aka rhizosphere; see Figure 1). In either case, the assembly and maintenance of the plant-associated microbiome is mediated in part by plant immune system itself, which involves phytoalexin, ETI, PTI, and other unknown approaches to shape the commensal microbiota selectively or unselectively (Hacquard *et al.*, 2017; Teixeira *et al.*, 2019; Vannier *et al.*, 2019). In return, as introduced next, the commensal microbiota perform critical functions in facilitating the development of immune system and plant morphogenesis extensively, potentially due to their signaling cross-talk (see Figure 6).

In the broadest sense, the nonpathogenic components of commensals influence the plant immune system in two primary ways. First, microbial communities can stimulate the development and maturation of the immune system (Vannier *et al.*, 2019). In an effort to identify the relationship(s) between microbiome function maturation of host immunity, a recent study utilized a germ-free (axenic) environment to evaluate immune system performance over the ontogenesis of the plant (Kremer, 2017; Kremer *et al.*, 2018). As

demonstrated, Arabidopsis grown in an axenic environment showed subtle, yet measurable, differences in shape and size as compared to plants in holoxenic (with a natural microbiome) environments. However, in terms of immune system maturation and performance, axenic plants showed significant deficiencies compared to holoxenic plants, because the axenic plants display an overall shut-down of defense gene expression and immune signaling processes (e.g., MAPK activity, ROS burst, and defense se hormone biosynthesis). Echoing this study, Dur an *et al*. also demonstrated that microbial community functions to enhance plant resistance against invading pathogens and identified certain components of protective microbes (Dur an *et al*., 2018). Collectively, these studies convincingly demonstrate that the development of immune system is indispensable to the engagement of plant-associated microbiome. Interestingly, while facilitating the development of plant immune system, commensal microbiome can also promote root morphogenesis in an auxin signaling-dependent manner (Zamioudis *et al*., 2013; Klikno and Kutschera, 2017). In agreement with current models that organ morphogenesis and immune system development are potentially coupled, as discussed above, these data supports the hypothesis that the plant-associated microbiome not only plays a critical role in promoting the development of plant immune system, but also contributes to additional aspects of whole plant ontogenesis.

A second process through which commensal microbes assist plant immunity is via inhibiting pathogen proliferation by direct microbe-microbe interaction(s) (Vannier *et al*., 2019). While a full mechanistic understanding is still lacking, significant insight has been gained through the identification and characterization of the role of secreted microbial anti-biotic compounds. For instance, a rhizosphere bacteria, *Streptomyces* sp. S4–7, was shown to secrete a thiopeptide that significantly inhibits the growth of *F. oxysporum* (a fungal pathogen causing Fusarium-wilt) during pathogenesis (Cha *et al*., 2016). In this study, mRNA-seq analysis revealed that the antimicrobial thiopeptide can impair RNA metabolism, cytoskeleton architecture, and cell wall biosynthesis in its targeted fungal pathogens. In a similar case, *Pseudomonas piscium*, isolated from wheat, can secrete an antimicrobial compound, phenazine-1-carboxamide, that suppresses *F. graminearum* via the inhibition of *Fg*Gcn5, a histone acetyltransferase, resulting in histone acetylation dysfunction (Chen *et al*., 2018). Another common mechanism that enhance immune system function and broad defense mechanisms is through the reduction of pathogenicity and abundance of potential pathogens. Recently, through a large-scale analysis of microcosms and greenhouse disease assays, it was shown that rhizosphere commensals with high diversity and a large niche overlap with *R. solanacearum* can suppress pathogen population growth and disease symptom development on tomato (Wei *et al*., 2015). In addition to these processes, several additional mechanisms have been described that have the potential to alleviate the threat of the pathogen over-taking the plant immune system, including nutrition interdependency, biofilm formation, endosymbiosis, quorum sensing, and predation (Hassani *et al*., 2018).

VI. Final thoughts

To date, numerous processes involved in plant-pathogen interactions have been identified, the analysis of which has greatly contributed to a better understanding of the molecular mechanisms underpinning plant immunity. However, while most research in this area has necessarily been guided by a reductionist approach, a full picture of plant immunity,

including the connectivity of different immune processes, requires higher resolution-based approaches and a systemic perspective. Moreover, it requires a full assessment and integration of the temporal and spatial events to generate a complete picture. In this review, we focused on the presentation of an integrative and dynamic process, one that is mediated by local and distal signaling of immunity, as well as plant ontogenesis and its interaction with the environment. However, on the frontier of this field of study still stand many old and new questions: *How do plants dynamically promote and attenuate immunity in a tradeoff? How do plant neuron-like transmission systems function? How do plants decode single (*e.g., Ca^{2+}*) and combined signals for robust signaling that is specific to and appropriate for the nature of the stimulus? Can growth and immunity be integrated synergistically rather than antagonistically?* These are just a few of the outstanding questions that remain on the battlefield of plant-pathogen interactions.

Acknowledgements

We appreciate Dr. Richard Hilleary (Michigan State University), Dr. Yunhao Sun (Sun Yat-sen University), and Yexin Yang (Yale University) for providing critical advice during the development of this review.

Funding

This work was supported by the National Science Foundation Plant-Biotic Interactions Program [IOS-1146128] and the National Institute of General Medical Sciences [1R01GM125743] awarded to BD.

References

Adachi H, Derevnina L, and Kamoun S 2019 NLR singletons, pairs, and networks: evolution, assembly, and regulation of the intracellular immunoreceptor circuitry of plants. Curr. Opin. Plant Biol 50:121–131. doi:10.1016/j.pbi.2019.04.007 [PubMed: 31154077]

Anderson JC, Bartels S, Besteiro MAG, Shahollari B, Ulm R, and Peck SC 2011 Arabidopsis MAP Kinase Phosphatase 1 (AtMKP1) negatively regulates MPK6-mediated PAMP responses and resistance against bacteria. Plant J 67:258–268. doi:10.1111/j.1365-313X.2011.04588.x [PubMed: 21447069]

Anjago WM, Zhou T, Zhang H, Shi M, Yang T, Zheng H, and Wang Z 2018 Regulatory network of genes associated with stimuli sensing, signal transduction and physiological transformation of appressorium in *Magnaporthe oryzae*. Mycol 9:211–222. doi:10.1080/21501203.2018.1492981

Arnaud D, and Hwang I 2015 A sophisticated network of signaling pathways regulates stomatal defenses to bacterial pathogens. Mol. Plant 8:566–581. doi:10.1016/j.molp.2014.10.012 [PubMed: 25661059]

Auyong AS, Ford R, and Taylor PW 2015 The role of cutinase and its impact on pathogenicity of *Colletotrichum truncatum*. Plant Pathol. Microbiol 6: 259–270.

Backer R, Naidoo S, and van den Berg N 2019 The NONEXPRESSOR OF PATHOGENESIS-RELATED GENES 1 (NPR1) and related family: mechanistic insights in plant disease resistance. Front Plant Sci 10:102. doi:10.3389/fpls.2019.00102 [PubMed: 30815005]

Baker CM, Chitrakar R, Obulareddy N, Panchal S, Williams P, and Melotto M 2010 Molecular battles between plant and pathogenic bacteria in the phyllosphere. Braz. J. Med. Biol. Res 43:698–704. doi:10.1590/S0100-879X2010007500060 [PubMed: 20602017]

Balint-Kurti P 2019 The plant hypersensitive response: concepts, control and consequences. Mol. Plant Pathol 20:1163–1178. doi:10.1111/mpp.12821 [PubMed: 31305008]

Bellincampi D, Cervone F, and Lionetti V 2014 Plant cell wall dynamics and wall-related susceptibility in plant-pathogen interactions. Front. Plant Sci 5:228. doi:10.3389/fpls.2014.00228 [PubMed: 24904623]

Beneloujaephajri E, Costa A, L'Haridon F, M etraux J-P, and Binda M 2013 Production of reactive oxygen species and wound-induced resistance in *Arabidopsis thaliana* against *Botrytis cinerea* are

preceded and depend on a burst of calcium. BMC Plant Biol 13:160. doi:10.1186/1471-2229-13-160 [PubMed: 24134148]

Berens ML, Berry HM, Mine A, Argueso CT, and Tsuda K 2017 Evolution of hormone signaling networks in plant defense. Annu. Rev. Phytopathol 55:401–425. doi:10.1146/annurev-phyto-080516-035544 [PubMed: 28645231]

Bernoux M, Moncuquet P, Kroj T, and Dodds PN 2014 A novel conserved mechanism for plant NLR protein pairs: the 'integrated decoy' hypothesis. Front. Plant Sci 5:606. [PubMed: 25506347]

Birkenbihl RP, Kracher B, Roccaro M, and Somssich IE 2017 Induced genome-wide binding of three Arabidopsis WRKY transcription factors during early MAMP-triggered immunity. Plant Cell 29:20–38. doi:10.1105/tpc.16.00681 [PubMed: 28011690]

Bonardi V, Tang S, Stallmann A, Roberts M, Cherkis K, and Dangl JL 2011 Expanded functions for a family of plant intracellular immune receptors beyond specific recognition of pathogen effectors. Proc. Natl. Acad. Sci. USA 108:16463–16468. doi:10.1073/pnas.1113726108 [PubMed: 21911370]

Boutrot F, and Zipfel C 2017 Function, discovery, and exploitation of plant pattern recognition receptors for broad-spectrum disease resistance. Annu. Rev. Phytopathol 55:257–286. doi:10.1146/annurev-phyto-080614-120106 [PubMed: 28617654]

Brandt B, Brodsky DE, Xue S, Negi J, Iba K, Kangasjärvi J, Ghassemian M, Stephan AB, Hu H, and Schroeder JI 2012 Reconstitution of abscisic acid activation of SLAC1 anion channel by CPK6 and OST1 kinases and branched ABI1 PP2C phosphatase action. Proc. Natl. Acad. USA 109:10593–10598. doi:10.1073/pnas.1116590109

Braun U, Shin H-D, Takamatsu S, Meeboon J, Kiss L, Lebeda A, Kitner M, and Götz M 2019 Phylogeny and taxonomy of *Golovinomyces orontii* revisited. Mycol. Progress 18:335–357. doi:10.1007/s11557-018-1453-y

Bredow M, and Monaghan J 2019 Regulation of plant immune signaling by calcium-dependent protein kinases. Mol. Plant Microbe Interact 32:6–19. doi:10.1094/MPMI-09-18-0267-FI [PubMed: 30299213]

Brooks DM, Bender CL, and Kunkel BN 2005 The *Pseudomonas syringae* phytotoxin coronatine promotes virulence by overcoming salicylic acid-dependent defences in *Arabidopsis thaliana*. Mol. Plant Pathol 6:629–639. doi:10.1111/j.1364-3703.2005.00311.x [PubMed: 20565685]

Burger M, and Chory J 2019 Stressed out about hormones: how plants orchestrate immunity. Cell Host Microbe 26:163–172. doi:10.1016/j.chom.2019.07.006 [PubMed: 31415749]

Campos ML, Kang JH, and Howe GA 2014 Jasmonate-triggered plant immunity. J. Chem. Ecol 40: 657–675. doi:10.1007/s10886-014-0468-3 [PubMed: 24973116]

Canales J, Henriquez-Valencia C, and Brauchi S 2018 The integration of electrical signals originating in the root of vascular plants. Front. Plant Sci 8:2173. doi:10.3389/fpls.2017.02173 [PubMed: 29375591]

Cha J-Y, Han S, Hong H-J, Cho H, Kim D, Kwon Y, Kwon S-K, Crüsemann M, Bok Lee Y, Kim JF, Giaever G, Nislow C, Moore BS, Thomashow LS, Weller DM, and Kwak Y-S 2016 Microbial and biochemical basis of a *Fusarium* wilt-suppressive soil. Isme J 10:119–129. doi:10.1038/ismej.2015.95 [PubMed: 26057845]

Chang H-X, Miller LA, and Hartman GL 2014 Melanin-independent accumulation of turgor pressure in appressoria of *Phakopsora pachyrhizi*. Phytopathol 104: 977–984. doi:10.1094/PHYTO-12-13-0335-R

Chappelka AH, and Grulke NE 2016 Disruption of the 'disease triangle' by chemical and physical environmental change. Plant Biol. J 18: 5–12. doi:10.1111/plb.12353

Chen Y, Wang J, Yang N, Wen Z, Sun X, Chai Y, and Ma Z 2018 Wheat microbiome bacteria can reduce virulence of a plant pathogenic fungus by altering histone acetylation. Nat. Comm 9:1–14.

Cheng F, Ma A, Zhuang X, He X, and Zhuang G 2016 N-(3-oxo-hexanoyl)-homoserine lactone has a critical contribution to the quorum-sensing-dependent regulation in phytopathogen *Pseudomonas syringae* pv. *tabaci* 11528. FEMS Microbiol. Lett 363:fnw265. [PubMed: 27864298]

Cheng YT, Zhang L, and He SY 2019 Plant-microbe interactions facing environmental challenge. Cell Host Microbe 26:183–192. doi:10.1016/j.chom.2019.07.009 [PubMed: 31415751]

Chisholm ST, Coaker G, Day B, and Staskawicz BJ 2006 Host-microbe interactions: shaping the evolution of the plant immune response. Cell 124:803–814. doi:10.1016/j.cell.2006.02.008 [PubMed: 16497589]

Choi J, Tanaka K, Liang Y, Cao Y, Lee SY, and Stacey G 2014a Extracellular ATP, a danger signal, is recognized by DORN1 in Arabidopsis. Biochem. J 463: 429–437. doi:10.1042/BJ20140666 [PubMed: 25301072]

Choi W-G, Hilleary R, Swanson SJ, Kim S-H, and Gilroy S 2016 Rapid, long-distance electrical and calcium signaling in plants. Ann. Rev. Plant Biol 67: 287–307. [PubMed: 27023742]

Choi WG, Miller G, Wallace I, Harper J, Mittler R, and Gilroy S 2017 Orchestrating rapid long-distance signaling in plants with Ca^{2+}, ROS and electrical signals. Plant J 90:698–707. doi:10.1111/tpj.13492 [PubMed: 28112437]

Choi W-G, Toyota M, Kim S-H, Hilleary R, and Gilroy S 2014b Salt stress-induced Ca2+ waves are associated with rapid, long-distance root-to-shoot signaling in plants. Proc. Natl. Acad. Sci. USA 111:6497–6502. doi:10.1073/pnas.1319955111 [PubMed: 24706854]

Claus LAN, Savatin DV, and Russinova E 2018 The crossroads of receptor-mediated signaling and endocytosis in plants. J. Integr. Plant Biol 60:827–840. doi:10.1111/jipb.12672 [PubMed: 29877613]

Coates ME, and Beynon JL 2010 *Hyaloperonospora arabidopsidis* as a pathogen model. Annu. Rev. Phytopathol 48:329–345. [PubMed: 19400636]

Conde A, Chaves MM, and Geros H 2011 Membrane transport, sensing and signaling in plant adaptation to environmental stress. Plant Cell Physiol 52:1583–1602. doi:10.1093/pcp/pcr107 [PubMed: 21828102]

Corredor-Moreno P, and Saunders DGO 2020 Expecting the unexpected: factors influencing the emergence of fungal and oomycete plant pathogens. New Phytol 225:118–125. doi:10.1111/nph.16007 [PubMed: 31225901]

Couto D, Niebergall R, Liang X, Bücherl CA, Sklenar J, Macho AP, Ntoukakis V, Derbyshire P, Altenbach D, Maclean D, Robatzek S, Uhrig J, Menke F, Zhou J-M, and Zipfel C 2016 The Arabidopsis protein phosphatase PP2C38 negatively regulates the central immune kinase BIK1. PLoS Pathog 12: e1005811. doi:10.1371/journal.ppat.1005811 [PubMed: 27494702]

Dangl JL, and Jones JDG 2019 A pentangular plant inflammasome. Science 364:31–32. doi:10.1126/science.aax0174 [PubMed: 30948541]

De Schepper V, De Swaef T, Bauweraerts I, and Steppe K 2013 Phloem transport: a review of mechanisms and controls. J. Exp. Bot 64:4839–4850. doi:10.1093/jxb/ert302 [PubMed: 24106290]

Ding Y, Sun T, Ao K, Peng Y, Zhang Y, Li X, and Zhang Y 2018 Opposite roles of salicylic acid receptors NPR1 and NPR3/NPR4 in transcriptional regulation of plant immunity. Cell 173:1454–1467. doi:10.1016/j.cell.2018.03.044 [PubMed: 29656896]

Frei dit Frey N, Garcia A, Bigeard J, Zaag R, Bueso E, Garmier M, Pateyron S, de Tauzia-Moreau M-L, Brunaud V, Balzergue S, Colcombet J, Aubourg S, Martin-Magniette M-L, and Hirt H 2014 Functional analysis of Arabidopsis immune-related MAPKs uncovers a role for MPK3 as negative regulator of inducible defences. Genome Biol 15:R87. doi:10.1186/gb-2014-15-6-r87 [PubMed: 24980080]

Dulla G, and Lindow SE 2008 Quorum size of *Pseudomonas syringae* is small and dictated by water availability on the leaf surface. Proc. Natl. Acad. Sci. USA 105:3082–3087. doi:10.1073/pnas.0711723105 [PubMed: 18287070]

Durán P, Thiergart T, Garrido-Oter R, Agler M, Kemen E, Schulze-Lefert P, and Hacquard S 2018 Microbial interkingdom interactions in roots promote Arabidopsis survival. Cell 175:973–983. e914. doi:10.1016/j.cell.2018.10.020 [PubMed: 30388454]

Durrant WE, and Dong X 2004 Systemic acquired resistance. Annu. Rev. Phytopathol 42:185–209. [PubMed: 15283665]

Duxbury Z 2016 Determining the molecular mechanism of plant disease resistance following pathogen effector perception by the resistance gene pair RPS4/RRS1 Thesis, University of East Anglia.

Eichmann R, and Schafer P 2015 Growth versus immunity-a redirection of the cell cycle? Curr. Opin. Plant Biol 26:106–112. doi:10.1016/j.pbi.2015.06.006 [PubMed: 26190589]

Evans MJ, Choi W-G, Gilroy S, and Morris RJ 2016 A ROS-assisted calcium wave dependent on the AtRBOHD NADPH oxidase and TPC1 cation channel propagates the systemic response to salt stress. Plant Physiol 171:1771–1784. doi:10.1104/pp.16.00215 [PubMed: 27261066]

Fawke S, Doumane M, and Schornack S 2015 Oomycete interactions with plants: infection strategies and resistance principles. Microbiol. Mol. Biol. Rev 79:263–280. doi:10.1128/MMBR.00010-15 [PubMed: 26041933]

Ficke A, Gadoury DM, and Seem RC 2002 Ontogenic resistance and plant disease management: a case study of grape powdery mildew. Phytopathol 92:671–675. doi:10.1094/PHYTO.2002.92.6.671

Forde BG, and Roberts MR 2014 Glutamate receptor-like channels in plants: a role as amino acid sensors in plant defence? F1000 Prime Rep 6:37.

Fu ZQ, Yan S, Saleh A, Wang W, Ruble J, Oka N, Mohan R, Spoel SH, Tada Y, Zheng N, and Dong X 2012 NPR3 and NPR4 are receptors for the immune signal salicylic acid in plants. Nature 486:228–232. doi:10.1038/nature11162 [PubMed: 22699612]

Gadoury DM, Seem RC, Ficke A, and Wilcox WF 2003 Ontogenic resistance to powdery mildew in grape berries. Phytopathol 93:547–555. doi:10.1094/PHYTO.2003.93.5.547

Gaquerel E, and Stitz M 2017 Insect resistance: an emerging molecular framework linking plant age and JA signaling. Mol. Plant 10:537–539. doi:10.1016/j.molp.2017.02.006 [PubMed: 28267597]

Geng X, Cheng J, Gangadharan A, and Mackey D 2012 The coronatine toxin of *Pseudomonas syringae* is a multifunctional suppressor of Arabidopsis defense. Plant Cell 24:4763–4774. doi:10.1105/tpc.112.105312 [PubMed: 23204405]

Geng X, Jin L, Shimada M, Kim MG, and Mackey D 2014 The phytotoxin coronatine is a multifunctional component of the virulence armament of *Pseudomonas syringae*. Planta 240:1149–1165. doi:10.1007/s00425-014-2151-x [PubMed: 25156488]

Gillmor CS, Silva-Ortega CO, Willmann MR, Buendia-Monreal M, and Poethig RS 2014 The Arabidopsis Mediator CDK8 module genes CCT (MED12) and GCT (MED13) are global regulators of developmental phase transitions. Dev 141:4580–4589. doi:10.1242/dev.111229

Gimenez-Ibanez S, Boter M, Fernandez-Barbero G, Chini A, Rathjen JP, and Solano R 2014 The bacterial effector HopX1 targets JAZ transcriptional repressors to activate jasmonate signaling and promote infection in Arabidopsis. PLoS Biol 12:e1001792. [PubMed: 24558350]

Gimenez-Ibanez S, Chini A, and Solano R 2016 How microbes twist jasmonate signaling around their little fingers. Plants (Basel) 5:9. doi:10.3390/plants5010009

Glander S, He F, Schmitz G, Witten A, Telschow A, and de Meaux J 2018 Assortment of flowering time and immunity alleles in natural *Arabidopsis thaliana* populations suggests immunity and vegetative lifespan strategies coevolve. Genome Biol. Evol 10:2278–2291. doi:10.1093/gbe/evy124 [PubMed: 30215800]

Guerra T, Schilling S, Hake K, Gorzolka K, Sylvester FP, Conrads B, Westermann B, and Romeis T 2020 Calcium-dependent protein kinase 5 links calcium signaling with N-hydroxy-l-pipecolic acid-and SARD 1-dependent immune memory in systemic acquired resistance. New Phytol 225:310–325. doi:10.1111/nph.16147 [PubMed: 31469917]

Guo J, Zeng W, Chen Q, Lee C, Chen L, Yang Y, Cang C, Ren D, and Jiang Y 2016 Structure of the voltage-gated two-pore channel TPC1 from *Arabidopsis thaliana*. Nature 531:196. doi:10.1038/nature16446 [PubMed: 26689363]

Gupta R, Lee SE, Agrawal GK, Rakwal R, Park S, Wang Y, and Kim ST 2015 Understanding the plant-pathogen interactions in the context of proteomics-generated apoplastic proteins inventory. Front. Plant Sci 6:352. doi:10.3389/fpls.2015.00352 [PubMed: 26082784]

Hacquard S, Spaepen S, Garrido-Oter R, and Schulze-Lefert P 2017 Interplay between innate immunity and the plant microbiota. Annu. Rev. Phytopathol 55: 565–589. doi:10.1146/annurev-phyto-080516-035623 [PubMed: 28645232]

Haffner E, Konietzki S, and Diederichsen E 2015 Keeping control: the role of senescence and development in plant pathogenesis and defense. Plants 4:449–488. doi:10.3390/plants4030449 [PubMed: 27135337]

Hander T, Fernández-Fernández ÁD, Kumpf RP, Willems P, Schatowitz H, Rombaut D, Staes A, Nolf J, Pottie R, Yao P, Gonçalves A, Pavie B, Boller T, Gevaert K, Van Breusegem F, Bartels S, and Stael S 2019 Damage on plants activates Ca^{2+}-dependent meta-caspases for release of

immunomodulatory peptides. Science 363:eaar7486. doi:10.1126/science.aar7486 [PubMed: 30898901]

Handley R, Ekbom B, and Ågren J 2005 Variation in trichome density and resistance against a specialist insect herbivore in natural populations of *Arabidopsis thaliana*. Ecol. Entomol 30:284–292. doi:10.1111/j.0307-6946.2005.00699.x

Hartmann M, and Zeier J 2018 l-lysine metabolism to N-hydroxypipecolic acid: an integral immune-activating pathway in plants. Plant J 96:5–21. doi:10.1111/tpj.14037 [PubMed: 30035374]

Hartmann M, Zeier T, Bernsdorff F, Reichel-Deland V, Kim D, Hohmann M, Scholten N, Schuck S, Brautigam A, Holzel T, Ganter C, and Zeier J 2018 Flavin monooxygenase-generated N-hydroxypipecolic acid is a critical element of plant systemic immunity. Cell 173:456–469. doi:10.1016/j.cell.2018.02.049 [PubMed: 29576453]

Hassani MA, Dur an P, and Hacquard S 2018 Microbial interactions within the plant holobiont. Microbiome 6:58. [PubMed: 29587885]

Heil M, and Land WG 2014 Danger signals - damaged-self recognition across the tree of life. Front. Plant Sci 5: 578. doi:10.3389/fpls.2014.00578 [PubMed: 25400647]

Henty-Ridilla JL, Li J, Day B, and Staiger CJ 2014 ACTIN DEPOLYMERIZING FACTOR4 regulates actin dynamics during innate immune signaling in Arabidopsis. Plant Cell 26:340–352. doi:10.1105/tpc.113.122499 [PubMed: 24464292]

Heo J.o., Roszak P, Furuta KM, and Helariutta Y 2014 Phloem development: current knowledge and future perspectives. Am. J. Bot 101:1393–1402. doi:10.3732/ajb.1400197 [PubMed: 25253700]

Hirakawa Y, Torii KU, and Uchida N 2017 Mechanisms and strategies shaping plant peptide hormones. Plant Cell Physiol 58:1313–1318. doi:10.1093/pcp/pcx069 [PubMed: 28961990]

Hou S, Liu Z, Shen H, and Wu D 2019 Damage-associated molecular pattern-triggered immunity in plants. Front. Plant Sci 10:646. doi:10.3389/fpls.2019.00646 [PubMed: 31191574]

Hu L, and Yang L 2019 Time to Fight: molecular mechanisms of age-related resistance. Phytopathol 109: 1500–1508.

Huang C-Y, Wang H, Hu P, Hamby R, and Jin H 2019 Small RNAs-Big Players in Plant-Microbe Interactions. Cell Host Microbe 26:173–182. doi:10.1016/j.chom.2019.07.021 [PubMed: 31415750]

Hubbard KE, and Webb AA 2015 Circadian rhythms in stomata: physiological and molecular aspects In: Rhythms in Plants Springer, pp 231–255.

Huffaker A, Pearce G, and Ryan CA 2006 An endogenous peptide signal in Arabidopsis activates components of the innate immune response. Proc. Natl. Acad. Sci. USA 103:10098–10103. doi:10.1073/pnas.0603727103 [PubMed: 16785434]

Huot B, Yao J, Montgomery BL, and He SY 2014 Growth-defense tradeoffs in plants: a balancing act to optimize fitness. Mol. Plant 7:1267–1287. doi:10.1093/mp/ssu049 [PubMed: 24777989]

Hussain A, Noman A, Khan MI, Zaynab M, Aqeel M, Anwar M, Ashraf MF, Liu Z, Raza A, Mahpara S, Bakhsh A, and He S 2019 Molecular regulation of pepper innate immunity and stress tolerance: an overview of WRKY TFs. Micro. Pathogen 135:103610. doi:10.1016/j.micpath.2019.103610

Jewell JB, Sowders JM, He R, Willis MA, Gang DR, and Tanaka K 2019 Extracellular ATP shapes a defense-related transcriptome both independently and along with other defense signaling pathways. Plant Physiol 179: 1144–1158. doi:10.1104/pp.18.01301 [PubMed: 30630869]

Jeworutzki E, Roelfsema MRG, Anschütz U, Krol E, Elzenga JTM, Felix G, Boller T, Hedrich R, and Becker D 2010 Early signaling through the Arabidopsis pattern recognition receptors FLS2 and EFR involves Ca^{2+}-associated opening of plasma membrane anion channels. Plant J 62:367–378. doi:10.1111/j.1365-313X.2010.04155.x [PubMed: 20113440]

Jiang L, Anderson JC, Besteiro MAG, and Peck SC 2017a Phosphorylation of Arabidopsis MAP kinase phosphatase 1 (MKP1) is required for PAMP responses and resistance against bacteria. Plant Physiol 175:1839–1852. doi:10.1104/pp.17.01152 [PubMed: 29070514]

Jiang L, Chen Y, Luo L, and Peck SC 2018 Central roles and regulatory mechanisms of dual-specificity MAPK phosphatases in developmental and stress signaling. Front. Plant Sci 9:1697. doi:10.3389/fpls.2018.01697 [PubMed: 30515185]

Jiang L, Wan Y, Anderson JC, Hou J, Islam SM, Cheng J, and Peck SC 2017b Genetic dissection of Arabidopsis MAP kinase phosphatase 1-dependent PAMP-induced transcriptional responses. J. Exp. Bot 68: 5207–5220. doi:10.1093/jxb/erx335 [PubMed: 29045691]

Jiang S, Yao J, Ma KW, Zhou H, Song J, He SY, and Ma W 2013 Bacterial effector activates jasmonate signaling by directly targeting JAZ transcriptional repressors. PLoS Pathog 9:e1003715. doi:10.1371/journal.ppat.1003715 [PubMed: 24204266]

Jones JD, and Dangl JL 2006 The plant immune system. Nature 444:323–329. doi:10.1038/nature05286 [PubMed: 17108957]

Jubic LM, Saile S, Furzer OJ, El Kasmi F, and Dangl JL 2019 Help wanted: helper NLRs and plant immune responses. Curr. Opin. Plant Biol 50:82–94. doi:10.1016/j.pbi.2019.03.013 [PubMed: 31063902]

Kadota Y, Shirasu K, and Zipfel C 2015 Regulation of the NADPH oxidase RBOHD during plant immunity. Plant Cell Physiol 56:1472–1480. doi:10.1093/pcp/pcv063 [PubMed: 25941234]

Kadota Y, Sklenar J, Derbyshire P, Stransfeld L, Asai S, Ntoukakis V, Jones JD, Shirasu K, Menke F, Jones A, and Zipfel C 2014 Direct regulation of the NADPH oxidase RBOHD by the PRR-associated kinase BIK1 during plant immunity. Mol. Cell 54:43–55. doi:10.1016/j.molcel.2014.02.021 [PubMed: 24630626]

Karasov TL, Chae E, Herman JJ, and Bergelson J 2017 Mechanisms to mitigate the trade-off between growth and defense. Plant Cell 29:666–680. doi:10.1105/tpc.16.00931 [PubMed: 28320784]

Kebdani N, Pieuchot L, Deleury E, Panabieres F, Le Berre JY, and Gourgues M 2010 Cellular and molecular characterization of *Phytophthora parasitica* appressorium- mediated penetration. New Phytol 185:248–257. doi:10.1111/j.1469-8137.2009.03048.x [PubMed: 19807870]

Kehr J, and Kragler F 2018 Long distance RNA movement. New Phytol 218:29–40. doi:10.1111/nph.15025 [PubMed: 29418002]

Kleemann J, Rincon-Rivera LJ, Takahara H, Neumann U, van Themaat EVL, van der Does HC, Hacquard S, Stüber K, Will I, and Schmalenbach W 2012 Sequential delivery of host-induced virulence effectors by appressoria and intracellular hyphae of the phytopathogen Colletotrichum higginsianum. PLoS Pathog 8: e1002643. [PubMed: 22496661]

Klikno J, and Kutschera U 2017 Regulation of root development in *Arabidopsis thaliana* by phytohormone-secreting epiphytic methylobacteria. Protoplasma 254: 1867–1877. doi:10.1007/s00709-016-1067-7 [PubMed: 28054231]

Kremer JM 2017 Characterization of axenic immune deficiency in *Arabidopsis thaliana*. Michigan State University.

Kremer JM, Paasch BC, Rhodes D, Thireault C, Froehlich JE, Schulze-Lefert P, Tiedje JM, and He SY 2018 FlowPot axenic plant growth system for microbiota research. bioRxiv 254953. doi:10.1101/254953

Krol E, Mentzel T, Chinchilla D, Boller T, Felix G, Kemmerling B, Postel S, Arents M, Jeworutzki E, Al-Rasheid KA, Becker D, and Hedrich R 2010 Perception of the Arabidopsis danger signal peptide 1 involves the pattern recognition receptor AtPEPR1 and its close homologue AtPEPR2. J. Biol. Chem 285: 13471–13479. doi:10.1074/jbc.M109.097394 [PubMed: 20200150]

Kus JV, Zaton K, Sarkar R, and Cameron RK 2002 Age-related resistance in Arabidopsis is a developmentally regulated defense response to *Pseudomonas syringae*. Plant Cell 14:479–490. doi:10.1105/tpc.010481 [PubMed: 11884688]

Latunde-Dada A, O'connell R, Nash C, and Lucas J 1999 Stomatal penetration of cowpea (*Vigna unguiculata*) leaves by a Colletotrichum species causing latent anthracnose. Plant Pathol 48:777–785. doi:10.1046/j.1365-3059.1999.00405.x

Lee J, and Lee I 2010 Regulation and function of SOC1, a flowering pathway integrator. J. Exp. Bot 61: 2247–2254. doi:10.1093/jxb/erq098 [PubMed: 20413527]

Lehmann S, Serrano M, L'Haridon F, Tjamos SE, and Metraux JP 2015 Reactive oxygen species and plant resistance to fungal pathogens. Phytochem 112:54–62. doi:10.1016/j.phytochem.2014.08.027

Lenzoni G, Liu J, and Knight MR, 2018 Predicting plant immunity gene expression by identifying the decoding mechanism of calcium signatures. New Phytol 217:2. 1598–1609. [PubMed: 29218709]

Leonard S, Hommais F, Nasser W, and Reverchon S 2017 Plant-phytopathogen interactions: bacterial responses to environmental and plant stimuli. Environ. Microbiol 19:1689–1716. [PubMed: 27878915]

Lewis LA, Polanski K, de Torres-Zabala M, Jayaraman S, Bowden L, Moore J, Penfold CA, Jenkins DJ, Hill C, Baxter L, Kulasekaran S, Truman W, Littlejohn G, Prusinska J, Mead A, Steinbrenner J, Hickman R, Rand D, Wild DL, Ott S, Buchanan-Wollaston V, Smirnoff N, Beynon J, Denby K, and Grant M 2015 Transcriptional dynamics driving MAMP-triggered immunity and pathogen effector-mediated immunosuppression in Arabidopsis leaves following infection with *Pseudomonas syringae* pv *tomato* DC3000. Plant Cell 27:3038–3064. doi:10.1105/tpc.15.00471 [PubMed: 26566919]

Leybaert L, and Sanderson MJ 2012 Intercellular Ca(2+) waves: mechanisms and function. Physiol Rev 92:1359–1392. doi:10.1152/physrev.00029.2011 [PubMed: 22811430]

Li B, Meng X, Shan L, and He P 2016 Transcriptional regulation of pattern-triggered immunity in plants. Cell Host Microbe 19:641–650. doi:10.1016/j.chom.2016.04.011 [PubMed: 27173932]

Li J, and Staiger CJ 2018 Understanding cytoskeletal dynamics during the plant immune response. Annu. Rev. Phytopathol 56:513–533. doi:10.1146/annurev-phyto-080516-035632 [PubMed: 29975609]

Li J, Cao L, and Staiger CJ 2017 Capping protein modulates actin remodeling in response to reactive oxygen species during plant innate immunity. Plant Physiol 173: 1125–1136. doi:10.1104/pp.16.00992 [PubMed: 27909046]

Li J, Henty-Ridilla JL, Staiger BH, Day B, and Staiger CJ 2015 Capping protein integrates multiple MAMP signalling pathways to modulate actin dynamics during plant innate immunity. Nat. Comm 6:7206.

Li P, and Day B 2019 Battlefield cytoskeleton: turning the tide on plant immunity. Mol. Plant Microbe Interact 32:25–34. doi:10.1094/MPMI-07-18-0195-FI [PubMed: 30355064]

Li Q, Wang C, and Mou Z 2020 Perception of Damaged Self in Plants. Plant Physiol 182:1545–1565. doi:10.1104/pp.19.01242 [PubMed: 31907298]

Li S, Han X, Yang L, Deng X, Wu H, Zhang M, Liu Y, Zhang S, and Xu J 2018 Mitogen-activated protein kinases and calcium-dependent protein kinases are involved in wounding-induced ethylene biosynthesis in *Arabidopsis thaliana*. Plant. Cell Environ 41:134–147. doi:10.1111/pce.12984 [PubMed: 28543054]

Liang X, Ding P, Lian K, Wang J, Ma M, Li L, Li L, Li M, Zhang X, Chen S, Zhang Y, and Zhou J-M 2016 Arabidopsis heterotrimeric G proteins regulate immunity by directly coupling to the FLS2 receptor. Elife 5:e13568. doi:10.7554/eLife.13568 [PubMed: 27043937]

Liao D, Cao Y, Sun X, Espinoza C, Nguyen CT, Liang Y, and Stacey G, 2017 Arabidopsis E3 ubiquitin ligase PLANT U-BOX13 (PUB 13) regulates chitin receptor LYSIN MOTIF RECEPTOR KINASE5 (LYK 5) protein abundance. New Phytol 214:2. 1646–1656. [PubMed: 28195333]

Linthorst HJM, and Van Loon LC 1991 Pathogenesis-related proteins of plants. Crit. Rev. Plant Sci 10: 123–150.

Liu J, Lenzoni G, and Knight MR 2020 Design principles for decoding calcium signals to generate specific gene expression via transcription. Plant Physiol 182: 1743–1761. doi:10.1104/pp.19.01003 [PubMed: 31744935]

Liu L, Sonbol F-M, Huot B, Gu Y, Withers J, Mwimba M, Yao J, and He SY, Dong DX 2016 Salicylic acid receptors activate jasmonic acid signalling through a non-canonical pathway to promote effector-triggered immunity. Nat. Comm 7:1–10.

Liu S, Kracher B, Ziegler J, Birkenbihl RP, and Somssich IE 2015 Negative regulation of ABA signaling by WRKY33 is critical for Arabidopsis immunity towards *Botrytis cinerea* 2100. Elife 4:e07295. doi:10.7554/eLife.07295 [PubMed: 26076231]

Liu Y, Du M, Deng L, Shen J, Fang M, Chen Q, Lu Y, Wang Q, Li C, and Zhai Q 2019 MYC2 regulates the termination of jasmonate signaling via an autoregulatory negative feedback loop. Plant Cell 31:106–127. doi:10.1105/tpc.18.00405 [PubMed: 30610166]

Lu D, Lin W, Gao X, Wu S, Cheng C, Avila J, Heese A, Devarenne TP, He P, and Shan L 2011 Direct ubiquitination of pattern recognition receptor FLS2 attenuates plant innate immunity. Science 332: 1439–1442. doi:10.1126/science.1204903 [PubMed: 21680842]

Lu T, Ke M, Lavoie M, Jin Y, Fan X, Zhang Z, Fu Z, Sun L, Gillings M, Penuelas J, Qian H, and Zhu YG 2018 Rhizosphere microorganisms can influence the timing of plant flowering. Microbiome 6:231. doi:10.1186/s40168-018-0615-0 [PubMed: 30587246]

Ludwig N, Löhrer M, Hempel M, Mathea S, Schliebner I, Menzel M, Kiesow A, Schaffrath U, Deising HB, and Horbach R 2014 Melanin is not required for turgor generation but enhances cell-wall rigidity in appressoria of the corn pathogen *Colletotrichum graminicola*. Mol. Plant Microbe Interact 27:315–327. doi:10.1094/MPMI-09-13-0267-R [PubMed: 24261846]

Lumbreras V, Vilela B, Irar S, Sol e M, Capellades M, Valls M, Coca M, and Pag es M 2010 MAPK phosphatase MKP2 mediates disease responses in Arabidopsis and functionally interacts with MPK3 and MPK6. Plant J 63:1017–1030. [PubMed: 20626661]

Luo X, Xu N, Huang J, Gao F, Zou H, Boudsocq M, Coaker G, and Liu J 2017 A lectin receptor-like kinase mediates pattern-triggered salicylic acid signaling. Plant Physiol 174:2501–2514. doi:10.1104/pp.17.00404 [PubMed: 28696275]

Mackey D, Belkhadir Y, Alonso JM, Ecker JR, and Dangl JL 2003 Arabidopsis RIN4 is a target of the type III virulence effector AvrRpt2 and modulates RPS2-mediated resistance. Cell 112:379–389. doi:10.1016/S0092-8674(03)00040-0 [PubMed: 12581527]

Mackey D, Holt BF, Wiig A, and Dangl JL 2002 RIN4 interacts with *Pseudomonas syringae* type III effector molecules and is required for RPM1-mediated resistance in Arabidopsis. Cell 108:743–754. doi:10.1016/S0092-8674(02)00661-X [PubMed: 11955429]

Manners JM, Penninckx IA, Vermaere K, Kazan K, Brown RL, Morgan A, Maclean DJ, Curtis MD, Cammue BP, and Broekaert WF 1998 The promoter of the plant defensin gene PDF1.2 from Arabidopsis is systemically activated by fungal pathogens and responds to methyl jasmonate but not to salicylic acid. Plant Mol. Biol 38:1071–1080. [PubMed: 9869413]

Mao G, Meng X, Liu Y, Zheng Z, Chen Z, and Zhang S 2011 Phosphorylation of a WRKY transcription factor by two pathogen-responsive MAPKs drives phytoalexin biosynthesis in Arabidopsis. Plant Cell 23:1639–1653. doi:10.1105/tpc.111.084996 [PubMed: 21498677]

Mao Y-B, Liu Y-Q, Chen D-Y, Chen F-Y, Fang X, Hong G-J, Wang L-J, and Wang J-W, Chen X-Y 2017 Jasmonate response decay and defense metabolite accumulation contributes to age-regulated dynamics of plant insect resistance. Nature Comm 8:1–13.

Marcec MJ, Gilroy S, Poovaiah B, and Tanaka K 2019 Mutual interplay of Ca2+ and ROS signaling in plant immune response. Plant Sci 283:343–354. doi:10.1016/j.plantsci.2019.03.004 [PubMed: 31128705]

Mbengue M, Bourdais G, Gervasi F, Beck M, Zhou J, Spallek T, Bartels S, Boller T, Ueda T, Kuhn H, and Robatzek S 2016 Clathrin-dependent endocytosis is required for immunity mediated by pattern recognition receptor kinases. Proc. Natl. Acad. Sci. USA 113: 11034–11039. doi:10.1073/pnas.1606004113 [PubMed: 27651493]

Mehlmer N, Wurzinger B, Stael S, Hofmann-Rodrigues D, Csaszar E, Pfister B, Bayer R, and Teige M 2010 The Ca^{2+}-dependent protein kinase CPK3 is required for MAPK-independent salt-stress acclimation in Arabidopsis. Plant J 63:484–498. doi:10.1111/j.1365-313X.2010.04257.x [PubMed: 20497378]

Melotto M, Underwood W, Koczan J, Nomura K, and He SY 2006 Plant stomata function in innate immunity against bacterial invasion. Cell 126:969–980. doi:10.1016/j.cell.2006.06.054 [PubMed: 16959575]

Melotto M, Zhang L, Oblessuc PR, and He SY 2017 Stomatal defense a decade later. Plant Physiol 174: 561–571. [PubMed: 28341769]

Meng X, and Zhang S 2013 MAPK cascades in plant disease resistance signaling. Annu. Rev. Phytopathol 51: 245–266. [PubMed: 23663002]

Miller G, Schlauch K, Tam R, Cortes D, Torres MA, Shulaev V, Dangl JL, and Mittler R 2009 The plant NADPH oxidase RBOHD mediates rapid systemic signaling in response to diverse stimuli. Sci. Signal 2: ra45–ra45. doi:10.1126/scisignal.2000448 [PubMed: 19690331]

Mine A, Berens ML, Nobori T, Anver S, Fukumoto K, Winkelmüller TM, Takeda A, Becker D, and Tsuda K 2017 Pathogen exploitation of an abscisic acid- and jasmonate-inducible MAPK phosphatase and its interception by Arabidopsis immunity. Proc. Natl. Acad. Sci. USA 114:7456–7461. doi:10.1073/pnas.1702613114 [PubMed: 28652328]

Mine A, Seyfferth C, Kracher B, Berens ML, Becker D, and Tsuda K 2018 The defense phytohormone signaling network enables rapid, high-amplitude transcriptional reprogramming during effector-triggered immunity. Plant Cell 30:1199–1219. doi:10.1105/tpc.17.00970 [PubMed: 29794063]

Mithoe SC, and Menke FL 2018 Regulation of pattern recognition receptor signalling by phosphorylation and ubiquitination. Curr. Opin. Plant Biol 45:162–170. doi:10.1016/j.pbi.2018.07.008 [PubMed: 30064038]

Monteiro F, and Nishimura MT 2018 Structural, functional, and genomic diversity of plant nlr proteins: an evolved resource for rational engineering of plant immunity. Annu. Rev. Phytopathol 56:243–267. doi:10.1146/annurev-phyto-080417-045817 [PubMed: 29949721]

Moore JW, Loake GJ, and Spoel SH 2011 Transcription dynamics in plant immunity. Plant Cell 23: 2809–2820. doi:10.1105/tpc.111.087346 [PubMed: 21841124]

Morris CE, Barny MA, Berge O, Kinkel LL, and Lacroix C 2017 Frontiers for research on the ecology of plant-pathogenic bacteria: fundamentals for sustainability: challenges in bacterial molecular plant pathology. Mol. Plant Pathol 18:308–319. doi:10.1111/mpp.12508 [PubMed: 27862839]

Müller DB, Vogel C, Bai Y, and Vorholt JA 2016 The plant microbiota: systems-level insights and perspectives. Annu. Rev. Genet 50:211–234. doi:10.1146/annurev-genet-120215-034952 [PubMed: 27648643]

Nandi D, Tahiliani P, Kumar A, and Chandu D 2006 The ubiquitin-proteasome system. J. Biosci 31:137–155. [PubMed: 16595883]

Návarová H, Bernsdorff F, Döring A-C, and Zeier J 2012 Pipecolic acid, an endogenous mediator of defense amplification and priming, is a critical regulator of inducible plant immunity. Plant Cell 24:5123–5141. doi:10.1105/tpc.112.103564 [PubMed: 23221596]

Notaguchi M, and Okamoto S 2015 Dynamics of long-distance signaling via plant vascular tissues. Front Plant Sci 6:161doi:10.3389/fpls.2015.00161 [PubMed: 25852714]

Nühse TS, Bottrill AR, Jones AM, and Peck SC 2007 Quantitative phosphoproteomic analysis of plasma membrane proteins reveals regulatory mechanisms of plant innate immune responses. Plant J 51:931–940. doi:10.1111/j.1365-313X.2007.03192.x [PubMed: 17651370]

Ortiz-Martín I, Thwaites R, Macho AP, Mansfield JW, and Beuzón CR 2010 Positive regulation of the Hrp type III secretion system in *Pseudomonas syringae* pv. *phaseolicola*. Mol. Plant Microbe Interact 23:665–681. doi:10.1094/MPMI-23-5-0665 [PubMed: 20367474]

Ortiz-Morea FA, Savatin DV, Dejonghe W, Kumar R, Luo Y, Adamowski M, Van den Begin J, Dressano K, Pereira de Oliveira G, Zhao X, Lu Q, Madder A, Friml J, Scherer de Moura D, and Russinova E 2016 Danger-associated peptide signaling in Arabidopsis requires clathrin. Proc. Natl. Acad. Sci. USA 113: 11028–11033. doi:10.1073/pnas.1605588113 [PubMed: 27651494]

Paccanaro MC, Sella L, Castiglioni C, Giacomello F, Martínez-Rocha AL, D'Ovidio R, Schäfer W, and Favaron F 2017 Synergistic effect of different plant cell wall-degrading enzymes Is important for virulence of *Fusarium graminearum*. Mol. Plant Microbe Interact 30: 886–895. doi:10.1094/MPMI-07-17-0179-R [PubMed: 28800710]

Padmanabhan MS, Ma S, Burch-Smith TM, Czymmek K, Huijser P, and Dinesh-Kumar SP 2013 Novel positive regulatory role for the SPL6 transcription factor in the N TIR-NB-LRR receptor-mediated plant innate immunity. PLoS Pathog 9:e1003235. doi:10.1371/journal.ppat.1003235 [PubMed: 23516366]

Pandey SP, Roccaro M, Schön M, Logemann E, and Somssich IE 2010 Transcriptional reprogramming regulated by WRKY18 and WRKY40 facilitates powdery mildew infection of Arabidopsis. Plant J 64:912–923. doi:10.1111/j.1365-313X.2010.04387.x [PubMed: 21143673]

Pearce G, Johnson S, and Ryan CA 1993 Structure-activity of deleted and substituted systemin, an 18-amino acid polypeptide inducer of plant defensive genes. J. Biol. Chem 268:212–216. [PubMed: 8416929]

Porter K, Shimono M, Tian M, and Day B 2012 Arabidopsis actin-depolymerizing Factor- 4 links pathogen perception, defense activation and transcription to cytoskeletal dynamics. PLoS Pathog 8:e1003006. doi:10.1371/journal.ppat.1003006 [PubMed: 23144618]

Preston JC, and Hileman L 2013 Functional evolution in the plant SQUAMOSA- PROMOTER BINDING PROTEIN-LIKE (SPL) gene family. Front. Plant Sci 4:80. doi:10.3389/fpls.2013.00080 [PubMed: 23577017]

Qi Z, Verma R, Gehring C, Yamaguchi Y, Zhao Y, Ryan CA, and Berkowitz GA 2010 Ca2 signaling by plant *Arabidopsis thaliana* Pep peptides+ depends on AtPepR1, a receptor with guanylyl cyclase activity, and cGMP-activated Ca^{2+} channels. Proc. Natl. Acad. Sci. USA 107:21193–21198. doi:10.1073/pnas.1000191107 [PubMed: 21088220]

Quiñones B, Dulla G, and Lindow SE 2005 Quorum sensing regulates exopolysaccharide production, motility, and virulence in *Pseudomonas syringae*. Mol. Plant Microbe Interact 18:682–693. doi:10.1094/MPMI-18-0682 [PubMed: 16042014]

Ranf S, Eschen-Lippold L, Pecher P, Lee J, and Scheel D 2011 Interplay between calcium signalling and early signalling elements during defence responses to microbe- or damage-associated molecular patterns. Plant J 68: 100–113. doi:10.1111/j.1365-313X.2011.04671.x [PubMed: 21668535]

Rekhter D, Ludke D, Ding Y, Feussner K, Zienkiewicz K, Lipka V, Wiermer M, Zhang Y, and Feussner I 2019 Isochorismate-derived biosynthesis of the plant stress hormone salicylic acid. Science 365:498–502. doi:10.1126/science.aaw1720 [PubMed: 31371615]

Robatzek S, Chinchilla D, and Boller T 2006 Ligand-induced endocytosis of the pattern recognition receptor FLS2 in Arabidopsis. Genes Dev 20:537–542. doi:10.1101/gad.366506 [PubMed: 16510871]

Ruan J, Zhou Y, Zhou M, Yan J, Khurshid M, Weng W, Cheng J, and Zhang K 2019 Jasmonic Acid Signaling Pathway in Plants. Int. J. Mol. Sci 20:2479.

Rusterucci C, Zhao Z, Haines K, Mellersh D, Neumann M, and Cameron R 2005 Age-related resistance to *Pseudomonas syringae* pv. tomato is associated with the transition to flowering in Arabidopsis and is effective against *Peronospora parasitica*. Physiol. Mol. Plant Pathol 66:222–231. doi:10.1016/j.pmpp.2005.08.004

Ryder LS, and Talbot NJ 2015 Regulation of appressorium development in pathogenic fungi. Curr. Opin. Plant Biol 26:8–13. doi:10.1016/j.pbi.2015.05.013 [PubMed: 26043436]

Sanabria N, Goring D, Nurnberger T, and Dubery I 2008 Self/nonself perception and recognition mechanisms in plants: a comparison of self-incompatibility and innate immunity. New Phytol 178:503–514. doi:10.1111/j.1469-8137.2008.02403.x [PubMed: 18346103]

Savatin DV, Gramegna G, Modesti V, and Cervone F 2014 Wounding in the plant tissue: the defense of a dangerous passage. Front. Plant Sci 5:470. doi:10.3389/fpls.2014.00470 [PubMed: 25278948]

Scheer JM, and Ryan CA 1999 A 160-kD systemin receptor on the surface of lycopersicon peruvianum suspension-cultured cells. Plant Cell 11:1525–1536. doi:10.1105/tpc.11.8.1525 [PubMed: 10449585]

Schuman MC, Meldau S, Gaquerel E, Diezel C, McGale E, Greenfield S, and Baldwin IT 2018 The active jasmonate JA-Ile regulates a specific subset of plant Jasmonate-Mediated Resistance to Herbivores in Nature. Front. Plant Sci 9:787doi:10.3389/fpls.2018.00787 [PubMed: 29963064]

Segonzac C, and Monaghan J 2019 Modulation of plant innate immune signaling by small peptides. Curr. Opin. Plant Biol 51:22–28. doi:10.1016/j.pbi.2019.03.007 [PubMed: 31026543]

Segonzac C, Macho AP, Sanmartín M, Ntoukakis V, S anchez-Serrano JJ, and Zipfel C 2014 Negative control of BAK1 by protein phosphatase 2A during plant innate immunity. Embo J 33:2069–2079. [PubMed: 25085430]

Serrano M, Wang B, Aryal B, Garcion C, Abou-Mansour E, Heck S, Geisler M, Mauch F, Nawrath C, and M etraux J-P 2013 Export of salicylic acid from the chloroplast requires the multidrug and toxin extrusion-like transporter EDS5. Plant Physiol 162:1815–1821. doi:10.1104/pp.113.218156 [PubMed: 23757404]

Sharma B, Joshi D, Yadav PK, Gupta AK, and Bhatt TK 2016 Role of ubiquitin-mediated degradation system in plant biology. Front. Plant Sci 7:806. doi:10.3389/fpls.2016.00806 [PubMed: 27375660]

Sharma D, and Kanneganti TD 2016 The cell biology of inflammasomes: mechanisms of inflammasome activation and regulation. J. Cell Biol 213:617–629. doi:10.1083/jcb.201602089 [PubMed: 27325789]

Sheard LB, Tan X, Mao H, Withers J, Ben-Nissan G, Hinds TR, Kobayashi Y, Hsu FF, Sharon M, Browse J, He SY, Rizo J, Howe GA, and Zheng N 2010 Jasmonate perception by inositol-

phosphate-potentiated COI1-JAZ co-receptor. Nature 468:400–405. doi:10.1038/nature09430 [PubMed: 20927106]

Shine MB, Xiao X, Kachroo P, and Kachroo A 2019 Signaling mechanisms underlying systemic acquired resistance to microbial pathogens. Plant Sci 279:81–86. doi:10.1016/j.plantsci.2018.01.001 [PubMed: 30709496]

Singh A, Sagar S, and Biswas DK 2017 Calcium dependent protein kinase, a versatile player in plant stress management and development. Crit. Rev. Plant Sci 36: 336–352. doi:10.1080/07352689.2018.1428438

Skamnioti P, and Gurr SJ 2007 *Magnaporthe grisea* Cutinase-2 mediates appressorium differentiation and host penetration and is required for full virulence. Plant Cell 19:2674–2689. doi:10.1105/tpc.107.051219 [PubMed: 17704215]

Smith JM, and Heese A 2014 Rapid bioassay to measure early reactive oxygen species production in Arabidopsis leave tissue in response to living *Pseudomonas syringae*. Plant Meth 10: 6. doi:10.1186/1746-4811-10-6

Stam R, Mantelin S, McLellan H, and Thilliez G 2014 The role of effectors in nonhost resistance to filamentous plant pathogens. Front. Plant Sci 5: 582. doi:10.3389/fpls.2014.00582 [PubMed: 25426123]

Staskawicz BJ 2001 Genetics of plant-pathogen interactions specifying plant disease resistance. Plant Physiol 125: 73–76. doi:10.1104/pp.125.1.73 [PubMed: 11154300]

Sukarta OC, Slootweg EJ, and Goverse A 2016 Structure-informed insights for NLR functioning in plant immunity. Semin. Cell Dev. Biol 56: 134–149. doi:10.1016/j.semcdb.2016.05.012 [PubMed: 27208725]

Sun T, Zhang Y, Li Y, Zhang Q, Ding Y, and Zhang Y 2015 ChIP-seq reveals broad roles of SARD1 and CBP60g in regulating plant immunity. Nat. Comm 6: 1–12.

Sun Y, Zhu Y-X, Balint-Kurti PJ, and Wang G-F 2020 Fine-tuning immunity: players and regulators for plant NLRs. Trends Plant Sci doi:10.1016/j.tplants.2020.02.008

Tang W, Gao C, Wang J, Yin Z, Zhang J, Ji J, Zhang H, Zheng X, Zhang Z, and Wang P 2018 Disruption of actin motor function due to MoMyo5 mutation impairs host penetration and pathogenicity in *Magnaporthe oryzae*. Mol. Plant Pathol 19:689–699. doi:10.1111/mpp.12554 [PubMed: 28378891]

Tang X, Xiao Y, and Zhou JM 2006 Regulation of the type III secretion system in phytopathogenic bacteria. Mol. Plant Microbe Interact 19:1159–1166. doi:10.1094/MPMI-19-1159 [PubMed: 17073299]

Teixeira PJP, Colaianni NR, Fitzpatrick CR, and Dangl JL 2019 Beyond pathogens: microbiota interactions with the plant immune system. Curr. Opin. Microbiol 49:7–17. doi:10.1016/j.mib.2019.08.003 [PubMed: 31563068]

Tena G, Boudsocq M, and Sheen J 2011 Protein kinase signaling networks in plant innate immunity. Curr. Opin. Plant Biol 14:519–529. doi:10.1016/j.pbi.2011.05.006 [PubMed: 21704551]

Thomma BP, Nürnberger T, and Joosten MH 2011 Of PAMPs and effectors: the blurred PTI-ETI dichotomy. Plant Cell 23:4–15. [PubMed: 21278123]

Tian W, Hou C, Ren Z, Wang C, Zhao F, Dahlbeck D, Hu S, Zhang L, Niu Q, Li L, Staskawicz BJ, and Luan S 2019 A calmodulin-gated calcium channel links pathogen patterns to plant immunity. Nature 572: 131–135. doi:10.1038/s41586-019-1413-y [PubMed: 31316205]

Torrens-Spence MP, Bobokalonova A, Carballo V, Glinkerman CM, Pluskal T, Shen A, and Weng J-K 2019 PBS3 and EPS1 complete salicylic acid biosynthesis from isochorismate in Arabidopsis. Mol. Plant 12: 1577–1586. doi:10.1016/j.molp.2019.11.005 [PubMed: 31760159]

Torres MA, Jones JD, and Dangl JL 2006 Reactive oxygen species signaling in response to pathogens. Plant Physiol 141:373–378. doi:10.1104/pp.106.079467 [PubMed: 16760490]

Toyota M, Spencer D, Sawai-Toyota S, Jiaqi W, Zhang T, Koo AJ, Howe GA, and Gilroy S 2018 Glutamate triggers long-distance, calcium-based plant defense signaling. Science 361:1112–1115. doi:10.1126/science.aat7744 [PubMed: 30213912]

Tsuda K, and Somssich IE 2015 Transcriptional networks in plant immunity. New Phytol 206:932–947. doi:10.1111/nph.13286 [PubMed: 25623163]

Underwood W 2012 The plant cell wall: a dynamic barrier against pathogen invasion. Front. Plant Sci 3:85. [PubMed: 22639669]

Van der Biezen EA, and Jones JD 1998 Plant disease-resistance proteins and the gene- for-gene concept. Trends Biochem. Sci 23:454–456. doi:10.1016/S0968-0004(98)01311-5 [PubMed: 9868361]

Van Ngo H, and Mostowy S 2019 Role of septins in microbial infection. J. Cell Sci 132:jcs226266. [PubMed: 31040222]

Van Vu B, Itoh K, Nguyen QB, Tosa Y, and Nakayashiki H 2012 Cellulases belonging to glycoside hydrolase families 6 and 7 contribute to the virulence of *Magnaporthe oryzae*. Mol. Plant Microbe Interact 25: 1135–1141. doi:10.1094/MPMI-02-12-0043-R [PubMed: 22852807]

Vannier N, Agler M, and Hacquard S 2019 Microbiota-mediated disease resistance in plants. PLoS Pathog 15: e1007740. doi:10.1371/journal.ppat.1007740 [PubMed: 31194849]

Verdeil JL, Alemanno L, Niemenak N, and Tranbarger TJ 2007 Pluripotent versus totipotent plant stem cells: dependence versus autonomy? Trends Plant Sci 12: 245–252. doi:10.1016/j.tplants.2007.04.002 [PubMed: 17499544]

Verma V, Ravindran P, and Kumar PP 2016 Plant hormone-mediated regulation of stress responses. BMC Plant Biol 16:86. doi:10.1186/s12870-016-0771-y [PubMed: 27079791]

Volkov AG, Lang RD, and Volkova-Gugeshashvili MI 2007 Electrical signaling in Aloe vera induced by localized thermal stress. Bioelectrochem 71:192–197.

Wang C, Zhou M, Zhang X, Yao J, Zhang Y, and Mou Z 2017 A lectin receptor kinase as a potential sensor for extracellular nicotinamide adenine dinucleotide in *Arabidopsis thaliana*. Elife 6: e25474. doi:10.7554/eLife.25474 [PubMed: 28722654]

Wang J, Grubb LE, Wang J, Liang X, Li L, Gao C, Ma M, Feng F, Li M, Li L, Zhang X, Yu F, Xie Q, Chen S, Zipfel C, Monaghan J, and Zhou J-M 2018 A regulatory module controlling homeostasis of a plant immune kinase. Mol. Cell 69:493–504. e496. doi:10.1016/j.molcel.2017.12.026 [PubMed: 29358080]

Wang J, Hu M, Wang J, Qi J, Han Z, Wang G, Qi Y, Wang HW, Zhou JM, and Chai J 2019a Reconstitution and structure of a plant NLR resistosome conferring immunity. Science 364:eaav5870. doi:10.1126/science.aav5870 [PubMed: 30948527]

Wang J, Wang J, Hu M, Wu S, Qi J, Wang G, Han Z, Qi Y, Gao N, Wang HW, Zhou JM, and Chai J 2019b Ligand-triggered allosteric ADP release primes a plant NLR complex. Science 364:eaav5868. doi:10.1126/science.aav5868 [PubMed: 30948526]

Wang L, Einig E, Almeida-Trapp M, Albert M, Fliegmann J, Mithöfer A, Kalbacher H, and Felix G 2018a The systemin receptor SYR1 enhances resistance of tomato against herbivorous insects. Nat. Plants 4: 152–156. doi:10.1038/s41477-018-0106-0 [PubMed: 29459726]

Wang L, Tsuda K, Sato M, Cohen JD, Katagiri F, and Glazebrook J 2009 Arabidopsis CaM binding protein CBP60g contributes to MAMP-induced SA accumulation and is involved in disease resistance against Pseudomonas syringae. PLoS Pathog 5:e1000301. [PubMed: 19214217]

Wang L, Tsuda K, Truman W, Sato M, Nguyen LV, Katagiri F, and Glazebrook J 2011 CBP60g and SARD1 play partially redundant critical roles in salicylic acid signaling. Plant J 67:1029–1041. doi:10.1111/j.1365-313X.2011.04655.x [PubMed: 21615571]

Wang L, Wilkins KA, and Davies JM 2018b Arabidopsis DORN1 extracellular ATP receptor; activation of plasma membrane K^+-and Ca^{2+}-permeable conductances. New Phytol 218:1301–1304. doi:10.1111/nph.15111 [PubMed: 29574778]

Wang X, Gao J, Zhu Z, Dong X, Wang X, Ren G, Zhou X, and Kuai B 2015 TCP transcription factors are critical for the coordinated regulation of isochorismate synthase 1 expression in *Arabidopsis thaliana*. Plant J 82:151–162. doi:10.1111/tpj.12803 [PubMed: 25702611]

Wei Z, Yang T, Friman V-P, Xu Y, and Shen Q, Jousset JA 2015 Trophic network architecture of root-associated bacterial communities determines pathogen invasion and plant health. Nat. Comm 6:1–9.

Wildermuth MC, Dewdney J, Wu G, and Ausubel FM 2001 Isochorismate synthase is required to synthesize salicylic acid for plant defence. Nature 414:562–565. doi:10.1038/35107108 [PubMed: 11734859]

Wilson DC, Kempthorne CJ, Carella P, Liscombe DK, and Cameron RK 2017 Age- Related Resistance in Arabidopsis thaliana Involves the MADS-Domain Transcription Factor SHORT VEGETATIVE PHASE and direct action of salicylic acid on *Pseudomonas syringae*. Mol. Plant Microbe Interact 30:919–929. doi:10.1094/MPMI-07-17-0172-R [PubMed: 28812948]

Winter CM, Austin RS, Blanvillain-Baufum e S, Reback MA, Monniaux M, Wu M-F, Sang Y, Yamaguchi A, Yamaguchi N, Parker JE, Parcy F, Jensen ST, Li H, and Wagner D 2011 LEAFY target genes reveal floral regulatory logic, cis motifs, and a link to biotic stimulus response. Dev. Cell 20:430–443. doi:10.1016/j.devcel.2011.03.019 [PubMed: 21497757]

Winter D, Vinegar B, Nahal H, Ammar R, Wilson GV, and Provart NJ 2007 An "Electronic Fluorescent Pictograph" browser for exploring and analyzing large-scale biological data sets. PLoS One 2:e718. doi:10.1371/journal.pone.0000718 [PubMed: 17684564]

Withers J, and Dong X 2017 Post-translational regulation of plant immunity. Curr. Opin. Plant Biol 38:124–132. doi:10.1016/j.pbi.2017.05.004 [PubMed: 28538164]

Withers J, Yao J, Mecey C, Howe GA, Melotto M, and He SY 2012 Transcription factor-dependent nuclear localization of a transcriptional repressor in jasmonate hormone signaling. Proc Natl Acad Sci USA 109: 20148–20153. doi:10.1073/pnas.1210054109 [PubMed: 23169619]

Wu D, Ding W, Zhang Y, Liu X, and Yang L 2015 Oleanolic acid induces the type III secretion system of *Ralstonia solanacearum*. Front. Microbiol 6:1466. doi:10.3389/fmicb.2015.01466 [PubMed: 26732647]

Wu F, Chi Y, Jiang Z, Xu Y, Xie L, Huang F, Wan D, Ni J, Yuan F, Wu X, Zhang Y, Wang L, Ye R, Byeon B, Wang W, Zhang S, Sima M, Chen S, Zhu M, Pei J, Johnson DM, Zhu S, Cao X, Pei C, Zai Z, Liu Y, Liu T, Swift GB, Zhang W, Yu M, Hu Z, Siedow JN, Chen X, and Pei Z-M 2020 Hydrogen peroxide sensor HPCA1 is an LRR receptor kinase in Arabidopsis. Nature 578:577–581. doi:10.1038/s41586-020-2032-3 [PubMed: 32076270]

Wu G, Park MY, Conway SR, Wang J-W, Weigel D, and Poethig RS 2009 The sequential action of miR156 and miR172 regulates developmental timing in Arabidopsis. Cell 138:750–759. doi:10.1016/j.cell.2009.06.031 [PubMed: 19703400]

Wu Y, Zhang D, Chu JY, Boyle P, Wang Y, Brindle ID, De Luca V, and Despr es C 2012 The Arabidopsis NPR1 protein is a receptor for the plant defense hormone salicylic acid. Cell Rep 1:639–647. doi:10.1016/j.celrep.2012.05.008 [PubMed: 22813739]

Xie K, Chen J, Wang Q, and Yang Y 2014 Direct phosphorylation and activation of a mitogen-activated protein kinase by a calcium-dependent protein kinase in rice. Plant Cell 26:3077–3089. doi:10.1105/tpc.114.126441 [PubMed: 25035404]

Xin X-F, Kvitko B, and He SY 2018 *Pseudomonas syringae*: what it takes to be a pathogen. Nat. Rev. Microbiol 16:316–328. doi:10.1038/nrmicro.2018.17 [PubMed: 29479077]

Xu X, Chen C, Fan B, and Chen Z 2006 Physical and functional interactions between pathogen-induced Arabidopsis WRKY18, WRKY40, and WRKY60 transcription factors. Plant Cell 18:1310–1326. doi:10.1105/tpc.105.037523 [PubMed: 16603654]

Xu YP, Lv LH, Xu YJ, Yang J, Cao JY, and Cai XZ 2018 Leaf stage-associated resistance is correlated with phytohormones in a pathosystem-dependent manner. J. Integr. Plant Biol 60:703–722. doi:10.1111/jipb.12661 [PubMed: 29704401]

Yamada K, Yamashita-Yamada M, Hirase T, Fujiwara T, Tsuda K, Hiruma K, and Saijo Y 2016 Danger peptide receptor signaling in plants ensures basal immunity upon pathogen-induced depletion of BAK1. Embo J 35:46–61. doi:10.15252/embj.201591807 [PubMed: 26574534]

Yamaguchi A, Wu M-F, Yang L, Wu G, Poethig RS, and Wagner D 2009 The microRNA-regulated SBP-Box transcription factor SPL3 is a direct upstream activator of LEAFY, FRUITFULL, and APETALA1. Dev. Cell 17: 268–278. doi:10.1016/j.devcel.2009.06.007 [PubMed: 19686687]

Yamaguchi Y, Huffaker A, Bryan AC, Tax FE, and Ryan CA 2010 PEPR2 is a second receptor for the Pep1 and Pep2 peptides and contributes to defense responses in Arabidopsis. Plant Cell 22:508–522. doi:10.1105/tpc.109.068874 [PubMed: 20179141]

Ye BB, Zhang K, and Wang JW 2019 The role of miR156 in rejuvenation in *Arabidopsis thaliana*. J. Int. Plant Biol doi:10.1111/jipb.12855

Yi M, and Valent B 2013 Communication between filamentous pathogens and plants at the biotrophic interface. Annu. Rev. Phytopathol 51:587–611. doi:10.1146/annurev-phyto-081211-172916 [PubMed: 23750888]

Yin H, Hong G, Li L, Zhang X, Kong Y, Sun Z, Li J, Chen J, and He Y 2019 miR156/SPL9 regulates reactive oxygen species accumulation and immune response in *Arabidopsis thaliana*. Phytopathol 109: 632–642. doi:10.1094/PHYTO-08-18-0306-R

Yuan P, Jauregui E, Du L, Tanaka K, and Poovaiah B 2017 Calcium signatures and signaling events orchestrate plant-microbe interactions. Curr. Opin. Plant Biol 38: 173–183. doi:10.1016/j.pbi.2017.06.003 [PubMed: 28692858]

Zamioudis C, Mastranesti P, Dhonukshe P, Blilou I, and Pieterse CM 2013 Unraveling root developmental programs initiated by beneficial *Pseudomonas* spp. bacteria. Plant Physiol 162:304–318. doi:10.1104/pp.112.212597 [PubMed: 23542149]

Zhang H, Zhang H, and Lin J 2020 Systemin-mediated long-distance systemic defense responses. New Phytol doi:10.1111/nph.16495

Zhang Y, Li J, Zhang W, Wang R, Qiu Q, Luo F, Hikichi Y, Ohnishi K, and Ding W 2017 Ferulic acid, but not all hydroxycinnamic acids, is a novel T3SS inducer of *Ralstonia solanacearum* and promotes its infection process in host plants under hydroponic condition. Front. Plant Sci 8:1595. doi:10.3389/fpls.2017.01595 [PubMed: 28955375]

Zheng C, Ye M, Sang M, and Wu R 2019 A regulatory network for miR156-SPL module in *Arabidopsis thaliana*. Ijms 20:6166. doi:10.3390/ijms20246166

Ziv C, Zhao Z, Gao YG, and Xia Y 2018 Multifunctional roles of plant cuticle during plant- pathogen interactions. Front. Plant Sci 9:1088. doi:10.3389/fpls.2018.01088 [PubMed: 30090108]

Zou Y, Wang S, Zhou Y, Bai J, Huang G, Liu X, Zhang Y, Tang D, and Lu D 2018 Transcriptional regulation of the immune receptor FLS2 controls the ontogeny of plant innate immunity. Plant Cell 30: 2779–2794. doi:10.1105/tpc.18.00297 [PubMed: 30337428]

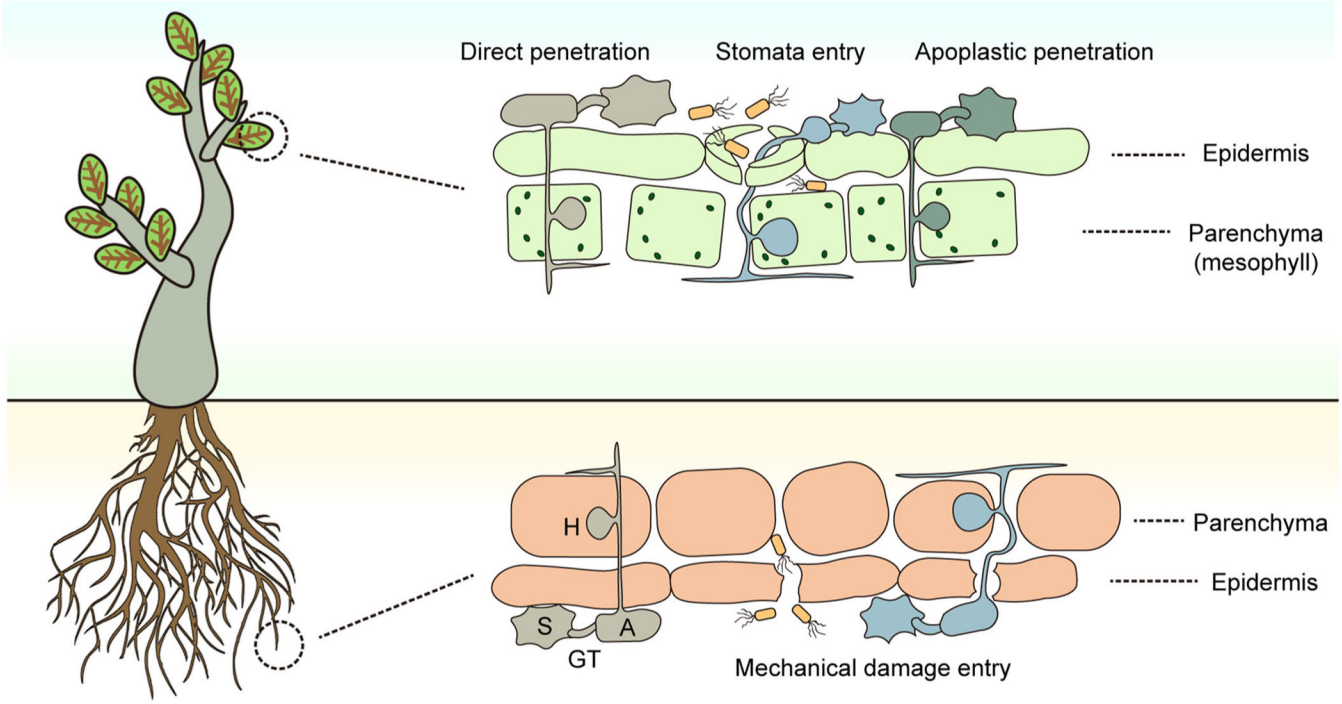

Figure 1.

Invasion strategies by phytopathogens. To promote infection, both phyllospheric and rhizospheric pathogens must overcome physical barriers on the plant surface. Filamentous pathogens typically infect their host using the appressorium to invade living cells. During infection, the germinating spore (S) forms an extended tube-like structure (i.e., germination tube, GT), which then develops into an appressoria (A) that promotes the entry into plant. Appressorium can either directly penetrate into epidermis cells by breaking through the cuticle surface and cell wall, or enter through the apoplast, the space between cells. Additionally, wounds or natural openings (i.e., stomata) on the plant surface provide easy entry into the intercellular space. Once inside the host, filamentous pathogens use a root-like structure (i.e., haustoria, H) to obtain host-derived nutrients, resulting in the establishment of the pathogen-host interface. The invasion of bacterial phytopathogens, unlike filamentous pathogen, highly depends on natural openings to enter the plant host.

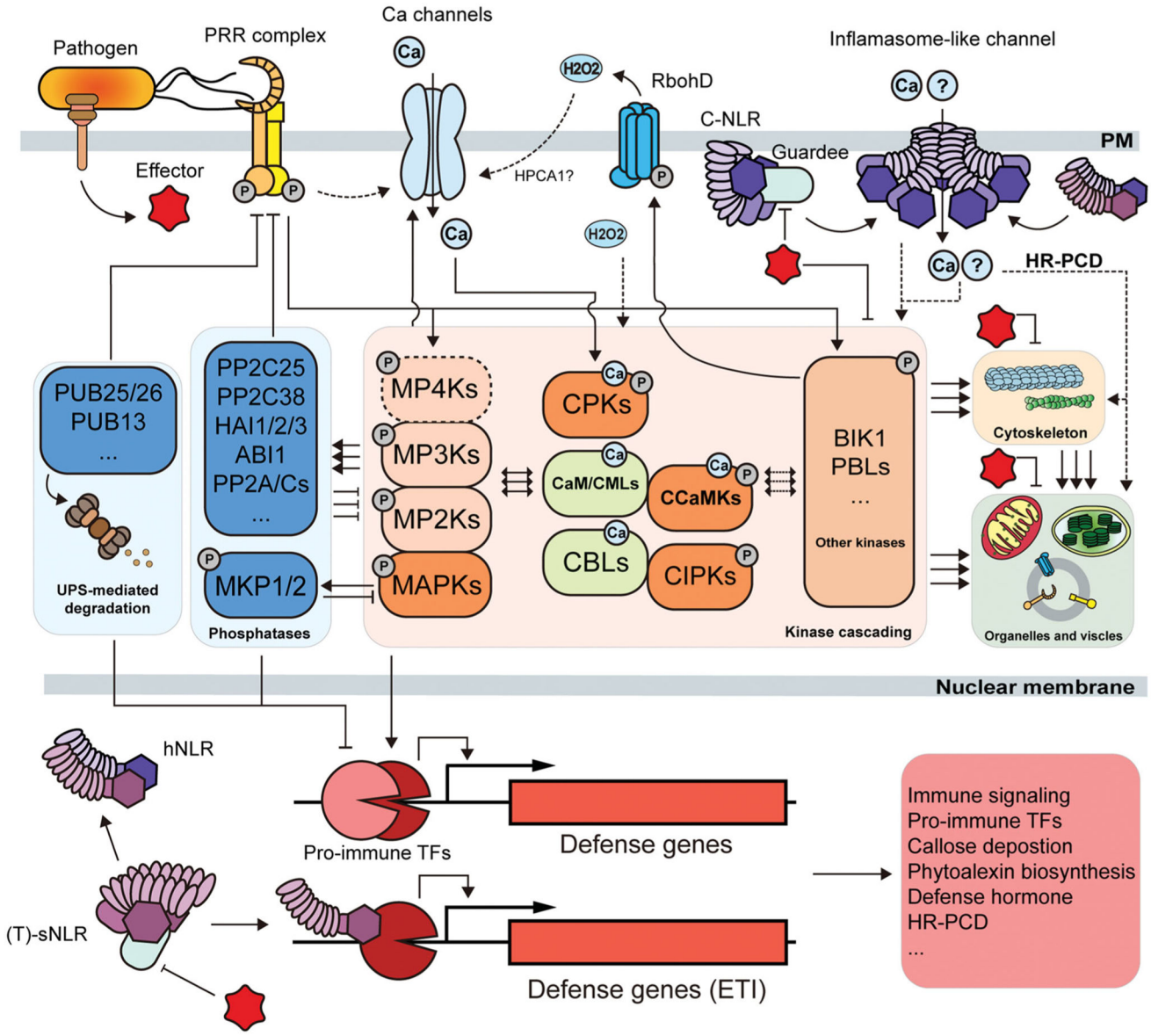

Figure 2.

A schematic map of plant local immunity. Invasive pathogens are recognized by plant PRR (pattern recognition receptor) proteins, which results in the activation of broad spectrum of downstream signaling, such as Ca^{2+} influx, the accumulation of H_2O_2 generated by RbohD (respiratory burst oxidase homolog protein D), and kinase cascading, which includes signaling pathways mediated by MAPKs, CPKs, and other additional kinases. As depicted, various kinases may also engage in a highly coordinated cross-talk during signal amplification and attenuation. These immune signals, amplified by kinase cascades, trigger a variety of defense responses, including cytoskeletal remodeling, activation of defense function in organelles, and transcriptional reprograming through the activity of pro-immune transcription factors (TF). In total, the sum of this highly coordinated signaling functions to promote plant defense signaling and pathogen resistance. Concomitant with the activation of defense signaling, the attenuation of key immune pathways occurs, a process hypothesized

to function in rebalancing of immunity and growth pathways occurs. To cope with plant immunity, pathogens have evolved mechanisms to deliver effector proteins into plant cell, which target and inhibits immune signaling, as well as to subvert immunity through targeting of critical host cellular processes. In response, plants utilize NLR (nucleotide-binding leucine-rich-repeat proteins) proteins to recognize certain effectors through sensing pathogen modification of surveilled host processes (i.e., guardee), resulting the activation of robust immune signaling and cell death (i.e., ETI; effector-triggered immunity). As a potential mechanism to activate ETI, cell membrane (PM)-associated NLRs (in most instances, possessing a coiled-coil domain, i.e, C-NLR), can form a channel-like structure following activation, which presumably functions to mobilize additional defense signaling molecules. NLRs containing a Toll/interleukin-1 receptor-like domain (T-NLRs) at the C-terminus are typically associated with a nuclear subcellular localization, and in large part, function as sensors (i.e., sNLR) that activate helper NLRs (hNLR) to form channels within the PM. As an additional hypothesized mechanism, activated nuclear NLRs may regulate specific defense genes functioning in ETI, by interacting with TFs. Dashed in indicate putative/hypothesized processes.

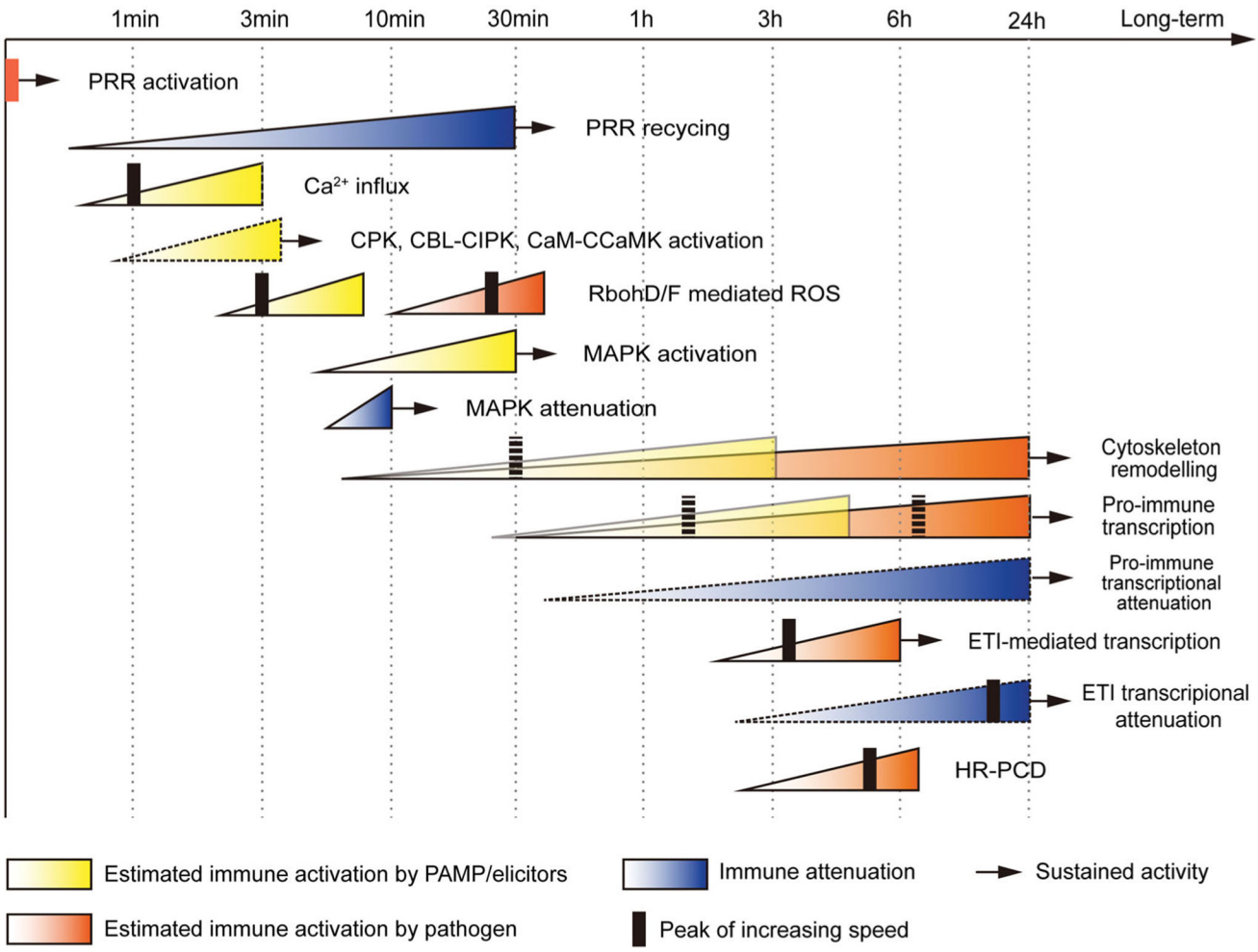

Figure 3.

Dynamics of signaling processes associated with local immunity. The signaling processes associated with local immune signaling can largely be described in a temporal fashion; for the sake of comparison, we suppose “Time 0” = PRR activation. To estimate the signaling dynamics (i.e., timing of initiation, sustained saturation, peak of increasing speed, and termination), pub lished data recording the development of immune processes following elicitor treatment or pathogen infection are collected, analyzed, and translated into this figure. Dashed lines in indicate estimation without direct evidence.

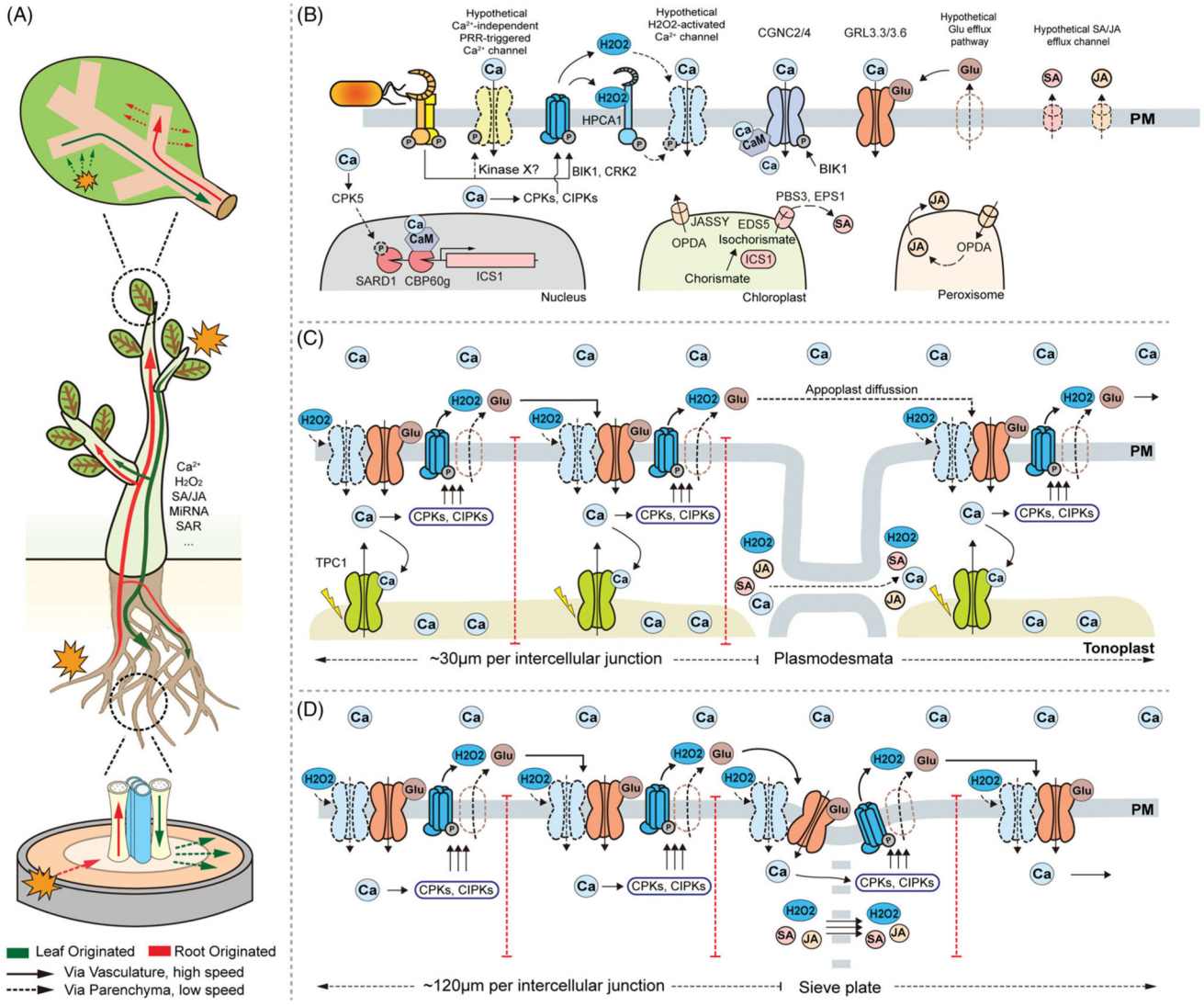

Figure 4.

The mechanism of distal immune signal transmission. (A) Biotic stresses trigger systemic immune signaling. Local immunity is induced following local pathogen perception, which results not only in the activation of local signaling, but also the induction of distal signaling within the root parenchyma and/or mesophyll. When the signal(s) reach the vasculature, signal transmission is substantially accelerated until it arrives at the site of distal parenchyma tissues in the leaf and/or root, after which time signal transmission decelerates. (B) Generation of distal signal molecules in local cells. Following immune activation, Ca^{2+} influx is initiated through an unknown Ca^{2+} channel(s) that are directly activated by PRR and/or RBOHD-synthesized H_2O_2. This initial influx activates Ca^{2+}-dependent signaling nodules as CPKs and CaM, which further activates additional Ca^{2+} channels such as CGNC2/4, rendering robust secondary Ca^{2+} influxes. Local defense response also leads to biosynthesis of immune hormones such as SA and JA, a partial of which will spread to distal tissues. (C) Transmission of distal signals in parenchyma cells (including mesophyll). Ca^{2+} influx at a given location can activate RbohD via CPKs and presumably CIPKs, a process

that results in the generation of H_2O_2 and the further activation of unknown H_2O_2-activated Ca^{2+} channels. Simultaneously, Ca^{2+} influx triggers an unknown glutamate (Glu) efflux pathway that activate glutamate-gated Ca^{2+} channel GRL3.3/3.6. Tonoplast membrane localized TPC1, a Ca^{2+} channel, gated by both Ca^{2+} and the resultant electrical potential may serve to amplify the Ca^{2+} signal. The transmission of signaling molecules is slowed at intercellular junctions as a result of the cell wall. (D) Transmission of distal signals within the vasculature. The mechanisms are the same as those in parenchyma (C), yet the gap of the intercellular junction is relieved via the action of the sieve plate, resulting in a faster speed of signal transmission.

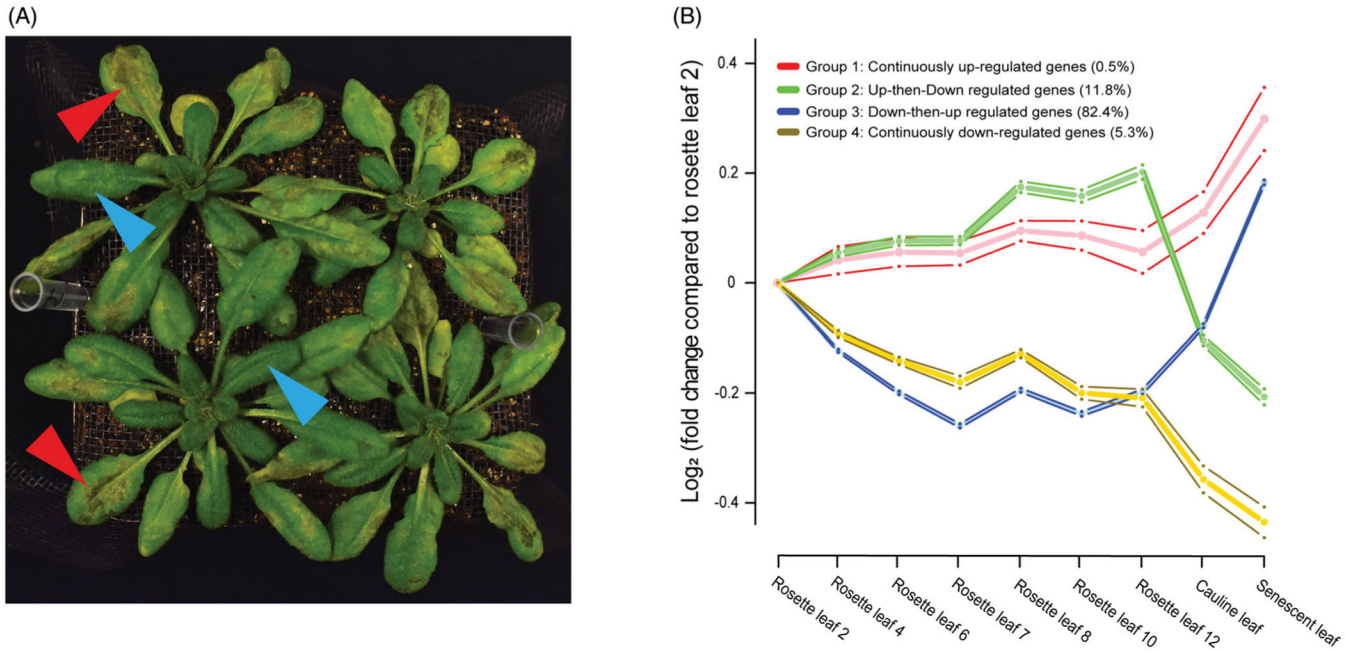

Figure 5.

Phenotypic example of age-related immunity in Arabidopsis. (A) Disease symptom varies in simultaneously inoculated rosette leaves of 5-week-old Arabidopsis Col-0 following dip-inoculation with *Pst* DC3000 (10^8 CFU/mL). While early-developed rosette leaves (red arrow) show severe disease symptoms (i.e., shrinking, chlorosis, and water-soaking), late-developed leaves (blue arrow) do not show disease symptoms in response to pathogen inoculation. (B) Immune-associated gene expression gradually changes among leaves in different development order. To illustrate this, we downloaded published RNA-microarray data (Winter *et al.*, 2007) reflecting the transcriptome of Arabidopsis rosette leaves 2, 4, 6, 7, 8, 10, 12, and both healthy and senescent cauline leaves. To screen for immune-associated genes, we selected genes within 10 key immune-associated categories: *immune, immunity, resistance, defense, biotic, chitin, fungus, flagellin, peptidoglycan*, and *bacterium*. As an output of this analysis, we identified 3901 genes with potential roles related to plant immunity. Next, we used a Pearson filter ($|r| > 0.5$ and P-value ≤ 0.05) to select and categorize genes whose expression pattern are corelated to the development order of different samples and determined 2104 immune-associated genes that can be categorized into 4 groups, with differing but significant trends, during development of Arabidopsis. The average pattern of each group of these genes is presented. Bold, tinted line: average. Thin, dark line: average ± se.

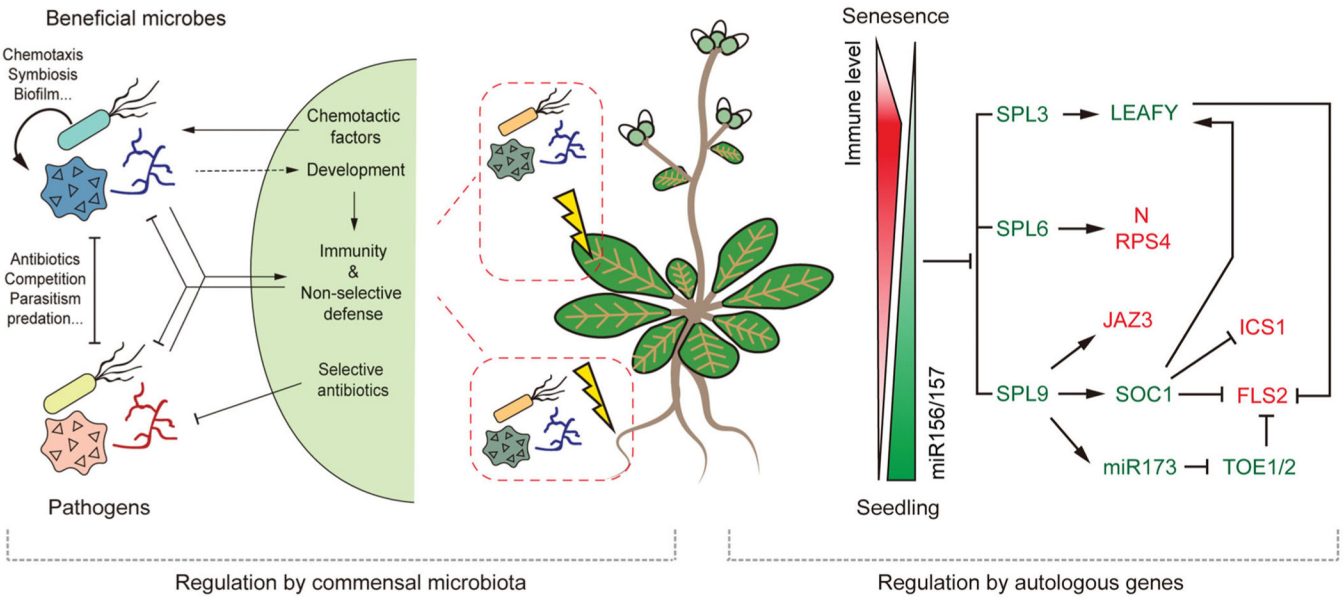

Figure 6.

The development of plant immune system is regulated by both autologous genes and commensal microbiota. Plant immune system maturation is correlated with the system development of the plant. In this process, miR156/157-SPLs plays a significant role in regulating the expression of genes functioning in immunity, including *JAZ3*, *N*, *RPS4*, *ICS*, and *FLS2*. As a central regulatory module of plant development, miR156/157-SPLs also play a key role in the synchronization of plant aging and organ morphogenesis. Additionally, the development of the plant immune system is indispensable to its commensal microbiota. While they do not necessarily cause disease, these microbes stimulate the development, maturation, and activity of the plant immune system, as well as the general development of the plant. To recruit a healthy microbiota, plants can selectively or nonselectively repel pathogens and attract beneficial microbes. Beneficial microbes can also inhibit the population growth of host associated pathogens, and as such, indirectly influence plant immunity.