

Making Sense of Hormone Crosstalk during Plant Immune Responses

Steven H. Spoel¹ and Xinnian Dong^{1,*}

¹Department of Biology, P.O. Box 90338, Duke University, Durham, NC 27708, USA

*Correspondence: xdong@duke.edu

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In response to biotic stress, crosstalk between plant hormonal signaling pathways prioritizes defense over other cellular functions. Some plant pathogens take advantage of this regulatory system by mimicking hormones that interfere with host immune responses to promote virulence. Here we discuss the various roles that crosstalk may play in response to pathogens with different infection strategies.

Introduction

Plants do not have specialized cells to carry out immune functions. Therefore, when challenged by a pathogen or an insect, plant cells undergo reprogramming to prioritize defense over their normal cellular functions. Programmed cell death at the site of invasion is a common plant defense mechanism against biotrophic pathogens and sucking insects, which rely on living host cells to provide nutrients. However, cell death is a prerequisite for the growth of necrotrophs, as these pathogens feed on dead tissue. It is therefore essential that plants activate the appropriate defense response according to the pathogen type. Salicylic acid (SA)-mediated resistance is effective against biotrophs, whereas jasmonic acid (JA)- or ethylene-mediated responses are predominantly against necrotrophs and herbivorous insects (Glazebrook, 2005). Intriguingly, some pathogens can induce multiple plant signal molecules and hormones, such as SA and JA. In such cases, crosstalk between these signaling pathways may be the mechanism that allows the plant to prioritize one response over the other. Pathogen infection also has profound effects on hormonal pathways involved in plant growth and development. As a virulence strategy, many pathogens have evolved mechanisms to tap into these hormonal signaling networks to interfere with host defense. In response, crosstalk may be used by the host as a direct defense mechanism against pathogen-triggered perturbation of hormone signaling. In this review, we will discuss these different roles of crosstalk in shaping the outcome of plant-pathogen interactions. Specifically, we will highlight recent advances made by studying the interaction between *Arabidopsis* and the bacterial pathogen *Pseudomonas syringae*.

Pathogens Employ Hormone Mimicry as a Virulence Strategy

A conspicuous feature of various pathogens is their ability to modify plant hormone signaling and hijack host hormonal crosstalk mechanisms as a virulence strategy.

Coronatine

P. syringae is a hemibiotrophic pathogen that causes a variety of diseases ranging from leaf spots to stem cankers. Some *P. syringae* strains produce a phytotoxin called coronatine (COR) (Bender et al., 1999) that structurally resembles JA derivatives, including JA-isoleucine (Staswick, 2008). COR is thought to affect JA homeostasis, as it induces cellular and physiological

changes in plants that are similar to those caused by methyl-JA (MeJA). Moreover, microarray analysis indicated a significant overlap in genes regulated by COR and MeJA (Uppalapati et al., 2005). Using COR-deficient *P. syringae* mutants and plants impaired in JA signaling, several research groups have shown that *P. syringae* employs COR to mimic JA signaling and thereby suppresses SA-mediated defense through antagonistic crosstalk (Koornneef and Pieterse, 2008).

Recently, Melotto et al. (2006) showed that COR also affects stomatal aperture. Some pathogens enter plant tissues through stomata, which are natural openings on the leaf surface. Recognition of microbe-associated molecular patterns (MAMPs) rapidly induces stomatal closure and prevents pathogen entry. This response requires components of the SA and abscisic acid (ABA) hormone signaling pathways. Wild-type *P. syringae* could inhibit the closure of stomata and rapidly induce its re-opening. In contrast, a COR-deficient *P. syringae* mutant failed to reopen the stomata, resulting in decreased virulence of this pathogen (Melotto et al., 2006). Moreover, exogenous COR application of plants strongly antagonized MAMP-induced stomatal closure. These data suggest that COR suppresses stomatal defense, allowing the pathogen to enter host tissue.

Auxin

COR also induces host gene transcription and physiological changes that are related to auxin signaling (Uppalapati et al., 2005). Auxin is an important plant hormone that affects almost all aspects of plant growth and development. Perturbing auxin homeostasis appears to be a common virulence mechanism, as many pathogens can synthesize auxin-like molecules. Loss of the ability to synthesize auxin-like molecules rendered these pathogens less virulent (Robert-Seilaniantz et al., 2007). Pathogens may also directly impact auxin biosynthesis of the host. Overexpression of the *P. syringae* effector protein AvrRpt2 in plants resulted in morphological phenotypes that are usually associated with modified auxin homeostasis (Chen et al., 2007). Indeed, AvrRpt2 overexpression promoted the biosynthesis of auxin and activated auxin-responsive gene expression. Furthermore, exogenous application of synthetic auxin to plants enhanced susceptibility to *P. syringae*, whereas mutant plants impaired in auxin signaling exhibited enhanced resistance (Chen et al., 2007; Navarro et al., 2006; Wang et al., 2007). These data strongly indicate that, like COR, auxin is involved in promoting pathogenesis.

How does auxin promote pathogen virulence? Intuitively, a growth-promoting hormone would be beneficial to biotrophic pathogens that feed on living host cells. Perhaps the best example is *Agrobacterium tumefaciens*, which uses auxin and other hormones to induce cell growth and division. This leads to the formation of galls that are “feeding factories,” providing the bacterium with a carbon and nitrogen source. An alternative mechanism by which auxin promotes virulence may be by suppressing host defense. Treatment of plants with synthetic auxin was recently demonstrated to repress SA-induced defense gene expression (Wang et al., 2007). Therefore, like COR, auxin may also promote biotroph invasion by suppression of SA-mediated defenses.

Abscisic Acid

In recent literature there is a new focus on the role of ABA in either promoting or suppressing resistance against various pathogens. For example, de Torres-Zabala et al. (2007) recently showed that *P. syringae* infection dramatically induced the biosynthesis of ABA. Moreover, genome-wide expression analysis revealed a substantial overlap between ABA- and pathogen-responsive genes. Subsequent disease tests with ABA-insensitive and ABA-hypersensitive mutants revealed enhanced resistance and susceptibility, respectively, indicating that ABA functions to promote virulence. It has been proposed that ABA suppresses the deposition of callose and lignin, both of which reinforce the cell wall to prevent pathogen invasion. Additionally, ABA inhibits the accumulation of SA and the expression of genes involved in basal resistance (de Torres-Zabala et al., 2007; Mohr and Cahill, 2007).

SA Antagonizes Pathogen-Induced Hormone Signaling through Crosstalk

While crosstalk can be exploited by pathogens to enhance virulence, its true function in plants may be to establish a new hormonal balance that favors host defense and survival in response to biotic stress. The best-studied example of hormonal crosstalk in plant defense is the interaction between SA- and JA-dependent signaling pathways. Experiments in which SA and JA are exogenously applied have shown that these hormones are antagonistic in various plant species, suggesting that it is an evolutionary conserved process. While COR is thought to mimic JA-Ile to suppress SA-dependent host defenses, plants may use SA to antagonize the inhibitory effects of COR. In wild-type plants, *P. syringae*-induced JA synthesis and signaling are rapidly repressed as SA accumulates in the plants (Spoel et al., 2003). But in SA-deficient NahG plants, *P. syringae*-induced JA synthesis and signaling are drastically increased, and the plants are hypersusceptible to infection by biotrophs. Furthermore, stomatal closure in response to *P. syringae* attack requires SA (Melotto et al., 2006), suggesting a possible role of SA in antagonizing COR in the regulation of stomatal aperture. As exogenous COR treatment suppressed MAMP-induced stomatal closure (Melotto et al., 2006), it will be interesting to assess whether the COR effect can be reversed by exogenous SA.

In *Arabidopsis*, SA-mediated suppression of JA signaling requires the regulatory protein NPR1 (Spoel et al., 2003). NPR1 may directly interfere with JA signaling or indirectly interfere through the transcriptional activation of WRKY transcription factor genes and/or glutaredoxin genes, both of which have been implicated in SA-JA crosstalk (Koornneef and Pieterse,

2008). The suppressive effects of COR on SA-mediated defense genes and stomatal closure appear to require the activity of the SCF^{COI1} ubiquitin ligase (Melotto et al., 2006; Staswick, 2008). The SCF^{COI1} complex relieves repression of JA-responsive genes by targeting repressor proteins, JAZs, for proteasome-mediated degradation. Thus, SA-activated NPR1 may suppress JA signaling by interfering with SCF^{COI1} function. Alternatively, NPR1-induced WRKY transcription factors and glutaredoxin-modified transcription factors may evade SCF^{COI1}-mediated degradation.

Besides antagonizing COR and JA signaling, SA inhibits auxin signaling during infection by *P. syringae* (Wang et al., 2007). Whole-genome transcription profiling showed that SA represses expression of many auxin-related genes, including those that encode for components of the SCF^{TIR1} ubiquitin ligase, which targets AXR transcription repressors for degradation. SA-mediated downregulation of SCF^{TIR1} dramatically stabilized AXR proteins. To demonstrate that this contributes to disease resistance, Wang et al. (2007) introduced the auxin-insensitive *axr2-1* mutation into NahG plants and found that it partially restored resistance to *P. syringae* in this SA-deficient background. These findings strongly argue that stabilization of the auxin repressor AXR2 by SA contributes significantly to SA-mediated resistance to this pathogen.

Taken together, SA plays a crucial role in establishing plant immunity against biotrophic pathogens not only by activation of antimicrobial genes, but also by antagonizing different pathogen-produced hormones or hormone mimics. It will be important to investigate if the antagonistic effect of SA is limited to only COR, JA, and auxin or also affects signaling pathways regulated by the hormones ABA, gibberellin, cytokinins, and brassinosteroids. A future challenge lies in dissecting the contribution of each activity of SA to disease resistance. Moreover, depending on the environment, crosstalk between SA and other hormones may allow plants to favor either stress responses or developmental processes.

The Effects of Crosstalk against Pathogens with Opposing Infection Strategies

While crosstalk may play an essential role in fine-tuning the plant's response to a single pathogen according to its infection strategy, it may be detrimental if the plant faces multiple pathogens with opposing infection strategies, i.e., biotrophs and necrotrophs. There are ample examples where the application of SA or SA analogs negatively affected JA-mediated resistance to necrotrophs and insects (Koornneef and Pieterse, 2008). Similarly, mutants or transgenic lines with constitutive or impaired SA signaling had reduced or enhanced resistance, respectively, to necrotrophs or insects. From an ecological perspective, however, the notion that infection by a biotroph would render plants more susceptible to a necrotroph or vice versa seems counterintuitive. Do trade-offs in resistance observed after hormonal treatment or in hormone-related mutants really occur in nature?

Spatial Regulation

Plant defense responses are often the strongest around the site of infection but taper off with increasing distance in systemic tissues. Surprisingly, few studies have assessed whether this gradient is correlated with the incidence of resistance trade-offs. Although studies of the effect of pathogen infection on insect

resistance have yielded conflicting results, i.e., enhanced versus reduced insect resistance, the local effects were generally much stronger than the systemic effects (Stout et al., 2006). This is in accordance with the finding that the hemibiotroph *P. syringae* suppressed JA-mediated resistance to the necrotrophic fungus *Alternaria brassicicola* in neighboring tissues, but not in systemic tissues (Spoel et al., 2007). Importantly, this correlated with strong SA-mediated suppression of JA signaling in local tissues, but weak SA-JA antagonism in systemic tissues (Spoel et al., 2007). Together, these studies suggest that low levels of antagonistic crosstalk in systemic tissues may not translate into a resistance trade-off. Resistance trade-offs may therefore require a threshold level of one hormone relative to another. This is supported by the observation that SA and JA acted synergistically when applied to the plant in low concentrations, whereas a high concentration of one hormone antagonized the other (Mur et al., 2005). Using spatial information to regulate the threshold of hormone crosstalk may therefore be a cost-efficient way of preventing undesirable resistance trade-offs (Figure 1).

Temporal Regulation

The threshold model can explain not only the spatial regulation of crosstalk but also the temporal effect of this response. Upon pathogen infection, the biosynthesis of various defense hormones is transiently induced. Thus, the time between invasion by the primary and secondary aggressors may determine whether a trade-off occurs. This is supported by the finding that activation of SA signaling suppressed JA-induced resistance against the herbivorous beet army worm *Spodoptera exigua* (Thaler et al., 2002), which was readily observed when SA and JA signaling pathways were activated simultaneously. In contrast, by temporally separating the activation of each pathway, antagonism between SA and JA was largely abolished. Temporal regulation of antagonistic crosstalk is therefore another key determinant for resistance trade-offs (Figure 1).

Pathogen-Type Effects

The specificity of a plant-pathogen interaction may also affect trade-offs. Biotrophic pathogens can produce effectors to suppress host defense responses and promote disease. Consequently, plants have evolved resistance (R) proteins to recognize the presence of effectors, turning them into avirulence signals for rapid activation of a plant defense response known as the hypersensitive response (HR). The HR is characterized by rapid programmed cell death of infected cells and strong activation of SA signaling. This response is extremely successful in combating biotrophic pathogens (Glazebrook, 2005). However, induction of programmed cell death may be detrimental when necrotrophic pathogens or insects are present. Studies in which the effect of the HR on insect resistance was examined have yielded inconsistent results (Stout et al., 2006). In some studies, the HR increased insect resistance, and in others it decreased insect resistance. There are also examples in which trade-offs were completely absent. Unfortunately, it is difficult to compare the results from these studies as the influences of pathogen- and insect-types were not clearly separated from spatial and temporal effects.

The effect of the HR on necrotrophic pathogen resistance has only been tested with *A. brassicicola*. Surprisingly, infection of *Arabidopsis* with avirulent strains of *P. syringae* had no effect on resistance against *A. brassicicola*, even in the neighboring tissues (Spoel et al., 2007). This was in contrast to the observation

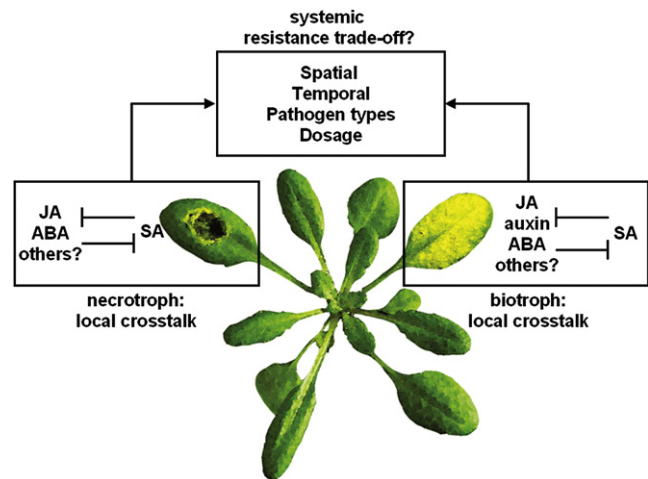


Figure 1. Model of Resistance Trade-Offs between Plant Defenses against Pathogens with Opposing Infection Strategies

This drawing shows a plant simultaneously invaded by two pathogens with opposing infection strategies (biotroph and necrotroph). Hormone crosstalk that occurs at one pathogen infection site may cause resistance trade-offs at the second site of infection. Spatial separation, timing, pathogen types, and dosage are all important factors that determine if a trade-off in resistance occurs. “Others” include the hormones gibberellin, brassinosteroids, and cytokinins.

that, in the absence of the avirulence signals, infection by the same bacterium caused enhanced susceptibility to *A. brassicicola*. Although both avirulent and virulent *P. syringae* strains are able to induce synthesis of SA to high levels, an active mechanism must be triggered during the HR to prevent SA-mediated crosstalk inhibition of resistance to *A. brassicicola*. The molecular basis for this lack of crosstalk remains unclear. It is plausible that this mechanism is in place to prevent necrotrophs from hijacking the HR (Govrin and Levine, 2000; Spoel et al., 2007). It is important to assess if similar results are found using other biotroph-necrotroph combinations, as different R-mediated responses may activate distinct signaling pathways.

Future Perspectives

Insight into hormone crosstalk is essential for our understanding of plant immune responses and for designing effective strategies of engineering disease resistance in crops. The mechanisms of hormone crosstalk deserve more in-depth investigation, as they are the ultimate regulatory steps that fine-tune the plant’s response to external stress. Advances in our understanding of individual hormone signaling pathways have laid the foundation for studying the crosstalk between them. The development of new approaches in systems biology may now greatly facilitate research of crosstalk mechanisms by allowing holistic views of the entire signaling network. This is especially important as studying crosstalk and associated resistance trade-offs requires knowledge of all the signals produced by the pathogen and the host that affect hormonal homeostasis. For example, infection of *Arabidopsis* by *P. syringae* pv. *tomato* DC3000 did not affect resistance against the herbivorous insect *Trichoplusia ni*, suggesting that trade-off was absent. However, when a COR-deficient mutant of this *P. syringae* strain was used, a significant increase in susceptibility to this insect was observed (Cui et al., 2005). Moreover, treatment of plants with purified COR induced

resistance against *T. ni*. These findings suggest that COR can directly influence resistance against this insect and possibly mask a resistance trade-off.

Plants appear to possess several regulatory mechanisms that together determine if hormone crosstalk results in a resistance trade-off against pathogens with opposing infection strategies. Hormone crosstalk that occurs at one pathogen infection site may or may not cause resistance trade-offs at a second site of infection by another pathogen (Figure 1). Spatial separation and timing between the primary and secondary pathogen challenges appear to be important factors. Dosage of the pathogens may also influence the outcome. Trade-off experiments are often performed with an unnaturally high inoculum. This is likely to result in hormone concentrations in local and systemic tissues that far exceed those observed in the field. Moreover, infection by one pathogen may result in an overall fitness reduction of the host plant, resulting in increased susceptibility to other diseases and pests. All these factors may lead to the observation of a resistance trade-off in the laboratory that is not found in the field or vice versa.

The use of controlled field experiments will be instrumental in understanding the physiological consequences of hormone crosstalk. Because plants and their aggressors have coevolved, a case-by-case assessment may be required to fully disclose the complexity of resistance trade-offs. At the same time, laboratory experiments will be critical in elucidating the molecular mechanisms that activate and restrict hormonal crosstalk. By combining laboratory and controlled field experiments, we may finally make sense of crosstalk during plant immune responses.

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