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Signal transduction in the plant immune response

John M. McDowell and Jeffery L. Dangl

Complementary biochemical and genetic approaches are being used to dissect the signaling network that regulates the innate immune response in plants. Receptor-mediated recognition of invading pathogens triggers a signal amplification loop that is based on synergistic interactions between nitric oxide, reactive oxygen intermediates and salicylic acid. Alternative resistance mechanisms in *Arabidopsis* are deployed against different types of pathogens; these mechanisms are mediated by either salicylic acid or the growth regulators jasmonic acid and ethylene.

PLANTS ARE EXPLOITED as a source of food and shelter by a wide range of parasites, including viruses, bacteria, fungi, nematodes, insects and even other plants. Plants have responded to this pressure by evolving mechanisms to recognize and counterattack prospective colonists. If a plant detects an invasion, then a set of inducible defense responses is deployed; these include programmed cell death (referred to as the hypersensitive response or HR), tissue reinforcement at the infection site, production of anti-microbial metabolites and induction of 'defense-associated' gene expression[1]. Activation of 'local' responses at the point of infection can be followed by establishment of secondary immunity throughout the plant ('systemic' acquired resistance or SAR), which is long lasting and effective against a broad spectrum of pathogens[2].

Activation of inducible defenses is contingent upon recognition of an invasion. Surveillance in the plant is the collective duty of a complex array of constitutively expressed *R* genes (for resistance). Individual *R* genes have narrow recognition capabilities and they trigger resistance only when the invading pathogen expresses a corresponding '*Avr* gene' (for avirulence). These genetic attributes have inspired a molecular model in which *Avr*-encoded proteins are delivered to the plant cell to facilitate invasion and there, they are recognized by the corresponding R protein as a signal that invaders have arrived. Studies arising from molecular cloning of numerous *Avr* and *R* genes have at least partially validated this 'receptor-elicitor' model. These efforts have been summarized in several excellent reviews, including a recent article in *TiBS*[3].

This review focuses on the molecular events that occur 'downstream' of pathogen recognition. For inducible defenses to be effective, they must be deployed rapidly; the ability of pathogens to outpace a tardy counterattack is well documented. On the other hand, these defenses cannot be unleashed with impunity, as they are resource-intensive and can inflict substantial collateral damage on host tissues. Thus, deployment must be confined to the proper place and time. These requirements suggest that a complex, highly integrated regulatory network controls defense responses. With these considerations in mind, we highlight recent insights into the mediatory signals between the initial recognition event and ultimate activation of defense responses.

Ion fluxes and reactive oxygen intermediates are the first messengers

Much effort has been devoted to identification of the earliest responses to pathogen invasion. These studies typically employ biochemical and physiological analysis of cultured cells or intact plants that have been challenged with pathogens or purified elicitors. The earliest detectable cellular events are ion fluxes across the plasma membrane and a burst of oxygen metabolism that produces reactive oxygen intermediates (ROIs), such as superoxide (O_2^-) and hydrogen peroxide (H_2O_2)[4]. A clear causal link between these events and defense induction was demonstrated in cultured cells of parsley, in which elicitor-induced ion fluxes are required for induction of the oxidative burst, but not vice versa[5] (Fig. 1). The oxidative burst is in turn required for activation of defense gene induction and production of antimicrobial metabolites.

Thus, receptor-dependent pathogen recognition triggers ion channel fluxes, which subsequently induce ROI production (Fig. 1). The molecules that connect these events have yet to be defined. Inhibitor studies suggest that receptor activation is linked to ion channel stimulation and ROI production by mechanisms involving protein phosphorylation and GTP-binding proteins[4], but genetic requirements for these steps have not been demonstrated. A recently identified pathogen-responsive calmodulin isoform[6]

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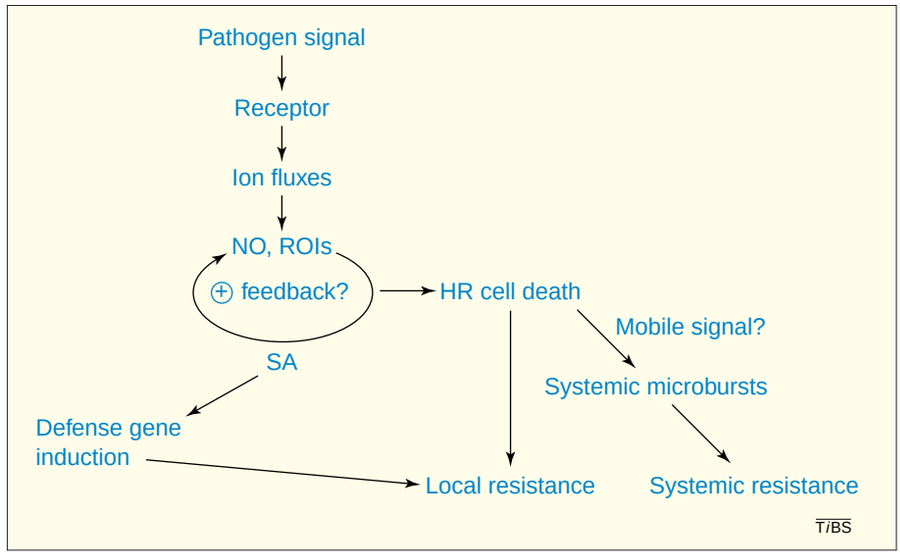

Figure 1

Regulation of local and systemic defense responses by ion fluxes, reactive oxygen intermediates (ROIs), nitric oxide (NO) and salicylic acid (SA). Receptor-mediated ion fluxes trigger localized production of NO and ROIs immediately after pathogen recognition. These second messengers synergistically induce cell death, defense gene expression, and production of SA and more ROIs, establishing a putative feedback loop in which the response is amplified. Defense responses in distal parts of the plant might be triggered by a similar mechanism, involving an unknown mobile signal.

might represent a downstream target of ion fluxes. Furthermore, several pathogen-responsive MAP kinase isoforms have been identified recently. In one case, the MAP kinase translocates to the nucleus after pathogen challenge, and activation and translocation occur independently, or upstream, of the oxidative burst[7]. Multiple pathogens as well as wounding and tissue damage activate a second MAP kinase[8]. Again, this activation is independent or upstream of the oxidative burst, suggesting a mechanism for pathogen-induced, but ROI-independent, transcriptional activation.

The role of ROIs in the defense response is particularly interesting and controversial at the moment. ROIs have been associated with apoptosis of mammalian cells, indicating a role in cell death during the HR in plants[9]. This analogy is supported by identification of a plant equivalent to the mammalian NADPH oxidase complex that produces ROIs in neutrophils. Plant homologs of two NADPH oxidase components ($gp91^{phox}$ and Rac) have been cloned. The phenotypes of transgenic plants expressing constitutively active or dominant negative variants of Rac indicate that it is a regulator of cell death in plants[10]. The plant $gp91^{phox}$ homologs contain Ca^{2+}-binding motifs that are not found in the mammalian homolog and could provide a regulatory connection to pathogen-induced ion fluxes[11]. However, no direct evidence exists currently to associate this protein with defense induction. Plant $gp91^{phox}$ homologs are encoded by a multi-gene family and might thus be functionally specialized or redundant, or both[12].

A requirement for NADPH oxidase in plant cell death is further supported by observations that pharmacological inhibitors of the NADPH oxidase complex can interfere with induction of the HR[9]. However, alternative sources of ROIs have been postulated in several studies, and the relative contributions of various oxidases must be clarified[13]. A more vexing question has arisen from reports that exogenously supplied ROIs are insufficient to activate HR cell death in some cell systems[5]. Although necessary, ROIs are not sufficient to trigger cell death and must therefore require accomplices.

Salicylic acid and nitric oxide potentiate ROI-dependent responses

Two putative accomplices have been identified in recent investigations of interactions between salicylic acid (SA), nitric oxide (NO) and ROIs. SA has long been associated with defense induction in plants: exogenous SA is sufficient to induce plant defense gene expression and systemic acquired resistance (but not cell death), whereas transgenic plants expressing an SA-degrading enzyme from bacteria (NahG) are unable to activate local or systemic defense responses[2]. NO collaborates with ROIs to trigger cell death in the mammalian immune response[14], and three recent studies provide evidence that NO also interacts with ROIs and SA in plants to induce the HR and defense gene expression. First, Shirasu *et al.* demonstrated that addition of exogenous SA 'primes'-cultured soybean cells to initiate cell death in response to pathogen challenge[15]. This potentiation occurs through an undefined, phosphorylation-dependent agonist that does not require translation. In this system, SA in combination with cantharidin (a protein phosphotase inhibitor) is sufficient to trigger ROI production in the absence of pathogens, but this oxidative burst is not sufficient to trigger cell death. However, a subsequent study demonstrated that exogenous NO in combination with SA plus cantharidin (or exogenous ROIs) is sufficient to induce cell death without pathogen challenge[16]. An independent study in intact tobacco plants demonstrated that exogenous NO triggers expression of pathogen-responsive genes, and that this gene induction is dependent on SA accumulation[17].

The physiological relevance of responses to exogenous NO was supported by indirect evidence in both studies for accumulation of endogenous NO in response to pathogen challenge. The effects of both exogenous and endogenous NO were counteracted by simultaneous addition of NO scavengers or pharmacological inhibitors of mammalian nitric oxide synthase (NOS). More significantly, induction of endogenous NO was dependent upon recognition of the challenging pathogen: NO accumulation does not occur in disease-susceptible tobacco lines[17] or in soybean cells that are challenged with an isogenic pathogen that does not express the appropriate *avr* gene[16]. This correlation provides strong, although still circumstantial, evidence for a link between NO and defense activation, and genetic confirmation of NOS involvement is awaited eagerly.

A model for signal amplification and propagation

How might these three small molecules interact to promote defense induction? An important observation in the above studies is that the contributions of NO, SA and H_2O_2 appear to be synergistic rather than additive, implying that they interact directly and cooperatively in a signal-amplification mechanism. Furthermore,

ROIs and NO stimulate SA biosynthesis, and SA in turn potentiates ROI–NO-dependent responses, as described above. These features suggest that receptor-dependent pathogen perception triggers a positive feedback loop of ROI–NO production and SA accumulation, which rapidly amplifies the initial signal and guarantees timely defense activation (Fig. 1). Culmination of this cycle in HR cell death could release ROIs, NO and SA into intercellular spaces, and these compounds could directly inhibit pathogen growth or 'warn' neighboring cells of an imminent invasion, or both.

A spatial extension of this scenario was provided in a report claiming that localized production of ROIs is sufficient to trigger SAR, and that establishment of SAR was correlated with oxidative 'microbursts' in distal tissues[18]. The implication is that systemic resistance can be induced by subsequent reiterations of the ROI–SA–cell-death cycle at lower amplitude throughout the plant. However, it is currently unclear whether ROIs themselves are the 'mobile signal' that induces distal microbursts and SAR. Other candidates for the mobile signal include SA (although the weight of available evidence argues against this) and nitrosylated glutathione[19].

The above model makes no predictions about the biochemical roles of SA, ROIs or NO. SA can bind to catalase (an H_2O_2-degrading enzyme) and other heme-containing enzymes *in vitro*, but the relevance of this capability *in planta* has not been demonstrated conclusively[20]. The literature on mammalian species provides an almost overwhelming number of candidate targets for NO and ROIs[14]. Perhaps the most intuitive scenario is that ROIs and NO interact to form cytotoxic radicals that could directly kill host or pathogen cells, or both. Alternatively, but not exclusively, ROIs and NO could serve as second messengers through redox-mediated alterations of downstream regulators. For example, an SA- and pathogen-inducible plant homolog of cyclooxygenase has been reported[21]. This enzyme is a prominent target of NO in mammalian cells and catalyses the production of lipid signal intermediates. Guanylate cyclase is a second target of NO, and Durner *et al.* provide evidence that cyclic GMP is an intermediate in defense-gene induction[17]. Durner *et al.* also demonstrate that defense-gene expression is induced by cyclic ADP ribose, which transduces NO-dependent signals in mammalian systems through modulation of ion channel activity. The obvious multiplicity of NO targets implies that dissection of NO signaling will be a challenging task requiring physiological and genetic approaches.

Another question not addressed in this model is the repression of defense responses. The apparent reiterative amplification of input signals that can kill plant cells implies the existence of key regulators that limit the propagation of the HR. SA can inhibit NOS in mammalian cells, suggesting a potential mechanism for self-limitation of the HR (Ref. 19). Similarly, H_2O_2 induces the expression of antioxidant enzymes in cells adjacent to the HR site[9]. Defense responses could also be under negative genetic control, as suggested by recessive mutants in which cell death or other defense responses are activated spontaneously. For example, the *lsd1* mutation renders the plant hypersensitive to cell-death-inducing stimuli, such as pathogens, SA or exogenous superoxide[22]. A particularly interesting aspect of this mutant is that cell death lesions, once initiated, can spread to engulf the entire leaf. This suggests that the wild-type gene somehow delimits the area of the HR. The *LSD1* gene encodes a zinc-finger protein, which raises the possibility that cell death is a default pathway under negative transcriptional regulation[23]. If true, this regulatory configuration might have arisen to meet a need for rapid defense activation. However, a critical need for future exploration clearly exists in this area.

SA-independent defense responses are triggered by distinct pathogens

The ROI–SA–cell-death response, described above, has received the majority of recent experimental attention. However, this response is not germane to every plant–pathogen interaction. For example, recent genetic studies in *Arabidopsis* have revealed resistance responses that operate independently of SA accumulation and are mediated by jasmonic acid (JA) and the gaseous hormone ethylene (ET)[24]. JA and ET are also plant growth regulators, suggesting overlap between the regulatory components of development and defense. *Arabidopsis* mutants, compromised in their ability to respond to JA or to produce SA, have been used elegantly to demonstrate that the SA-dependent and ET–JA-dependent responses are utilized differentially against pathogens with contrasting modes of attack[25] (Fig. 2). The ET–JA-dependent defense response is activated by pathogens that kill plant cells to obtain nutrients. In contrast, the SA-dependent response is triggered by a pathogen that obtains nutrients from living plant tissue. This observation raises the intriguing possibility (yet to be generalized) that plants can activate distinct defense responses tailored to specific types of parasites. Several studies have also suggested that the ET–JA and SA responses are mutually inhibitory[24]. Such potential cross talk is again suggestive of a capacity for selective defense deployment. It will be of great interest to define and compare the executioners in SA- and ET–JA-dependent responses, and to determine whether both responses are triggered by the same perception or early-response components.

Why such complexity?

A clear theme in this review is that simple linear pathways are insufficient to explain the regulation of inducible defenses in plants. Indeed, the branched models that we have presented in this review will soon be obsolete, as more regulatory components are defined. This apparent complexity probably reflects a requirement for tight regulation of an important, but costly response. Regulatory complexity might also have

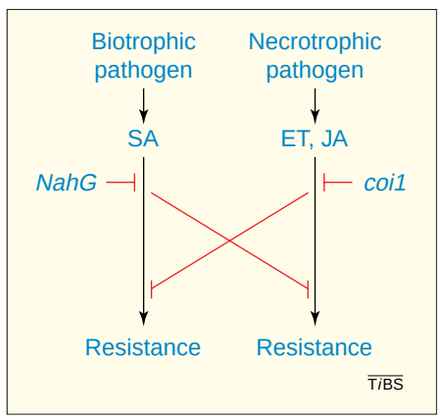

Figure 2
Genetically defined relationships between salicylic acid (SA)-dependent and ethylene–jasmonic acid (ET–JA)-dependent defense responses in *Arabidopsis*. The SA-dependent response is deployed against a biotrophic pathogen that obtains nutrients from living cells, whereas the ET–JA response is activated by necrotrophic pathogens that kill plant tissue. Resistance to necrotrophs (but not the biotroph) is compromised in an *Arabidopsis* mutant (*coi1*) that does not respond to JA. Expression of a transgene (*NahG*) that degrades SA compromises resistance to the biotroph but not the necrotrophs. These pathways appear to be mutually inhibitory.

arisen, in part, as a consequence of co-evolution between plants and pathogens. It is tempting to speculate that pathogens have evolved mechanisms to suppress defense responses by interfering with key downstream regulators, thereby forcing the plant in turn to evolve bypass mechanisms. In fact, some pathogens might have learned to turn defense responses to their own nefarious ends. For example, induction of the ROI–SA–cell-death response could work to the advantage of pathogens that live by killing host cells and perhaps, the plant has evolved the ET–JA response (with a concomitant repression of the SA response) to avoid turning the gun on itself unnecessarily. If these scenarios are valid, then a thorough comparison of defense response circuitry in several plant species could provide valuable insight into the molecular mechanisms of response regulation and the evolutionary mechanisms by which alternate regulatory configurations could have arisen.

Agricultural applications

A final and perhaps most significant payoff from endeavors in this area could come in the form of strategies for engineering resistance in crops. Several promising approaches have already been initiated from our relatively limited knowledge base. For example, an analog of SA is being marketed as an alternative to costly and environmentally harmful fungicides[26]. Cross-species transgenic approaches might also prove useful, as overexpression of the SAR regulatory gene *NPR1* in *Arabidopsis* confers resistance to two different pathogens without a discernible growth penalty to the plant[27]. Finally, Molina and co-workers demonstrate that one commonly used fungicide induces host defense responses[28], suggesting that already are we unwittingly manipulating plant defense responses to our own ends.

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