New Weapons and a Rapid Response against Insect Attack[1]

John Browse and Gregg A. Howe*

Institute of Biological Chemistry, Washington State University, Pullman, Washington 99164–6340 (J.B.); and U.S. Department of Energy Plant Research Laboratory, Department of Biochemistry and Molecular Biology, Michigan State University, East Lansing, Michigan 48824–1312 (G.A.H.)

Jasmonates (JAs) comprise a class of related oxylipin signaling molecules that have overlapping roles in regulating both stress responses and development in plants. Stress responses that depend on JA signaling include not only defense against insects but also defense against microbial pathogens, as well as responses to UV radiation, ozone, and other abiotic stresses (Glazebrook, 2005; Wasternack, 2007; Balbi and Devoto, 2008; Howe and Jander, 2008). In healthy, nonstressed plant tissue, JAs are involved in carbon partitioning, mechanotransduction, senescence, and reproductive development (Browse, 2005). The JA-dependent responses are associated with large-scale reprogramming of gene expression; hundreds of downstream JA-regulated and JA-coregulated genes have been identified (Reymond et al., 2000; Schenk et al., 2000; Devoto et al., 2005; Mandaokar et al., 2006).

A substantial proportion of our knowledge about the synthesis and function of JAs has come from studies of plants that are defective in the biosynthesis of the hormone. These include mutants blocked in synthesis of linolenic acid (18:3), the fatty acid precursor of JA (McConn and Browse, 1996; Li et al., 2003) and mutants deficient in enzymes of the JA synthesis pathway (Schaller et al., 2005). These mutants have been particularly useful for investigating the role of JA in regulating the outcome of plant-insect interactions because application of exogenous JA typically restores wild-type function (McConn et al., 1997; Halitschke and Baldwin, 2003; Schilmiller et al., 2007). Other mutants are defective in JA perception and have also been instrumental for the study of induced resistance to herbivory (Stintzi et al., 2001; Li et al., 2004; Reymond et al., 2004; Chen et al., 2005; Mewis et al., 2005; Paschold et al., 2007). The *coi1* mutant of Arabidopsis (*Arabidopsis thaliana*) was isolated on the basis of its resistance to the phytotoxin coronatine (Fig. 1), which induces many of the same responses as JA in plants (Feys et al., 1994). The contributions of *coi1* and other mutants to understanding the mechanism of JA signaling are discussed below.

The ability of methyl-JA (MeJA) to induce anti-insect proteinase inhibitors (PIs) in tomato (*Solanum lycopersicum*) was first reported in 1990 (Farmer and Ryan, 1990); articles describing the induction of some pathogen-defense genes followed (Glazebrook, 2005). The critical requirement for JA to induce defense against insects was established through investigations of JA synthesis mutants in tomato (Howe et al., 1996; Li et al., 2005) and Arabidopsis (McConn et al., 1997). Similarly, mutant analysis was central to establishing the importance of JA in defense against pathogens—particularly necrotrophic fungi and oomycetes (Vijayan et al., 1998; Glazebrook, 2005)—and to the recognition that JA is essential during the final stages of flower development in Arabidopsis (McConn and Browse, 1996) and tomato (Li et al., 2004).

In this Update, we will focus on the role of JAs in plant defense against arthropod herbivores, but the other processes controlled by the hormone will be discussed to the extent that they have provided new insight into the molecular mechanism of JA signaling.

PLANTS SYNTHESIZE NUMEROUS JA DERIVATIVES

JA is synthesized from membrane-derived 18:3 via the octadecanoid pathway (Browse, 2005; Schaller et al., 2005; Wasternack, 2007; Fig. 1). Newly synthesized JA, also known as (+)-7-*iso*-JA, epimerizes to the more stable (−)-JA isomer in which the side chains on the cyclopentane ring are in the thermodynamically more stable trans-configuration. JA is subject to various enzymatic transformations to generate a suite of derivatives that differ in their biological activities (Fig. 1). Among the major routes of JA metabolism are: (1) methylation of C-1 to yield volatile MeJA, which was originally identified as a fragrant component of jasmine flowers; (2) decarboxylation of C-1 to form another volatile compound, cis-jasmone; (3) hydroxylation at C-12 (or C-11) yielding tuberonic acid and related derivatives that can be modified further by sulfation or glycosylation; (4) reduction of C-6 to yield

[1] This work was supported by the U.S. Department of Energy (grant nos. DE–FG02–99ER20323 and DE–FG02–91ER20021), the National Institutes of Health (grant no. GM57795), and the Agricultural Research Center at Washington State University.

* Corresponding author; e-mail howeg@msu.edu.

The author responsible for distribution of materials integral to the findings presented in this article in accordance with the policy described in the Instructions for Authors (www.plantphysiol.org) is: Gregg A. Howe (howeg@msu.edu).

www.plantphysiol.org/cgi/doi/10.1104/pp.107.115683

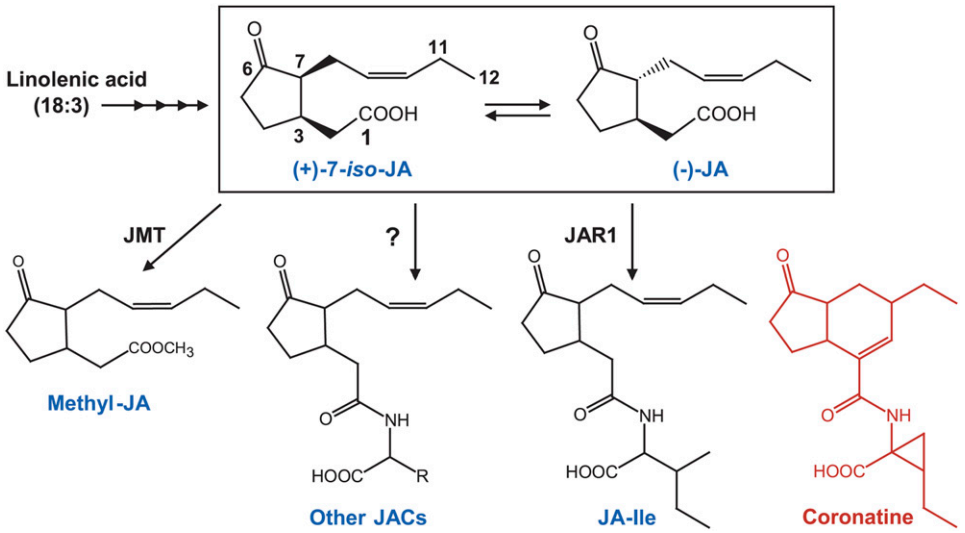

Figure 1. Biosynthesis and metabolism of JA. The octadecanoid pathway converts 18:3 to (+)-7-*iso*-JA, which epimerizes to the more stable (−)-JA isomer. It is generally assumed that (+)-7-*iso*-JA is the biologically relevant and more active form of the hormone. JA is metabolized to a wide range of derivatives (see text for details). For example, JA carboxyl methyltransferase (JMT in the image) converts JA to the volatile compound MeJA. Conjugation of JA to Ile by JAR1 produces JA-Ile, which promotes COI1 interaction with the JAZ1 repressor protein. Other enzymes, presumably related to JAR1, catalyze the formation of other JA-amino acid conjugates (JACs in the image). Coronatine (in red) is a phytotoxin produced by virulent strains of *Pseudomonas syringae*. The toxin functions as a JA mimic and is structurally related to JA-Ile.

cucurbic acid, which may also be esterified to sugar residues; and (5) amide-linked conjugation of the carboxyl group to Ile and other amino acids, yielding jasmonoyl-L-Ile (JA-Ile) and other jasmonoyl-amino acid conjugates, respectively (Fig. 1).

An important question in JA research concerns the biological function of the many chemical derivatives of the hormone. Here, we define bioactive JAs as compounds that bind to a receptor to evoke a physiological response. Nonbioactive JAs are either precursors or deactivated forms of active JAs. For example, hydroxylation of JA was recently implicated as a mechanism to deactivate the JA signaling pathway (Miersch et al., 2007). Although JA (i.e. jasmonic acid) and MeJA are generally considered to be bioactive, receptors that bind JA/MeJA have not been identified. Analysis of *jar1* mutants that fail to convert JA to JA-Ile indicates that JA-Ile is a primary signal for at least some defense responses to pathogens and insects (Staswick et al., 1998; Staswick and Tiryaki, 2004; Kang et al., 2006). At present, JA-Ile is the only derivative known to be active at the molecular level (see below).

JA IS A UBIQUITOUS SIGNAL FOR PLANT DEFENSE AGAINST ARTHROPOD HERBIVORES

The JA pathway constitutes a highly conserved mechanism for promoting plant defense responses to many, if not most, arthropod herbivores (Howe and Jander, 2008). Among the known phytophagous arthropods whose performance is negatively affected by this branch of the plant immune system are caterpillars (*Lepidoptera*), beetles (*Coleoptera*), thrips (*Thysanoptera*), leafhoppers (*Homoptera*), spider mites (*Acari*), fungal gnats (*Diptera*), and mirid bugs (*Heteroptera*). The widespread occurrence and diversity of JA-based defenses in the plant kingdom may reflect the fact that induced defenses have lower resource allocation costs than constitutive defense traits (Baldwin, 1998). Although ethylene, salicylic acid, and other phytohormones are clearly important for the control of plant-insect interactions, the contribution of these signals to host resistance appears to be relatively minor in comparison to JA (Bodenhausen and Reymond, 2007; von Dahl and Baldwin, 2007; Koornneef and Pieterse, 2008; Zheng and Dicke, 2008).

Several lines of experimental evidence support the assertion that JAs are master signals for regulating plant resistance to insect herbivores. Most herbivorous insects, upon feeding, betray their presence to the host plant by activating JA synthesis at or near the insect bite zone. Mechanical tissue damage is often, but not always, sufficient to trigger JA production and subsequent defense responses. Increasing evidence indicates that chemical elicitors in insect oral secretions play an important role in shaping the host response, both quantitatively and qualitatively (Howe and Jander, 2008; Mithöfer and Boland, 2008). It is well established that treatment of plants with JA results in major reprogramming of gene transcription, expression of defensive traits, and enhanced resistance to challenge by insect herbivores (Farmer and Ryan, 1990; Schenk et al., 2000; Kessler and Baldwin, 2002; Mandaokar

et al., 2006; Howe and Jander, 2008). Many JA-responsive genes are induced by herbivory, and proteins encoded by many of these genes have a confirmed role in anti-insect defense. The strongest evidence for JA as a positive regulator of defense is that mutants defective in the synthesis or perception of the hormone are compromised in resistance to arthropod attackers (Howe et al., 1996; McConn et al., 1997; Stintzi et al., 2001; Halitschke and Baldwin, 2003; Li et al., 2004; Reymond et al., 2004; Mewis et al., 2005; Paschold et al., 2007; Zarate et al., 2007). Conversely, genetic alterations that cause constitutive activation of the JA pathway result in enhanced resistance to herbivores (Ellis et al., 2002; Li et al., 2002a; Chen et al., 2005).

Our work with JA mutants of Arabidopsis and tomato led to the serendipitous discovery that these plants are highly susceptible to fungal gnats (*Bradysia impatiens*) and two-spotted spider mites (*Tetranychous urticae*), respectively (McConn et al., 1997; Li et al., 2004). That these herbivores are not serious pests of the respective wild-type plants indicates that the JA pathway provides effective protection against a spectrum of herbivores that extends well beyond the few insect species typically used in laboratory feeding trials. Thus, genetic removal of the JA pathway is sufficient to transform a nonhost plant into a food source for opportunistic insects. The profound ecological consequence of this phenomenon was clearly illustrated by field studies showing that JA-based defenses shape herbivore community composition (Kessler et al., 2004).

The wide variety of biotic aggressors dissuaded by JA-based immunity is consistent with the notion of the hormone as a relatively nonspecific sentinel of tissue injury (Howe and Jander, 2008). An increasing number of DNA microarray studies have concluded that the JA pathway plays a dominant role in controlling plant transcriptional responses to herbivory (Reymond et al., 2000, 2004; De Vos et al., 2005; Bodenhausen and Reymond, 2007; Vogel et al., 2007). Several microarray studies provide evidence for herbivore-specific transcriptional responses. In general, insects from different feeding guilds tend to elicit distinct (but overlapping) patterns of gene expression, whereas attackers from the same guild evoke similar responses. Herbivore-specific patterns of host gene expression may be attributed to variation in the quantity and quality of mechanical tissue damage inflicted by herbivores with different feeding habits or mouthpart architecture, as well as eliciting compounds delivered to plant cells via insect oral secretions (Howe and Jander, 2008). Phloem-feeding insects, for example, avoid JA-based defenses by employing feeding strategies that minimize tissue injury, or that produce signals (e.g. salicylic acid) that repress JA responses (Walling, 2008). A better understanding of the signaling pathways that connect herbivory to transcriptional processes in the plant is needed to explain the molecular basis of herbivore-specific defense responses.

Many JA-regulated compounds that exert direct toxic or antinutritional effects on arthropod herbivores have been identified. A survey of the literature shows that the synthesis of compounds belonging to nearly all the major classes of plant secondary metabolites is stimulated in response to exogenous JA, and that many of these compounds have a confirmed defensive function (Howe, 2005). In addition to secondary metabolites, which traditionally have been viewed as the major determinants of host plant utilization by insects (Berenbaum and Zangerl, 2008), proteins are also an important component of the plant's defensive arsenal. Wound-inducible PIs that impair digestive proteases in the insect gut provide one of the best examples of a defensive protein whose synthesis is tightly regulated by the JA pathway (Farmer and Ryan, 1990; Steppuhn and Baldwin, 2007). Other defensive proteins whose expression is dependent on the JA/COI1 pathway include polyphenol oxidase, Thr deaminase, arginase, and vegetative storage proteins (Zhu-Salzman et al., 2008). Many anti-insect proteins exhibit high stability and activity in the protease-rich environment of the insect digestive system (Wang and Constabel, 2004; Chen et al., 2005, 2007). The accumulated level of tomato defensive proteins in the lepidopteran midgut correlates with the abundance of the corresponding transcripts in insect-damaged tomato leaves (Li et al., 2004; Chen et al., 2007). This observation suggests that JA-induced transcription and protein stability provide complementary mechanisms to maximize the efficacy of protein-based defenses.

The sporadic phylogenetic distribution of anti-insect proteins in the plant kingdom suggests that the evolution of these compounds is governed by many of the same forces that have shaped the diversity of specialized plant metabolites. Because new defensive compounds evolve in the context of the plant's existing complement of chemical weaponry, anti-insect proteins and metabolites should not be viewed as separate entities, but rather as components of a multilayered defensive system in which individual compounds interact synergistically. An elegant demonstration of this synergism was achieved via genetic manipulation of PI and nicotine production in *Nicotiana attenuata* and demonstration that compensatory feeding by *Manduca sexta* in response to PIs was attenuated by the presence of nicotine in the leaf diet (Steppuhn and Baldwin, 2007).

In addition to orchestrating direct defenses, JAs also play a role in regulating indirect plant defenses to herbivore attack. This self-protection strategy involves the interaction of organisms at three trophic levels: plant host, herbivore, and natural enemy (parasitoid or predator) of the herbivore. One of the best examples of indirect defense is the production of plant volatiles in response to elicitors in the oral secretions of lepidopteran herbivores (Erb et al., 2008). These emitted compounds, which include terpenoids and short-chain aldehydes, enable parasitic wasps to locate leaf-eating caterpillars. Recent studies indicate that volatile organic compounds released from herbivore-damaged plant tissue also function to prepare, or prime, plants

for defense against future attacks (Frost et al., 2008). An increasing body of literature indicates that the synthesis of volatiles that mediate plant-insect interactions is regulated, at least in part, by the JA pathway (Erb et al. 2008; Zheng and Dicke, 2008).

THE ROLE OF JA IN INDUCED SYSTEMIC RESISTANCE TO HERBIVORY

In a seminal article published more than 35 years ago, Green and Ryan (1972) demonstrated that wounding of a single leaf of a tomato or potato plant results in systemic expression of defensive PIs. Their discoveries implied the existence of signals that are transported from the site of wounding to distal undamaged leaves. Wound-induced systemic responses, which have been documented in a wide range of plant species, provide effective resistance to future insect attacks (Karban and Baldwin, 1997). Considerable research effort has been devoted to the identification of systemic wound signals and the underlying mechanisms by which they are produced, transported, and perceived. Grafting experiments in tomato showed that systemic PI expression depends on JA synthesis at the site of wounding and on JA perception (i.e. COI1) in distal undamaged leaves (Li et al., 2002b). These experiments indicate that JA or a JA derivative is an essential component of the long-distance signal (Schilmiller and Howe, 2005; Wasternack et al., 2006). A recent study (Wang et al., 2008) showed that JA-Ile synthesis is required for induced systemic defense responses in *N. attenuata*. Metabolic labeling experiments, however, suggested that JA-Ile is not the mobile signal, but rather is synthesized de novo in undamaged leaves following the arrival of this signal. In addition to JA, various other signals, including oligogalacturonides, H_2O_2, abscisic acid, peptide signals, and elicitors in insect regurgitant, are implicated in the regulation of wound-induced systemic defense responses (Ryan, 2000; Narvaez-Vasquez et al., 2007; Wu et al., 2007).

DISCOVERY OF THE JAZ REPRESSORS AND THE MECHANISM OF JA SIGNALING

The discovery that *COI1* encodes an F-box protein led to the suggestion that core JA signaling depends on the action of the E_3 ubiquitin ligase, SCF^{COI1} (Xie et al., 1998). Specifically, it was proposed that JA signaling involves ubiquitination of specific target proteins by the SCF^{COI1} complex and their subsequent degradation by the 26S proteasome. Extensive genetic screens for positive effectors, negative effectors, and components downstream of COI1, as well as searches for COI1-interacting proteins failed to identify verifiable SCF^{COI1} targets (Thines et al., 2007; Balbi and Devoto, 2008). Recently, substrates of SCF^{COI1} were discovered through transcriptional profiling of stamen development in response to JA treatment (Mandaokar et al., 2006; Thines et al., 2007). This approach enabled us to identify several JA-inducible early response genes that encode proteins of unknown function and contain a so-called ZIM motif. These genes were thus named *JASMONATE ZIM-DOMAIN* (*JAZ*) genes (Chini et al., 2007; Thines et al., 2007).

The JAZ family of proteins in Arabidopsis consists of 12 members, which have been classified as a subgroup of the larger family of tify proteins that share a conserved TIFY×G sequence within the ZIM motif (Vanholme et al., 2007). Overall, homology among the JAZ proteins is confined to three regions: the N-terminal region exhibits the weakest sequence similarity; the central region contains the recognized ZIM motif; and the C-terminal region contains a highly conserved $SLX_2FX_2KRX_2RX_5PY$ stretch of amino acids referred to previously as "Domain 3" (Thines et al., 2007) or the "CT domain" (Chini et al., 2007). Here, we refer to the C-terminal signature sequence as the Jas motif (Yan et al., 2007). Several JAZ proteins have been localized to the nucleus but, unlike the Arabidopsis ZIM and ZIM-like proteins, which have zinc-finger DNA-binding domains (Shikata et al., 2003), none of the JAZ proteins contains a known DNA-binding domain.

Null mutations in four *JAZ* genes (*jaz2*, *jaz5*, *jaz7*, and *jaz9*) did not cause male sterility or other strong JA-related phenotypes (Thines et al., 2007). These results suggest that the JAZ proteins may have overlapping functions, and this could explain the failure of forward-genetic screens to identify recessive mutations in the *JAZ* genes. However, these results do not preclude the possibility that mutations in these or other *JAZ* genes cause subtle JA-related phenotypes, or that production of multiple-mutant lines will provide informative phenotypes. Although overexpression of genes encoding full-length JAZ proteins also failed to induce any phenotype, expression of a truncated JAZ1 protein (JAZ1Δ3A) lacking residues 202 to 228, which includes the Jas motif, yielded plants that were male sterile. The sterile *35S-JAZ1Δ3A* plants showed other phenotypes typical of JA-response mutants, including resistance to JA-mediated inhibition of root growth, resistance to infection by a coronatine-producing strain of *Pseudomonas syringae*, and weak induction of JA-responsive genes (Thines et al., 2007).

The dominant action of JAZ1Δ3A in blocking JA responses suggested a model in which JAZ proteins are repressors that prevent transcription of JA-responsive genes. In this scenario, bioactive JAs facilitate interaction between JAZ and COI1, leading to degradation of the JAZ substrates through the ubiquitination-26S proteasome pathway; destruction of JAZ repressors in response to a bioactive JA would allow rapid expression of early response genes (Fig. 2). Deletion of the C-terminal region of JAZ prevents the protein's degradation and allows continued suppression of JA-responsive genes. Experiments with plants expressing JAZ1-GUS reporters support this model. For example, it was shown that JA-induced turnover of JAZ1-GUS requires COI1 and the 26S proteasome, as well as the

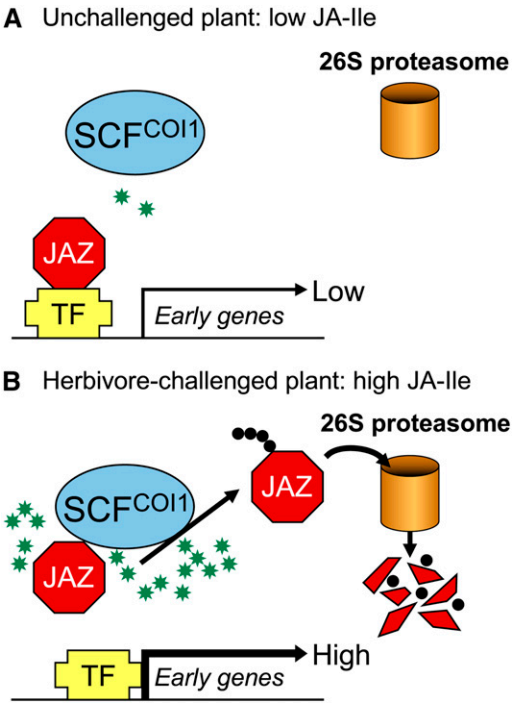

Figure 2. Model of JA signaling in response to herbivore attack. A, Low intracellular levels of JA-Ile (green stars) favor the accumulation of JAZ proteins, which repress the activity of transcription factors (TF in the image) such as MYC2 that positively regulate JA-responsive genes. B, Tissue injury caused by insect herbivores results in rapid accumulation of bioactive JAs, which promote SCF^{COI1}-mediated ubiquitination and subsequent degradation of JAZ proteins via the 26S proteasome. JA-induced removal of JAZ proteins causes derepression of TF and the activation of early response genes. Although this model depicts JA-Ile as the active signal for triggering JAZ degradation, current evidence does not exclude the possibility that other JAs are also active. See text for more details.

C-terminal region of JAZ1 (Thines et al., 2007). These results imply that the C-terminal region of JAZ contains the sequence determinants for JA-promoted interaction with COI1.

Direct evidence for COI1-JAZ1 interaction has come from both yeast (*Saccharomyces cerevisiae*) two-hybrid experiments and protein pull-down assays. The later assay took advantage of a transgenic line of tomato expressing a functional c-Myc-tagged tomato COI1 and recombinant tomato JAZ1 containing a 6×-His tag (Thines et al., 2007). Strikingly, these experiments showed that JA-Ile is highly active in promoting COI1-JAZ1 interaction in a dose-dependent manner. JA-Leu exhibited weak activity in the pull-down assay, whereas nonconjugated JAs, including jasmonic acid, MeJA, and 12-oxo-phytodienoic acid, were inactive. These findings provide direct evidence that JA-Ile is an active form of the hormone. It will be interesting to determine whether the JA-Ile-dependent interaction with COI1 is unique to JAZ1 or is more generally applicable to other members of the JAZ family. It is possible, for example, that jasmonic acid and other nonconjugated JAs promote COI1 interaction with other members of the JAZ family. With the identification of JAZ proteins as substrates for SCF^{COI1}, protein-protein interaction assays can now be used to study the mechanism of JA perception in vitro. A major advantage of this approach is that it minimizes the extent to which exogenous hormone is metabolized by intact plant cells prior to receptor binding.

Interestingly, a common splice variant of *JAZ10* (also known as *JAS1*; At5g13220.3) encodes a protein that lacks part of the Jas motif and, when overexpressed in Arabidopsis, provides partial resistance to the effects of JA on seedling growth (Yan et al., 2007). Also, the dominant JA-insensitive phenotype of the *jai3-1* mutant is caused by expression of a truncated JAZ3 protein (also known as JAI3) that lacks the Jas-motif-containing C-terminal region (Chini et al., 2007). Characterization of the wild-type and truncated derivatives of JAZ3 showed the C-terminal region interacts with the transcription factor MYC2. This is an important finding because MYC2 activity is central to JA responses in plants, particularly those involved in wounding and defense against pathogen attack (Lorenzo et al., 2004). MYC2 also positively regulates the expression of many of the *JAZ* genes (Chini et al., 2007). The interaction of MYC2 with JAZ3 was proposed to inhibit the activity of MYC2 as a transcriptional activator. Removal of the JAZ repressors via the SCF^{COI1}/26S proteasome pathway, in response to a bioactive JA signal, would then allow MYC2 to transcribe early response genes.

Chini et al. (2007) reported that, even in the absence of JA, COI1 interacts with the ZIM-motif-containing N-terminal region of JAZ3, but not with the C-terminal region that contains the Jas motif. This observation led them to propose a model in which the C-terminally truncated form of JAZ3 binds to and inhibits the ubiquitin ligase activity of SCF^{COI1}, thus allowing for the persistence of other JAZ proteins and continued repression of MYC2 activity, even in the presence JA. However, their studies did not address whether the COI1-JAZ interaction that they observed was promoted by JA. In light of other results showing that the COI1-JAZ1 interaction is stimulated by JA-Ile (Thines et al., 2007), additional work is needed to define the sequence determinants that target JAZ proteins to SCF^{COI1}, and how this interaction is influenced by JAs. Although there is much to be done, it is clear that discovery of the JAZ proteins has provided researchers with new tools to understand the molecular mechanism of JA signaling.

The emerging picture of JA signaling is remarkably similar to that of the plant hormone auxin. Recent studies have shown that auxin regulates gene expression by stimulating SCF^{TIR1}-ubiquitin-ligase-catalyzed degradation of Aux/IAA transcriptional repressors, and that direct binding of auxin to TIR1 (the auxin receptor) mediates TIR1 interaction with Aux/IAA substrates (Dharmasiri et al., 2005; Kepinski and Leyser, 2005). The x-ray crystal structure of the TIR1-Aux/IAA-auxin complex revealed that auxin binding to TIR1 promotes substrate recruitment by creating a

surface that facilitates Aux/IAA binding (Tan et al., 2007). Based on the predicted structural similarity between COI1 and TIR1 (Tan et al., 2007), is it conceivable that COI1 functions as a receptor for JA-Ile. The ability of JA-Ile to promote COI1-JAZ1 interaction in the yeast two-hybrid system (i.e. in the absence of any other plant factors) is consistent with this idea. The molecular mechanism of JA perception, however, remains to be determined.

Identification of the JAZ family of proteins will facilitate experiments to understand how the many different responses to JA, including defense responses to herbivory, are regulated by the action of a single E_3 ubiquitin ligase, SCF^{COI1}. It is possible that the myriad induced plant responses to herbivory are determined by the specificity of interactions between distinct JAs, COI1, JAZ proteins, and the downstream transcription factors that JAZ proteins act on. Although it is clear that JA-Ile is a bioactive signal for certain COI1-dependent defense responses (Staswick et al., 1998; Kang et al., 2006; Thines et al., 2007), there is evidence to indicate that other JAs, including 12-oxo-phytodienoic acid and jasmonic acid, may also be active per se as signals for defense against insects (Stintzi et al., 2001; Wang et al., 2008). An important challenge for future studies will be to identify the JA receptor and its cognate ligands, and to determine how receptor occupation is coupled to the destruction of specific JAZ proteins. Such studies promise to reveal the molecular mechanisms underlying the many physiological processes involved in plant resistance to arthropod herbivores.

ACKNOWLEDGMENTS

We thank Paul Staswick and members of the Howe lab for critical reading of the manuscript and helpful discussions. We also acknowledge Marlene Cameron for assistance with figures, and Karen Bird for editorial assistance.

Received December 28, 2007; accepted January 8, 2008; published March 6, 2008.

LITERATURE CITED

Balbi V, Devoto A (2008) Jasmonate signalling network in *Arabidopsis thaliana*: crucial regulatory nodes and new physiological scenarios. New Phytol **177:** 301–318

Baldwin IT (1998) Jasmonate-induced responses are costly but benefit plants under attack in native populations. Proc Natl Acad Sci USA **95:** 8113–8118

Berenbaum MR, Zangerl AR (2008) Facing the future of plant-insect interaction research: le retour à la ''raison d'être''. Plant Physiol **146:** 804–811

Bodenhausen N, Reymond P (2007) Signaling pathways controlling induced resistance to insect herbivores in Arabidopsis. Mol Plant Microbe Interact **20:** 1406–1420

Browse J (2005) Jasmonate: an oxylipin signal with many roles in plants. Vitam Horm **72:** 431–456

Chen H, Gonzales-Vigil E, Wilkerson CG, Howe GA (2007) Stability of plant defense proteins in the gut of insect herbivores. Plant Physiol **143:** 1954–1967

Chen H, Wilkerson CG, Kuchar JA, Phinney BS, Howe GA (2005) Jasmonate-inducible plant enzymes degrade essential amino acids in the herbivore midgut. Proc Natl Acad Sci USA **102:** 19237–19242

Chini A, Fonseca S, Fernandez G, Adie B, Chico JM, Lorenzo O, Garcia-Casado G, Lopez-Vidriero I, Lozano FM, Ponce MR, et al (2007) The JAZ family of repressors is the missing link in jasmonate signalling. Nature **448:** 666–671

De Vos M, Van Oosten VR, Van Poecke RMP, Van Pelt JA, Pozo MJ, Mueller MJ, Buchala AJ, Metraux JP, Van Loon LC, Dicke M, et al (2005) Signal signature and transcriptome changes of *Arabidopsis* during pathogen and insect attack. Mol Plant Microbe Interact **18:** 923–937

Devoto A, Ellis C, Magusin A, Chang HS, Chilcott C, Zhu T, Turner JG (2005) Expression profiling reveals COI1 to be a key regulator of genes involved in wound- and methyl jasmonate-induced secondary metabolism, defence, and hormone interactions. Plant Mol Biol **58:** 497–513

Dharmasiri N, Dharmasiri S, Estelle M (2005) The F-box protein TIR1 is an auxin receptor. Nature **435:** 441–445

Ellis C, Karafyllidis I, Turner JG (2002) Constitutive activation of jasmonate signaling in an Arabidopsis mutant correlates with enhanced resistance to *Erysiphe cichoracearum*, *Pseudomonas syringae*, and *Myzus persicae*. Mol Plant Microbe Interact **15:** 1025–1030

Erb M, Ton J, Degenhardt J, Turlings TCJ (2008) Interactions between arthropod-induced aboveground and belowground defenses in plants. Plant Physiol **146:** 867–874

Farmer EE, Ryan CA (1990) Interplant communication: airborne methyl jasmonate induces synthesis of proteinase inhibitors in plant leaves. Proc Natl Acad Sci USA **87:** 7713–7716

Feys B, Benedetti CE, Penfold CN, Turner JG (1994) *Arabidopsis* mutants selected for resistance to the phytotoxin coronatine are male sterile, insensitive to methyl jasmonate, and resistant to a bacterial pathogen. Plant Cell **6:** 751–759

Frost CJ, Mescher MC, Carlson JE, De Moraes CM (2008) Plant defense priming against herbivores: getting ready for a different battle. Plant Physiol **146:** 818–824

Glazebrook J (2005) Contrasting mechanisms of defense against biotrophic and necrotrophic pathogens. Annu Rev Phytopathol **43:** 205–227

Green TR, Ryan CA (1972) Wound-induced proteinase inhibitor in plant leaves: a possible defense mechanism against insects. Science **175:** 776–777

Halitschke R, Baldwin IT (2003) Antisense LOX expression increases herbivore performance by decreasing defense responses and inhibiting growth-related transcriptional reorganization in *Nicotiana attenuata*. Plant J **36:** 794–807

Howe GA (2005) Jasmonates. Kluwer Academic Publishers, Dordrecht, The Netherlands

Howe G, Jander G (2008) Plant immunity to insect herbivores. Annu Rev Plant Biol **59:** (in press)

Howe GA, Lightner J, Browse J, Ryan CA (1996) An octadecanoid pathway mutant (JL5) of tomato is compromised in signaling for defense against insect attack. Plant Cell **8:** 2067–2077

Kang JH, Wang L, Giri A, Baldwin IT (2006) Silencing threonine deaminase and JAR4 in *Nicotiana attenuata* impairs jasmonic acid-isoleucine-mediated defenses against *Manduca sexta*. Plant Cell **18:** 3303–3320

Karban R, Baldwin IT (1997) Induced Responses to Herbivory. University of Chicago Press, Chicago

Kepinski S, Leyser O (2005) The Arabidopsis F-box protein TIR1 is an auxin receptor. Nature **435:** 446–451

Kessler A, Baldwin IT (2002) Plant responses to insect herbivory: the emerging molecular analysis. Annu Rev Plant Biol **53:** 299–328

Kessler A, Halitschke R, Baldwin IT (2004) Silencing the jasmonate cascade: induced plant defenses and insect populations. Science **305:** 665–668

Koornneef A, Pieterse CMJ (2008) Cross talk in defense signaling. Plant Physiol **146:** 839–844

Li C, Liu G, Xu C, Lee GI, Bauer P, Ling HQ, Ganal MW, Howe GA (2003) The tomato *Suppressor of prosystemin-mediated responses2* gene encodes a fatty acid desaturase required for the biosynthesis of jasmonic acid and the production of a systemic wound signal for defense gene expression. Plant Cell **15:** 1646–1661

Li C, Schilmiller AL, Liu G, Lee GI, Jayanty S, Sageman C, Vrebalov J, Giovannoni JJ, Yagi K, Kobayashi Y, Howe GA (2005) Role of β-oxidation in jasmonate biosynthesis and systemic wound signaling in tomato. Plant Cell **17:** 971–986

Li C, Williams MM, Loh YT, Lee GI, Howe GA (2002a) Resistance of cultivated tomato to cell content-feeding herbivores is regulated by the octadecanoid-signaling pathway. Plant Physiol **130:** 494–503

Li L, Li C, Lee GI, Howe GA (2002b) Distinct roles for jasmonate synthesis

and action in the systemic wound response of tomato. Proc Natl Acad Sci USA **99:** 6416–6421

Li L, Zhao Y, McCaig BC, Wingerd BA, Wang J, Whalon ME, Pichersky E, Howe GA (2004) The tomato homolog of CORONATINE-INSENSITIVE1 is required for the maternal control of seed maturation, jasmonate-signaled defense responses, and glandular trichome development. Plant Cell **16:** 126–143

Lorenzo O, Chico JM, Sanchez-Serrano JJ, Solano R (2004) JASMONATE-INSENSITIVE1 encodes a MYC transcription factor essential to discriminate between different jasmonate-regulated defense responses in *Arabidopsis.* Plant Cell **16:** 1938–1950

Mandaokar A, Thines B, Shin B, Lange BM, Choi G, Koo YJ, Yoo YJ, Choi YD, Choi G, Browse J (2006) Transcriptional regulators of stamen development in Arabidopsis identified by transcriptional profiling. Plant J **46:** 984–1008

McConn M, Browse J (1996) The critical requirement for linolenic acid is pollen development, not photosynthesis, in an *Arabidopsis* mutant. Plant Cell **8:** 403–416

McConn M, Creelman RA, Bell E, Mullet JE, Browse J (1997) Jasmonate is essential for insect defense in Arabidopsis. Proc Natl Acad Sci USA **94:** 5473–5477

Mewis I, Appel HM, Hom A, Raina R, Schultz JC (2005) Major signaling pathways modulate Arabidopsis glucosinolate accumulation and response to both phloem-feeding and chewing insects. Plant Physiol **138:** 1149–1162

Miersch O, Neumerkel J, Dippe M, Stenzel I, Wasternack C (2007) Hydroxylated jasmonates are commonly occurring metabolites of jasmonic acid and contribute to a partial switch-off in jasmonate signaling. New Phytol **177:** 114–127

Mithöfer A, Boland W (2008) Recognition of herbivory-associated molecular patterns. Plant Physiol **146:** 825–831

Narvaez-Vasquez J, Orozco-Cardenas ML, Ryan CA (2007) Systemic wound signaling in tomato leaves is cooperatively regulated by systemin and hydroxyproline-rich glycopeptide signals. Plant Mol Biol **65:** 711–718

Paschold A, Halitschke R, Baldwin IT (2007) Co(i)-ordinating defenses: NaCOI1 mediates herbivore- induced resistance in *Nicotiana attenuata* and reveals the role of herbivore movement in avoiding defenses. Plant J **51:** 79–91

Reymond P, Bodenhausen N, Van Poecke RM, Krishnamurthy V, Dicke M, Farmer EE (2004) A conserved transcript pattern in response to a specialist and a generalist herbivore. Plant Cell **16:** 3132–3147

Reymond P, Weber H, Damond M, Farmer EE (2000) Differential gene expression in response to mechanical wounding and insect feeding in *Arabidopsis.* Plant Cell **12:** 707–720

Ryan CA (2000) The systemin signaling pathway: differential activation of plant defensive genes. Biochim Biophys Acta **1477:** 112–121

Schaller F, Schaller A, Stintzi A (2005) Biosynthesis and metabolism of jasmonates. J Plant Growth Regul **23:** 179–199

Schenk PM, Kazan K, Wilson I, Anderson JP, Richmond T, Somerville SC, Manners JM (2000) Coordinated plant defense responses in Arabidopsis revealed by microarray analysis. Proc Natl Acad Sci USA **97:** 11655–11660

Schilmiller AL, Howe GA (2005) Systemic signaling in the wound response. Curr Opin Plant Biol **8:** 369–377

Schilmiller AL, Koo AJ, Howe GA (2007) Functional diversification of Acyl-CoA oxidases in jasmonic acid biosynthesis and action. Plant Physiol **143:** 112–124

Shikata M, Takemura M, Yokota A, Kohchi T (2003) Arabidopsis ZIM, a plant-specific GATA factor, can function as a transcriptional activator. Biosci Biotechnol Biochem **67:** 2495–2497

Staswick PE, Tiryaki I (2004) The oxylipin signal jasmonic acid is activated by an enzyme that conjugates it to isoleucine in *Arabidopsis.* Plant Cell **16:** 2117–2127

Staswick PE, Yuen GY, Lehman CC (1998) Jasmonate signaling mutants of Arabidopsis are susceptible to the soil fungus *Pythium irregulare.* Plant J **15:** 747–754

Steppuhn A, Baldwin IT (2007) Resistance management in a native plant: nicotine prevents herbivores from compensating for plant protease inhibitors. Ecol Lett **10:** 499–511

Stintzi A, Weber H, Reymond P, Browse J, Farmer EE (2001) Plant defense in the absence of jasmonic acid: the role of cyclopentenones. Proc Natl Acad Sci USA **98:** 12837–12842

Tan X, Calderon-Villalobos LI, Sharon M, Zheng C, Robinson CV, Estelle M, Zheng N (2007) Mechanism of auxin perception by the TIR1 ubiquitin ligase. Nature **446:** 640–645

Thines B, Katsir L, Melotto M, Niu Y, Mandaokar A, Liu G, Nomura K, He SY, Howe GA, Browse J (2007) JAZ repressor proteins are targets of the SCF^{COI1} complex during jasmonate signalling. Nature **448:** 661–665

Vanholme B, Grunewald W, Bateman A, Kohchi T, Gheysen G (2007) The tify family previously known as ZIM. Trends Plant Sci **12:** 239–244

Vijayan P, Shockey J, Levesque CA, Cook RJ, Browse J (1998) A role for jasmonate in pathogen defense of Arabidopsis. Proc Natl Acad Sci USA **95:** 7209–7214

Vogel H, Kroymann J, Mitchell-Olds T (2007) Different transcript patterns in response to specialist and generalist herbivores in the wild Arabidopsis relative *Boechera divaricarpa.* PLoS ONE **2:** e1081

von Dahl CC, Baldwin IT (2007) Deciphering the role of ethylene in plant-herbivore interactions. J Plant Growth Regul **26:** 201–209

Walling LL (2008) Avoiding effective defenses: strategies employed by phloem-feeding insects. Plant Physiol **146:** 859–866

Wang JH, Constabel CP (2004) Polyphenol oxidase overexpression in transgenic *Populus* enhances resistance to herbivory by forest tent caterpillar (*Malacosoma disstria*). Planta **220:** 87–96

Wang L, Allmann S, Wu J, Baldwin IT (2008) Comparisons of LIPOXYGENASE3- and JASMONATE-RESISTANT4/6-silenced plants reveal that jasmonic acid and jasmonic acid-amino acid conjugates play different roles in herbivore resistance of *Nicotiana attenuata*. Plant Physiol **146:** 904–915

Wasternack C (2007) Jasmonates: an update on biosynthesis, signal transduction and action in plant stress response, growth and development. Ann Bot (Lond) **100:** 681–697

Wasternack C, Stenzel I, Hause B, Hause G, Kutter C, Maucher H, Neumerkel J, Feussner I, Miersch O (2006) The wound response in tomato—role of jasmonic acid. J Plant Physiol **163:** 297–306

Wu J, Hettenhausen C, Meldau S, Baldwin IT (2007) Herbivory rapidly activates MAPK signaling in attacked and unattacked leaf regions but not between leaves of *Nicotiana attenuata.* Plant Cell **19:** 1096–1122

Xie DX, Feys BF, James S, Nieto-Rostro M, Turner JG (1998) COI1: an Arabidopsis gene required for jasmonate-regulated defense and fertility. Science **280:** 1091–1094

Yan Y, Stolz S, Chetelat A, Reymond P, Pagni M, Dubugnon L, Farmer EE (2007) A downstream mediator in the growth repression limb of the jasmonate pathway. Plant Cell **19:** 2470–2483

Zarate SI, Kempema LA, Walling LL (2007) Silverleaf whitefly induces salicylic acid defenses and suppresses effectual jasmonic acid defenses. Plant Physiol **143:** 866–875

Zheng S-J, Dicke M (2008) Ecological genomics of plant-insect interactions: from gene to community. Plant Physiol **146:** 812–817