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Review

Top hits in contemporary JAZ: An update on jasmonate signaling

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ABSTRACT

The phytohormone jasmonate (JA) regulates a wide range of growth, developmental, and defense-related processes during the plant life cycle. Identification of the JAZ family of proteins that repress JA responses has facilitated rapid progress in understanding how this lipid-derived hormone controls gene expression. Recent analysis of JAZ proteins has provided insight into the nature of the JA receptor, the chemical specificity of signal perception, and cross-talk between JA and other hormone response pathways. Functional diversification of JAZ proteins by alternative splicing, together with the ability of JAZ proteins to homo- and heterodimerize, provide mechanisms to enhance combinatorial diversity and versatility in gene regulation by JA.

Contents

1. Introduction

Methyl jasmonate (MeJA), a fragrance from the jasmine flower, has long been used as a common ingredient in perfumes. The attractiveness of MeJA and related members of the jasmonate (JA) family of compounds (referred to as JAs) is underscored by the recent identification of *cis*-jasmone as a ligand for insect olfactory receptors (Tanaka et al., 2009) and the identification of JAs as potent anti-cancer agents (Cohen and Flescher, this issue). Plants, of course, produce JAs not only to manipulate animal behavior but also as hormonal signals for controlling broad aspects of plant development and metabolism. Among the many stress-related processes controlled by JA are host resistance to insects and pathogens

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(Kessler and Baldwin, 2002; Glazebrook, 2005; Howe and Jander, 2008; Browse and Howe, 2008), production of specialized metabolites (Pauwels et al., 2009), and responses to ultraviolet radiation, ozone, and salt stress (Fujita et al., 2004; Moons, 2005; Ma et al., 2006; Conconi et al., 1996; Rao et al., 2000). JAs are also involved in the control of carbon partitioning (Mason and Mullet, 1990), vegetative growth rate (Staswick et al., 1992; Yan et al., 2007; Zhang and Turner, 2008), senescence (Xiao et al., 2004), trichome patterning (Yoshida et al., 2009), and reproductive development (McConn and Browse, 1996; Stintzi and Browse, 2000; Li et al., 2001, 2004). These broad activities of the hormone highlight the versatility of JAs as small-molecule modulators of protein function in diverse biological systems.

As with other plant hormones, our understanding of JA function comes principally from the characterization of mutants that are deficient in JA synthesis or perception (Browse, this issue). A major advance in our understanding of the molecular mechanism of JA action was the identification of the Jasmonate ZIM-Domain (JAZ) family of proteins that negatively regulate JA responses (Chini et al., 2007; Thines et al., 2007; Yan et al., 2007). Although these initial discoveries have been the focus of several recent reviews (Chico et al., 2008; Katsir et al., 2008; Staswick, 2008; Browse, 2009), the field of JA signaling continues to advance at rapid pace. Here, we compile available information on JAZs, review the most recent advances in understanding JA signaling mechanisms, and highlight major challenges that lie ahead.

2. COI1 links JA signaling to the ubiquitin/26S proteasome pathway

The *Arabidopsis* mutant *coronatine insensitive 1* (*coi1*) has been the focus of intensive research efforts for more than a decade. The mutant was identified in a screen for plants that are insensitive to the phytotoxin coronatine (Feys et al., 1994). Coronatine is produced by several pathovars of the bacterial pathogen *Pseudomonas syringae*; its structure resembles that of the (3*R*, 7*S*) stereoisomer of jasmonoyl-L-isoleucine (JA–Ile), which is an active form of the hormone (Staswick, 2008; see below). *coi1* mutants of Arabidopsis (Feys et al., 1994), tomato (Li et al., 2004), and tobacco (Paschold et al., 2007) are highly insensitive to JA and are defective in most JA responses. When the *COI1* locus was identified by map-based cloning, it was found to encode an F-box protein that associates with other proteins, including SKP1 and CULLIN (CUL), to form the SCF^{COI1} complex (Xie et al., 1998; Turner et al., 2002; Xu et al., 2002). The SCF complex represents one class of E_3 ubiquitin ligase in the ubiquitin/26S proteasome pathway. The complex uses the F-box protein to bind target substrates, which are then polyubiquitined and degraded by the 26S proteasome (Moon et al., 2004). The severe JA-insensitive phenotype of *coi1* mutants suggested that SCF^{COI1}–mediated protein ubiquitination is pivotal for the activation of JA responses, thus establishing an important link between JA signaling and the ubiquitin/26S proteasome pathway.

The participation of SCF complexes in hormone responses is not restricted to JA signaling, but also includes auxin signaling (via SCF^{TIR1}), gibberellin signaling ($SCF^{SLY/GID2}$), and ethylene signaling ($SCF^{EBF1/2}$) (Gray et al., 1999; Guo and Ecker, 2003; McGinnis et al., 2003; Sasaki et al., 2003; Santner et al., 2009). SCF^{TIR1} was the first identified and is also the best characterized SCF complex in plants. This pioneering work culminated in the discovery that the F-box protein TIR1 is an auxin receptor (Dharmasiri et al., 2005; Kepinski and Leyser, 2005). Auxin acts as a "molecular glue" to enhance the interaction between TIR1 and the auxin/indole-3-acetic acid (Aux/IAA) proteins, which are the substrates of SCF^{TIR1} (Tan et al., 2007). Aux/IAA proteins repress auxin responses by binding to the auxin-response transcription factors (ARFs) and recruiting corepressors (e.g., TOPLESS) to the transcription initiation complex (Szemenyei et al., 2008). Auxin-mediated SCF^{TIR1}–substrate interaction promotes the degradation of Aux/IAA proteins by the ubiquitin/26S proteasome pathway.

In *Arabidopsis*, there are approximately 700 F-box proteins, which constitute one of the largest superfamilies of plant proteins (Gagne et al., 2002). Notably, COI1 is homologous to TIR1 and closely related F-box proteins that function as auxin receptors (Dharmasiri et al., 2005). SCF^{COI1} and SCF^{TIR1} also share other components and regulators of the SCF complex, including ASK1 and ASK2 (Arabidopsis SKP1 homologues), CUL1, AXR1 (Auxin Resistant 1), and RBX1 (Ring-box1) (Tiryaki and Staswick, 2002; Xu et al., 2002; Lorenzo and Solano, 2005; Ren et al., 2005). As one might expect, mutations in these genes cause pleiotropic phenotypes affecting both JA and auxin responses. The apparent similarity between the auxin and JA response pathways led to the idea that SCF^{COI1} interacts with proteins that repress JA responses and that such repressors are targeted for degradation by the ubiquitin/26S proteasome pathway in response to a JA signal. Until recently, this hypothesis was not testable because the substrates of SCF^{COI1} were unknown (Browse, 2009).

3. Discovery of JAZ

Genes encoding the SCF^{COI1} targets were first identified from transcription profiling studies as having transcripts that accumulate rapidly during JA signaling. One set of experiments investigated gene expression in stamens of the *Arabidopsis* JA-synthesis mutant (*opr3*) that is defective in 12-oxo-phytodienoic acid (OPDA) reductase3. The *opr3* mutant is male sterile, but application of JA to *opr3* mutant flower buds can restore fertility with extreme stage specificity (Stintzi and Browse, 2000). To study JA-responsive transcription in stamens, *opr3* flowers were treated with JA and transcriptional profiles were determined at various times thereafter. In total, 1296 genes were identified with specifically altered expression by JA treatment over the time course (Mandaokar et al., 2006). At the earliest sampling time (0.5 h) after JA application, only 31 genes were specifically induced by JA. Seven of these genes, as well as one additional gene induced at a later time point, were predicted to encode proteins of unknown function (Thines et al., 2007). All eight of these proteins contained a ~28-amino acid motif (ZIM) that was previously identified in a putative transcription factor in *Arabidopsis* (Shikata et al., 2004) (see below). JA-activated transcription of these genes is not confined to stamens, but was also observed in JA-treated seedlings (Table 1) (Thines et al., 2007). Four additional genes encoding ZIM-domain proteins were identified based on their high sequence similarity to the eight gene products, but transcription of these genes is not significantly induced by JA. These 12 genes are now known as *Jasmonate ZIM-Domain* (*JAZ*) genes (Table 1).

In an independent study, transcription profiling of wild-type and JA-deficient *Arabidopsis* plants identified a novel wound-inducible gene called *Jasmonate-Associated1* (*JAS1*), which is synonymous with *JAZ10* (Yan et al., 2007). These workers identified an alternative splice variant (At5g13220.3) of *JAZ10* that encodes a protein lacking 12 amino acids from the C-terminus, including a portion of the conserved Jas domain (see below). Overexpression of this truncated splice variant (JAZ10.3), but not the full-length JAZ10.1 isoform, in *Arabidopsis* conferred partial insensitivity to JA and mechanical wounding. This investigation also identified other members of the JAZ protein family by homology to JAZ10 (Yan et al., 2007).

Forward genetic analysis was also important to the discovery of the JAZ proteins and their role in JA signaling. The *jasmonate-insensitive3*-1 (*jai3*-1) mutant is unique among JA-response mutants be-

Table 1
Expression of *JAZ* genes in *Arabidopsis*.

Gene	Induction by MeJA		Induction by wounding[c]	G or T/G-box in promoter (−1500 bp)	MYC2-dependent gene expression[d]	Alternatively spliced transcripts[e]
	opr3 stamens[a]	WT seedlings[b]				
JAZ1	+	+[f]	++	Yes	Yes	2
JAZ2	+	++	++	Yes	Yes	1
JAZ3	–	+	+	Yes	Yes	3
JAZ4	–	nd	–	No	No	3
JAZ5	++	++	++	Yes	Yes	1
JAZ6	+	+	++	Yes	No	1
JAZ7	++	++	++	Yes	No	1
JAZ8	+	++	++	Yes	No	1
JAZ9	+	++	++	Yes	Yes	3
JAZ10	+	++	+	Yes	Yes	4
JAZ11	nd[g]	nd[g]	–	No	No	1
JAZ12	–	–	+	Yes	No	1

nd, not determined.
[a] Thines et al. (2007).
[b] Expression data obtained from the *Arabidopsis* Information Resource (TAIR) submission number ME00337.
[c] Yan et al. (2007) and Chung et al. (2008).
[d] Indicates *JAZ* genes that are repressed in *myc2/jin1* mutants and induced in *35S-MYC2* (Chini et al., 2007).
[e] Alternatively spliced transcripts annotated in TAIR9.
[f] JAZ1 expression in *Arabidopsis* seedlings is also induced by auxin (Grunewald et al., 2009).
[g] Not represented on Affymetrix ATH1 arrays used in the experiments.

cause it exhibits a dominant JA-resistant phenotype (Lorenzo et al., 2004). Genetic mapping of the *jai3* locus constrained its position to a region of chromosome 3 (Chini et al., 2007). The location of one *JAZ* gene (At3g17860, *JAZ3*) within this map window made it a candidate for *JAI3*. Sequencing of the *jai3-1* allele of At3g17860 demonstrated that the point mutation alters an intron acceptor site so that the encoded protein lacks the conserved Jas domain (Chini et al., 2007). The identification of the JAZ proteins in these three studies (Chini et al., 2007; Thines et al., 2007; Yan et al., 2007) opened the way to additional investigations that have provided considerable information about the molecular mechanism of JA signaling.

4. JAZs are members of the TIFY family

The term ZIM is derived from an *Arabidopsis* gene (At4g24470) named *Z*inc-finger protein expressed in *I*nflorescence *M*eristem (Nishii et al., 2000). *ZIM* and *ZIM-like* (*ZML*) genes encode putative transcription factors that contain a C-terminal GATA-type zinc-finger domain presumably involved in DNA binding, a CCT (CONSTANS/CO-like/TOC1) domain implicated in protein–protein interaction (Robson et al., 2001), and a novel ~28-amino-acid sequence motif located near the N-terminus (Shikata et al., 2004). Pfam (http://pfam.sanger.ac.uk/) and InterPro (http://www.ebi.ac.uk/Databases/) databases annotated the latter conserved sequence as a plant-specific domain called ZIM, after the founding member (At4g24470) in which the sequence motif was described. Database searches show that the ZIM domain is present in many plant proteins, including JAZ and PEAPOD (PPD) proteins, which lack a GATA-type zinc-finger or any other recognizable DNA-binding motif. Use of the term ZIM to describe both the transcription factor encoded by At4g24470 and the ZIM domain has led to the inclusion of JAZ and PPD proteins in various plant transcription factor databases (Guo et al., 2005; Rushton et al., 2008; Riaño-Pachón et al., 2007). These considerations prompted Vanholme et al. (2007) to rename the family TIFY, a term that reflects the ZIM domain's most highly conserved TIFYXG motif. Here, we use TIFY as a general term for proteins that contain a ZIM domain but we retain the use of the gene symbols *JAZ*, *PPD*, and *ZIM/ZML* because they are prominent in the literature and because they convey information relevant to protein function (Nishii et al., 2000; Shikata et al., 2003; White, 2006; Chini et al., 2007; Thines et al., 2007).

Sequences encoding TIFY proteins are found in higher and lower (i.e., moss) plants, but not in green algae or non-photosynthetic eukaryotes (Vanholme et al., 2007; Katsir et al., 2008a; Chico et al., 2008). In *Arabidopsis*, TIFY proteins are encoded by 18 genes (Fig. 1A and Table 2). The TIFY family in rice is composed of 20 members, 15 of which are annotated as JAZ proteins (Ye et al., 2009). Family members can be classified into two major subgroups depending on the presence or absence of the GATA-type zinc-finger domain (Vanholme et al., 2007). Based on existing knowledge of member function, phylogeny, and domain architecture, members may also be distinguished according to whether they contain a CCT domain (3 ZIM/ZMLs) or a divergent CCT domain (see below) previously referred to as Domain 3 (Thines et al., 2007) or the C-terminal (CT) domain (Chini et al., 2007), but now referred to as the Jas domain (Yan et al., 2007). *Arabidopsis* proteins containing the Jas domain or slight variations of this motif include 12 JAZ and 2 PPD proteins (Fig. 1A). The protein encoded by At4g32570 is unique in that it contains a ZIM domain but lacks a recognizable CCT or Jas domain.

As described below, current models indicate that JAZs exert their effects on gene expression through physical interaction with transcription factors (Fig. 2). However, a recent study showed that PPD2 can bind to the promoter region of a gene from a plant virus (Lacatus and Sunter, 2009). This result is interesting and unexpected because PPDs and JAZs, which constitute the so-called group II class of TIFY proteins (Vanholme et al., 2007), do not contain a known DNA-binding motif. Additional work is needed to determine what region of PPD2 interacts with DNA, and whether PPDs or JAZ proteins bind to *cis*-regulatory elements in plant genes.

5. Functional domains of JAZ proteins

5.1. The Jas domain

5.1.1. Protein–protein interaction

A distinguishing feature of JAZ proteins is the highly conserved Jas domain located near the C-terminus (Fig. 1A and B). It is now well established that this sequence participates in protein–protein interaction with both transcription factors (e.g., MYC2; see below)

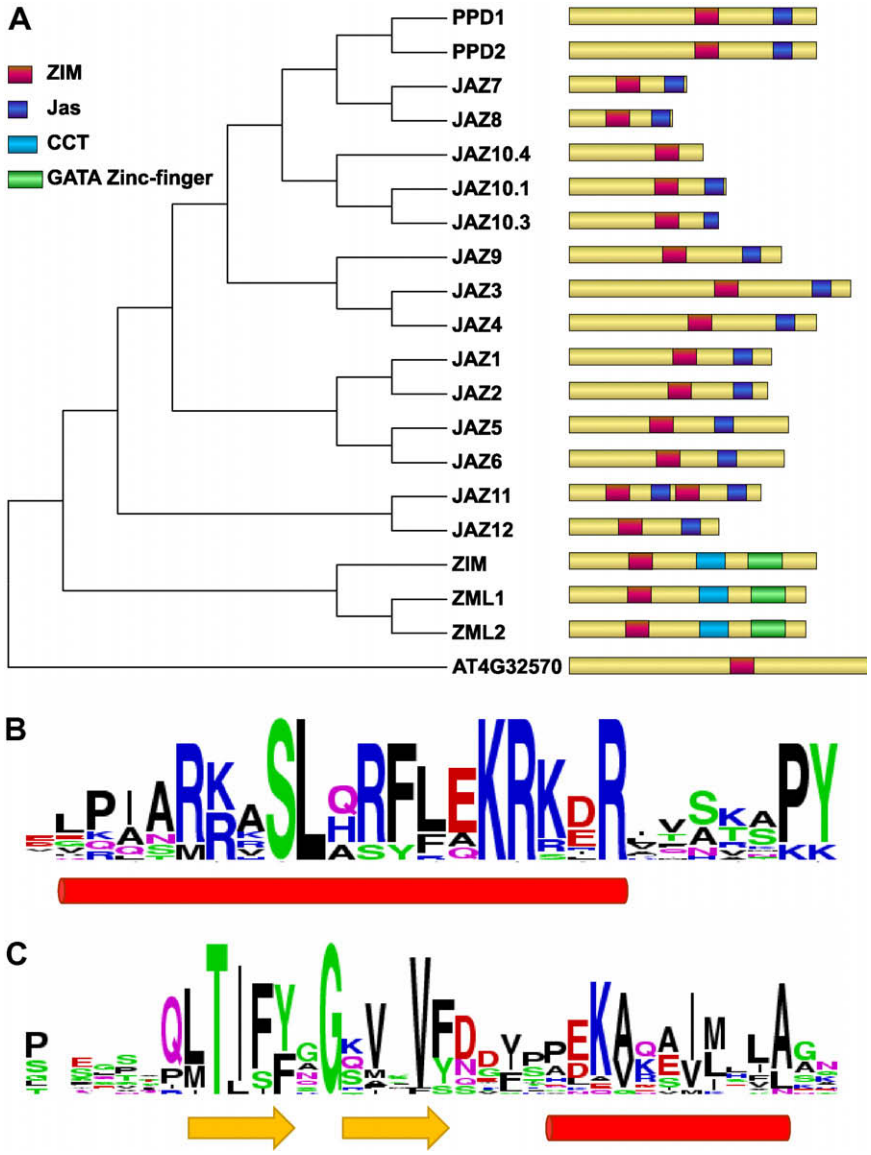

Fig. 1. The TIFY protein family in Arabidopsis. (A) The phylogenetic tree includes all known *Arabidopsis* TIFY proteins, including 12 members of the JAZ subfamily and splice variants of JAZ10.1. Full-length amino acid sequences were aligned using ClustalW, and the tree was constructed by the Neighbor Joining (NJ) method. The relative positions of the conserved domains in each protein are shown in color. (B) Sequence logo (Crooks et al., 2004) of the Jas (B) and ZIM (C) domains. PPD proteins contain a diverged Jas domain that lacks the conserved PY at the C-terminal end (Fig. 3). Sequences used to create the ZIM and Jas domain logos were 36 and 27 amino acids, respectively, in length. Secondary structure (α-helix, red; β-sheet, yellow) in each domain was predicted by Jpred3 (Cole et al., 2008; http://www.compbio.dundee.ac.uk/~www-jpred/) and is depicted below the logo.

and COI1. With respect to the latter, several independent lines of evidence indicate that the Jas domain destabilizes JAZ proteins by promoting their hormone-dependent interaction with SCF^{COI1}. First, JAZ proteins fused to GUS or GFP/YFP reporters are degraded in JA-treated cells in a manner that depends on COI1 and the 26S proteasome (Thines et al., 2007; Chini et al., 2007; Chung and Howe, 2009). Second, truncated JAZ proteins (referred to as JAZΔ Jas) that lack the Jas domain are stable in the presence of JA (Chini et al., 2007; Thines et al., 2007; Shoji et al., 2008; Chung and Howe, 2009). And third, ectopic expression of JAZΔJas proteins impairs the plant's sensitivity to JA. Among the JA-insensitive phenotypes observed in JAZΔJas-expressing plants are reduced expression of JA-response genes, reduced production of specialized metabolites (e.g., nicotine), resistance to JA-mediated inhibition of root growth, susceptibility to insect feeding, enhanced resistance to coronatine-producing strains of *P. syringae*, and male sterility (Thines et al., 2007; Chini et al., 2007; Chung et al., 2008; Melotto et al., 2008; Shoji et al., 2008; Chung and Howe, 2009). These observations support current models (Fig. 2) indicating that JAZ proteins are negative regulators of JA signaling, and that hormone-dependent JAZ degradation by the SCF^{COI1}/26S proteasome pathway is mediated by the Jas domain. Mass spectrometric analysis of ubiquitin-protein conjugates has provided direct evidence for ubiquitination of JAZ proteins (Saracco et al., 2009).

Additional insight into the biochemical function of the Jas domain came from yeast two-hybrid and *in vitro* protein pull-down experiments showing that JAZ proteins bind directly to COI1 in a hormone-dependent manner (Thines et al., 2007). Subsequent studies from several laboratories established that the Jas domain is necessary and sufficient for COI1–JAZ interaction in the presence of bioactive JAs (Katsir et al., 2008b; Melotto et al., 2008; Chini et al., 2009; Fonseca et al., 2009). Melotto et al. (2008) identified two adjacent basic amino acids near the N-terminal end of the Jas domain of JAZ1 and JAZ9 (R205R206 and R223K224, respec-

Table 2
The TIFY protein family in *Arabidopsis*.

Protein	TIFY name[a]	AGI number	TIFY motif	Localization	Homo-dimerization[b,c]	Interaction with COI1[d]	Interaction with MYC2[e]
ZIM	TIFY1	AT4G24470	TISFRG	Nuclear	Yes/nd	nd	nd
ZML1	TIFY2a	AT1G51600	TLSFQG	nd	Yes/nd	nd	nd
ZML2	TIFY2b	AT3G21175	TLSFQG	nd	Yes/nd	nd	nd
JAZ1	TIFY10a	AT1G19180	TIFYAG	Nuclear	Yes/Yes	Yes	Yes
JAZ2	TIFY10b	AT1G74950	TIFYGG	nd	Yes/No	nd	Yes
JAZ3	TIFY6b	AT3G17860	TIFYAG	Nuclear	Yes/Yes	Yes	Yes
JAZ4	TIFY6a	AT1G48500	TIFYAG	nd	Yes/Yes	nd	Yes
JAZ5	TIFY11a	AT1G17380	TIFFGG	nd	Yes/No	nd	Yes
JAZ6	TIFY11b	AT1G72450	TIFFGG	Nuclear	Yes/No	nd	Yes
JAZ7	TIFY5b	AT2G34600	TIFYNG	nd	No/No	nd	No
JAZ8	TIFY5a	AT1G30135	TIFYNG	nd	No/No	nd	Yes
JAZ9	TIFY7	AT1G70700	TIFYGG	nd	No/Yes	Yes	Yes
JAZ10.1	TIFY9	AT5G13220.1	TIFYNG	Nuclear	Yes/No	Yes	Yes
JAZ10.3		AT5G13220.3	TIFYNG	Nuclear	Yes/nd	Yes (weak)	Yes
JAZ10.4		AT5G13220.4	TIFYNG	Nuclear	Yes/nd	No	Yes
JAZ11	TIFY3a	AT3G43440	TIIFGG	nd	No/No	nd	Yes
JAZ12	TIFY3b	AT5G20900	TIFFGG	nd	No/No	nd	Yes
PPD1	TIFY4a	AT4G14713	TIFYSG	nd	nd/nd	nd	nd
PPD2	TIFY4b	AT4G14720	TIFYSG	nd	nd/nd	nd	nd
Unknown	TIFY8	AT4G32570	TIFYGG	nd	nd/nd	nd	nd

nd, not determined.
[a] Vanholme et al. (2007).
[b,c] Homodimerization capacity as determined by yeast two-hybrid analysis in two different studies (Chung and Howe, 2009; Chini et al., 2009). Differences in the JAZ–JAZ interactions reported in these studies may reflect the use of different yeast two-hybrid systems.
[d] Indicates JA–Ile- or coronatine-dependent JAZ interaction with COI1 in yeast two-hybrid and/or *in vitro* pull-down assay (Thines et al., 2007; Melotto et al., 2008; Chung and Howe, 2009; Chini et al., 2009).
[e] As determined by yeast two-hybrid and/or *in vitro* pull-down assay (Chini et al., 2007, 2009; Melotto et al., 2008; Chung and Howe 2009).

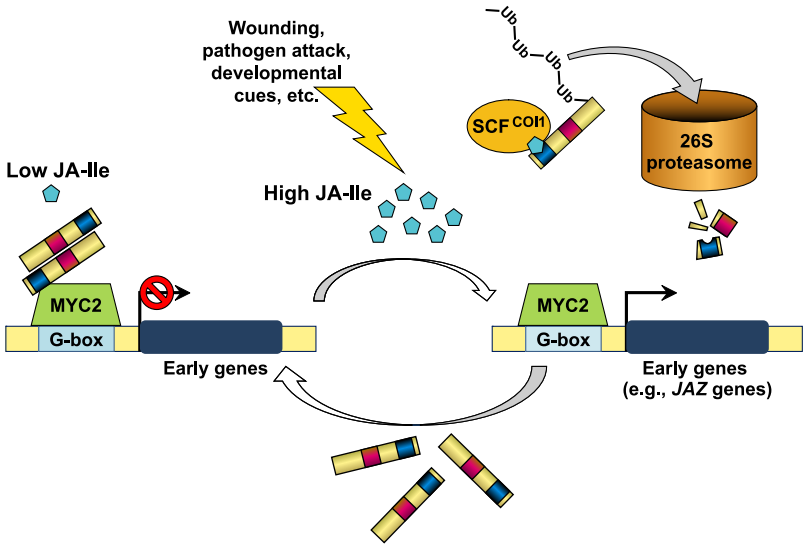

Fig. 2. Model for jasmonate signaling. Low levels of JA–Ile (blue pentagons) permit the accumulation of JAZ proteins that repress the activity of the bHLH transcription factor MYC2 (green) and likely other transcription as well. In response to developmental or environmental cues that activate JA synthesis, high levels of JA–Ile promote SCF^{COI1}-mediated ubiquitination (ub) and subsequent degradation of JAZs via the 26S proteasome. Following its release from JAZ-mediated repression, MYC2 positively regulates the expression of primary JA-responsive genes, including *JAZ* genes, which contain a G-box in the promoter region (Table 1). Newly synthesized JAZ repressors (lower arrow) presumably establish a negative feedback loop by binding MYC2 and attenuating expression of JA response genes. JAZ homo- or heteromeric complexes may be the functional unit for MYC2 repression (Chung and Howe, 2009).

tively; Fig. 1B) that are required for interaction with COI1. Alanine substitution of these residues blocked hormone-induced interaction with COI1 and, in the case of JAZ1, conferred dominant JA insensitivity in transgenic plants. Functional analysis of JAZ10 splice variants has provided evidence that the C-terminal seven amino acids of the domain ending in the conserved PY motif (Fig. 1B) plays a role in promoting JAZ interaction with COI1 (Yan et al., 2007; Chung and Howe, 2009; see below). Given the pivotal role of JAZ accumulation in controlling JA signal output, additional work is clearly needed to define the sequence determinants within the Jas domain that promote JA-dependent recruitment of JAZs to COI1. X-ray crystallography studies, similar to those performed with the auxin receptor TIR1 (Tan et al., 2007), are expected to provide important insight into this question.

5.1.2. Nuclear localization

Several studies have shown that JAZ-GFP/YFP fusion proteins accumulate preferentially in the plant nucleus (Chini et al., 2007; Thines et al., 2007; Yan et al., 2007; Chung and Howe, 2009). However, sequence analysis programs did not identify an obvious nuclear localization signal (NLS) in any JAZ (Thines et al., 2007). Grunewald et al. (2009) recently reported that the Jas domain con-

tains an NLS. This study showed that a 24-amino-acid segment of the Jas domain of JAZ1, excluding six amino acids (ELPIA) at the N-terminus (Fig. 1B), is sufficient to target GFP to the nucleus of tobacco BY-2 cells. Removal of five amino acids, including the conserved PY motif, from the C-terminal end of the domain resulted in loss of the strong nuclear localization pattern. These findings suggest that the Jas domain is involved in both protein–protein interaction and subcellular protein sorting. It should be noted that JAZ proteins lacking either the entire Jas domain or the C-terminal portion of the domain (e.g., JAZ10.3) localize to the nucleus and functionally interact with the JA signaling apparatus (Chini et al., 2007; Thines et al., 2007; Yan et al., 2007; Chung and Howe, 2009). It therefore appears that although the Jas domain may play a role in nuclear localization, it is not strictly required for protein entry into the nucleus. The high p*I* (>9) of JAZ proteins may also facilitate partitioning to the nucleus.

5.1.3. Functional diversification by alternative splicing

Alternative splicing is a fundamental process for expanding protein diversity and the functional complexity of eukaryotic organisms. Recent studies indicate that 95% of multiexon transcripts encoded by the human genome undergo alternative splicing (Pan et al., 2008). Although our understanding of alternative splicing in plants is still in its infancy, increasing evidence indicates that this process promotes plant adaptation to stress (Barbazuk et al., 2008; Reddy, 2007). The recent identification of functionally distinct splice variants of JAZ10 has broadened our appreciation of the role of post-transcriptional regulation in JA signaling (Yan et al., 2007; Chung and Howe, 2009). Alternative splicing of *JAZ10* pre-mRNA generates three splice variants that differ in the sequence of the Jas domain (Fig. 1A). The full-length JAZ10.1 protein contains an intact Jas domain, strongly interacts with COI1 in a JA-dependent manner, and is degraded via the 26S proteasome pathway in response to JA treatment (Chung and Howe, 2009). JAZ10.3, which lacks seven amino acids from the C-terminal end of the of Jas domain, interacts weakly with COI1 in a ligand-dependent manner and is degraded *in planta* in response to high concentrations of exogenous JA. Consistent with the intermediate stability of JAZ10.3, overexpression of this splice variant in *Arabidopsis* confers partial insensitivity to JA (Yan et al., 2007; Chung and Howe, 2009). A third splice variant (JAZ10.4) lacks the entire Jas domain. As predicted, this protein does not interact with COI1, is highly resistant to JA-mediated degradation, and confers strong JA insensitivity when overexpressed *in planta*. An important conclusion of this work is that alternative splicing events affecting the Jas domain expand the functional diversity of JAZ proteins in *Arabidopsis*. It will be interesting to determine whether alternative splicing of *JAZ10* pre-mRNA is regulated in a manner that favors the accumulation of a particular transcript in specific cell types, and whether alternative splicing of *JAZ* genes is widespread in the plant kingdom.

A common feature of hormone signaling pathways in all eukaryotes is that prolonged stimulation decreases responsiveness to the signal, a phenomenon called desensitization. JAZ10.3 and JAZ10.4 appear to function in this capacity. According to this hypothesis, synthesis of bioactive JAs in response to inductive cues would trigger rapid destruction of unstable JAZs (e.g., JAZ10.1). Depletion of these relatively unstable proteins would lead to transcriptional activation of the *JAZ10* gene, which itself is controlled by the SCF^{COI1}/JAZ pathway (Chini et al., 2007; Yan et al., 2007; Chung et al., 2008). Alternative splicing of *JAZ10* pre-mRNA generates transcripts for *de novo* synthesis of all three JAZ10 splice variants but, owing to differences in protein stability, only the JAZ10.3 and JAZ10.4 proteins are predicted to accumulate and eventually attenuate the signal output. In this manner, JA-stimulated cells would become desensitized to elevated hormone levels through the synthesis of JAZ10.3/JAZ10.4 and perhaps other stable JAZ proteins. This model implies that the increased sensitivity of *jaz10* loss-of-function mutants (Yan et al., 2007) results mainly from the absence of JAZ10.3/10.4 rather than from reduced production of the more labile JAZ10.1. The ability of JAZ10.3/10.4 to desensitize the signaling pathway may be important for curtailing JA responses that are energetically demanding or potentially toxic to the cell, or for modulation of JA responses involved in resource allocation between growth- and defense-related processes (Herms and Mattson, 1992). JAZ proteins such as JAZ10.3/10.4 that are stabilized against hormone-induced degradation may also play a role in counteracting the virulence activity of the *P. syringae* toxin coronatine (see below).

5.1.4. Similarity to the CCT domain

The amino acid sequence of the Jas domain is similar to the N-terminal portion of the CCT domain that was first identified in the plant proteins TOC1 and CONSTANS (CO) (Strayer et al., 2000; Robson et al., 2001) (Fig. 3). Based on this similarity, the Jas domain is described in the Pfam database as a divergent CCT motif called CCT2 (PF09425). CONSTANS and other CCT domain-containing proteins have a well-established role in regulating plant responses to light, temperature, and the circadian clock. Although the C-terminal portion of the CCT domain has been implicated in protein–protein interactions that regulate gene expression, the biochemical function of the N-terminal CCT sequence resembling the Jas domain is not known (Wenkel et al., 2006). It will be interesting to further investigate the evolutionary and functional relationship between the Jas (CCT2) and CCT domains.

5.2. The ZIM domain

Although the ZIM domain is the defining feature of the TIFY family (Vanholme et al., 2007), a molecular or biochemical function for this signature sequence was not described for any member of the family until recently. Two independent laboratories employed a yeast two-hybrid (Y2H) approach to demonstrate that Arabidopsis JAZ proteins form homomeric complexes (Chung and Howe, 2009; Chini et al., 2009). Both studies showed that JAZ1, JAZ3, and JAZ4 homodimerize, whereas JAZ7, JAZ8, JAZ11, and JAZ12 do not (Table 2). The two studies yielded inconsistent results concerning the ability of the remaining five JAZs to self interact. These discrepancies likely reflect the use of different Y2H systems, and highlight the need to use independent protein–protein interaction assays for validating Y2H results. Indeed, bimolecular fluorescence complementation assays showed that JAZ3 (Chung and Howe, 2009) and JAZ3ΔJas (Chini et al., 2009) homodimerize in the plant nucleus. Self interaction of JAZ3 was also confirmed with an *in vitro* pull-down assay (Chini et al., 2009).

JAZ proteins also form heteromeric complexes. Using the LexA Y2H system to test all 66 possible heteromeric combinations between the 12 Arabidopsis JAZs, Chung and Howe (2009) reported 38 combinatorial interactions involving most but not all isoforms. The Gal4 Y2H system employed by Chini et al. (2009) identified seven unique heteromeric interactions, all but one (JAZ4–JAZ9) of which was identified with the LexA system. *In vitro* pull-down assays showing that JAZ3ΔJas binds to eight of the 12 JAZs (Chini et al., 2009) supports the existence of a larger network of JAZ heterodimers, as suggested by Chung and Howe (2009). Removal of the Jas domain from JAZ3 by artificial truncation (Chini et al., 2009) or from JAZ10.1 by alternative splicing (Chung and Howe, 2009) appears to broaden the range of JAZ partners in comparison to the corresponding full-length proteins, suggesting that the Jas domain may inhibit some JAZ–JAZ interactions. The ability of ZIM and ZML proteins to homo- and heterodimerize in yeast further indicates that protein–protein interaction is a common theme among TIFY proteins (Chung and Howe, 2009). In fact, systematic

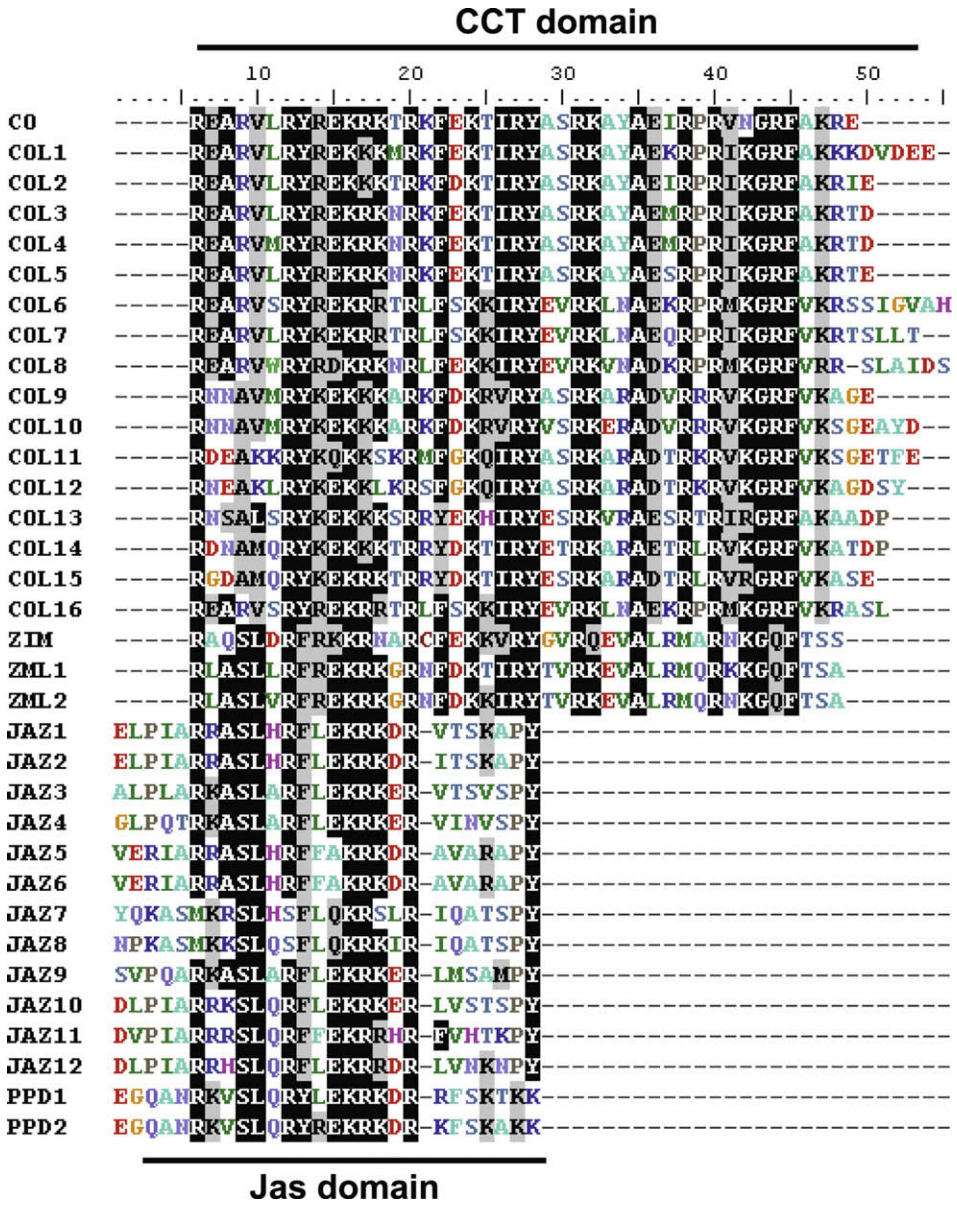

Fig. 3. Sequence similarity between the Jas and CCT domains. Sequence alignment of the Jas domain of *Arabidopsis* JAZ and PPD proteins with the CCT domain of *Arabidopsis* ZIM, ZIM-like (ZML), CONSTANS (CO), and CO-like (COL) proteins using ClustalW. Sequences used for the alignment consisted of 50 amino acids that span the domain. Sequences less than 50 amino acids in length were used for those JAZ proteins that terminate shortly after the Jas domain.

analysis of JAZ3 deletion constructs demonstrated that the ZIM domain is necessary and sufficient for JAZ homo- and heterodimerization (Chung and Howe, 2009; Chini et al., 2009). Site-directed mutagenesis showed that the invariant G residue in the conserved TIFYxG motif of JAZ3 and JAZ10.1 is critical for homo- and heterodimerization of these JAZs.

Evidence that JAZ–JAZ interaction affects JA signaling has come from molecular genetic analysis of JAZ10.4, which confers a strong JA-insensitive phenotype when overexpressed in Arabidopsis (Chung and Howe, 2009). Point mutations within the TIFYXG motif that block JAZ10.4 interaction with other JAZs suppress this JA-insensitive phenotype to various degrees, depending on the mutation; the strength of suppression correlated with the extent to which the point mutation impaired JAZ10.4 interaction with other JAZs. These findings indicate that the dominant negative action of JAZ10.4 likely depends on ZIM domain-mediated interaction with another TIFY protein or JAZ10.4 itself. Grunewald et al. (2009) showed that full-length JAZ1 localizes as discrete speckles within the nucleoplasm of tobacco BY-2 cells. These so-called nuclear bodies have been implicated in various signaling processes in plants, most notably phytochrome-mediated light responses (Chen, 2008). Removal of the ZIM domain from JAZ1 abolished nuclear body formation and also mislocalized the protein to the nucleolus. These findings led to the suggestion that proper localization of JAZ1 in the nucleoplasm may involve protein–protein interaction mediated by the ZIM domain (Grunewald et al., 2009). In summary, these findings not only establish the ZIM domain as a new protein–protein interaction motif in plants, but also indicate that JAZ–JAZ interactions are integral to the mechanism by which JAZs negatively regulate JA signaling (see below).

6. (3*R*, 7*S*)-JA–Ile is the bioactive form of the hormone

Bioactive JAs can be defined as JA derivatives that directly promote the formation of COI1–JAZ complexes (Katsir et al., 2008a). Non-bioactive JAs are either precursors or deactivated forms of the bioactive compounds. The development of cell-free and

yeast-based COI1–JAZ interaction assays led to the discovery that interaction of COI1 with several JAZ proteins is promoted by the JA-amino acid conjugate JA–Ile (Table 2) (Thines et al., 2007; Katsir et al., 2008b; Melotto et al., 2008). That JA, MeJA, OPDA, and several other JA-amino acid conjugates failed to promote these protein–protein interactions indicates that these compounds are not active, at least for the particular COI1–JAZ combinations tested. Identification of JA–Ile as a causal signal for COI1–JAZ binding (Thines et al., 2007) extends the pioneering work of Staswick and colleagues showing that conjugation of JA to Ile by the enzyme JASMONATE RESISTANT1 (JAR1) is a key event in JA signaling (Staswick and Tiryaki, 2004; Staswick, 2008).

Plants contain two stereoisomers of JA that differ with respect to the orientation of the pentenyl side chain at position C7 of the cyclopentanone ring (Creelman and Mullet, 1997; Wasternack, 2007). For the purposes of this discussion, we refer to these isomers as (3*R*, 7*S*)-JA (also known as (+)-7-*iso*-JA or *cis*-JA) and (3*R*, 7*R*)-JA (also known to as (−)-JA or *trans*-JA). These terms are useful because they provide explicit information about the configuration of the C7 side chain, as well as the acetyl side chain at position C3. (3*R*, 7*S*)-JA is the initial biosynthetic product of the octadecanoid pathway (Schaller and Stintzi, this issue). The C3 and C7 bonds of this isomer are in the *cis* orientation with respect to one another. This stereochemistry is generated in a highly specific manner by allene oxide cyclase (Ziegler et al., 2000). Epimerization of the pentenyl side chain of (3*R*, 7*S*)-JA via keto-enol tautomerization yields the *trans* (3*R*, 7*R*)-isomer, which is generally considered to be less active than the (3*R*, 7*S*)-isomer (Creelman and Mullet, 1997; Holbrook et al., 1997; Lauchli and Boland, 2003). We refer to the L-Ile conjugates of (3*R*, 7*S*)-JA and (3*R*, 7*R*)-JA as (3*R*, 7*S*)-JA–Ile and (3*R*, 7*R*)-JA–Ile, respectively (see Koo and Howe, this issue). Although (3*R*, 7*S*)-JA is the predominant isomeric form of JA in wounded leaves (Schulze et al., 2006), the relative abundance of JA–Ile stereoisomers in plant tissues is unclear. It is also unclear whether JAR1 exhibits specificity for (3*R*, 7*S*)-JA or (3*R*, 7*R*)-JA.

Standard synthetic preparations of so-called (−)-JA–Ile (Kramell et al., 1988, 1999; Staswick and Tiryaki, 2004) yield predominantly (3*R*, 7*R*)-JA–Ile in equilibrium (~94:6) with minor amounts of (3*R*, 7*S*)-JA–Ile (Miersch et al., 1986; Creelman and Mullet, 1997; Wasternack, 2007; Fonseca et al., 2009). This mixture of the two native isomers promotes JA-dependent physiological responses, including expression of JA-response genes (Kramell et al., 1997, 2000; Wasternack et al., 1998; Staswick and Tiryaki, 2004; Wang et al., 2008). Initial COI1–JAZ interaction studies used this mixed isomeric preparation to demonstrate that JA–Ile is the chemical mediator of COI1–JAZ binding (Thines et al., 2007; Katsir et al., 2008b). These studies did not draw conclusions or make assumptions about the relative activity of (3*R*, 7*S*)-JA–Ile and (3*R*, 7*R*)-JA–Ile. It is generally assumed that *cis* stereoisomers of JA are more active than the corresponding *trans* (3*R*, 7*R*)-isomers (Farmer, 1994; Lauchli and Boland, 2003). Moreover, the stereochemical similarity of (3*R*, 7*S*)-JA–Ile to coronatine, a highly potent agonist of the JA receptor (Katsir et al., 2008b; see below), suggested that (3*R*, 7*S*)-JA–Ile is likely to be the bioactive isomer (Staswick, 2008).

Recent studies performed with purified stereoisomers provided direct evidence that (3*R*, 7*S*)-JA–Ile is highly active in comparison to (3*R*, 7*R*)-JA–Ile. This work showed that (3*R*, 7*S*)-JA–Ile strongly promotes JA-dependent phenotypic responses in *Arabidopsis* and *in vitro* interaction of COI1 with JAZ9, whereas the corresponding (3*R*, 7*R*) *trans* isomer is inactive (Fonseca et al., 2009). These findings highlight the importance of the C3 and C7 bond configuration in signal activity. The low activity of (3*R*, 7*R*)-JA–Ile in these assays led to the proposal that (3*R*, 7*S*)-JA–Ile is inactivated *in vivo* by epimerization to (3*R*, 7*R*)-JA–Ile. Testing of this idea will require analytical methods (e.g., Schulze et al., 2006) to quantify the endogenous level of specific stereoisomers of JA–Ile in plant tissues. It was also shown that (3*S*, 7*S*)-JA–Ile, a non-native synthetic isomer, is highly active in promoting COI1–JAZ interaction (Fonseca et al., 2009). This finding suggests that the 7*S* configuration of the pentenyl side chain is critical for interaction of the hormone with its cognate receptor. Elucidation of the structure of COI1/JA–Ile/JAZ complexes promises to resolve many of the remaining questions concerning the chemical specificity of JA signaling.

Several studies have provided evidence that OPDA and other non-conjugated JA derivatives elicit distinct JA-related responses without their prior conversion to JA–Ile (Hopke et al., 1994; Blechert et al., 1999; Miersch et al., 1999; Stintzi et al., 2001; Wang et al., 2008; Ribot et al., 2008). These observations, together with the fact that *jar1* mutants possess residual JA/COI1-dependent signaling activity (Chung et al., 2008; Suza and Staswick, 2008; Wang et al., 2008; Koo et al., 2009; Yoshida et al., 2009), raises the interesting possibility that ligands other than JA–Ile promote COI1 binding to different JAZ isoforms. Despite the attractiveness of this hypothesis for explaining the diversity of JA responses, only JA–Ile and structurally related JA-amino acid conjugates (e.g., JA-Val/Leu) have been reported to promote COI1–JAZ binding (Thines et al., 2007; Katsir et al., 2008b; Fonseca et al., 2009). The occurrence of JA responses in *jar1* mutants may be explained by the ability of these mutants to synthesize residual amounts of JA–Ile, presumably by JAR1-related enzymes that operate in the absence of JAR1 (Chung et al., 2008; Suza and Staswick, 2008). Consistent with this view, recent studies provided evidence that low levels of JA–Ile in a *jar1* null mutant are sufficient to activate systemic wound responses through the SCF^{COI1}/JAZ pathway (Koo et al., 2009; Koo and Howe, this issue). Generation and characterization of mutants that completely lack JA–Ile will provide an important test of the idea that JA–Ile is the main endogenous signal for triggering COI1–JAZ interaction and subsequent JA responses.

7. The JA receptor

The ability of JAZ proteins to interact with COI1 in the presence of JA–Ile is integral to the mechanism of hormone perception. Our current understanding of how JA–Ile is perceived by plant cells has been facilitated by the use of the *P. syringae*-derived toxin coronatine which, as mentioned above, is a close structural mimic of (3*R*, 7*S*)-JA–Ile. Biochemical studies performed with COI1 and JAZ proteins from tomato showed that ^{3}H-coronatine binds reversibly to COI1-JAZ complexes with high affinity ($K_d \sim 20$ nM) and specificity (Katsir et al., 2008b). *In vitro* binding assays were used to address the question of whether the receptor for coronatine is COI1, JAZ, or a COI1-JAZ complex (Katsir et al., 2008b). This study showed that specific binding of the toxin to the complex requires COI1, and that purified JAZ alone does not bind coronatine. The finding that excess unlabeled JA–Ile (an isomeric mixture) competes with ^{3}H-coronatine for binding indicates that coronatine and JA–Ile are recognized by the same receptor. Binding of coronatine to the COI1-JAZ3 complex of tomato was disrupted by a point mutation in the putative ligand binding site within the leucine-rich repeat (LRR) portion of COI1 (Katsir et al., 2008b). Y2H assays showed that hormone-induced binding of JAZ by COI1 does not require plant proteins other than COI1 and JAZ (Thines et al., 2007; Melotto et al., 2008; Chini et al., 2009). Consistent with this finding, ligand-dependent COI1-JAZ interactions were observed following partial purification of COI1 from plant extracts (Fonseca et al., 2009). These collective results indicate that COI1 is an essential component of the coronatine/JA–Ile receptor. This conclusion is supported by extensive genetic evidence showing that loss of COI1 function severely impairs plant responses to JA and coronatine (Feys et al., 1994; Li et al., 2004; Paschold et al., 2007;

Browse, 2009b). The ability of coronatine to efficiently target JAZ proteins for destruction via the SCF^{COI1}/ubiquitin pathway provides a compelling example of how pathogens exploit hormone signaling pathways in the host plant to promote disease (Katsir et al., 2008a, b; Melotto et al., 2008; Grant and Jones, 2009).

Recently, Yan et al. (in press) employed various biochemical and modeling approaches to further investigate JA binding. First, these workers used surface plasmon resonance spectroscopy to confirm that the interaction between Arabidopsis COI1 and JAZ1 is stimulated by coronatine and JA–Ile, and that these compounds do not bind JAZ in the absence of COI1. Second, a photoaffinity labeling strategy was used to demonstrate that a biotin-tagged coronatine probe binds directly to COI1 in the absence of JAZ or any other plant protein. Finally, the authors constructed a structural model of COI1 (based on the crystal structure of TIR1; Tan et al., 2007), and performed molecular docking simulations to study the interaction of various ligands with the putative binding pocket of COI1. Consistent with biochemical studies (e.g., Thines et al., 2007), these simulations predicted that binding of coronatine/JA–Ile to COI1 is energetically more favorable than binding of JA, MeJA, and OPDA. Based on these results, Yan et al. (in press) proposed a molecular mechanism for binding of coronatine/JA–Ile to COI1. A potential limitation of this approach is the lack of consideration for how ligand binding is affected by JAZ substrates or putative co-factors (see below) that may contribute to the structural integrity of the receptor complex (Tan et al., 2007). Modeling approaches are inherently problematic for predicting structure, especially for bimolecular interactions. The X-ray crystal structure of the COI1-ligand-JAZ ternary complex will provide a crucial test of the accuracy of the predictions described by Yan et al.

Although the weight of biochemical and genetic evidence leaves little room for doubt that COI1 is a receptor for JA–Ile, several important questions remain to be addressed. For example, what biochemical events are involved in the formation of the COI1-ligand-JAZ ternary complex? Available information is consistent with a model (Katsir et al., 2008a; Yan et al., in press) in which formation of a low-affinity COI1-ligand complex precedes the assembly of a high-affinity COI1-JAZ co-receptor complex. Further development of this hypothesis will benefit from quantitative ligand binding assays performed in the presence and absence of JAZ substrates, as well as the demonstration that pure stereoisomers of JA–Ile bind specifically, saturably, and reversibly to purified COI1 or COI1-JAZ complexes. It will also be important to determine how different JAZ substrates affect ligand binding to COI1, and to define the stoichiometry of ligand and protein molecules within these complexes. Finally, future work is needed to identify additional factors involved in the assembly and disassembly of the receptor complex.

The emerging view of the core JA signaling module has striking similarity to the mechanism of auxin action (Fig. 2) (Katsir et al., 2008a); both hormones work by stabilizing the interaction between an F-box protein and its cognate substrates. Elegant structural studies have revealed that auxin binding to the LRR region of the auxin receptor (TIR1) promotes substrate recruitment by creating a hydrophobic binding surface for Aux/IAA proteins (Tan et al., 2007). Homology models indicate that the general architecture of COI1 is likely to be similar to that of TIR1 (Tan et al., 2007; Katsir et al., 2008a; Yan et al., 2009). Moreover, most amino acid residues involved in positioning the inositol-hexakisphosphate (IP_6) cofactor at the center of the TIR1-LRR solenoid are conserved in COI1 (Tan et al., 2007). These recent advances in understanding JA perception extend the paradigm of F-box proteins as sensors of small molecules that regulate transcription.

8. Transcriptional control by JAZ proteins

The basic helix-loop-helix (bHLH) transcription factor, MYC2, plays a key role in regulating the expression of early JA-response genes. *MYC2* (also known as *JIN1*) was originally identified in genetic screens for mutants that exhibit reduced sensitivity to JA-mediated root growth inhibition (Berger et al., 1996; Lorenzo et al., 2004). Molecular genetic analyses showed that MYC2 differentially regulates two branches of JA-mediated responses; it positively regulates wound-responsive genes, but represses the expression of certain pathogen-responsive genes (Boter et al., 2004; Lorenzo et al., 2004). Mutant analysis in *Arabidopsis* has also revealed that MYC2 acts as an integrator of light, abscisic acid, and JA signaling pathways (Abe et al., 1997, 2003; Lorenzo et al., 2004; Yadav et al., 2005; Dombrecht et al., 2007). Clearly, regulation of MYC2 activity by JA is an important control point within a larger signaling network for integrating responses to diverse environmental cues.

JAZ proteins do not contain a known DNA-binding domain, but rather are hypothesized to regulate gene expression through interaction with DNA-binding transcription factors. Because MYC2 is a key transcription factor in JA responses, Chini et al. considered MYC2 to be a potential candidate for interacting with JAZ proteins. Indeed, Y2H and pull-down assays showed MYC2 physically associates with most JAZ proteins (Table 2) (Chini et al., 2007, 2009; Melotto et al., 2008; Chung and Howe, 2009). Promoter analysis revealed that the MYC2-binding motif G-box or its variant T/G-box is over-represented in *JAZ* promoters (Table 1), and experiments confirmed that MYC2 directly binds to these motifs in the *JAZ3* promoter (Chini et al., 2007). Furthermore, expression of many *JAZ* genes is attenuated in mutants that lack MYC2. These and other recent results support a model (Fig. 2) in which MYC2-mediated expression of early JA-response genes, including *JAZ* genes, is repressed by direct interaction with JAZ proteins (Chini et al., 2007, 2009; Chung et al., 2008; Melotto et al., 2008; Chung and Howe, 2009).

A complete understanding of how JAZs repress MYC2 activity will require knowledge of sequence motifs that mediate the MYC2–JAZ interaction. JAZ3 interacts with the N-terminal region of MYC2 (Chini et al., 2007). Interestingly, this portion of the MYC2 protein contains plant-specific sequence motifs conserved in a small subgroup of plant bHLH proteins (Heim et al., 2003). Further characterization of sequence determinants within MYC2 that bind JAZ3 may provide clues for identifying other transcription factors that are controlled by JAZs. MYC2 cannot be the only transcription factor targeted by JAZ repressors because *myc2*/*jin1* loss-of-function mutants are unaffected in some JA responses, including male fertility and trichome initiation (Berger et al., 1996; Lorenzo et al., 2004; Laurie-Berry et al., 2006; Yoshida et al., 2009).

Initial insight into regions within JAZ that interact with MYC2 is also beginning to emerge. The fact that nearly all *Arabidopsis* JAZs bind MYC2 in Y2H assays indicates a relative lack of biochemical specificity in MYC2–JAZ pairings (Chini et al., 2007, 2009; Melotto et al., 2008; Chung and Howe, 2009). Pull-down and Y2H assays showed that the Jas domain is necessary and sufficient for interaction of JAZ3 with MYC2 (Chini et al., 2007, 2009). Thus, the Jas domain of JAZ3 interacts with COI1 in the presence of JA–Ile, and with MYC2 in a hormone-independent manner. Mutations in the Jas domain that block hormone-dependent interaction of JAZ9 with COI1 did not affect MYC2 binding, leading to the suggestion that the COI1 and MYC2 interaction surfaces of JAZ9 are not identical (Melotto et al., 2008). In contrast to the role of the Jas domain in mediating MYC2 interaction with JAZ3, the JAZ10.4 splice variant that lacks the Jas domain interacts with MYC2 in yeast (Chung and

Howe, 2009). This finding raises the possibility that a JAZ10.4 homo- or heterodimer represses JA signaling through direct binding to MYC2. It will be important to define the regions outside the Jas domain that interface with MYC2, and to determine whether JAZ10.4 and other JAZΔJas proteins interact directly with MYC2 in plant cells.

Initial studies (Chini et al., 2007; Thines et al. 2007) clearly established that at least some JAZ proteins act both as transcriptional repressors of JA signaling and as substrates for COI1. The precise mechanism by which JAZs repress transcription factor activity, however, remains to be determined. The well-established role of the Jas domain in mediating JA-dependent COI1–JAZ interaction provides a partial explanation for how JAZΔJas proteins act in a dominant fashion; by escaping destruction via the SCF^{COI1}/proteasome pathway, these proteins continue to repress target transcription factors such as MYC2. The paradox in this model lies in the fact that because the Jas domain mediates JAZ3 interaction with MYC2 (Chini et al., 2007, 2009), JAZ3ΔJas cannot repress signaling through direct binding to MYC2. Chini et al. (2007) reported two findings that appeared to resolve this paradox. First, they showed that JAZ3ΔJas interacts with COI1 in a hormone-independent manner. Although potentially interestingly in the context of a mechanism to facilitate recruitment of JAZs to COI1, the relevance of this interaction in JA signaling remains to be shown (Chico et al., 2008). Second, *in vitro* protein turnover assays provided evidence that JAZ3ΔJas prevents SCF^{COI1}/proteasome-dependent degradation of full-length JAZ proteins. Based on these observations, it was proposed that binding of JAZ3ΔJas to COI1 poisons the ability of SCF^{COI1} to target endogenous JAZs for JA-induced degradation (Chini et al., 2007).

The discovery of the ZIM domain as a determinant for JAZ–JAZ partnering (Chung and Howe, 2009; Chini et al., 2009) provides an alternative model that is more parsimonious with the existing data. In this "JAZ dimer" model, JAZΔJas proteins repress JA signal output by dimerizing with full-length JAZs that can interact with MYC2 via the Jas domain. This hypothesis is consistent with the finding that JAZ10.4-mediated repression of JA signaling depends on a functional ZIM domain (Chung and Howe, 2009), and does not evoke the need for a Jas domain-independent COI1–JAZ interaction. The fact that bHLH transcription factors typically function as homo- or heterodimers (Heim et al., 2003) is also consistent with the idea that JAZ–JAZ complexes are the functional unit for MYC2 repression. Such complexes may prevent MYC2 from binding target promoter regions or by recruiting co-repressors to the transcription pre-initiation complex. The necessary tools are now available to test these ideas.

9. Conclusions and future directions

The recent identification of JAZ proteins has facilitated rapid progress in our understanding of the molecular mechanism of JA action. Nevertheless, several important challenges remain to be addressed. One major question is how the diversity of JA responses is controlled in specific organs and cell types. The existence of a single *COI1* gene in most plants, together with mounting evidence that (3*R*, 7*S*)-JA–Ile is the major bioactive form of the hormone, supports the idea that JAZ proteins and the transcription factors they interact with are largely responsible for the diversity of JA responses. An important area of future research will be to link specific JAZ proteins to specific physiological and metabolic processes. The ability of JAZ proteins to functionally interact with one another via the ZIM domain adds a new layer of complexity to this problem. Further understanding the mechanism of JAZ action will be facilitated by protein structure studies, analysis of JAZ post-translational modifications, and the identification of additional JAZ-interacting proteins. Progress in this direction will assist efforts to decipher the molecular basis of cross-talk between JA and other signaling pathways. Recent advances in understanding how JA signaling is integrated with the gibberellin (Navarro et al., 2008), salicylate (Koornneef and Pieterse, 2008), auxin (Nagpal et al., 2005; Grunewald et al., 2009; Sun et al., 2009), and phytochrome (Moreno et al., 2009; Kidd et al., in press) response pathways provide important steps in this direction. The availability of complete genome sequences for diverse plant species provides an opportunity to address these questions from an evolutionary perspective, and should thus provide insight into how F-box proteins evolved as small-molecule sensors.

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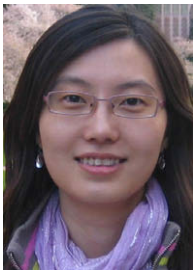

Yajie Niu received her B.S. in Biological Science from the Fudan University in China in 2003. She obtained her Ph.D. in Molecular Plant Sciences from Washington State University in June 2009. Her thesis focused on characterization of key transcriptional regulators of jasmonate signaling in Arabidopsis, including Jasmonate ZIM-Domain (JAZ) repressor proteins and their interacting transcription factors. She will soon join Dr. Jen Sheen's group at Massachusetts General Hospital as a postdoctoral fellow, and will study MAPK cascades in Arabidopsis.

John Browse received his bachelor's degree and Ph.D. in Plant Physiology (1977) from the University of Auckland. He is now a Fellow in the Institute of Biological Chemistry and Professor of Molecular Plant Sciences at Washington State University. The research program in his laboratory encompasses a diverse set of projects that have at their base investigations of the biosynthesis and function of membrane and storage lipids in plants using *Arabidopsis* as a model. The projects include the isolation and characterization of genes that control the elongation, desaturation or other modifications of fatty acids. These genes have been used to produce transgenic plants with alterations in membrane lipid composition or the fatty acid composition of seed oils. Several research projects focus on the roles of membrane lipids in the cell biology and physiology of plants using a large number of mutants with alterations in the lipid composition of their membranes. The isolation of mutants of *Arabidopsis* deficient in the synthesis of the plant hormone, jasmonate, has resulted in discoveries about the involvement of jasmonate in stamen and

pollen development, insect defense and non-host resistance against fungal pathogens. Most recently, transcriptional profiling of jasmonate responses in stamens led to the identification of Jasmonate ZIM-Domain (JAZ) proteins that are repressors acting in core jasmonate signaling. These discoveries have wide implications for plant biology in areas ranging from hybrid breeding to crop protection.

Gregg Howe received his B.A. and M.S. degrees in Biology from East Carolina State University. After working in the plant biotechnology industry for two years, he pursued Ph.D. studies in Sabeeha Merchant's lab (University of California, Los Angeles) on the role of metals in the assembly of the photosynthetic apparatus in *Chlamydomonas reinhardtii*. He then worked as an NIH Postdoctoral Fellow in Clarence Ryan's lab at Washington State University. His postdoctoral research was focused on the identification and characterization of wound response mutants of tomato, many of which were subsequently shown to be defective in the synthesis or perception of jasmonate. In 1997, he joined the faculty of the Department of Energy-Plant Research Laboratory and Department of Biochemistry and Molecular Biology at Michigan State University. Research in the Howe lab is currently focused on understanding the mechanisms of jasmonate synthesis and perception, as well as the role of jasmonates in the wound response and plant–insect interactions.