Insight

Unwinding JAZ7 – enigma to harmony

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JASMONATE ZIM-DOMAIN (JAZ) proteins are primary transcriptional repressors in the jasmonate (JA) signaling pathway that regulate a broad range of JA-dependent responses. A mechanistic mode of action is well established, but what are the biological roles of individual JAZs, and how do they contribute to the specification of JA signaling outputs? Two recent articles in Journal of Experimental Botany reveal roles of JAZ7 in regulating dark-induced senescence and susceptibility to fungal pathogens.

Jasmonates (JAs) regulate a broad range of processes, from growth and development to biotic and abiotic stress responses. JASMONATE ZIM-DOMAIN (JAZ) proteins are key regulators in controlling JA signaling outputs. Since the discovery of JAZs in 2007, over 100 research articles have been published on the characterization of JAZ functions, genomic or transcriptomic analyses of JAZs, or the identification of down-stream transcription factors in the JA signaling pathway through yeast two-hybrid screens using JAZs as bait (reviewed in Pauwels and Goossens, 2011; Shyu and Brutnell, 2015). Biochemical and molecular functions of JAZ proteins have been extensively studied, but surprisingly little is known about the biological role of JAZs and how they contribute to specific JA signaling outputs.

Enigmatic JAZ7

JAZs were identified in 2007 as the missing link between the F-box protein CORONATINE INSENSITIVE 1 (COI1) and the transcription factor MYC2, which mediates JA responses (Chini et al., 2007; Thines et al., 2007; Yan et al., 2007). JAZ proteins function as transcriptional repressors that repress gene expression by bridging co-repressors NOVEL INTERACTOR OF JAZ (NINJA)/TOPLESS and transcription factors to repress JA-responsive gene expression. Upon accumulation of bioactive JA, JAZs form a co-receptor complex with COI1 to perceive JA. This interaction leads to ubiquitination and degradation of JAZ proteins, resulting in activation of JAZ-interacting transcription factors and JA-responsive gene expression (reviewed in Pauwels and Goossens, 2011).

Among the 13 JAZ proteins in Arabidopsis, JAZ7 is one of the more enigmatic members of the family. Unlike most JAZs, JAZ7 does not form homo or heterodimers with other JAZ members, and interacts with very few transcription factors, namely MYC3 and MYC4 (Chung and Howe, 2009; Fernandez-Calvo

Box 1. Model for JAZ specification of diverse JA-mediated responses

Lines are drawn based on phenotypes reported in loss-of-function, overexpression, or dominant-negative lines of the JAZ proteins indicated. JAZX represents JAZs other than those specified; dashed lines indicate speculative interactions.
et al., 2011; Thatcher et al., 2016). JAZ7’s closest homolog is JAZ8, which shares an N-terminal ERF-associated amphipathic repression (EAR) domain and was characterized to be a stable transcriptional repressor even in the presence of JA (Shyu et al., 2012). Similar to JAZ8, JAZ7 does not interact with the co-repressor NINJA (Pauwels et al., 2010). It was speculated that JAZ7 would have similar molecular functions as JAZ8, but no biochemical or genetic characterization was reported.

In Yu et al. (2016), it is shown that jaz7 has a loss-of-function phenotype – something that has only been detected with jaz9 and jaz10 loss-of-function alleles and not with the other 10 JAZ genes to date (Cerrudo et al., 2012; Demianski et al., 2012; Yang et al., 2012; Leone et al., 2014; Yu et al., 2016). Working independently, Thatcher et al. (2016) characterized the same T-DNA insertion allele, which they named jaz7-1D, along with an activation-tagged allele jaz7-1A. These studies mutually reveal the biological function of JAZ7 in Arabidopsis.

Dark-induced senescence

Using detached leaves, Yu et al. (2016) showed that transcripts from JAZ7 are rapidly induced in the dark, along with several other JAZ genes. Screening of multiple jaz T-DNA insertion lines then demonstrated that the loss-of-function jaz7 allele was more sensitive to dark-induced senescence, with enhanced leaf yellowing and reduced chlorophyll content compared to wild type. This phenotype was complemented by 35S::JAZ7 in the jaz7 background. Overexpression of JAZ7 in the wild-type background showed no significant senescence phenotype, but had reduced H2O2 content compared to wild-type plants. These results collectively suggest that JAZ7 plays a role in inhibiting dark-induced senescence. Genetic analysis of jaz7 crossed with coi1 or myc2 indicated that JAZ7-regulated senescence is dependent on COII and the regulation is likely through MYC2. A microarray analysis was then performed in WT and jaz7 in both control and dark conditions to identify genes regulated by JAZ7 in response to dark. The authors concluded that JAZ7 plays a role in inhibiting dark-induced senescence by controlling senescence, cell death, and defense-responsive gene expression.

Fungal resistance

Thatcher et al. (2016) found JAZ7 in a very different way. Using the agriculturally destructive fungal pathogen Fusarium oxysporum, jaz T-DNA insertion alleles were screened for altered susceptibility to F. oxysporum. The jaz7-1D line was identified among other alleles to be more susceptible to F. oxysporum treatment. Interestingly, the T-DNA insertion in jaz7-1D was in the promoter region of JAZ7 and led to increased expression of JAZ7, mimicking a JAZ7 overexpression line. In contrast to the JAZ8 overexpression line and other dominant-negative JAZ lines that are less sensitive to JA (Thines et al., 2007; Chung and Howe, 2009; Shyu et al., 2012), jaz7-1D showed hypersensitive phenotypes to JA including increased JA-induced root growth inhibition and increased JA-responsive gene expression in response to stress in addition to increased susceptibility to F. oxysporum. These results suggest that jaz7-1D either functions as a dominant-negative allele, or that JAZ7 plays a positive role in promoting JA responses, which is contradictory to biochemical characterization of JAZs published to date. Even more puzzling, transgenic lines overexpressing JAZ7 did not reproduce phenotypes observed in jaz7-1D. With careful genetic characterization of jaz7-1D along with sequencing of the JAZ7 transcripts in both WT and jaz7-1D and analysis of RNAseq data from Yan et al. (2014), the authors ruled out possibilities of additional T-DNA insertions, mutations in the JAZ7 transcript, or alternative splice forms causing the jaz7-1D phenotype.

To clarify the molecular function of JAZ7, Thatcher et al. conducted a series of biochemical and molecular studies and concluded that JAZ7 is indeed a transcriptional repressor. JAZ7 interacted with co-repressor TOPLESS likely through its N-terminus EAR motif, and repressed target gene expression in transient transcriptional activity assays in an EAR-dependent manner. Though these studies support the hypothesis of JAZ7 functioning similarly to the stable transcriptional repressor JAZ8, the jaz7-1D hypersensitive phenotype is still hard to explain. The authors speculate that jaz7-1D phenotypes may be due to ectopic cell- or tissue-specific expression caused by the T-DNA insertion, or that jaz7-1D may have hypersensitive phenotypes due to sequestering transcriptional repressors such as JAM1 (Nakata et al., 2013). Interestingly, microarray and qRT-PCR analyses of jaz7-1D showed that expression of genes involved in senescence such as SAG12 and DIN11 were induced in jaz7-1D and that the jaz7-1 loss of function allele exhibited wild-type Fusarium-induced senescence responses. This seems contradictory to the observation of JAZ7 negatively regulating senescence as reported by Yu and colleagues. Taken together, it is hard to rationalize the overexpression and jaz7-1D phenotypes with the loss-of-function allele.

JAZ harmony – who does what?

Though biochemical and molecular functions of JAZ proteins are fairly conserved within the JAZ family, it has become clear that specific JAZs contribute to different biological functions that JA regulates (Box 1). Mutants in jaz7 are more responsive to dark-induced leaf senescence while jaz6 mutants are less sensitive (Yu et al., 2016). The jaz9 mutant is late flowering (Yang et al., 2012) and jaz13/jaz7/jaz8/jaz10 mutants are hypersensitive to JA-induced root growth inhibition (Thierry et al., 2015). In addition to loss-of-function phenotypes, divergent biological functions of each JAZ have been proposed based on JAZ-interacting transcription factors that regulate specific JA responses. For example, yeast two-hybrid screens using JAZ1 as bait led to the identification of AP2 transcription factors – TARGET OF EAT 1 (TOE1) and TOE2 – that target FLOWERING LOCUS T to delay flowering time (Zhai et al., 2015). Among all JAZs tested, TOE1 and TOE2 only interact with JAZ1, JAZ3, JAZ4 and JAZ9, suggesting that these specific JAZs are involved in JA-mediated flowering time regulation. These interaction-based discoveries are informative, but it is worth mentioning that most of these interactions are deduced from heterologous systems, and phenotypic
characterizations are generally performed on alleles that have altered expression of down-stream transcription factors. Most JAZ overexpression lines have no phenotypes, presumably due to their instability in the presence of bioactive JA. This is with the exception of JAZ8 and JAZ13, where overexpression of JAZ8 and JAZ13 leads to decreased sensitivity to JA-induced root growth inhibition and increased susceptibility to insect feeding (Shyu et al., 2012; Thireault et al., 2015). Overexpression of alternative splice forms of JAZ10 that produce a stabilized JAZ10 variant also leads to male sterility in addition to decreased sensitivity to JA-induced root growth inhibition (Chung and Howe, 2009). Though jaz7-1D is hypersensitive to JA-induced root growth inhibition, it is interesting that both jaz7-1D and JAZ9 overexpression lines are early flowering (Thatcher et al., 2016; Yang et al., 2012). This suggests overlapping functions for JAZ7 and JAZ9 to promote flowering.

Future perspectives

We are just starting to get a glimpse of the biological function of JAZ proteins in Arabidopsis. Characterization of jaz mutants needs to be more comprehensive and detailed to get a broader view of JAZ function. JAZ proteins are grouped into different clades based on sequence similarity (Chico et al., 2008), and thus higher order mutants are also desirable given the high degree of redundancy among JAZs (Thireault et al., 2015). The contradicting phenotypes of the jaz7-1D mutant also suggest that the COI-JAZ-TF signaling pathway may be more complex. Therefore biochemical approaches to identify JAZ complexes in vivo are needed to provide more pieces of the JA puzzle. In addition to Arabidopsis JAZs, there is a much more diverse pool of JAZ genes in monocots, particularly grasses. JA mutants in rice and maize also reveal unique roles for JA in regulating spikelet formation, stem elongation and sex determination (reviewed in Shyu and Brutnell, 2015). Given the rapid development of CRISPR technology and other genome editing tools (reviewed in Liu et al., 2016), it will become increasingly straightforward to manipulate JAZ sequences to study the biological function of JAZs in different species. This will help us decipher the complex network of JA biology in plants, and provide possibilities to fine-tune JA responses in economically important crop species.

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