Molecular basis for jasmonate and ethylene signal interactions in *Arabidopsis*

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Abstract

Jasmonate (JA) and ethylene (ET) are considered to be two essential plant hormones helping plants to tolerate infections by necrotrophic fungi. Phenotypic observations and marker gene expression analysis suggest that JA and ET act synergistically and interdependently in these defence responses. However, JA and ET also interact in an antagonistic way. JA represses ET-induced apical hook formation, while ET inhibits JA-controlled wounding responses. Although these physiological observations have been reported for more than a decade, only recently have the underlying molecular mechanisms been uncovered. Here, I review the recent advances in the understanding of these two hormone interactions and further discuss the biological significance of these apparently opposite interactions between these two hormones in orchestrating plant growth and development.

Key words: EBF1, EIL1, EIN3, ethylene, jasmonate, MYC2, phytohormone.

Introduction

As sessile organisms, plants produce different plant hormones to cope with sudden environmental changes and integrate the external information with internal hormone responses to coordinate plant growth, development, and defence. Among approximately a dozen plant hormones identified so far, jasmonate (JA) and ethylene (ET) are recognized as two crucial plant hormones, which primarily regulate plant resistance to infections by necrotrophic fungi and root hair development (Lorenzo et al., 2003; Zhu et al., 2006). Individually both JA and ET induce pathogen-responsive gene expression, while the simultaneous application of JA and ET conveys the highest expression (Xu et al., 1994; Penninckx et al., 1998). The expression of pathogen-responsive genes helps plants tolerate infections. However, in both ET- and JA-insensitive mutants, the application of JA and ET, individually and in combination, could not induce expression of these genes (Penninckx et al., 1998; Lorenzo et al., 2003). This result indicates that ET and JA control plant defence in an interdependent manner. Moreover, it is also reported that JA and ET mutually antagonize certain of each others’ functions. ET promotes apical hook formation in etiolated seedlings, which is considered to be essential for the protection of the shoot apical meristem during germination (Guzman and Ecker, 1990). JA greatly reduces apical hook angles even in the presence of ET (Turner et al., 2002). On the other hand, ET suppresses JA-induced plant resistance to herbivores (Rojo et al., 1999; Bodenhausen and Reymond, 2007). Although these synergistic and antagonistic interactions between ET and JA have been described for more than a decade, only recently have the underlying molecular mechanisms been uncovered. Here,
I will highlight the molecular basis of their interactions and discuss the biological significance of their dual interactions.

ET signal is transmitted by the bridging molecule ETHYLENE INSENSITIVE 2 (EIN2) from endoplasmic reticulum (ER)-localized ET receptors to the nuclear-localized transcription factors ETHYLENE INSENSITIVE 3 (EIN3) and its homologue EIN3 LIKE 1 (EIL1) (Zhu and Guo, 2008; Ji and Guo, 2013). EIN2 contains 12 transmembrane domains in its N-terminus and is localized on the ER membrane. The ein2 mutant is completely insensitive to ET (Alonso et al., 1999). In the presence of ET, the C-terminal end (CEND) of EIN2 is dephosphorylated and cleaved, by an unknown mechanism, to translocate into the nucleus to activate EIN3/EIL1 (Ju et al., 2012; Qiao et al., 2012; Wen et al., 2012). EIN3/EIL1 are two central transcription factors that control the majority of ET responses (Zhong et al., 2009). ET activates EIN3/EIL1 by promoting their protein accumulation through inhibiting two F-box proteins, EIN3 BINDING F-BOX PROTEIN 1 (EBF1) and EBF2, which target EIN3/EIL1 for degradation in the absence of ET (Guo and Ecker, 2003; Potuschak et al., 2003; Gagne et al., 2004; An et al., 2010). It is hypothesized that the EIN2 CEND is involved in the inhibition of EBF1/EBF2 and stabilization of EIN3/EIL1 in the nucleus (Wen et al., 2012).

JA activates its downstream transcription factors (e.g. MYC2) via a derepression mechanism. Without JA, MYC2 is repressed by interacting with a family of proteins named JASMONATE ZIM-DOMAIN PROTEINS (JAZs). JAZs recruit TOPELL co-repressor directly or through the adaptor protein NOVEL INTERACTOR of JAZ (NINJA) to inhibit MYC2 functions (Chini et al., 2007; Thines et al., 2007; Pauwels et al., 2010; Shyu et al., 2012). The bioactive JA in planta is JA-Ile, an isoleucine-conjugated form, whose formation is catalysed by a JA-amino synthetase JASMONATE RESISTANT 1 (JAR1) (Staswick and Tiryaki, 2004). JA-Ile acts as a ‘molecular glue’ to promote the interaction between JAZs and the F-box protein CORONATINE INSENSITIVE 1 (COI1), which brings about JAZ degradation to relieve their repression of MYC2. JAZ–COI1 proteins are hence known as JA co-receptors (Katsir et al., 2008; Yan et al., 2009; Sheard et al., 2010). MYC2 is the first reported primary transcription factor in JA signalling, which regulates plant wounding responses, metabolism, and root growth (Boter et al., 2004; Lorenzo et al., 2004; Dombrecht et al., 2007). Besides MYC2, JAZs also interact with several other transcription factors to control various JA responses (Pauwels and Goossens, 2011; Wager and Browse, 2012).

**JA–ET synergistic interaction**

As defence hormones, both JA and ET are required for proper plant resistance to infections by necrotrophic fungi. JA and ET interdependently and synergistically induce the expression of pathogen-responsive genes, such as PLANT DEFENSIN 1.2 (PDF1.2), to support plant tolerance against infections. Two APETALA 2/ETHYLENE RESPONSE FACTOR (AP2/ERF) domain transcription factors, ETHYLENE RESPONSE FACTOR 1 (ERF1) and OCTADECANOID-RESPONSIVE ARABIDOPSIS AP2/ERF 59 (ORA59), are required for the induction of PDF1.2 expression by the direct association of the GCC box in the PDF1.2 promoter (Brown et al., 2003; Lorenzo et al., 2003; Pre et al., 2008; Zarei et al., 2011). Although it has been suggested that ERF1 and ORA59 are essential molecules for integrating JA and ET signalling in controlling the expression of pathogen-responsive genes (Lorenzo et al., 2003; Pre et al., 2008), the direct molecular links from the JA and ET signalling molecules to ERF1 and ORA59 are still unknown.

**ERF1** and **ORA59** are direct target genes of EIN3 (Solano et al., 1998; Zander et al., 2012). Zhu et al. (2011) tested whether or not EIN3/EIL1 are the direct links for JA and ET signalling synergy. It was found that ein3 eil1 double mutants are insensitive to both JA and ET treatment in the induction of pathogen-responsive gene expression and root hair development. Furthermore, it was shown that EIN3/EIL1 physically interact with at least three JAZ members (JAZ1/3/9), which suggests that EIN3/EIL1 are another branch of JAZ-interacting transcription factors. Overexpression of stabilized JAZ1 protein suppresses the EIN3ox (EIN3 overexpression) phenotype, indicating that JAZ protein inhibits EIN3 functions in planta. In addition, chromatin immunoprecipitation (ChIP) assay shows that JA or ET treatment enhances EIN3 DNA binding ability. Next, it was shown that HISTONE DEACETYLASE 6 (HDA6) interacts with JAZs and EIN3/EIL1 as a co-repressor, while JA treatment reduces the HDA6–EIN3 interaction to release its inhibition. Consistent with this result, it was observed that the histone acetylation levels on the **ERF1** promoter are enhanced after JA treatment (Zhu et al., 2011). Taken together, this work demonstrates that JA directly activates EIN3/EIL1 through a derepression mechanism, while ET activates EIN3/EIL1 via the reported post-transcriptional regulation. These two different activation approaches are both required for fully activating EIN3/EIL1. EIN3/EIL1 protein levels are very low in ein2, whereas JAZ repressors are over-accumulated in coil, so neither ET nor JA treatment could induce pathogen-responsive gene expression in coil or ein2 due to the lack of functional EIN3/EIL1 (Fig. 1).

In addition, the mitogen-activated protein kinase (MAPK) cascade has been extensively studied in plant hormone biosynthesising and signalling pathways (Schweighofer and Meskiene, 2008). It was reported that JA activates the M KK3–MPK6 cascade to regulate pathogen-responsive gene expression positively, and MYC2-governed wounding-related gene expression and root elongation negatively. More interestingly, it has been shown that ein2 or ein3 can suppress the JA- or ET-induced higher PDF1.2 expression in the M KK3 overexpression background (Takahashi et al., 2007). This suggests that the M KK3–MPK6 cascade might play a positive role in modulating EIN3/EIL1 activities, but the identity of their kinase substrates has yet to be determined (Fig. 2).

**JA–ET antagonistic interaction**

The next challenging question is how JA and ET mutually inhibit each other’s functions. Two independent groups have
revealed fairly similar mechanisms in their recent reports that elucidate how JA/ET antagonistic regulation occurs. Surprisingly, their conclusion is that JA–ET antagonistic regulation relies on EIN3/EIL1.

We know that ET-induced apical hook formation is caused by the induction of HOOKLESS1 (HLS1) expression, which encodes a protein similar to N-acetyltransferase. Although the biochemical nature of HLS1 is still a mystery, it has been shown that HLS1 modulates auxin distribution and signalling to initiate differential elongation of hypocotyl cells and trigger hook formation (Lehman et al., 1996; Raz and Koornneef, 2001). Further studies demonstrate that EIN3 directly associates with the HLS1 promoter and induces its transcription (An et al., 2012; Chang et al., 2013).

Given the central role of HLS1 in controlling apical hook development, it is hypothesized that JA antagonizes ET’s positive role in hook development through the modulation of HLS1. Experimental results show that the hls1 mutant is insensitive to JA treatment, which suggests that HLS1 is required for JA antagonism (Song et al., 2014; Zhang et al., 2014). Furthermore, quantitative reverse transcription–PCR and HLS1 promoter-driven glucuronidase expression results show that JA down-regulates HLS1 mRNA expression even in the presence of ET. Consistent with the previous report...
that HLS1 is a direct target gene of EIN3, the expression of HLS1 in ein3-1 eil1 is lower than in the wild type, and JA could not further reduce its expression in ein3-1 eil1. Overall, it is concluded that JA antagonizes ET-induced hook formation by repressing EIN3/EIL1-controlled HLS1 expression (Song et al., 2014; Zhang et al., 2014).

In order to look into how JA represses EIN3/EIL1 functions, Song et al. (2014) focused on MYC2, the first identified but most prominent JAZ-interacting transcription factor that regulates the majority of JA responses (Kazan and Manners, 2013). It was shown that MYC2 and its closest homologues MYC3 and MYC4 function redundantly in mediating JA’s inhibition of ET-induced hook formation. MYC3 and MYC4 are two reported JAZ-interacting transcription factors and act additively with MYC2 in controlling JA responses (Cheng et al., 2011; Fernandez-Calvo et al., 2011; Niu et al., 2011). The expression level of HLS1 in myc234 (referring to the myc2 myc3 myc4 triple mutant) is higher than in the wild type and JA could not repress HLS1 expression in myc234 (Song et al., 2014). This result implies that MYC2 is a negative regulator of HLS1 expression and JA negatively regulates HLS1 expression through MYC2 family proteins. It is further revealed that MYC2 physically interacts with EIN3 and EIL1. Through a dual luciferase reporter assay, it was shown that the expression of EIN3 or EIL1 protein induces HLS1 promoter-driven luciferase (P_{HLS1-LUC}) expression; however, simultaneously expressing MYC2 greatly inhibits P_{HLS1-LUC} expression (Song et al., 2014). This result indicates that MYC2 directly represses EIN3 transcriptional activity. Zhang et al. (2014) also found that MYC2 interacts with EIN3/EIL1 and further reported that MYC2 abrogates EIN3 DNA binding ability in an in vitro electrophoretic mobility shift assay (EMSA). In support of these in vitro studies, in vivo co-immunoprecipitation and ChIP assay results show that although MYC2 interacts with EIN3, MYC2 could not associate with the EIN3 binding site (EBS) on the HLS1 promoter, which further proves that the interaction between MYC2 and EIN3 blocks the association between EIN3 protein and the HLS1 promoter (Zhang et al., 2014). In addition to the repression of HLS1, MYC2 also represses the expression of ERF1 through the inhibition of EIN3/EIL1 (Song et al., 2014). Taken together, these findings demonstrate that JA-activated MYC2 interacts with EIN3/EIL1 and represses their DNA binding ability, thereby reducing the expression of EIN3/EIL1 target genes, such as HLS1 and ERF1 (Fig. 2).

Moreover, Song et al. (2014) also found that EIN3/EIL1 repress the expression of a MYC2-controlled wounding-responsive gene on the basis of their reciprocal interactions. It was reported that ET suppresses JA-induced wounding responses, and the expression of wounding-responsive genes is higher in the ein2 mutant (Rojo et al., 1999; Lorenzo et al., 2004). Although it is not clear whether EIN3 directly abrogates MYC2 DNA binding ability or recruits chromatin remodelling factors to repress MYC2 functions, the study of Song et al. (2014) at least provides a clue for further exploring how ET antagonizes JA functions.

Besides this layer of transcriptional regulation, Zhang et al. (2014) additionally uncovered a post-transcriptional regulation model for JA-stimulated inhibition of EIN3 functions. They found that EIN3 and EIL1 protein abundance is decreased after JA treatment even in the presence of ET, while JA cannot alter the EIN3 protein level when epitagged EIN3 is expressed in the ebf1 ebf2 background. This result indicates that JA controls EIN3/EIL1 protein accumulation in an SCF^{EBF1/EBF2}-dependent manner. It was further reported that JA induces the expression of EBF1 but not EBF2, and this induction is diminished in the myc2 mutant. There are two putative MYC2 binding sites (MBS1 and MBS2; CACATG) located in the EBF1 promoter. MBS1 is in the –1032 to –1072 region and MBS2 is in –341 to –336 region (base pairs before of the start codon). EMSA and ChIP experiments demonstrate that MYC2 preferentially associates with the MBS1 region. The JA-triggered EIN3 protein degradation is diminished in ebf1 and myc2 mutants (Zhang et al., 2014). These observations demonstrate that JA induces EBF1 expression through MYC2 to promote EIN3/EIL1 turnover.

Taken together, these two separate studies reveal that JA-activated transcription factor MYC2 negatively regulates EIN3/EIL1 functions through two distinct mechanisms: (i) MYC2 physically interacts with EIN3/EIL1 to inhibit their transcriptional ability mutually; and (ii) MYC2 induces the expression of the F-box gene EBF1 to trigger EIN3/EIL1 degradation. These two mechanisms work together to completely repress EIN3/EIL1 and their downstream gene expression to suppress hook development and defence, which nicely explains how JA antagonizes ET’s physiological responses (Fig. 2).

Chemical modification is another effective form of regulation for balancing hormone homeostasis. 1-Aminocyclopropane-1-carboxylic acid (ACC) is the limiting precursor for ET synthesis. Gas chromatography–mass spectrometry analysis shows that JA–ACC conjugates exist in Arabidopsis leaf extracts. Physiological experiments demonstrate that in contrast to JA or ACC, JA–ACC is inactive for inhibiting root elongation (Staswick and Tiryaki, 2004). The conjugation or hydrolysis of JA–ACC can therefore control the supply of JA or ACC for further conversion into the active JA-Ile or ET, thus adding an additional layer of regulation of JA–ET signal interactions (Fig. 2).

**Perspective**

Why do both synergistic and antagonistic JA–ET interactions co-exist in plants, and why do these complex interactions occur on EIN3/EIL1 transcription factors? JA and ET are two essential hormones regulating defence and various growth responses. When necrotrophic fungi infect plants, both JA and ET are quickly synthesized as a protective mechanism. The cooperative interactions between JA and ET ensure that plants express plant defensins (such as PDF1.2) and other pathogen-responsive secondary metabolites under precise control. Furthermore, the induction of pathogen-responsive genes must be tightly controlled to avoid their excessive effects because some pathogen-responsive gene products are associated with cell death for defence purposes.
and the long-term activation of EIN3/EIL1 inhibits cell elongation (Solano et al., 1998). For instance, in some constitutively activated JA or ET signalling mutants (cev1, cev1, or ctrl), although the defence genes are strongly expressed, those plants have stunted growth most probably due to the higher activation of EIN3/EIL1 (Kieber et al., 1993; Ellis and Turner, 2001; Xu et al., 2001; Jensen et al., 2002). The most significant evidence comes from ebf1 ebf2 double mutants, which are lethal because of the overaccumulation of EIN3/EIL1 proteins. It has been shown that ein3 eil1 completely rescue ebf1 ebf2 in the ebf1 ebf2 ein3 eil1 quadruple mutants, suggesting the importance of controlling EIN3/EIL1 levels (Gagne et al., 2004; An et al., 2010).

For plants, one clever strategy for balancing defence and growth trade-off is to have an intrinsic fine-tuning mechanism that gradually turns off the defence machinery when the defence war starts. JA or ET rapidly induce ERF1 expression and extend its expression to the peak at 6 h, but reduce its expression after 10 h (Lorenzo et al., 2003). Now we know that the mechanism is the repression of EIN3/EIL1 after they are activated by the stimulus.

EIN3/EIL1 and MYC2 are two classes of JA-activated transcription factors. After JA application, both of them are activated through the removal of JAZ repressors. In order to orchestrate plant growth and defence in the most energy-efficient way, plants take advantage of the already activated MYC2 to suppress its partner (EIN3/EIL1) and vice versa. However, which factor determines the final output is still a mystery. As discussed above, JA constantly suppresses EIN3/EIL1 functions in the apical hook to down-regulate HSL1 expression but initially activates EIN3/EIL1 for ERF1 induction. It is speculated that hormone–hormone interactions may have tissue type- or cell type-specific preferences. It has already been reported that the co-repressor protein NINJA mainly functions in roots (Acosta et al., 2013), which suggests that the JA signalling pathway differs in various tissues. It will be worthwhile exploring whether or not the JAZ–HDA6 modules have cell type specificity for the inhibition of EIN3/EIL1.

Although these recent studies greatly advance our understanding of JA–ET signal interactions, there are still several questions waiting to be answered. (i) Does EIN3/EIL1 directly affect MYC2 DNA binding ability? (ii) Since the phosphorylation and degradation of MYC2 is required for its activity (Zhai et al., 2013), do EIN3/EIL1 affect MYC2 protein modifications? (iii) Given that both MYC2 and EIN3/EIL1 interact with JAZs and DELLA proteins (transcriptional regulators in gibberellin signalling) and that JAZs also interact with DELLA (Chini et al., 2007; Thines et al., 2007; Hou et al., 2010; Zhu et al., 2011; An et al., 2012; Hong et al., 2012), are there any competitive interactions among these regulatory proteins for coordinating different hormone responses? (iv) Given that MYC2 and EIN3/EIL1 are able to bind different cis-elements, do their protein–protein interactions alter their DNA binding preferences in the genome? (v) Which factor controls JA–ACC conjugation or hydrolysis when plants are under stress? (vi) How does JA activate MKK3 and what is the substrate protein for MPK6 in JA signalling?

Overall, the studies of JA–ET interactions shed new light on our understanding of hormone signalling. With greater advancements in next-generation sequencing techniques and single cell detection approaches, we can expect progressively more exciting achievements in plant hormone studies. For instance, it will be possible to dissect transcriptome profiling and transcription factor binding preferences through single cell RNA-seq and ChIP-seq methods to compare the differences in hormone signalling in different tissue types.

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References


