Regulation of ORA59, a key modulator of disease resistance in Arabidopsis

Körbes, A.P.

Citation

Körbes, A. P. (2010, June 24). *Regulation of ORA59, a key modulator of disease resistance in Arabidopsis*. Retrieved from https://hdl.handle.net/1887/15722

Version:	Corrected Publisher's Version
License:	Licence agreement concerning inclusion of doctoral thesis in the Institutional Repository of the University of Leiden
Downloaded from:	https://hdl.handle.net/1887/15722

Note: To cite this publication please use the final published version (if applicable).

CHAPTER 1

General introduction

Ana Paula Körbes and Johan Memelink

Stress signaling in plants

Plants are exposed to many forms of stress, including pathogen and herbivore attack, or adverse light, water, temperature, nutrient or salt conditions. Due to their sessile life style, plants are only able to survive by the ability to build up fast and highly adapted responses to these diverse environmental stresses. To effectively avoid invasion by microbial pathogens and herbivorous insects, plants have evolved sophisticated mechanisms to provide several strategic layers of constitutive and induced defenses. Preformed physical and biochemical barriers constitute the first line of defense and fend off the majority of pathogens and insects. However, when a pathogen or herbivore overcomes or evades these constitutive defenses, recognition of pathogen-derived or insect-induced signal molecules by plant receptors leads to the activation of a concerted battery of defense responses designed to prevent further pathogen spread or plant damage.

Perception of stress signals often results in the biosynthesis of one or more of the major secondary signaling molecules jasmonates (JAs; Balbi and Devoto, 2008; Turner et al., 2002; Wasternack, 2007), ethylene (ET; Wang et al., 2002; Guo and Ecker, 2004) and salicylic acid (SA; Shah et al., 2003). Production of one or more of these hormones generates signal transduction networks that lead to a cascade of events responsible for the physiological adaptation of the plant to the external stress. In general, it can be stated that defense against pathogens with a biotrophic lifestyle is mediated by the SA signal transduction route, whereas responses to wounding and insect herbivory are mediated by JA and attack by necrotrophic pathogens triggers JA/ET-dependent responses (Dong, 1998; Glazebrook, 2005; Howe and Jander, 2008). Over the past decade, it has become increasingly clear that a plant's resistance to attack is not brought about by the isolated activation of parallel, linear hormonal pathways, but rather is the consequence of a complex regulatory network that connects the individual pathways, enabling each to assist or antagonize the others (Grant and Jones, 2009; Pieterse et al., 2009). The JAs, ET and SA signal transduction pathways act synergistically or antagonistically in a variety of responses, leading to fine-tuning of the complex defense response (Kunkel and Brooks, 2002; Pieterse et al., 2009). These signaling pathways affect each other through extensive cross-talk occurring at different levels (Pieterse et al., 2009). Whereas SA works mainly antagonistically to jasmonates, ET can have either synergistic or antagonistic effects on certain subsets of genes regulated by jasmonates. Genes encoding proteins involved in defense against necrotrophic pathogens, such as the anti-microbial plant defensin PDF1.2, are synergistically induced by a combination of JA and ET, whereas genes encoding proteins involved in defense against herbivorous insects, such as the acid phosphatase VSP1, are strongly induced by JA alone and ET has a strong negative effect on the JA response. In addition other factors, such as growth conditions, tissue type and age, and other hormones such as abscisic acid, affect the response output to JAs and ET (Pauwels et al., 2008; De Vleesschauwer et al., 2010).

Jasmonate signal perception

Jasmonic acid (JA) and its cyclic precursors and derivatives, collectively referred to as jasmonates (JAs), constitute a family of bioactive oxylipins that regulate plant responses to environmental and developmental cues (Balbi and Devoto, 2008; Turner et al., 2002; Wasternack, 2007). These signaling molecules affect a variety of plant processes including fruit ripening (Creelman and Mullet, 1997), production of viable pollen (Feys et al., 1994; McConn and Browse, 1996; Sanders et al., 2000; Stintzi and Browse, 2000), root elongation (Staswick et al., 1992), tendril coiling (Devoto and Turner, 2003), response to wounding (Zhang and Turner, 2008) and abiotic stresses, and defense against insects (McConn et al., 1997) and necrotrophic pathogens (Thomma et al., 1999). There is evidence that the jasmonates 12-oxo-phytodienoic acid (OPDA), JA, and methyl-jasmonic acid (MeJA) act as active signaling molecules (Wasternack, 2007). It now appears that a highly active jasmonate is (+)-7-*iso*-Jasmonoyl-L-Isoleucine (Fonseca et al., 2009), which is perceived by the receptor CORONATINE INSENSITIVE1 (COI1; Fonseca et al., 2009; Katsir et al., 2008; Yan et al., 2009). (+)-7-*iso*-JA-L-Ile (in short JA-Ile) is synthesized from (+)-7-*iso*-JA, which is produced by the octadecanoid pathway for jasmonate biosynthesis (Wasternack, 2007), by conjugation to Isoleucine by the enzyme JASMONATE RESISTANT1 (JAR1; Staswick and Tiryaki, 2004). Remarkably, many COI1-dependent, wound-responsive genes are expressed normally in a *jar1* mutant (Chung et al., 2008; Suza and Staswick, 2008). The *jar1* mutant still produces 10-25% of wild-type levels of JA-Ile, which may be sufficient to support gene expression. Alternatively other jasmonates might serve as signaling molecules. It remains to be determined whether COI1 serves as a receptor for other jasmonates besides JA-Ile.

JA-responsive promoter elements

The expression of a gene is determined by the *cis*-acting DNA elements located in the vicinity of the gene and the *trans*-acting protein factors that interact with them. In general, these *cis*-acting elements are concentrated in a relatively small promoter region of a few hundred nucleotides upstream of the transcriptional start site, although there are examples of regulatory sequences located at a distance of several thousands of nucleotides from the gene they control. Several *cis*-acting elements in various gene promoters that mediate jasmonate responsiveness have been identified. The most common jasmonate-responsive promoter sequences are the GCC motif and the G-box. In addition several other jasmonate-responsive promoter elements have been reported.

In the promoter of the terpenoid indole alkaloid biosynthesis gene *strictosidine synthase* (*STR*) from *Catharanthus roseus* a jasmonate- and elicitor-responsive element (JERE) has been identified (Menke et al., 1999). Mutation or deletion of this JERE results in an inactive and unresponsive *STR* promoter derivative. A tetramer of the JERE fused to a minimal promoter confers MeJA-responsive gene expression on a reporter gene, showing that the JERE is an

autonomous MeJA-responsive sequence (Menke et al., 1999). Within this JERE a GCC-box-like sequence is present. In *Arabidopsis*, a GCC motif (GCCGCC) plays a role in conferring JA responsiveness to the *PDF1.2* promoter (Brown et al., 2003). The GCC motif has also been shown to function autonomously as an ethylene-responsive element (Ohme-Takagi and Shinshi, 1995; Fujimoto et al., 2000). The *PDF1.2* gene is synergistically induced by a combination of JA and ET (Penninckx et al., 1998), which is likely caused by a convergent action of both signals on the GCC motif. However, not all GCC motifs confer JA- and ET-responsive gene expression, since the *STR* gene does not respond to ET (Memelink, unpublished results). This may be due to the sequence of the *STR* GCC motif (GACCGCC), which differs slightly from the consensus sequence.

The G-box (CACGTG) or G-box-like sequences (e.g. AACGTG) that are essential for the jasmonate response were found in the promoters of the potato *PIN2* gene (Kim et al., 1992), the soybean vegetative storage protein B gene (*VSPB*; Mason et al., 1993), the Arabidopsis *VSP1* gene (Guerineau et al., 2003), the tomato leucine aminopeptidase gene (*LAP*; Boter et al., 2004), the tobacco putrescine N-methyltransferase 1a gene (PMT1a; Xu and Timko, 2004) and the Octadecanoid-derivative Responsive *Catharanthus* AP2-domain gene (*ORCA3*; Vom Endt et al., 2007). Also, analysis of the promoters of JA-responsive genes showed that the G-box element was statistically significantly over-represented (Mahalingam et al., 2003). In the tomato *LAP* promoter, the G-box-like sequence is flanked by another sequence characterized by a GAGTA repeat, which is also essential for JA-responsive expression (Boter et al., 2004). In the *NtPMT1a* promoter, the G-box is flanked by a GCC motif, and both sequences are essential for MeJA-responsive promoter activity (Xu and Timko, 2004). In the *ORCA3* promoter the G-box-like sequence is flanked by an A/T-rich sequence which is important for the expression level (Vom Endt et al., 2007).

Several additional JA-responsive promoter sequences have also been reported. TGACG (*as-1*-type) sequences were found to be essential for JA inducibility of the promoter of the *Agrobacterium tumefaciens* T-DNA nopaline synthase (*nos*) gene in tobacco (Kim et al., 1993, 1994) and of the barley lipoxygenase 1 gene promoter (*LOX1*; Rouster et al., 1997). A monomer or a tetramer of the *as-1* sequence from the Cauliflower Mosaic Virus (*CaMV*) *35S* promoter also conferred JA-responsive expression to a reporter gene in transgenic tobacco (Xiang et al., 1996). Two jasmonate-responsive elements, JASE1 (5’-CGTCAATGAA-3’) and JASE2 (5’-CATACGTCGTCAA-3’), were identified in the promoter of the *OPR1* gene in *Arabidopsis* (He and Gan, 2001). JASE1 is a new motif without any signature sequence so far reported, whereas JASE2 possesses an ACGT core which is also found in the G-box and in *as-1*-type elements. In the Long Terminal Repeat (LTR) promoter of the tobacco retrotransposon *Tto*1 a 13 bp element, which contains a box L/AC-I or H-box-like motif, is involved in responsiveness to MeJA (Takeda et al., 1998).

In conclusion, a variety of jasmonate-responsive elements appear to exist. The best characterized elements are the G-box and closely related variants, which are commonly found in promoters that respond to jasmonates and are negatively affected by ET, and the GCC motif, which is commonly present in promoters that respond in a synergistic manner to JAs combined

with ET. It has been well established that the JAs-responsive activity of promoters containing the GCC motif (e.g. *PDF1.2*; Lorenzo et al., 2003) or the G-box (e.g. *VSP*; Benedetti et al., 1995) is dependent on COI1. For promoters containing other elements COI1 dependency has not been established. The *OPR1* gene for example, containing the JASE1/2 motifs in its promoter, has been shown to be wound-inducible in a *coi1* mutant background (Reymond et al., 2000), and is inducible by OPDA but not by JA in an *opr3* mutant background (Stintzi et al., 2001). Therefore it remains to be established whether so-called JA-responsive elements other than the GCC motif and the G-box confer responses to bioactive JAs via COI1.

Transcription factors and JA responses

JAZ repressors and COI1 control the activity of AtMYC2

To identify molecular components of jasmonate signal transduction, screenings for *Arabidopsis* mutants that are insensitive to (Me)JA or to coronatine (a bacterial toxin which is a structural and functional analogue of JA-Ile) or that show constitutive jasmonate responses have been performed (Lorenzo and Solano, 2005; Browse, 2009).

The *coi1* mutant was isolated in a screen for *Arabidopsis* mutants insensitive to root growth inhibition by coronatine (Feys et al., 1994). The *coi1* mutant is also insensitive to JAs (Feys et al., 1994), is defective in resistance to certain insects and pathogens and fails to express jasmonate-regulated genes (Turner et al., 2002). The *COI1* gene encodes an F-box protein (Xie et al., 1998). F-box proteins associate with cullin, Skp1 and Rbx1 proteins to form an E3 ubiquitin ligase known as the SCF complex, where the F-box subunit functions as the specificity determinant targeting proteins for ubiquitin-mediated proteolysis by the 26S proteasome (Del Pozo and Estelle, 2000). Co-immunoprecipitation experiments showed that COI1 associates *in vivo* with Skp1, cullin and Rbx1 proteins to form the SCF^{COI1} complex (Devoto et al., 2002; Xu et al., 2002). Plants that are deficient in other components or regulators of SCF complexes, including AXR1, COP9 and SGT1b, also show impaired jasmonate responses (Lorenzo and Solano, 2005). The existence of a *COI1* function in species other than *Arabidopsis* was demonstrated in tomato (Li et al., 2004), soybean (Wang et al., 2005), *Nicotiana attenuata* (Paschold et al., 2007), tobacco (Shoji et al., 2008) and potato (Halim et al., 2009). COI1 is a component that is specific to the JA pathway, whereas SGT1b and AXR1 are shared by other signaling pathways. Mutations in *AXR1* or *SGT1b* have pleiotropic effects that impair plant responses not only to JA but also to auxin and pathogens, suggesting that both SGT1b and AXR1 are regulators of SCF complexes and are involved in several different signaling pathways (Austin et al., 2002; Azevedo et al., 2002; Gray et al., 2003).

A particularly effective screen for jasmonate signaling mutants has been described by Lorenzo et al. (2004). Screening for mutants affected in JA-induced root growth inhibition in an *ethylene-insensitive*3 (*ein3*) background resulted in the identification of 5 loci called *JA-insensitive* (*JAI*) 1-5. The *JAI1* locus corresponds to the *AtMYC2* gene (Lorenzo et al., 2004), encoding a basic-

Helix-Loop-Helix (bHLH) transcription factor which regulates a subset of jasmonate-responsive genes involved in wounding responses and resistance against insects (Boter et al., 2004; Lorenzo et al., 2004; Dombrecht et al., 2007). Recombinant AtMYC2 binds *in vitro* to the G-box and related sequences (De Pater et al., 1997; Chini et al., 2007; Dombrecht et al., 2007). The *JAI2* locus corresponds to the previously characterized *JAR1* gene (Staswick et al., 1992), encoding an enzyme that couples JA to amino acids with a preference for isoleucine (Staswick and Tiryaki, 2004). The *JAI4* locus corresponds to the *SGT1b* gene (Lorenzo and Solano, 2005). The *JAI5* locus corresponds to the *COI1* gene (Lorenzo et al., 2004).

Recently, the gene affected in the *jai3* mutant was identified. It encodes a protein with a zinc finger-like ZIM motif (Chini et al., 2007). There are several related genes in *Arabidopsis* forming a gene family called ZIM or TIFY (Vanholme et al., 2007). The 12 members that are induced at the gene expression level by JAs are called Jasmonate ZIM domain (JAZ) proteins (Chini et al., 2007; Thines et al., 2007). They contain in addition to the highly conserved central ZIM domain a highly conserved C-terminal Jas domain and a less conserved N-terminal region. In the *jai3* mutant an aberrant protein is expressed with a deletion of the C-terminal region including the Jas domain. The wild-type JAI3 (or JAZ3) protein is rapidly degraded in response to JA in a COI1-dependent manner, whereas the jai3 mutant protein is stable. The JAI3/JAZ3 protein was shown to interact *in vitro* and in yeast with AtMYC2. Based on these findings it was postulated that JAI3 is a repressor of AtMYC2 which is rapidly degraded in response to JA thereby activating AtMYC2 (Figure 1; Chini et al., 2007). However it remains to be experimentally demonstrated that the full-length JAI3/JAZ3 protein is indeed a repressor of gene expression. There are many examples of full-length transcription factors which have opposite activities to certain deletion derivatives (e.g. Gill and Ptashne, 1988; Fan and Dong, 2002; Miao and Lam, 1995).

JAZ variants lacking effective Jas domains also occur naturally in *Arabidopsis*. For JAZ10.1, two more stable variants have been described which are translated from alternatively spliced mRNAs. JAZ10.3 misses a few amino acids at the C terminus, making it more stable in response to jasmonate (Chung and Howe, 2009), and therefore it has dominant-negative effects on jasmonate responses when overexpressed (Yan et al., 2007). The splice variant JAZ10.4 lacks the entire Jas domain, rendering it completely stable and turning it into a strong dominant-negative repressor when overexpressed (Chung and Howe, 2009).

In independent studies, members of the *JAZ* gene family in *Arabidopsis* were characterized as being predominant among genes induced in anthers after 30 min of JA treatment (Mandaokar et al., 2006). Subsequent study of the family member JAZ1 demonstrated that it is rapidly degraded in response to JA in a COI1-dependent manner (Thines et al., 2007). On the other hand a deletion derivative of JAZ1 lacking the C-terminal region including the Jas domain is stable. Interestingly, these authors were able to detect interaction between JAZ1 and COI1 in a yeast two-hybrid assay in the presence of JA conjugated to Ile (JA-Ile) in the yeast growth medium or in an *in vitro* pull-down assay in the presence of JA-Ile (Thines et al., 2007). No interaction was detected in the presence of OPDA, JA, MeJA or JA conjugated to Trp or Phe, whereas JA-Leu was about 50-fold less effective in promoting interaction between COI1 and JAZ1 than JA-Ile. JA-Ile

and JA-Leu are products of the JAR1-mediated conjugation reaction (Staswick and Tiryaki, 2004). JA-Ile and coronatine were also shown to promote the interaction between JAZ3 and JAZ9 in a yeast two-hybrid assay, whereas JA or MeJA are ineffective (Melotto et al., 2008). The C-terminal regions containing the conserved Jas domain of tomato JAZ1 (Katsir et al., 2008) and *Arabidopsis* JAZ1, JAZ3, JAZ9 (Melotto et al., 2008) and JAZ10.1 (Chung and Howe, 2009) were shown to be necessary for binding to COI1 in a JA-Ile or coronatine dependent manner. In addition it was shown that the Jas domains of tomato JAZ1 (Katsir et al., 2008) and *Arabidopsis* JAZ1, JAZ3, and JAZ9 (Melotto et al., 2008) are sufficient for binding to COI1 in a JA-Ile or coronatine dependent manner.

Using tomato SlCOI1 and SlJAZ1, it was shown that the complex binds radiolabeled coronatine (Katsir et al., 2008). Binding can be displaced with unlabeled coronatine or JA-Ile. Combined with the coronatine-dependent interaction between COI1 and JAZ proteins in yeast, these experiments provide strong evidence that COI1 is the receptor for at least certain JAs including JA-Ile, as well as for the microbial JA-Ile mimic coronatine. They do not exclude, however, the possibility that proteins co-purified with COI1 from the plant extracts function as JA receptor. A recent report combining purified COI1 (expressed *in vitro* in insect cell lysates) with surface plasmon resonance and photoaffinity labelling technology provided more evidence that COI1 directly binds JA-Ile and coronatine (Yan et al., 2009).

The expression of the *JAZ* genes in *Arabidopsis* is induced by JA (Mandaokar et al., 2006; Chini et al., 2007; Thines et al., 2007; Yan et al., 2007) and is controlled by AtMYC2 (Chini et al., 2007). The model is therefore that AtMYC2 and JAZ proteins form a jasmonate-responsive oscillator, where JAZ proteins negatively regulate AtMYC2 activity at the protein level, JAs cause JAZ degradation and AtMYC2 activation, and AtMYC2 switches on the expression of JAZ repressors at the gene level (Figure 1). Homo- and heterodimerization of JAZ proteins likely play important roles in AtMYC2 gene repression and in the interaction with COI1 (Chini et al., 2009; Chung and Howe, 2009), although it remains to be formally proven that the complexes are dimers and not higher order complexes. Although there are some discrepancies in the two reports (Chini et al., 2009; Chung and Howe, 2009), it can be concluded that most JAZ proteins are able to form homo- and heterodimers. Specific amino acids in the TIFY motif are important for dimer formation mediated by the ZIM domain (Chung and Howe, 2009). Interestingly, the dominant-negative effect of the naturally occurring splice variant JAZ10.4, which is stable due to the absence of the Jas domain, depends on a functional ZIM domain (Chung and Howe, 2009), which implies that the functional repressing unit is a JAZ (hetero)dimer. It has been reported that expression of the jai3 (JAZ3ΔJas) protein stabilizes other full-length JAZ proteins *in trans* (Chini et al., 2007). This phenomenon can be explained by assuming that the jai3 protein heterodimerizes with other JAZ proteins and thereby stabilizes them, although the molecular mechanism for such stabilization remains to be determined.

The picture that emerges for jasmonate signal transduction is highly reminiscent of auxin signal transduction. In the absence of auxin, auxin-responsive gene expression is inhibited by the action of Auxin/Indole-3-Acetic Acid (Aux/IAA) repressors which bind to ARF (Auxin

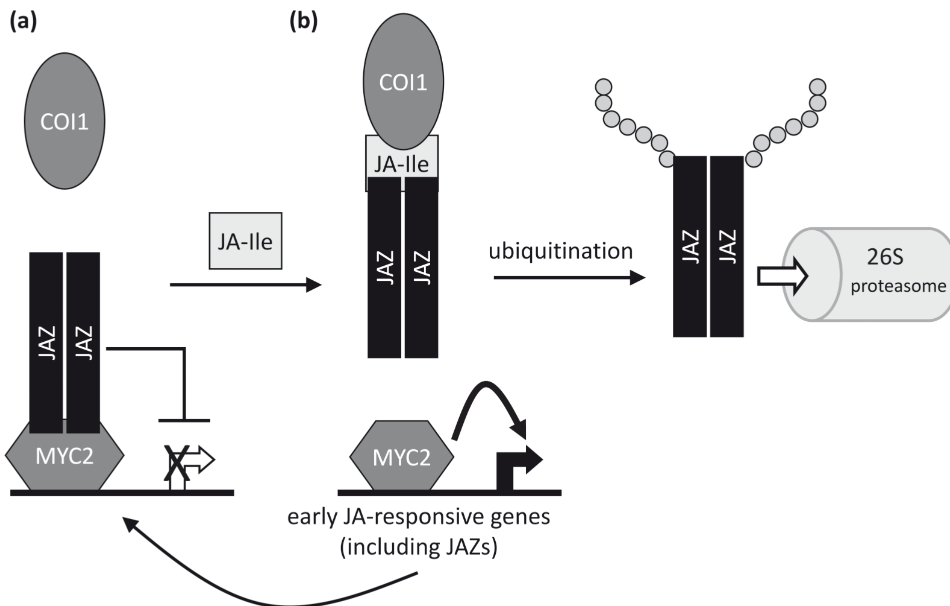

Figure 1. Model for regulation of jasmonate-responsive gene expression by AtMYC2 and JAZ proteins. Although depicted as a single protein, COI1 forms part of the putative E3 ubiquitin ligase SCF^{COI1}. **(a)** In the absence of JA-Ile, a (hetero)dimer of JAZ proteins interacts with AtMYC2 maintaining this transcription factor inactive. **(b)** JA-Ile promotes the interaction between JAZ and COI1. SCF^{COI1} causes the ubiquitination of JAZ resulting in degradation by the 26S proteasome. AtMYC2 is liberated and activates transcription of target genes, including the genes encoding JAZ proteins, resulting in a negative feedback loop.

Response factor) transcriptional activators. The F-box protein TRANSPORT INHIBITOR RESPONSE PROTEIN1 (TIR1) is the auxin receptor (Kepinski and Leyser, 2005; Dharmasiri et al., 2005). Auxin acts as the molecular glue between TIR1 and Aux/IAA proteins (Tan et al., 2007), resulting in their ubiquitination (Maraschin et al., 2009) and degradation (Guilfoyle, 2007). COI1 is the closest relative to TIR1 that is not related to auxin perception among the about 700 members of the *Arabidopsis* F-box protein family (Gagne et al., 2002).

AP2/ERF-domain transcription factors and jasmonate responses

In *Arabidopsis*, the AP2/ERF-domain transcription factor family comprises 122 members (Nakano et al., 2006). The expression of the AP2/ERF gene *ORA59* is induced by JA or ET, and is synergistically induced by both hormones (Pré et al., 2008). Genome-wide microarray analysis showed that overexpression of the *ORA59* gene resulted in increased expression of a large number of JA- and ET-responsive defense genes, including *PDF1.2*. Plants overexpressing *ORA59*

were more resistant to infection by the necrotrophic fungus *Botrytis cinerea*.

Plants overexpressing ERF1, a closely related member of the AP2/ERF-domain family, were previously shown to have an elevated *PDF1.2* expression level (Solano et al., 1998; Lorenzo et al., 2003) and to be more resistant to *B. cinerea* (Berrocal-Lobo et al., 2002). Similar to *ORA59* expression, the *ERF1* gene is synergistically induced by JA and ET (Lorenzo et al., 2003). These similarities in gene expression patterns and in target gene sets, as well as the fact that they are close homologues in the AP2/ERF-domain family, suggest that ORA59 and ERF1 have redundant functions in JA and ET signal transduction. However, an essential role for ORA59 as an integrator of the JA and ET signals leading to regulation of defense genes was demonstrated with plants where the *ORA59* gene was silenced via an RNAi approach (Pré et al., 2008). In response to JA and/or ET, or after infection with the necrotrophic fungi *B. cinerea* or *Alternaria brassicicola,* expression of *PDF1.2* and other defense genes was blocked in *ORA59*-silenced plants. As expected from the dramatic effect on defense gene expression, the silenced plants were also more susceptible to *B. cinerea* infection. The results demonstrate that ORA59 integrates JA and ET signal inputs to coordinate the appropriate gene expression response directed against pathogen attack (Figure 2). An evaluation of whether ERF1 has essential roles or whether it is a dispensable functionally redundant transcription factor awaits analysis of *ERF1* knock-out mutants.

The transcription factor AtERF2, encoded by a JA-inducible gene, has also been reported to control the expression of JA/ET-responsive genes including *PDF1.2* (Brown et al., 2003; McGrath et al., 2005). In addition, overexpression of the related transcription factor AtERF1 (which is also encoded by a JA-inducible gene, and which is different from ERF1) led to increased levels of *PDF1.2* expression (Pré et al., 2008). These observations apparently contradict the finding that loss-of-function of ORA59 by RNAi abolishes *PDF1.2* expression in response to JA, to combined JA/ET treatment or to infection with *B. cinerea* or *A. brassicicola* (Pré et al., 2008), indicating that no other AP2/ERF domain transcription factor or member of another class of transcriptional regulators was able to activate the expression of *PDF1.2* in response to these treatments. In experiments where transcription factors were inducibly expressed in transgenic plants or transiently expressed in protoplasts AtERF1 and AtERF2 failed to activate *PDF1.2* expression in contrast to ORA59 and ERF1 (Pré et al., 2008). One possible explanation for these observations is that overexpression of AtERF1 or AtERF2 causes a stress condition leading non-specific expression of defense genes including *PDF1.2*, whereas ORA59 and ERF1 are *bona fide* direct regulators of *PDF1.2*.

AtERF4 differs from the AP2/ERF-domain transcription factors encoded by JA-responsive genes described above by the presence of an ERF-associated amphiphilic repression (EAR) motif in the C-terminal part of the protein. The EAR motif has been shown to function as an active repressor of transcription (Ohta et al., 2001). The *AtERF4* gene is induced by JA (McGrath et al., 2005; Yang et al., 2005), ET (Fujimoto et al., 2000; Yang et al., 2005), infection with *Fusarium oxysporum* (McGrath et al., 2005) or wounding (Cheong et al., 2002). Overexpression of *AtERF4* had no effect on the basal transcript level of several JA-responsive genes in untreated plants. However, upon JA and/or ET treatment, *AtERF4*-overexpressing plants showed significantly

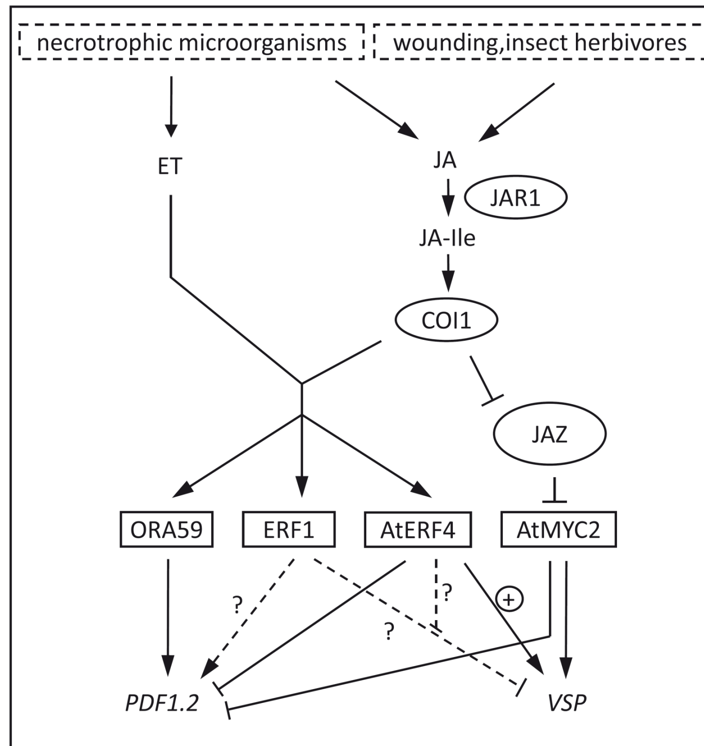

Figure 2. Model for the role of transcription factors in the stress-responsive network involving the JA and ET signaling pathways. Different types of biotic or abiotic stress, including wounding, attack by herbivorous insects and infection with necrotrophic pathogens, induce the synthesis of JA and related oxylipins. JAR1 converts JA into the biologically active JA-Ile. Some stress signals such as infection with necrotrophic pathogens simultaneously induce ET biosynthesis. JAs induce the expression of several genes encoding transcription factors, including ORA59, ERF1, AtERF4 and AtMYC2 via COI1, an F-box protein that is the receptor for JA-Ile. Binding of JA-Ile results in COI1-mediated degradation of JAZ repressors via the ubiquitin/proteasome pathway, thereby releasing AtMYC2 from repression. The bHLH-type transcription factor AtMYC2 positively regulates the expression of wound-responsive genes (e.g. *VSP*) and represses other genes, including *PDF1.2*. The JA and ET signals cooperate to induce the expression of genes encoding the AP2/ERF-domain transcription factors ORA59, ERF1 and AtERF4. ORA59 is the key regulator of JA/ET-responsive genes including *PDF1.2*, whereas the role of ERF1 in gene regulation remains unclear and awaits analysis of a knockout mutant (indicated by dashed lines and question marks). Conversely, AtERF4 represses the induction of JA/ET-responsive genes including *PDF1.2*. AtERF4 also enhances the JA-induced expression of AtMYC2 target genes including *VSP* (circled plus), possibly by repressing the negative effect of ET executed by ERF1 (dashed bar line and question mark).

lower induction of a subset of JA- and ET-responsive genes, including *PDF1.2,* compared to control plants (McGrath et al., 2005; Pré, 2006). On the other hand, plants in which *AtERF4* expression was silenced via T-DNA insertion (McGrath et al., 2005) or via RNAi (Pré, 2006)

showed increased *PDF1.2* transcript levels after JA- and/or ET-treatment compared to control plants, corroborating the complementary results obtained with *AtERF4*-overexpressing plants. This demonstrates that AtERF4 plays a role in JA and ET signaling by repressing the expression of a number of genes in response to JA and/or ET. The same genes were shown to be positively regulated by ORA59 (Pré et al., 2008) and ERF1 (Lorenzo et al., 2003).

In addition, overexpression of the *AtERF4* gene resulted in enhanced JA-induced expression of a distinct subset of JA-responsive genes, including *VSP1* and *CYP79B2* (Pré, 2006). This indicated that the presence of AtERF4 positively regulated the expression of these genes in response to JA treatment. It is not clear how the positive effect of *AtERF4* overexpression on JA signaling for this gene subset is operating at the molecular level, but assuming that AtERF4 always acts as a repressor, the positive effect is hypothesized to be caused by the repression of a repressor. The ET signaling pathway was shown to repress the wound-induced expression of several wound-responsive genes, including the *VSP1* and *CYP79B2* genes (Rojo et al., 1999; Mikkelsen et al., 2000). Overexpression of the ET-responsive *ERF1* gene has been shown to inhibit the expression of the *VSP2* gene in response to JA (Lorenzo et al., 2004). JA-induced expression of the *VSP2* gene is controlled by AtMYC2 (Figure 2; Boter et al., 2004; Lorenzo et al., 2004). It was therefore suggested that the negative regulation of the *VSP2* gene by ET is executed through ERF1, although the molecular relationships between the activator AtMYC2 and the repressor ERF1 on JA-responsive *VSP2* expression remains to be characterized. It is possible that AtERF4 antagonizes the ERF1-mediated negative effect of ET on the expression of a subset of JA-responsive genes, including *VSP* genes (Figure 2). AtERF4 and AtMYC2 seem to positively regulate the same subset of JA-responsive genes. However, overexpression of *AtMYC2* is sufficient to activate *VSP2* expression in the absence of JAs (Lorenzo et al., 2004), which is not the case in *AtERF4*-overexpressing plants (Pré, 2006).

Therefore, JA and ET synergistically induce both activators (ORA59 and ERF1) and repressors (AtERF4) acting on the same set of genes. The functional importance of the simultaneous induction of both positive and negative regulators by JA and ET remains unclear. The balance between AP2/ERF-domain activators and repressors on common target promoters may provide a mechanism for switch-like transcriptional control. Additionally or alternatively, such a mechanism might be necessary to coordinate the response output to JAs and ET with other signals, such as growth conditions, tissue type and age, and other hormones (Pauwels et al., 2008).

Conclusions

Frequently occurring jasmonate-responsive promoter sequences are the GCC motif, which is commonly found in promoters activated synergistically by jasmonate and ethylene, and the G-box, which is commonly found in promoters activated by jasmonates and repressed by ethylene. Important transcription factors conferring jasmonate-responsive gene expression in

Arabidopsis are ORA59 (Pré et al., 2008) and AtMYC2 (Boter et al., 2004; Lorenzo et al., 2004), with other transcription factors acting as positive (e.g. ERF1, ANAC019/055) and negative (e.g. AtERF4) modulators of the gene expression response. ORA59 controls the expression of genes that are synergistically induced by jasmonates and ethylene, whereas AtMYC2 interacts *in vitro* with the G-box and related sequences (Zarei, 2007; Chini et al., 2007; Dombrecht et al., 2007), and controls genes activated by jasmonate alone.

The activity of AtMYC2 is postulated to be controlled by JAZ proteins, which are hypothesized to act as repressors (Chini et al., 2007). The bioactive jasmonate (+)-7-*iso*-JA-L-Ile promotes the interaction between JAZ proteins and the putative ubiquitin ligase complex SCF^{COI1} (Fonseca et al., 2009), presumably leading to ubiquitination of JAZ proteins and resulting in their degradation by the 26S proteasome. It has been proposed that different biologically active JAs could promote the binding between COI1 and specific JAZ family members, and that these family members could act as repressors of different downstream targets, presumably other transcription factors (Thines et al., 2007). However there is no evidence to support that notion, and in fact 10 of the 12 JAZ proteins were shown to interact with AtMYC2 (Chini et al., 2009). While this leaves open the possibility that JAZ4 and JAZ7 repress other transcription factors, the question remains whether and how other jasmonate-responsive transcription factors such as ORA59 are activated by jasmonates in a COI1-dependent manner. It is conceivable that JA-Ile or other biologically active JAs enhance binding between COI1 and hitherto unidentified repressors distinct from the JAZ proteins.

Thesis outline

Jasmonic acid is a plant signaling molecule that plays an important role in defense against wounding, insects and necrotrophic pathogens. Depending on the stress situation and on the simultaneous induction of ET and SA biosynthesis, JA induces the expression of specific subsets of genes encoding defense-related proteins and/or enzymes involved in biosynthesis of protective secondary metabolites. Many aspects concerning the mode of action of JA on the regulation of gene expression are poorly understood. Several transcription factors involved in JA-responsive gene expression have been identified, including ORA59, ERF1, AtERF4, and AtMYC2. Identification of the mechanisms whereby these transcription factors are activated by JA at the protein level and of the interaction between these transcription factors and the binding sites in the promoters of their target genes is of major importance to understand how JA acts.

The studies described in this thesis are focused on the functional analysis of the JA/ET-responsive transcription factor ORA59 in Arabidopsis.

Chapter 2 describes studies aiming at the dissection of the interaction of ORA59 and the related transcription factor ERF1 with the *PDF1.2* promoter. Two GCC boxes in the *PDF1.2* promoter were found to be equally important for trans-activation by ORA59 and ERF1 in transient assays and for *in vitro* binding. Application of the chromatin immunoprecipitation technique

showed that ORA59 bound to the *PDF1.2* promoter *in vivo*. Interestingly, mutation of either GCC box was sufficient to completely abolish the expression of the *PDF1.2* promoter in response to JA alone or in combination with the ET-releasing agent ethephon in stably transformed plants.

Chapter 3 focuses on studies to determine whether JA has an activating effect on ORA59 at the protein level. The results show that JA caused stabilization as well as nuclear localization of ORA59 protein. Interestingly, JA-responsive nuclear localization of ORA59 did not require a functional COI1 protein. Based on the findings it is postulated that there is a jasmonate receptor distinct from COI1, an F-box protein that targets ORA59 for degradation, and a repressor protein that sequesters ORA59 in the cytoplasm.

Chapter 4 describes the characterization of the ORA59-interacting CCCH zinc finger protein ZFAR1 identified by yeast two-hybrid (Y2H) screening. Using Bimolecular Fluorescent Complementation (BiFC) it was shown that ORA59 and ZFAR1 interact in the cytoplasm of Arabidopsis cell suspension protoplasts. Consistent with this interaction ZFAR1 repressed ORA59 transcriptional activation of *PDF1.2* in protoplast trans-activation assays and had a negative effect on *Botrytis* resistance based on studies with plants with altered *ZFAR1* expression levels. Taken together the results indicate that ZFAR1 is a repressor protein that sequesters ORA59 in the cytoplasm.

References

Austin MJ, Muskett P, Kahn K, Feys BJ, Jones JDG, Parker JE (2002) Regulatory role of SGT1 in early R gene-mediated plant defenses. Science **295**: 2077-2080

Azevedo C, Sadanandom A, Kitagawa K, Freialdenhoven A, Shirasu K, Schulze-Lefert P (2002) The RAR1 interactor SGT1, an essential component of R gene-triggered disease resistance. Science **295**: 2073-2076

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

Benedetti CE, Xie D, Turner JG (1995) COI1-dependent expression of an Arabidopsis vegetative storage protein in flowers and siliques and in response to coronatine or methyl jasmonate. Plant Physiol **109**: 567-572

Berrocal-Lobo M, Molina A, Solano R (2002) Constitutive expression of ETHYLENE-RESPONSE-FACTOR1 in Arabidopsis confers resistance to several necrotrophic fungi. Plant J **29**: 23-32

Boter M, Ruiz-Rivero O, Abdeen A, Prat S (2004) Conserved MYC transcription factors play a key role in jasmonate signaling both in tomato and Arabidopsis. Genes Dev **18**: 1577-1591

Brown RL, Kazan K, McGrath KC, Maclean DJ, Manners JM (2003) A role for the GCC-box in jasmonate-mediated activation of the *PDF1.2* gene of Arabidopsis. Plant Physiol **132:** 1020-1032

Browse J (2009) Jasmonate passes muster: a receptor and targets for the defense hormone. Annu. Rev. Plant Biol **60:** 183-205

Cheong YH, Chang H-S, Gupta R, Wang X, Zhu T, Luan S (2002) Transcriptional profiling reveals novel interactions between wounding, pathogen, abiotic stress, and hormonal responses in Arabidopsis. Plant Physiol **129:** 661-677

Chini A, Fonseca S, Fernández G, Adie B, Chico JM, Lorenzo O, García-Casado G, López-Vidriero I, Lozano FM, Ponce MR, Micol JL, Solano R (2007) The JAZ family of repressors is the missing link in jasmonate signaling. Nature **448:** 666-671

Chini A, Fonseca S, Chico JM, Fernández-Calvo P, Solano R (2009) The ZIM domain mediates homo- and

heteromeric interactions between Arabidopsis JAZ proteins. Plant J **59:** 77-87

Chung HS, Koo AJ, Gao X, Jayanty S, Thines B, Jones AD, Howe GA (2008) Regulation and function of Arabidopsis JASMONATE ZIM-domain genes in response to wounding and herbivory. Plant Physiol **146:** 952-964

Chung HS, Howe GA (2009) A critical role for the TIFY motif in repression of jasmonate signaling by a stabilized splice variant of the JASMONATE ZIM-domain protein JAZ10 in Arabidopsis. Plant Cell **21:** 131-145

Creelman RA, Mullet JE (1997) Biosynthesis and action of jasmonates in plants. Annu. Rev. Plant Physiol Plant Mol Biol **48:** 355-381

De Vleesschauwer D, Yang Y, Vera Cruz C, Höfte M (2010) Abscisic acid-induced resistance against the brown spot pathogen *Cochliobolus miyabeanus* in rice involves MAPK-mediated repression of ethylene signaling. Plant Physiology Preview DOI:10.1104/pp.109.152702

De Pater S, Pham K, Memelink J, Kijne J (1997) RAP-1 is an *Arabidopsis* MYC-like R protein homologue, that binds to G-box sequence motifs. Plant Mol Biol **34:** 169-174

Del Pozo JC, Estelle M (2000) F-box proteins and protein degradation: an emerging theme in cellular regulation. Plant Mol Biol **44:** 123-128

Devoto A, Nieto-Rostro M, Xie D, Ellis C, Harmston R, Patrick E, Davis J, Sherratt L, Coleman M, Turner JG (2002) COI1 links jasmonate signaling and fertility to the SCF ubiquitin-ligase complex in Arabidopsis. Plant J **32:** 457-466

Devoto A, Turner JG (2003) Regulation of jasmonate-mediated plant responses in Arabidopsis. Ann. Bot **92:** 329-337

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

Dombrecht B, Xue GP, Sprague SJ, Kirkegaard JA, Ross JJ, Reid JB, Fitt GP, Sewelam N, Schenk PM, Manners JM, Kazan K (2007) MYC2 differentially modulates diverse jasmonate-dependent functions in Arabidopsis. Plant Cell **19:** 2225-2245

Dong X (1998) SA, JA, ethylene, and disease resistance in plants. Curr Opin Plant Biol **1**:316-323

Fan W, Dong X (2002) In vivo interaction between NPR1 and transcription factor TGA2 leads to salicylic acid-mediated gene activation in Arabidopsis. Plant Cell **14:** 1377-1389

Feys BF, 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

Fonseca S, Chini A, Hamberg M, Adie B, Porzel A, Kramell R, Miersch O, Wasternack C, Solano R (2009) (+)-7-*iso*-Jasmonoyl-L-isoleucine is the endogenous bioactive jasmonate. Nat Chem Biol **5:** 344-350

Fujimoto SY, Ohta M, Usui A, Shinshi H, Ohme-Takagi M (2000) Arabidopsis ethylene-responsive element binding factors act as transcriptional activators or repressors of GCC box-mediated gene expression. Plant Cell **12:** 393-404

Gagne JM, Downes BP, Shiu SH, Durski AM, Vierstra RD (2002) The F-box subunit of the SCF E3 complex is encoded by a diverse superfamily of genes in Arabidopsis. Proc Natl Acad Sci USA **99:** 11519-11524

Gill G, Ptashne M (1988) Negative effect of the transcriptional activator GAL4. Nature **334:** 721-724

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

Gray WM, Muskett PR, Chuang HW, Parker JE (2003) Arabidopsis SGT1b is required for SCF^{TIR1}-mediated auxin response. Plant Cell **15:** 1310-1319

Guerineau F, Benjdia M, Zhou DX (2003) A jasmonate-responsive element within the *A. thaliana vsp1* promoter. J. Exp Bot **54:** 1153-1162

Guilfoyle T (2007) Sticking with auxin. Nature **446:** 621-622

Guo H, Ecker JR (2004) The ethylene signaling pathway: new insights. Curr. Opin. Plant Biol **7:** 40-49

Halim VA, Altmann S, Ellinger D, Eschen-Lippold L, Miersch O, Scheel D, Rosahl S (2009) PAMP-induced defense responses in potato require both salicylic acid and jasmonic acid. Plant J **57:** 230-242

He Y, Gan S (2001) Identical promoter elements are involved in regulation of the *OPR1* gene by senescence

and jasmonic acid in Arabidopsis. Plant Mol Biol **47:** 595-605

Howe GA and Jander G (2008) Plant immunity to insect herbivores. Annu Rev Plant Biol **59**:41-66

Katsir L, Schilmiller AL, Staswick PE, He SY, Howe GA (2008) COI1 is a critical component of a receptor for jasmonate and the bacterial virulence factor coronatine. Proc Natl Acad Sci USA **105:** 7100-7105

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

Kim SR, Choi JL, Costa MA, An G (1992) Identification of G-box sequence as an essential element for methyl jasmonate response of potato *proteinase inhibitor II* promoter. Plant Physiol **99:** 627-631

Kim SR, Kim Y, An G (1993) Identification of methyl jasmonate and salicylic acid response elements from the *nopaline synthase (nos)* promoter. Plant Physiol **103:** 97-103

Kim Y, Buckley K, Costa MA, An G (1994) A 20 nucleotide upstream element is essential for the *nopaline synthase (nos)* promoter activity. Plant Mol Biol **24:** 105-117

Kunkel BN, Brooks DM (2002) Cross talk between signaling pathways in pathogen defense. Curr. Opin. Plant Biol **5:** 325-331

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

Lorenzo O, Piqueras R, Sanchez-Serrano JJ, Solano R (2003) ETHYLENE RESPONSE FACTOR1 integrates signals from ethylene and jasmonate pathways in plant defense. Plant Cell **15:** 165-178

Lorenzo O, Solano R (2005) Molecular players regulating the jasmonate signaling network. Curr. Opin. Plant Biol **8:** 532-540

Mahalingam R, Gomez-Buitrago A, Eckardt N, Shah N, Guevara-Garcia A, Day P, Raina R, Fedoroff NV (2003) Characterizing the stress/defense transcriptome of Arabidopsis. Genome Biol **4:** R20

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

Maraschin F dos S, Memelink J, Offringa R (2009) Auxin-induced, SCF^{TIR1}-mediated poly-ubiquitination marks AUX/IAA proteins for degradation. Plant J **59:** 100-109

Mason HS, DeWald DB, Mullet JE (1993) Identification of a methyl jasmonate-responsive domain in the soybean *vspB* promoter. Plant Cell **5:** 241-251

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

McGrath KC, Dombrecht B, Manners JM, Schenk PM, Edgar CI, Maclean DJ, Scheible WR, Udvardi MK, Kazan K (2005) Repressor- and activator-type ethylene response factors functioning in jasmonate signaling and disease resistance identified via a genome-wide screen of Arabidopsis transcription factor gene expression. Plant Physiol **139:** 949-959

Melotto M, Mecey C, Niu Y, Chung HS, Katsir L, Yao J, Zeng W, Thines B, Staswick P, Browse J, Howe GA, He SY (2008) A critical role of two positively charged amino acids in the Jas motif of Arabidopsis JAZ proteins in mediating coronatine- and jasmonoyl isoleucine-dependent interactions with the COI1 F-box protein. Plant J **55:** 979-988

Menke FLH, Champion A, Kijne JW, Memelink J (1999) A novel jasmonate- and elicitor-responsive element in the periwinkle secondary metabolite biosynthetic gene *Str* interacts with a jasmonate- and elicitor-inducible AP2-domain transcription factor, ORCA2. EMBO J **18:** 4455-4463

Miao ZH, Lam E (1995) Construction of a trans-dominant inhibitor for members of the TGA family of transcription factors conserved in higher plants. Plant J **7:** 887-896

Mikkelsen MD, Hansen CH, Wittstock U, Halkier BA (2000) Cytochrome P450CYP79B2 from Arabidopsis catalyzes the conversion of tryptophan to indole-3-acetaldoxime, a precursor of indole glucosinolates

and indole-3-acetic acid. J Biol Chem **275:** 33712-33717

Nakano T, Suzuki K, Fujimura T, Shinshi H (2006) Genome-wide analysis of the ERF gene family in Arabidopsis and rice. Plant Physiol **140:** 411-432

Ohme-Takagi M, Shinshi H (1995) Ethylene-inducible DNA-binding proteins that interact with an ethylene-responsive element. Plant Cell **7:** 173-182

Ohta M, Matsui K, Hiratsu K, Shinshi H, Ohme-Takagi M (2001) Repression domains of class II ERF transcriptional repressors share an essential motif for active repression. Plant Cell **13:** 1959-1968

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

Pauwels L, Inzé D, Goossens A (2008) Jasmonate-inducible gene: What does it mean? Trends Plant Sci **14:** 87-91

Penninckx IAMA, Thomma BPHJ, Buchala A, Metraux JP, Broekaert WF (1998) Concomitant activation of jasmonate and ethylene response pathways is required for induction of a plant defensin gene in Arabidopsis. Plant Cell **10:** 2103-2113

Pieterse CMJ, Leon-Reyes A, Van der Ent S, Van Wees SCM (2009) Networking by small-molecule hormones in plant immunity. Nature Chem Biol **5**: 308-316

Pré M (2006) ORA EST: Functional analysis of jasmonate-responsive AP2/ERF-domain transcription factors in *Arabidopsis thaliana*. PhD thesis. Leiden University, Leiden, The Netherlands

Pré M, Atallah M, Champion A, de Vos M, Pieterse CMJ, Memelink J (2008) The AP2/ERF domain transcription factor ORA59 integrates jasmonic acid and ethylene signals in plant defense. Plant Physiol **147:** 1347-1357

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

Rojo E, Titarenko E, León J, Berger S, Vancanneyt G, Sánchez-Serrano JJ (1998) Reversible protein phosphorylation regulates jasmonic acid-dependent and -independent wound signal transduction pathways in *Arabidopsis thaliana*. Plant J **13:** 153-165

Rojo E, Leon J, Sanchez-Serrano JJ (1999) Cross-talk between wound signaling pathways determines local versus systemic gene expression in *Arabidopsis thaliana*. Plant J **20:** 135-142

Rouster J, Leah R, Mundy J, Cameron-Mills V (1997) Identification of a methyl jasmonate-responsive region in the promoter of a *lipoxygenase 1* gene expressed in barley grain. Plant J **11:** 513-523

Sanders PM, Lee PY, Biesgen C, Boone JD, Beals TP, Weiler EW, Goldberg RB (2000) The Arabidopsis *DELAYED DEHISCENCE1* gene encodes an enzyme in the jasmonic acid synthesis pathway. Plant Cell **12:** 1041-1061

Shah J (2003) The salicylic acid loop in plant defense. Curr. Opin. Plant Biol **6:** 365-371

Shoji T, Ogawa T, Hashimoto T (2008) Jasmonate-induced nicotine formation in tobacco is mediated by tobacco *COI1* and *JAZ* genes. Plant Cell Physiol **49:** 1003-1012

Solano R, Stepanova A, Chao Q, Ecker JR (1998) Nuclear events in ethylene signaling: a transcriptional cascade mediated by ETHYLENE-INSENSITIVE3 and ETHYLENE-RESPONSE-FACTOR1. Genes Dev **12:** 3703-3714

Staswick PE, Su W, Howell SH (1992) Methyl jasmonate inhibition of root growth and induction of a leaf protein are decreased in an *Arabidopsis thaliana* mutant. Proc Natl Acad Sci USA **89:** 6837-6840

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

Stintzi A, Browse J (2000) The Arabidopsis male-sterile mutant, *opr3*, lacks the 12-oxophytodienoic acid reductase required for jasmonate synthesis. Proc Natl Acad Sci USA **97:** 10625-10630

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

Suza WP, Staswick PE (2008) The role of JAR1 in Jasmonoyl-L-isoleucine production during *Arabidopsis* wound response. Planta **227:** 1221-1232

Takeda S, Sugimoto K, Otsuki H, Hirochika H (1998) A 13-bp *cis*-regulatory element in the LTR promoter

of the tobacco retrotransposon *Tto*1 is involved in responsiveness to tissue culture, wounding, methyl jasmonate and fungal elicitors. Plant J **18:** 383-393

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 signaling. Nature **448:** 661-665

Thomma BPHJ, Nelissen I, Eggermont K, Broekaert WF (1999) Deficiency in phytoalexin production causes enhanced susceptibility of *Arabidopsis thaliana* to the fungus *Alternaria brassicicola*. Plant J **19:** 163-171

Turner JG, Ellis C, Devoto A (2002) The jasmonate signal pathway. Plant Cell **14:** Suppl., S153-S164

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

Vom Endt D, Soares e Silva M, Kijne JW, Pasquali G, Memelink J (2007) Identification of a bipartite jasmonate-responsive promoter element in the *Catharanthus roseus ORCA3* transcription factor gene that interacts specifically with AT-hook DNA-binding proteins. Plant Physiol **144:** 1680-1689

Wang KL, Li H, Ecker JR (2002) Ethylene biosynthesis and signaling networks. Plant Cell **14:** Suppl., S131-S151

Wang Z, Dai L, Jiang Z, Peng W, Zhang L, Wang G, Xie D (2005) GmCOI1, a soybean F-box protein gene, shows ability to mediate jasmonate-regulated plant defense and fertility in Arabidopsis. Mol Plant Microbe Interact **18:** 1285-1295

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 **100:** 681-697

Xiang C, Miao ZH, Lam E (1996) Coordinated activation of *as-1*-type elements and a tobacco *glutathione S-transferase* gene by auxins, salicylic acid, methyl-jasmonate and hydrogen peroxide. Plant Mol Biol **32:** 415-426

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

Xu L, Liu F, Lechner E, Genschik P, Crosby WL, Ma H, Peng W, Huang D, Xie D (2002) The SCF^{COI1} ubiquitin-ligase complexes are required for jasmonate response in Arabidopsis. Plant Cell **14:** 1919-1935

Xu B, Timko M (2004) Methyl jasmonate induced expression of the tobacco putrescine *N* -methyltransferase genes requires both G-box and GCC-motif elements. Plant Mol Biol **55:** 743-761

Yan Y, Stolz S, Chételat 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

Yan J, Zhang C, Gu M, Bai Z, Zhang W, Qi T, Cheng Z, Peng W, Luo H, Nan F, Wang Z, Xie D (2009) The Arabidopsis CORONATINE INSENSITIVE1 Protein Is a Jasmonate Receptor. Plant Cell **21**: 2220–2236

Yang Z, Tian L, Latoszek-Green M, Brown D, Wu K (2005) Arabidopsis ERF4 is a transcriptional repressor capable of modulating ethylene and abscisic acid responses. Plant Mol Biol **58:** 585-596

Zarei A (2007) Functional analysis of jasmonate-responsive transcription factors in *Arabidopsis thaliana*. PhD thesis. Leiden University, Leiden, The Netherlands

Zhang Y, Turner JG (2008) Wound-induced endogenous jasmonates stunt plant growth by inhibiting mitosis. PLoS One **3:** e3699