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Jasmonate-Responsive Gene Expression

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Abstract

Jasmonic acid (JA) and its volatile methyl ester (MeJA) belong to a family of lipid-derived signalling molecules that affect many aspects of plant life, including defense against certain pathogens and insects and some developmental processes. JA signal transduction leads to modulation of the expression of primary response genes, the products of which lead to the expression of secondary response genes. The ORCA3 transcription factor from *Catharanthus roseus* is a good candidate for a terminal component of the JA signal transduction pathway. To our knowledge, not a single component of the primary JA signal transduction pathway has been characterized to date in *Arabidopsis*. Many transcriptional components of secondary JA response pathways have been described in this model plant species, and are reviewed here. Our review advocates a strong adherence to signal transduction terminology as employed in the animal research field and in molecular biology textbooks, to simplify and correct current models about JA signal transduction leading to gene expression.

Key words: *Arabidopsis*; Defense; Jasmonic acid; Promoter; Signal transduction; Transcription factor

Introduction

Plant growth and adaptation to the environment are controlled by the perception of signals which are converted in a response via a process known as ''signal transduction''. The signal is first perceived by a specific receptor protein. Interaction with the ligand changes the activity state of the receptor, which then initiates a cascade of events. An important and general primary response to any signal, whether it is a hormone, a pathogen-related elicitor or an abiotic signal, is the modulation of the expression of a specific set of genes called ''primary response genes'' or ''immediate-early genes''. Concentration changes in the translation products of the primary transcripts lead to secondary responses, including modulation of the expression of secondary response genes (Figure 1). To which class genes expressed in response to a signal belong can be determined by combining the signal with the 80S ribosome inhibitor cycloheximide (CHX). Primary response genes will be expressed in a CHX-insensitive manner, whereas secondary response genes will not be expressed, because their expression depends on *de novo* protein synthesis (Figure 1) (Alberts and others 2002). This difference has important consequences for signal transduction research. For example, transcription factors regulating primary response genes can be expected to be activated in response to the signal, for example by posttranslational modifications such as phosphorylation. Because secondary response genes are regulated by

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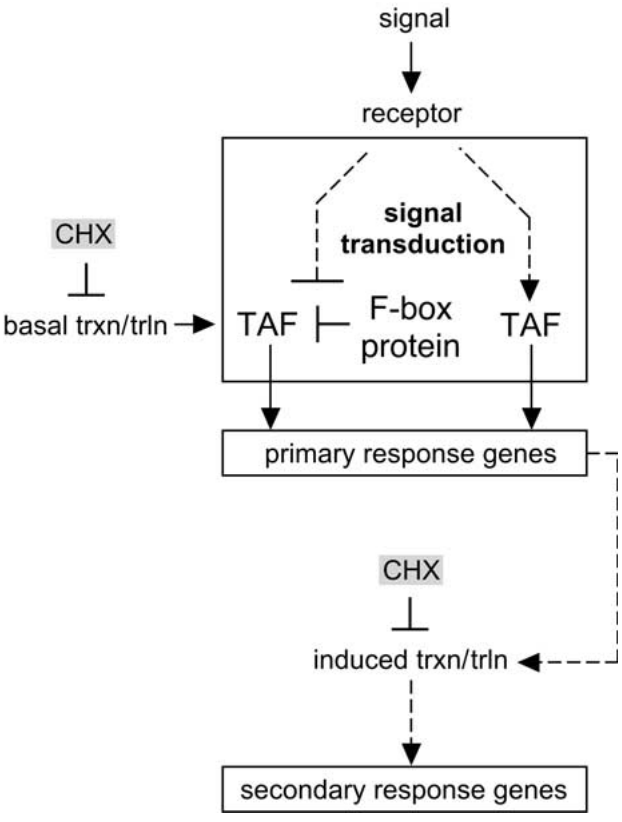

Figure 1. Signal transduction leading to gene expression. The signal transduction pathway is boxed. Secondary response genes, and primary pathways involving changes in the stability of a transcription activating factor (TAF), are dependent on transcription (trxn) and translation (trln). The latter process is inhibited by cycloheximide (CHX).

transcription factors that are synthesized *de novo, a priori* there is no reason to expect that such proteins are posttranslationally modified in a signal-responsive manner, although it can not be excluded. In any case, the involvement of posttranslational modifications of such secondary transcription factors in response to the signal cannot be studied via pharmacological approaches using specific chemical inhibitors if these inhibitors also affect the translation process. This would, for example, be the case for inhibitors of (de)phosphorylation, because translation involves a large amount of proteins, many of which are (de)phosphorylated during the process.

In the plant research field, the term ''signal transduction'' is used loosely, which leads to much confusion and even incorrect experimental approaches and faulty interpretations of experiments. A common notion in the plant research field is that signal transduction is complex with many cross-talk connections. This may or may not be so, but the picture is certainly unnecessarily complicated by the confused use of signal transduction terminology. In the animal research field and in authoritative molecular biology textbooks (Alberts and others 2002), the term signal transduction, when applied to a hormone or hormone-like signals, is reserved exclusively for processes that affect the activity states of preexisting proteins. If such a protein is a transcription factor, signal transduction changes the transcriptional state of primary response genes. According to this view, signal transduction is a CHX-insensitive process. In the plant research field, this distinction between primary and secondary responses is usually not made. For example, NPR1 is commonly called a component of the salicylic acid (SA) signal transduction pathway, whereas it is required for the expression of secondary response genes but not primary response genes (Uquillas and others 2004). NPR1 is therefore, by definition, not a component of the SA signal transduction pathway.

However, it must be noted that the simple notion of CHX insensitivity of primary response genes is complicated by the discovery of protein degradation as a major regulatory mechanism in signal transduction (Alberts and others 2002). The cellular abundance of many regulatory proteins, including transcription factors, in many species turns out to be regulated at the level of protein turnover rather than by transcriptional regulation. Such conditionally short-lived regulatory proteins are recognized by specific proteins, which are part of ubiquitin-protein ligases that mark the proteins for degradation by the 26S proteasome. A prominent class of such specificity determinants of the protein degradation process are the F-box proteins. The *Arabidopsis* genome, for example, encodes 694 F-box proteins. As exemplified by the role of the transcription factor EIN3 in ethylene-responsive gene expression (Figure 2) (Potuschak and others 2003; Guo and Ecker 2003), a signal transduction pathway can inhibit the F-box protein-mediated degradation of a regulatory protein, as a result of which the latter becomes more abundant and then initiates downstream processes. Although such a signal transduction process would qualify as a primary response pathway, it would be CHX-sensitive, because the increased abundance of the regulator would require at least a basal level of translation (Figure 1). Nevertheless, the notion of primary and secondary responses remains an important one, as illustrated below, even if CHX sensitivity may not be the ultimate discriminatory determinant.

Jasmonic acid (JA) and its volatile methyl ester (MeJA) are cyclopentanone derivatives, which are

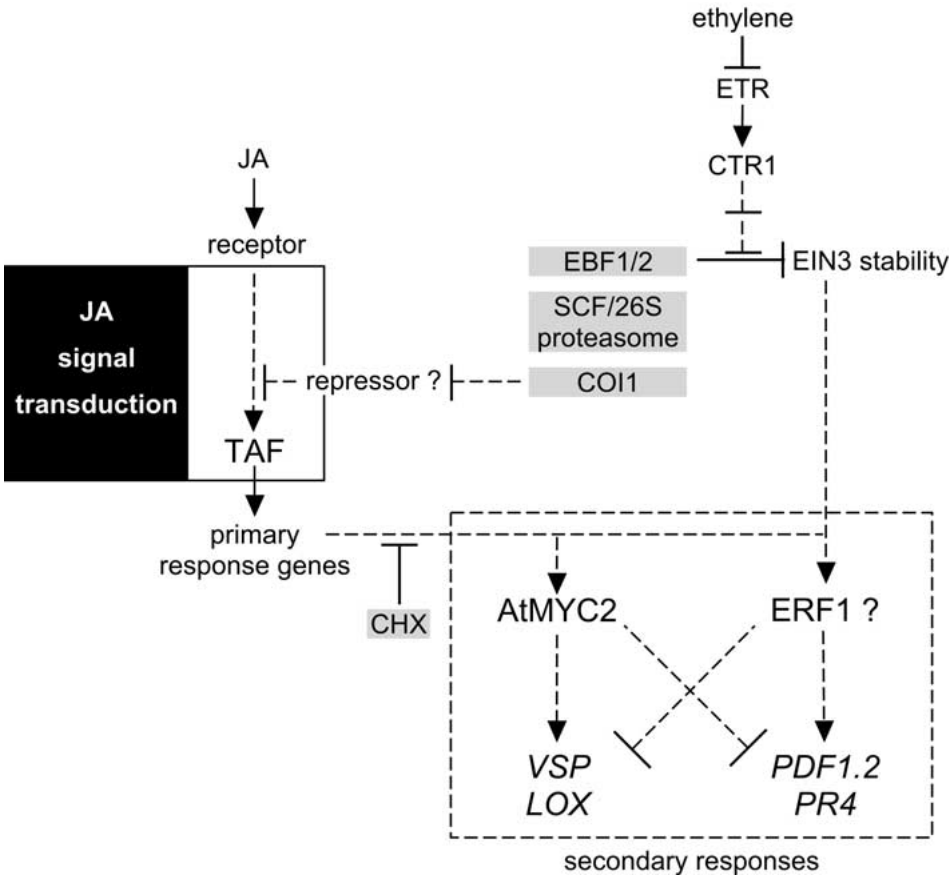

Figure 2. Jasmonate and ethylene responses in *Arabidopsis*. The JA signal transduction pathway is boxed with solid lines. Solid lines indicate single steps, white broken lines indicate (possible) multiple steps. TAF: transcription activating factor. For other abbreviations, see text. The question mark indicates that ERF1 function has not been confirmed by analysis of loss-of-function mutants.

synthesized via the octadecanoid pathway from linolenic acid (Turner and others 2002; Schaller and others this issue). JA and MeJA affect many aspects of plant growth and adaptation to the environment, including seed germination, regulation of carbon and nitrogen storage, photosynthesis, senescence, pollen development, fruit ripening, wounding responses and resistance to insects and pathogens (Creelman and Mullet 1997). In the regulation of defense responses, JA often acts synergistically with, or antagonistically to, two other plant hormones, salicylic acid (SA) (Shah 2003) and ethylene (Guo and Ecker 2004), respectively, leading to fine-tuning of the defense response (Kunkel and Brooks 2002).

Here we review recent advances in the identification of JA-responsive genes, the JA-responsive elements in their promoters, and transcription factors interacting with such elements. We focus on the distinction between primary response genes, which are targets of JA signal transduction, and secondary response genes, which are expressed as a consequence of primary responses. We propose several mechanisms by which JA may regulate transcription factors and/or gene expression.

JA-Responsive Genes

Exogenous application of (Me)JA results in major reprogramming of gene expression. A number of genes that are known to be involved in plant stress responses are induced by JA treatment. JA induces the expression of genes encoding proteinase inhibitors (PIN), which are involved in the protection of plants from insect damage (Farmer and Ryan 1992; Howe this issue; Halitschke and Baldwin this issue). Also, JA increases the transcript levels of genes encoding the antimicrobial proteins defensin (PDF1.2) (Penninckx and others 1998) and thionin (THI2.1)(Epple and others 1995). Furthermore, JA induces the expression of biosynthesis genes leading to the accumulation of antimicrobial secondary metabolites, including alkaloids, terpenoids, flavonoids, anthraquinones, and glucosinolates in different plant species (Memelink and others 2001; Blechert and others 1995). Micro-array analysis showed that MeJA induces transcription of genes involved in the oxidative burst and programmed cell death, such as those encoding catalase, glutathione S-transferase, and cysteine protease (Schenk and others 2000). Interestingly, in this micro-array

experiment a significant number of genes that were induced by MeJA treatment were also induced by SA treatment. This is in contrast to previous notions that SA and JA might act antagonistically. However, in the same experiment other genes that were significantly induced by SA were significantly repressed by MeJA, indicating that the antagonistic activities of SA and JA are specific to a particular set of genes.

cDNA macro-array analysis revealed that MeJA treatment induced several genes involved in JA biosynthesis, such as *AOC, OPR1, OPR3, LOX2* and *AOS* (Sasaki and others 2001). This analysis confirms the results presented in other reports, which show that JA induces transcription of the (Me)JA biosynthesis genes, *LOX2, AOS, OPR3, DAD1, JMT, and AOC* (Bell and Mullet 1993; Laudert and Weiler 1998; Mussig and others 2000; Ishiguro and others 2001; Seo and others 2001; Stenzel and others 2003). Together, these results indicate the existence of a positive feedback regulatory mechanism for JA biosynthesis.

Besides genes that are known to be involved in stress responses, genes involved in cell maintenance, amino acid and sugar metabolism, and senescence are also induced by (Me)JA (Schenk and others 2000; Sasaki and others 2001). For example, one class of well known JA-responsive genes are vegetative storage proteins (VSPs), vacuolar glycoproteins that are abundant in flowers and young seed pods (Mason and others 1993; Guerineau and others 2003).

A limited number of studies have been carried out to determine which genes respond to (Me)JA in a CHX-insensitive manner. The majority of the genes studied, including *VSP, LOX2* (Rojo and others 1998; Jensen and others 2002) and *PDF1.2* (Champion and Memelink unpublished results), do not respond to JA in the presence of CHX, demonstrating that they are not primary JA response genes. In *Arabidopsis* only the *JR3* gene, which is similar to a gene family encoding auxin-conjugate hydrolases (Rampey and others 2004), was demonstrated to be a primary response gene (Rojo and others 1998). The alkaloid biosynthesis genes *Tryptophan decarboxylase* and *Strictosidine synthase (STR)* from *Catharanthus roseus* are also primary JA response genes (van der Fits and Memelink 2001). In addition, several genes encoding transcription factors were induced by JA in a CHX-insensitive manner. These include the *ORCA3* gene from *C. roseus* encoding an AP2-domain transcription factor (van der Fits and Memelink 2001), and a gene encoding a rice MYB transcription factor (Lee and others 2001).

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 factors that interact with them. In general, these *cis*-acting elements are concentrated in a relatively small promoter region of a few hundred to a few thousand nucleotides upstream of the transcriptional start site.

Again, a distinction should be made here between JA-responsive elements derived from primary response genes and those from secondary response genes. *A priori*, these two types of elements bind two different classes of transcription factors. Primary response elements interact with preexisting transcription factors, which are activated as terminal components of the JA signal transduction pathway, whereas secondary response elements are bound by transcription factors, which are synthesized *de novo* in response to JA (Figure 1).

Several *cis*-acting elements in various gene promoters that mediate the JA responsiveness have been identified. In the promoter of the terpenoid indole alkaloid biosynthesis gene *STR* from *C. roseus*, a jasmonate-and elicitor-responsive element (JERE) has been identified (Menke and others 1999b). Mutation or removal of this JERE results in an inactive and unresponsive *STR* promoter derivative. A tetramer of the JERE fused to a minimal promoter confers JA-responsive gene expression on a reporter gene, showing that the JERE is an autonomous JA-responsive sequence (Menke and others 1999b). Two AP2-domain transcription factors called ORCA2 and ORCA3 from *C. roseus* bind to the JERE (see below; Menke and others 1999b; van der Fits and Memelink 2001). JA-responsive expression of the *STR* gene is CHX-insensitive (van der Fits and Memelink 2001), demonstrating that the JERE is a primary JA-responsive element. Within this JERE a GCC-box-like sequence is present. In *Arabidopsis*, a GCC-box (GCCGCC) plays a role in conferring JA-responsiveness to the *PDF1.2* promoter (Brown and others 2003). However, since the *PDF1.2* gene is not a primary JA response gene (Champion and Memelink unpublished results), this GCC box is not a primary response element. The GCC-box functions autonomously as an ethylene-responsive element, which interacts *in vitro* with AP2-domain transcription factors (Ohme-Takagi and Shinshi 1995; Fujimoto and others 2000). The *PDF1.2* gene is synergistically induced by a combination of JA and ethylene (Penninckx and others 1998), which is likely caused by a convergent action of both signals on the GCC box.

G-box sequences (CACGTG) or G-box-like sequences (AACGTG) that are essential for the JA response were found in the promoters of the potato *PIN2* gene (Kim and others 1992), the soybean *VSPB* gene (Mason and others 1993), the *Arabidopsis VSP1* gene (Guerineau and others 2003) and the tomato *LAP* gene (Boter and others 2004). Also, analysis of the promoters of JA-responsive genes showed that the G-box element was statistically significantly over-represented in these promoters (Mahalingam and others 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 and others 2004). The *Arabidopsis VSP* gene is not a primary response gene (Rojo and others 1998), and therefore this G-box-like element is not a primary response element. A G-box sequence can be bound by G-box-binding factor (GBF)-type of bZIP proteins or basic helix-loop-helix (bHLH) transcription factors (see below).

TGACG (*as-1*-type) sequences were essential for JA inducibility of the promoter of the *Agrobacterium tumefaciens* T-DNA nopaline synthase *(nos)* gene (Kim and others 1993 1994), the Cauliflower Mosaic Virus 35S promoter (Xiang and others 1996) and the barley *LOX1* gene (Rouster and others 1997). TGA-type of bZIP *trans*-acting factors can bind to an *as-1*-type sequence (bZIP research group 2002).

Two JA-responsive elements, JASE1 (5′-CGTCAATGAA-3′) and JASE2 (5′-CATA-CGTCGTC-AA-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 a mixed A/C box, which is a putative binding site for bZIP transcription factors (He and Gan 2001).

A 13-bp element, which contains a box L/AC-I or H-box-like motif, in the LTR promoter of the tobacco retrotransposon *Tto1* is involved in responsiveness to MeJA (Takeda and others 1998). This element was shown to interact with tobacco MYB-related DNA-binding proteins including NtMYB2 (Sugimoto and others 2000).

In conclusion, a variety of JA-responsive elements appear to exist. However, due to the lack of distinction between primary and secondary response elements, it is unclear whether the JA signal transduction pathway is targeted to several distinct response elements, or whether the diversity is created in the secondary responses.

Regulation of Transcription Factor mRNA Abundance by JA

Cis-acting elements present in promoters are recognized by transcription factors, which are sequence-specific DNA-binding proteins. After binding to these *cis* elements, transcription factors modulate the rate of initiation of mRNA synthesis by RNA polymerase II. The most direct way by which JA can control gene expression is by the regulation of transcription factor abundance via adjustment of the production of the encoding mRNA.

Several examples of induction of mRNAs encoding transcription factors by JA have been described. The expression of two genes encoding the transcription factors ORCA2 and ORCA3 is rapidly induced by MeJA in *C. roseus* (Menke and others 1999b; van der Fits and Memelink 2001). ORCA2 and ORCA3 belong to the AP2-domain family of transcription factors, which are unique to plants and are characterized by the AP2 DNA-binding domain. The ORCAs *trans*-activate expression of the alkaloid biosynthesis gene *STR* via specific binding to the JERE (see above; Menke and others 1999b; van der Fits and Memelink 2001). Overexpression of ORCA3 increased the expression levels of several genes involved in primary as well as secondary metabolism including *STR* (van der Fits and Memelink 2000). Although *ORCA* gene expression is JA-responsive, suggesting that target genes are switched on as a result of increased transcription factor abundance, the *STR* gene is a primary response gene (van der Fits and Memelink 2001). Therefore, increased *ORCA* gene expression is not necessary for JA-responsive expression of target genes.

In *C. roseus* JA also induces the transcription of a gene encoding the basic helix-loop-helix (bHLH) transcription factor CrMYCl, which binds specifically to the G-box element in yeast (Chatel and others 2003). Interestingly, G-box and G-box-like elements were essential for the JA-responsiveness of several promoters, some of which belonged to genes that were secondary response genes (see above). Therefore, binding of bHLH factors synthesized *de novo* in response to JA to the G-boxes of these promoters may confer the JA-responsive expression of these secondary response genes (see below).

The expression of the *Arabidopsis* AP2-domain transcription factor *ERF1* is induced by JA or ethylene and is synergistically induced by both hor-

mones (Lorenzo and others 2003). Micro-array analysis indicates that overexpression of ERF1 upregulates the expression of a large number of genes that are induced simultaneously by ethylene and JA, including *PDF1.2* and *PR4* (Lorenzo and others 2003). However, overexpression downregulates the expression of JA-responsive genes that are inhibited by simultaneous treatment with ethylene, such as *VSP* (Lorenzo and others 2004). Conversely, loss of function mutation in the *JASMONATE-INSENSITIVE1* locus encoding the bHLH transcription factor AtMYC2 severely reduces the JA-responsive level of *VSP* gene expression and causes an increased expression level of *PDF 1.2* in response to JA (Lorenzo and others 2004; Boter and others 2004). Both the *ERF1* gene (Lorenzo and others 2003) as well as the *AtMYC2* gene are induced by JA in a COI1-dependent manner (Lorenzo and others 2004). Therefore, it seems that the differential action of ethylene on two subsets of secondary JA-responsive genes is executed by the antagonistic action of ERF1 and AtMYC2, which are both synthesized *de novo* in response to JA. It should be noted here that confirmation of the hypothesized role of ERF1 in regulation of JA-responsive gene expression awaits analysis of ERF1 loss-of-function mutants. Two tomato homologues of AtMYC2, i. e. JAMYC2 and JAMYC10, bind *in vitro* to the JA-responsive G-box-like sequence in the *LAP* gene (Boter and others 2004). These transcription factors also bound *in vitro* to the G-box from the potato *PIN2* gene (Boter and others 2004), which was involved in JA-responsive expression (Kim and others 1992). Overexpression of these tomato transcription factors in transgenic potato did not induce the expression of *StLAP* or *StPIN2*, but lowered the induction threshold at subsaturating doses of JA (Boter and others 2004). A likely explanation for this observation is that JA induces the expression of an accessory rate-limiting transcription factor. This is supported by the discovery of a second element consisting of two GAGTA repeats flanking the G-box-like sequence that is required for JA-responsive expression of the *LAP* promoter (Boter and others 2004). Within this scenario, it is still entirely possible that AtMYC2 and its homologues in other plant species are not targets at all of primary or secondary responses to JA signal transduction. The available evidence merely indicates that their basal expression is necessary for modulation of the expression of the genes studied.

The expression levels of 5 other *Arabidopsis* genes encoding AP2-domain transcription factors, *AtERF2*, *AtERF3*, *AtERF4*, *AtERF13* and *RAP2.10*, are also increased by MeJA treatment (Onate-Sanchez and Singh 2002; Brown and others 2003). Overexpression of AtERF2 upregulates *PDF1.2*, *PR4* and *THI2.1* expression, suggesting that these genes may be potential targets of AtERF2 (Brown and others 2003). *PDF1.2* and *PR4* were also upregulated in ERF1-overexpressing plants, whereas *THI2.1* was downregulated in these plants (Lorenzo and others 2003), indicating that multiple AP2-domain proteins can potentially regulate JA-responsive genes. This may reflect the natural situation, or it may be an artifact of transcription factor overexpression. Loss-of-function studies should give more insight in this issue. Because the ERF1 (Solano and others 1998) and AtERF2 (Fujimoto and others 2000) proteins bind *in vitro* to a GCC-box sequence, these proteins may regulate JA-responsive gene expression through binding to a GCC-box in the promoters of their target genes. However, it is possible that ERF1 and AtERF2 bind to another *cis* element as well. Chromatin immuno-precipitation experiments showed that the AP2-domain transcription factor Pti4 binds *in vivo* to both GCC-box containing DNA sequences as well as to DNA sequences that do not contain a GCC box (Chakravarthy and others 2003). The latter interaction was hypothesized to be mediated by interaction with another DNA-binding transcription factor.

In addition to activating the expression of certain transcription factor genes, JA downregulates the expression of the *Arabidopsis* gene encoding the zinc-finger transcription factor WRKY70 (Li and others 2004). Antisense suppression of *WKRY70* upregulates several JA-responsive genes, including *VSP* and *COR1*, indicating that this transcription factor is involved in the repression of JA-responsive genes (Li and others 2004). WRKY70 probably does not bind directly to the promoters of JA-responsive genes, because not all of them contain the W-box binding site for WRKY proteins, but may activate another negative regulator, for instance AtMYC2. Interestingly, this WRKY70 transcription factor seems to act as an activator of SA-induced genes, indicating that this transcription factor may be involved in the execution of the antagonistic action of JA and SA (Li and others 2004).

JA induces genes encoding transcription factors from several different classes. In addition to the transcription factor genes mentioned, a gene encoding a rice MYB transcription factor (Lee and others 2001) and two genes encoding petunia Cys_2/His_2 zinc-finger transcription factors were induced by JA (van der Krol and others 1999; Sugano and others 2003). This shows that the JA signal transduction pathway induces a number of primary response genes, some of which encode transcription

factors, leading to downstream branching and expression of a diverse set of secondary response genes. Some of the transcription factor genes mentioned may be primary response genes, such as the *ORCA3* gene (van der Fits and Memelink 2001) and the rice *MYB* gene (Lee and others 2001), whereas others may be secondary response genes, leading to further downstream branching.

Regulation of Regulatory Protein Abundance by JA

In addition to the regulation of cellular concentrations of transcription factors by transcriptional control, JA could regulate the abundance of these factors by adjusting the protein turnover rate. Protein stability is regulated via covalent modifications such as phosphorylation and/or ubiquitination.

The *coi1* mutation defines an *Arabidopsis* gene that is important for all JA responses, including pollen development and defense against pathogens (Feys and others 1994). The *COI1* gene encodes a protein containing leucine-rich repeats and an F-box motif (Xie and others 1998). Co-immunoprecipitation experiments show that COI1 forms part of an SCF (Skpl, cullin, F-box protein)-type ubiquitin ligase complex (Xu and others 2002; Devoto and others 2002). Yeast two-hybrid screening with COI1 as a bait detected interaction with a histone deacetylase (HDAC; Devoto and others 2002). Histone deacetylation represses transcription by decreasing the accessibility of chromatin to the transcription machinery (Alberts and others 2002). Therefore, it is possible that the COI1-interacting HDAC suppresses the transcription of JA-responsive genes under normal conditions. After JA treatment, the HDAC may be degraded via recruitment by the SCF^{COI1} complex, leading to expression of JA-responsive genes.

In reviews about JA signaling (for example, Turner and others 2002), COI1 is commonly a central player in JA signal transduction models. This does not conform to models employed in animal research and in molecular biology textbooks (Alberts and others 2002), where F-box proteins (β-TrCP in NF-κB-mediated gene expression), (for example Silverman and Maniatis 2001, Alberts and others 2002) are placed outside of the forward signaling pathway (Figure 2). In the case of COI1, it is even possible that it is involved in the constitutive degradation of a repressor independent of JA signalling. In that case, even its target repressor would not be part of the JA signal transduction pathway.

The role of COI1 in JA responses is always explained by targeting of a repressor for ubiquitin-mediated proteolysis (Turner and others 2002). Alternatively, COI1 may operate via the ''suicide model'' proposed for, among others, the well-known human MYC onco-protein (Kim and others 2003; von der Lehr and others 2003). According to this model, F-box-mediated ubiquitination stimulates the activity of the transcription factor and at the same time targets it for destruction (Bach and Ostendorff 2003).

Regulation of Transcription Factor Protein Activity by JA

In addition to the regulation of cellular concentrations of transcription factors, JA may also regulate the activity of certain transcription factors by post-translational modifications, such as phosphorylation, acetylation, hydroxylation, nitrosylation, glutathiolation, intra- and intermolecular S-S bridge formation between cysteines, myristoylation, farnesylation, ubiquitination, or glycosylation. These modifications may alter protein conformation, allow interaction with other regulatory proteins, or affect subcellular localization. These changes in turn can affect the DNA binding affinity, activation potential, nuclear localization and/or protein stability of the transcription factors.

An example of the induction of gene expression by JA via posttranslational modification is the JA-induced expression of the alkaloid biosynthesis gene *STR* mediated by the ORCA3 transcription factor (Figure 3). Expression of the *STR* and *ORCA3* genes is induced by MeJA in a CHX-insensitive manner (van der Fits and Memelink 2001), demonstrating that the induction of both genes depends on activation of pre-existing transcription factors. Apparently, ORCA3 is activated by JA signalling via posttranslational modification and/or protein-protein interaction. One of the possible posttranslational modifications is phosphorylation. Reversible phosphorylation is a prevalent mechanism by which the activity of eukaryotic transcription factors is regulated in response to changes in the cellular environment (Holmberg and others 2002). The induction *of STR* expression by JA is sensitive to protein kinase inhibitors (Menke and others 1999a), which is compatible with the possibility that ORCA3 is phosphorylated, although any protein in the signal transduction pathway leading to ORCA3 activation could be a phosphoprotein. In addition to activating ORCA3, JA also activates an upstream

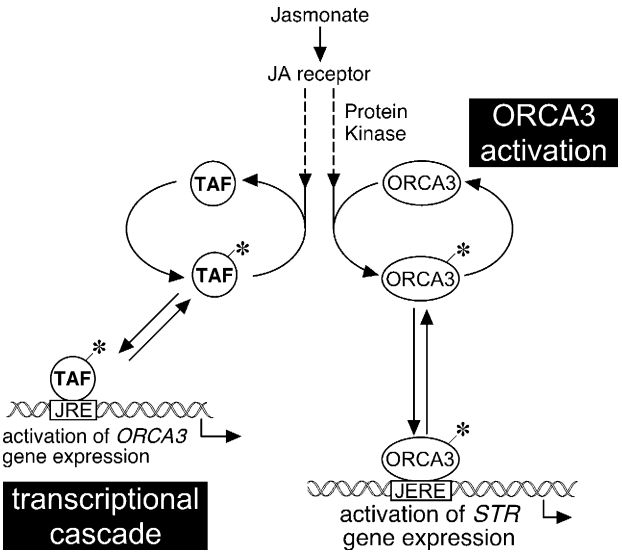

Figure 3. Model for JA-induced *STR* gene expression mediated by the ORCA3 transcription factor in *Catharanthus roseus*. JA directly activates the ORCA3 protein by posttranslational modification, indicated by an asterisk. Active ORCA3 stimulates *STR* gene expression by binding to the jasmonate- and elicitor-responsive element (JERE). JA also activates an upstream transcription factor by posttranslational modification, which then induces *ORCA3* gene expression via binding to a jasmonate-responsive element (JRE). ORCA3, octadecanoid-responsive Catharanthus AP2-domain protein 3; TAF, transcription activating factor; *STR*, *strictosidine synthase*.

transcription factor distinct from the ORCAs, which then induces *ORCA3* gene expression via binding to a JA-responsive element in its promoter (Figure 3)(Vom Endt and Memelink unpublished results).

Pharmacological studies using inhibitors of (de)phosphorylation have also been carried out in *Arabidopsis* with the intention of elucidating the role of protein phosphorylation in JA-responsive gene expression (Rojo and others 1998; Jensen and others 2002). Indeed, some conclusions were drawn by these authors, the main one being that JA signal transduction leading to the expression of the *JR1, JR2, VSP*, and *LOX* genes depends on one or more protein dephosphorylation steps. However, because these publications also showed that these genes are not primary response genes, the conclusions are not supported by the evidence. These reports are illustrative of a common caveat of many plant signal transduction studies, which simply copy experimental approaches from animal signal transduction research without paying attention to the theoretical framework necessary for correct interpretation. Translation involves a large number of proteins, many of which are (de)phosphorylated during the process. The apparent sensitivity of the JA-responsive expression of the genes studied to protein phosphatase inhibitors could, for example, be due to the fact that the recycling of translation initiation factor eIF-2 into new rounds of translation requires its dephosphorylation (Alberts and others 2002).

Nonetheless, there are some indications that phosphorylation may play a role in JA signal transduction. In the *Arabidopsis mpk4* mutant, in which the gene encoding the mitogen-activated protein kinase AtMPK4 is disrupted, the induction of *PDF1.2* and *THI2.1* expression by JA was blocked, suggesting that this MAP kinase functions downstream of JA in regulating *PDF1.2* and *THI2.1* expression (Petersen and others 2000). However, because *PDF1.2* is not a primary response gene, AtMPK4 may also operate on secondary responses. For example, it could phosphorylate one or more JA-responsive transcription factors synthesized *de novo* in response to JA. ERF1 (Lorenzo and others 2003) and/or AtERF2 (Brown and others 2003), which upregulated *PDF1.2* expression when overexpressed, could be phosphorylation targets of AtMPK4. Phosphorylation of AP2-domain transcription factors may increase their DNA binding affinity, as demonstrated for *in vitro* binding of Pti4 upon phosphorylation by the receptor kinase Pto (Gu and others 2000).

Conclusion and Perspectives

Several studies including micro- and macro-array analyses, have shown that JA induces the transcription of genes involved in a variety of processes, including defense responses against microbial pathogens and insects, wounding responses, jasmonic acid biosynthesis, secondary metabolite biosynthesis, cell maintenance, amino acid and sugar metabolism, and senescence. A distinction between primary and secondary response genes has in general not been made. In the promoters of these genes various JA-responsive elements have been identified, without distinction between primary and secondary response elements. The isolation of *trans*-acting factors that bind to primary JA-responsive promoter elements should give more information about the mechanisms by which JA regulates the expression of these genes. Such transcription factors have not been isolated yet from *Arabidopsis*. The ORCA transcription factors from *C. roseus* are the only regulatory proteins described for which the available evidence strongly indicates that their activity states are modulated by the JA signal transduction pathway. In *Arabidopsis*, several tran-

scription factors have been identified whose gene expression levels are increased by JA. Overexpression analysis of some of these JA-responsive transcription factors identified several putative target genes. The actual binding of these JA-responsive transcription factors to these targets *in vivo* needs to be confirmed, for example, by using chromatin immuno-precipitation experiments. All these putative target genes are secondary response genes, indicating that the corresponding transcription factors are not targets of JA signal transduction, but are synthesized *de novo* in response to JA. This also implies that there is no evidence so far for the popular notion of cross-talk between JA signal transduction and other signalling pathways, because all these synergistic and antagonistic interactions occur at the level of secondary responses.

The induction of gene expression by JA probably occurs via posttranslational modification of pre-existing transcription factors. Research on JA-responsive gene expression in *C. roseus* cells indicates that protein phosphorylation and specific AP2-domain transcription factors are elements in a JA signal transduction pathway regulating the expression of primary response genes. In *Arabidopsis* not a single component of the forward primary JA signal transduction pathway has been isolated. Current challenges are to identify these pre-existing transcription factors in *Arabidopsis*, to define the nature of the posttranslational modifications and the enzymes involved in it. One common modification of transcription factors is phosphorylation. Due to the conceptual confusion in plant signal transduction research, at the moment even the role of phosphorylation, if any, in JA signal transduction in *Arabidopsis* is unknown.

The main important idea that *Arabidopsis* research has contributed to unraveling the primary JA signal transduction pathway is that protein degradation mediated by the F-box protein COI1 seems to be of vital importance. The SCF^{COI1} complex putatively targets negative regulators of JA-responsive gene expression for proteolytic destruction, although examples for an alternative explanation exist. Although one of the putative substrates for the SCF^{COI1} complex has been identified, it will be interesting to determine its *in vivo* role, and to identify other substrates for this complex. It is also important to determine whether the activity of COI1 towards its substrates is constitutive or modulated by JA. In the latter case, a major challenge is to determine the mechanism by which JA modulates recruitment of potential substrates by the SCF^{COI1} complex. By analogy to recruitment of substrates by mammalian F-box proteins, post-translational modifications such as phosphorylation or oxidation, or interaction with other proteins may be involved.

As a final message, we would like to advocate a signal transduction terminology in the plant research field that conforms to definitions used in animal research and in textbooks, to eliminate the current confusion and to obtain a clear separation between causes and consequences in descriptions of JA signal transduction.

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