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Review

Jasmonate-regulated Arabidopsis stress signalling network

Alessandra Devoto* and John G. Turner

School of Biological Sciences, University of East Anglia, Norwich NR4 7TJ, Norfolk, UK
**Corresponding author, e-mail: a.devoto@uea.ac.uk*

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Plants respond to biotic and abiotic factors in the external environment. These include wounding, pathogen and pest attack, and changes in light and temperature. They also respond to internal signals produced during development, such as during the formation of flowers. These responses generally involve the re-programming of gene expression. Biotic and abiotic stress signal perception responses have commonly been represented as three pathways, which can be distinguished by the production of jasmonates, ethylene or salicylic acid. Each of these pathways therefore involves the perception of the stress, the synthesis of the signal molecule, the perception of the signal molecule and the ensuing response. An added complication is that these signalling pathways act both locally, at the point of the stress, and systemically. Much of what we know about these signalling pathways has come from studies of responses to the signal molecules, and of mutants altered in the production of, or sensitivity to, these signal molecules. This has provided clear indications that the pathways interact significantly. Because biotic and abiotic stresses induce the production of all three of these signal molecules, albeit in differing amounts, it is more appropriate to view the response output as the integration of a signalling network that involves the production of jasmonates, salicylic acid and ethylene.

In this review, we provide an update on the regulation of jasmonic acid biosynthesis and the suggested roles for different biologically active intermediates in this pathway in Arabidopsis, and describe the jasmonate signalling mutants identified so far. We also examine how outputs from the jasmonate, salicylic acid and ethylene signalling pathways are integrated in the regulation of stress response and plant development. We use Boolean gates as a tool to represent the molecular networks and provide a qualitative description of the transmission of the signals. Finally, we illustrate how protein degradation, a common mechanism regulating many plant processes, may act as the ultimate level of integration between signalling pathways.

Jasmonate signals have their own specific identity

(+)-7-*iso*-JA is commonly referred to as jasmonic acid (JA), and is one of the final products of the octadecanoid pathway (Fig. 1). This molecule functions as a signal, together with other intermediates in this pathway and with biologically active derivatives (referred to collectively as jasmonates), in response to stimuli of a biotic or abiotic nature. Jasmonates are fundamental to the mediation of responses to stress, such as wounding and elicitor molecules (Doares et al. 1995; Kramell et al. 1995; Parchmann et al. 1997; Leon et al. 2001), ultraviolet light and ozone exposure (Overmyer et al. 2000; Rao et al. 2000), drought (Reymond et al. 2000; Sugano et al. 2003), defences against insects (McConn et al. 1997) and pathogens (Thomma et al. 1999; Kloek et al. 2001). Jasmonates also elicit protective alkaloid production in *Eschscholtzia californica* cell cultures (Byun 2000). JA, its precursor 12-oxo-phytodienoic acid (OPDA) and other oxylipins (Krumm et al. 1995; Bate and Rothstein 1998) act as signals for defence (Fig. 1), suggesting that an entire cohort of molecules regulates host responses to attackers. For example, OPDA (a precursor of JA; Fig. 1) acts as a signal for defence, and induces broad-spectrum resistance in the absence of JA (Stintzi et al. 2001). The methylation of JA to methyl jasmonate (MeJA) is catalysed by an *S*-adenosyl-L-methionine:jasmonic acid carboxyl methyltransferase (JMT;

Abbreviations – axr, auxin resistant; coi, coronatine insensitive; CSN, COP9 signalosome-proteasome; ET, ethylene; JA, jasmonate; jai, jasmonate insensitive; JMT, *S*-adenosyl-l-methionine: jasmonic acid carboxyl methyltransferase; LOX, lipoxygenase; MCP, 1-methylcyclopropene; MeJA, methyl jasmonate; OPDA, 12-oxo-phytodienoic acid; OPR3, OPDA reductase3; SA, salicylic acid; SCF, SKP1-Cullin-Fbox protein; vsp, vegetative storage protein.

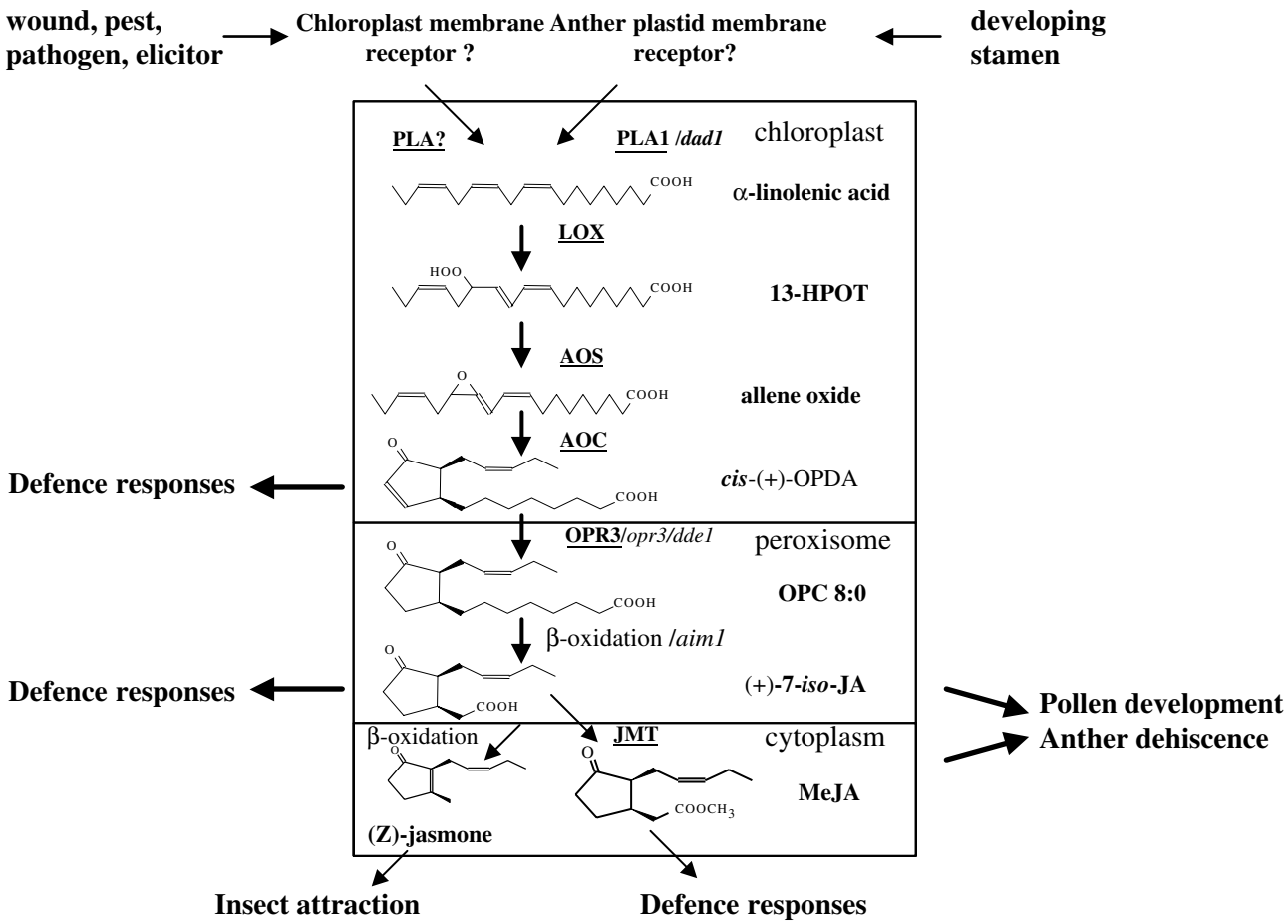

Fig. 1. Biosynthesis of jasmonates in Arabidopsis. The existence of a membrane receptor that is activated by elicitors remains to be demonstrated. All enzymes catalysing OPDA formation are localized in the chloroplast (Mueller 1997; Stintzi and Browse 2000). AOC, allene oxide cyclase; AOS, allene oxide synthase; DAD1, defective anther dehiscence1; 13-HPOT, 13-hydroperoxylinolenic acid; JA, jasmonic acid; JMT, *S*-adenosyl-L-methionine:jasmonic acid carboxyl methyltransferase; LOX, lipoxygenase; MeJA, methyl jasmonate; OPC 8:0, 3-oxo-2(2′pentenyl)-cyclopentane-1-octanoic acid; OPDA, 12-oxo-phytodienoic acid; OPR3, OPDA reductase3; PL, phospholipase; abbreviations for enzyme names are underlined; abbreviations for names of intermediates are in bold; mutants identified are in italic.

Fig. 1): overexpression of a JA methyltransferase gene increases resistance to the necrotroph *Botrytis cinerea*, strengthening the role for MeJA in the induction of pathogen defence responses (Seo et al. 2001). So far, genetic screens have not identified a JA receptor: it remains to be elucidated whether different stimuli interact with a single common receptor, or whether different receptors regulate signalling pathways that converge on the pathway for JA responses (Fig. 1). Jasmonates also influence many plant processes, including fruit ripening, root growth (Staswick et al. 1992), pollen development and anther dehiscence (Feys et al. 1994; McConn and Browse 1996; Sanders et al. 2000; Stintzi and Browse 2000).

Plant responses to the activation of JA signalling eventually involve the induction of genes, such as those encoding the vegetative storage proteins (*VSPs*; Benedetti et al. 1995), thionin (*Thi2.1*; Epple et al. 1995; Vignutelli et al. 1998) and a plant defensin (*PDF1.2*; Penninckx et al. 1998). JAs also enhance the transcription of genes involved in JA synthesis (Fig. 1), such as *DAD1*, *LOX2*, *AOS*, *OPR3* and *JMT* (Heitz et al. 1997; Laudert and Weiler 1998; Mussig et al. 2000; Ishiguro et al. 2001; Seo et al. 2001). Microarray analyses have confirmed the MeJA inducibility and organ-specific expression of genes required for JA biosynthesis, corroborating the existence of a positive feedback regulatory system for JA biosynthesis (Sasaki et al. 2001).

Several reviews have already described in detail the regulation of jasmonate biosynthesis and the biological effects of JAs (Creelman and Mullet 1997; Berger et al. 2002; Turner et al. 2002; Weber 2002; Devoto and Turner 2003; Devoto et al. 2003). We therefore focus mainly on the latest findings, placing them in the context of signalling 'cross-talk'.

Mutants identified in the jasmonate biosynthetic and signal transduction pathways

All the mutants depicted below are listed in Table 1 where all abbreviations are given.

Table 1. Arabidopsis mutants in the jasmonate biosynthesis, perception and signal transduction pathways. aim, abnormal inflorescence meristem; axr, auxin resistance; coi, coronatine insensitive; cos, coi1-suppressor; dad, delayed anther dehiscence; dde, delayed dehiscence; GC, gas chromatography; JA, jasmonic acid; jai, jasmonate insensitive; jar, jasmonate resistant; jin, jasmonate insensitive; joe, jasmonate overexpressing; jue, jasmonate underexpressing; LOX, lipoxygenase; luc, luciferase; MeJA, methyl jasmonate; PCR, polymerase chain reaction; thi, thionin; vsp, vegetative storage protein.

Mutant	Screen	Gene	Reference
JA biosynthesis			
fad3-2, *7-1*, *8*	Altered fatty acid composition of single leaf by GC	*FAD3*, *7* and *8* fatty acid desaturases	(Browse et al. 1986, 1993; McConn et al. 1994)
dad1	Altered male fertility	*DAD1* – chloroplastic phospholipase A1	(Ishiguro et al. 2001)
dde2	Altered male fertility	*AOS* – allene oxide synthase	(Von Malek et al. 2002)
aos	PCR-based reverse genetics – TDNA insertion (TJ1180)	*CYP74A* (cytochrome P450 – allene oxide synthase, *AOS*)	(Park et al. 2002)
opr3, *dde1*	Altered male fertility	*OPR3* – 12-oxophytodienoic acid reductase	(Sanders et al. 2000; Stintzi and Browse 2000)
aim1	Altered male fertility	nd	(Richmond and Bleecker 1999)
Perception and signal transduction			
cev1	Constitutive expression of the transgene *pVSP1-luc*	*AtCesA3* – cellulose synthase	(Ellis and Turner 2001; Ellis et al. 2002a)
cet1 to *cet9*	Constitutive expression of *Thi2.1*	nd	(Hilpert et al. 2001)
cex1	Stunted phenotype in absence of JA	nd	(Xu et al. 2001)
joe1 and *2*	Aberrant expression of *pLOX2-luc* transgene	nd	(Jensen et al. 2002)
jue1 to *3*	Aberrant expression of *pLOX2-luc* transgene	nd	(Jensen et al. 2002)
cas1	Constitutive *AOS* expression	nd	(Kubigsteltig and Weiler 2003)
coi1-1	Insensitivity to coronatine	*COI1* – Fbox-LRR	(Feys et al. 1994; Xie et al. 1998)
coi1-16	Lacks *pVSP1-luc* transgene expression in presence of JA	*COI1* – Fbox-LRR	(Ellis and Turner 2002)
coi1-20	Enhanced resistance to *P. syringae*	*COI1* – Fbox-LRR	(Kloek et al. 2001)
coi1/*jai5* (7 alleles)	Insensitivity to MeJA	*COI1* – Fbox-LRR	(Lorenzo et al. 2004)
cos1	Suppression of *coi1* root JA insensitivity	*COS1* – lumazine synthase	(Xiao et al. 2004)
jai1/*jin1*	Insensitivity to MeJA	*AtMYC2* – nuclear localized bHLHzip transcription factor	(Berger et al. 1996; Lorenzo et al. 2004)
jai3 and *4*	Insensitivity to MeJA	nd	(Lorenzo et al. 2004)
jar1-1/*jin4*/*jai2*	Insensitivity to MeJA	JA adenylase/luciferase superfamily	(Staswick et al. 1992; Berger et al. 2002; Lorenzo et al. 2004)
mpk4	Transposon tagging – dwarf phenotype	*MPK4* – mitogen-activated protein kinase	(Petersen et al. 2000)
axr1-24	Insensitivity to MeJA	*AXR1* – Nedd8/RUB1-activating enzyme subunit	(Leyser et al. 1993; Tiryaki and Staswick 2002)

JA biosynthesis

The analysis of JA biosynthetic mutants has demonstrated that jasmonates contribute to pollen maturation, anther dehiscence and wound-induced defence against biotic attacks.

Fatty acid metabolism is affected and JA synthesis is totally impaired in the triple mutant *fad3-2*, *7-1*, *8* (Browse et al. 1986, 1993). It shows enhanced sensitivity to *Bradysia impatiens*, which can be rescued by exogenous application of JA (McConn et al. 1997). The involvement of a phospholipase A1 in JA biosynthesis during pollen and anther development has been demonstrated following the isolation and characterization of *DAD1* (Ishiguro et al. 2001; Turner et al. 2002; Fig. 1). Similarly, the identification of the mutant *dde2* (Von Malek et al. 2002) clearly demonstrated a role for *AOS* in Arabidopsis male fertility. AOS is a cytochrome P450 enzyme (CYP74A) that catalyses the first committed step in JA synthesis (Fig. 1). A knock-out mutant defective in *CYP74A*, isolated by Park et al. (2002), contained reduced amounts of JA and, like *dde2*, was male sterile. Plants in which *AOS* was overexpressed had enhanced wound-induced induction of *Arabidopsis thaliana* vegetative storage protein 2 (*AtVSP2*), demonstrating the role of AOS as a modulator of the wound signal. The *opr3*/*dde1* mutants are blocked in JA biosynthesis after OPDA. The mutants are impaired in pollen development and show delayed anther dehiscence, leading to male sterility which cannot be rescued by OPDA (Sanders et al. 2000; Stintzi and Browse 2000). However, in contrast with *fad3-2*, *7-1*, *8* and *coi1* (see below), *opr3* is more resistant to *Bradysia impatiens* (Stintzi et al. 2001). Reduced fertility is also shown by *aim1*, which is defective in an acyl-CoA hydratase and possibly in a β-oxidation step (Richmond and Bleecker 1999; Fig. 1). In all the mutants described above, male sterility could be restored by exogenous application of MeJA.

Perception and signal transduction

Mutants with constitutive or enhanced JA responses

The *cev1* mutant overproduces OPDA, JA and ethylene and shows constitutive expression of *VSP*, *PDF1.2*, *Thi2.1* and the chitinase *CHI-B* (Ellis and Turner 2001; Ellis et al. 2002a). *cev1* plants are stunted and accumulate anthocyanins. This mutant has enhanced defences against both the biotrophic fungal pathogen, *Erysiphe cichoracearum*, and the bacterial pathogen, *Pseudomonas syringae*, and increased resistance to the aphid, *Myzus persicae* (Ellis et al. 2002b). Activation of the JA pathway may therefore contribute to the reduction of susceptibility to both necrotrophic (JMT, see above) and biotrophic pathogens. Ellis et al. (2002a) have shown that *cev1* acts at an early step in the stress perception/transduction pathway, and may act as a negative regulator of JA and ethylene pathways. The *cev1* mutant phenotype is partially suppressed in the *coi1* (see below) and in the *ethylene resistant1* (*etr1*) mutant backgrounds, and the triple mutant, *cev1;coi1;etr1*, appears as the wild type except for slightly shorter roots (Ellis et al. 2002a). *CEV1* encodes the cellulose synthase *CeSA3* (Ellis et al. 2002a), indicating involvement of the cell wall in mediating JA- and ethylene-dependent stress and defence responses. The mutation in *CeSA3* in *cev1* also alters the cellulose content of the roots and leads to ectopic lignification.

Nine *cet* mutants have been isolated (Hilpert et al. 2001). Most of the mutants show spontaneous leaf necrotic lesions and two (*cet1* and *cet3*) contain high levels of OPDA and JA. *cet4*-1 contains wild-type levels of these molecules and may therefore affect the sensitivity to JA or transduction of the signal downstream of JA production. The *cex1* mutant (Xu et al. 2001) overexpresses *Thi2.1*, *VSP* and *PDF1.2*, and may encode a negative regulator of JA signalling. Like the *cev1* mutant, *cex1* plants are also stunted. It remains to be elucidated whether the similarity of phenotypes between the *cet*, *cex1* and *cev1* mutants can be explained by allelism.

Lipoxygenases (LOXs) catalyse the oxygenation of fatty acids to their hydroperoxy derivatives (Fig. 1). In a screen for aberrant expression of the transgene *pLOX2-luc*, Jensen et al. (2002) isolated three recessive mutants that underexpress the reporter, designated *jue*1, 2 and 3, and two recessive mutants that overexpress the reporter, designated *joe*1 and 2. Reporter overexpression in the *joe* mutants requires *COI1* (see below), suggesting that they act prior to *COI1* to regulate *LOX2* expression. *joe1* responded to MeJA with increased anthocyanin accumulation, while *joe2* responded with decreased root growth inhibition. In addition, wild-type induction of the reporter and endogenous *LOX2* expression by the serine-threonine protein kinase inhibitor, staurosporine, were deficient in *joe2*. The *joe2* mutation may lead to inactivation of a kinase or its substrate, while *joe1* may act prior to a phosphorylation event in the JA signalling pathway. The *cas1* mutant shows elevated levels of AOS, OPDA and JA and a flower phenotype similar to the biosynthetic mutant *dad1* (Kubigsteltig and Weiler 2003; Fig. 1). Whilst *joe1* maps in the same region on chromosome V as *cev1* (Ellis and Turner 2001), *cas1* and *joe2* may affect the same or related locus.

Mutants with reduced sensitivity to jasmonate

JA-insensitive mutants have been isolated by screening for insensitivity to coronatine (a structural analogue of MeJA), to MeJA itself (Staswick et al. 1992; Feys et al. 1994; Berger et al. 1996; Lorenzo et al. 2004) and in a screen for mutants that do not express a *VSPA::luc* transgene in the presence of JA (Ellis and Turner 2001). These screens identified multiple alleles of the gene *coi1* (Xie et al. 1998) and the mutants *jin* (*1* and *4*; Berger et al. 1996), *jar1* (Staswick et al. 1998) and *jai1–5* (Lorenzo et al. 2004). Allelic relationships are indicated in Table 1. The *COI1*, *JAR1* and *JAI1*/*JIN1* genes have been isolated, but none of them defines a receptor for JA, corroborating the hypothesis that there is redundancy amongst the JA receptors.

COI1 is required for all the JA-dependent responses tested so far. The *coi1* mutants exhibit enhanced susceptibility to powdery mildew (Ellis and Turner 2002) and to several necrotrophic pathogens, such as *A. brassicicola* (Penninckx et al. 1996; Thomma et al. 1998), *Botrytis cinerea* (Thomma et al. 1998) and *Erwinea carotovora* (NormanSetterblad et al. 2000). *coi1* is also more susceptible to aphid infestation (Ellis et al. 2002b) and, contrary to *cev1*, its resistance cannot be further increased following JA treatment. Recent results have also shown diminished resistance of *coi1* to the sciarid fly, *Bradysia paupera*, and to the diamond back moth, *Plutella xylostella* (A. Devoto and J. G. Turner 2004, unpublished results). On the contrary, Stotz et al. (2000) demonstrated that *jin1* and *4* have wild-type sensitivity to this diptera. That the *coi1* mutation confers resistance to various strains of *P. syringae* (Feys et al. 1994; Kloek et al. 2001; Ellis et al. 2002b) is apparently difficult to reconcile with the above. However, the finding that resistance to this particular pathogen in *coi1* occurs through a salicylic acid (SA)-independent inability to develop disease symptoms (Kloek et al. 2001) is consistent with the hypothesis that the *P. syringae* phytotoxin, coronatine, acts to promote virulence by inhibiting host defence responses. Enhanced *PR1* (*pathogenesis related1*) expression in *coi1*-20 plants is compromised in the double mutants, *coi1*-20;*npr1*-1 and *coi1*-20;*nahG*, implying activation of the SA-dependent defence pathway in the *coi1* mutants (Fig. 2), which results in restriction of pathogen growth. Significantly, *coi1* is male sterile: this phenotype is shared with other mutants involved in JA biosynthesis, reinforcing the importance of JA in reproduction. However, a number of JA signalling mutants, such as *jar1* and *jin1*, still produce fertile flowers. The recent isolation of the tomato mutant *jai1* (Li L et al. 2004), a homologue of *COI1*, revealed that JA has multiple roles in the reproductive development of different plant species. Whether these roles are mediated by different signalling components remains to be elucidated. On the contrary, several defence-related phenotypes of *jai1* are similar to those of *coi1*.

The Arabidopsis *COI1* gene encodes one of the 694 Arabidopsis F-box proteins (Gagne et al. 2002), and is closely related to the auxin signalling *TIR1* gene (Ruegger et al. 1998). The COI1 protein has been shown to be a component of an SCF^{COI1} complex (Devoto et al. 2002;

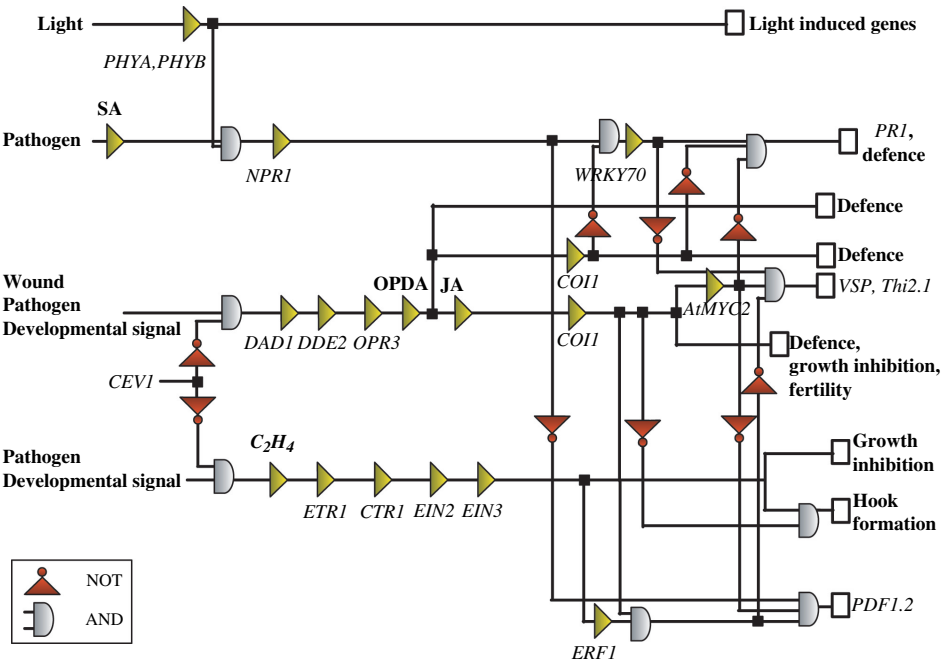

Fig. 2. Boolean representation of the signal transduction network between jasmonate (JA), ethylene (ET), salicylic acid (SA) and light signalling pathways. A wound signal may induce the production of JA which will stimulate the expression of JA-responsive genes, such as *Thi2.1* and *VSP*. JAs, via *COI1*, and ET, via *ETR1* and *CTR1*, act synergistically and in an *ERF1*-dependent manner to induce the expression of *PDF1.2*, but act antagonistically with respect to the expression of *VSP* and *Thi2.1*. *AtMYC2* antagonistically regulates two branches of the JA signalling pathway in a *COI1*-dependent manner. *AtMYC2* positively regulates genes such as *VSP* and negatively regulates pathogen response genes such as *PR1* and *PDF1.2*. *cev1* has constitutive JA and ET signalling. *COI1*-dependent *PR1* repression represents antagonism between JA and SA signalling. The antagonistic effect of SA on JA signalling requires *NPR1*. At the same time, the induction of *PR1* by SA correlates strictly with the activity of the signalling pathway controlled by both phyA and phyB photoreceptors. *WRKY70* is downstream of *NPR1* in the SA-dependent signalling pathway, and analysis of overexpressing lines has revealed that it acts as an activator of SA-induced genes and as a repressor of JA-responsive genes. Abbreviations for gene names are in italics and given below the symbols; abbreviations for the names of signalling molecules are in bold and given above the symbols. *CEV1*, constitutive expressor of *VSP*; *COI1*, coronatine insensitive1; *CTR1*, constitutive response1; *DAD1*, delayed anther dehiscence1; *DDE1*, delayed dehiscence1; *EIN1*, ethylene insensitive1; *ERF1*, ethylene response factor1; *ETR1*, ethylene resistant1; *NPR1*, nonexpressor of *PR1*; OPDA, 12-oxo-phytodienoic acid; *OPR3*, OPDA reductase3; *PDF1.2*, plant defensin1; *PR1*, pathogenesis related1; *Thi2.1*, thionin2.1; *VSP*, vegetative storage protein.

Xu et al. 2002), one of the six families of Arabidopsis E3 ubiquitin-ligases. These are the specificity determinants mediating the final transfer of ubiquitin molecules to target proteins that will undergo proteolytic degradation in the 26S proteasome (Hochstrasser 1996; Deshaies 1999). Feng et al. (2003) have demonstrated direct in planta interaction between COI1 and subunits of the COP9 signalosome-proteasome (CSN). CSN and SCF^{COI1} also appear to collaboratively mediate JA responses.

The *coi1* suppressor, *cos1*, restores JA-dependent senescence and the expression of some JA-inducible, senescence-associated genes to the *coi1* mutant (Xiao et al. 2004). This mutant also rescues resistance to *B. cinerea*. *COS1* encodes a lumazine synthase, the enzyme involved in the penultimate step of vitamin B2 biosynthesis, suggesting a role for the riboflavin pathway in JA signalling.

jar1-1 is involved in protection against the opportunistic soil fungus, *Pythium irregulare* (Staswick et al. 1998). This is in accordance with the evidence that defects in JA sensitivity or biosynthesis enhance the susceptibility to necrotrophic pathogens. *jar1*-1 also has a role in systemic resistance against various other pathogens (van Loon et al. 1998; Clarke et al. 2000) and in limiting damage from ozone exposure (Overmyer et al. 2000; Rao et al. 2000). *JAR1* belongs to a multigene family that includes the auxin-induced soybean *GH3* (Abel and Theologis 1996; Staswick et al. 2002). Fold prediction modelling and an in vitro biochemical assay have revealed that *JAR1* is structurally related to the firefly luciferase superfamily of adenylate-forming enzymes. This suggests that adenylation of JA is required for some but not all JA responses. Recently, Lorenzo et al. (2004) have identified five *jai1* alleles and have cloned the product encoded by *JAI1*/*JIN1*, AtMYC2, a nuclear localized bHLHzip transcription factor that antagonistically regulates two branches of the JA signalling pathway in a *COI1*-dependent manner. *AtMYC2* positively regulates genes such as *VSP* and *LOX3*, previously shown to be activated by wounding or JA alone (Reymond et al. 2000; Cheong et al. 2002), and negatively regulates pathogen response genes such as *PR1* and *PDF1.2* (Fig. 2). In contrast, ethylene, through *ERF1* (*ethylene response factor1*), negatively regulates the set of genes activated by wounding or JA (Lorenzo et al. 2004; Fig. 2).

The dwarf, JA-insensitive mutant *mpk4* also contains high levels of SA and shows constitutive expression of *PR1* (Petersen et al. 2000). The mitogen-activated protein kinase MPK4 therefore seems to be involved in the regulation of cross-talk between the two signalling molecules by concurrent suppression of SA biosynthesis and promotion of JA perception/response.

The auxin-resistant mutant *axr1*-24 has been isolated in screens for decreased sensitivity to JA (Tiryaki and Staswick 2002) and ethylene (Lincoln et al. 1990). This mutant also shows reduced sensitivity to other inhibitors of root growth, such as the ethylene precursor 1-aminocyclopropane-1-carboxylic acid, 6-benzylaminopurine, epi-brassinolide and abscisic acid. *AXR1* is also necessary for resistance to *Pythium irregulare* in Arabidopsis. JA-responsive genes *LOX2*, *AOS* and *AtVSP* were also induced by IAA in *axr1*-24. Root growth inhibition and *VSP* transcript induction are impaired in *axr1*-3 mutants, and the allele *axr1*-12 has reduced pollen yield and shorter anther filaments (Tiryaki and Staswick 2002).

How jasmonate interconnects with ethylene and SA signalling pathways

Genoud, Metraux and coworkers (Genoud and Metraux 1999; Genoud et al. 2001, 2002) have adapted Boolean language in a very elegant way to represent and analyse the interactions between pathways. They have demonstrated using Boolean gates that signalling processes may be represented more accurately as network-like structures than with linear sequences of events in intuitive formalism. In this language, interfering input signals reach a Boolean gate (or switch with a molecular identity), generating an output signal that results from the combination of all inputs going through the gate (Arkin and Ross 1994). By using digital simulation programs, as described by Genoud et al. (2001), it is possible to predict the outputs of the logical gates by activating or inactivating input signals. We focus here on representing the integration of the JA pathway with the SA and ethylene signalling pathways (Fig. 2). The existence of multiple interferences and intersections between the JA, SA and ethylene signalling pathways can be inferred from the characteristics of the mutants described above. The discussion here highlights elements of this complex 'circuit'.

The *cev1* mutant has been used to investigate the transfer of signals between the JA, ethylene and SA signalling pathways (Ellis et al. 2002a). Treatment of *cev1* with SA suppresses expression of *PDF1.2* and enhances expression of *PR1*, although less so than in wild-type plants. *coi1* mutants, which are deficient in JA response, have slight but significant *PR1* expression, indicating that a *COI1*-dependent signal normally suppresses *PR1* in untreated plants. The double mutant *cev1;coi1* expresses neither *PDF1.2* nor *Thi2.1*, confirming that expression of these genes requires the JA perception response pathway regulated by *COI1*. In the double mutant *cev1;etr1* (*etr1*, ethylene resistant), *PDF1.2* expression is absent, confirming a requirement for an ethylene signal for *PDF1.2* transcription (Ellis and Turner 2001). *Thi2.1* is constitutively expressed in this double mutant, indicating that ethylene signalling suppresses the transcription of *Thi2.1*.

ERF1 is clearly a convergence point between ethylene and jasmonate pathways (Fig. 2), encoding a transcription factor which, when overexpressed, regulates pathogen response genes that prevent disease progression (Lorenzo et al. 2003, 2004). Overexpression of *ERF1* can rescue the defence response defects of both *coi1*

and *ein2* (*ethylene insensitive2*). Transcription profiling of *35S:ERF1* plants also confirms and extends the overlap between sets of ERF1 and ethylene/jasmonate up- and downregulated genes.

It is notable that different signalling pathways may regulate, synergistically or antagonistically, the same developmental process. Clearly, the specific responses to the various signalling molecules are caused, at least in part, by the physiological state of the cell upon which they act. Genetic analysis of the JA biosynthetic mutants highlighted the role of JA in the production of pollen and in anther dehiscence. There is evidence that JA produced in the stamen synchronizes the later stages of flower development (Sanders et al. 2000; Ishiguro et al. 2001). Rieu et al. (2003) have shown, by expressing the Arabidopsis mutant *etr1*-1 receptor or treatment with the ethylene perception inhibitor 1-methylcyclopropene (MCP), that ethylene insensitivity delays anther dehiscence in tobacco. These findings suggest that ethylene, like JA, promotes the final steps of flower development. It remains to be elucidated whether they have or have not redundant functions. The phenotype of Arabidopsis *dde1* would be in agreement with the second possibility. The balance between ethylene and JA signalling influences the development of the apical hook in dark-grown seedlings of mutants in the JA and ethylene signalling pathways. As a result, JA suppresses the apical hook in wild-type and *ctr1* (*constitutive response1*) seedlings, but not in *coi1* seedlings, and ethylene increases the formation of the apical hook in wild-type and *coi1* seedlings (Ellis and Turner 2002; Turner et al. 2002). The response of the *cev1* mutant to JA and ethylene is similar to that of wild-type plants, and the response of the double mutant *cev1;etr1* is similar to that of *etr1* mutants. These results reveal an antagonistic relationship between the JA and ethylene signalling pathways in apical hook development. Significantly, the auxin signalling mutant *axr1*-12 is deficient in hypocotyl hook formation. It is therefore possible that, through *COI1*, JA affects the formation of the apical hook by antagonizing ethylene or auxin function.

Ethylene, SA and JA signalling pathways are all induced by ozone (Overmyer et al. 2000; Rao et al. 2000). However, it has recently been demonstrated that ethylene and JA have opposite effects on ozone-induced spreading cell death (Tuominen et al. 2004), where JA acts as a protecting agent. The mutants *jar1*, *coi1* and *fad3-2;fad7-2;fad8* show hypersensitivity to ozone. This observation correlates with the increased susceptibility of JA mutants to necrotrophic pathogens, whose growth is facilitated by cell death. Incidentally, the initiation of the cell death response also exhibits a strict dependence on both the presence and amplitude of a phytochrome-elicited signal. The growth of an incompatible strain of *P. syringae* was enhanced in the light perception double mutant *phyA;phyB* and decreased in the light signal processing *psi2* (*phytochrome signalling2*) mutant (Genoud et al. 2002).

There is significant communication between JA and SA pathways in different plant species, such as Arabidopsis and tomato (Stout et al. 1999; Moran and Thompson 2001). JA and SA signal cascades may activate different sets of plant defence genes (Thomma et al. 1998) or act antagonistically on the same genes (Doares et al. 1995; Felton et al. 1999). Several genetic studies provide evidence that JA signalling negatively regulates the expression of SA-responsive genes in Arabidopsis (Petersen et al. 2000; Kloek et al. 2001). Transduction of the SA signal requires *NPR1*/*NIM1* (nonexpressor of *PR1*/non-immune), a regulatory protein sharing structural similarity with IkB, a target protein that undergoes ubiquitin-mediated protein degradation in the proteasome during anti-inflammatory responses in animals (Regnier et al. 1997). Similarly, the antagonistic effect of SA on JA signalling requires *NPR1*. At the same time, the induction of *PR1* by SA and functional analogues has been found to correlate strictly with the activity of the signalling pathway controlled by both phyA and phyB photoreceptors (Genoud et al. 2002). Although nuclear localization of NPR1 is essential for the induction of *PR* genes (Kinkema et al. 2000), interaction between SA and JA is modulated through NPR1 in the cytosol (Spoel et al. 2003). Another common component in SA- and JA-mediated signalling pathways is the transcription factor *WRKY70* (Li et al. 2004). *WRKY70* is downstream of *NPR1* in the SA-dependent signalling pathway, and analysis of overexpressing lines reveals that it acts as an activator of SA-induced genes and as a repressor of JA-responsive genes. *WRKY70* mRNA levels are enhanced in a *coi1*-1 background, suggesting that JA represses its expression.

The regulation of protein turnover is a control element common to many plant signalling pathways

A plethora of reviews have been published on the mechanisms of ubiquitin-mediated protein degradation in both animals and plants. In ubiquitin-mediated proteolysis, activated ubiquitin is bound to the substrate protein by a ubiquitin-protein ligase (E3). Polyubiquitinated proteins are then recognized and degraded by the 26S proteasome (Fig. 3). E3 ligases control the specificity of substrate ubiquitination. Several different classes of E3 ligases have been identified (Hershko and Ciechanover 1998; Patton et al. 1998; Deshaies 1999; Pickart 2001). The importance of SCF-type E3 ligase activities in regulating plant signalling pathways has also been recognized (Ellis et al. 2002c; Devoto et al. 2003; Sullivan et al. 2003; Vierstra 2003).

SKP1 (S-PHASE KINASE-ASSOCIATED PROTEIN1), Cullin (CUL1), F-box containing proteins and Rbx (RING-box protein1) are the characterized components of the SCF (SKP1-CDC53p/CUL1-F-box) complex (Fig. 3). In Arabidopsis, there are at least 21 Skp1 homologues (ASK, Arabidopsis Skp1), 11 cullin homologues and two Rbx homologues (Farras et al. 2001; Lechner et al. 2002; Shen et al. 2002). Recent work has identified the superfamily of 694 putative

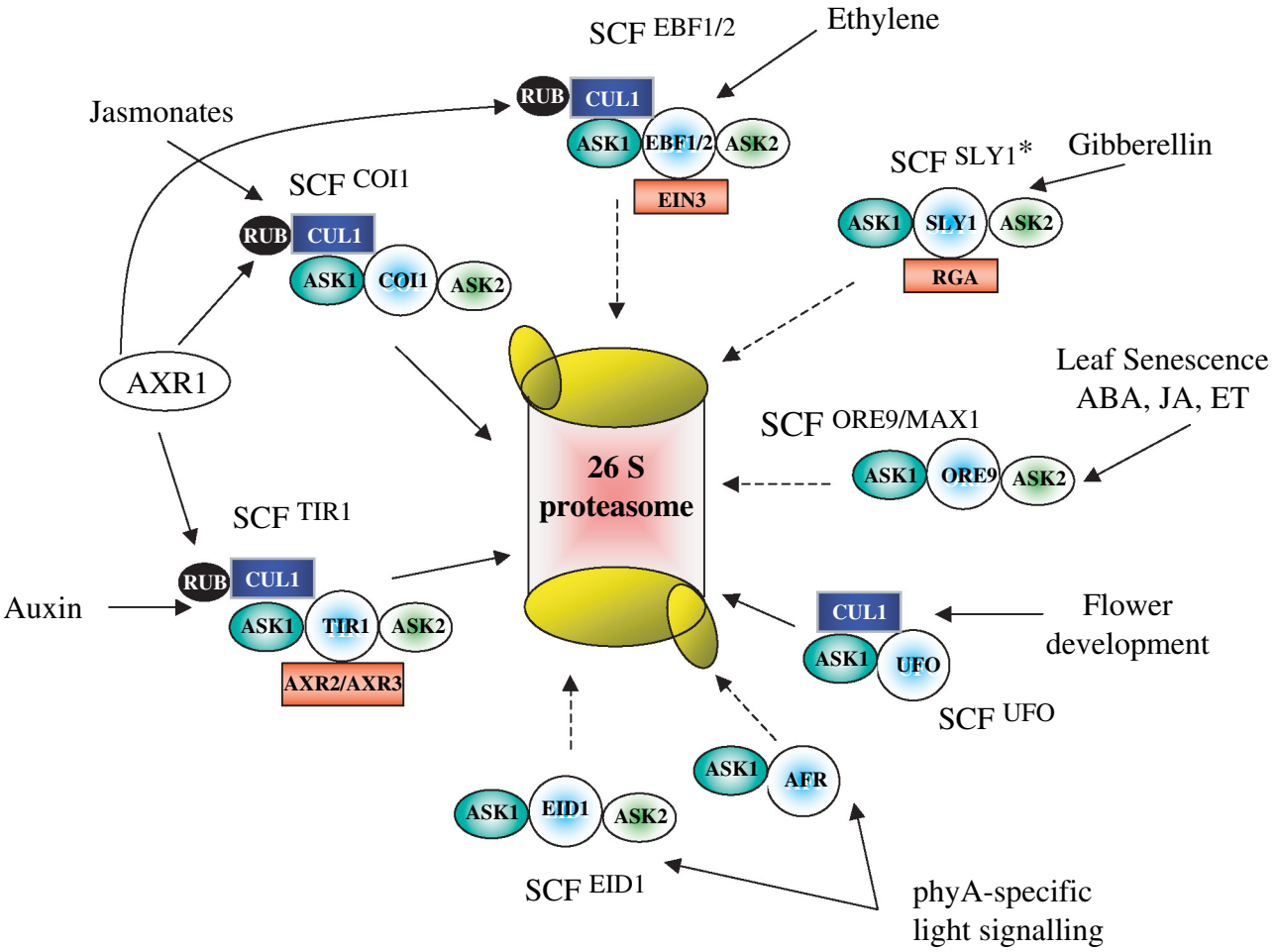

Fig. 3. The regulation of protein turnover is a control element common to many plant processes. Only interactions that have been demonstrated by direct binding studies are depicted. Arrows indicate the existence of a link but do not necessarily represent positive regulation. *Binding to ASK1 and 2 has been identified for related family members (Gagne et al. 2002). The COP9 signalosome (CSN) shows homology to the 19S lid subcomplex of the 26S proteasome. Broken arrows indicate that the existence of a direct link with CSN subunits has not yet been demonstrated. Targets of SCF complexes are represented by rectangular shaded boxes. ABA, abscisic acid; AFR, attenuated far-red response; ASK1 and 2, Arabidopsis Skp1; AXR2/AXR3, auxin-regulated proteins (AUX/IAA); COI1, coronatine insensitive1; CUL1, cullin1; EBF1 and 2, EIN3 binding F-box 1 and 2, EID1, empfindlicher im dunkelroten Licht/more sensitive to far-red light; EIN3, ethylene insensitive3; ET, ethylene; JA, jasmonate; MAX1, more axillary growth1; ORE9, oresara9-delayed senescence; RGA, repressor of *ga1*-3; RUB, related to ubiquitin; SCF, SKP1-CDC53p/CUL1-F-box; SKP1, S-phase kinase-associated protein1; SLY1, SLEEPY1; TIR1, transport inhibitor response1; UFO3, unusual floral organs3.

F-box protein genes in Arabidopsis (Gagne et al. 2002; Kuroda et al. 2002). The F-box proteins provide specificity to the E3 complex. Only a very limited number of the identified F-box proteins have been functionally characterized (Gray et al. 1999; Samach et al. 1999; Dieterle et al. 2001; Woo et al. 2001; Devoto et al. 2002; Xu et al. 2002; Guo and Ecker 2003; Harmon and Kay 2003; Potuschak et al. 2003; Dill et al. 2004; Fu et al. 2004; Gagne et al. 2004). For these proteins, direct binding to components of the SCF complex has been demonstrated (Fig. 3).

The COP9 signalosome (CSN) is an evolutionarily conserved multiprotein complex that acts in photomorphogenesis, auxin response and floral organ formation. It shows homology to the 19S lid subcomplex of the 26S proteasome. The F-box proteins TIR1 (transport inhibitor response1), COI1 and UFO3 (unusual floral organs3) have been demonstrated to associate directly with CSN subunits. In these three cases, CSN not only interacts with, but also modulates, the activities of these SCF complexes and the developmental responses they regulate (Schwechheimer et al. 2001; Feng et al. 2003; Wang et al. 2003). The binding of SCF complexes to regulators that are targeted for degradation in the proteasome and their functional involvement in specific signalling pathways following a stimulus have been demonstrated only for TIR1, EBF1/2 (EIN3 binding F-box 1 and 2) and SLY1 (SLEEPY1) (Gray et al. 1999; Guo and Ecker 2003; Dill et al. 2004; Fu et al. 2004; Gagne et al. 2004).

The degradation of specific proteins following a hormonal stimulus has been demonstrated for auxin signalling. Auxin stimulates physical interaction between

SCF^{TIR1} and AUX/IAA proteins (auxin-regulated proteins) and promotes their degradation. Both treatment with a proteasome inhibitor and mutations affecting the SCF^{TIR1} complex increase the stability of AUX/IAA proteins (Gray et al. 2001). EBF1 and 2 repress ethylene action and promote growth by interacting with the transcriptional regulator ethylene insensitive3 (EIN3, Fig. 3), and directing its degradation (Guo and Ecker 2003; Gagne et al. 2004; and, for a review, Kepinski and Leyser 2003). In Arabidopsis, the DELLA proteins are encoded by a family of five genes, *GA1* (gibberellic acid insensitive), *RGA* (repressor of *ga1*-3) and three different repressors of *ga1*-3-like genes (*RGL1*, *RGL2* and *RGL3*) (Peng et al. 1997; Silverstone et al. 1998; Dill et al. 2001; Lee et al. 2002). *SLEEPY1*/*SLY1* (McGinnis et al. 2003) belongs to the C2 family of F-box proteins (Gagne et al. 2002). GA derepresses its signalling pathway through SCF^{SLY1} by inducing proteolysis of RGA, a DELLA protein (Dill et al. 2004).

AXR1 is a positive regulator of auxin response that modulates SCF^{TIR1} activity (Leyser et al. 1993) and, in addition, may be considered as a connection point regulating the activity of different SCF complexes. AXR1 is a subunit of the heterodimeric Nedd8/RUB1-activating enzyme that mediates the first step in the conjugation of the ubiquitin-like modification Nedd8/RUB1 to Cullin. Deconjugation (deneddylation) is one of the biochemical activities that has been attributed to CSN (Schwechheimer and Deng 2001; del Pozo et al. 2002). Dharmasiri et al. (2003) have shown that disruption of the RUB conjugation pathway in Arabidopsis causes a seedling lethal phenotype characteristic of a defect in auxin signalling. RUB modification of CUL1 is also required for normal SCF^{COI1} function.

As a result of the similarities in the mode of action of COI1 and TIR1, it is likely that jasmonate and auxin might use analogous signalling mechanisms sharing common components such as AXR1 (Fig. 3). In fact, whilst *axr1* mutants are insensitive to JA, *coi1* is not altered in its response to auxin (Feys et al. 1994). *axr1* mutants also exhibit resistance to exogenous ethylene (Tiryaki and Staswick 2002). Involvement of AXR1 in different pathways that are controlled by E3-mediated protein degradation has been provided by Schwechheimer et al. (2002) (Fig. 3). AXR1 also participates in the repression of photomorphogenesis in the dark, a process that requires the activity of a non-SCF-type E3 consisting of the RING finger protein COP1. Xu et al. (2002) have also demonstrated that *AXR1*-dependent modification of the AtCUL1 subunit of SCF^{COI1} complexes is important for JA signalling. Mutations in *AXR1* decrease the abundance of the modified AtCUL1 of SCF^{COI1} and lead to a reduction in JA response.

Concluding remarks

Plant responses to stress can be viewed as being orchestrated through a network that integrates signalling pathways characterized by the production of JA, SA, ethylene and, to a lesser extent, auxin and gibberellin. No doubt other pathways characterized by the production of abscisic acid, brassinosteroids and cytokinin also map onto this network. The identified regulatory steps in the network involve transcription, protein–protein interaction and targeted protein destruction. Stress and hormone receptors, transcription factors and crucial connection points which would lead to a consistent mechanistic explanation of the signalling pathways all await identification. Depiction of the network through Boolean gates leads to testable hypotheses of the outputs of signal interaction.

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