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REVIEW

Hormone Signaling Pathways in Plants: The Role of Jasmonic Acid in Plant Cell Signaling

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Abstract: Plant growth and metabolism are affected by various biotic and abiotic stimuli including microorganisms and insects attack as well as light and environmental stresses. Such a diverse plant response requires a communication system that uses a group of chemical messengers called hormones. Hormones promote, inhibit, or qualitatively modify plant growth and development. This complex process requires a signal transduction that defines a specific information pathway within a cell that translates an intra- or extracellular signal into a specific cellular response. The characterization of mutants in the hormone response pathway provides not only an excellent opportunity to understand hormone action in plant physiology and development, but also helps to dissect the molecular genetics of hormone signaling pathways and to isolate the corresponding genes. This paper will introduce plant hormone signaling and mutants involved in signaling pathways in general, and will review recent progress in the molecular genetics of jasmonic acid signaling.

Key Words: Plant hormones, jasmonate signaling, elicitors, hormone receptors, hormone mutants

Bitkilerde Hormon Sinyal Akış Ağları: Bitki Hücre Sinyalizasyonunda Jasmonic Asitlerin Rolü

Özet: Bitki büyüme ve metabolizması, mikroorganizmalar, böcekler, ışık ve benzeri çevre etmenlerini içine alan çok farklı biyotik ve abiyotik etmenler tarafından etkilenmektedir. Bu çok farklı bitki tepkileri hormon olarak adlandırılan bir grup kimyasal haberci tarafından gerçekleştirilir. Hormonlar bitki büyüme ve gelişmesini teşvik edici, önleyici, veya modifiye edici etkilere sahiptirler. Bitki büyüme ve gelişmesinde böylesine kompleks etkilerin oluşumu "sinyal akış ağı" olarak isimlendirilen bir sistemin varlığına ihtiyaç duymaktadır. Sinyal akış ağları, hücre içerisinde hücre içi ve/veya hücre dışından gelen spesifik bir sinyali hücresel bir cevaba dönüştüren özel bilgi ağlarıdır. Hormon tepkilerine karşı mutasyona uğramış mutant bitkilerin karekterizasyonu, hormonların bitki fizyolojisi ve gelişmesinde nasıl çalıştıklarını anlamak için büyük önem taşımaktadırlar. Aynı zamanda bu mutant bitkiler, kompleks hormon sinyal akış ağlarının moleküler genetiğini küçük parçalara ayırmak suretiyle anlamaya ve bu sinyal akış ağlarında görev alan genlerin tespit edilerek klonlanması çalışmalarına da büyük katkılar sağlamaktadırlar. Bu makale bitkilerde hormon sinyalizasyonu ve bu sinyal akış ağlarıyla ilgili mutantları genel olarak tanıtıp, jasmonic asit sinyal akışının moleküler genetiği ile ilgili meydana gelen son gelişmelere değinilecektir.

Anahtar Sözcükler: Bitki hormonları, jasmonat sinyal akış ağları, elisitörler, hormone reseptörler, hormon mutantlar

Plant hormones control a diverse array of plant responses affecting growth and development, as well as defense against microorganisms and insects, and protection from abiotic stresses (Hildmann et al., 1992; McConn et al., 1997; Reymond and Farmer, 1998; Overmyer et al., 2000; Steudle, 2000). This complex process requires a communication system that can operate over relatively long distances among different plant organs as well as different organelles within a single cell. In such a system, cells of different tissues and organs are not only capable of detecting signals they receive from other parts of the plant, but also of responding and transmitting those signals in their own characteristic way (Klumpp and Krieglstein, 2002). In higher organisms like plants, such diverse communication is performed by a group of chemical messengers called hormones (Salisbury and Ross, 1992; Gray and Estelle, 1998).

A plant hormone is generally described as a naturally occurring organic compound that is active at very low concentrations (e.g., <1 mM, often 1 uM). A hormone is

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often formed in certain parts of the plant and then translocated to other sites where it evokes specific biochemical, physiological, and/or morphological responses (Salisbury and Ross, 1992; Davies, 1995). These organic compounds promote, inhibit, or qualitatively modify plant growth and development in the tissues where they are produced as well as in distant tissues to which they are translocated. Therefore, the synthesis and action of plant hormones are not necessarily localized to a specific tissue, as with animal hormones, but occur in a wide range of tissues (Davies, 1995). In addition, plants respond to biotic and abiotic external stimuli such as pathogen and insect attack, drought, and salt stress using hormone signal transduction pathways that cause changes in the hormone metabolism and distribution within the plant.

The commonly recognized classes of plant hormones are auxin (IAA), gibberellin (GA), cytokinin (CK), abscisic acid (ABA), and ethylene (ACC). More recently recognized molecules involved in plant signaling include brassinosteroids (BR), jasmonic acid (JA), and salicylic acid (SA).

Hormone Signal Transduction Pathway

The induction of plant responses to any exogenous or endogenous stimuli requires a perception by the plant via different types of signal molecules collectively known as elicitors (Keen, 1975). Elicitors can be classified in 3 groups: (i) chemical signals such as hormones and phytotoxins, (ii) physical signals such as blue and red light, and (iii) biotic signals such as fungal elicitors (Aducci, 1997). The chemical nature of these elicitors may vary from large molecules such as polypeptides, carbohydrates, glycoproteins, and fatty acids, to low molecular weight compounds such as hormones (Ebel and Cosio, 1994).

Another group of signal molecules that induce plant response to pathogens are those that can trigger defense responses at a distance from the inoculation site. Among the long-distance mobile signals, salicylic acid, jasmonic acid, and systemin are the most studied. Exogenous application of these compounds induces defense responses at a distance, and with SA there is an induction of protection against some challenge pathogens (Pennazio et al., 1987; Enyedi et al., 1992; Malamy and Klessig, 1992).

Signal transduction defines a specific information pathway within a cell that translates an intra- or extracellular signal into a specific cellular response (McCourt, 1999). If the initial signal is a hormone, such as SA, GA, or ethylene, the first step in signaling involves the interaction of that hormone with a specific cellular recognition protein called a receptor (Figure 1). The initial phase of signal transduction requires high-affinity binding of the hormone to the receptor(s), which causes the receptor to undergo a conformational change that initiates a sequence of downstream events called signal transduction (Figure 1). After the signal is activated, the receptor may alter gene expression directly by acting as a transcription factor without transducing the activated signal to the pathway as in mammalian glucocorticoid receptors (Bohen et al., 1995) (Figure 1). Alternatively, the receptor may pass the signal to the nucleus through a series of intermediary steps acting as a molecular switch (Stone and Walker, 1995; Palme et al., 1997) (Figure 1). In the pathway, the signaling components are generally modified by phosphorylation or by the activation of low molecular weight GTP-binding proteins (Stone and Walker, 1995; Palme et al., 1997; Engelberth et al., 2004). For instance, activation of nuclear factor-KB (NF-KB) requires phosphorylation of a family of inhibitory proteins, IKBs via ubiquitination-dependent proteolysis, SCF E3Rsas$^{IKBs/TrCP}$, which frees NF-KB to translocate to the nucleus where it regulates gene transcription in mammals (Karin and Ben-Neriah, 2000). Similarly, SCF^{TIR} in auxin response suggests that similar phosphorylation-based signaling pathways might be involved (Del Pozo and Estelle, 2000). On the other hand, phosphorylation on a hydroxyl group of serine (Ser), threonine (Thr), or tyrosine (Tyr) residues is predominantly used in animals (Klumpp and Krieglstein, 2002). In contrast to animal signal induced phosphorylation, a nitrogen atom of a histidine (His) residue and an acyl group of an aspartate (Asp) residue are predominantly used for phosphorylation in bacteria (Klumpp and Krieglstein, 2002).

Of the plant-specific signaling molecules including hormones, elicitors, and secondary metabolites, plants share some signaling agents with animals such as nitric oxide, reactive oxygen species, and other regulators function in both kingdoms. For instance, Glu, which was previously known as an animal signaling agent, is now regarded as a likely plant signaling compound (Dennison and Spalding, 2000), and genes encoding putative Glu

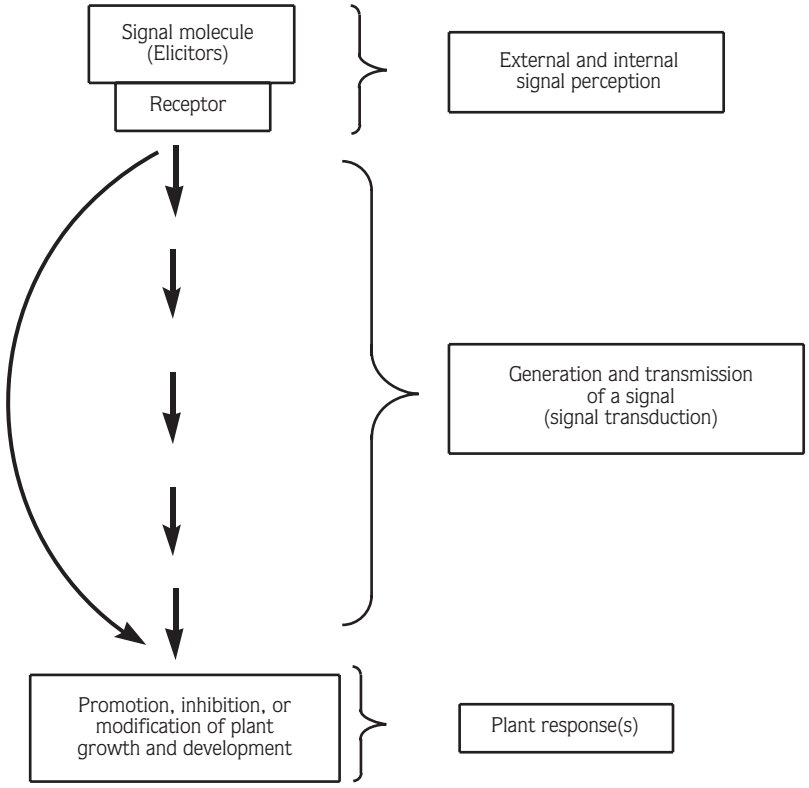

Figure 1. The phases of the hormone signaling pathway in plants. Three steps including signal perception, signal transduction, and plant response(s) are shown. An alternative pathway in which the receptor could alter gene expression directly by acting as a transcription factor without transducing the activated signal to the pathway is also shown.

receptor subunits have been identified in the *Arabidopsis* genome (Lacombe et al., 2001). This finding suggested that other low molecular weight compounds such as extracellular ATP (eATP) could be signaling agents in plants (Demidchik et al., 2003; Tang et al., 2003).

Since a signaling cascade can be a complex process, transduction pathways also require sensitivity and specificity that are coordinated and integrated with the related signaling components (Moller and Chua, 1999). Depending on the components of the pathway, the stimulation of the receptor must activate (positive) or inactivate (negative) relay components of the pathway through some type of cascading mechanism. In this case, the receptor acts as a molecular switch. These changes in signaling proteins not only permit a rapid response to the hormone signal but also allow recycling of components of the signaling system so that they can receive further signals (McCourt, 1999). As a result, signal transduction not only modulates the enzyme activity in target cells, but also alters the rates of synthesis of existing proteins or triggers the synthesis of new ones.

Although some details of hormone signaling are known described above, there are still several intricacies that need to be revealed. For instance, do different hormone pathways use similar, or even the same signaling molecules? Do different cells, tissues, or even species of plants use the same steps in a particular hormone signaling pathway? How does cross talk among different hormone signaling pathways occur? The application of genetic analysis to hormone mutants helps us to answer these questions. The characterization of mutants in hormone responses provides an excellent opportunity to understand hormone action in plant physiology and development. Mutants can be used to

study hormone biosynthesis, to dissect the molecular genetics of hormone signaling pathways, and to isolate the corresponding genes. The recent availability of the whole Arabidopsis genome sequence has made this easier and faster. Therefore, this paper also introduces hormone mutants involved in hormone signaling for a comprehensive understanding of hormone signaling pathways in plants.

Hormone Mutants Involved in Hormone Signaling Pathways

Plant hormone mutants can be classified into 2 main groups; (i) those that influence hormone levels by altering biosynthesis, generally termed biosynthesis mutants including (a) auxotrophs and (b) over accumulation mutants, and (ii) those that influence the response to hormones, generally termed response mutants including (a) insensitive and (b) hypersensitive mutants (Reid, 1993). Most auxotrophic mutants show a reduction in hormone level, and exogenous hormone application restores the mutant phenotype to its wild type. However, not all auxotrophs necessarily exhibit a reduction in the hormone biosynthesis. In some cases biosynthesis mutants may also overproduce hormones (Normanly et al., 1993; Ross et al., 1993; Hirayama et al., 1999; Woeste et al., 1999; Woeste and Kieber, 2000; Gibson et al., 2001). On the other hand, response mutants appear to be insensitive to their own endogenous hormone levels or resistant to toxic or growth inhibiting levels of exogenous hormone. The main difference between a hormone response (insensitive or hypersensitive) mutant and a hormone biosynthesis (deficient) mutant is that the response mutant phenotype cannot be restored to the wild type by exogenous hormone application.

Another useful type of mutant in the investigation of complex hormone signaling is a secondary mutation that suppresses the effect of one of the mutations described above. Suppressors demonstrating their own phenotypes and partially suppressing an earlier gene mutation are useful not only for identifying new gene functions but also for identifying new mutations in previously characterized genes. Genes encoding components of a particular signaling pathway may have other functions that may be missed by direct screening but that can be identified genetically among suppressor mutations of signaling mutants (McCourt, 1999). Recent studies have shown that this technique can identify novel genes functioning in the hormone signaling pathway in plants (Reed et al., 1998; Steber et al., 1998; Peng et al., 1999; Hsieh et al., 2000). For instance, a screen for suppressors of the auxin resistant mutant *axr1* in *Arabidopsis thaliana* has identified a second site suppressor locus called *SAR1* (Suppressor of Auxin Resistance 1). Genetic analysis of this mutant indicated that *sar1* partially suppresses every aspect of *axr1* and functions in the same or overlapping signaling pathway in auxin signaling (Cernac et al., 1997; Tiryaki and Staswick, unpublished results).

To identify mutations in genes related to a specific hormone signaling pathway, the simplest and most used method is to assay a mutagenized plant population for an altered response to a specific hormone that is supplied exogenously. This should reveal a clear and reproducible phenotypic difference between wild type and mutant. However, in screens where seeds and seedlings are exposed to higher concentrations of hormone than those a plant experiences under normal growth conditions, mutations that confer insensitivity to such conditions may not always be specific to the hormone dependent pathway of interest. For instance, the *iba1* (indole-3-butyric acid resistant 1) mutant of *Nicotiana plumbaginifolia* was recovered in a screen for resistance to a very low concentration of auxin, but was later found to be resistant to ABA and paclobutrazol, an inhibitor of gibberellic acid (GA) biosynthesis (Bitoun et al., 1990). In addition, not all hormone mutant genes determined in hormone screenings are necessarily directly involved in hormone signal transduction pathways. It is possible that mutations identified in a screen mark genes whose functions are necessary for a signaling event to occur, but which are not directly involved in the regulation of the signal transduction pathway. For instance, it has been suggested that early germination and the wilty phenotype of *iba1* mutant are due to a change in the ABA/GA ratio; auxin may have a secondary effect on *iba1* phenotype (Bitoun et al., 1990). A similar result was also reported in Arabidopsis (Koornneef and Veen, 1980).

Mutants in hormone signaling genes can modulate (i) the level of receptors, (ii) the affinity of the receptor protein for the hormone, or (iii) the magnitude of the response. Insensitivity to a particular hormone may be attributed to a receptor that is uncoupled from the activating ligand, such as *ETR1* (Gamble et al., 1998;

Imamura et al., 1998), to the effect of genes encoding biosynthetic enzymes that alter intracellular hormone levels, or to the effect of other genes whose actions in an unexpected activation of the hormone signal transduction chain such as in *iba1* mutant (Bitoun et al., 1990). On the other hand, mutants that affect multiple hormones can shed light on the complex mechanisms through which hormone signaling is integrated in the plant.

It needs to be mentioned that, in addition to the forward genetics approaches mentioned above, (i.e. beginning with a mutant phenotype and ending with the genetic sequence that causes the altered phenotype), the recent availability of the whole Arabidopsis genome sequence may provide an opportunity to use reverse genetics, such as insertional mutagenesis to resolve complex signaling pathways in plants. Reverse genetics begins with a mutant gene sequence and tries to identify the resulting change in the phenotype. Gene knockouts, or null mutations, provide a direct route to determining the function of a gene product in situ. New studies have shown that this approach can successfully identify novel mutants in plants (Sanders et al., 2000; Ellis and Turner, 2001; Stintzi et al., 2001; Alonso et al., 2003a; Alonso et al., 2003b). This approach usually involves the use of either transposable elements or T-DNA as a mutagen. The foreign DNA not only disrupts expression of the gene into which is inserted, but also acts as a marker for subsequent identification of the mutation because of its known sequence (Krysan et al., 1999). An important aspect of this insertional mutation is that it permits the identification of genes that would have been missed in traditional mutagenesis screens (Sundaresan et al., 1995) because the success of traditional mutagenesis strictly depends on the selection methods applied to detect desired mutants (Harten, 1998). For instance, if a gene is functionally redundant, a reduction or loss of function of the gene may result in no obvious or only subtle phenotypic changes that cannot be identified in screens for mutant phenotypes but may be detected by expression pattern in enhancer-trap or gene-trap screens (Sundaresan et al., 1995; McCourt, 1999). In most traditional screens, since seeds and seedlings are exposed to higher concentrations of hormone than those plants experience, gene mutations that are homozygous lethal are usually missed, but can be maintained in the heterozygous plant populations with insertional mutagenesis (Krysan et al., 1999).

Since jasmonate signaling has been one of the most extensively studied signaling pathways during the last decade, it was used an example to show how hormone mutants can be used to reveal complex hormone signaling in plants. Recent developments regarding the molecular genetics of jasmonate signaling are also discussed.

Molecular Genetics of Jasmonate Signaling

Jasmonate signaling plays a critical role in plant reproductive development (McConn and Browse, 1996; Sanders et al., 2000; Stintzi and Browse, 2000), in protecting plants from pathogens and insects (Farmer and Ryan, 1990; Penninckx et al., 1996; McConn et al., 1997; Staswick et al., 1998; Engelberth et al., 2004; Huang et al., 2004), and in limiting damage from abiotic agents (Overmyer et al., 2000; Rao et al., 2000; Traw and Bergelson, 2003; Huang et al., 2004). In Arabidopsis, 3 mutants defective in JA response (i.e. *jar1*, *coi1*, and *jin1*) (Staswick et al., 1992; Feys et al., 1994; Berger et al., 1996), and 1 triple mutant defective in JA biosynthesis (*fad3-2/fad7-2/fad8*) (McConn and Browse, 1996) were isolated in order to better understand how JA works in plants. More recently, additional mutants related to JA response have been characterized; the Arabidopsis T-DNA mutants *dde1* (for delayed dehiscence 1), *dad1* (anther dehiscence1), *opr3* (for oxo-phytodienoic acid reductase 3), which is shown to be allelic to *dde1,* and *cev1* (for the constitutive expression of vegetative storage protein 1) (Sanders et al., 2000; Ellis and Turner, 2001; Ishiguro et al., 2001; Stintzi et al., 2001). One mutant in the tomato, *def1* (defenseless 1), is deficient in jasmonate biosynthesis and fails to accumulate proteinase inhibitors (PI) (Howe et al., 1996).

Molecular and genetic analysis of JA biosynthesis or perception mutants revealed that JA is required for male fertility (McConn and Browse, 1996; Stintzi and Browse, 2000). For instance, *coi1, fad3-2/fad7-2/fad8*, and *opr3/dde1* mutants are male sterile. Fertility is restored by the application of jasmonic acid in all these mutants, except for *coi1.* JA would not be expected to complement *coi1*, which is a signaling rather than a biosynthetic mutant. Therefore, development of the stamen and pollen does require jasmonic acid (Sanders et al., 2000; Stintzi and Browse, 2000). Further results with *dde1* and *dad1* also showed that jasmonic acid is required for

development of the filament, development of pollen grains, and dehiscence of the anthers (Sanders et al., 2000). However, male sterility is not a general phenotype of JA mutants because *jar1, jin1,* and *def1* are male fertile (Staswick et al., 1998). There are 2 possible explanations for this discrepancy. First, the part of the signaling network that is affected in *jar1*, *jin1,* and *def1* is not necessary for proper flower fertility. Second, *jar1* and *jin1* show a less pronounced phenotype than *coi1* in several respects, i.e. root growth and gene expression, suggesting that these mutants are weak alleles that allow some JA perception and signaling that is sufficient for proper reproduction. More recent evidence showed that *JAR1* does not encode a signal transduction component, but rather an enzyme that biochemically modifies JA, suggesting that although required for some aspects of JA response, this modification is apparently not necessary for pollen fertility (Staswick et al. 2002). More detailed molecular characterization of *def1, jin1,* and other mutants is needed to assess the role of JA in plants.

Defects in JA response or disruptions of the JA biosynthetic pathway result in susceptibility of plants to various pathogens and insects (Farmer and Ryan, 1990; Howe et al., 1996; Penninckx et al., 1996; McConn et al., 1997; Staswick et al., 1998; Engelberth et al., 2004). For example, *jar-1* has been shown to be susceptible to the fungal pathogen *Pythium irregulare* (Staswick et al., 1998) and *coi1* is susceptible to *Alternaria brassicicola* and *Pythium mastophorum* (Drechs.), but is resistant to *Pseudomonas syringeae* (Feys et al., 1994). The triple mutant (*fad3-2/fad7-2/fad8*) that contains negligible levels of JA is also susceptible to the same fungal root pathogens as *jar1,* and *coi1* shows susceptibility (Staswick et al., 1998; Vijayan et al., 1998). The *fad3-2/fad7-2/fad8* mutant is also more susceptible to attack by larvae of a saprophagous fungal gnat, *Bradysia impatiens* (Stintzi et al., 2001). Unlike the response of the triple mutant, *fad3-2/fad7-2/fad8* and *coi1*, the *opr3* plants show the same resistance as wild types in the face of attack by *Bradysia* larvae as well as the fungal pathogen *A. brassicicola* (Stintzi et al., 2001). Collectively, these results indicate that the regulation of resistance or susceptibility of the plant by JA-dependent signaling pathways is determined by the type of pathogen as well as the type of pathogenicity.

The result in *opr3*, which carries a mutation that blocks JA biosynthesis beyond the JA biosynthetic precursor OPDA (12-oxo-phytodienoic acid), in response to *Bradysia* larvae and the fungal pathogen *A. brassicicola* is particularly important because it shows that resistance to insect and fungal attack can be observed in the absence of JA (Stintzi et al., 2001). This suggests that JA and MeJA may not be required for all jasmonate responses, and that OPDA can signal defense against *Bradysia* larvae, as well as the fungal pathogen *A. brassicicola* in Arabidopsis (Stintzi et al., 2001). Other intermediates of JA biosynthesis, dinor oxo-phytodienoic acid (dnOPDA), which is synthesized from hexadecatrienoic acid (16:3), and JA conjugates such as JA-amino acid and JA-glucosyl, may also be important signaling molecules of JA pathways (Staswick et al., 2002). Furthermore, emerging evidence has shown that the biochemical modification of JA may also be an important part of jasmonate signaling (Staswick et al., 2002). Identifying new mutant plants that disrupt the JA biosynthesis at each intermediate of the pathway such as allene oxide cyclase (AOC), allene oxide synthase (AOS), and lipoxygenase (LOX), or further biochemical tests related to JA modification will help to reveal the complex interaction between jasmonate family members and their role in response to different stimuli.

The initial characterization of the JA response mutants *jar1*, *coi1*, and *jin1* suggested that these loci might affect jasmonate signal trunsduction (Staswick et al., 1992; Feys et al., 1994; Berger et al., 1996). This has been confirmed for *coi1* by subsequent cloning and biochemical characterization. *COI1* encodes an F-box protein that is related to the auxin response factor *TIR1*, a component of the ubiquitin-like E3 complex called SCF that is involved in plant auxin response (Xie et al., 1998). The SCF complex including cullin, SKP1, RBX1 and an F-box protein is involved in the transfer of ubiquitin from ubiquitin ligase to target proteins in the ubiquitin conjugation pathway. In this pathway, the ubiquitination specificity is determined by unique F-box proteins that contain an F-box motif (~45 amino acids) and sequences required for target protein recognition. Recognition elements can include leucine-rich repeats (LRRs), WD40 repeats, or protein-protein interaction motifs (Del Pozo and Estelle, 2000). In the case of auxin signaling the F-box protein is *TIR1* (a complex known as SCF^{TIR1}), which is closely related to the jasmonate response factor encoded by *COI1* (Xie et al., 1998). This suggests that

jasmonate signaling also involves an SCF-mediated ubiquitination pathway (Gray et al., 1999). Indeed, new emerging evidence shows that imminoprecipitates of epitope-tagged *COI1* from transgenic Arabidopsis plants co-precipitate with cullin and SKP1 proteins to form an E3 ubiquitin ligase, confirming that *COI1* forms an SCF^{COI1} complex in vivo (Turner et al., 2002). Furthermore, we and others also demonstrated that this pathway is dependent on a component of the RUB-activating enzyme, AXR1, which is shared with the auxin proteasome signaling pathway (Staswick et al., 2002; Tiryaki and Staswick, 2002; Xu et al., 2002; Feng et al., 2003).

Our current understanding of JA signaling and its interaction with other signaling pathways such as auxin, imperfect as it is, reveals an enormous complexity. However, biochemical approaches and screens for new mutants via insertional mutagenesis such as T-DNA and transposable elements will provide new opportunities to discover multiple control sites and to dissect the complexity of the pathway.

Conclusion

Intensive studies with hormone mutants have indicated that plant hormone signaling pathways are not linear but rather a network interacting with each other to make a coordinated plant response(s) during growth and development. In addition to forward genetics approaches, the recent availability of the whole Arabidopsis genome sequence now provides another opportunity to use reverse genetics to dissect these complex signaling pathways. Gene knockouts, or null mutations, may therefore provide a direct route to determining the function of a gene product in situ. Current challenges would be to define those networks and understand how plants use this pathway(s) to respond to biotic and abiotic stresses.

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