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Auxin Signaling Involves Regulated Protein Degradation by the Ubiquitin-Proteasome Pathway

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Abstract

Ubiquitin-mediated protein degradation plays a critical role in the regulation of diverse biological processes in eukaryotes (Hershko and Ciechanover 1998). Recent studies in *Arabidopsis* also implicate the ubiquitin proteolytic system in auxin response. A combination of genetic and molecular approaches has resulted in the identification of components of a common auxin-response pathway. Mutations in either *AXR1* or *TIR1* result in decreased auxin response and a variety of auxin-related growth defects. The *AXR1* gene encodes a subunit of a RUB-activating enzyme analogous to the E1 ubiquitin-activating enzyme (del Pozo and others 1998). AXR1 functions as a heterodimer with ECR1 to activate RUB, a ubiquitin-related protein. RUB is conjugated to the cullin CUL1 in an AXR1-dependent manner. CUL1 is a component of an E3-ubiquitin ligase SCF complex along with a Skp1-like gene (ASK1), RBX1, and an F-box protein (Gray and others 1999). The *TIR1* gene encodes an F-box protein, and recent data have demonstrated that the role of SCF^{TIR1} is to degrade one or more negative regulators of auxin response. Further, RUB modification of CUL1 is required for normal SCF^{TIR1} function. The *Aux/IAA* genes encode short-lived nuclear proteins that repress auxin-regulated gene expression, possibly through interaction with members of the ARF family of transcription factors. Genetic and biochemical studies have revealed that members of the Aux/IAA family are substrates for SCF^{TIR1} and that auxin regulates transcription of downstream genes by promoting degradation of the Aux/IAA proteins.

Key words: Protein degradation; Ubiquitin; Auxin; RUB; SCF complex; Aux/IAA proteins; ARF

Introduction

Protein degradation is an integral component of plant cell physiology (Callis and Vierstra 2000; Estelle 2001). In eukaryotes ubiquitin-mediated degradation of regulatory proteins is essential for regulating biological processes including cell cycle control, transcriptional regulation, endocytosis, and signal transduction (Hershko and Ciechanover 1998). In this article we will review recent studies that implicate the ubiquitin proteolytic system in the auxin-response pathway.

The Ubiquitin-Conjugation Pathway

Ubiquitin is a 76 amino acid polypeptide that is highly conserved among all eukaryotes. The attachment of ubiquitin to a target protein is a process involving the activity of three enzymes or protein

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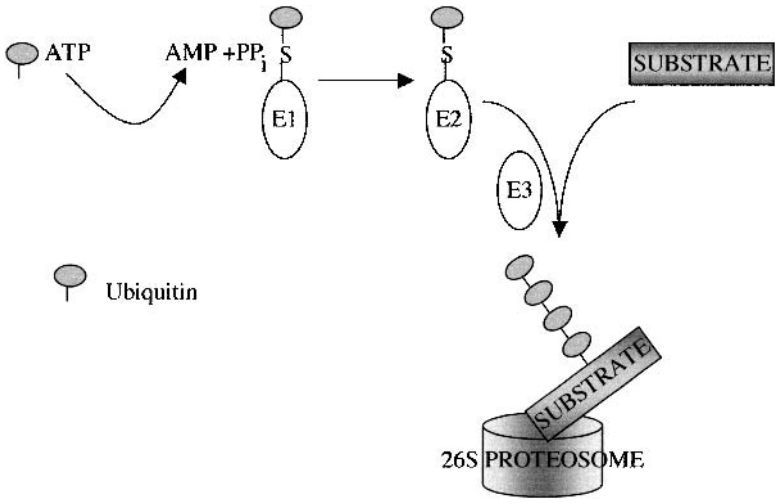

Figure 1. The ubiquitin pathway. The E1 ubiquitin-activating enzyme activates ubiquitin by forming a thiol-ester bond with ubiquitin. Ubiquitin is subsequently transesterified to the E2 ubiquitin-conjugating enzyme before being attached to the lysine residue of a target protein with the aid of an E3 ubiquitin-ligase. Multiple ubiquitin subunits are attached to the target protein, which is subsequently degraded by the 26S proteosome.

complexes (Herschko and Ciechanover 1998). The C-terminal conserved glycine residue of ubiquitin is activated in an ATP-dependent manner to form a thiol-ester linkage with a cysteine residue in the ubiquitin-activating enzyme, E1 (Figure 1). The activated ubiquitin is then transferred to a cysteine residue in a ubiquitin-conjugating enzyme or E2. Finally, ubiquitin is ligated to a target protein in a reaction that typically requires a ubiquitin protein ligase or E3. Linkage occurs through an isopeptide bond between the C-terminal glycine residue of ubiquitin and an internal lysine ϵ-amino group on the target protein. The substrate protein can be multiply modified, generating intermediates with many ubiquitin molecules attached either at several sites within the protein or as a chain of ubiquitin subunits. A protein with a ubiquitin chain of at least four subunits is recognized by the 26S proteosome and degraded (Hershko and Ciechanover 1998).

The substrate specificity of this pathway is the responsibility of the E3 ubiquitin-ligase. The E3 can be a single subunit or a multisubunit complex. Thus far, four types of E3s have been identified in animals and yeast that are also in plants including HECT-domain E3s and N-end-rule E3s. Two different E3 complexes called SCF and APC have been described (Hershko and Ciechanover 1998; Callis and Vierstra 2000). E3s bind both the E2 enzyme and the substrate protein and facilitate the transfer of ubiquitin from one to the other. In the case of HECT domain E3s, the E3 directly participates in the reaction by forming a thiol-ester intermediate between ubiquitin and an internal cysteine. Other types of E3s appear to function as a scaffold that promotes close interaction between the E2 and the substrate.

The SCF E3s are named after the three original components identified in yeast: *S*kp1, *C*dc53 (cullin in other species), and an *F*-box protein. The core subunits Skp1p and Cdc53p interact with different F-box proteins to confer target specificity and thus the physiological role of the E3. Each F-box protein binds to the core complex through an interaction between Skp1p and the F-box domain. Skp1p also binds Cdc53, forming a bridge between the two proteins (Patton and others 1998). There are many F-box proteins present in the yeast, human, and *Arabidopsis* sequence databases. In the predicted *Arabidopsis* proteome, there are at least six cullin proteins (Hellmann and Estelle unpublished), 20 SKP1-related proteins (called ASKs) (Crosby, pers. comm.), and greater than 500 F-box-containing proteins (J. Gagne and R. Vierstra pers. comm.). The function of each cullin and ASK is not clear, but it is possible that different cullin-ASK combinations recruit specific F-box proteins. The most recently identified member of the complex is RBX1, a RING-H2 finger protein, that is involved in recruiting the E2 to the complex and promoting transfer of ubiquitin to the substrate protein (Seol and others 1999; Skowyra and others 1999). The E2 also binds to the core complex by binding to a conserved motif at the C-terminal end of the cullin (Patton and others 1998). Substrate binding appears to be through protein-protein interaction domains found within the F-box such as WD-40 and leucine-rich repeats.

Ubiquitin-Like Proteins

A number of ubiquitin-like proteins have been identified in recent years, including Sentrin-1 (SUMO-1, PIC1, or Smt3p) and Rub1 (Nedd8 in mammals) (Yeh and others 2000). Sentrin-1 is only 18% identical to ubiquitin. Nedd8 and its yeast and plant

orthologues Rub1 are much more closely related to ubiquitin (50%–60%) and appear to play a role in the ubiquitin pathway. Ubiquitin-like proteins are conjugated to lysine residues within target proteins in a manner similar to that for ubiquitin. However, the result of this modification is strikingly different. A single ubiquitin-like protein is conjugated to the target protein rather than a chain, and the modification may affect the subcellular location or biological function rather than metabolic stability. Stability may, however, be affected indirectly, for example, SUMO-1 modification of IκBα at the same lysine residue as ubiquitin prevents ubiquitination and thus blocks signal-induced activation of NF-κB (Desterro and others 1998). Identified substrates for SUMO-1/PIC1/Smt3 include RanGAP1, PML, IκBα in mammals (Bischoff and others 1995; Sternsdorf and others 1997; Desterro and others 1998) and the yeast septin proteins (Johnson and Blobel 1999; Takahashi and others 1999). To date, the only identified targets for Nedd8 or Rub1 modification are members of the cullin family (Lammer and others 1998; Osaka and others 1998; Wada and others 1999). RUB conjugation also depends on a specific E2 enzyme (Ubc12 in yeast, RCE1 in *Arabidopsis,* see below). A recent study by Kamura and co-workers (1999) has demonstrated that Rub/Nedd8 modification of Cdc53 and the human cullin Cul2 requires Rbx1. As Rub1 modification also involves an E2 enzyme it is possible that Rbx1 recruits this E2 to the complex.

RUB modification of the cullin subunit of the SCF appears to regulate its function in some way. In budding yeast, mutations in the Rub1p conjugation pathway confer no obvious phenotype. However, a synthetic lethal phenotype is observed in double mutants between genes in the pathway and conditional alleles of components of SCF^{CDC4}, suggesting that Rub1p modification of Cdc53 is important for activity of this pathway (Lammer and others 1998).

In *Arabidopsis,* three members of the RUB family have been identified, RUB1 and RUB2 differ by a single amino acid. The more divergent RUB3 is approximately 77% identical to the other two family members (Rao-Naik and others 1998). *In vitro* all three RUB proteins can form a thiol-ester linkage with the RUB activating-enzyme (Gray and Estelle 2000). It is unclear as yet whether they have overlapping functions.

Auxin-Response Mutants

Two approaches have been employed to identify components of auxin-signaling pathways. The first involves screening for mutants that have altered responses to applied auxin and the second involves the biochemical characterization of genes rapidly induced upon the application of auxin. Recently these two approaches have converged on a common pathway.

A number of *Arabidopsis* mutants have been identified that display increased resistance to normally inhibitory concentrations of auxin. Both loss-of-function (*axr1, axr4* and *tir1*) and gain-of-function (*axr2* and *axr3*) mutations have been recovered in these screens. These mutants display pleiotropic phenotypes associated with a decrease in auxin response (Estelle and Klee 1994; Ruegger and others 1998). Mutations in the *AXR1* gene were the first to be characterized in detail. These mutants have defects in apical dominance, cell division and elongation, tropic responses, and auxin-induced gene transcription (Estelle and Klee 1994; Timpte and others 1995). *AXR1* was cloned several years ago and shown to encode a protein similar to the N-terminal half of the E1 ubiquitin-activating enzyme (Leyser and others 1993). However, it lacks the active site cysteine required for thiol-ester bond formation with ubiquitin. Other such genes have been found in yeast and mammals (Chow and others 1996; Shayeghi and others 1996; Johnson and others 1997). A breakthrough in our understanding of these proteins came when it was discovered that the ubiquitin-like proteins are activated by a heterodimeric E1 enzyme. In yeast, activation of the ubiquitin-like Smt3p is performed by the E1-like heterodimer Aos1p/Uba2p rather than a monomeric E1-like activity (Johnson and others 1997). Another AXR1-like protein, yeast Enr2p, functions as a bipartite enzyme in partnership with Uba3p to conjugate Rub1p to Cdc53 (Lammer and others 1998). An *Arabidopsis* database search for homology to Uba2p identified a gene, *ECR1,* that encodes a protein containing the active site cysteine required for E1 activity (del Pozo and others 1998). Experiments by del Pozo and coworkers (1998) demonstrated that the AXR1-ECR1 bipartite E1 enzyme was able to activate RUB1 *in vitro.* In addition, an *Arabidopsis* RUB-conjugating enzyme, RCE1, was identified through homology to the yeast Ubc12p. RCE1 was able to form a thiol-ester linkage with RUB1 when incubated with AXR1-ECR1, confirming its function as a RUB E2 (del Pozo and Estelle 1999).

The *Arabidopsis* genome contains at least six cullin genes (Hellmann and Estelle unpublished). Del Pozo and Estelle (1999) demonstrated that CUL1 was modified in an *in vitro* reaction mixture containing recombinant AXR1, ECR1, RCE1, RUB1, and ATP. Modification was abolished in a mutant CUL1 with a

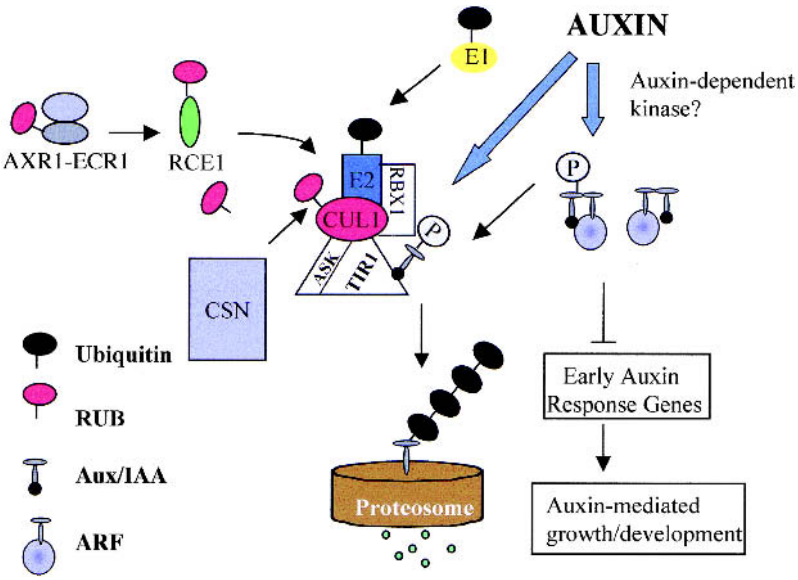

Figure 2. Model for auxin response. In response to auxin, the negative acting Aux/IAA proteins are targeted for degradation by the ubiquitin ligase SCF^{TIR1} thus releasing the ARF proteins to activate downstream transcription of early-response genes involved in auxin-mediated growth and development. AXR1-ECR1-dependent RUB1 modification of CUL1 is required for activity of SCF^{TIR1}. Auxin stimulates the interaction between domain II of the Aux/IAA proteins and TIR1 and may also regulate the activity of a protein kinase which phosphorylates targets of this pathway. The COP9 signalosome cleaves RUB1 from the modified CUL1 (Swechheimer and others 2001). Abbreviations; RCE1, RUB-conjugating enzyme; E1, ubiquitin-activating enzyme; E2, ubiquitin-conjugating enzyme.

K722M substitution. This particular lysine residue is conserved in all cullins and appears to be the site for attachment of RUB. Although AXR1-ECR1 is capable of forming a thiol-ester bond with all three RUBs *in vitro,* it is not known whether all three can be attached to CUL1. It is also not known whether an E3 ligase activity is required for CUL1 modification. Clearly mutations in *AXR1* that reduce RUB activation and thus modification of CUL1 have dramatic consequences for the plant. These studies highlight the important role of the AXR1-ECR1 RUB conjugation pathway in response to the plant hormone auxin.

Further evidence for the importance of protein degradation in the auxin-response pathway comes from studies of the *Arabidopsis tir1* mutant. Mutations in *TIR1* result in an auxin-resistant root phenotype that is associated with decreased numbers of lateral roots and defects in cell division and elongation (Ruegger and others 1998). TIR1 encodes a protein containing an F-box domain and a series of leucine-rich repeats similar to the yeast proteins Cdc4p and Grr1p (Ruegger and others 1998). Gray and others (1999) showed that TIR1 interacts with the SKP1-like proteins ASK1 and ASK2 and the cullin CUL1 to form SCF^{TIR1}. This complex can also interact with RBX1 (WM Gray and M Estelle unpublished). That AXR1 and TIR1 converge on a common auxin-response pathway is confirmed by the observation that the *tir1/axr1-12* double mutant has a synergistic phenotype (Ruegger and others 1998). There are at least three *TIR1*-like genes in *Arabidopsis,* one of which is identical to the *COI1* gene (Xie and others 1998). The recessive *coi1* mutants are completely insensitive to jasmonic acid suggesting that the SCF and ubiquitin-mediated protein degradation are essential for jasmonate signaling (Feys and others 1994).

Genetic data have shown that two other auxin-response mutants, *axr4* and *sar1,* also function in the same pathway as *axr1.* Double mutant combinations among *axr4, tir1,* and *axr1* all display synergistic interactions (Hobbie and Estelle 1995; Ruegger and others 1998). *Sar1* was identified as a recessive suppressor of the auxin-resistant root phenotype of *axr1-3* (Cernac and others 1997). It is epistatic to *axr1* and can suppress nearly all aspects of the *axr1* phenotype. Work is ongoing to positionally clone these two genes and thus establish their roles in the *AXR1* auxin-response pathway (S Dharmaseri, S Ward and M Estelle unpublished).

The AXR1-TIR1 Pathway and Its Targets

The double mutant analysis of *axr1tir1* combined with the observations that loss of function mutations in components of SCF^{TIR1} or the RUB-conjugation pathway confer a reduced response to auxin, clearly implicates *AXR1* in an auxin-response pathway that includes *TIR1* (Figure 2). One of the most important questions about this pathway is the nature of the proteins targeted for degradation by TIR1. The strongest candidates are proteins involved in auxin-regulated gene expression, the best characterized of which are the *Aux/IAA* genes (Abel and Theologis 1996). In *Arabidopsis* there are at least

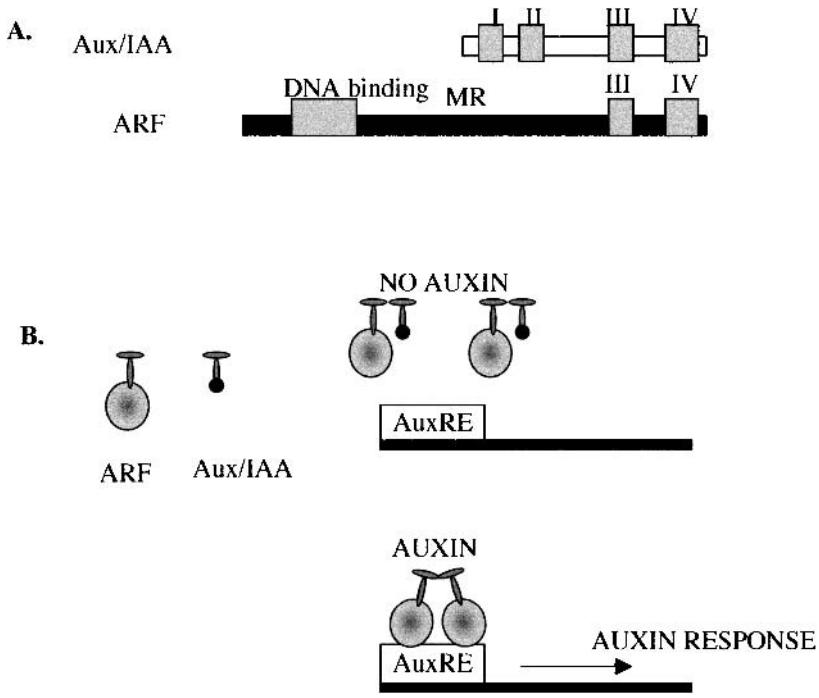

Figure 3. The Aux/IAA and ARF protein families. (A) Schematic diagram of the domain organization of the Aux/IAA and ARF protein families. Aux/IAA proteins contain four highly conserved domains. The ARF family share the AUX/IAA domains III and IV, involved in dimerization. At the carboxy-terminal end, the ARFs have a domain similar to the DNA binding domains in maize VP1 transcription factors. The middle region (MR) may be biased to a specific amino acid in some ARFs, for example, glutamines, serines, and leucines in ARFs 5 through 8. This region may function as a transcription activation/repression domain (Ulmasov and others 1996). (B) In the absence of auxin, basal levels of Aux/IAA proteins repress the auxin response pathway by forming heterodimers with ARF proteins and preventing binding to auxin-response elements in the promoters of downstream genes and thus activating their transcription. When stimulated by auxin, the negative acting Aux/IAA proteins are degraded, thus allowing the ARFs to dimerize and bind the auxin-response elements.

twenty-four *Aux/IAA* genes that all share a common structure with four highly conserved domains known as domains I, II, III and IV (Figure 3A). Domain III is related to the βαα-fold in β-ribbon DNA-binding domains of the Arc and MetJ prokaryotic transcriptional repressor proteins although the proteins have not been shown to bind DNA directly. Members of the *Aux/IAA* family are capable of forming homo- and heterodimers through domains III and IV (Kim and others 1997). Some members of the family can also regulate transcription by forming dimers with members of a second protein family known as the ARFs (Ulmasov and others 1997). The ARFs bind auxin-response elements (AuxRE) adjacent to auxin-regulated genes and either activate or repress transcription depending on the ARF (Figure 3A). These proteins also contain sequences similar to domains III and IV of the Aux/IAA proteins and can dimerize either with themselves or other members of the ARF and Aux/IAA protein families (Kim and others 1997). Over-expression of some Aux/IAA proteins in carrot protoplasts repressed transcription through an AuxRE containing promoter. At present there is no evidence that the Aux/IAA proteins bind DNA, suggesting that repression occurs through interaction with an ARF protein (Ulmasov and others 1997).

These data suggest that in the absence of auxin, heterodimers can form between negative acting Aux/IAA proteins and ARFs thus preventing the ARFs from binding to auxin-response-elements and activating the transcription of downstream genes (Figure 3B). When auxin is present the Aux/IAA proteins are rapidly degraded, allowing the formation of ARF dimers and downstream transcription. As the Aux/IAA proteins can regulate their own transcription, levels of the repressors would rapidly increase again, shutting off the pathway by binding to ARFs and inhibiting their interactions with AuxREs. Auxin regulates a widely different set of responses throughout the plant and this may be reflected in the different expression patterns and response kinetics of the members of the *Aux/IAA* and *ARF* gene families.

Several of the *Aux/IAA* genes have been identified in genetic studies. Dominant gain-of-function mutations in *AXR2/IAA7, AXR3/IAA17, SHY2/IAA3,* and *IAA28* all cause defects in auxin response (Rouse and others 1998; Nagpal and others 2000; Tian and Reed 1999; Rogg and others 2001). These mutations all lie within a small highly conserved region within domain II. It has been suggested that these mutations act to stabilize the Aux/IAA protein. This model has been confirmed in recent studies showing that domain II contains a transferable destabilization determinant (Worley and others 2000). Further, the do-

main II mutations act to stabilize the affected protein (Worley and others 2000; Ouellet and others 2001). For *IAA3, IAA7,* and *IAA28,* the effects of stabilization are to reduce auxin response, suggesting that most Aux/IAA proteins are negative regulators of auxin signaling. However, stabilization of AXR3/IAA17 appears to increase auxin response in some tissues, suggesting that individual members of the family may act to either activate or repress auxin response (Leyser and others 1996).

Two additional classes of genes have been identified that display genetic interactions with *AXR1* and/or *TIR1* and may be targets of the *AXR-TIR1* auxin-response pathway. *NAC1* is a member of a family of genes with diverse developmental roles that are proposed to act as transcriptional activators (Xie and others 2000). The *NAC1* gene is induced by auxin and is primarily expressed in the root meristem and lateral root initiation sites. Transgenic plants expressing antisense *NAC1* cDNA with a decrease in the endogenous level of *NAC1* transcript, displayed a decrease in the number of lateral roots initiated. Over-expression of *NAC1* leads to an increase in the number of lateral roots initiated even in the absence of endogenous applied auxin. Conversely, the *tir1* mutant displays a decrease in the number of lateral roots initiated (Ruegger and others 1997). Over-expression of *NAC1* in a *tir1* background can rescue this lateral root defect. This result, coupled with the observation that *NAC1* transcript level is greatly reduced in a *tir1* background, led the authors to propose that *TIR1* regulates *NAC1* transcription. This could occur through the degradation of a negative regulator of *NAC1* (Xie and others 2000).

The auxin efflux carrier EIR1 (AGR/PIN2) is implicated in mediating gravitropic response in *Arabidopsis* roots (Luschnig and others 1998; Chen and others 1998; Galweiler and others 1998; Utsuno and others 1998). A translational fusion containing the entire *EIR1* genomic coding region fused to the amino terminus of the reporter gene β-glucuronidase was expressed in both wild-type and *axr1–3* plants. The authors found that in wild-type seedlings, the construct was unstable in response to changes in auxin homeostasis (Sieberer and others 2000). When expressed in the *axr1–3* background, however, the construct became stabilized, suggesting that AXR1 is required for degradation of EIR1. Proteolytic degradation of EIR1 via activation of AXR1 could be essential for the establishment of an auxin gradient in response to gravitropic stimuli.

Another possible target for AXR1-mediated proteolysis is the previously mentioned *SAR1,* suppressor of *axr1.* Genetic data place *SAR1* downstream of *AXR1* in the auxin-response pathway (Cernac and others 1997). However, double mutant analysis of *sar1tir1* showed that they function in separate pathways (A Cernac and M Estelle unpublished), leaving open the possibility that *SAR1* may be a substrate for one of the TIR1 related F-box proteins.

Auxin, Light and the COP9 Signalosome

There is increasing evidence of links between light and auxin signaling pathways. Gain-of-function mutations in domain II of *AXR2/IAA7* and *AXR3/IAA17* (Rouse and others 1998; Nagpal and others 2000) as well as *shy2/iaa3,* which was identified independently as a suppressor of the chromophore-deficient, long hypocotyl, *hy2* mutant, and as a suppressor of phyB (Kim and others 1996; Reed and others 1997; Tian and Reed 1999), induce ectopic light responses in dark-grown seedlings. In addition, two members of the auxin-regulated *GH3* family display phenotypes consistent with a role in mediating light responses when either mutated or over-expressed (Hsieh and others 2000; Nakazawa and others 2001). In one of the most direct connections between light and auxin signaling, the red light photoreceptor phytochrome A has recently been shown to phosphorylate some of the Aux/IAA family members *in vitro* (Colón-Carmona and others 2000). This *in vitro* phosphorylation of Aux/IAA proteins by recombinant oat phytochrome A was not light-dependent and it remains to be seen what effect phosphorylation has on function of these proteins. The authors speculate that because the potential phosphorylation sites map to domains I and II, stability of the proteins may be affected by phosphorylation (Colón-Carmona and others 2000).

Another connection between light and auxin was recently revealed in studies of the COP9 signalosome (CSN). The CSN is an eight-protein complex that represses photomorphogenesis in the dark. Each of its eight subunits is related to one of the eight subunits that form the cap of the 26S proteosome, leading to the exciting idea that the CSN may be involved in protein degradation via the ubiquitin-proteosome pathway (Schwechheimer and others 2001). Two recent papers reveal that the CSN plays a role in removing RUB/NEDD8 from CUL1. Lyapina and coworkers (2001) identified all eight subunits of the CSN as CUL1 interacting proteins in mouse cells. Continuing their studies in fission yeast, they observed that in a CSN-deficient background, 100% of CUL1 (Pcul in *S. pombe*) was modified by Nedd8, whereas in wild-type cells, very few

molecules were in the modified form. This result suggests that the CSN is responsible for removal of Nedd8.

The most compelling demonstration of this activity comes from *in vitro* studies in which the addition of purified CSN to modified cullin led to rapid removal of Nedd8. A physiological role for this "deneddylation" was suggested by studies in *Arabidopsis.* As in the previous study, *Arabidopsis* SCF components were shown to interact with CSN components in two hybrid and immunoprecipitation assays. Schwechheimer and coworkers (2001) created an antisense *CSN5* plant that has decreased amounts of CSN. The plant displayed many phenotypes associated with decreased auxin-response, including reduced apical dominance and stature, leaf epinasty, and reduced transcription of *Aux/IAA* genes. Confirmation of the requirement for both AXR1 and the CSN for auxin-response was obtained by introducing the antisense *CSN5* construct into *axr1-3.* The resulting plants displayed a synergistic phenotype. Just as in mammalian and fission yeast cells, reduced CSN function resulted in an increase in the amount of modified CUL1. Thus, both decreased (*axr1*) and increased (*CSN-5* co-suppression lines) RUB-CUL1 result in defects in SCF functions.

These results indicate that RUB modification is a dynamic process, and raise the possibility that SCF activity involves a cycle of RUB modification and removal. The RUB/Nedd8 deconjugating activity is the first well-characterized biochemical activity of the CSN. However, given the complexity of the CSN it is unlikely to be the only activity. The CSN also interacts with the proteosome (Karniol and Chamovitz 2000), and earlier studies showed that the complex has protein kinase activity (Bech-Otschir and others 2001). It will be interesting to see how these observations relate to SCF function and auxin response.

How Does Auxin Regulate the AXR1/TIR1 Pathway?

Until very recently it has been unclear if auxin directly regulates the AXR1-TIR1 auxin response pathway. However, recent results from the Estelle and Leyser labs (Gray and others, in press) suggest that auxin regulates the interaction between SCF^{TIR1} and the Aux/IAA proteins. Experiments with Aux/IAA-GUS fusion proteins showed that proteolytic degradation of the Aux/IAA proteins was directly mediated by the AXR1-TIR1 auxin response pathway. Targeted degradation occurred as a consequence of an interaction between TIR1 and the highly conserved domain II of Aux/IAA proteins. Most excitingly the interaction between TIR1 and the Aux/IAA proteins was stimulated by auxin application. As a consequence of this interaction the Aux/IAA proteins are ubiquitinated and targeted for degradation by the 26S proteosome.

This is the first example of a direct interaction between an *Arabidopsis* SCF and its substrate. The finding that the interaction between SCF^{TIR1} and its substrate is auxin-regulated leads to the interesting possibility that COI1 interactions with its substrates may be stimulated by jasmonic acid. It remains to be seen how auxin exerts this effect. For most other SCF complexes where the substrates have been identified, substrate-SCF interaction depends on substrate phosphorylation (Gray and Estelle 2000). This leads to the intriguing possibility that there is an auxin-responsive protein kinase that phosphorylates the target proteins thus enabling their recruitment by TIR1. A good candidate is the PINOID kinase, which has been implicated in auxin responses (Christensen and others 2000). Auxin-responsive MAP kinases have also been identified (Mizoguchi and others 1994; Mockiatis and Howell 2000). At present, the targets of these kinases are unknown.

Conclusion

Many genetic and molecular studies have now converged into a single coherent model. There are several clear challenges ahead. One is to understand the complexity of Aux/IAA and ARF protein function. Both protein families are large and it is likely that the complexity of their interactions is a reflection of the complex nature of auxin responses. Another challenge is to define those proteins that function upstream of the IAA proteins to regulate SCF substrate interaction. A third focus will be the identification of ARF-Aux/IAA-regulated genes and their function in auxin response. Finally, our knowledge of auxin signaling, and other signaling pathways, should permit new insight into how the various signals interact and integrate during plant growth and development.

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