REVIEW: BOTANY

Plant Development: Regulation by Protein Degradation

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Many aspects of eukaryotic development depend on regulated protein degradation by the ubiquitin-proteasome pathway. This highly conserved pathway promotes covalent attachment of ubiquitin to protein substrates through the sequential action of three enzymes called a ubiquitin-activating enzyme (E1), a ubiquitin-conjugating enzyme (E2), and a ubiquitin-protein ligase (E3). Most ubiquitinated proteins are then targeted for degradation by the 26S proteasome. Recent studies have also shown that the ubiquitin-related protein RUB/Nedd8 and the proteasome-related COP9 signalosome complex cooperate with the ubiquitin-proteasome pathway to promote protein degradation. Most of these components are conserved in all three eukaryotic kingdoms. However, the known targets of the pathway in plants, and the developmental processes they regulate, are specific to the plant kingdom.

Regulated protein degradation plays a crucial role during development in all organisms. One mechanism for proteolysis in eukaryotes is the ubiquitin-proteasome pathway (*1*). The pathway begins with formation of a thiolester bond between ubiquitin and a ubiquitin-activating enzyme (E1). Ubiquitin is transferred to a cysteine residue within a ubiquitin-conjugating enzyme (E2). The E2 interacts with a ubiquitin-protein ligase (E3) and transfers ubiquitin to E3-bound substrates. Finally, proteins with polyubiquitin chains are recognized and degraded by the 26*S* proteasome, a complex that consists of a 20*S* core and two 19*S* regulatory particles (*1*). The importance of this pathway in plants becomes apparent upon examination of the *Arabidopsis* genome (*2*). On the basis of sequence homology to known components, it has been estimated that about 1200 proteins, or 5% of the predicted 25,000 proteins in *Arabidopsis*, function in the ubiquitin-proteasome pathway (*3*).

The Ubiquitin-Protein Ligases

Most eukaryotes have one or two E1 isoforms and a larger family of E2 proteins. There are at least 36 E2 isoforms in *Arabidopsis* that cluster into 12 groups (*4*). The physiological function of these groups is not clear at present, but (as in animals and fungi) different E2s are probably specialized for specific E3 enzymes and/or cellular functions. The E3 ubiquitin ligases are the specificity components of the pathway and have therefore attracted the most attention. E3s bind the E2 enzyme and the protein substrate and promote ubiquitin transfer by bringing the two proteins into close proximity. E3s are diverse but can be divided into two groups on the basis of the presence of a HECT domain (homologous to E6-AP C-terminus) or a RING-finger domain. HECT-domain E3s are present in plants, but so far there is no information on their substrates or functions (*4, 5*). RING-finger E3s can be divided into several subgroups: single-subunit RING E3s, the anaphase-promoting complex (APC), SCF-type E3s, and the VCB-Cul2 complex (VBC). Members of each subgroup except the VBC are found in plants (*4*). In this review, we focus on E3s that have been clearly implicated in a developmental process.

Single-subunit RING E3s. RING E3 proteins have a characteristic set of cysteine and histidine residues (the RING domain) that bind two zinc ions (*6*). The function of this domain is to bind the E2 enzyme, whereas other regions of the protein are responsible for binding substrate proteins and ancillary factors. An examination of sequenced genomes reveals a very large number of putative RING proteins. More than 300 RING proteins are encoded by the *Arabidopsis* genome, whereas the *Drosophila* and *Caenorhabditis elegans* genomes encode about 100 and 150 such proteins, respectively (*2*). Many RING proteins have also been identified in mammals and implicated in diverse aspects of cellular regulation (*7*).

Arabidopsis researchers are just beginning to reveal the multifarious functions of this huge family of proteins. The best-characterized plant RING protein, COP1 (constitutive photomorphogenesis), is required to repress light-regulated development in the dark (*8–10*). The *cop1* mutant is one of several *cop* mutants discussed below. In the absence of COP1, dark-grown *Arabidopsis* seedlings display characteristics of seedlings grown in the light, including shortened hypocotyls, leaf development, and formation of the photosynthetic apparatus. In addition to the RING motif, COP1 contains seven WD-40 repeats (i.e., the repeat length is about 40 residues and the last two amino acids are usually Trp and Asp) as well as a coiled-coil domain presumably required for binding of substrates and other regulatory factors.

Because the phenotype of loss-of-function *cop1* mutants is constitutive photomorphogenesis, the COP1 substrates probably promote light-regulated development. Several potential substrates have been identified, most notably the bZIP transcription factors HY5 (hypocotyl 5) and HYH (HY5 homolog) (*11–13*). HY5 accumulates in the light and is required for light-regulated gene expression, whereas in the dark the protein is degraded by the proteasome (*14*) (Fig. 1). In *cop1* mutants HY5 accumulates in the dark, indicating that COP1 is required for its degradation. Experiments showing that COP1 interacts with HY5 through the WD-40 repeats both in vitro and in vivo confirm that COP1 is a component of an E3 required for HY5 degradation (*15*). Similar results were recently obtained for HYH. Indeed, overexpression of HYH can suppress the *hy5* mutation, which suggests that these two proteins have similar functions (*13*). Despite these results, COP1-mediated ubiquitination of HY5 has not been demonstrated in vitro. In a recent report (*16*), a COP1-interacting protein called CIP8, itself a RING-finger protein, was shown to ubiquitinate HY5 in vitro. Thus, it is possible that a COP1-CIP8 heterodimer is the HY5 E3.

Light regulation of COP1 activity and HY5 levels is complex (Fig. 1). One component is movement of COP1 between the nucleus and the cytoplasm. In darkness, when the photomorphogenic program is repressed, COP1 is in the nucleus and HY5 is degraded. In the light, COP1 is absent from the nucleus and HY5 levels increase, promoting the transcription of light-regulated

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genes (*17*). How light regulates the localization of COP1 is a fascinating and largely unanswered question. Curiously, another RING finger protein called HOS1 may also be translocated between cellular compartments in response to a stimulus. HOS1, a negative regulator of low-temperature signal transduction, is found in the cytoplasm at normal temperatures (*18*). At low temperatures it accumulates in the nucleus, possibly to promote the degradation of transcription factors involved in cold-induced changes in gene expression.

A second component of COP1 regulation has been revealed through studies of photoreceptor proteins. Light is perceived by two major groups of photoreceptors in plants: the red/far red–absorbing phytochromes, and the blue light–absorbing cryptochromes (CRY1 and CRY2 in *Arabidopsis*). On the basis of their similarity to flavin-binding photolyases (*19*), the CRY proteins are thought to function via a light-dependent redox reaction. In addition to the conserved flavin-binding domain, the CRY proteins have a COOH-terminal region that is not found in the photolyases. Overexpression of this COOH-terminal fragment (CCT) leads to a *cop* phenotype, suggesting that CRY photoreceptors may negatively regulate COP1 function (*20*). This hypothesis is strongly supported by the recent discovery that CRY1 and CRY2 each bind COP1 through their COOH-terminal domains (*9, 21*) (Fig. 1). In addition, overexpression of CCT1 or CCT2 results in stabilization of HY5, consistent with reduced activity of COP1 (*9*).

The CRY-COP1 interaction occurs in both the light and the dark, which suggests that binding is not sufficient to inhibit COP1 (*21*). Instead, CRY1 and CRY2 may mediate a blue light–dependent redox reaction that results in a change in the activity of bound COP1 (*21*). Phytochrome may also regulate COP1 function because the COOH terminus of phytochrome B (PhyB) interacts with COP1 in a yeast two-hybrid test (*21*). However, further study is required to confirm a role for PhyB in COP1 regulation. Two additional candidate COP1 substrates have been identified through yeast two-hybrid screens. Both COP1-interacting proteins (CIP4 and CIP7) are nuclear proteins that may function as transcriptional coactivators (*10, 22*). Like HY5, CIP4 and CIP7 are required for aspects of photomorphogenesis. Further studies are required to determine whether COP1 directly regulates CIP4 and/or CIP7 levels.

Loss of the COP10 protein also results in constitutive photomorphogenesis (*14*). COP10 is a variant E2 enzyme that lacks the active-site cysteine required for ubiquitin conjugation (*23*). Studies on related enzymes in yeast and mammals, called UEV proteins, indicate that they form an active E2 enzyme in association with a second traditional E2 protein (*24*). At least in some cases this heterodimer promotes the assembly of a novel ubiquitin chain that has regulatory activity (*24, 25*). COP10 is part of a ~300-kD nuclear complex, and a truncated form of COP10 interacts with COP1 in a two-hybrid test. On the basis of these results, Suzuki *et al.* proposed that the COP10 complex acts as an E2, either in the ubiquitination of COP1 targets or in the formation of a novel ubiquitin chain on COP1 (*23*).

SCF E3s. For the APC, the VBC, and diverse SCF complexes, the RING protein is just one subunit of an E3 complex. In animal and fungal systems, the APC is required for degradation of key cell cycle regulators. The APC is conserved in plants, but at present little is known about its substrates or regulation. SCF E3s are involved in diverse aspects of cellular regulation in animals, and the large number of putative SCFs in *Arabidopsis* (see below) suggests that the same is probably true in plants. SCFs consist of four subunits: a cullin, SKP1 (S-phase kinase-associated protein 1), a RING finger protein (RBX1/HRT1/ROC1, here referred to as RBX1), and an F-box protein (*26*) (Fig. 2). The F-box proteins confer specificity to the SCF, each interacting with a specific substrate or set of substrate proteins. F-box proteins have a SKP1-interacting domain (the F-box) and novel substrate recognition sequences often consisting of a series of repeats such as leucine-rich repeats or WD-40 repeats.

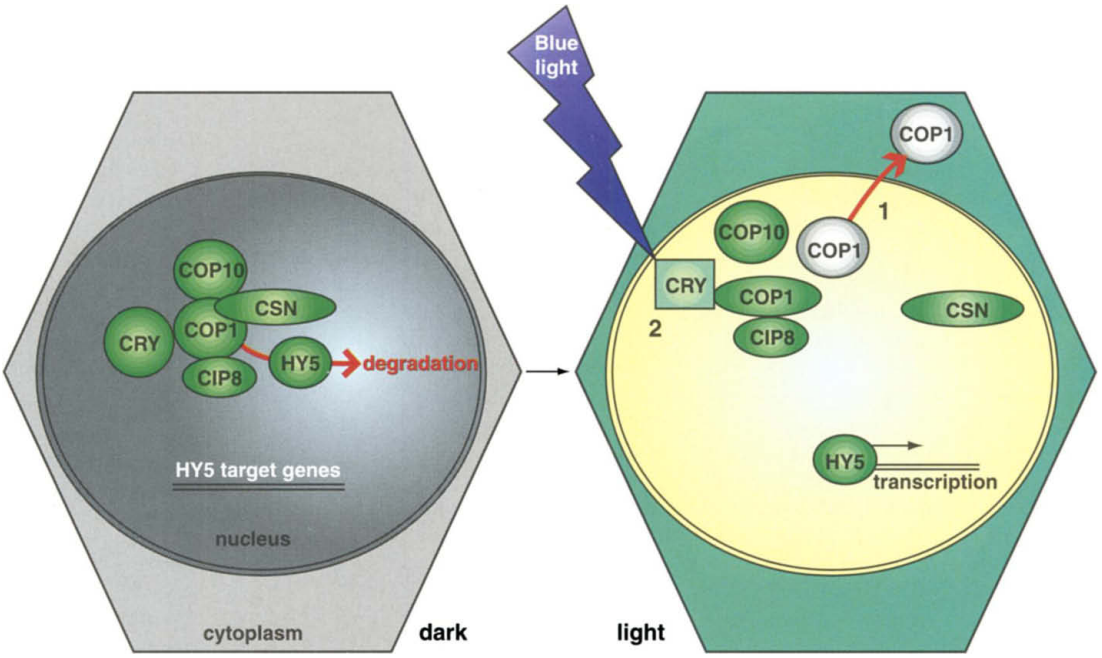

Fig. 1. Light-dependent regulation of COP1 activity. COP1 interacts in dark-grown plants with the COP10 complex, the CSN, blue light–absorbing cryptochromes (CRY), and the b-ZIP transcription factor HY5. COP1-HY5 interaction results in degradation of HY5. The COP10 complex may provide E2 function. At least two light-dependent mechanisms stabilize HY5 and promote transcription of HY5 target genes: White light induces translocation of COP1 from the nucleus to the cytoplasm (1); blue light alters COP1 function in a CRY-dependent fashion (2).

Recent estimates indicate that the *Arabidopsis* genome encodes about 700 F-box proteins (*27*). Although some of these proteins are likely to be redundant, this impressive number provides a preview of the likely impact of SCF-mediated proteolyses on plant development. The high number of F-box proteins is especially striking when compared to fungi and animals. There are an estimated 14, 24, and 337 F-box proteins encoded by the budding yeast, *Drosophila*, and *C. elegans* genomes, respectively (*2*). Given the size of the family, it is not surprising that the number of plant mutants affected in F-box proteins is rapidly increasing. Genetic approaches using a variety of different

mutant screens have already revealed a role for F-box proteins in senescence (*28*), apical dominance (*29*), circadian rhythm (*30, 31*), flower and meristem development (*32–34*), phytochrome A signaling (*35*), and phytohormone signaling (*36–38*). For most of these proteins, participation in an SCF complex is suggested by the presence of the F-box motif. In some cases, interaction with SKP1 has also been demonstrated (*28*). Only in the case of SCF^{TIR1} are the substrates of the E3 known. The complexity of the SCF E3 subgroup is further increased by the presence of multiple cullin and SKP1 isoforms. In *Arabidopsis*, there are at least six cullin proteins and 19 SKP1 proteins (*27*). One of the SKP1 proteins, *Arabidopsis* SKP1-related 1 (ASK1), has been implicated in both hormone response and male gametogenesis (*37, 39*). The possibility that some or all of the other members of these families have unique functions is now being explored.

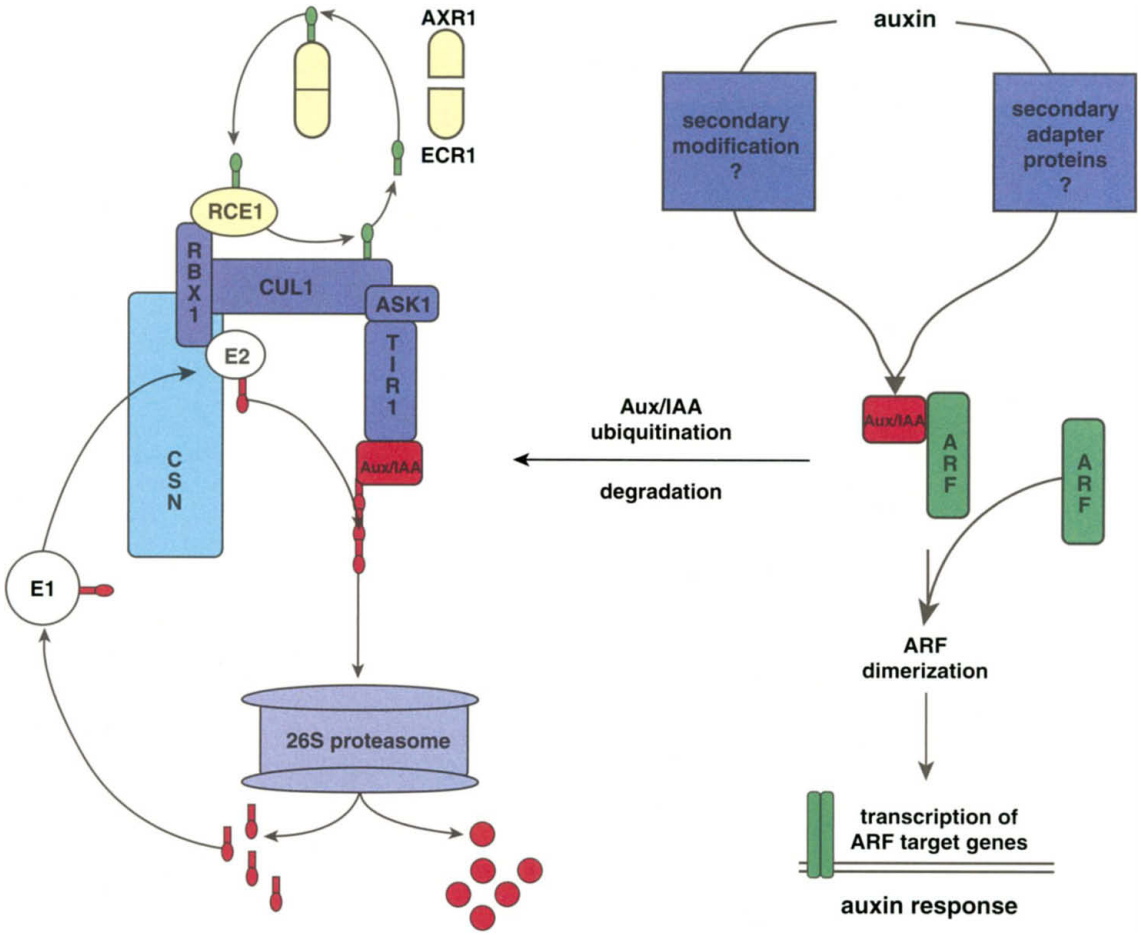

Fig. 2. Model for auxin-dependent Aux/IAA protein degradation. The E3 ubiquitin ligase SCF^{TIR1} (ASK1, CUL1, TIR1, and RBX1) mediates ubiquitination of Aux/IAA proteins by transferring ubiquitin (red cones) from an E2 to its substrate. SCF^{TIR1} activity is dependent on a dynamic cycle of conjugation and removal of the ubiquitin-related protein RUB1 (green cones) to CUL1. RUB1 conjugation is promoted by the bipartite E1-like enzyme AXR1-ECR1, the E2-like enzyme RCE1, and RBX1. Removal of RUB1 requires the CSN. The Aux/IAA proteins repress transcription of auxin-responsive genes by binding to ARF transcription factors. Auxin promotes modification of either Aux/IAA proteins or an adapter protein, resulting in binding of Aux/IAA by SCF^{TIR1} and consequent ubiquitination and degradation. Removal of Aux/IAAs permits the formation of ARF homodimers and transcription of auxin-responsive genes.

SCF^{TIR1} mediates auxin response via AUX/IAA protein degradation. The best-characterized plant SCF complex is SCF^{TIR1} from *Arabidopsis* (*37*) (Fig. 2). Mutations in *TIR1* or the *ASK1* gene result in decreased response to the phytohormone auxin, a key regulator of diverse aspects of plant development ranging from embryogenesis to senescence (*37–39*). The phenotype of loss-of-function mutations in components of SCF^{TIR1} suggests that its substrates are negative regulators of auxin response. Indeed, recent studies indicate that this is the case and that the negative regulators in question are the Aux/IAA proteins (*40*). The *Arabidopsis* genome encodes at least 24 of these small nuclear-localized proteins (20 to 35 kD), all of which share four conserved domains (*41*). Domains III and IV are required for homo- and heterodimerization of Aux/IAA proteins and for dimer formation between Aux/IAA proteins and members of a second large family of proteins called auxin response factors (ARFs). There are 23 *ARF* genes in the *Arabidopsis* genome (*41–43*). A typical ARF protein has conserved domains III and IV similar to the Aux/IAAs, as well as a DNA binding domain and a transcriptional activation domain. The ARF proteins bind to a conserved DNA element called the AuxRE. Most members of the family appear to activate auxin-regulated gene expression (*44, 45*). In contrast, the Aux/IAA proteins appear to repress ARF function, probably by dimerization with the ARFs (*43*).

The Aux/IAA proteins are extremely unstable, with reported half-lives ranging from 6 to 80 min (*46*). The importance of this instability for auxin response has been amply demonstrated through genetic and biochemical studies conducted in a number of labs (*41*). Genetic screens have recovered gain-of-function mutations in 10 members of the Aux/IAA family. Each mutation results in a substitution within another conserved region of the protein, called domain II, and a reduction in auxin response (*41, 47*). In those instances where it has been tested, the mutation stabilizes the affected protein, indicating that the defect in auxin response is caused by accumulation of the repressor (*40, 43, 48, 49*). Further, the fusion of a 13–amino acid subfragment of domain II to firefly luciferase (LUC) destabilized LUC, showing unequivocally that this sequence is a transferable degradation signal (*49–51*). LUC stability was further decreased in the presence of auxin, suggesting that the hormone acts directly to stimulate degradation of the IAA proteins (*50*).

Three lines of evidence indicate that auxin stimulates degradation by promoting an interaction between SCF^{TIR1} and the IAA proteins. First, proteasome inhibitors stabilize the Aux/IAA proteins (*40, 51*). Second, the *tir1* mutation stabilizes both AXR2/IAA7 and AXR3/IAA17 (*40*).

Third, AXR2/IAA7, AXR3/IAA17, and AXR5/IAA1 interact with SCF^{TIR1} through domain II in an auxin-dependent fashion (*40, 47*). The domain II mutations prevent this interaction. Together, these results suggest a compelling model in which auxin regulates gene expression by promoting the interaction between SCF^{TIR1} and the Aux/IAA repressors, resulting in their degradation (Fig. 2).

Although this model is a major advance, the question remains: How does auxin promote the interaction between the Aux/IAA proteins and SCF^{TIR1}? In animals and fungi, the interaction between an F-box protein and its substrates is typically dependent on phosphorylation of the substrate (*26*). Hence, one consequence of auxin signal transduction might be phosphorylation of Aux/IAA proteins. Indeed, members of the family interact and are phosphorylated in vitro by oat phytochrome A (phyA), and in vivo phosphorylation has been demonstrated for IAA3/SHY2 (*52*). On the other hand, phyA-dependent phosphorylation occurs at the NH_2-terminal part of AUX/IAA proteins outside of domain II. In addition, substitution of potential phosphorylation sites within domain II does not affect degradation (*51*). This indicates that Aux/IAA phosphorylation cannot be the decisive factor for interaction with TIR1. Instead, auxin might signal the phosphorylation of an adapter or bridge protein that mediates the interaction between the SCF and the Aux/IAA protein (Fig. 2). Alternatively, the interaction may depend on a different post-translational modification. For example, binding of the VBC to its substrate, the transcription factor HIFα, depends on hydroxylation of a key proline residue within the substrate (*53, 54*). This observation is of special interest because there are two conserved prolines within domain II that are required for interaction with SCF^{TIR1} (*40, 51*).

Ubiquitin-Related Proteins and E3 Function

Eukaryotic genomes encode several ubiquitin-related proteins. The best-characterized of these, SUMO/Smt3 and RUB/Nedd8, are, like ubiquitin, conjugated to other proteins through the action of specific activating and conjugating enzymes (*55*). Diverse SUMO conjugates have been identified in animals and fungi (*56*), and it is likely that this will be true in plants as well (*57*). The consequences of SUMO modification are also diverse and include cellular localization, activation, and protection from ubiquitin-mediated proteolysis (*56*). In contrast, the only known RUB/Nedd8-modified proteins are the cullins, subunits of the SCF, VCB, and other cullin-based E3s (*55*). A single RUB molecule is conjugated to a conserved lysine near the COOH terminus of the cullin (*58–62*). Except for the cullin-related protein found in the APC complex, RUB/Nedd8 modification appears to be a general feature of all cullins. Several possible functions of the modification have been proposed, including localization, recruitment of the E2 protein, and/or recruitment of the F-box protein (*63–65*). However, compelling evidence for any of these possibilities is lacking so far.

Regardless of the specific biochemical function, it is clear that RUB modification has an essential role in most eukaryotes, the one exception being budding yeast. In this species, the deletion of components of the RUB conjugation pathway has no effect on viability, but does reduce SCF function in certain sensitized genetic backgrounds (*66*). In contrast, the pathway is essential in fission yeast, *C. elegans*, mouse, and *Arabidopsis* (*61, 67–69*). In *Arabidopsis*, mutations in several components of the RUB modification pathway have been identified, including both subunits of the RUB activating enzyme (AXR1 and ECR1) as well as a RUB E2 called RCE1 (*68, 70, 71*) (Fig. 2). In each case, the mutation results in reduced RUB-CUL1 levels, stabilization of Aux/IAA proteins, and reduced auxin response, indicating that the modification is required for normal SCF^{TIR1} function (*40, 70*) (Fig. 2). For both *AXR1* and *RCE1*, a closely related gene is present in the *Arabidopsis* genome. Mutations in the *AXR1*-related gene (called *AXL1*) have no apparent phenotype. However, double mutants deficient in both *AXR1* and *AXL1* die as young seedlings, indicating that the RUB pathway is essential for viability in *Arabidopsis* (*71*).

In the case of SUMO, recent studies have identified several classes of E3s (*72, 73*) that mediate SUMO conjugation to specific substrates. For RUB/Nedd8, the SCF subunit RBX1 may function as the E3. In budding yeast and human cells, RBX1 promotes RUB/Nedd8 modification of cullins (*74*). We have obtained similar results in *Arabidopsis*, and in addition demonstrated that RBX1 interacts with the RUB E2 enzyme RCE1. Overexpression of RBX1 in *Arabidopsis* results in increased levels of RUB-CUL1. Curiously, this change causes a phenotype that is very similar to the *axr1* mutants, including stabilization of the Aux/IAA proteins and decreased auxin response. Thus, both an increase and a decrease in the relative amounts of RUB-CUL1 results in reduced SCF^{TIR1} function. These results suggest that dynamic changes in RUB/Nedd8 modification of cullins are important for SCF function. A similar conclusion was reached in earlier studies of the role of the COP9 signalosome in SCF function (see below).

The COP9 Signalosome and Proteolysis

The COP9 signalosome (CSN) is a nuclear-enriched protein complex first identified in *Arabidopsis* and also present in other eukaryotes (*75, 76*). The complex consists of eight subunits, all of which are related to proteins of the 19*S* regulatory particle (19*S* RP) of the proteasome. In addition, 19*S* RP and CSN subunits share two common domains with subunits of the eukaryotic translation initiation factor 3 (eIF3), a complex of at least 11 subunits (*75*). Studies in plants, fission yeast and mammals have revealed that the CSN functions in various physiological processes such as cell cycle, transcriptional control, and hormone-dependent pathways (*75, 77*). The biochemical functions of the CSN are not fully understood, but accumulating evidence indicates that it has one or more important roles in ubiquitin-mediated protein degradation (*65, 78, 79*).

The CSN interacts with the cullin and the RBX1 subunits of SCF E3s (Fig. 2). This was demonstrated in human cells, fission yeast, and *Arabidopsis* (*65, 79*). In two-hybrid experiments, the CSN2 subunit interacts with *Arabidopsis* CUL1, whereas CSN1 and CSN6 interact with RBX1. In addition, a reduction in CSN levels leads to stabilization of Aux/IAA proteins and strongly impaired auxin-dependent transcription of Aux/IAA genes (*78*), which suggests that the CSN is required for SCF^{TIR1} activity. Because CUL1 and RBX1 are presumably subunits of many different SCF complexes, the CSN is likely to have a broad role in mediating SCF function. In fact, a reduction in CSN levels produces a highly pleiotropic phenotype (*80, 81*).

One function of the CSN appears to be regulation of RUB/Nedd8-cullin levels. In both plants and fungi, a reduction in CSN levels causes an increase in the levels of modified cullin, and in vitro experiments indicate that the CSN is associated with a RUB/Nedd8 deconjugating activity. As mentioned above, increased RUB-CUL1 is associated with stabilization of SCF^{TIR1} substrates, presumably because of an effect on the SCF. However, more detailed dissection of CSN subunits illustrates the complexity of CSN function. In *Arabidopsis*, loss of any of the CSN subunits, including CSN1, results in loss of the CSN complex and seedling lethality. Expression of truncated forms of CSN1 in a *csn1* mutant permits assembly of an intact CSN and restores normal levels of RUB-CUL1. However, the truncated CSN1 proteins do not rescue seedling viability, indicating that loss of RUB deconjugating activity is not the only factor contributing to seedling le-

thality. A similar complexity is observed in fission yeast. Null *csn1* and *csn2* mutations result in slow growth and increased Nedd8-Pcu1, the SCF cullin subunit in this species (*82, 83*). The *csn4* and *csn5* mutants also have increased levels of Nedd-Pcu1 but, in these mutants, growth is not affected.

The CSN is also important for the function of other E3s. For example, degradation of the transcription factor HY5 requires both COP1 and a functional CSN (Fig. 1). Again, the situation is complex because the CSN is required for dark-dependent localization of COP1 to the nucleus (*76*). How the CSN affects COP1 localization and whether this is the only requirement of the CSN in HY5 degradation are important questions that are likely to be answered in the near future.

Future Prospects

Researchers investigating the ubiquitin-proteasome pathway have made tremendous progress in recent years, revealing a complex regulatory network controlling protein stability. So far the basic principles are conserved among all eukaryotes. Two major challenges can be identified. The first is to determine the functions of RUB/Nedd8 conjugation and the CSN in protein degradation. The second and more monumental challenge, particularly with the huge groups of RING and F-box proteins in plants, is to place each E2 and E3 in its correct biological context. Only a few members of these families have been studied, so it is probably too soon to identify any functional themes. However, early indications are that regulated protein degradation is particularly important for plant hormone signaling.

In addition to auxin and jasmonic acid, cytokinin, gibberelic acid (GA), and brassinosteroid (BR) action may all depend on protein degradation. In *Arabidopsis*, the loss of the proteasome subunit RNP12 results in a cytokinin defect, suggesting that cytokinin response may depend on degradation of one or more proteins (*84*). In the case of GA action, the *Arabidopsis* RGA protein is a repressor of GA response that is degraded upon GA treatment (*85, 86*). Deletion of a motif in RGA called the DELLA sequence stabilizes the protein and reduces GA response (*87*). In rice, the RGA ortholog SLR1 is also degraded in a GA-dependent manner (*88*).

A related story may be emerging in the case of BR signaling. The BZR1 and BES proteins are related nuclear proteins that promote BR response (*89*, *90*); the BIN2 protein is a glycogen synthase kinase 3–like kinase that functions as a negative regulator (*91*, 92). BR has two effects on BZR1 and BES: It induces their dephosphorylation and results in their accumulation. The proteasome inhibitor mg132 also stabilizes BZR1, which suggests that BR may act to prevent the proteasome-dependent degradation of these proteins. BIN2, on the other hand, phosphorylates BZR1 and prevents its accumulation. Apparently BR acts, at least in part, by regulating the phosphorylation status and hence the stability of BZR1 and BES. It will be interesting to learn the identity of the E3 enzyme responsible for their degradation.

References and Notes

1. A. Ciechanover, *EMBO J.* **17**, 7151 (1998).
2. The *Arabidopsis* Genome Initiative, *Nature* **408**, 796 (2000).
3. R. Vierstra, personal communication.
4. J. Callis, R. D. Vierstra, *Curr. Opin. Plant Biol.* **3**, 381 (2000).
5. P. W. Bates, R. D. Vierstra, *Plant J.* **20**, 183 (1999).
6. C. M. Pickart, *Annu. Rev. Biochem.* **70**, 503 (2001).
7. C. A. Joazeiro, A. M. Weissman, *Cell* **102**, 549 (2000).
8. C. S. Hardtke *et al.*, *EMBO J.* **19**, 4997 (2000).
9. H. Wang, L. G. Ma, J. M. Li, H. Y. Zhao, X. W. Deng, *Science* **294**, 154 (2001).
10. Y. Y. Yamamoto, X. W. Deng, M. Matsui, *Plant Cell* **13**, 399 (2001).
11. S. Chattopadhyay, L. H. Ang, P. Puente, X. W. Deng, N. Wei, *Plant Cell* **10**, 673 (1998).
12. T. Oyama, Y. Shimura, K. Okada, *Genes Dev.* **11**, 2983 (1997).
13. M. Holm, L.-G. Ma, L.-J. Qu, X. W. Deng, *Genes Dev.* **16**, 1247 (2002).
14. M. T. Osterlund, C. S. Hardtke, N. Wei, X. W. Deng, *Nature* **405**, 462 (2000).
15. L. H. Ang *et al.*, *Mol. Cell* **1**, 213 (1998).
16. C. S. Hardtke, H. Okamoto, C. Stoop-Myer, X. W. Deng, *Plant J.* **30**, 385 (2002).
17. A. G. von Arnim, X. W. Deng, *Cell* **79**, 1035 (1994).
18. H. Lee *et al.*, *Genes Dev.* **15**, 912 (2001).
19. A. R. Cashmore, J. A. Jarillo, Y. J. Wu, D. Liu, *Science* **284**, 760 (1999).
20. H. Q. Yang *et al.*, *Cell* **103**, 815 (2000).
21. H. Q. Yang, R. H. Tang, A. R. Cashmore, *Plant Cell* **13**, 2573 (2001).
22. Y. Y. Yamamoto, M. Matsui, L. H. Ang, X. W. Deng, *Plant Cell* **10**, 1083 (1998).
23. G. Suzuki, Y. Yanagawa, S. F. Kwok, M. Matsui, X. W. Deng, *Genes Dev.* **16**, 554 (2002).
24. R. M. Hofmann, C. M. Pickart, *Cell* **96**, 645 (1999).
25. L. Deng *et al.*, *Cell* **103**, 351 (2000).
26. R. J. Deshaies, *Annu. Rev. Cell Dev. Biol.* **15**, 435 (1999).
27. J. M. Gagne, B. P. Downes, S.-H. Shiu, A. M. Durski, R. D. Vierstra, *Proc. Natl. Acad. Sci. U.S.A.*, in press.
28. H. R. Woo *et al.*, *Plant Cell* **13**, 1779 (2001).
29. P. Stirnberg, K. van De Sande, H. M. Leyser, *Development* **129**, 1131 (2002).
30. D. E. Somers, T. F. Schultz, M. Milnamow, S. A. Kay, *Cell* **101**, 319 (2000).
31. D. C. Nelson, J. Lasswell, L. E. Rogg, M. A. Cohen, B. Bartel, *Cell* **101**, 331 (2000).
32. A. Samach *et al.*, *Plant J* **20**, 433 (1999).
33. D. Zhao, M. Yang, J. Solava, H. Ma, *Dev. Genet.* **25**, 209 (1999).
34. G. C. Ingram *et al.*, *EMBO J.* **16**, 6521 (1997).
35. M. Dieterle, Y. C. Zhou, E. Schafer, M. Funk, T. Kretsch, *Genes Dev.* **15**, 939 (2001).
36. D. X. Xie, B. F. Feys, S. James, M. Nieto-Rostro, J. G. Turner, *Science* **280**, 1091 (1998).
37. W. M. Gray *et al.*, *Genes Dev.* **13**, 1678 (1999).
38. M. Ruegger *et al.*, *Genes Dev.* **12**, 198 (1998).
39. M. Yang, Y. Hu, M. Lodhi, W. R. McCombie, H. Ma, *Proc. Natl. Acad. Sci. U.S.A.* **96**, 11416 (1999).
40. W. M. Gray, S. Kepinski, D. Rouse, O. Leyser, M. Estelle, *Nature* **414**, 271 (2001).
41. J. W. Reed, *Trends Plant Sci.* **6**, 420 (2001).
42. J. Kim, K. Harter, A. Theologis, *Proc. Natl. Acad. Sci. U.S.A.* **94**, 11786 (1997).
43. S. B. Tiwari, X. J. Wang, G. Hagen, T. J. Guilfoyle, *Plant Cell* **13**, 2809 (2001).
44. T. Ulmasov, G. Hagen, T. J. Guilfoyle, *Science* **276**, 1865 (1997).
45. ———, *Proc. Natl. Acad. Sci. U.S.A.* **96**, 5844 (1999).
46. S. Abel, P. W. Oeller, A. Theologis, *Proc. Natl. Acad. Sci. U.S.A.* **91**, 326 (1994).
47. X. Yang, J. So, L. Hobbie, M. Estelle, unpublished data.
48. F. Ouellet, P. J. Overvoorde, A. Theologis, *Plant Cell* **13**, 829 (2001).
49. C. K. Worley *et al.*, *Plant J.* **21**, 553 (2000).
50. N. Zenser, A. Ellsmore, C. Leasure, J. Callis, *Proc. Natl. Acad. Sci. U.S.A.* **98**, 11795 (2001).
51. J. A. Ramos, N. Zenser, H. M. Leyser, J. Callis, *Plant Cell* **13**, 2349 (2001).
52. A. Colon-Carmona, D. L. Chen, K. C. Yeh, S. Abel, *Plant Physiol.* **124**, 1728 (2000).
53. M. Ivan *et al.*, *Science* **292**, 464 (2001).
54. P. Jaakkola *et al.*, *Science* **292**, 468 (2001).
55. M. Hochstrasser, *Nature Cell Biol.* **2**, E153 (2000).
56. S. Muller, C. Hoege, G. Pyrowolakis, S. Jentsch, *Nature Rev. Mol. Cell Biol.* **2**, 202 (2001).
57. R. D. Vierstra, J. Callis, *Plant Mol. Biol.* **41**, 435 (1999).
58. T. Hori *et al.*, *Oncogene* **18**, 6829 (1999).
59. D. Liakopoulos, T. Busgen, A. Brychzy, S. Jentsch, A. Pause, *Proc. Natl. Acad. Sci. U.S.A.* **96**, 5510 (1999).
60. M. Morimoto, T. Nishida, R. Honda, H. Yasuda, *Biochem. Biophys. Res. Commun.* **270**, 1093 (2000).
61. F. Osaka *et al.*, *EMBO J.* **19**, 3475 (2000).
62. J. C. del Pozo, M. Estelle, *Proc. Natl. Acad. Sci. U.S.A.* **96**, 15342 (1999).
63. E. Freed *et al.*, *Genes Dev.* **13**, 2242 (1999).
64. K. Wu, A. Chen, P. Tan, Z. Q. Pan, *J. Biol. Chem.* **277**, 516 (2002).
65. C. Schwechheimer, X. W. Deng, *Trends Cell Biol.* **11**, 420 (2001).
66. D. Lammer *et al.*, *Genes Dev.* **12**, 914 (1998).
67. K. Tateishi, M. Omata, K. Tanaka, T. Chiba, *J. Cell Biol.* **155**, 571 (2001).
68. J. C. del Pozo, C. Timpte, S. Tan, J. Callis, M. Estelle, *Science* **280**, 1760 (1998).
69. T. Kurz *et al.*, *Science* **295**, 1294 (2002).
70. J. C. del Pozo *et al.*, *Plant Cell* **14**, 421 (2002).
71. M. Dharmasiri, M. Estelle, unpublished data.
72. P. K. Jackson, *Genes Dev.* **15**, 3053 (2001).
73. A. Pichler, A. Gast, J. S. Seeler, A. Dejean, F. Melchior, *Cell* **108**, 109 (2002).
74. T. Kamura, M. N. Conrad, Q. Yan, R. C. Conaway, J. W. Conaway, *Genes Dev.* **13**, 2928 (1999).
75. N. Wei, X. W. Deng, *Trends Genet.* **15**, 98 (1999).
76. D. A. Chamovitz *et al.*, *Cell* **86**, 115 (1996).
77. D. Bech-Otschir, M. Seeger, W. Dubiel, *J. Cell Sci.* **115**, 467 (2002).
78. C. Schwechheimer *et al.*, *Science* **292**, 1379 (2001).
79. S. Lyapina *et al.*, *Science* **292**, 1382 (2001).
80. Z. Peng, G. Serino, X. W. Deng, *Development* **128**, 4277 (2001).
81. X. Wang *et al.*, *Mol. Biol. Cell* **13**, 646 (2002).
82. C. Zhou *et al.*, *BMC Biochem.* **2**, 7 (2001).
83. K. E. Mundt, C. Liu, A. M. Carr, *Mol. Biol. Cell* **13**, 493 (2002).
84. J. Smalle *et al.*, *Plant Cell* **14**, 17 (2002).
85. A. L. Silverstone *et al.*, *Plant Cell* **13**, 1555 (2001).
86. A. L. Silverstone, P. Y. Mak, E. C. Martinez, T. P. Sun, *Genetics* **146**, 1087 (1997).
87. A. Dill, H. S. Jung, T. P. Sun, *Proc. Natl. Acad. Sci. U.S.A.* **98**, 14162 (2001).
88. H. Itoh, M. Ueguchi-Tanaka, Y. Sato, M. Ashikari, M. Matsuoka, *Plant Cell* **14**, 57 (2002).
89. Z.-Y. Wang *et al.*, *Dev. Cell* **2**, 505 (2002).
90. Y. Yin *et al.*, *Cell* **109**, 181 (2002).
91. J. Li, K. H. Nam, D. Vafeados, J. Chory, *Plant Physiol.* **127**, 14 (2001).
92. J.-X. He, J. J. M. Gendron, Y. Yang, J. Li, Z.-Y. Wang, *Proc. Natl. Acad. Sci. U.S.A.* **99**, 10185 (2002).

93. We thank K. Mockaitis for critical reading of the manuscript. Supported by Deutsche Forschungsgemeinschaft grant HE 3224/1-1 (H.H.), NIH grant GM43644, and U.S. Department of Energy grant DE-FG02-98-ER20313.