The Ubiquitin-Proteasome Pathway and Plant Development

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INTRODUCTION

The importance of the ubiquitin-proteasome pathway to cellular regulation in eukaryotes has become increasingly apparent during the last several years. This fact was formally acknowledged recently by the awarding of the 2004 Nobel Prize in Chemistry to Aaron Ciechanover, Avram Hershko, and Irwin Rose for the discovery of ubiquitin-mediated protein degradation. In plants, regulated protein degradation by the ubiquitin/26S proteasome contributes significantly to development by affecting a wide range of processes, including embryogenesis, hormone signaling, and senescence. In Arabidopsis thaliana more than 1400 genes (~5% of the proteome) encode components of the ubiquitin/26S proteasome (Ub/26S) pathway (Smalle and Vierstra, 2004). Approximately 90% of these genes encode subunits of the E3 ubiquitin ligases, which confer substrate specificity to the pathway. Two of the primary objectives in the field are to identify the substrates of the Ub/26S proteasome pathway and to characterize the E3 ubiquitin ligases. This review will discuss how the Ub/26S proteasome affects plant development by focusing primarily on the action of the E3 ubiquitin ligases.

THE Ub/26S PATHWAY

The general function of the ubiquitination pathway is to conjugate ubiquitin to Lys residues within substrate proteins, thus targeting them for degradation by the proteasome (Smalle and Vierstra, 2004). Although regulation of the pathway is complex, its early steps can be described fairly simply. The 76–amino acid ubiquitin protein is attached to a substrate through the action of three enzymes: the ubiquitin activating enzyme (E1), ubiquitin conjugating enzyme (E2), and ubiquitin protein ligase (E3). The E1 forms a thioester bond with the C terminus of ubiquitin (Ub) in an ATP-dependent manner and transfers the activated Ub to an E2 enzyme. The E2 either transfers ubiquitin directly to the E3 in the case of HECT (for homology to E6-AP C terminus) E3s (see next section) or binds the E3 and transfers the ubiquitin to the substrate (Figure 1). In either case, the E3 enzyme specifies the substrate. Typically, this process is then repeated several times to attach multiple ubiquitin molecules to the substrate, and polyubiquination has been shown to be necessary for degradation of the substrate by the 26S proteasome (Wilkinson, 2000; Doherty et al., 2002; Smalle and Vierstra, 2004).

The 26S proteasome is a multisubunit complex that consists of a cylindrical 20S core protease capped on each end by a 19S regulatory particle (Groll and Huber, 2003). The 19S regulatory particle can be further divided into lid and base components. The lid contains nine subunits and is responsible for recognizing ubiquitinated substrates and for removing the Ub chains. The base contains several subunits that work to unfold the substrate. As a whole, the regulatory particle serves as the gate into the interior of the core protease. The core protease consists of a stack of proteolytic α and β subunits surrounding a narrow chamber. It is here that the substrates are finally degraded into short peptides, after which the constituent amino acids can be recycled (Voges et al., 1999; Yang et al., 2004).

Subunits of the regulatory particle are encoded by gene families (Yang et al., 2004). The loss of some of these subunits can lead to stabilization of specific targets. For example, Arabidopsis plants deficient in the rpn10 gene, a base component, show defects in ABA signaling. This defect can be explained, at least in part, by stabilization of the transcription factor ABI5 (Smalle et al., 2003). Furthermore, a mutation in rpn12a, a lid component, results in the upregulation of two cytokinin-induced genes, CYCD3 and NIA1 (Smalle et al., 2002). It is unclear whether these regulatory particle subunits act in a specific manner or whether the effects on these signaling pathways are because of a general defect in proteasome activity. However, in both cases, other proteins that are degraded by the Ub/26S proteasome pathway remain unaffected.

THE FAMILY OF E3 UBIQUITIN LIGASES

The E3 ubiquitin ligases comprise a large and diverse family of proteins or protein complexes containing either a HECT domain or a RING/U-box domain (Figure 1). The RING domain E3s can be further divided into single subunit RING/U-box E3s, such as Constitutive Photomorphogenesis1 (COP1), SEVEN IN ABSENTIA IN ARABIDOPSIS THALIANA 5 (SINAT5), and Arm Repeat-Containing1 (ARC1), and multisubunit RING E3s, which include the SCF, CUL3-BTB, and APC complexes. Whereas the

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Article, publication date, and citation information can be found at www.plantcell.org/cgi/doi/10.1105/tpc.104.161220.
Arabidopsis RING domain–containing family includes several hundreds of proteins, the HECT family is relatively small.

**HECT E3s**

HECT E3s are large proteins, typically 100 400 kD. The HECT domain is a 350–amino acid motif and contains both a ubiquitin binding site and a Ub E2 binding site (Pickart, 2001). In addition to the HECT domain, these E3s have a protein–protein interaction domain, such as an SH3 domain, RING-finger, or coiled-coil domain. This part of the protein is probably involved in target recognition and/or localization. Exceptional among ubiquitin ligases, HECT E3s form a covalent bond with ubiquitin before transferring it to the substrate protein (Pickart, 2001). Although many HECT E3s have been identified in animals (up to 50 putative HECT E3s in humans, for example), only seven HECT E3s are encoded by the Arabidopsis genome (Schwarz et al., 1998; Downes et al., 2003).

The first examples of plant HECT E3s, **UPL1** (for **Ubiquitin Protein Ligase1**) and **UPL2**, were reported in 1999 (Bates and Vierstra, 1999). In Arabidopsis, the RING finger genes number >400, although it is not clear if all RING finger genes are E3 ubiquitin ligases.

**SINGLE SUBUNIT RING E3s**

The RING (for Really Interesting New Gene) proteins are characterized by the presence of a zinc binding motif, or RING finger. The ~70–amino acid RING finger binds the E2 (Seol et al., 1999; Freemont, 2000). In Arabidopsis, the RING finger genes number >400, although it is not clear if all RING finger genes are E3 ubiquitin ligases.

**COP1**

Of the single subunit RING E3 ubiquitin ligases, COP1 is the most extensively studied. Originally identified in a mutant screen for defects in photomorphogenesis, COP1 is best known as a negative regulator of the light response. Dark-grown cop1 mutant seedlings display characteristics of light-grown seedlings, including short hypocotyls, leaf development, and photosynthetic activity (Deng et al., 1991). Subsequent work has shown that COP1 represses light-regulated development by targeting activators of light response for degradation (Osterlund et al., 2000). In the light, COP1 is depleted from the nucleus so these activators are no longer degraded (von Arnim and Deng, 1994; Osterlund et al., 2000). A microarray analysis found that >20% of the genome, representing more than 28 pathways, is regulated as exhibiting a defect in trichome development (Downes et al., 2003; El Refy et al., 2003). Indeed, upl3 mutants display increased elongation of hypocotyls on GA, consistent with a hypersensitive response to GA. Other GA-dependent responses, such as germination or flowering, are not disrupted. This study provides a description of HECT E3 function in plants, but thus far, the UPL3 substrates are unknown.

Figure 1. Classes of E3 Ubiquitin Ligases in Plants.

Cullin and cullin-like proteins are green, RING proteins are red, and substrate specificity factors are purple.
by COP1 in the dark (Ma et al., 2002). This gene set includes 20% of all putative transcription factors in Arabidopsis. As such, it is likely that most light-activated gene expression is repressed directly or indirectly by COP1 activity (Ma et al., 2002).

Repression of these genes is the consequence of COP1-dependent degradation of the βZIP transcription factor HY5 (for Long Hypocotyl5), phytochrome A (phyA), and perhaps other factors (Figure 2). The influence of COP1 activity on HY5 in particular is illustrated by microarray data showing that a large number of genes repressed by COP1 are activated by HY5 (Ma et al., 2002). The hypothesis that COP1 contributes to the degradation of HY5 is supported by the finding that HY5 accumulates in the dark in cop1 mutants, thereby activating the light response pathway (Osterlund et al., 2000). The affinity of COP1 for HY5 is regulated by the phosphorylation state of HY5 (Hardtke et al., 2000). Because phyA (see below) has been shown to have kinase activity in vitro, it is possible that this represents another level of control among COP1, phyA, and HY5 (Fankhauser et al., 1999; Wang and Deng, 2003).

Interestingly, only monoubiquitination of HY5 by COP1 has been demonstrated in vitro (Sajjo et al., 2003). The physiological significance of this is unknown. Because HY5 is polyubiquitinated in vivo, it is believed that other factors must be required for COP1-dependent ubiquitination of HY5 (Sajjo et al., 2003). One such factor may be the RING-finger protein CIP8 (for COP1 Interacting Protein 8). CIP8 interacts with the Ub E2 enzyme AtUBC8, forming a module that can interact with COP1 through the RING motif present in both CIP8 and COP1 (Figure 2). This complex may target HY5 for degradation, although it has been shown that CIP8 itself is sufficient for ubiquitination of HY5 in vitro (Hardtke et al., 2002). More genetic data on the function of CIP8 may help clarify its role in this pathway.

PhyA, a photoreceptor that mediates photomorphogenesis, is also degraded in a COP1-dependent pathway, and cop1 mutants and dominant negative cop1 transgenic plants exhibit higher phyA levels. Furthermore, COP1 can polyubiquitinate phyA in vitro, and COP1 and phyA colocalize to nuclear bodies in transiently transformed onion (Allium cepa) epidermal cells (Seo et al., 2004). Therefore, the authors suggest that phyA is ubiquitinated by COP1 after exposure to far-red light when phyA migrates from the cytosol to the nucleus (Figure 2). The existence of a cytosolic pool of phyA even after exposure to light suggests that additional mechanisms are required for phyA's complete removal (Nagy and Schaffer, 2002; Seo et al., 2004) (see below).

Another substrate for COP1 is the transcriptional activator LAF1 (for Long After Far Red Light1), which is required for the phyA response (Seo et al., 2003). Many lines of evidence indicate that LAF1 is a target of COP1 (Figure 2). Tagged versions of LAF1 and COP1 colocalize to nuclear bodies, and the two proteins interact in coimmunoprecipitation experiments. Furthermore, overexpression of COP1 leads to reduced levels of LAF1, an effect that can be reversed by treatment with the proteasome inhibitor MG132. Finally, COP1 can polyubiquitinate LAF1 in vitro. The results suggest that COP1 ubiquitinates LAF1 without

**Figure 2.** COP1 and the Light Response.

(A) In the dark, the level of COP1 in the nucleus is high. After a light stimulus, the level decreases as COP1 moves into the cytoplasm. (B) COP1 interacts with many other proteins to facilitate protein degradation. The RING-finger protein CIP8 associates with the E2 enzyme Ubc8 as well as with COP1. COP10 has been shown to interact with both COP1 and the CSN. The four SPA proteins all interact with COP1. SPA1 may aid ubiquitination of targets when in the presence of lower concentrations of COP1. (C) Known light-regulated targets of COP1 include HY5, PHYA, and LAF. The degradation of each of these proteins appears to be dependent on the interaction of COP1 with different subsets of accessory proteins. Defined interactions are shown with arrows. See text for references.
the need for an accessory protein like CIP8, as in the case of HY5 (Seo et al., 2003).

In addition to its activity in the dark, COP1 may also target HY5 and LAF1 in the light through its interaction with members of the SPA family of proteins (Suppressor of phyA-105). SPA1 is a light-dependent repressor of photomorphogenesis that attenuates the light response. Several light-regulated genes are upregulated in spa1 mutants (Hoecker and Quail, 2001). One of these genes is HY5. Indeed, the upregulation of HY5 may explain much of the light hypersensitivity seen in spa1 mutants (Saijo et al., 2003). SPA1 interacts with both HY5 and COP1 in vitro and also in yeast two-hybrid assays and has an intriguing effect on the activity of COP1. In vitro experiments show that SPA1 prevents the mono-ubiquitination of HY5 by COP1 (Saijo et al., 2003). It is possible that this activity sets the stage for the polyubiquitination of HY5 by COP1/CIP8. By contrast, in vitro experiments using only the coiled-coil domain of SPA1 seem to stimulate LAF1 ubiquitination by COP1 when COP1 is present in low levels (Seo et al., 2003). After a light stimulus, COP1 migrates out of the nucleus, and it is perhaps during this migration that nuclear SPA1 binds to COP1, possibly in a manner dependent on the concentration of COP1. The binding of SPA1 and COP1 would result in the ubiquitination and degradation of LAF1 facilitating an attenuation of light response (Seo et al., 2003) (Figure 2). The different effects of SPA1 may be attributable to the substrates involved or perhaps the differences in experimental design. In either case, it is clear that SPA1 alters the action of COP1.

SPA1 is a member of a family of closely related genes. SPA2 is mostly closely related to SPA1, whereas SPA3 and SPA4 are less similar to SPA1 (58 to 68% identity across WD repeats) but exhibit 74% identity with each other (Laubinger and Hoecker, 2003). Like SPA1, SPA2, SPA3, and SPA4 may also function in a COP1-containing complex to repress photomorphogenesis (Figure 2). SPA1 appears to play the most prominent role in light-grown seedlings, whereas in dark-grown seedlings both SPA1 and SPA2 have a significant impact on COP1-dependent development. By contrast, the activity of SPA3 and SPA4 appears to be most important for light-adapted growth in adult plants (Laubinger and Hoecker, 2003; Laubinger et al., 2004). Quadruple spa1 spa2 spa3 spa4 mutants exhibit a very severe dwarf phenotype. Double or triple spa mutants are healthier, demonstrating a degree of functional redundancy between SPA proteins (Laubinger et al., 2004).

Aside from its role in the light response, COP1 has been implicated in the control of flowering. Shin and coworkers found that MYB21, a transcription factor that is involved in floral development, is present in higher amounts in cop1 mutants (Shin et al., 2002). Overexpression of MYB21 results in dwarfed plants, malformed flowers, and sterility. The MYB21 overexpression lines also display partial photomorphogenesis in the dark, although MYB21 is not usually expressed during seedling development. It is possible that COP1 is required to repress MYB21 in seedlings. A direct interaction between COP1 and MYB21 has yet to be shown (Shin et al., 2002).

Because of the pleiotropic phenotype of cop1 mutants, COP1 may function in all photoreceptor signaling pathways. Perhaps it is through COP1’s interaction with other proteins, such as members of the SPA family, that the light response is fine tuned (Hoecker and Quail, 2001). For example, in the presence of light, phyA response may be attenuated by the degradation of both phyA and HY5 via the E3 ubiquitin ligase COP1-SPA1 heterodimer. No targets of the COP1-SPA2, COP1-SPA3, or COP1-SPA4 heterodimers have been identified, but one can imagine that these heterodimers may degrade factors important for light response and development. Indeed, the term “single subunit” E3 could be misleading particularly because COP1 has been shown to reside within a large 700-kD complex in vivo (Saijo et al., 2003). Although capable of ubiquitinating substrates by itself, COP1 probably interacts with accessory proteins to achieve a broader range of specificity.

Clearly, COP1 plays a central role in plant development, but its influence is also significant in other organisms. Human COP1 (HsCOP1) is 52% identical to AtCOP1 and is required for degradation of the tumor suppressor protein p53, and depletion of HsCOP1 causes cell cycle arrest (Dorman et al., 2004). It will be interesting to learn the full extent of COP1’s influence on growth and development.

**SINAT5**

SINAT5 is another example of a single subunit RING E3 (Figure 1). It was isolated in a yeast two-hybrid screen for proteins that interacted with NAC1 (for NAM/CUC-like protein1), a transcriptional activator involved in lateral root formation in response to auxin (Xie et al., 2002). Physiological data support the SINAT5–NAC1 interaction. Overexpression of SINAT5 results in fewer lateral roots, consistent with its role in the degradation of NAC1, a lateral root activator. In addition, the typical low protein levels of NAC1 in lateral roots can be increased by treatment with the proteasome inhibitor MG132. Finally, NAC1 can be directly ubiquitinated by SINAT5 in vitro (Xie et al., 2002). The degradation of NAC1 is induced by auxin, attenuating the auxin signal. In the absence of auxin, SINAT5 is expressed at low levels in the vasculature of the root. However, upon application of auxin, both SINAT5 and NAC1 are induced and colocalize to lateral root initials. At present, it is unclear if auxin promotes NAC1 degradation by regulating the ubiquitination activity of SINAT5.

**ARC1**

The ARC1 protein contains a U-box domain rather than a RING-finger domain (Stone et al., 2003). The U-box domain is similar in structure to the RING-finger–like motif, but does not use zinc ions to stabilize the motif. Although there are 37 such proteins in Arabidopsis, ARC1 is the only U-box E3 characterized to date (for review, see Azevedo et al., 2001). ARC1 was identified from *Brassica napus* in a yeast two-hybrid screen for proteins that interact with SRK, an S-Receptor Kinase that functions as the pistil-specific determinant of self-incompatibility (for review, see Newbigin and Vierstra, 2003; Stone et al., 2003). Suppression of ARC1 led to a partial breakdown of the self-incompatibility response, meaning that self-pollen was allowed to proceed with pollination. MG132 also inhibited the self-incompatibility pathway, consistent with a role for the 26S proteasome. ARC1 interacts with SRK in a phosphorylation-dependent manner and is then phosphorylated by SRK. In addition, the presence
of active SRK results in the movement of ARC1 from the cytosol to the nucleus where it colocalizes with subunits of the 26S proteasome (Stone et al., 2003). Based on these results, it seems likely that ARC1 destroys SRK, causing rejection of self-pollen on the surface of the stigma.

The proteins originally designated as single subunit E3s target a diverse group of substrates involved in environmental and hormone response (COP1), organ morphogenesis (SINAT5), and reproduction (ARC1). However, there are some common themes emerging from the study of these proteins. Both SINAT5 and COP1 form homodimers via the coiled-coil domain. COP1 also uses this domain in interactions with other proteins, and it is possible that SINAT5 forms heterodimers with the four other members of the SINAT family in Arabidopsis (Xie et al., 2002). Will we find that ARC1 has an accessory protein as well? There is evidence in animal systems that U-box E3s interact with chaperones to target misfolded proteins for degradation (Hatakeyama and Nakayama, 2003). These single subunit E3s may be more similar to multisubunit E3s than initially imagined. Interactors, such as SPA1, may provide substrate specificity, not unlike the F-box proteins of the multisubunit E3 ubiquitin ligases.

MULTISUBUNIT RING E3s

With the exception of the ECS (for Elongin C-SOCS box-CUL2), all multisubunit E3s are present in plants: the APC (for Anaphase Promoting Complex), SCF (for Skp-Cullin-F-box), and the CUL3-based BTB (for Broad-complex, Tramtrack, Bric-a-Brac) complexes. All of these complexes contain a cullin (or cullin-like) protein and a RING-finger protein. Although we are just beginning to understand the function of CUL3-BTB complexes in plants, there have been many exciting discoveries concerning the functions of the APC and especially the SCF in recent years.

THE SCF

The SCF class of E3 ligases is the most thoroughly studied in plants (Figure 1). The name is derived from three of its four subunits: SKP1 (ASK in plants for Arabidopsis SKP1), CDC53 (or Cullin), and the F-box protein. The fourth subunit is the RING finger protein RBX1 (for Ring-Box 1). In this complex, the scaffold-like cullin binds both RBX1 and the linker protein ASK (Zheng et al., 2002b). ASK proteins in turn bind a diverse array of substrate specificity factors called F-box proteins (Pickart, 2001; Smalle and Vierstra, 2004). In Arabidopsis, there are five canonical cullins, but of these only the highly related CUL1 and CUL2 have been shown to participate in SCF complexes (see below). There are two RBX1 genes in Arabidopsis, 21 ASKs (Marrocco et al., 2003; Risseeuw et al., 2003; Cheng et al., 2004b; Takahashi et al., 2004), and >700 F-box proteins (Andrade et al., 2001; Gagne et al., 2002; Risseeuw et al., 2003). The combinatorial possibilities of ASKs and F-box proteins can result in remarkable variability and the potential to target a very large number of substrates. Known targets of the SCF include transcription factors, cell cycle regulators, and factors involved in development and signal transduction (Hershko and Ciechanover, 1998; Dharmasiri and Estelle, 2002; Devoto et al., 2003; Itoh et al., 2003; Wang, 2003; Pagano, 2004).

F-box proteins, named for the conserved 60-amino acid motif responsible for binding to ASK/SKP, represent the largest superfamily in Arabidopsis, comprising 2.7% of the Arabidopsis genome (Gagne et al., 2002). In almost all F-box proteins, the N terminus of the protein contains the F-box motif, and the remainder of the protein contains the protein–protein interaction domains required for substrate binding. There are 13 classes of the interaction domains represented by the family in Arabidopsis, including Leu-rich repeat, Kelch, WD-40, and Armadillo (Arm). The Leu-rich repeat or Kelch repeats are most widely represented; they are present in ~200 of the 700 F-boxes proteins (Gagne et al., 2002). There is evidence from yeast (Kominami et al., 1998), animals (Suzuki et al., 2000), and plants (Risseeuw et al., 2003) that some F-box proteins form heterodimers with other F-box proteins, which would lead to an even higher level of complexity.

So far, there is only limited information on the function of most of the ASK proteins. In yeast two-hybrid experiments, some Arabidopsis ASKs interact with a subset of F-box proteins, and others interact with a more diverse group of F-box proteins. For example, ASK1, 2, 11, and 19 interact with most of the F-box proteins investigated, whereas ASK5 and ASK16 interact with only a few F-box proteins (Gagne et al., 2002; Risseeuw et al., 2003). If the yeast two-hybrid data are representative of the in vivo function of these proteins, this suggests that ASK1 and ASK2 participate in the majority of SCF complexes (Risseeuw et al., 2003). Promoter–β-glucuronidase fusions, RT-PCR, and in situ hybridization show that ASK1 is the most strongly and widely expressed of all the ASKs (Marrocco et al., 2003; Zhao et al., 2003). As one might expect, the ask1 mutant has a pleiotropic phenotype with defects in vegetative and floral development (Yang et al., 1999). The mutant is impaired in both jasmonic acid (JA) and auxin response, consistent with the presence of ASK1 in two well-characterized SCFs known to be involved in JA and auxin signaling, SCFCOI1 and SCFTIR1, respectively (Gray et al., 1999; Xu et al., 2002). Because ask1 plants are viable, it is possible that the ASK genes have partially overlapping function. Single ask2 mutants have no phenotype, but ask1 ask2 double mutant plants exhibit severe defects in seedling development, suggesting that ASK1 can compensate in seedlings that lack ASK2 (Liu et al., 2004).

In Arabidopsis, the five canonical cullins are CUL1, CUL2, CUL3A, CUL3B, and CUL4. The Arabidopsis CUL1 and CUL2 genes do not appear to be orthologous to the animal CUL1 or any other known cullin in animal and yeast systems, based on phylogenetic analysis (Risseeuw et al., 2003). However, like the animal CUL1, AtCUL1 and AtCUL2 are subunits of SCF complexes (Gray et al., 1999; Risseeuw et al., 2003; J. Moon and M. Estelle, unpublished data). In plants, there is no ortholog of the animal CUL2. This may explain why there is no evidence of the E3 ligase ECS. Of the cullins studied so far, CUL1 is the most important role in general plant development. Null mutants in cul1 are embryonic lethal (Shen et al., 2002). Further characterization of CUL1 has been aided by the discovery that homozygous lethal mutant axr6 is affected in CUL1 protein function (Hobbie et al., 2000; Hellmann et al., 2003). The dominant axr6 alleles were isolated in a screen for auxin resistance, and axr6 plants produce a full-length CUL1 with a single amino acid
change within the ASK binding site. Therefore, the axr6 mutation affects the ability of CUL1 to bind ASK1 and form an active SCF complex, thus leading to lethality at the two cotyledon stage (Hellmann et al., 2003). Creation of transgenic plants that result in cosuppression of CUL1 highlights the broad role of CUL1 throughout development. In a small percentage of these lines, the apical meristem forms a pin-like structure, indicating that CUL1 is important for organ initiation (Hellmann et al., 2003).

The Arabidopsis CUL3A, CUL3B, and CUL4 proteins have been grouped with the animal CUL3 and CUL4 clades, respectively (Risseeuw et al., 2003). Animal studies indicate that CUL3 interacts with members of a family of proteins called BTB proteins (Furukawa et al., 2003). The CUL3/BTB complex is similar to the SCF in that a cullin serves as a scaffold. However, the SKP and F-box protein subunits have been replaced by a single BTB protein (Figure 1). Therefore, the adapter protein interacts directly with the cullin subunit (Pintard et al., 2004). Although many BTB proteins have been identified in animals, the only known target of this complex is the heterodimer MEI1/MEI2 (Kurz et al., 2002), which is important for mitotic spindle function (for review, see Pintard et al., 2004). In plants, the BTB protein ETO1 plays a role in the control of ethylene production (Figure 3D). This is achieved by facilitating the degradation of the ACC synthase protein ACS5, an enzyme that controls the rate-limiting step in ethylene formation (Wang et al., 2004). In vitro assays have determined that the N-terminal portion of ETO1 containing the BTB domain binds CUL3, whereas the C-terminal part of the protein is required for interaction with ACS5 (Wang et al., 2004). The authors predict that ETO1 acts as an adapter to bring ACS5 into proximity with the ubiquitination apparatus. Further analysis of BTB proteins in Arabidopsis will determine whether each has a specific function and whether all BTB proteins are members of CUL3-containing E3 ligases.

THE ROLE OF SCFS IN PLANT DEVELOPMENT

The participation of SCFs in plant development is extensive, affecting processes such as hormone response, photomorphogenesis, circadian rhythms, floral development, and senescence. At present, information exists on the functions of a relatively small number of F-box proteins. Most of these are involved in regulation of hormone signaling pathways. For some responses, the role of the SCF is to degrade repressors of hormone response (auxin, GA, and JA), whereas in response to ethylene, the SCF degrades positive regulators in the absence of the hormone. The following sections will discuss some of the recent progress in determining the role of the SCF in these signaling pathways.

Figure 3. Control of Signaling Pathways by Protein Degradation.

(A) Light prevents degradation of HY5 by COP1, resulting in gene expression of certain AUX/IAA proteins.
(B) Auxin promotes degradation of AUX/IAA proteins by SCFTIR1, thus releasing the inhibition of ARF-mediated gene expression.
(C) Auxin and GA promote degradation of RGA by SCFSLY.
(D) BTBETO controls ethylene production by facilitating degradation of ACS5. Ethylene prevents degradation of EIN3 by SCFEBF1/2, subsequently allowing gene expression. Rectangles denote E3 complexes, and circles denote their substrates.
SCFTIR1 and Auxin

SCFTIR1 was the first SCF complex identified in plants. Loss-of-function mutations in SCF components (ask1, tir1, and rbx1) confer resistance to auxin, suggesting that targets of SCFTIR1 are negative regulators of auxin response (Gray et al., 1999, 2001; Hellmann et al., 2003). This hypothesis was confirmed when members of the AUX/IAA family of proteins, short-lived transcriptional repressors of auxin response, were shown to be substrates of SCFTIR1 (Figure 3B) (Gray et al., 2001). A direct auxin-dependent interaction between the F-box protein TIR1 and several AUX/IAA proteins has been demonstrated, and two AUX/IAA proteins (IAA7 and IAA17) are stabilized in the tir1 mutant (Gray et al., 2001; Dharmasiri et al., 2003a; Tian et al., 2003).

AUX/IAA proteins contain four conserved regions called domains I through IV (Abel et al., 1995). Domains III and IV are necessary for dimerization with other AUX/IAA proteins and with members of another family of transcriptional regulators called AUXIN RESPONSE FACTORS (ARFs) (Guilfoyle et al., 1998). Earlier studies showed that AUX/IAA proteins repress ARF function in a way that requires dimerization between the two proteins. More recently, it has been shown that domain I of the AUX/IAA proteins contains a Leu-rich region that can act as a general transcriptional repressor (Tiwari et al., 2003, 2004). When transfected into carrot (Daucus carota) cell suspension cultures, domain I alone will repress transcription mediated by either the VP16 or ARF transcriptional activators. These studies suggest that after formation of an AUX/IAA-ARF dimer, domain I of the AUX/IAA actively represses ARF action by an as yet unknown mechanism. In response to auxin, the AUX/IAA proteins are degraded, allowing ARFs to function.

It is unclear at present how auxin regulates the interaction between the SCF and its substrates. In animal and fungal systems, SCF-substrate recognition typically requires phosphorylation of the substrate (Pickart, 2001). By contrast, several studies indicate that the SCFTIR1–AUX/IAA interaction is not regulated by phosphorylation (Ramos et al., 2001; Dharmasiri et al., 2003a; Kepinski and Leyser, 2004). In addition, auxin promotes the interaction in plant extracts that have been cleared of membranes (Dharmasiri et al., 2003a; Kepinski and Leyser, 2004), indicating that the auxin receptor and other signaling proteins required for this response are soluble. Most recently, Kepinski and Leyser (2004) present data suggesting that auxin acts on TIR1 or a closely associated protein to promote substrate recognition.

Some aspects of the auxin response appear to be controlled indirectly by COP1 (Figure 3A). The promoters of the AUX/IAA genes IAA7 and IAA14 contain domains that are bound by the transcription factor HY5 (Cluis et al., 2004). Therefore, the auxin response can be negatively regulated in the light by the expression of AUX/IAA proteins. This finding may explain the auxin and cytokinin-resistant phenotype of hy5 roots as well as the defect in lateral root formation (Cluis et al., 2004).

SCF E3s and GA

An SCF complex was recently shown to play a key role in GA signaling. GA response is negatively regulated by members of a family of nuclear proteins called the DELLA proteins (Peng and Harberd, 1997; Silverstone et al., 1998; Dill et al., 2001). This family includes GAI and RGA plus three additional members. The gai (for gibberellic acid-insensitive) mutant was isolated as a dominant GA-insensitive mutant, whereas the rga (for repressor of ga1-3) mutants were identified in a suppressor screen using the GA-deficient mutant ga1-3 (Silverstone et al., 1997). GA promotes the degradation of both GAI and RGA, leading to the occurrence of various GA-dependent processes (Cheng et al., 2004a; Fu et al., 2004; Tyler et al., 2004; Yu et al., 2004). Recent studies indicate that GAI and RGA degradation occurs via the E3 SCFSLY (McGinnis et al., 2003; Dill et al., 2004; Fu et al., 2004) (Figure 3C). Mutations in the F-box gene SLEEPY1 (SLY1) result in stabilization of GAI and GAI even the presence of GA (Silverstone et al., 2001). Furthermore, loss-of-function rga and gai mutants partially suppress the sly1-10 phenotype (McGinnis et al., 2003; Dill et al., 2004). These results suggest that SCFSLY targets the DELLA proteins for degradation, which alleviates DELLA-mediated inhibition of GA-regulated growth (McGinnis et al., 2003). This model is supported by yeast two-hybrid assays and pull-downs, demonstrating that SLY1 directly binds RGA and GAI (Dill et al., 2004; Fu et al., 2004).

In barley (Hordeum vulgare), the likely RGA ortholog is called SLN (for Slender). Like RGA, SLN is rapidly degraded in response to GA via the 26S proteasome pathway (Fu et al., 2002). The RGA ortholog in rice (Oryza sativa) is called SLR1 (for Slender Rice1) (Ikeda et al., 2001; Itoh et al., 2002). Characterization of a GA-insensitive dwarf mutant in rice led to the identification of the F-box gene, GID2 (for GA-insensitive dwarf 2), which is a putative ortholog of Arabidopsis SLY (Sasaki et al., 2003). To determine if SLR1 is a target for SCFSLY, the authors examined SLR1 levels and found it to be more abundant in gid2 mutants than in the wild type. Recently, GA-induced phosphorylation of SLR1 was shown to increase the affinity between SLR1 and SCFGID2 (Sasaki et al., 2003; Gomi et al., 2004). In Arabidopsis, this finding is supported by experiments showing that SCFSLY interacts more strongly with the phosphorylated DELLA proteins (Hsiung et al., 2001; Fu et al., 2004). Although phosphorylation of substrates is typically required for SCF recognition in animals, this would be the first example of such a mechanism in plants (Pickart, 2001).

One of the most interesting recent developments in the GA field is the discovery that the DELLA proteins are a point of intersection for several hormone-signaling pathways. Auxin promotes GA-dependent degradation of the DELLA proteins in the root, whereas ethylene inhibits DELLA protein degradation (Achard et al., 2003; Fu and Harberd, 2003). Furthermore, axr1 plants (see below) also have a defect in GA-mediated degradation. However, in this case it is not clear if this effect is related to auxin signaling or to the likely role of AXR1 in SCFSLY function. Regardless, these results indicate that we are beginning to understand the molecular basis for the diverse hormone interactions that occur during plant growth and development.

SCFCOI1 and JA

JA signaling is also mediated by an SCF. The F-box gene COI1 (for Coronatine Insensitive) was identified in a mutant screen for root elongation on medium containing coronatine (Benedetti
et al., 1998; Xie et al., 1998). Coronatine is a toxin normally produced by *Pseudomonas syringae* that is similar to methyl jasmonate in structure. The *coi1* mutants are male sterile and resistant to JA. Yeast two-hybrid and comammunoprecipitation experiments showed that COI1 is part of an SCF complex that includes ASK1 or ASK2 and CUL1 (Xu et al., 2002). In another study, a yeast two-hybrid screen with COI1 as bait resulted in the recovery of a histone deacetylase called RPD3b. COI1 and RPD3b comammunoprecipitate from plant extracts, suggesting that the histone deacetylase may be a COI1 substrate (Devoto et al., 2002). However, this has not been confirmed.

**SCFEBF1/EBF2 and Ethylene**

The ethylene signaling pathway is known to include a family of endoplasmic reticulum–localized receptors (ETR1, ETR2, and EIN4), the Raf-like kinase CTR1 (for Constitutive Triple Response1), and the enigmatic EIN2 (for Ethylene Insensitive2) protein (Guo and Ecker, 2004). At the genomic level, this pathway acts to promote transcription of ethylene-regulated genes through the action of the transcription factor EIN3 (Guo and Ecker, 2003, 2004). However, until recently it was not known how the pathway affected EIN3. Now several groups have shown that ethylene stabilizes EIN3 and that in the absence of ethylene, the protein is degraded by the proteasome. The E3 responsible for the degradation is SCFEBF1/2 (Guo and Ecker, 2003; Potuschak et al., 2003; Gagne et al., 2004) (Figure 3D). In the double mutant *ebf1 ebf2*, EIN3 is stabilized, resulting in a constitutive ethylene response. Furthermore, these plants exhibit a constitutive triple response (Guo and Ecker, 2003). In summary, there is strong evidence that SCFEBF1/2 degrades the transcriptional activator EIN3 in the absence of ethylene. Because a MAP kinase cascade has been implicated in ethylene signaling (Novikova et al., 2000; Ouaked et al., 2003), it will be interesting to see if phosphorylation of EIN3 is required for its stabilization.

**SCFs and the Light Response**

The role of SCF E3 ligases in light response is supported by the discovery of EID1 (for Empfindlicher Im Dunkelroten Licht) and AFR (for Attenuated Far-Red Response), both of which are F-box proteins involved in phyA-mediated light signaling (Buche et al., 2000; Dieterle et al., 2001; Harmon and Kay, 2003). Knockdown *afr* lines show impaired phyA response, and expression of *AFR* seems to be regulated by the circadian clock, so it is possible that SCFAFR degrades a repressor of light response in preparation for light signals at dawn (Harmon and Kay, 2003). *eid1* mutant seedlings exhibit increased sensitivity to far-red light in a phyA-specific manner but show no changes in the level of phyA protein (Buche et al., 2000). Therefore, the function of SCFEID1 is most likely to degrade proteins that positively regulate the phyA pathway (Dieterle et al., 2001). Efforts are now underway to determine the substrates for both SCFEID1 and SCFAPR. *eid1 spa1* double mutants are extremely sensitive to far-red light, which suggests that COP1 and SCFEID1 play distinct roles in the control of light signaling (Zhou et al., 2002).

The circadian clock is also under the influence of regulated protein degradation. The *ZEITLUPE* (*ZTL*) gene was identified in a screen for period length mutants and shown to encode an F-box protein (Mas et al., 2003). Intriguingly, ZTL contains a LOV domain, previously implicated in photoreceptor function. Thus, it is possible that ZTL functions as a light sensor, perhaps mediating photoperiod regulation of the circadian clock and other light-regulated processes. One role of ZTL has recently become clear with the demonstration that TOC1 (for Timing of CAB Expression1), a key regulator of circadian rhythm, is a substrate of SCFZTL (Schultz et al., 2001). In a different study, a detailed genetic analysis indicates a role for ZTL in photomorphogenesis and flowering (Somers et al., 2004). Based on their analysis, this group proposes that degradation of TOC1 is not sufficient to explain the *ztl* phenotype, suggesting that there must be additional SCFZTL substrates. The functions of two close relatives of ZTL have also been characterized. Overexpression of the LKP2 (for LOV kelch protein 2) results in an arrhythmic phenotype, suggesting that this protein has a similar function to that of ZTL (Schultz et al., 2001). Indeed, recent results indicate that LKP2 also interacts with TOC1 (Yasuura et al., 2004). By contrast, another close relative of ZTL called FKF (for FLAVIN binding, KELCH REPEAT, F-BOX) does not appear to regulate circadian rhythm. However, *FKF* is itself clock regulated and required for the floral transition at least in part through regulation of the flowering gene *CONSTANS* (Nelson et al., 2000; Imaizumi et al., 2003).

**SCFs and the Self-Incompatibility Response**

As detailed above, the U-box E3 ARC1 is involved in the sporophytic self-incompatibility system found in *B. napus*. Recent work suggests that SCFs are also involved in the gametophytic incompatibility system. S-locus–linked F-box genes have been identified in almond (*Prunus dulcis*) (Ushijima et al., 2003), Japanese apricot (*Prunus mume*) (Entani et al., 2003), cherry (*Prunus avium*) (Yamane et al., 2003), *Antirrhinum majus* (Qiao et al., 2004), and petunia (*Petunia hybrida*) (Sijacic et al., 2004). These genes are expressed in the pollen, suggesting that they are pollen self-incompatibility determinants. In petunia and *Antirrhinum*, this has been confirmed by transgenic studies (Qiao et al., 2004; Sijacic et al., 2004). Furthermore, studies indicate that the substrate of SCFANSLF-S2 is the S-RNase that is the style determinant in this system (Qiao et al., 2004). These results suggest that the S-RNase is degraded through the action of SCFANSLF-S2 during a compatible interaction. It will be interesting to see if this model holds for the other plant species mentioned above.

**SCFUFO and Floral Development**

The Arabidopsis F-box gene *UFO* (for *UNUSUAL FLORAL ORGANS*) and its likely *Antirrhinum* ortholog *FIM* (for *FIMBRIATA*) were the first F-box genes to be identified in plants (Levin and Meyerowitz, 1995; Ingram et al., 1997; Samach et al., 1999). Mutations in either gene result in a variety of floral defects, suggesting a complex role for SCFUFO. In both species, genetic studies have shown that UFO/FIM is a positive regulator of B function gene expression and that this activity requires the LFY protein (Lee et al., 1997; Laufs et al., 2003; Ni et al., 2004). In
addition, UFO appears to act antagonistically to the C function
gene AGAMOUS in the second whorl, allowing petals to develop
(Durfee et al., 2003; Ni et al., 2004). Substrate(s) of SCFUFO have
not been identified, but it is possible that different substrates are
involved in these distinct aspects of UFO action.

SCFORE9/MAX2

As the name(s) suggests the ORE9/MAX2 gene was identified in
two different genetic screens. The ore9 mutant (for ORESARA,
which means “long living” in Korean) displays delayed senes-
cence, and the max2 (for more auxiliary growth2) mutant was
identified in a screen for plants with increased lateral branching
(Woo et al., 2001; Stirnberg et al., 2002). Control of lateral branch
growth is thought to be mediated by an interaction between auxin
and an as yet unidentified compound (Sorefan et al., 2003).
ORE9/MAX2 acts solely in the shoot, so the current working
hypothesis is that the SCF promotes the degradation of a protein
that stimulates lateral branching (Ward and Leyser, 2004). In the
case of senescence, ORE9/MAX2 presumably degrades a pro-
tein that inhibits leaf senescence (Woo et al., 2001). Whether this
is the same substrate as that which promotes lateral branching is
unknown.

REGULATION OF SCF FUNCTION

Given the importance of SCFs to cellular regulation, it is not
surprising that SCF assembly and function are highly regulated.
So far, three proteins or protein complexes have been implicated
in SCF regulation. These are the ubiquitin related protein RUB (for
Related to Ubiquitin), the COP9 signalosome (CSN), and a re-
cently discovered protein called Cullin Associated Neddylation
Dissociated1 (CAND1).

The RUB Conjugation Pathway

The RUB protein (also called Nedd8 in animals) is conjugated to
the cullin subunit of the SCF through the action of RUB-specific
E1 and E2 enzymes (Parry and Estelle, 2004). In Arabidopsis, the
AXR1/ECR1 (for Auxin Resistant1, E1 C-terminal1) dimer func-
tions as the E1, whereas the E2 is called RCE1 (for RUB
conjugating enzyme1) (del Pozo et al., 2002; Dharmasiri et al.,
2003b). Recent evidence suggests that RBX1, a component of
the SCF ubiquitin E3, is also the E2 signalosome (CSN), and a
recently discovered protein called Cullin Associated Neddylation
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2003b). Recent evidence suggests that RBX1, a component of
the SCF ubiquitin E3, is also the E2 for RUB conjugation to the
cullins (Kamura et al., 1999; Gray et al., 2002; Dharmasiri et al.,
2003b). The highly conserved site of RUB conjugation is present
in all cullins, the only known targets of RUB modification.

The RUB conjugation pathway was first discovered in plants
by screening for mutants resistant to auxin. The screen identified
the axr1 mutants (Lincoln et al., 1990; Leyser et al., 1993). Loss of
the AXR1 protein results in a variety of auxin-related defects, all
of which can be explained by a reduction in the level of RUB-
modified CUL1. This in turn affects the function of SCFTIR1,
resulting in stabilization of the AUX/IAA proteins and reduction in
auxin-induced gene expression. The significance of the RUB1
modification has also been confirmed by studies of the RUB E2
enzyme, RCE1 (Dharmasiri et al., 2003b). Mutant rce1 plants are
also affected in SCFTIR1 function and exhibit a pleiotropic
phenotype similar to that of axr1 plants. Arabidopsis axr1 rce1
double mutants die as young seedlings and are unable to
develop more than a single cotyledon (Dharmasiri et al., 2003b).

Given what we now know about the involvement of the SCF
in many developmental processes, it is likely that a mutation
in the RUB conjugation pathway would lead to a pleiotropic
phenotype. This is the case as axr1 plants are resistant to
ethylene (Timpte et al., 1995), JA, abscissic acid (Tiryaki and
Staswick, 2002), and brassinosteroid (Nakamura et al., 2003) in
addition to auxin. However, it is worth noting that these defects
have not been directly related to stabilization of a known SCF
substrate. In fact, the situation is likely to be complex, as
illustrated by a consideration of the ethylene response.
Because EIN3 is an SCF substrate, it may be stabilized in an axr1
background. However, EIN3 is a positive regulator of ethylene
signaling, and the axr1 mutants are resistant. The relative
importance of RUB conjugation to the function of various
SCFs is unknown.

Despite the importance of the RUB pathway for SCF function,
the precise biochemical function of the modification remains
uncertain. One possible role is to promote and stabilize the
interaction between the SCF and the ubiquitin E2, increasing
the efficiency of the ubiquitination process (Wu et al., 2002).
Alternatively, emerging evidence suggests that RUB may play an
important role in the cycle of SCF assembly/dissassembly (see
below).

The CSN

Originally identified as a repressor of photomorphogenesis, the
CSN has since been shown to have much broader role in plant
development (Serino and Deng, 2003). Consisting of eight
subunits, the CSN shows intriguing structural and sequence
homology to the 19S Lid of the proteasome. Because of this, it is
believed that the CSN and 26S proteasome lid evolved from
a common ancestor (Serino and Deng, 2003). A mutation in
a single CSN subunit can destabilize the entire complex
(Schwechheimer et al., 2002). csn mutants are highly pleiotropic,
and strong csn alleles are lethal. Biochemical studies indicate
that the CSN interacts with a variety of E3s, including COP1,
SCFGO1, SCFTIR1, and SCFTIR2 (Schwechheimer et al., 2001; Feng
et al., 2003; Wang, 2003).

So far the best-characterized biochemical function of the CSN
is removal of RUB from CUL1. RUB deconjugation is accom-
plished by the Csn5 subunit (also called JAB1 or AJH), a metal-
lloprotease (Cope et al., 2002). A weak csn5 antisense line has
increased levels of RUB-modified CUL1. This change is associ-
ated with a phenotype that is very similar to axr1, including curled
leaves, decreased apical dominance, and stabilization of AUX/
IAA proteins (Schwechheimer et al., 2002). The fact that both
increased and decreased levels of RUB modification have
a similar affect on SCF function implies that a cycle of RUB
conjugation and deconjugation is important for activity of the
complex. This view is also supported by studies in which the
putative RUB E3, RBX1, is overexpressed in transgenic plants.
Like the csn5 lines, these lines exhibit increased levels of RUB-
CUL1 and increased stability of AUX/IAA protein, suggesting a
defect in SCFTIR1 function (Gray et al., 2001; Lechner et al.,
2002).
In addition to its interaction with SCFs, the CSN is required for localizing COP1 to the nucleus (Cope and Deshaies, 2003). As such, the CSN can interact with at least three types of E3 ligases: SCFs, CUL3/BTB1, and COP1 (Serino and Deng, 2003). Because the CSN seems to be a point of convergence for several protein degradation pathways, it is possible that the CSN brings together several components for processing by derubylatation, deubiquitination, substrate binding, and other modifications. Recent in vivo evidence suggests that there exists a very large and dynamic supercomplex consisting of the CSN, the 26S proteasome, and the SCF. This “one-stop shop” means ubiquitination and degradation of targets may be important for very efficient processing of unstable proteins (Serino and Deng, 2003).

**CAND1 and the SCF Cycle**

The paradoxical effects of changes in the level of RUB-CUL1 may be partially explained by the discovery of CAND1. This protein was first discovered in animals (also called TIP120A; Oshikawa et al., 2003) and shown to selectively bind unmodified CUL1 (Liu et al., 2002; Zheng et al., 2002a). CAND1 binding inhibits CUL1/RBX1 binding to SKP1, thus preventing the formation of active SCF. Reducing the amount of CAND1 in cells leads to an increase in the number of complexes containing CUL1 and SKP1. However, RUB modification of CUL1 dissociates CAND1 from the CUL1/RBX1 complex. Based on these results, it has been proposed that CAND1, the RUB/Nedd8 conjugation pathway, and the CSN together regulate a cycle of SCF assembly and disassembly (Cope and Deshaies, 2003; Pintard et al., 2003; Parry and Estelle, 2004). This process would presumably permit rapid changes in the cells repertoire of SCFs as the requirements of the cell change.

In Arabidopsis, the cand1 mutants were recovered in a screen for sirtinol resistance and as an enhancer of tir1 (Cheng et al., 2004b; Chuang et al., 2004). In addition, the gene was identified based on sequence similarity in the Arabidopsis genome (Feng et al., 2004). cand1 plants (also called eta2 for enhancer of tir1 auxin resistance) (Chuang et al., 2004) have a pleiotropic phenotype with altered responses to several plant hormones, including auxin and GA. The defects in auxin and GA signaling are the result of increased stability of AUX/IAA proteins and RGA, respectively (Cheng et al., 2004b; Feng et al., 2004). As in animal systems, AtCAND1 preferentially binds unmodified CUL1 in vivo (Feng et al., 2004), and cand1 mutants disrupt the formation of SCF complexes, such as SCFTRI1 (Chuang et al., 2004), indicating that many of the eta2 phenotypes can be attributed to a reduction in SCF activity.

At present, the role of RUB, CAND1, and the CSN in the function of other cullin-based E3s in plants is largely unexplored. However, the fact that all cullins are modified by RUB/Nedd8 suggests that the CSN and CAND1 are also important for the activity of these other E3s (Parry and Estelle, 2004).

**The APC**

The anaphase-promoting complex/cyclosome (APC/C) is a highly conserved complex consisting of eleven subunits (Gieffers et al., 2001; Capron et al., 2003a) (Figure 1). Two of the 11 components have homology to subunits in the SCF: APC2 (related to cullin) and APC11 (a RING protein) (Tang et al., 2001). In plants, the APC2, APC/NOMEGA, and APC3/HOBBIT (HBT) genes have been recently characterized. Both apc2 and apc6/nomega mutant plants exhibit defects in gametogenesis (Capron et al., 2003b; Kwee and Sundaresan, 2003). The hbt mutants have severe defects in meristem organization, indicating that HBT is important for cell division and differentiation (Biliou et al., 2002). The existence of another APC3/CDC27 homolog in Arabidopsis may explain why HBT, unlike APC2 and NOMEGA, does not appear to play a central role in gametogenesis (Capron et al., 2003b).

In plants, most of the known targets of the APC are cyclins. Like animal cyclins, a conserved D-box motif, or destruction box, has been identified in many plant cyclins (Renaudin et al., 1996; Fang et al., 1998; Hames et al., 2001). However, HBT, and by extension, the APC, also appears to function in an auxin response pathway that may regulate cell division and differentiation early in development (Biliou et al., 2002). The auxin-responsive promoter construct DRS5:β-glucuronidase is not induced in the root meristem of hbt mutants exposed to auxin (Biliou et al., 2002). Furthermore, AXR3/IAA17, one of the AUX/IAA proteins, is stabilized in hbt mutants. Whether this effect is direct or indirect is still unclear.

**Emerging Complexes**

A recent study in human cells demonstrated the existence of a novel E3 consisting of components of a single subunit E3 and a multisubunit E3. This E3 consists of HsCOP1, HsDET1, a DNA damage binding protein (DDB1), CUL4A, and RBX1. The complex is thought to degrade c-jun, a proto-oncogene transcription factor that is involved in countless cellular processes (Wertz et al., 2004). The presumed Arabidopsis DDB1 homolog is known to interact with AtDET1, suggesting that DDB1 and DET1 may also link COP1 to a cullin-containing multisubunit E3 in plants. However, so far the story seems to be somewhat different. In a recent study, DET1 and DDB1 were shown to form a complex with COP10 called the CDD (for COP10 DDB1 DET1) (Yanagawa et al., 2004). Both the det1 and the cop10 mutants have a phenotype similar to cop1 (Pepper et al., 1994; Suzuki et al., 2002). COP10 is related to ubiquitin E2s (Suzuki et al., 2002) but lacks a key Cys residue necessary for E2 function. In the latest work, COP10 was shown to enhance the activity of several E2s in vitro either by itself or as part of the CDD complex. The complex interacts with both COP1 and the CSN. Based on these results and the phenotypes of det1 and cop10, these workers propose that the CDD complex promotes the degradation of positive regulators of photomorphogenesis like HY5 (Yanagawa et al., 2004). At this point, it is not clear if Arabidopsis CUL4 is also present in a complex containing DET1 and COP1.

**SUMMARY**

During the last several years there has been a remarkable explosion of information on the proteins of the ubiquitin/proteasome system and their roles in cellular regulation. Biochemical studies have shown that these proteins function together in several
complexes and supercomplexes to regulate the degradation of diverse proteins. The complexity and dynamic nature of these complexes is just beginning to be evident, and one of the major challenges of the immediate future is to understand how their assembly and function is regulated. In the specific area of plant development, a growing number of E3s have been implicated in a variety of developmental processes. Given the very large number of E3s encoded by the Arabidopsis genome, it is possible that soon every plant developmental biologist will have their favorite F-box or RING protein to study. The discovery of the ubiquitin/proteasome pathway occurred relatively recently. Nonetheless, it is clear that rapid and irreversible protein inactivation by degradation is a common and, therefore, highly successful way to regulate cellular processes.

ACKNOWLEDGMENTS

The authors would like to thank Esther Lechner for critical reading of the manuscript. Research in the authors’ laboratory is supported by grants from the National Institutes of Health (GM43644-17), the National Science Foundation (DBI-0115870), and the Department of Energy (DE-FG02-02ER15312).

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*Plant Cell* 2004;16;3181-3195
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