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REVIEW ARTICLE

Roles of Various Cullin-RING E3 Ligases Involved in Hormonal and Stress Responses in Plants

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Abstract Post-translational modification plays an important role in the regulation of protein stability, enzyme activity, and the cellular localization of proteins. Ubiquitination is a representative post-translational modification in eukaryotes that is mainly responsible for protein degradation. There have been a number of reports on the role of ubiquitination in various cellular responses in plants, such as regulation of the cell division cycle, stress responses and hormonal signaling. Among the three types of ubiquitination-related enzymes, E3 ubiquitin ligase is critical in determining substrate specificity. The importance of cullin-RING E3 ligase (CRL), a type of E3 ligase, has been emphasized during the recent decade due to its large number and its involvement in various plant cellular processes. Here, we describe how CRL E3 ligase complexes are involved in cellular events mediated by plant hormones and during plant stress adaptation while focusing on their substrate receptors.

Keywords Cullin-RING E3 ligase, Plant hormones, Stress adaptation, Ubiquitination

Introduction

Ubiquitin (Ub) is a small eukaryotic regulatory protein that functions in maintaining cellular homeostasis and enables the effective adaptation to environmental changes. Ubiquitination, the attachment of ubiquitins to a target protein, plays a variety of important roles in protein stability, cellular localization and gene regulation (Smalle and Vierstra 2004).

Ubiquitin contains 76 amino acids, including seven lysine residues (K6, K11, K27, K29, K33, K48 and K63). Even though all lysines are involved in ubiquitin chain formation, five lysines (K6, K11, K29, K48 and K63) appear to be the primary target sites, with ubiquitin-Lys48 (K48) and -Lys63 (K63) being mainly responsible for the polyubiquitination process. While ubiquitin chains from K48 ubiquitination primarily trigger the entry of target ubiquitinated substrates into the 26S proteasomes for degradation via what is known as the ubiquitin-proteasome system (UPS), the chains from K63 ubiquitination are involved in cellular processes such as DNA repair, endosomal sorting, and autophagy of misfolded proteins, rather than proteasomal degradation (Thrower et al. 2000; Olzman and Chin 2008; Piper and Lehner 2011). Based on the presence of ubiquitin chains, polyubiquitination is distinguished from monoubiquitination which is involved in modulating endocytosis of membrane proteins, DNA repair and histone activity (Haglund et al. 2003; Huang and D'Andrea 2006; Mukhopadhyay and Riezman 2007; Salmena and Pandolfi 2007) (Fig. 1).

The ubiquitination process requires a three-step enzymatic cascade: E1 (ubiquitin-activating enzyme), E2 (ubiquitin-conjugating enzyme), and E3 (ubiquitin ligase) (Hershko and Ciechanover 1998). Initially, E1 forms a thioester bond with ubiquitin for its activation. Thereafter, the activated ubiquitin is transferred to E2, and is finally transferred to the target protein by E3. Based on the large number of E3 ligases present in plants, substrate specificity for ubiquitination is believed to be determined by the E3 genes. Specifically, the rice genome encodes six E1 genes, 49 E2 and E2-like genes, and over 1300 E3 genes (Craig et al. 2009). Similarly, the *Arabidopsis* genome is predicted to encode 2 E1 isoforms (AtUBA1 and AtUBA2), around 37 E2 enzymes, and 1,415 putative E3 ligases (Smalle and Vierstra 2004; Kraft et al. 2005).

In eukaryotes, E3 ligases are classified into two major types based on their subunit components and action modes, namely, single-subunit types such as the HECT (homologous

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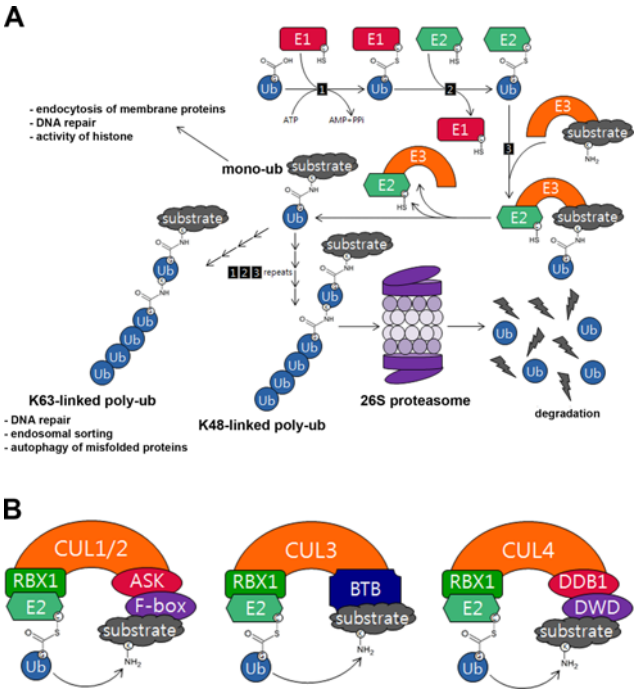

Fig. 1. Ubiquitination process and the structure of plant cullin-RING E3 ligases.
A. Overview of ubiquitination. Ubiquitination commonly requires a series of enzymes such as E1 ubiquitin-activating enzyme, E2 ubiquitin-conjugating enzyme and E3 ubiquitin ligase. While K48-linked polyubiquitination is primarily responsible for protein degradation, K63-linked polyubiquitination and monoubiquitination is involved in a wide variety of cellular responses, including DNA repair, protein sorting and endocytosis, rather than protein destruction. B. A type of plant CRL complexes. CRL1/2 and CRL4 possess ASK1 and DDB1 as an adaptor, and F-box and DWD (DCAF) as a substrate receptor, while CRL3 only uses BTB protein as a substrate receptor without an adaptor. All CRL complexes commonly utilize RBX1, a RING protein for recruiting E2.

to E6-Associated carboxyl terminus) domain and RING (really interesting new gene)/Ubox domain containing E3 ligases, and multi-subunit types such as SCF (Skp-Cullin-F-box), CUL3-BTB (Broad-complex, Tramtrack, Bric-a-Brac) domain and CUL4-DDB1 (DNA damage binding 1)- DWD (DDB1-BINDING WD40 Protein)/DCAF (DDB1 and CUL4-ASSOCIATED Factor) domain containing E3 ligases (Vierstra 2009).

HECT is the smallest domain within the E3 subfamily, consisting of 350 amino acids. Seven members of the HECT-domain containing proteins exist in the *Arabidopsis* genome (Downes et al. 2003). HECT forms a thioester bond with ubiquitin before the final transfer of ubiquitin to the target protein. (Deshaies and Joazeiro 2009). In *Arabidopsis*, the largest gene family of single-subunit E3 ligases is characterized by the presence of RING finger proteins, in which eight conserved metal-binding cysteins and histidine residues coordinate two zinc atoms (Barlow et al. 1994). Rice and *Arabidopsis* are known to harbor at least 425 and 477 RING domain-containing proteins, respectively (Viestra 2009). U-box type proteins are encoded by 64 predicted genes in the *Arabidopsis* genome, and by 77 genes in the rice genome (Zeng et al. 2008; Yee and Goring 2009). Unlike HECT E3 ligases, RING and U-box ligases mediate the transfer of ubiquitin directly from the E2-Ub to the target protein (Deshaies and Joazeiro 2009).

Cullin-RING E3 ligases (CRL) are a major type of multi-subunit E3 ligases. In *Arabidopsis*, five cullin proteins (CUL1, CUL2, CUL3a, CUL3b and CUL4) have been shown to be central components of CRLs. Among these, CUL1 and CUL2 are subunits of SCF complexes (Zheng et al. 2002). Both CUL3a and CUL3b interact directly with substrate receptor proteins that contain BTB/POZ (Pox virus and Zinc finger) domains, forming CUL3-BTB ubiquitin ligases (Thomann et al. 2005). CRL4 utilizes DDB1 as an adaptor and DWD/DCAF as substrate receptors to assemble E3 ligase complexes (Viestra 2009).

The ubiquitin-proteasome system (UPS) triggered by ubiquitination can be directed by a series of Ub-like/Ub-associated (UBL/UBA) proteins (Finley 2009). RADIATION SENSITIVE23 (RAD23), DOMINANT SUPPRESSOR OF KAR2 (DSK2) and DNA DAMAGE-INDUCIBLE1 (DDI1) are known members of UBL/UBA proteins in yeast (Lambertson et al. 1999; Funakoshi et al. 2002; Gabriely et al. 2008), and their orthologue proteins in plants have been reported by Farmer et al. (2010). These proteins commonly possess an N-terminal UBL domain, are structurally similar to ubiquitin, and contain one or more UBA domains responsible for binding ubiquitins from ubiquitylated proteins (Finley 2009; Farmer et al. 2010). Their function is mainly shuttling ubiquitylated proteins to the 26S proteasome. Core components of the 19S regulatory particle (RP) of the 26S proteasome complex, such as RPN10 and RPN13, are involved in recognizing ubiquitylated protein-UBL/UBA proteins as the ubiquitin receptor and tethering them to the protease complex (Deveraux et al. 1994; Husnjak et al. 2008).

Sumoylation, a type of ubiquitin-like post-translational modification, also acts as a crucial modification in eukaryotes. This process is performed by the conjugation of small ubiquitin-related modifier (SUMO), a protein that is structurally related to ubiquitin (Miura and Hasegawa 2010). Despite only ~20% amino acid similarity between SUMO and ubiquitin, the two proteins share a similar 3D structure. Sumoylation is also controlled by the activity of a series of E1, E2 and E3 enzymes, although the enzymes that participate in this process are specialized. The conjugation of SUMO to target proteins is involved in a wide variety of cellular responses including enzymatic activity, cell cycle control, protein stability, DNA repair and cellular localization (Ulrich

2005; Verger et al. 2003).

Due to the large number of E3 ligases in plants and their diverse roles in plant cellular responses, in this review, we focus on the biological role of CRL E3 ligases, in terms of their involvement in hormonal responses and stress adaptation processes.

CRLs in Plant Hormone Responses

The usage of F-box proteins as substrate receptors for CRL1 is closely related to plant hormonal signaling (Yu et al. 2007). TRANSPORT INHIBITOR RESPONSE 1 (TIR1) is the best-characterized F-box protein involved in auxin signaling. TIR1 acts as an auxin receptor and mediates the degradation of AUX/IAA protein, the repressor of early auxin response genes (Kepinski and Leyser 2005; Dharmasiri et al. 2005). Several auxin-signaling F-box proteins (AFBs), which are structurally similar to TIR1, also bind and perceive auxin and participate in auxin response. While TIR1 and AFB2 are positive regulators of auxin response in seedling roots (Parry et al. 2009), AFB4 acts as a negative regulator of auxin signaling in seedlings (Greenham et al. 2011). S-Phase Kinase-Associated Protein 2A (SKP2A) has been reported as another type of auxin binding protein. SKP2A functions as a regulatory protein in the cell cycle that modulates the protein stability of D SITE OF ALBUMIN PROMOTER BINDING PROTEIN (DPB), a transcription factor for cell division (del Pozo et al. 2006). It has been suggested that auxin acts as a molecular 'glue' for SKP2A and DPB based on the finding that auxin binds to SKP2A and enhances the interaction between SKP2A and DPB (Jurado et al. 2010). A recently identified F-box protein, AUXIN UP-REGULATED F-BOX PROTEIN1 (AUF1), is involved in the cross talk between auxin transport and cytokinin signal transduction (Zheng et al. 2011). AUF1 is a positive regulator of root elongation that functions by tethering auxin movements to cytokinin signaling. Epistasis analyses suggest that the cytokinin regulator, ARABIDOPSIS RESPONSE REGULATOR 1 (ARR1), or its effector is the substrate of SCF^{AUF1} complex.

In the case of the gaseous plant hormone, ethylene, ETHYLENE INSENSITIVE 3 (EIN3)-binding F-box protein 1 (EBF1) and EBF2 have been reported as repressors in the ethylene signal transduction pathway. A mutation in either *EBF1* or *EBF2* leads to the enhanced accumulation of EIN3, resulting in the ethylene-hypersensitive phenotype. Based on the fact that EBFs are components of the SCF E3 ligase complex and interact with EIN3, EBF1 and EBF2 both have been suggested to directly target EIN3 for degradation (Guo and Ecker 2003; Potuschak et al. 2003). Two other F-box proteins, EIN2 TARGETING PROTEIN1 and 2 (ETP1 and 2), negatively regulate the stability of the ethylene signaling protein, EIN2. Accordingly, decrease in the level of both ETP1 and ETP2 leads to the enhanced accumulation of EIN2. ETP1 and ETP2 also interact with EIN2 and their overexpression impairs EIN2 accumulation (Qiao et al. 2009). While SCF (CRL1) complexes have been implicated in ethylene signaling, CRL3 is thought to function in ethylene biosynthesis. *eto1* (ethylene-overproducer mutants 1) mutant produces at least 40 times more ethylene than the wild-type. Its gene product, ETO1, is a BTB-domain containing protein component of CRL3 that directly interacts with and negatively regulates the ACC synthase enzyme, ACS5. Ectopic expression of *ETO1* results in the inhibition of ethylene production and decreases ACS5 protein stability via a proteasome-dependent pathway (Wang et al. 2004). Christians et al. recently investigated two additional BTB proteins, ETO1-like 1 (EOL1) and EOL2, which are closely related to ETO1. Similar to ETO1, these proteins play a negative role in ethylene biosynthesis and are involved in the stability of type-2 ACC synthases. Phenotype analyses of *eto1 eol1 eol2* mutants have indicated that the three proteins act together as members of CRL3 E3 ligases and target type-2 ACC synthases for degradation (Christians et al. 2009).

In *Arabidopsis*, SLEEPY1 (SLY1) and its homolog SNEEZY (SNE) are representative F-box proteins that participate in gibberellin (GA) signaling (McGinnis et al. 2003; Dill et al. 2004; Strader et al. 2004). SLY1 interacts with DELLA proteins (via the DELLA domain at the N-terminal) to repress the expression of GA early response genes, and positively regulates GA signaling by triggering the subsequent degradation of DELLA proteins through the 26S proteasome (Thomas and Sun 2004). Ectopic expression of *SNE* partially rescued the dwarf phenotype of *sly1* and restored the protein level of REPRESSOR OF ga1-3 (RGA, as one of DELLAs) to wild type level (Ariizumi et al. 2011; Strader et al. 2004). Therefore, SNE can functionally replace SLY1 via the GA-induced proteolysis of RGA and act as a redundant positive regulator in GA signaling. In rice, GIBBERELLIN-INSENSITIVE DWARF 2 (GID2), a homolog of *Arabidopsis* SLY1, is responsible for the negative regulation of SLENDER RICE-1 (SLR1), a rice DELLA protein, through the 26S proteasome dependent pathway, indicating that the SLY1/GID2-mediated function of the SCF complex is highly conserved in plants (Itoh et al. 2003).

Similar to the role of TIR1 in auxin signaling, an F-box protein, CORONATINE INSENSITIVE1 (COI1), positively regulates JA-dependent responses through ubiquitination-mediated destruction of negative regulators in JA signaling (Xie et al. 1998; Xu et al. 2002). COI1 and its interacting partner, JAZ, together form a coreceptor complex that recognizes JA-Ile (the endogenous bioactive JA) as a repressor of JA signaling. Specifically, COI1 first binds to JA-Ile, and this interaction leads to the recruitment of JAZ

(Yan et al. 2009). The binding of JA-Ile with COI1-JAZ then triggers the ubiquitination of JAZ by COI1. In the absence of JA, JAZ interacts with MYC2, a positive regulator of JA responses, and inhibits MYC2 activity by recruiting corepressors such as TOPLESS (TPL) and TPL-Related proteins via interactions with the adaptor protein, NINJA (Novel Interactor of JAZ) (Pauwels et al. 2010). Interestingly, Feng et al. reported that COP9 signalosome (CSN) physically interacts with SCF^{COI1} and modulates jasmonate responses. The reduction of CSN inhibited JA-dependent root growth and JA-induced gene expression, resulting in a phenotype similar to that produced by *coi1-1*. Therefore, COI1 and CSN are thought to work together as positive regulators in JA-mediated cellular processes (Feng et al. 2003).

A number of studies have examined the relationship between CRL and ABA signaling. Three types of DWD hypersensitive to ABA (DWA) proteins, DWA1-3, function as substrate receptors for CRL4 and play a negative role in ABA responses. *dwa1*, *dwa2* and *dwa3* exhibited hypersensitive phenotypes in response to ABA and salt stresses. In mutants, various ABA-responsive genes such as *rd29a*, *rd29b* and *rd22* were hyper-induced when compared to the wild-type. Moreover, all mutants had higher levels of the ABA-responsive transcription factor ABA INSENSITIVE5 (ABI5) than the wild-type in response to ABA. Although the substrate of DWA3 has yet to be identified, DWA1 and DWA2 were able to associate with ABI5 *in vivo*, and were directly responsible for ABI5 degradation mediated by the 26S proteasome-dependent pathway. This implies that ABI5 is a direct target of CRL4 complexes, which utilize DWA1 and DWA2 as substrate receptors (Lee et al. 2010; Lee et al. 2011). Interestingly, DWA1 and DWA2 can directly interact with each other and act together in ABA response, presenting a novel mechanism that enables two independent (non-homologous to each other) substrate receptors for the CRL complex act together in plant hormone signaling (Lee et al. 2010).

Several F-box genes are also involved in ABA hormone signaling. DROUGHT TOLERANCE REPRESSOR (DOR) was shown to act as a receptor for CRL1 complex and specifically interact with ARABIDOPSIS SKP1-LIKE 14 (ASK14) and CUL1. Furthermore, a knock-out mutant for the *DOR* gene exhibited ABA-hypersensitive phenotype in terms of stomatal closing and drought resistance. Based on these data, DOR is thought to function as a negative regulator of ABA-induced guard cell signaling related to ubiquitination (Zhang et al. 2008). EID1-LIKE PROTEIN 3 (EDL3) was initially characterized as a homolog of EMPFINDLICHER IM DUNKELROTEN LICHT 1 (EID1), an F-box protein involved in phytochrome A-dependent light signal transduction (Marrocco et al. 2006). *EDL3* is highly up-regulated by ABA treatment, indicating that it is involved in responses regulated by ABA. Based on BiFc analysis, EDL3 has been reported to directly interact with ASK proteins, confirming its role as an F-box protein for the SCF complex. Subsequent analyses using conditional knock-down and over-expresser lines for *EDL3* demonstrated that this gene product positively regulates ABA-dependent processes such as germination and root growth (Koops et al. 2011). Although potential targets for the SCF complex mediated by EDL3 have not yet been identified, it is believed that EDL3 negatively regulates the repressor protein by degradation via the ubiquitin-dependent proteasome. Recently, F-BOX-OVEREXPRESSED/OPPRESSED ABA SIGNALING 1 (FOA1) was suggested as another negative regulator of the ABA signal transduction pathway. *foa1* mutant exhibits a decreased germination rate, shorter root length, and increased ABA-hypersensitivity (Peng et al. 2012).

In addition to CRL1 and CRL4, CRL3 complexes composed of several BTB proteins have been reported to play roles in ABA response. ARM REPEAT PROTEIN INTERACTING WITH ABF2 (ARIA) was initially identified as a protein that interacts with ABF2, a transcription factor that controls the expression of ABA-responsive genes through the G-box type ABA-responsive elements (Kim et al. 2004). ARIA possesses a BTB domain in its C-terminal, implying that it functions as a member of the CRL3 complex. A study examining the expression level of ARIA in plants revealed that ARIA positively regulates the transcriptional activity of ABF2. An N-terminal BTB domain-containing protein, BTB AND TAZ DOMAIN PROTEIN 2 (BT2), was originally shown to promote telomerase activity. Further studies of BT2 showed that its constitutive expression imparted resistance to both sugars and ABA at germination, while the impaired expression of *BT2* led to a hypersensitive response to both sugar- and ABA-mediated inhibition of germination, raising the possibility that BT2 inhibits cellular responses for sugar and ABA signaling (Mandadi et al. 2009). A recent study conducted by Lechner et al. contributed to further understanding the role of CRL3 in ABA signaling (Lechner et al. 2011). Six *Arabidopsis* MATH-BTB proteins, BTB-POZ AND MATH DOMAIN-CONTAINING PROTEIN 1-6 (BPM1-6), participate in the CRL3 complex as $CUL3^{BPM}$. Reducing $CUL3^{BPM}$ function results in inhibited plant growth and fertility, and alterations in ABA responses such as germination and stomatal closing. This group of proteins target ATHB6, a transcription factor crucial in ABA signaling, for ubiquitination and degradation. In rice, overexpression of *OsDRF1* leads to ABA-hypersensitivity, suggesting that OsDRF1 plays a role as a positive regulator in ABA signaling (Cao et al. 2008).

CRLs in Plant Stress Responses

Among the stresses that a plant is subject to, drought has the greatest effect on productivity, and therefore, its signal transduction pathway has been studied intensively. To avoid growth defects triggered by this stress, plants have developed various mechanisms to sense water limitation and overcome stress during drought. Drought-stress signaling is largely composed of ABA-independent and ABA-dependent pathways (Yamaguchi-Shinozaki and Shinozaki 2005). The ABA-dependent pathway is divided into two main types, one controlled by bZIPs such as ABA INSENSITIVE5/ABRE binding factors/ABRE binding proteins (ABI5/ABF/AREB) and ABA-responsive element (ABRE), and another regulated by MYC/MYB and MYC-/MYB-recognition sequences (MYCRS/MYBRS) (Abe et al. 1997; Shinozaki and Yamaguchi-Shinozaki 2000; Uno et al. 2000; Agarwal et al. 2006). The ABA-independent pathway uses a different regulatory system where dehydration-responsive element/C-repeat (DRE/CRT) works as a *cis*-acting element and the AP2/ERF family members, C-REPEAT BINDING FACTOR/DRE-BINDING PROTEIN 1 (CBF/DREB1) and DREB2, act as *trans*-acting factors (Yamaguchi-Shinozaki and Shinozaki 2005). Interestingly, most of the reported CRL receptor proteins (such as DWA1, DWA2, DOR, EDL3 and BPMs) involved in drought stress signaling are components related to ABA-dependent drought signaling, while the relationship between CRL and ABA-independent drought signaling is not well known. Therefore, it would be meaningful to identify CRL complexes that can modulate the stability of CBF/DREB1 and/or DREB2 proteins.

Recently, there has been a significant increase in the amount of UV that passes through the ozone layer. This has made UV a serious environmental stress for plants. There have been several studies on the effect of UV-B (280 to 320 nm) signaling on the action of CRL4 complexes, an effect that is mediated by COCKAYNE SYNDROME A-LIKE PROTEINS 1A (CSAat1A, also named as ATCSA-1), CSAat1B, and DAMAGE-SPECIFIC DNA BINDING PROTEIN 2 (DDB2) (Biedermann and Hellmann 2010; Zhang et al. 2010). CSAat1A and B can form heterotetramers and associate with the CUL4-DDB1 complex. This association is thought to play an important role in plant response against DNA damage caused by UV-B (Zhang et al. 2010). Moreover, Biedermann and Hellmann (2010) reported that CSAat1A, along with DDB2, is a key component for UV-B-induced damage repair. REPRESSOR OF UV-B PHOTOMORPHOGENESIS 1 (RUP1) and RUP2 are likely negative regulators of the UV-B specific response, possibly via direct interaction with UVR8, a crucial positive regulator in UV-B signaling. Although there is currently no evidence that both RUP1 and RUP2 interact with CUL4-DDB1, they might act as components of CRL4 since they commonly possess the DWD domain (Gruber et al. 2010).

Several F-box proteins have been reported to be involved in plant defense responses against insects and pathogens. COI1, previously described as part of JA signaling, is well-known for its role in wound healing and defense processes (Xie et al. 1998). Since *cos1* (*coi1 suppressor1* mutant) mutation restores the *coi1*-related phenotypes, COI1 is thought to act upstream of COS1, a crucial riboflavin pathway component, in the JA-mediated defense signaling pathway (Xiao et al. 2004). Suppressor screens carried out on mutagenized plants of *nim1-1*, the mutant defective of *NONINDUCIBLE IMMUNITY 1*, the gene regulating systemic acquired resistance, led to the isolation of *son1*. *son1* imparted pathogen resistance regardless of the up-regulation of SAR-associated genes, indicating that *son1*-mediated resistance is independent of SAR. Since SON1 possesses an F-box, it is thought to negatively regulate defense signaling independently of SAR via ubiquitination (Kim and Delaney 2002). CONSTITUTIVE EXPRESSOR OF PR GENES 30 (CPR30) is known as another negative regulator of both SA-dependent and SA-independent defense responses (Gou et al. 2009). *cpr30* shows constitutive resistance to *Pseudomonas syringae* and hyper-induction of various defense-response genes. The fact that CPR30 associated and colocalized with the adaptor proteins of SCF (ASK1 and/or ASK2) raises the possibility that it is a member of SCF used for repression of defense signaling against pathogen attacks through the ubiquitin-proteasome process.

Regulation of Activity of the CRL Complex

Although CUL4 typically associates with DDB1, RBX1 and DWD (DCAF) to form CRL4, this protein can also connect with another COP10-DET1-DDB1 (CDD) complex (Chen et al. 2006). Indeed, the existence of this atypical cullin-based complex is surprising. To elucidate the possible role of the CDD complex for CUL4, Chen et al. showed that the purified CDD complex promoted autoubiquitination of RBX1, a RING protein, as a component of CRL4 by increasing the E3 activity of CRL4 (Chen et al. 2006). Therefore, the CDD complex has been suggested to be involved in the positive regulation of CRL4 activity. Another factor reported to control CRL activity is the covalent modifications of cullins. The ubiquitin-like molecules, Related to Ubiquitin (RUB)/Neural precursor cell-Expressed Developmentally Downregulated 8 (NEDD8), are conjugated with cullin, a process referred to as 'rubylation', and enhance the activity of CRL by promoting E2 recruitment (Hotton and Callis 2008). Moreover, CSN has been reported to participate in the modulation of CRL activity through detachment of

RUB/NEDD9 (derubylation) (Lyapina et al. 2001; Schwechheimer et al. 2001). Additionally, it has been reported that CULLIN-ASSOCIATED NEDD8-DISSOCIATED 1 (CAND1) negatively regulates the action of CRL1 by binding to unmodified cullin and inhibiting the interaction of adaptor proteins with cullin (Feng et al. 2004; Petroski and Deshaies 2005).

Conclusion

Plant hormones regulate a variety of cellular processes during a plant life cycle. In most cases, the cross-communication and balance between multiple hormones determine the direction of cellular events such as tolerance to environmental stresses and developmental processes. To adapt their growth and survive under unfavorable environments, plants frequently need to remove detrimental proteins or negative regulators via processes such as ubiquitination, and effectively transduce the related signals downstream. Although the cellular function of single subunit E3 ligases in the protein degradation process has been widely studied, only a small portion of CRL complexes have been investigated for their roles in plant cellular processes.

One strategy to elucidate the possible involvement of CRL complexes in plant hormonal response and stress adaptation is to monitor the transcriptional levels of CRL substrate receptor genes in response to plant hormones and stress treatments. The change in expression of certain genes in response to a specific stimulus would strongly imply the gene's potential role for such stimuli. For example, our preliminary analyses using microarray data from the AtGenExpress Visualization Tool (AVT) (Kilian et al. 2007) showed that 2.1% of the entire F-box genes, 10% of BTBs and 4.2% of DWD/DCAFs are up-regulated by more than two-times in response to ABA application (data not shown). Determining whether loss-of-function mutants for these candidate genes exhibit altered sensitivity in response to ABA would be a good starting point to explore the functional relationship between CRL-mediated ubiquitination and ABA signaling. Interestingly, the functional relationship between CRL and cytokinin/brassinosteroid has not been thoroughly investigated to date. The aforementioned approach may help elucidate any possible connections between CRL and these hormones.

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