Minireview

Regulation of Abiotic Stress Signal Transduction by E3 Ubiquitin Ligases in *Arabidopsis*

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Ubiquitination is a unique protein degradation system utilized by eukaryotes to efficiently degrade detrimental cellular proteins and control the entire pool of regulatory components. In plants, adaptation in response to various abiotic stresses can be achieved through ubiquitination and the resulting degradation of components specific to these stress signalings. *Arabidopsis* has more than 1,400 E3 enzymes, indicating E3 ligase acts as a main determinant of substrate specificity. However, as only a minority of E3 ligases related to abiotic stress signaling have been studied in *Arabidopsis*, the further elucidation of the biological roles and related substrates of newly identified E3 ligases is essential in order to clarify the functional relationship between abiotic stress and E3 ligases. Here, we review the current knowledge and future prospects of the regulatory mechanism and role of several E3 ligases involved in abiotic stress signal transduction in *Arabidopsis*. As another potential approach to understand how ubiquitination is involved in such signaling, we also briefly introduce factors that regulate the activity of cullin in multi-subunit E3 ligase complexes.

UBIQUITINATION AND E3 LIGASES

Ubiquitination is a post-translational modification of proteins that plays an important role in several processes in plants such as biotic and abiotic stress responses, cell cycle progression and hormone response. Ubiquitin, so named because of its ubiquitous presence in all eukaryotic species, is a highly conserved 8-kDa protein. This small protein attaches to lysine residues on the target protein by three successive enzymes; Ub-activating enzyme (E1), Ub-conjugating enzyme (E2), and Ub-ligase (E3) (Kraft et al., 2005; Mukhopadhyay and Riezman, 2007; Smalle and Viestra, 2004; Stone et al., 2005) (Fig. 1A). The target protein can be tagged with a single ubiquitin molecule (mono-ubiquitination), multiple ubiquitin molecules (multiubiquitination), or a ubiquitin chain (polyubiquitination). In general, monoubiquitination and multiubiquitination function in the regulation of protein activity, protein-protein interaction, and subcellular localization. In contrast, polyubiquitination, the most frequently found type of ubiquitination, mainly triggers the transfer of the ubiq-

uityinated target protein to the 26S proteasome complex, resulting in further degradation (Mukhopadhyay and Riezman, 2007; Pickart and Eddins, 2004).

Arabidopsis has 2 E1, at least 37 E2, and more than 1,400 E3 enzymes (Downes et al., 2003; Dreher and Callis, 2007; Gagne et al., 2002; Gingerich et al., 2005; Hatfield et al., 1997; Kraft et al., 2005; Vierstra, 2009). The large diversity of E3 ligases suggests that E3 is important in determining substrate specificity, that is, identifying which protein will be ubiquitinated. E3 ligases can be largely divided into two groups based on their structure: single-subunit E3 ligases and multi-subunit E3 ligases.

Single-subunit E3 ligases

Single-subunit E3 ligases are further categorized into distinct families based on the presence of specific domains, such as homology to E6AP C terminus (HECT), U-box, and really interesting new gene (RING) domains (Fig. 1B).

HECT E3 ligases contain a conserved C-terminal HECT domain that was first detected in human E6AP (Huibregtse et al., 1995). While other single-subunit E3 ligases are indirectly involved in the transfer of ubiquitin from E2 to the target protein, HECT E3 ligases generate an intermediate form of Ub-E3 through a cysteine residue that pre-exists in the HECT domain. This intermediate form acts as a proximal ubiquitin donor for the ligation of ubiquitin to the substrate protein. Although approximately 7 HECT E3 ligases have been identified in *Arabidopsis* (UPL1-7), there are only a few reports on their roles in plant signalings so far. For instance, UPL3 has been suggested to be involved in trichome development and UPL5 was shown to play a negative role in leaf senescence through degradation of the transcription factor WRKY53 (Downes et al., 2003; Miao and Zentgraf, 2010; Vierstra, 2003).

RING finger-containing proteins are the most abundant group among the single-subunit ligases. It is estimated that more than 460 RING proteins exist in the *Arabidopsis* genome. This group is defined by the presence of a RING finger motif, a zinc-binding motif composed of approximately 70 amino acids. Most RING motifs coordinate two zinc ions so that it forms a platform that can bind E2 (Freemont et al., 1991). RING finger-containing proteins also function as a component of the cullin-based multi-subunit E3 ligase complex (Santner and Estelle, 2010).

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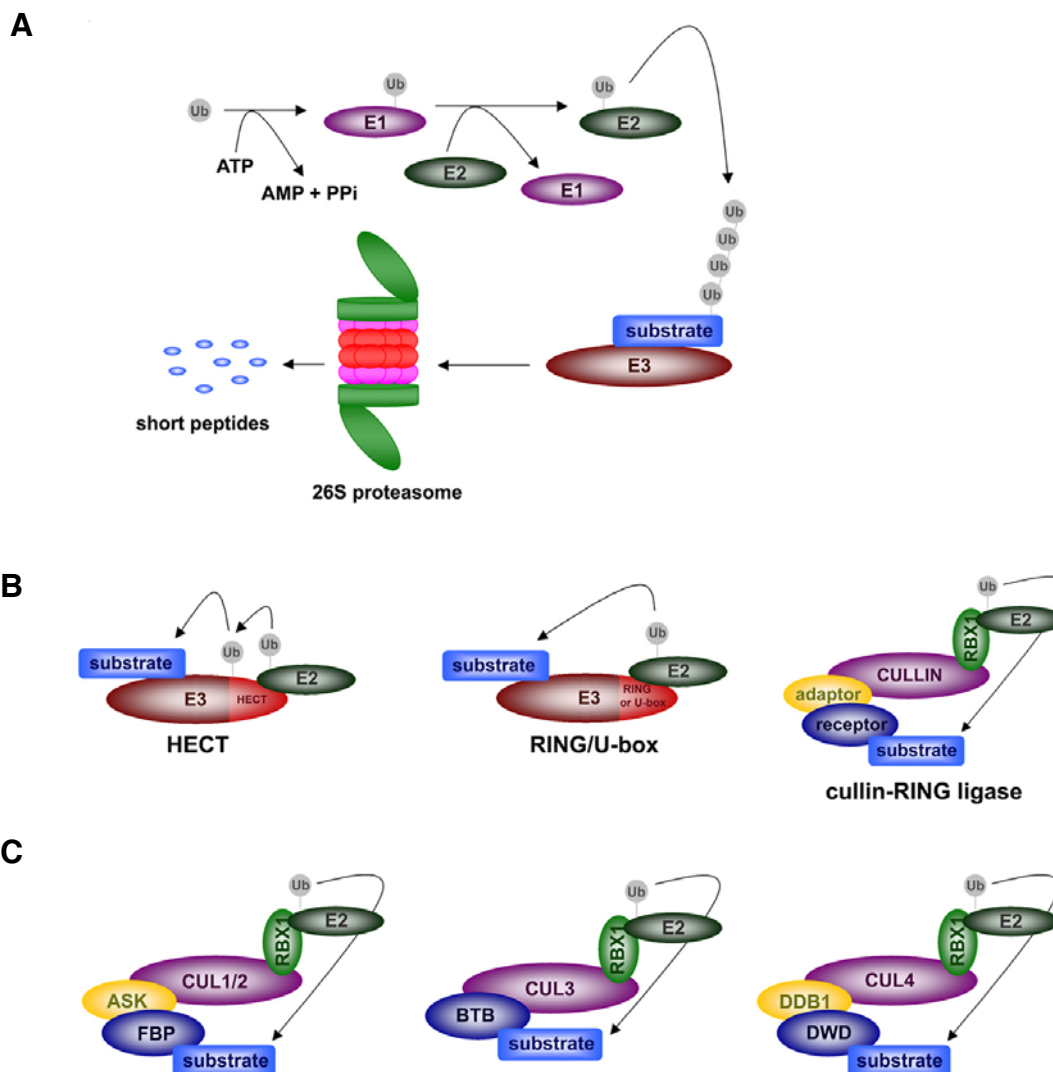
Fig. 1. Ubiquitination process and E3 ligases. (A) Overview of the polyubiquitination process. Ubiquitination requires the sequential action of three enzymes; ubiquitin-activating enzyme (E1), ubiquitin-conjugating enzyme (E2) and ubiquitin ligase (E3). Ubiquitinated substrates are targeted to the 26S proteasome for subsequent degradation. (B) Single subunit and multi-subunit E3 ligases. Based on the E2-interacting region, single subunit E3 ligases can be largely categorized into two types; HECT-type and RING/U-box E3 ligases. Cullin-based E3 ligases are composed of several components; cullin, RBX1, adaptor, and substrate receptor. The resulting complex acts as an E3 ligase in the ubiquitination of substrates. (C) Several types of cullin-based E3 ligases in plants. Cullin 1 assembles into the SCF complex with ASK as an adaptor and F-box protein as a substrate receptor. As cullin 2 was reported to interact with F-box proteins, cullin 2 may be another subunit of the SCF complex (Risseeuw et al., 2003). In the case of cullin 4 complex, DDB1 and DWD proteins are utilized as adaptors and substrate receptors, respectively. Unlike cullin 1 and cullin 4, cullin 3 only requires BTB-containing proteins as substrate receptors without additional adaptor proteins.

U-box domain is composed of 75 amino acids (Hatakeyama et al., 2001) and is structurally related to RING domain. But unlike RING, zinc is not required for the stabilization of the U-box structure (Aravind and Koonin, 2000). Similar to RING domain-containing proteins, U-box proteins participate in several events in the life of a plant such as hormone and biotic/abiotic stress signalings (Yee and Goring, 2009). There are 64 U-box genes in the *Arabidopsis* genome, a significantly larger number than that in human or yeast (21 and 2, respectively). As other single subunit E3 ligases share a similar number of gene families among eukaryotes, the diversity of plant U-box genes suggests that the U-box domain is important in determining substrate specificity for various cellular processes in plants (Aravind

and Koonin, 2000; Koegl et al., 1999; Ohi et al., 2003; Wiborg et al., 2008).

Multi-subunit E3 ligases: cullin-based E3 ligases
Among the multi-subunit E3 ligases, cullin-based E3 ligases have been best characterized to date. In *Arabidopsis*, five cullins, namely, cullin 1, cullin 2, cullin 3a, cullin 3b, and cullin 4 have been identified as components of the cullin-based E3 ligases. Cullin proteins serve as a scaffold that provides a framework for assembling the ubiquitination machinery. In addition to cullin proteins, two other modules are required for the complex: the RING finger protein, RBX1, which recruits E2, and the substrate-recognition module that contains the adaptor and substrate

receptor. After all proteins are assembled, the complex acts as an E3 ligase for subsequent target modification (Petroski and Deshaies, 2005; Schwechheimer and Calderon Villalobos, 2004; Thomann et al., 2005) (Fig. 1B).

At least three types of cullin-based E3 ligases exist in plants (Fig. 1C). In the well-known Skp1-Cullin-F-box (SCF) complex, cullin 1 directly interacts with Arabidopsis-SKP1-like (ASK), the counterpart of SKP1 in animals, and ASK serves as an adaptor protein that connects cullin 1 and the F-box protein. There are more than 700 different F-box proteins in *Arabidopsis*. As F-box proteins act as substrate receptors in the SCF complex, such diversity ensures that a specific target protein is recruited to the complex (Cardozo and Pagano, 2004; Cope and Deshaies, 2006; Schulman et al., 2000; Zheng et al., 2002). In *Arabidopsis*, it has been reported that cullin 2 also interacts with F-box proteins, suggesting cullin 2 could also be a subunit of the SCF complex (Risseeuw et al., 2003).

The function and expression pattern of cullin 3a and cullin 3b largely overlap with each other and both proteins are essential for embryo development in *Arabidopsis* (Figueroa et al., 2005). Cullin 3a and cullin 3b can assemble into E3 ligase complexes in the absence of adaptor proteins because BTB/POZ-domain proteins directly associate with CUL3 and also act as receptors for substrate recognition (Gingerich et al., 2005; Pintard et al., 2004). It appears that *Arabidopsis* possesses at least 37 BTB/POZ-domain proteins (*from* Protein Families Database of Alignments, PFAM) (Bateman et al., 2004).

Cullin 4 utilizes damaged DNA binding 1 (DDB1) protein as an adaptor to assemble the E3 ligase complex. *Arabidopsis* has two closely related forms of DDB1; DDB1a and DDB1b. The expression pattern of DDB1a and DDB1b overlap and the protein sequences of the two proteins are highly similar (91% amino acid identity) (Schroeder et al., 2002). Nevertheless, the fact that *ddb1b* mutants are lethal while *ddb1a* does not show any distinct phenotype indicates each protein also has a unique function during plant development (Bernhardt et al., 2010). As a substrate receptor for the cullin 4 E3 ligase complex, DWD domain-containing proteins have been identified (Angers et al., 2006; He et al., 2006; Higa et al., 2006; Jin et al., 2006; Lee et al., 2008). DWD proteins contain WD40 repeats that, in turn, share a conserved motif, the DWD box, that is usually composed of 16 amino acids. In general, the 16th arginine (or lysine) of the DWD domain acts as the major determinant for the direct interaction with CUL4-DDB1 (Angers et al., 2006; He et al., 2006; Jin et al., 2006). There have been 85 and 78 DWD proteins reported in *Arabidopsis* and rice, respectively (Lee et al., 2008). In some cases, the WDxR motif in the WD40 region rather than the entire DWD box is sufficient to bind DDB1. This suggests that an additional 34 *Arabidopsis* proteins and 32 rice proteins that contain the WDxR motif may also be potential substrate receptors for the E3 complex (Zhang et al., 2008a). Interestingly, besides the typical CUL4-DDB1-DWD (or WDxR) complex, *Arabidopsis* CUL4 is able to associate with a different complex named CDD (COP10-DET1- (COP10-DET1-DDB1) as well, and the resulting CUL4-CDD complex mediates the repression of photomorphogenesis (Bernhardt et al., 2006; Chen et al., 2006).

FUNCTIONAL RELATIONSHIP BETWEEN ABIOTIC STRESS SIGNALING AND E3 LIGASES

Water-deficit stress signaling and the related E3 ligases
Water-deficit stress such as drought and high salinity results in a marked reduction of crop productivity on as much as half of the world's irrigated land (Boyer, 1982; Cushman and Bohnert, 2000). This stress brings about a variety of cellular processes such as stomatal closure and changes in cell wall/membrane composition which, in turn, enable plants to overcome the unfavorable conditions (Bohnert et al., 2006). As shown in Fig. 2A, signal transduction pathways triggered by drought and high salt content share a number of signaling components that transduce the related signalings into downstream processes that endow resistance to such stresses.

Drought/salt stress signaling can be divided into two types of pathways based on the dependence on the plant hormone, abscisic acid (ABA). A large number of ABA-inducible genes are up-regulated by drought and high-salinity, indicating ABA acts as a positive regulator in such stress signal transduction pathways (Takahashi et al., 2004). The ABA-dependent pathway is mediated by two different systems (Agarwal et al., 2006). First, in the basic leucine zipper (bZIP)/ABA-responsive element (ABRE) system, leucine zipper transcription factors such as ABRE binding protein (AREB), ABRE BINDING FACTOR (ABF), ABA INSENSITIVE 5 (ABI5) and *Dc3* promoter binding factor (DPBF) bind to ABRE, a *cis*-acting element for ABA-responsive genes such as *RD29B*, *AIL1* and *RAB18*, and transactivate the responding genes (Fujita et al., 2005; Nakashima et al., 2009). Nine types of proteins have been identified as members of the AREB/ABF/ABI5/DPBF bZIP subfamily in *Arabidopsis*. Among them, AREB1/ABF2, AREB2/ABF4, and ABF3/DPBF5 are inducible in response to dehydration, high-salt treatments, and ABA in vegetative tissues (Fujita et al., 2005). The second system is controlled by MYC/MYB transcription factors. These proteins bind to *cis*-acting elements such as MYCRS and MYBRS (MYC- and MYB-recognition sequence, respectively) and activate transcription of the drought-responsive gene, *RD22* (Abe et al., 1997).

The first insight into ABA-independent dehydration stress signaling came from findings that various dehydration stress-responsive genes were upregulated in ABA-deficient (*aba*) and ABA-insensitive (*abi*) mutants under drought stress (Shinozaki and Yamaguchi-Shinozaki, 2000). This also led to the discovery of dehydration-responsive element (DRE), a *cis*-acting element responsible for dehydration signal transduction (Yamaguchi-Shinozaki and Shinozaki, 1994). Although DRE binding 1 (DREB1) and DREB2, transcription factors that belong to the APETALA2 (AP2)/ethylene response factor (ERF) family, both interact with DRE, DREB2 functions as a transcription factor only under drought/salt stresses and DREB1 only under low temperature (Liu et al., 1998).

Several reports have demonstrated that single E3 ligases are involved in ABA-dependent or -independent pathways in drought/salt stress responses. In *Arabidopsis*, the RING finger E3 ligase, SALT-AND DROUGHT-INDUCED RING FINGER1 (SDIR1) was identified as a positive regulator in ABA response, as loss of *SDIR1* resulted in ABA-insensitive phenotypes. Although *SDIR1* is induced by drought and salt stress, and not by ABA, transgenic plants that overexpress *SDIR1* display ABA-hypersensitive phenotypes such as enhanced ABA-induced stomatal closure and drought tolerance. The heterologous overexpression of *Arabidopsis SDIR1* in tobacco and rice also confers enhanced drought tolerance, suggesting the role of SDIR1 in drought-tolerance is conserved across dicotyledons and monocotyledons (Zhang et al., 2008b). Since *SDIR1* fails to rescue *abi5-1*, whereas *ABI5*, *ABF3* and *ABF4* rescue the ABA-insensitive phenotype of *sdir1-1*, SDIR1 may function upstream of the bZIP proteins in ABA signaling (Fig. 2B) (Zhang et al., 2007).

The U-box containing E3 ligase, AtCHIP, is also involved in ABA-mediated stress response. AtCHIP, named because of its structural resemblance to animal CHIP proteins, was originally
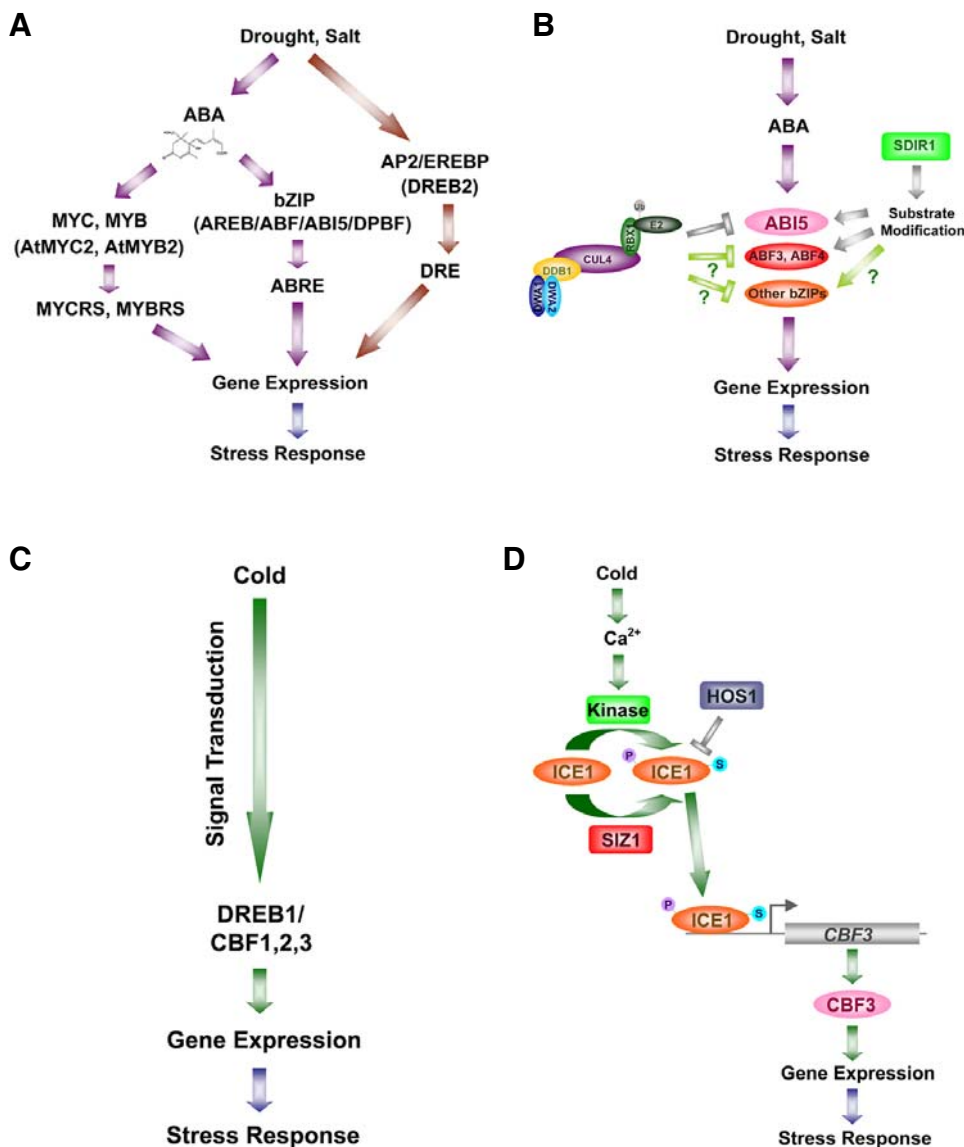
Fig. 2. Abiotic stress signalings and the related E3 ligases. (A, B) Schematic representation of water-deficit stress signaling in the presence or absence of ABA (A) and the involvement of E3 ligases in ABA-mediated drought signaling via AREB/ABF/ABI5/DPBF bZIP subfamily (B). 'Substrate modification' indicates either negative regulation by polyubiquitination or positive regulation by monou-biquitination. (C, D) Simplified scheme of cold signaling pathway (C) and the negative regulation of ICE1 by HOS1 E3 ligase (D). The protein stability of ICE1 is negatively regulated by ubiquitination, whereas its activity is positively regulated by phosphorylation and sumoylation. The activated ICE1 binds to MYCRS in the promoter region of *CBF3* and then positively regulates the expression of *CBF3* and its downstream genes. 'P' and 'S' indicates phosphate and small ubiquitin-related modifier (SUMO), respectively.

reported to play a crucial role in tolerance against low temperature stress (Yan et al., 2003). Interestingly, AtCHIP monoubiquitylates PP2A *in vitro* and the activity of PP2A increases in AtCHIP-overexpressing plants under low-temperature conditions. Moreover, these overexpressing plants are more sensitive to ABA treatment in terms of stomatal aperture and germination rate (Luo et al., 2006). Since monoubiquitination can regulate substrate activity (Pickart, 2001; Wojcik, 2001), this suggests that the activity of PP2A is increased by AtCHIP and that the increase in PP2A activity leads to enhanced ABA sensitivity.

Recently, Ryu et al. (2010) acquired 100 different T-DNA insertion mutants for RING E3 ligase genes in *Arabidopsis* and screened for mutants that exhibited altered ABA-sensitivity phenotypes. Here, the loss-of-function mutant of AtAIRP1 (*Arabidopsis thaliana* ABA Insensitive Ring Protein 1) displayed ABA-insensitive phenotypes, while *AtAIRP1*-over-expressors were significantly more resistant to drought stress. These results implicate that AtAIRP1 acts as a positive regulator in drought-stress responses mediated by ABA (Ryu et al., 2010).

PUB22 and PUB23 were first identified as *Arabidopsis* proteins most closely related to the hot pepper (*Capsicum annuum*) CaPUB1, a U-box-containing E3 ligase induced by drought stress (Cho et al., 2006; 2008). In a previous report, transgenic plants harboring *35S::PUB22* and *35S::PUB23* were hypersensitive to drought, while *pub22* and *pub23* mutants displayed drought-tolerance, and *pub22 pub23* double mutants showed even stronger tolerance to drought. From these results, PUB22 and PUB23 are thought to act as negative regulators in response to water-deficit stress (Cho et al., 2008). Interestingly, PUB22 and PUB23 interact with and ubiquitinate RPN12a, a subunit of the 19S regulatory particle (RP) in the 26S proteasome. Moreover, RPN12a dissociates from the 19S RP in wild-type and PUB-overexpressing plants under water stress. Thus, PUB22 and PUB23 have been proposed to function in the fine-tuning of drought response by ubiquitinating cytosolic RPN12a and then altering the characteristics of the 19S RP in response to water-deficit stress. Similar to *PUB22* and *PUB23* overexpressors and mutants, the overexpression of *CaPUB1* in *Arabidopsis* does not alter ABA-sensitivity (Cho et al., 2006). Thus, it

is most likely that PUB22 and PUB23 function is independent of ABA.

The hot pepper Rma1H1 is a homolog of human RING membrane-anchor 1 E3 ligase and possesses E3 ligase activity *in vitro*. Rma1H1 overexpression in plants inhibits PIP2;1 trafficking from the ER to the plasma membrane and increases tolerance to drought stress. The reduction of PIP2;1 in Rma1H1-overexpressing plants is inhibited by MG132, a 26S proteasome inhibitor. In addition, Rma1H1 interacts with an aquaporin isoform PIP2;1 *in vitro* and ubiquitinates it *in vivo*. Rma1, an *Arabidopsis* homolog of Rma1H1, also inhibits PIP2;1 trafficking and the reduced expression of Rma1 leads to an increase in the level of PIP2;1. Taken together, it has been suggested that Rma1H1 and Rma1 play a positive role in drought tolerance by repressing the trafficking of aquaporin and decreasing its protein stability (Lee et al., 2009). On the other hand, an *Arabidopsis* homolog AtRma2 interacts auxin binding protein 1, suggesting that Rma1 homologs are associated not only with abiotic stress responses but also with hormone responses (Son et al., 2009; 2010).

Cullin-RING ubiquitin E3 ligases (CRLs) are also involved in ABA-mediated water-deficit stress in *Arabidopsis*. Drought tolerance repressor (DOR) is a F-box protein that interacts with CUL1, and is, therefore, a substrate receptor for Cullin1-RING ubiquitin E3 ligases (CRL1). Loss of *DOR* results in ABA-hypersen-sitive stomatal closing and increased drought tolerance, and overexpression of *DOR* leads to higher susceptibility to drought. Thus, DOR is thought to function as a negative regulator in ABA-mediated guard cell signaling (Zhang et al., 2008c).

DWD hypersensitive to ABA1 (DWA1) and DWA2 have also been reported as negative regulators in ABA signal transduction pathways (Lee et al., 2010). Both proteins possess DWD domains, the conserved domain found in most substrate receptors for Cullin4-RING ubiquitin E3 ligases (CRL4), and interact with components of the CRL4 complex such as DDB1 and CUL4 *in vivo*, indicating they function as substrate receptors for CRL4. DWA1 and DWA2 directly interact with each other and both associate with and mediate the degradation of ABI5, a key component for ABA responses such as inhibition of seed germination and post-germinational growth arrest (Fig. 2B). However, *dwa1 dwa2* mutants exhibit drought tolerance in adult plants as well as ABA-hypersensitivity in seed germination. Thus, DWA1 and DWA2 have been speculated to be involved in the destruction of other positive regulators of ABA response, such as additional AREB/ABF/DPBF bZIP proteins that are highly expressed in adult plants (Nakashima et al., 2009). In a recent report, DWA3 was also reported as a negative regulator in ABA response (Lee et al., 2011). Loss of *DWA3* resulted in increased ABA sensitivity and, in particular, *dwa3* leaves lost less water than those of wild-type after detachment. Unlike DWA1 and DWA2, DWA3 does not appear to be directly involved in the destruction of ABI5 even though ABI5 level is higher in *dwa3* than in wild-type, indicating DWA3 may not act together with other DWA proteins in ABA signaling.

Cold stress signaling and HOS1
In response to low temperature, C-repeat (CRT)-binding factors (CBFs), namely CBF1 (DREB1b), CBF2 (DREB1c) and CBF3 (DREB1a), bind to the *cis*-acting element, CRT/DRE, and function as key regulators of cold-responsive (COR) genes (Liu et al., 1998; Stockinger et al., 1997; Yamaguchi-Shinozaki and Shinozaki, 1994) (Fig. 2C). It is thought that CBFs are differentially regulated during cold acclimation as CBF2 is induced later than CBF1 and CBF3 (Novillo et al., 2004). There have been

reports on upstream regulators of CBF3 that are involved in low temperature signaling. Inducer of CBF expression 1 (ICE1) is a MYC-like basic helix-loop-helix (bHLH) transcription activator that binds to MYCRS within the promoter of *CBF3*. *ice1* mutants display impaired cold-induced transcription of *CBF3* and its downstream genes and exhibit decreased chilling and freezing tolerance (Chinnusamy et al., 2003). Phosphorylation and sumoylation of ICE1 by an unknown kinase and SIZ1, respectively, are required for its activity in cold signaling (Chinnusamy et al., 2007). In contrast, ICE1 activity is inhibited through ubiquitination. The RING finger E3 ligase, HOS1, interacts with and ubiquitinates ICE1 (Fig. 2D). Mutations in *HOS1* lead to the hyper-induction of both the *CBF*s and their downstream cold-responsive genes in response to cold treatment (Lee et al., 2001). The overexpression of *HOS1* lowers the level of ICE1 protein and reduces the expression of CBFs and their downstream target genes involved in freezing tolerance (Dong et al., 2006). Thus, HOS1 functions as a negative regulator in low temperature signaling by mediating the degradation of ICE1 and inhibiting the expression of ICE1 target genes.

The role of COP1 and CRL4 in UV-B signaling
In the past years, there has been an astonishing increase in the amount of UV-B (280 to 320 nm) that penetrates the ozone layer and reaches the surface of the Earth. Due to this phenomenon, UV-B has become a major source of stress to plants. While high fluence UV-B generates reactive oxygen species (ROS) that damage macromolecules such as DNA, low fluence UV-B acts as a signal that initiates regulatory responses involved in repairing UV damage. In addition, low fluence UV-B also triggers photomorphogenic processes such as anthocyanin accumulation, inhibition of hypocotyls, and cotyledon opening (Jenkins, 2009). Although little is known about the UV-B signaling pathway, there have been several reports on UV RESISTANCE LOCUS 8 (UVR8), a key component that positively regulates UV-B response. UVR8 rapidly accumulates in the nucleus upon UV-B irradiation and associates with chromatin regions that contain UV-B-responsive genes such as HY5 (ELONGATED HYPOCOTYL5) and HY5 HOMOLOG (HYH). This association may function in recruiting transcription factors that positively modulate UV-B signaling (Brown et al., 2005; Cloix and Jenkins, 2008). In the presence of UV-B, UVR8 also interacts with the RING finger E3 ligase, CONSTITUTIVELY PHOTOMORPHOGENIC 1 (COP1) (Favory et al., 2009). In white light conditions, COP1 negatively regulates the level of HY5 protein. However, under UV-B, the COP1-mediated protein degradation of HY5 is inhibited and, instead, COP1 contributes in the increase of HY5 expression. Therefore, it has been proposed that UVR8 is involved in the inhibition of HY5 degradation under UV-B and that UVR8-COP1 interaction is important to properly tranduce UV-B signaling into downstream pathways (Favory et al., 2009; Oravecz et al., 2006). Although the mechanism of action of COP1 under UV-B is still poorly understood, it is thought that the E3 ligase activity of COP1 contributes to the positive regulation of UV-inducible gene expression.

Recently, two DWD genes, an ortholog of the mammalian Cockayne Syndrome type-A (*AtCSA-1*) and *damage-specific DNA binding protein 2 (DDB2)*, were reported as crucial components in UV-B response (Biedermann and Hellmann, 2010; Zhang et al., 2010). As AtCSA-1 and DDB2 form CRL4 complexes with DDB1 and CUL4, it is possible that CRL4 E3 ligase acts as an important regulator in UV-B response (Biedermann and Hellmann, 2010; Zhang et al., 2010).

CONCLUSION AND FUTURE PERSPECTIVES

Higher plants are frequently affected by diverse stresses from the environment throughout their life cycles. This stress can have a huge impact on the growth of plants. In particular, abiotic stresses such as drought, cold and salt greatly impair plant growth and development (Boyer, 1982). Although many genes involved in such stress signalings have been studied by biochemical and genetic methods, the physiological role of these genes in relation to stress tolerance or sensitivity is largely unknown in higher plants (Bohnert et al., 2006; Oono et al., 2003; Vij and Tyagi, 2007; Zhu, 2002). Therefore, a more detailed understanding of stress signal transduction pathways would serve as a platform to increase the quality and productivity of agronomically useful plants.

Although only a minor part of E3 genes in the entire *Arabidopsis* genome have been studied, the rapidly growing amount of novel findings on E3 ligases has significantly advanced our knowledge in this field over the last decade. However, in spite of several reports on E3 ligases in abiotic stress signaling in this study, there is only a limited amount of information on the associated target proteins. Further studies on E3 ligase substrates will offer a broader insight into how plants specifically modulate the activity and/or stability of substrates via E3 ligases and utilize the ubiquitination system to adapt to unfavorable conditions. Comparative genomics and proteomics with mutants or over-expressors of E3 ligases of interest would also help elucidate the related substrates.

In some cases, the target proteins destined for ubiquitination are also under the control of additional post-translation modifications. For example, phosphorylation is required for the increased activity of ICE1 (as previously mentioned) and ABI5, which are negatively modulated via ubiquitination (Chinnusamy et al., 2007; Lopez-Molina et al., 2001). The proper balance between these modifications would most likely act as a determining factor in how plants transduce upstream signals into downstream pathways. Thus, it would be interesting to further investigate whether ubiquitination substrates found by comparative genomics and proteomics are dependent on other modification processes, and if so, how the different modifications are regulated during abiotic stress signaling.

In CRL E3 ligases, cullin is another factor that determines the activity of the complex. The ubiquitin-like protein, RUB (Related to Ubiquitin)/NEDD8 (Neural precursor cell-Expressed Developmentally Downregulated 8) can bind to cullin and this binding (named rubylation) seems to stimulate the activity of CRL *in vitro* by promoting E2 recruitment (Kawakami et al., 2001; Read et al., 2000; Wu et al., 2000). Moreover, it has been reported that COP9 signalosome (CSN) modulates the activity of CRL via the derubylation/deneddylation of cullin (Lyapina et al., 2001; Schwechheimer et al., 2001). Therefore, future studies on the role of CSN and rubylation in the aspect of cullin in abiotic stress signaling would help us understand how ubiquitination mediated by multi-subunit E3 complexes contribute to abiotic stress tolerance.

Here, we described the recent advances and future prospects in abiotic stress signaling mediated by E3-dependent ubiquitination in *Arabidopsis*. Because an enormous amount of E3 ligases still remain to be identified, the further pursuit of E3 enzymes involved in abiotic stress transduction could provide a functional connection between this signaling and the ubiquitination process, and furthermore, aid the generation of stress-resistant plants.

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