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Roles of ubiquitination-mediated protein degradation in plant responses to abiotic stresses

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ABSTRACT

Ubiquitination is a major modifier of signaling in all eukaryotes that results in the conjugation of ubiquitin to the lysine residues of acceptor proteins. The targeted protein is then subjected to degradation by the 26S proteasome, the major protein degradation system in eukaryotes. The ubiquitin–proteasome system (UPS) greatly influences plant growth and development by modulating the activity, localization, and stability of proteins. Plants are frequently exposed to various abiotic stresses during their life cycles; they rely on proteomic plasticity achieved by the UPS to adapt to unfavorable environmental conditions. In stress signal pathways, a large number of components are modified by specific ubiquitination machinery. In this review, we highlight recent advances in understanding the roles of ubiquitination in plant responses to abiotic stresses, including salt and drought, temperature, ultraviolet (UV), and nutrient availability. The review focuses primarily on the roles of the UPS. In salt and/or drought stress signaling, a number of E3 ligases mediate the stress response in both abscisic acid (ABA)-dependent and ABA-independant pathways. The UPS-mediated regulation of several key ABA-regulated transcriptional factors, e.g. ABI3 and ABI5, has been well documented. In cold signaling, the transcription factor ICE1 is targeted by E3 ligase HOSI for proteosomal degradation. Under UV stress, CUL4-DDB1A-DDB2 E3 ligase participates in DNA excision repair, and COP1 interacts with the UVR8 mediated UV response. The UPS is also involved in the uptake, transport, and homeostasis of nutrients such as iron, phosphorus, and nitrogen. SIZ1-mediated sumoylation, a ubiquitin-like modification, is necessary for a number of processes involved in plant responses to abiotic stresses. A challenge moving forward for researchers is to define more UPS components and to characterize their functions in plant responses to stress conditions; there is particular interest in identifying the ubiquitination targets that function in specific stress signaling pathways.

1. Introduction

Ubiquitin (Ub) is a highly conserved protein found in all eukaryotic species. This small protein is involved in the destruction of endogenous targets via the ubiquitin 26S proteasome system, the major proteolysis mechanism in eukaryotic cells (Hershko et al., 2000). Ubiquitination plays an important role in many processes in plants, including organ development, photomorphogenesis, hormone responses, as well as biotic and abiotic stress responses (Hershko et al., 2000; Lyzenga and Stone, 2012; Pokhilko et al., 2011; Santner and Estelle, 2010; Sonoda et al., 2009; Spoel and Dong, 2012).

Abiotic stresses such as high salinity, drought, low temperature, UV radiation, and nutrient deprivation have adverse impacts on plant growth, development, and reproduction. Plants rely on proteomic plasticity to remodel themselves to maximize their chances of survival under varying environmental conditions (Fujita et al., 2005; Hirayama and Shinozaki, 2010; Lee and Kim, 2011). A variety of abiotic stresses affect plant growth and development throughout their life cycles. As such, plants have needed to evolve diverse strategies to combat all these various forms of abiotic stress (Lyzenga and Stone, 2012). The ubiquitin system is one of the most important stress response systems, as it functions across numerous signaling pathways. In this review, we address recent advances to our understanding of the role of the ubiquitin–proteasome system (UPS) during plant abiotic stress signaling.

2. The UPS and UPS enzymes

Ubiquitin is a highly conversed protein of 76 amino acids found in all eukaryotes. Ubiquitin modifies target proteins to alter various aspects of their regulation via the UPS (Jentsch and Pyrowolakis,

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2000; Sadanandom et al., 2012). It has been supposed that ubiquitin has a compact structure called a 'Ub fold' with a five-strand mixed β-sheet that forms a cavity into which a single α-helix fits diagonally (Vierstra, 1996). Ubiquitination is mediated by the sequential action of at least three enzymes: the E1 (ubiquitin-activating enzyme, UBA), E2 (ubiquitin-conjugating enzyme, UBC), and E3 (ubiquitin ligase) (Kraft et al., 2005; Mukhopadhyay and Riezman, 2007; Smalle and Vierstra, 2004) (Fig. 1A). The E1 enzymes initiate the Ub conjugation cascade in an ATP-dependent reaction. Ubiquitin activated by an E1 enzyme forms what is known as an E1-ubiquitin intermediate. The activated ubiquitin is then transferred from the intermediate to the cysteine residue of the E2 enzyme. Finally, the E2–ubiquitin intermediate binds with E3 to deliver ubiquitin onto the substrate or E3 enzyme (Sadanandom et al., 2012; Smalle and Vierstra, 2004). The 3-step pathway creates an isopeptide bond between the C-terminal glycine of ubiquitin and a lysine residue on the target protein. A variety of substrate modifications are possible in the conjugation cascade, including the addition of a single ubiquitin molecule (monoubiquitination), the attachment of multiple ubiquitin molecules to different lysines on the same target protein (multiubiquitination), or the addition of different types of polyubiquitin chains (polyubiquitination). There are seven lysine residues (K6, K11, K27, K29, K31, K48, and K63) in ubiquitin, any of which are available for ubiquitin attachment to produce polyubiquitin chains (Kim et al., 2007; Kirkpatrick et al., 2006). The structure of the attached polyubiquitin chains seems to affect the fate of the target protein. K48-linked chains are the best-characterized type of polyubiquitin chains; they target substrate proteins for proteasomal degradation (Pickart and Fushman, 2004; Vierstra, 1996, 2009). Recent evidence indicates that K11 linked chains also commit target proteins for proteasomal degradation (Matsumoto et al., 2010). K63-linked ubiquitin chains are associated with both proteasome-independent cellular processes such as DNA repair, signal transduction, and receptor endocytosis (Hicke, 2001; Löfke et al., 2013; Pickart and Fushman, 2004), and proteasome-dependent pathways and serve as targeting signals for the 26S proteasome (Saeki et al., 2009).

In *Arabidopsis* (*Arabidopsis thaliana*), there are 2 E1s, more than 37 E2s, and 1400 E3s. *Arabidopsis* E1s are encoded by two genes (*AtUBA1* and *AtUBA2*) that synthesize approximately 123-kDa proteins with 81% amino acid sequence identity; both contain a cysteine residue in the putative active site for forming the ubiquitin thio-ester intermediate (Hatfield et al., 1997). They transfer the activated ubiquitin to E2 with near equal specificity and are similarly expressed in almost all tissues (Hatfield et al., 1997). E2 enzymes contain a 140-amino-acid UBC domain with a conserved cysteinyl residue required for accepting the ubiquitin from E1 to form the E2–ubiquitin intermediate (Kraft et al., 2005; Wu et al., 2003). E2s interact with E3s via their UBC domains (Kraft et al., 2005; Wu et al., 2003). The vast number and diversity of E3 ubiquitin ligases in the *Arabidopsis* genome facilitate the identification of specific substrates by the UPS (Lee and Kim, 2011). There are seven known types of E3 ligases in plants which can be divided into two groups: the single-subunit E3 ligases and the multi-subunit E3 ligases (Lyzenga and Stone, 2012) (Fig. 1B and C). The single-subunit E3 ligases can be further classified based on the presence of one of three domains: the Homology to E6AP C-terminus (HECT) domain, the U-box domain, or the Really Interesting New Gene (RING) domain (Fig. 1B).

The HECT-type E3 enzymes comprise the smallest of the E3 subfamilies. In the *Arabidopsis* and rice (*Oryza sativa*) genomes, only seven and eight to nine HECT-type E3 genes, respectively, have been identified (Downes et al., 2003). HECT E3 ligases have a conserved C-terminal HECT domain with 350-residues that was initially identified in human E6AP (Huibregtse et al., 1995). HECT E3 ligases differ from other E3s during the Ub transfer processes. During Ub transfer, HECT E3 ligases generate an Ub–E3 intermediate on a conserved cysteine residue of the HECT domain, then the Ub–E3 intermediate

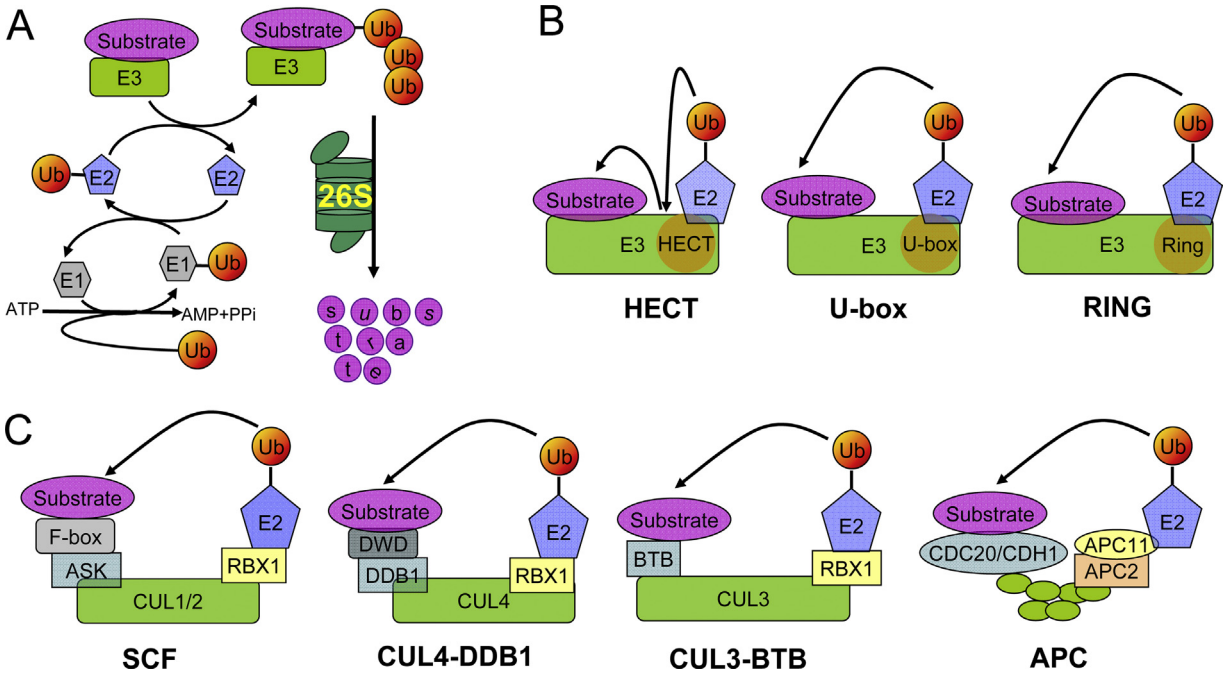

Fig. 1. The ubiquitination pathway and ubiquitin E3 ligases. (A) a ubiquitin activating enzyme (E1) activates ubiquitin in an ATP dependent manner. Ubiquitin is then tansferred to a ubiquitin conjugating enzyme (E2), forming an E2-ubiquitin intermediate. Ubiquitin ligase (E3) then recruits the substrate and attaches to the E2-ubiquitin intermediate, forming a complex. Ubiquitin is finally transferred from the E2–ubiquitin intermediate to an internal lysine of the substrate. After the initial ubiquitination of the substrate, additional ubiquitin can be added onto the already attached ubiquitin. (B) Single-subunit E3 ligases possess one of the three domains: Homology to E6-AP C-Terminus (HECT), U-box, or Really Interesting New Gene (RING). (C) Four types of cullin-based E3 ligases in plants. Cullins provide a platform around which the various complex subunits can assemble. ASK and DDB1 serve as adaptor proteins, attaching to the F-box and DWD substrate-recruiting proteins in CUL1/2- and CUL4-based E3s, respectively. APC11 provides the RING domain in the Anaphase Promoting Complex (APC) complex.