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REVIEW

Ubiquitin and Ubiquitin-like Modifiers in Plants

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Abstract Posttranslational modifications of proteins by small polypeptides including ubiquitination, neddylation (related to ubiquitin (RUB) conjugation), and sumoylation are implicated in plant growth and development, and they regulate protein degradation, location, and interaction with other proteins. Ubiquitination mediates the selective degradation of proteins by the ubiquitin (Ub)/proteasome pathway. The ubiquitin-like protein RUB is conjugated to cullins, which are part of a ubiquitin E3 ligase complex that is involved in auxin hormonal signaling. Sumoylation, by contrast, is known for its involvement in guiding protein interactions related to abiotic and biotic stresses and in the regulation of flowering time. ATG8/ATG12-mediated autophagy influences degradation and recycling of cellular components. Other ubiquitin-like modifiers (ULPs) such as homology to Ub-1, ubiquitin-fold modifier 1, and membrane-anchored Ub-fold are also found in *Arabidopsis*. ULPs share similar three-dimensional structures and a conjugation system, including E1 activating enzymes, E2 conjugation enzymes, and E3 ligases, as well as proteases for deconjugation and recycling of the tags. However, each of the ULP posttranslational modifications possesses its own specific enzymes and modifies its specific targets selectively. This review discusses recent findings on ubiquitination and ubiquitin-like modifier processes and their roles in the posttranslational modification of proteins in *Arabidopsis*.

Keywords *Arabidopsis* · Posttranslational modification · Ubiquitination · Ubiquitin-like modifiers

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Introduction

A major component of the organization and regulation of cell structure and function, as well as the development of the entire organism, is the contribution of a large number of posttranslational modifications to proteins. Such modifications introduce a superior level of complexity that is not achievable by gene content and gene expression characteristics alone, and more than 200 different ways of altering proteins are known to date. Many of these posttranslational modifications are also transient, and the dynamics of the introduced changes, which are genetically determined, generate yet another level of control. Posttranslational modifications can alter the activity, location, or lifetime of individual proteins, as well as their state of aggregation (Faye et al. 2005; Huffaker 1990; Kirschner 1999).

Posttranslational modifications of proteins include: (1) the addition of small functional groups like methane, acetate, and phosphate; (2) the attachment of fatty acids, sugars, or amino acids as well as other chemical groups; and (3) the covalent attachment of polypeptides such as ubiquitin (Ub) and/or other ubiquitin-like modifiers (Table 1). The modification of proteins causes rapid changes in biological function by affecting protein structure, activity, stability, and protein interactions (Ytterberg and Jensen 2010; Wold 1981). Ubiquitination and other ubiquitin-like modifier (ULM)-based posttranslational modifications such as sumoylation and neddylation are present in plants. The focus of this review is ubiquitination and the more recently identified and

Table 1 Common and important posttranslational modifications

PTM type	ΔMass (Da)	Stability[a]	Function and notes
Phosphorylation pTyr pSer, pThr	 80 80	+++ +/++	Activate (On) or deactivate (Off) enzymes reversibly. Modulate signaling transduction and pathway, regulation of enzyme activity. Involved in protein–protein and protein–ligand interactions. Phosphorylation and sulfation have the same Δm (80 Da), but can be distinguished by positive- or negative-ion mass-spectrometry analysis.
Acetylation (N-terminal Lys)	42	+++	Protein stability, protection of N terminus. Regulation of protein–DNA interactions (histone acetylation) leading to controlling gene expression.
Methylation Lys mono-, di-, and tri-, Arg mono and dimethylation	14 28 42	+++	Regulation of gene expression
Acylation Farnesyl Myristoyl Palmitoyl	 204 210 238	 +++ +++ +/++	Fatty acid modification involved in cellular localization and targeting to the membrane, protein–protein interactions.
Glycosylation N-linked O-linked GPI anchor	 >800 203, >800 >1,000	+/++ +/++ ++	Excreted proteins, cell–cell recognition/signaling O-GlcNAc, reversible, regulatory functions. Glycosylphosphatidylinositol (GPI) anchor, membrane tethering of enzymes and receptors, mainly to outer leaflet of plasma membrane. Protein stability, solubility, secretion signal, regulator of interactions, extracellular recognition and interactions; modification by a GPI anchor is coupled to protein processing.
Hydroxyproline	16	+++	Protein stability and protein–ligand interactions.
Sulfation (sTyr)	80	+	Modulator of protein–protein and receptor–ligand interactions.
Disulfide bond (Cys)	–2	++	Intra- and intermolecular crosslink, protein stability. Stabilizes protein structure and activity, involved in redox processes.
Deamidation (Asn, Gln)	1	+++	Possible regulator of protein–ligand and protein–protein interactions

Modified after Mann and Jensen (2003)

GPI glycosylphosphatidylinositol

[a] Stability: + labile in tandem mass spectrometry, ++ moderately stable, +++ stable

now intensely studied ULM-dependent posttranslational modifications in plants.

Ubiquitination

Ubiquitination consists of the reversible addition of the 76-amino acid (9 KDa) molecule ubiquitin in a system discovered in the early 1970s, for which a Nobel Prize was awarded in 2004 (Hershko and Ciechanover 1998). Ubiquitin targets lysine residues in many proteins, leading to different outcomes depending on the target protein, the number of added ubiquitin molecules, and the specific amino acids modified by ubiquitin in the target protein.

The best known fate of polyubiquitination is target degradation by the 26S proteasome, a 2.5-MDa ATP-dependent protease complex found in the cytoplasm and nucleus. The 26S proteasome consists of a 20S-core particle structure and two 19S regulatory caps that form part of the ubiquitin–proteasome system (UPS). The proteasome is composed of a cylindrical core that contains the proteolytic active sites and surrounds an empty space within which proteins are degraded. The two ends of the core associate with a 19S regulatory cap that includes ATPase active sites and ubiquitin binding sites. At least four ubiquitin molecules in the form of a polyubiquitin chain must be attached to a protein to target it for degradation. The activity of the UPS is mediated by the sequential action of three enzymes, namely E1, E2, and E3. The E1, or ubiquitin-activating, enzyme hydrolyzes ATP and adenylates ubiquitin, forming a thioester bond between a cysteine residue in E1 and the carboxy terminal glycine of ubiquitin. The activated ubiquitin is transferred to a cysteine residue of E2, the ubiquitin-conjugating enzyme, by a trans-esterification reaction. In the final step, a specific E3 ubiquitin ligase recognizes the target to be ubiquitinated and catalyzes the transfer of ubiquitin from E2 to the target protein. E3 ligases are a diverse class of proteins that play a key role in the determination of substrate specificity. Ubiquitination is also associated with non-

proteolytic cellular functions including vesicular trafficking and sorting, endocytosis, regulation of transcription, and chromatin structure modifications (Kerscher et al. 2006).

As a reversible process, ubiquitination also involves a ubiquitin deconjugation system composed of a number of de-ubiquitinating enzymes (DUBs). DUBs function to catalyze the removal of Ub from Ub-conjugated substrates. The *Arabidopsis thaliana* genome includes more than 27 DUBs that have been grouped into 17 subfamilies (Yan et al. 2000).

E3 ubiquitin ligases in plants

A fraction of 6% of the proteome was predicted to be related to the UPS in *Arabidopsis* based on sequence data showing two E1, more than 37 E2, and over 1,400 E3 genes, as well as more than 700 F-box subunits of the SCF E3 and more than 477 RING domain proteins (see below). Some of E3 enzymes found in plants are plant specific. The development of an extensive UPS system in plants may be a consequence of their sessile nature, which determines the need for increased regulation at the posttranslational level for the modulation of growth and development and for the physiological processes of plants (Bachmair et al. 2001; Vierstra 2009). Substrate-specific E3 ligases can be divided into two major groups distinguished by a homology to E6-AP C-terminus (HECT) or a RING/U-box domain (Moon et al. 2004; Pickart 2001). RING domain E3 enzymes can be divided into two classes: single subunit RING/U-box E3s, such as constitutive photomorphogenesis 1 (COP1), seven in absentia in *A. thaliana* 5 (SINAT5), and arm repeat-containing 1 (ARC1), and multisubunit RING E3 enzymes, including SCF, CUL3-bric-a-brac-tramtrack-broad complex (BTB), and anaphase-promoting complex (APC).

HECT E3 ligases

HECT-E3s are large proteins (100–400 KDa) that contain ubiquitin-binding and E2-binding sites. Ubiquitin forms a thioester intermediate with a cysteine in the HECT domain and is then transferred to a lysine residue in the target protein. HECT-E3s catalyze the covalent attachment of ubiquitin to its substrates, while other E3s interact non-covalently with the E2-ubiquitin intermediate. *Arabidopsis* has seven HECT-E3s named UPL1–UPL7 (ubiquitin protein ligase) (Downes et al. 2003). UPL1 and UPL2 were the first shown to have E3 ligase activities (Bates and Vierstra 1999), followed by the addition of an additional five UPLs (UPL3–7) to the group (Downes et al. 2003). The *upl3* and *kak*s (KAKTUS) mutants were shown to develop branched trichomes (Downes et al. 2003; Refy et al. 2003), and the *upl3* mutants develop longer hypocotyls than wild-type plants when grown in gibberellic acid-3 (GA_3) containing media. In addition, *upl3* plants show hypersensitivity to gibberellic acid (GA). These results are consistent with the fact that GA regulates trichome development (Downes et al. 2003). UPL4 was isolated as a small Ub-like modifier (SUMO)-binding protein, and the null mutant *upl4* phenotype was not different from that of wild-type plants (unpublished data).

Single unit RING E3 ligases

RING E3 ligases contain the really interesting new gene (RING) finger domain, which is composed of a series of histidine and cysteine residues with a characteristic spacing that coordinate two zinc ions (a characteristic termed C3HC4 or C3H2C3). The U-box includes a modified RING finger domain that does not contain the scaffold-stabilizing, zinc-chelating cysteine and histidine residues conserved in the RING domain (Yee and Goring 2009).

COP1, which contains a RING finger domain, a coiled coil domain, and seven WD40 repeats, was identified as a negative regulator of light responses in plants (Deng et al. 1991). Similar proteins are found in vertebrates as well (Yi and Deng 2005). COP1 directly interacts with elongated hypocotyl 5 (HY5), a bZIP transcription factor that functions as a positive regulator of photomorphogenesis in the nucleus, and promotes its proteasome-mediated degradation in the dark. HY5 accumulates to high levels in light-grown seedlings and becomes degraded via proteasome-mediated proteolysis during the light to dark transition. HY5 degradation during the light to dark transition is impaired in *cop1* seedlings. COP1-mediated ubiquitination targets long after far-red light 1 (LAF1), a myb transcription factor and positive regulator or phytochrome A-mediated far-red light signaling. It also controls long hypocotyl in far-red (HFR1), a basic helix–loop–helix transcription factor involved in far-red and blue light signaling. COP1 is localized to the nucleus and targets several photomorphogenesis promoting transcription factors (HY6, HYH, LAF1, and HFR1) for ubiquitination and subsequent degradation. Upon light exposure, COP1 levels in the nucleus are reduced, and the transcription factors are protected from degradation, leading to the activation of transcription of downstream genes (Yi and Deng 2005).

Another RING E3 ligase, SINAT5, which is homologous to SINA (seven in absentia) from *Drosophila* and seven in absentia homolog in humans, catalyzes the ubiquitination of NAC1, which is a NAM/CUC family transcription factor that functions downstream of the auxin receptor transport inhibitor response 1 (TIR1), to transduce the auxin signal for lateral root development (Xie et al. 2002). Overexpression of SINAT5 led to fewer lateral roots and

overexpression of a dominant negative mutant of SINAT5 generated excess lateral roots. The low level of NAC1 in the root was increased by treatment with MG132, a proteasome inhibitor.

ARC1 is a U-box E3 ligase from *Brassica*. Whether all 64 of the predicted *Arabidopsis* U-box proteins have E3 ligase activity is not yet known (Yee and Goring 2009). ARC1 was isolated as an interacting partner of the S receptor kinase SRK, which functions as a pistil-specific determinant of self-incompatibility (Stone et al. 2003). During self-incompatibility, SRK is degraded via ARC1-mediated ubiquitin-dependent proteolysis to promote pollen rejection.

Multisubunit E3 ligases

Multisubunit E3 ligases are cullin-RING ligases (CRLs) composed of a cullin, which is a hydrophobic protein that acts as a scaffold for E3 ligases, and a C-terminal RBX1 (RING Box 1)-binding domain including the RING domain and another component that functions in the recognition of target proteins. The RING proteins that form multisubunit E3 ligases are RBX1a and RBX1b (RING Box 1) (Gray et al. 2002). Eleven different cullins work as a scaffold in different CRLs, namely CUL1/2 in SCF (S phase kinase-associated protein 1/2 (SKP1))-cullin1 (CUL1)-F-box), CUL3 in the BTB, CUL4 in the DNA damage binding (DDB) E3, and a distinct cullin protein named APC2 in APC containing >11 subunits (Thomann et al. 2005; Vierstra 2009). APC is required for the degradation of key cell cycle regulators such as securin, cyclin A, cyclin B, and many of the mitotic regulatory kinases in animals and fungi that control metaphase to anaphase transition and exit from mitosis (Harper et al. 2002). However, the plant APC has not been well studied (Capron et al. 2003). CRLs can be classified according to their target recognition modules: F-Box proteins for SCF E3s, BTB proteins for BTB E3s, DWD (WD40 domain-containing proteins) for DDB (Moon et al. 2004; Vierstra 2009).

SCF [SKP1-cullin1 (CUL1)-F-box] contains three components and includes yet another subunit, the RING finger protein, RBX1. The *Arabidopsis* SKP is called *Arabidopsis* SKP (ASK). The *Arabidopsis* genome contains five cullins, 21 ASKs, two RBXs, and approximately 700 F-box proteins. F-box proteins are named for the F-box motif and the protein–protein interacting domains such as the leucine-rich repeat, WD40, and Armadillo (Arm) repeats for substrate binding. They are involved in plant growth and development, hormone homeostasis, photomorphogenesis, circadian rhythms, floral development, and senescence (see references in Dreher and Callis 2007; Moon et al. 2004; Smalle and Vierstra 2004; Vierstra 2009). Target proteins of SCR-mediated proteasome degradation include transcription factors, cell cycle regulators, among others. A few examples are detailed below.

$SCR^{TIR1/AFB1\text{-}3}$ (the name of the F-box protein is indicated in uppercase) is the first SCF complex found in *Arabidopsis*. Transport inhibitor response 1/auxin signaling F-Box protein 1–3 (TIR1/AFB1-3) belongs to the F-Box protein family and is a positive regulator of auxin responses. In the absence of auxin, the auxin/indole-3-acetic acid (AUX/IAA) protein acts as a transcriptional repressor of the auxin response by forming a heterodimer with the auxin response factor (ARF) transcription factor, which recognizes specific promoter elements in auxin response genes, leading to the inhibition of ARF's transcriptional activity. In the presence of auxin, AUX/IAA (domain II) binds to TIR1 (TIR1 pocket), resulting in its ubiquitination and rapid degradation. Free ARF can activate the expression of auxin-response and auxin-inducible genes (see Dreher and Callis 2007).

SCF^{SLY} (*Arabidopsis*) and SCF^{GID1} (rice) are involved in gibberellin signaling. DELLA proteins, which are named after their conserved N-terminal amino acid sequence (aspartic acid–glutamic acid–leucine–leucine–alanine), localize to the nucleus, where they act as negative regulators of GA responses in a process similar to that described for AUX/IAA in auxin responses. There are five DELLA proteins in *Arabidopsis* (GA insensitive; repressor of GA1-3,1; GRA like1 (RGL1); RGL2; and RGL3), one DELLA protein (slender rice1 (SLR1)) in rice and one in barley (Slender1 (SLN1)). Upon GA application, the rapid degradation of DELLA proteins is dependent upon their phosphorylation and subsequent proteolysis mediated by the SCF E3 complex and the 26S proteasome pathway. Sleepy (SLY) in *Arabidopsis* and gibberellins insensitive dwarf2 (GID2) in rice are the responsive F-box proteins for each SCF complex that recognize and ubiquitinate DELLA proteins in the presence of GA (see Dreher and Callis 2007; Moon et al. 2004).

SCF^{COI1} E3 ligases function in the ubiquitination and targeting of the repressor of jasmonate signaling JAZ (jasmonate ZIM domain protein) for degradation. JAZ proteins bind to and work as repressors of MYB2, a key transcriptional activator of jasmonate-regulated gene expression (Chini et al. 2007; Katsir et al. 2008; Thines et al. 2007).

$SCF^{EBF1/2}$ is the E3 ligase responsible for EIN3 degradation. Ethylene insensitive 3 (EIN3), which is a positive transcriptional regulator of ethylene regulated genes, is stabilized by ethylene, but in the absence of ethylene, EIN3 is degraded (Dreher and Callis 2007; Moon et al. 2004).

The CUL3-based E3 ubiquitin ligase $CUL3^{ETO1}$ targets ACS5 (1-aminocyclopropane-1-carboxylic acid (ACC) synthase 5), which is one of 12 ACS family genes. Ethylene overproducer 1 (ETO1) encodes a protein containing a BTB domain at the N-terminus and six predicted tetratricopep-

tide repeat) motifs and a coiled coil motif at the C-terminus, through which it directly interacts with ACS5. Overexpression of ETO1 in transgenic plants suppresses cytokinin-induced ethylene production and promotes the proteasome-dependent degradation of ACS5. ETO1 interacts with CUL3A in a ubiquitin ligase complex and plays a role as a substrate-specific adaptor for ACS5 (Wang et al. 2004). These findings show that the direct interaction of ETO1 with ACS5 inhibits its enzyme activity, and that ACS5 stability is regulated by its proteasome-dependent degradation through interaction with ETO1, which also interacts with CUL3-based ubiquitin E3 ligases (Wang et al. 2004). ETO1 interacts with other type 2 ACS members, namely LE-ACS5 and AtACS5 (type 2), but not with LE-ACS2 (type 1 ACS) and LE-ACS4 (type 3 ACS), indicating that the consensus type 2-specific C-terminal sequence of ACS proteins is important for the interaction with ETO1 (Yoshida et al. 2005).

Ubiquitin like modifiers in plants

Eukaryotic genomes encode several ubiquitin-related or ubiquitin-like proteins. They include related to Ub-1/neural precursor cell expressed, developmentally downregulated 8 (RUB1/NEDD8), SUMO, autophagy 8 (ATG8), ATG12, fau ubiquitin-like protein (FUB1), URM1, Ub-fold modifier-1 (UFM1), and homology to Ub-1 (HUB1). These proteins share a similar three-dimensional structure termed the Ub fold, which consists of a globular core with a pocket of four interacting β-strands within which α-helices are arranged diagonally. Although the structures of these proteins are remarkably conserved, their amino acid sequence identities with ubiquitin can be remarkably low. Other common features of ubiquitin-like proteins include a flexible C-terminus and a nearly invariant C-terminal glycine that targets lysine residues in the substrate proteins (Fig. 1). The only exception, lacking the terminal glycine, is HUB1 (see below). Furthermore, similar to the ubiquitin conjugation system, each ubiquitin-like modifier conjugation system contains its own E1 activating enzymes and E2 conjugating enzymes, as well as specific proteases that deconjugate the tags (ULM) from the targets and recycle the free tags (Fig. 2) (Table 2). Most tags (except for ATG12) are synthesized de novo as larger precursors that require processing by specific proteases to expose the C-terminal active glycine (Downes and Vierstra 2005).

RUB/NEDD8

Related to ubiquitin/neural precursor cell expressed, developmentally downregulated 8 (RUB/NEDD8) are 76-amino acid polypeptides that are conserved in all eukaryotes and show 52–63% identity to ubiquitin. *Arabidopsis* encodes three RUBs that share the Ub fold: AtRUB1 and AtRUB2 are nearly identical, showing a sequence difference of only one amino acid, and AtRUB3 is 77.6% identical to the other two RUBs (Callis et al. 1995; Rao-Naik et al. 1998). While AtRUB1 and AtRUB2 are expressed in most organs, the expression of AtRUB3 is restricted to stems and flower buds, and it is absent in leaves, mature flowers, and seedlings (Rao-Naik et al. 1998).

Similar to the ubiquitin conjugation system, RUB conjugation/modification is catalyzed by the RUB-activating E1 and the RUB-conjugating E2 enzymes (del Pozo et al. 1998). *Arabidopsis* heterodimeric RUB E1 activating enzyme is composed of auxin-resistant 1 (AXR1) and E1 C-terminal-related 1 (ECR1). The RUB-conjugating enzyme, RCE is expressed throughout the plant life cycle in all organs. Particularly high expression is seen in growing tissues such as the elongation zone in roots, shoot–hypocotyl junction, root hairs and root tips, as well as vascular tissue trichomes and flowers (Dharmasiri et al. 2003; del Pozo et al. 2002). Single homozygous T-DNA mutants of AtRUB1 and AtRUB2 show a wild-type phenotype, but double mutants are embryo lethal. Following dsRNAi knockdown of AtRUB1 and AtRUB2, viable plants were small, lost apical dominancy, were insensitive to auxin, and displayed the triple response in the dark without ethylene treatment (Bostick et al. 2004). AXR1 was originally screened and characterized based on its *axr1* auxin-deficient phenotype (Leyser et al. 1993). A mutant named *rce1-1* that contains a Ds insert 371 nucleotides upstream of RCE1 exhibited the *axr1* phenotype of an auxin resistance mutant. The *axr1/rce1* double mutant developed two cotyledons but lacked hypocotyls and did not develop roots. The *rce1-1* phenotype is reminiscent of *mp* and *bdl* mutants, which are deficient in auxin signaling (Dharmasiri et al. 2003). AtCUL1, a component of the cullin-based SCR^{TIR} ubiquitin E3 ligase, is a target of AXR1/ECR1-mediated RUB conjugation (del Pozo and Estelle 1999; del Pozo et al. 2002). Consistently, an AUX/IAA fusion protein was stabilized in mutant *ecr1-1* (Woodward et al. 2007). Another *rce1* allele mutant, *rce1-2*, with a 49-bp deletion in RCE1, developed very small plants and showed the triple response phenotype without ethylene in the dark. The phenotype resulted from increased ACO [ACC (1-aminocyclopropane-1carboxylic acid) oxidase] activity. In addition, the ethylene-inducible genes chiB (basic chitinase) and PDF1.2 (plant defensin 1.2), and jasmonic acid-inducible PDF1.2 were downregulated in the *rce1* mutants, suggesting that RUB might be involved in ethylene biosynthesis and induction of plant pathogen defense-related genes (Larsen and Cancel 2004). Recently, AXL (AXR1-like) was characterized for its function in rescuing the *axr1-3* auxin resistant pleiotropic phenotype. AUX/IAA proteins showed greater stability in double mutant

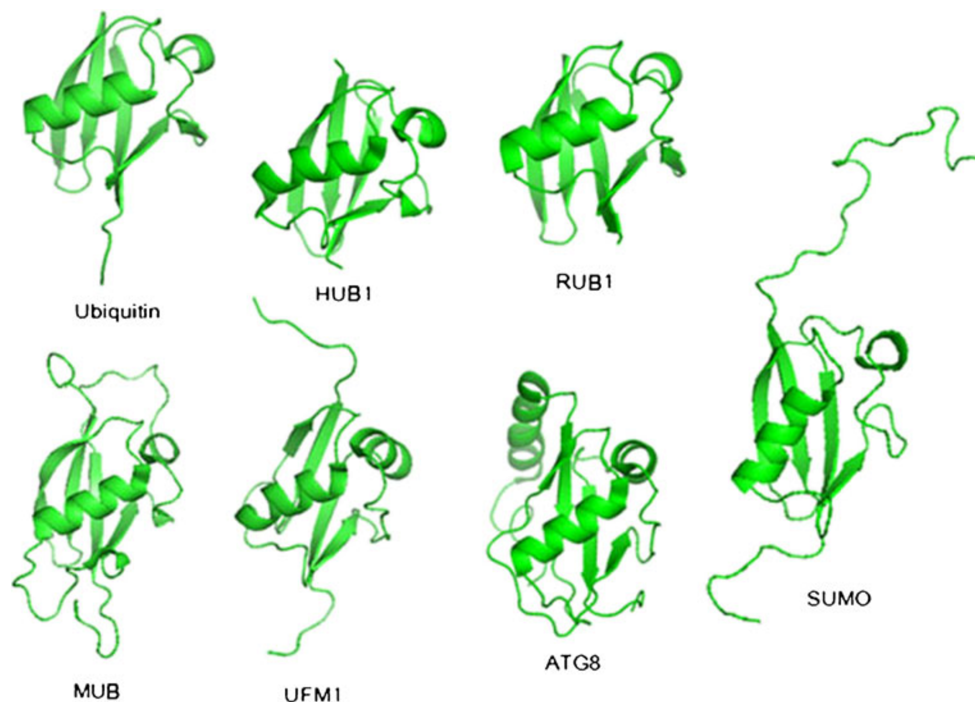

Fig. 1 Three-dimensional ribbon diagrams of several peptide tags for ubiquitin, SUMO1, UFM1, and ATG8. The structures of full-length (unprocessed) proteins are shown: human ubiquitin (1UBQ) (Vijay-kumar et al. 1987), yeast HUB1 (1M94) (Ramelot et al. 2003), *Arabidopsis* RUB1 (1BT0) (Rao-Naik et al. 1998), *Arabidopsis* MUB (1SE9) (Vinarov et al. 2004), human UFM1 (1WXA) (Sasakawa et al. 2006), yeast ATG8 (2KWC) (Kumeta et al. 2010), human SUMO (1A5R) (Bayer et al. 1998). pdb files were downloaded from PDB (http://www.rcsb.org/pdb/home/home.do), and diagrams of ubiquitin like modifiers were made using PyMol (www.pymol.org) (The PyMOL Molecular Graphics System, Version 1.3, Schrödinger, LLC)

axl-1axr1-12 than *axr1-12* plants, which accumulate more AUX/IAA proteins than wild-type plants (Dharmasiri et al. 2007).

With regard to E3 ligases, RBX1 in SCF functions as an E3 ligase by promoting the conjugation of RUB to a lysine residue in the VRIMK sequence motif of cullin subunits in cullin-based RING E3 ligases (Hotton and Callis 2008). The COP9 signalosome (CSN), which is conserved in eukaryotes and is related to the 19S lid complex of the 26S proteasome, deconjugates RUB from cullins. CSN consists of eight subunits, six PCI (proteasome, COP9, eIF3; CSN1–4, CSN 7–8) and two Mov34, Pad1 N-terminal (MPN; CSN 5 and 6) domain-containing proteins. Loss of function mutations in any of the subunits of CSN impairs its activity and results in the accumulation of RUB modified cullins, causing constitutive photomorphogenic/de-etiolated/fus phenotypes. These phenotypes include light-grown morphology even in the dark (constitutive expression of light-induced genes), anthocyanin accumulation, de-etiolation, and early seedling lethality (Hotton and Callis 2008; Serino and Deng 2003; Wei and Deng 2003). The cullin-associated and neddylation disassociated 1 (CAND1) protein binds to cullin-RBX1, blocking the binding between CUL1 and SKP1. CAND1 binds free deneddylated CUL1, and RUB conjugation to CUL1 promotes dissociation of CAND1 and association of SKP and F-box proteins to cullin, and the formation of an active SCF complex (Hotton and Callis 2008).

SUMO

SUMOs are approximately 12 KDa in size and are only approximately 15% identical to ubiquitin. Irrespective of the low sequence conservation, SUMOs are structurally similar to ubiquitin with the exception of a flexible N-terminus.

SUMO proteins are present in all eukaryotic organisms (van den Burg et al. 2010; Melchior 2000). A single gene encoding a SUMO protein is present in budding yeast, nematodes, and the fruit fly, while four isoforms have been identified in animals. *Arabidopsis* includes genes for eight isoforms of SUMO (AtSUM1–8) but only four of them, AtSUM1, 2, 3 and 5, have been found among expressed sequence tags. AtSUM1 and AtSUM2 are 99% identical. The fact that only the homozygous double mutant *sum1sum2* shows a phenotype, namely embryo lethality, indicates that AtSUM1 and AtSUM2 are functionally redundant (van den Burg et al. 2010).

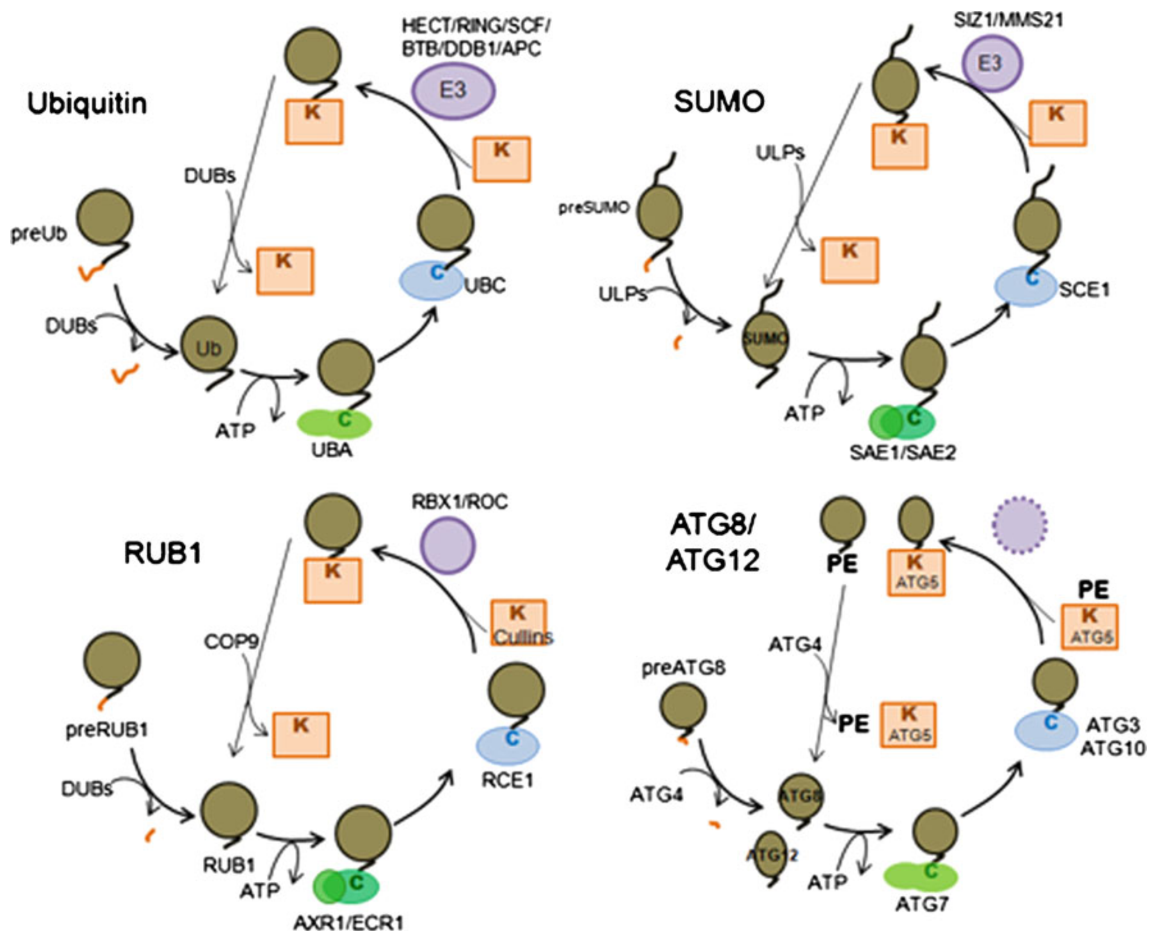

Fig. 2 The proposed functions of the seven polypeptide tags found in plants and components required for their processing, conjugation and disassembly. C, active-site cysteine; K, lysine acceptor site within the target that binds to the tag; PE, phosphatidylethanolamine. Whereas polymers of both ubiquitin and SUMO can be attached to targets by reiterative rounds of conjugation, the other tags appear to be attached as monomers. For SUMO and RUB1, the E1 consists of a heterodimer of two proteins. For RUB1, ATG8, ATG12, UFM1, and HUB1 conjugation, it remains unclear if an E3 enzyme is required. For ATG8 and ATG12, the only single targets that have been identified to date are PE and the ATG5 protein, respectively

The *Arabidopsis* SUMO-activating E1 enzyme is a heterodimer of SAE1 and SAE2. SAE1 is encoded by two genes, SAE1a, and SAE1b while SAE2 is encoded by a single gene. SAE1a/b and SAE2 are 28% to 33% identical to their

Table 2 Post-translational modification systems in plants[a]

Modifier	E1	E2	E3	Protease	Substrate and Function
Ubiquitin	UBA	UBCs	Many	DUBs	diverse
SUMO	SAE1 SAE2	SCE1	SIZ1, HPY2/MMS21	ULPs, ESD4	see the text
RUB	ECR1 AXR1	RCE	RBX1/ROC	COP9 signalosome	Regulation of cullin for plant development, including responses to auxin.
ATG8	ATG7	ATG3	ATG12–ATG5	ATG4	PE
ATG12	ATG7	ATG10	n.d.	n.d.	ATG5

[a] UBA (ubiquitin-activating enzymes), UBC (ubiquitin-conjugating enzyme), DUB (deconjugating enzyme), SAE (SUMO-activating enzyme), SCE (SUMO-conjugating enzyme), SIZ (SAP and Miz), HPY2 (high ploidy 2), MMS21 (methylmethanesulfonate-sensitive 21), ULP (ubiquitin-like protein-specific protease), ESD4 (early in short day 4), ECR1 (E1-conjugating enzyme-related 1), AXR1 (auxin resistant 1), RCE1 (RUB1-conjugating enzyme 1), RBX1/ROC (RING box 1/regulator of cullins 1), COP9 (constitutive photomorphogenic 9), ATG (autophagy); PE (phosphatidylethanolamine); n.d., not determined.

animal orthologs, but share conserved domains including the active site (Kurepa et al. 2003). SAE1a and SAE1b are 81% identical at the amino acid sequence level, suggesting functional redundancy (Saracco et al. 2007). The SUMO conjugation E2 enzyme is SCE1. Null T-DNA insertion mutants of SAE2 and SCE1 (*sae2* and *sce1*) are embryo lethal, showing arrest at the early embryonic stages (e.g., globular, heart, early torpedo), indicating that the E1 and E2 are essential for SUMO modification (Saracco et al. 2007).

In *Arabidopsis*, SIZ1 and methyl methanesulfonate-sensitive 21 (MMS21)/HYP2 (high ploidy 2) constitute the E3 SUMO ligases. Loss of function SIZ1 mutations result in dwarfism, elevated SA levels with increased PR1(pathogen-related gene 1) expression associated with resistance to bacterial pathogens, early flowering in short days, and hypersensitivity to cold and phosphorus-limited conditions. This combination of phenotypes has led to the discovery of several SIZ1 targets, among them phosphate starvation response 1, ABA insensitive 5 (ABI5), inducer of CBF/ DREB1 expression (ICE1), and FLD (flowering locus D) (Catala, et al. 2007; Lee et al. 2006; Jin et al. 2008; Miura et al. 2009; Yoo et al. 2006). The second SP-RING domain E3 ligase in *Arabidopsis*, AtMMS21/HYP2, is involved in cell proliferation and root meristem maintenance (Huang et al. 2009; Ishida et al. 2009).

The deconjugation of SUMO substrates or desumoylation is catalyzed by ubiquitin-like SUMO specific proteases (ULP). Seven SUMO specific proteases have been found to date, but only four of these *Arabidopsis* SUMO-specific proteases have been functionally characterized: AtEDS4, ULP1a/ELS1, ULP1c, and ULP1d (Chosed et al. 2006; Colby et al. 2006; Hermkes et al. 2011; Kurepa et al. 2003; Miura et al. 2007a; Miura et al. 2007b; Miura and Hasegawa 2010).

ATG8 and ATG12

Autophagy is a protein degradation process that leads to the recycling of cellular components and nutrients under stress conditions such as nutrient starvation, and also during senescence and programmed cell death. The two autophagic pathways that have been characterized are microautophagy and macroautophagy. In microautophagy, invaginations of the tonoplast membrane envelop cytoplasmic constituents that pinch off to form autophagic bodies to be deposited into the vacuolar lumen. Macroautophagy starts with the formation of cup-shaped membranes in the cytoplasm. The double membranes elongate/expand and eventually fuse together forming a double membrane vesicle called the autophagosome, which surrounds cytoplasmic material. The autophagosome fuses with vacuoles, resulting in an autolysome (Bassham 2007, 2009). Yeast autophagy (ATG) genes were initially found that constitute the molecular machinery of autophagosome formation, and ATG genes have now been identified in all eukaryotes including plants (Ohsumi and Mizushima 2004; Thompson and Vierstra 2005). Two ubiquitin-like conjugation (ATG8 and ATG12) systems are involved in autophagosome formation. ATG12 does not show amino acid sequence homology to ubiquitin, but shares the three-dimensional structure of the ubiquitin fold (Suzuki et al. 2005). The ATG12 (21 KDa) system consists of ATG12 (a ubiquitin-like tag), ATG7, ATG10, and ATG5 (targets). The C-terminal glycine of ATG12 is activated by the E1-like enzyme ATG7, and transferred to ATG10, which is the corresponding E2-like enzyme. ATG12 is conjugated to ATG5 forming a stable ATG12-ATG5 complex that subsequently develops into a multimeric complex with ATG16.

The ATG8 system consists of ATG8, ATG7, ATG3, ATG4, and the lipid phosphatidylethanolamine (PE). ATG8 is processed by the ATG4 protease exposing the C-terminus glycine, and it is then conjugated to PE in a process mediated by the E1-like enzyme ATG7 and the E2-like enzyme ATG3 (Bassham 2007). Although the ATG8 and ATG12 conjugation pathways share the E1-like enzyme ATG7, the two systems utilize different E2-like enzymes, which is probably due to their different targets. The ATG12-ATG5 conjugate, which promotes the formation of ATG8-PE conjugates, is a ubiquitin protein ligase E3-like enzyme that is essential for ATG8-mediated autophagy (Chung et al. 2010; Fujioka et al. 2008; Hanada et al. 2007). Loss of function mutations in the genes involved in these pathways fail to form autophagic bodies in the vacuole and show early senescence as well as hypersensitivity to nitrogen and/or carbon starvation (Chung et al. 2010; Doelling et al. 2002; Phillips et al. 2008; Thompson et al. 2005; Yoshimoto et al. 2004).

Homology to Ub-1

HUB1 was first found in budding yeast by sequence analyses querying for ubiquitin-related proteins (Dittmar et al. 2002). Irrespective of its low sequence identity to ubiquitin (22%), HUB1 shows a three-dimensional structure nearly identical to the Ub-fold. Unlike other ubiquitin-like modifiers, HUB1 lacks a glycine residue at its C-terminus conjugation site. The E1 or E2 enzymes in the HUB conjugation pathway have not yet been identified. HBT1 (HUB1 target, YDL223c) and Sph1 are known as target proteins that are involved in the formation of mating projections (Dittmar et al. 2002). In hub1Δ mutants, the subcellular localization of HBT1 and Shp1 was disrupted, suggesting that HUB1 conjugation is important for polarization during mating in yeast (Dittmar et al. 2002). The *hub1* mutation leads to splicing defects and mislocalization of nuclear splicing factors (Wilkinson et al. 2004; Yashiroda and Tanaka 2004). HUB1 homologs are present in

Arabidopsis based on sequence searches, but it is unknown whether a functional HUB1 conjugation pathway exists in plants (Downes and Vierstra 2005; Miura and Hasegawa 2010).

Ubiquitin-fold modifier 1

The 9.1 KDa small protein UFM1 shows only 14% sequence identity to ubiquitin but contains the typical Ub-fold (Sasakawa et al. 2006). UFM1 is synthesized as a precursor protein with two amino acids at its C-terminus that are cleaved to expose a glycine residue for conjugation by the UFM1-specific proteases UfSP1 and UfSP2 in yeast (Kang et al. 2007). UFM1 conjugation involves a specific E1-like protein, UBA5, and E2-like UFC1 enzymes for activation and conjugation, respectively (Komatsu et al. 2004). A recent report described the identification of an E3 ligase for UFM1 conjugation (ufmylation) termed UFL1 (Tatsumi et al. 2010). Yeast C20ofg116, which is the only known target of UFM1, is modified by UFM1 in a reversible conjugation process. In contrast to the components of other ubiquitin-like modifier conjugation reactions, which are nucleus localized, the E3 ligase for UFM1 modification, UFL1, and its target C20orf116 mainly localize to the endoplasmic reticulum. UFM1, UBA5 (E1), and UFC1 (E2) have been identified in the *Arabidopsis* genome and a single protease homologous to UfSP2 was found in *Arabidopsis* (Kang et al. 2007; Komatsu et al. 2004). It is predicted, but not yet proven, that UFM1 modification occurs in plants as well.

Membrane-anchored Ub-fold

AtMUB1, whose gene was found in *Arabidopsis* database searches for Ub-fold proteins only recently (Downes et al. 2006), shows 20% amino acid sequence identity with ubiquitin but includes the signature Ub-fold. The human membrane-anchored Ub-fold (MUB) ortholog, HsMUB, was discovered in 1999, and originally named ubiquitin-like protein 3 (Chadwick et al. 1999; de la Cruz et al. 2007). MUB proteins are conserved in other plants as well, and they are found in fish, fruit flies, worms, and filamentous fungi. However, the MUB gene sequence is not detected in budding and fission yeasts, suggesting that MUBs are restricted to multicellular eukaryotes. MUBs include the domain cysteine–aliphatic–aliphatic–any amino acid (CAAX) at the C-terminus, which can be prenylated with either a farnesyl or geranylgeranyl moieties. *Arabidopsis* has six MUBs: five terminate with CAAX, while a sixth ends in a cysteine-rich sequence distinct from the CAAX box. However, all MUBs are membrane-anchored on the plasma membrane. AtMUBs are expressed in all plant tissues, and single homozygous T-DNA inserted mutations in AtMUB2 and AtMUB3 behave like wild type under standard growth conditions, suggesting the functional redundancy of AtMUBs (Downes et al. 2006). AtMUBs interact noncovalently with ubiquitin E2 conjugation enzymes (UBCs), which they seem to recruit to the plasma membrane (Dowil et al. 2011).

Conclusion

Posttranslational modifications of proteins including Ub-like modifications allow transient or/and rapid changes to existing proteins. These changes result in the modulation of biochemical properties such as protein interactions, activities, and cellular location. Thus, posttranslational modifications provide a means for regulating signaling and biochemical pathways, development and responses to the environment in plants. Posttranslational modifications therefore impose an additional control circuit that extends transcriptional control and the tissue and cell specificity of biological processes.

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